Oceanography Committee

ICES CM 1998/C:3
Ref.: ACME

REPORT OF THE

WORKING GROUP ON PHYTOPLANKTON ECOLOGY

Lisbon, Portugal
19–24 March 1998

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International Council for the Exploration of the Sea
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1 OPENING OF THE MEETING

The meeting was opened by the chairman, Prof. Franciscus Colijn at 10.00 hrs on 19 March 1998. The chairman welcomed the members and participants of the Working Group meeting and thanked the local organiser Dr Maria-Antonia Sampayo for her help in making hotel reservations, guiding the participants to the hotel and to the IPIMAR facilities. Then the chairman passed to Dr Carmen Lima, the director of IPIMAR, who welcomed the participants on behalf of the institute. In her address she stressed the importance of phytoplankton for the productivity of the sea, and the problems of some phytoplankton species because they affect the quality of seafood for human consumption. She wished the participants a fruitful meeting and mentioned the Annual Science Conference later this year at Estoril hosted by Portugal.

The chairman started the meeting with a series of announcements mainly regarding ICES business and new questions for the Working Group on Phytoplankton Ecology which came up recently, and which will be dealt with in the agenda.

A few members have informed the chairman that they were unable to attend the meeting: Sakshaug (Norway), Richardson (Denmark), Bode (Spain); no members are attending from the Netherlands, Belgium, the Baltic States, Poland, Ireland and France. Still effort should be given to try to get more scientists involved in the work of the Working Group on Phytoplankton Ecology, mainly because a series of interesting scientific and applied problems are discussed (eutrophication, marine food web structure and regulation, global change etc.).

The chairman mentioned that the following papers are available for discussion:

Check list of phytoplankton species of the Northwestern Iberian Atlantic (1948–1996) by M. Varela, A. Bode and J. Lorenzo (Annex 4)

Phytoplankton species composition in the Southern Iberian coast, by A. Bode and M. Varela (Annex 5)

Working manual on the use of a standard incubator-technique for primary production measurements, by F. Colijn and L: Edler; (Annex 7)

Flowcytometry as a tool for counting and identification of phytoplankton (groups) and other applications by G. Dubelaar and R. Jonker; (Annex 8)

Extract from the 1998 Draft Report of the marine Chemistry Working Group, Stockholm, 2–6 March 1998, 8.3.3.d. Quality assurance aspects in the determination of chlorophyll a in sea water (Annex 9)

Determination of chlorophyll a by spectroscopic methods: overview and recommendations for quality assurance by A. Aminot (this paper originates from the Marine Chemistry Working Group and will be available through Marine Chemistry Working Group or ICES; it will not be annexed to this report)

The chairman then informs the members on the status of the proceedings of the Kiel 1997 Variability Symposium, held just one year ago. The physical problems of the chairman after a second Achilles tendon rupture, have caused a delay in the acceptation procedure of many manuscripts. However about 15 have been accepted and are with the final editor of the ICES J. of Marine Science, Niels Daan in the Netherlands. Another 15 are on its way of being accepted or rejected. It will take another 2 months to finalise all manuscripts. There is good hope that the volume can be printed this year.

The chairman shortly informed about a recent study at his institute in commission for the German Environmental Ministry on the preparation of phytoplankton samples for intercomparison studies between institutes and companies which like to be involved in phytoplankton monitoring studies. The high degree of homogeneity of samples to be counted and identified by participants is a prerequisite for an intercomparison.

An ICES/HELCOM workshop/training course on phytoplankton (WPHYT) will be held under the chairmanship of A. Andersson-Nordström in Klaipeda (Lithuania) from 24 to 28 August 1998.

During the meeting the chairman will insert an agenda item (TOR i) on (EURO)GOOS and its role in ICES and the ideas of the Working Group on Phytoplankton Ecology on this item. (the terms of reference on this item can be found under the steering group on GOOS, chairman R. Saetre, stating that the chairmen under the newly formed Oceanography committee have to comment and support R. Saetre by correspondence).

Another item which needs to be discussed is the 5- year plan of the newly formed Oceanography Committee (Chairman Harald Loeng, IMR, Bergen, Norway), to which the Working Group on Phytoplankton Ecology will report. We need to come up with a plan what we are actually going to do in the next years. Moreover we should discuss the possibility to build stronger links to other WG's under the Oceanography committee like Working Group on Zooplankton Ecology, Working Group on Shelf Seas Oceanography, ICES/IOC Working Group on Harmful Algal Bloom Dynamics etc. A first step will be the joint meeting with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics during the meeting in Lisbon on the 24th of March.

The contents of the 2001 ICES variability symposium was asked for. Apart from the Convenors and the venue no official information is yet available. The symposium will have structure comparable to the one held in Mariehamm in 1991 on decadal variability. New results of plankton long term variability studies could well be presented there.

After these announcements a short introduction round was made because a few new members and colleagues of IPIMAR and the University of Algarve were attending the meeting.

After adoption of the agenda (Annex 1), Dave Mills was appointed as rapporteur. A few final arrangements about coffee/tea and lunch breaks were settled. Then a coffee break was held before the discussion on the TORs started.

2 TORS

a) review progress in the preparation of a practical check-list of all phytoplankton occurring in the ICES area, with special emphasis on toxic species and species known to cause harm;

b) propose a mesocosm experiment to investigate new approaches in phytoplankton ecology, in a joint meeting with the ICES/IOC ICES/IOC Working Group on Harmful Algal Bloom Dynamics;

c) identify and discuss methods for the measurement of phytoplankton biomass, production and growth rate in situ, and its identification, including QA procedures;

d) discuss and exemplify effects of anthropogenic inputs of nutrients including changed nutrient ratios over time on the phytoplankton community, with special emphasis on phytoplankton bloom development and phytoplankton community changes;

e) assess monitoring strategies of the pelagic ecosystem and their practical outcome in monitoring programmes within the ICES area;

f) review in a joint session with ICES/IOC Working Group on Harmful Algal Bloom Dynamics on 24 March the results of the Workshop on Development of in situ growth rate measurements of Dinoflagellates held in Kristineberg;

g) review in a joint session with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics on 24 March the status of taxonomic coding systems with a view to recommend the adoption of a single coding system for use in ICES;

h) consider the future work programme in relation to the remit of the Oceanography Committee and the development of the ICES five year plan, including cooperation with other working groups.

i) (new/added) discuss the role of ICES in (EURO)GOOS and report suggestions to the chairman of Steering Group on the Global Ocean Observing System, R. Saetre inter alia to prepare an action plan for how ICES should take an active and leading role in the further development and implementation of GOOS at a North-Atlantic regional level with special emphasis on operational fisheries oceanography.

3 DISCUSSION OF TOR'S

a) review progress in the preparation of a practical check-list of all phytoplankton occurring in the ICES area, with special emphasis on toxic species and species known to cause harm;

This TOR was discussed in a general sense. The chairman opened the discussion by stating that he had tried to prepare this TOR through a discussion with M. Elbrächter. The result of this discussion was a proposal to be discussed with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics on setting up a meeting of a few days with taxonomic experts to check available checklists for most of the ICES subregions.

A list of known checklists and phytoplankton identification literature was produced during last years meeting of the Working Group on Phytoplankton Ecology. This list was extended further (Annex 10). The WG decided that also species lists of phytoplankton that are not published, but in use in different laboratories should be made available, as they are of great value in the development of lists covering the entire ICES area. In case these lists were not yet available they could be before the experts meeting in winter 1998–1999. A discussion with Henrik Enevoldsen (IOC) showed that

IOC is also interested in this activity and might be willing to support it. The checklists however should be available before the experts convene. Speed is needed for this activity because some experts will be retired soon (e.g., Drebes) and working up material later is difficult and not guaranteed. The chairman will ask permission from the RIKZ in the Netherlands to use their material to compile a Dutch checklist with the help of TRIPOS.

The Working Group on Phytoplankton Ecology then discussed possible ways of preparing a practical „ICES Checklist of Phytoplankton" and its contents. It was agreed that *practical* in this case means - what is possible to produce now. When having made the „ICES Checklist of Phytoplankton" several smaller checklists, covering regions should be extracted. Examples of such regions are: the Baltic Sea, the Kattegat and Skagerrak, the North Sea, the waters around the British Isles, the French waters, Iberian-Atlantic waters, Icelandic waters, St. Lawrence Estuary, Canadian Atlantic waters, north-east USA waters, south-east USA waters.

The possible role of the ETI in Amsterdam (Expert Taxonomic Institute) which had been involved in setting up the former Linnaeus CD Rom was mentioned several times. Upgrading and extension of the work done would be very appreciated. Several members suggested to try to encompass much more than before electronic media for exchange purposes (electronic www checklist; transmission of electronic/ video pictures etc.). Accreditation of phytoplankton analysis requires reference material. This is accomplished by saving computerised pictures which may be checked by taxonomic experts regularly. A further suggestion was to add to the electronic species list eco-physiological details on species. However this would certainly enhance the amount of work considerably.

The work of compiling, adding synonyms and checking the validity of the checklist is a heavy workload, the idea of applying for an EU project was therefore discussed. As several bodies, such as IOC/UNESCO, ICES, OSPAR and HELCOM have a considerable interest in a useful checklist support from these bodies can be anticipated.

It was further agreed that the coding of species is a technical and not a taxonomic problem and should be solved by computer experts in consultation with phytoplanktologists.

Lars Edler will try to compile during the meeting any further extensions to the list given in last year report (see Annex 10). That should be the basis for the material to be checked by the experts. Several members reported about lists which were under construction. Finally publication of the list in the ICES Journal of Marine Science was strongly supported.

All members will give input to this TOR. Recommendations on the proposed meeting were formulated and are given in Section 6.

b) propose a mesocosm experiment to investigate new approaches in phytoplankton ecology, in a joint meeting with the ICES/IOC ICES/IOC Working Group on Harmful Algal Bloom Dynamics (see report section under 4);

Before discussing this item with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics the chairman invited the members to come up with suggestions to formulate such a mesoscosm experiment, which not necessarily needs to be performed together with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics. However to improve cooperation and use expertise from both groups it would be useful to discuss it together. Several suggestions were made on the principal questions to be raised and which could function as a hypothesis to be tested. One of the possibilities which obtained a lot of support was to test the causes and meaning of diel variability in phytoplankton processes. By setting up this experiment with a species relevant for the ICES/IOC Working Group on Harmful Algal Bloom Dynamics it might well be possible to perform this together. The outline of a possible experiment were set by T. Smayda. His suggestions are the following:

Proposal for an experiment

Diel periodicity in flagellates - causes and consequences

Background

The periodicity observed in natural populations of phytoplankton is largely a result of entrained signals which have both a biological and a physical component. Diel periodicity in diatom dominated population results primarily from the interaction between fluctuation at the cellular level and physical forcing. Periodicity in flagellate dominated populations are further complicated because of the additional behavioural component and our understanding of flagellate bloom dynamics is therefore limited.

Objectives

- to identify the sources of variability in observed diel periodicity in flagellates
- to determine critical rate processes and activities
- to determine and critically evaluate the role of physical forcing with special emphasis on light

Rationale and approach

Observed diel variability with a biological cause include motility and migration, circadian rhythms in cell divisions, photosynthetic parameters, cell cycle (e.g., C:chl), changes in specific gravity and grazing. Physical forcing likely to impact upon short term changes include, light, and mixing and also nutrient regime.

In particular, the role of light is seen as critical with a particular advantage of mesocosm approach being natural irradiance. The natural light field has a number of elements, intensity, photo-period and quality (wavelength). Irradiance level is critical for photosynthesis, photo-period for cell division and light quality impacts upon both. Light also has other unique attributes in contrast to all other physical factors. It cannot be mixed, it is the only variable to fall to zero (excluding moonlight) and it is 'recharged' daily but not always to the same level.

An important aspect of this study will be to ensure that a sufficiently intense vertical gradient in light and nutrient (macro and micro) concentration are developed to allow development of a range of growth conditions (niches). This will allow the major behavioural component (vertical migration) to be studied.

A key element of our approach will be to ensure that sampling frequency is sufficient to resolve the variability of critical parameter in time and space. This implies some measurements will have time scales of < 1 minute and vertical resolution of < 10 cm.

Benefits

Such a study would benefit both observational and modelling studies of flagellate (possibly harmful) bloom dynamics. Many observational programmes rely upon simple, low frequency measurements to characterise the state of growth and the associated physical-chemical environment of phytoplankton populations. Our confidence in such measurements is limited by our awareness of rapid change in some of these parameters in space (depth) and time (sub-daily). Future observational programmes could, therefore, be better designed in terms of sample frequency (both in space and time) and choice of parameters.

Better understanding of rate processes and activity would lead to improved diagnostic models of flagellate bloom dynamics with 'fingerprinting' (identifying external conditions which select for a particular species) contributing to the development of prognostic models with the potential to predict development of flagellate blooms.

A spin-off of this work is that we will use the opportunity to evaluate a range of newly emerging (bio-optical) techniques to provide information on state (e.g., chlorophyll biomass, accessory pigment concentration) and rate (ϕ, α, Pb) variables. These techniques also have the advantage of providing the high frequency (spatial and temporal) measurements already identified as of particular interest in this study.

Problems to be addressed

Based on initial feedback from the Working Group on Phytoplankton Ecology members a number of issues have been raised and are outlined below.

- how do you collect large samples with out disrupting vertical structure
- how do we ensure the development of a population of flagellates (natural vs cultured)
- the presence of a nutrient rich aphotic zone is desirable
- dimensions of the mesocosm are critical if naturally generated turbulence is desired
- how will the work be funded and where will it be carried out

A table is attached outlining the possible sites for such an experiment. The list is not exhaustive and will be completed later. Funding for travel and subsistence could be secured by opting to use a mesocosm designated as a Large Scale Facility by the EU.

Although an exhaustive literature search is still outstanding it is clear that new developments in technology for making measurements in the sea (and therefore mesocosms) and emergence of new disciplines (e.g., molecular biology) will enable us to carry out our investigation with advantages over previous work. These developments are particularly significant in biology and chemistry where through the use of novel sampling (íntelligent´ water samplers), high frequency electronic sensors and (bio-) optical techniques (e.g., FRRF, UV nitrate sensors) we can resolve differences in the vertical of < 10 cm. These technologies, in particular, bring us into the same realm as physicists in being able to resolve very small vertical differences. Furthermore, these fine scale measurements will include rate and state variables, critical in the approach to this study. A further advantage of such techniques is there ability to resolve rapid changes in time when used at a fixed point. Judicious use of such techniques in this proposed work will provide detailed and matching spatial and temporal variability of physical, biological and chemical parameters within our mesocosm. It is unlikely that such an approach has been taken elsewhere.

Although many questions remain to be addressed the nature of the mesocosm and its potential vertical physical structure are crucial. Clearly we envisage a mesocosm with vertical gradients that allow the development of specific niches for flagellates. Dimensions of the mesocosm are likely to be critical in facilitating the development of such gradients and we can learn from previous work. Although, we would wish to allow the natural development of vertical gradients we may have to consider intervening to artificially generate such conditions. These considerations will play an important part in determining the feasibility of the mesocosm approach and require attention at an early stage in the design of the experiment.

In deciding what constitutes a successful outcome to the proposed work we need to consider whether we require specific information about targeted species or whether we are more interested in a generic approach. Perhaps the answer to this question will reflect the interests of the two working groups. There are obvious advantages and disadvantages for both approaches. Generic conclusions may have wider significance although interpretation with respect to particular species is likely to be more difficult. In contrast, targeted work may yield valuable insights on particular species but may be of limited value to the wider community. Clearly, our strategy need to be thought through carefully. Finally, it is worth noting that a successful experiment could be carried out with either a toxic or non-toxic species.

Another suggestion made by Wolfgang Hickel was to use a mesocosm to control growth conditions in such a way that potential toxic species do form toxins. *Chrysochromulina* would be a good candidate for such a study.

Before making recommendations on a repeated mesocosm experiment under ICES responsibility/flag our ideas were discussed in a joint session with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics, where several comments on the mesocosm experiment were made (see Report on Joint Meeting including Agenda, Topic 4, and Annex 12).

c) identify and discuss methods for the measurement of phytoplankton biomass, production and growth rate in situ, and its identification, including QA procedures;

The Working Group on Phytoplankton Ecology has been active in this field over the last years. The chairman had listed in the annotated agenda the main items on which the WG had worked: the standardisation of the ^{14}C method for monitoring purposes; the standardisation of the chlorophyll a measurement and introduction of new promising techniques in phytoplankton ecology like fluorescence methods to study photosynthesis and flowcytometry to study phytoplankton species identification.

-1. Measurement of phytoplankton biomass: chlorophyll a.

The Working Group on Phytoplankton Ecology has again discussed the matters related to the development of a standard procedure for the measurement of chlorophyll *a* in seawater, although it was not on the meeting's terms of references. This was due to a communication from the ICES Marine Chemistry Working Group, including a document on the measurement of chlorophyll *a* prepared by Alan Aminot from IFREMER, that was made available to the Working Group on Phytoplankton Ecology for comments,. The document agreed in most part with the recommendations from the Working Group on Phytoplankton Ecology report form 1997, except on the subject of storage of the filters. The Working Group on Phytoplankton Ecology recognised the fact that storage at -20 ° C is indeed a good storage procedure easily to recommend when only chlorophyll *a* is being measured since no phaeopigments were produced during the different forms of storage. This fact was already recognised in the 1997 Working Group on Phytoplankton Ecology meeting but it did not come clearly forward in the report. Some others comments were also made, mainly regarding different suggestions on the Quality Assurance procedures.

At the meeting it was also reported that contact have been made with Dr Shirley Jeffrey from the SCOR WG 78, who was the main scientist behind the UNESCO book (Phytoplankton pigments in Oceanography, 1997) on which most of

the information that leaded to the recommendations of both ICES WG was based on. Dr Jeffrey has promised to continue her work on the development of an equation for chlorophyll *c* in methanol. This together with recently improved equations for chlorophyll *a* and *b* will make it possible to develop the same kind of trichromatic equation as for 90 % acetone and provide the means to carry out a thorough comparison of both extract solvents as suggested by the 1997 meeting.

The Working Group on Phytoplankton Ecology recommends also to establish contact with the Marine Chemistry Working Group in order to propose working together by mail in producing a common document that includes the recommendations and opinions of both groups, specially regarding QA procedures. This work should start as soon as possible in order to have the document ready for the Oceanography Committee well ahead of the Annual Science Meeting to be held in September in Portugal. After approval by ICES, the document will also be made available for other scientific communities as OSPARCOM and HELCOM.

-2. Measurement of primary production: a standard incubator protocol and device.

Within the ICES community there is a very strong interest in ^{14}C production measurements with the newly developed incubator and standard measuring protocol. The protocol has once more been distributed and a few additional remarks were made for improvement. However, the manual was not meant for further additions in the procedure because it would bring us away from the 'simple and inexpensive incubator`. However Lars Edler and the chairman were willing to incorporate some of the remarks as alternative options in the manual. Also a few other details will be reconsidered. Together with the Annexes the manual will go to ICES for publication; a strong plea was held to combine the procedure with a standard calculation based on software available for everyone using the method. Addresses of interest for users of the method will be made available by the editors of the method. Drafts of the Manual and annexes will be made available through photocopies and if possible through the FTZ website, as long as the procedure has not been published officially (The Manual and Protocol are printed as Annex 7)

-3. Measurement of species composition: identification by flowcytometry.

Based on last years report the chairman had invited two Dutch colleagues (Dubelaar and Jonker) to write a state of the art paper on the possibilities of flowcytometry in the identification of phytoplankton. The paper is presented as Annex 8.

-4. Advanced plankton monitoring and smart moorings.

Dave Mills presented information on attempts to improve the monitoring of plankton, by updating the continuous plankton recorder with new sensors, and by using smart moorings. The information is copied as Annex 11 (two pages).

Finally it was decided to prepare for next years meeting a document on the state of the art of fluorescence measurements (F. Colijn) and on the growth of phytoplankton (T. Smayda).

d) discuss and exemplify effects of anthropogenic inputs of nutrients including changed nutrient ratios over time on the phytoplankton community, with special emphasis on phytoplankton bloom development and phytoplankton community changes;

The American East coast

A clear linkage between increased algal bloom events and nutrient enrichment has not been established for the coastal waters of the eastern United States. Certainly, novel blooms and harmful and benign species have occurred, just as nutrient-enriched coastal waters are recognisable. But these blooms and nutrification sites are not identical. There is currently growing concern, however, over a possible linkage between agriculturally derived nutrification and blooms and the notorious ichthyotoxic, and associated hazard to public health, species, *Pfiesteria piscicida* in Chesapeake Bay and North Carolinean estuaries. However, the evidence at best, is anecdotal and awaits verification in the form of a quantitative, year-round study in representative nutrient enriched and pristine habitats within the known geographical range of bloom events of *Pfiesteria*. (e.g., Burkholder & Glasgow, 1997).

The Baltic Sea

In spite of all protection measures taken by all countries around the Baltic Sea, the sea reflects the changes very slowly. Based on the results of the Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea, some improvement of the environmental situation was observed.

In the beginning of the 1970s a strong increase in phosphorus and nitrogen concentrations was observed. By the early 1980s the results of rapid nutrient increase became a problem in many areas around the Baltic Sea. Intensive algal blooms indicating increasing eutrophication appeared to occur more frequently. There was evidence that phytoplankton primary production had doubled in the area from the Kattegat to the Baltic Proper, with a similar doubling of phytoplankton biomass and its subsequent sedimentation. Consequently, low oxygen concentrations in late summer and autumn were often observed in the southern Kattegat, the Belt Sea, the Sound and the Arkona Basin in the 1980s, resulting in increased mortality of demersal fish and benthic organisms.

Nitrogen inputs display considerable year-to-year variations since they depend on river run-off and atmospheric deposition. The land-based inputs are assumed to have decreased slightly since the 1980s while the atmospheric inputs are still increasing. The phosphorus inputs have decreased significantly during the past decade and this tendency is continuing in most areas of the Baltic Sea.

However, despite first indications of decreasing winter nutrient concentrations in the Arkona and Bornholm Seas, in the Gulf of Riga and, in particular, in several coastal regions, the drastic reduction in fertiliser usage since 1989/1990 has not yet been significantly reflected in the Baltic Proper. The symptoms of eutrophication have decreased in some coastal areas where the reduction of nutrient inputs has been substantial. However, in the open sea areas no clear changes have so far been observed.

With respect to long-term variations, there were no major differences in the dominance of phytoplankton species between the three assessment periods 1979–1983, 1984–1988 and 1989–1993. There are indications that the frequency and spatial coverage of harmful blooms in the Baltic Sea may have increased. This may be partly due to changes in the seasonal availability and relative proportions of nutrients.

Nitrogen or nitrogen and phosphorus together are proved to be the limiting nutrients for the phytoplankton production in the Baltic Sea except the Bothian Bay, where phosphorus is the main limiting nutrient. Silicate limitation is also reported. this is expected to be the main reason for the observed dominance of diniflagellates in spring. Low N:P ratios are promoting the development of nitrogen fixing blue-green algal blooms.

Model calculations and experimental studies in laboratory and in the sea show that both nitrogen and phosphorus inputs are to be reduced in order to counteract eutrophicatioin in the Baltic Sea.

The Swedish coast - Baltic Sea

In the Archipelago south of Stockholm (Himmerfjärden) in the Baltic, large scale and long-term experiments have been carried out studying the effects of changing nutrient loadings and ratios on the ecosystem (Elmgren and Larsson, 1997). The results on the phytoplankton community demonstrated that some of the dominating species reacted with a large increase in abundance when nutrients were increased. Further, the species diversity was affected so that the occurrence of rare species became much more variable compared to the occurrence of the dominating species. The main result of the input of nutrients was an increased phytoplankton biomass. The Himmerfjärden study also demonstrated very clearly the importance of the weather on the variability of the phytoplankton community. Several of the observed changes in the phytopankton variability were observed both on the nutrient impacted and the control station, supporting the idea that weather can pose much on the variability. Therefore the importance of long time series with frequent sampling including reference areas is stressed. Only in this way effects of weather variability and anthropogenic effects on phytoplankton communities can be separated.

The Sweden coast - Kattegat/Skagerrak

In autumn 1997 – winter 1998 the worst situation regarding the deep oxygen concentration and the negative effects on the benthic fauna was observed in the archipelago along the north western coast. This situation was probably the result of a high organic load in combination with the unusual warm and nice summer of 1997. As a result, there is an ongoing debate on how important the anthropogenic input described above has been in relation to the weather and long term climatological variability. The long term decline in oxygen concentrations in the deep water (Rosenberg, 1990) in combination with the results on increasing annual primary production during the period 1985 – 1995 (Lindahl, 1995) most likely indicates that the input of anthropogenic compounds is too large, at least during periods of negative influence of weather conditions and/or long term climatological variability. However, at present there are no results on the phytoplankton biomass and the species variability/occurrence which directly can support the effects of the anthropogenic nutrient inputs.

The German Bight

From long term time series in the German Bight (Helgoland) carried out since 1962, it appears that:

- eutrophication could be clearly measured, in the way as summarised in the 1997 Working Group on Phytoplankton Ecology report. The main features were a 3–4 times increase in nitrate concentrations at Helgoland (inner German Bight) but only a doubling of phosphate concentrations. While phosphate decreased again since 1984, nitrate is still on the rise. A large increase in N:P ratio resulted.

- the effects of increasing levels and N:P ratios on phytoplankton could not be shown at Helgoland to the expected level, however. Diatoms did not increase, only flagellates did. When separating size classes, only nannoplankton < 20 micron, mainly < 10 micron, was responsible for the increase.

- large phytoplankton blooms in the outer German Bight suggested that a large impact of eutrophication was found there, because of improved light conditions in the water column. These locations however, are outside the reach of the daily sampling scheme at Helgoland.

- this leads to the recommendation that a more efficient monitoring program must be based on the hydrographical structure of the German Bight, not only on the appropriate sampling frequency in time. This is particularly true considering the sites of the other German monitoring stations, which are in the narrow coastal strip which receives the river water and is permanently mixed due to tidal currents.

Waters around the UK

A 3 year programme assessing the offshore effect of anthropogenic nutrient input to UK coastal waters (JONUS II) will be completed in April 1999. The work focuses upon the Thames and Southern Bight of the North Sea and the Irish Sea and will examine the response of the pelagic ecosystem to nutrient input.

In the Irish Sea 3 areas have been sampled during 1996 - 97 which differ in their nutrient loading, Liverpool Bay, Dundalk Bay and a deep seasonally stratified site in the north western Irish Sea. Highest biomass and productivity during the spring bloom were associated with the highest winter input of nutrients (approx. 30 µM) in Liverpool Bay. Next highest biomass levels were found in Dundalk Bay (15.0 µM). Copepod abundance showed an inverse relationship with peak biomass such that lowest numbers were found in Liverpool Bay. Size fractionated biomass and production measurements showed that large cells (> 5.0 µm) dominated biomass (> 95 %) and productivity in Liverpool Bay. Diatoms and later Phaeocystis sp. dominated the spring bloom in Liverpool Bay, whilst diatoms dominated in Dundalk Bay. At the stratified site silicoflagellates appeared to dominate the spring bloom in 1997.

In the southern North Sea our 3 sample sites lay along a gradient of dissolved inorganic nutrient loading (about 30 - 15 µM) from the mouth of the R. Thames to the southern Bight. There is a clear decline in chlorophyll biomass and production along this gradient with small (< 5.0 µm) phytoplankon contributing < 30 % of the productivity at the most inshore site and > 70 % at the most offshore site. We also made measurements during the winter and demonstrated the presence of viable photosynthesising populations at our most turbid and cold (< 1.0^{o}C) inshore site that were capable of gross photosynthesis. We were unable to detect respiration raising the interesting possibility of net production occurring under some circumstances in the winter.

Dutch coastal waters

Based on the manuscript for the ICES J. Mar. Science (Plankton Variability Symposium Kiel, March 1997) a summary of the most recent findings for the Dutch coastal zone are included (by courtesy of de Vries *et al.*).

In the Dutch coastal zone, nutrient and chlorophyll concentrations show gradients up to one order of magnitude perpendicular to the coast within a zone of 0 km to 30–50 km offshore. Time series analysis reveals significant decreasing trends for dissolved inorganic phosphorus (40 %) and total-phosphorus (35 %) and an increase of the dissolved inorganic N/P -ratio from 25–30 to 40–55 mol/mol in the period 1988–1995. Other trends, e.g., nitrogen (-15 %), silicate (stable), and chlorophyll are smaller and mostly not statistically significant. The trends in phosphorus reflect a proportional and immediate response to decreasing riverine inputs.

The observed trends, the spatial gradients and long term seasonal patterns are simulated quite well with a detailed coupled physical — ecological model for the coastal zone. The model results indicate no effect of decreasing phosphorus but an important role of both nitrogen and light climate for primary production and algal biomass.

Above results have been reproduced in mesocosm experiments. Moreover, these experiments indicate a strong response of primary production and chlorophyll to decreasing nitrogen load, whereas secondary production (macrobenthos) remains stable. Ecological efficiency of secondary production increases from 7 % to > 10 % with decreasing nitrogen.

Due to the absence of a significant nitrogen trend in the coastal zone, the mesocosm results cannot be related to field data as yet. However, it is to be expected that coastal eutrophication can be combated effectively by reducing nitrogen inputs without affecting productivity at higher trophic levels.

e) assess monitoring strategies of the pelagic ecosystem and their practical outcome in monitoring programmes within the ICES area;

A compilation of existing monitoring programmes were listed by the participants. Therefore they cover mainly those countries present in the meeting. At the same time this stresses the need for participation from countries not represented in the WG. Nevertheless the compilation gives a broad but incomplete, overview of pelagic monitoring programmes.

1. *Narragansett Bay Monitoring (USA)*

This program was started for scientific purposes and results from a series of investigations initiated to evaluate the regulation of phytoplankton blooms and species succession The main topics studied were: the effects of nutrients and grazing on population dynamics; in situ growth rates; modelling of species successional and bloom patterns; experimental, ecophysiological studies on the nutritional requirements and growth at various combinations of temperature-irradinace for all major phytoplankton species; mesocosm experiments; grazing rates of the dominant copepods and ctenophore *Mnemiopsis leidyi*, and mathematical modelling of blooms These results have been reported in > 100 papers and theses.

The study required measurements at a fixed station (?). The parameters measured varied with the changing objectives and the various research projects, but always included measurements of light, secchi disc, temperature, salinity, phytoplankton species composition and numerical abundance and macro-nutrients. In addition, measurements of chlorophyll, primary production, zooplankton species composition, numerical abundance, dryweight, C, N and ctenophore abundance were also measured., but these time-series are less extensive. Less frequent measurements of nitrate reductase and alkaline phosphatase were also made. Sampling frequency was weekly with samples collected from three depths located within the 10 m water column. These data have been used in various publications and are currently being evaluated for analyses of long term trends and patterns in environmental properties and phytoplankton-zooplankton dynamics in Narragansett Bay. The data are computer entered. The time series extends from January 1959 through June 1997.

2. *Canadian Atlantic Coast*

The Department of Fisheries and Oceans Canada (DFO) is developing an integrated monitoring program for the Canadian Atlantic coast (including the Gulf of St. Lawrence and the Bay of Fundy). The main objectives of the monitoring program are to collect and analyse biological, chemical and physical data in order to characterise and understand the causes of the ocean variability at seasonal, interannual and decadal scales and provide the multidisciplinary datasets that can be used to establish relationships among the biological, chemical, and physical variability. An additional but no less important objective is to ensure the protection of the marine environment by providing adequate data to support the sound development of ocean activities in the Canadian Atlantic zone. The principal coordinator of this program is J.-C. Therriault (Institut Maurice Lamontagne, Mont-Joli, P.Q.) and includes several scientists in 3 DFO regions (Laurentian, Maritime, Newfoundland regions).

The monitoring program is based on 1) seasonal/opportunistic sampling in order to obtain information on the variability of the physical environment for the whole Northwest Atlantic region, 2) higher frequency temporal sampling at accessible fixed sites in order to monitor the finer time scale dynamics in representative areas, and 3) Remote sensing of physical and biological variables in order to provide a broader spatial coverage.

Overview of current monitoring activities (short list: biological variables only)

Nearshore sampling

- Toxic algae monitoring: Initiated in 1989, this monitoring program provides multidisciplinary data (temperature, salinity, dissolved oxygen, Secchi disk, phytoplankton identification and enumeration (particularly for harmful algae) collected biweekly on average (from May to October) for the nearshore zone (14 fixed stations in the Estuary and Gulf of St. Lawrence, and in the Bay of Fundy). This program is currently active in 2 DFO regions (Laurentian and Maritime regions) under supervision of M. Levasseur and J. Martin.

Fixed stations

- Anticosti Gyre and Gaspe Current. Initiated in the fall of 1995, two stations one in the north-west of the Gulf of St. Lawrence and one in the Gaspe current. Sampling of these stations is to be conducted at least on a bi-weekly basis from February to October and a monthly basis at other times. These stations provide information on two major hydrological regimes in the Gulf of St. Lawrence, the output waters from the Gaspe Current and the more oceanic type waters from Anticosti gyre. Measured variables are; chlorophyll *a*, phytoplankton species composition, secchi disk, PAR, nutrient concentrations, temperature and salinity (J.-C. Therriault, DFO-maritime region).

- Newfoundland shelf environment: Initiated in 1996, a long-term monitoring program is being carried out at 47^0 32.8` N, $52^0$ 35` W. This station is representative of the Newfoundland shelf environment. Sampling of this station is to be conducted at least on a bi-weekly basis from February to October Variables are; chlorophyll a, phytoplankton species composition, secchi disk, PAR, nutrient concentrations, temperature and salinity (DFO staff, Newfoundland region).

Bay of Fundy: A long term monitoring program is being carried out at India Point (Malone Bay) and Sambro and 4 sites in Passamaquody Bay. Sampling is carried out about 26 times per year for temperature, salinity, dissolved oxygen, PAR, nutrients, chlorophyll a and phytoplankton analyses. Thermographes are maintained and plankton tows carried out. Initiated in 1995. (DFO staff, Maritime region).

Remote sensing:

The remote sensing groups of the Bedford Institute (DFO-maritime region) and the Maurice-Lamontagne Institute (DFO-Laurentian region) are planning projects to issue maps of the distribution of chlorophyll and primary production on the whole east coast every two weeks using Sea Wifs data. Sea Wifs became fully operational on September 18, 1997. The group also processes AVHRR/SST images regularly since 1994. Surface chlorophyll maps should be available for operational purposes shortly (T. Platt; BIO, Halifax; P. Larouche; MLI, Mont-Joli).

CPR lines:

The CPR program dates back to 1957 in the western North Atlantic and in the western north Atlantic and is the only time series for phytoplankton and zooplankton that measures decadal scale changes. The monthly samples provide data on phytoplankton and zooplankton along two sections, one between Iceland and Newfoundland and the other between Nova Scotia and Georges Bank (DFO staff, Maritime region)

Data management:

DFO is developing a data management plan to centralise access to the monitoring data, data products and derived information collected and generated by the monitoring program via an Internet web site (J.C. Therriault, Laurentian region).

3. The German Monitoring Program

This is organised in the BLMP (Bund-Laender-Messprogramm), which is the national German contribution to JAMP. It comprises the following programs and institutions:

1) BSH (the German Hydrographic Institute, formerly DHI) chemical investigations of the German Bight, using own research vessels.

- including inorganic nutrients in water, hydrographic measurements on vertical profiles, German Bight every 5 yr.

2) Biologische Anstalt Helgoland (BAH), since January 1998 incorporated in the Alfred-Wegener-Institute for Polar- and Sea Research (AWI).

2 a) Helgoland time-series (since 1962). Daily (Monday-Friday) surface sampling at Helgoland Roads by boat. Scientific time-series, later also used in the frame of official monitoring program.

- Parameters: Inorganic nutrients, S, t, Secchi, phytoplankton species or groups, biomass. (Dr Hickel), N.N. – Zooplankton 3 times per week: species composition. Dr Greve (now with BSH).

2 b) 3 transects from Helgoland to the Elbe and Eider mouths and to the northwest. Once a month.

- Parameters: vertical series of S, t, Secchi, nutrients (not all depths), phytoplankton (partly). From 1962–1970 and again since 1980. Scientific purpose, but in cooperation with the BSH.

2 c) List (island of Sylt): German Wadden Sea.

- 1–3 stations in the Wadden Sea of Sylt, 1–2 times per week.

- Parameters: S, t, nutrients, seston weight, POC, PN, Mesozooplankton quantitatively and qualitatively. Since 1972. Scientific time-series. Dr Martens.

3) Landesamt fur Umwelt und Naturschutz (LANU), Kiel.

- Biological Monitoring program, not primarily scientific motivated.

- Phytoplankton species composition, on a number of stations off the Schleswig-Holstein West Coast and (Baltic) East Coast, Primary production, chlorophyll a and inorganic nutrients.

4) Niedersaechsisches Landesamt fur Oekologie (NLO), Norderney.

- Biological and chemical monitoring of the Wadden Sea of Norderney (26x /yr), and transects in the whole German East Frisian Wadden Sea, 17 stations, (2x /yr), nutrients, chlorophyll a.

Further details in the new BLMP-program from February, 1998. The BLMP-office is with the BSH, Hamburg (Dr Heinrich, Dr Rolke).

4. Icelandic waters

The objective of the annual phytoplankton observations is to map the phytoplankton biomass distribution around Iceland during the late spring and to evaluate the conditions for further growth. The observations are part of a biological oceanographic monitoring program for observations of the environmental conditions in the waters around Iceland. The program started in the fifties along with research of the Atlanto-Scandinavian herring stock, at that time found during its feeding migrations in the waters north of Iceland. The environmental program was expanded in the sixties in order to cover the waters all around Iceland as the herring stock disappeared after a major change in the environmental conditions in the area.

The vessels of the Marine Research Institute (MRI) are used for the cruises. A grid of about 80 stations distributed over 12 standard transects are covered in late May and early June. A vertical profile of the ambient temperature, salinity and fluorescence is taken at each station as well as a plankton net sample in the uppermost 5 meters, and the Secchi depth whenever the stations are taken during the daytime. Furthermore water samples are taken at standard depths (0, 10, 20 and 30m for the phytoplankton) and subsampled for analysis of chlorophyll *a*, ^{14}C uptake at light saturation, concentrations of inorganic nitrate, phosphate and silicate, as well as a sample for phytoplankton counting. Additionally P *vs.*I experiments are made at selected stations, 1 -3 on each transect. During the whole cruises measurements of fluorescence, temperature and salinity in a flow-through system is observed whenever possible during the growth season.

The results are presented in an annual report, and comparisons are made with earlier findings, taking into account the simultaneous measurements of the hydrographic, chemical and biological parameters. A review of previous findings is presented occasionally. The annual observations, as well as comparable measurements performed at other times of the

year, are filed in a database (Oracle) at the MRI. The filed records on ^{14}C uptake for the standard depths, along with the Secchi depths and the time and place of the observations started in 1958. The chlorophyll *a* records are also included since these measurements were adopted to the routine in 1974. The P *vs.*I experiments started in the eighties and are filed separately.

The above mentioned observations on the phytoplankton in the area, *i.e.,* the annual observations covering approximately 50 nautical miles around Iceland are taken care of by MRI. The person presently in charge of the phytoplankton observations is Kristinn Gudmundsson. (e.g., Gudmondsson, 1998).

5. Norwegian waters

In Norwegian waters a series of monitoring programmes are running which have different goals.

5.a.

- Reason for the program: Scientific
- Location: Different oceanographic sections in the central and northern North Sea
- Strategy: Research vessel: Oceanographic sections and stations
- Parameters: CTD, nutrients (nitrite, nitrate, phosphate and silicate), oxygen
- Frequency: Once a year in November/December
- Use of results: Internal, international projects
- Start/End of the program: Started in 1978 (?), ongoing.
- Responsible institution/person: Institute of Marine Research, Bergen/ Lars Føyn

5.b.

- Reason for the program: Scientific
- Location: 27 major Norwegian fjords along the whole Norwegian coast
- Strategy: Research vessel: Oceanographic sections along the fjords (4 to 8 stations each)
- Parameters: CTD, nutrients (nitrite, nitrate, phosphate and silicate), oxygen.
- Frecuency: Once a year in November/December
- Use of results: Internal, national reports (in Norwegian)
- Start/End of the program: Started in 1976, ongoing
- Responsible institution/person: Institute of Marine Research, Bergen/Lars Føyn.

5.c.

- Reason for the program: Governmental (Monitoring of trophic status of coastal waters)
- Location: Southeastern Norway
- Strategy: Research vessel: Five coastal stations
- Parameters: CTD, oxygen. nutrients(nitrate, nitrite, ammonium, phosphate, silicate), Tot-N, Tot-P, chlorophyll, POC/PON, phytoplankton, zooplankton (since 1995), yellow substances(since 1996).
- Frecuency: 22 times a year
- Use of results: Reports to the State Pollution Control Authority (JAMP, OSPARCOM)
- Start/End of the program: 1990, ongoing.
- Responsible institution/person: Norwegian Institute of Water Research, Oslo/Jan Magnusson and Institute of Marine Research, Bergen/Jan Aure and Institute of Marine Research, Flødevigen Station/Einar Dahl

5.d.

- Reason for the program: Scientific
- Location: Oceanographic section between Torungen (Norway) and Hirtshals (Denmark)
- Strategy: Research vessel: oceanographic stations
- Parameters: CTD, nutrients, oxygen, chlorophyll, phytoplankton.
- Frecuency: Monthly
- Use of results: Internal, ICES database.
- Start/End of the program: 1951 (temperature, salinity, partly oxygen), since 1980 (nitrate, nitrite, phosphate, ammonium for a shorter period, silicate since 1988), oxygen, chlorophyll, phytoplankton.
- Responsible institution/person: Institute of Marine Research, Flødevigen Station/Didrik Danielssen

5.e.

- Reason for the program: Scientific
- Location: Weather Station Mike, Norwegian Sea, 66°N, 02°E
- Strategy: Weather ship, hydrocasts, vertical profiles.
- Parameters: Temperature, salinity, nutrients (nitrite, nitrate, phosphate and silicate), oxygen, chlorophyll, phytoplankton, secchi depth
- Frequency: Weekly (secchi readings, once a day)
- Use of results: Internal, national environmental assessment.
- Start/End of the program: Started in 1990, ongoing

- Responsible institution/person: Institute of Marine Research, Bergen/ F. Rey

5.f.

- Reason for the program: Scientific
- Location: Flødevigen Bay, the Norwegian Skagerrak Coast
- Strategy: Surface samples taken from the pier (0–3 m)
- Parameters: Temperature, salinity, chlorophyll, phytoplankton
- Frequency: three times a week (Monday, Wednesday, and Friday)
- Use of results: Internal, weekly Algae report through IMR`s website.
- Start/End of the program: 1981, ongoing.
- Responsible institution/person: Institute of Marine Research, Flødevigen Station/ E. Dahl

5.g.

- Reason for the program: Political(Service to aquaculture and fisheries)
- Location: Outer Oslofjord and the Norwegian Skagerrak Coast.
- Strategy: Small boat, in situ fluorometer, surface samples and fluorescence maximum.
- Parameters: CTD, in situ fluorormeter, phytoplankton
- Frequency: variable, most intensively from March to October
- Use of results: warning to fish farmers and fishermen, contribution to Algae report through IMR
- Start/End of the program: Started in 1994, ongoing
- Responsible institution/person: Directorate of Fisheries/ Gunnar Larsen

5.h.

- Reason for the program: Commercial (fish farming, insurance companies, mussel plants)
- Location: Different fjords along the Norwegian coast
- Strategy: Surface observations at the fish farms
- Parameters: phytoplankton, secchi depth, temperature
- Frequency: variable
- Use of results: internal, contribution to Algae report through IMR
- Start/End of the program: Started in 1990(?), ongoing
- Responsible institution/person: Oceanor, Trondheim/ Karl Tangen

5.i.

- Reason for the program: Political(public service)
- Location: Different fjords along the Norwegian coast
- Strategy: surface observations at selected sites
- Parameters: DSP and PSP producing organisms, toxicity test of mussels(by the Norwegian College of Veterinarian Medicine/ Tore Aune
- Frequency: weekly from the end of March to the beginning of October
- Use of results: internal, public warning (TV-text, mussel-phone), contribution to Alge report through IMR
- Start/End of the program: 1992(?), ongoing
- Responsible institution/person: The Norwegian State Food Control Authority/ Gesche Torp Varran

6. The Dutch coastal waters.

A monitoring programme for the Dutch coastal zone is in use since 1975. Then a programme started in which standard hydrographic parameters were measured on a series of transects perpendicular to the coast. This program has been adapted and modified several times based on statistical analyses. A reduction of the number of transects has occurred whereas at the same time other parameters have been introduced. Since 1989 phytoplankton species composition is part of the standard program. Other parameters which are measured include inorganic dissolved nutrients, secchi disc, salinity, temperature, turbidity. During some years primary production has been measured but not as part of the monitoring effort. Data management is with Rijkswaterstaat (RIKZ, National institute for Coastal and Marine Management). They also publish the most important data in an annual review. All data are stored in a large RWS database. Information can be achieved from Dr P. Bot, RIKZ, the Hague.

7.The Baltic Monitoring Programme (Finland)

The Finnish Baltic Sea Environment monitoring programme is carried out by the Finnish Institute of Marine Research in the open sea areas and by the Finnish Environment Institute with the Regional Environment Centers in the coastal regions.

In order to assess the environmental state of the Baltic Sea and to detect the trends, an effective monitoring programme based on key parameters supported by appropriate research activities will be carried out by Finland.

- The **mandatory monitoring programme** covers the *long-term trend approach* of a few key parameters on hydrography, nutrients and biology in open sea and coastal regions. It also provides *rapid information* on sudden and exceptional events.
- The **supporting programme** includes studies giving *background information* for the assessment purposes.

Mandatory plankton programme in the open sea

The Finnish monitoring of phytoplankton is mainly based on unattended recording and sampling on merchant ships and on satellite imagery. On the merchant ships, chlorophyll *a* fluorescence, temperature and salinity are recorded quasi-continuously with spatial resolution of 100-300 m while the ferries are moving. The temporal resolution for the recordings varies between 1 and 3 days. Concurrently, water samples are collected for the microscopic analysis of phytoplankton species composition and for the quantitative determination of chlorophyll *a* and nutrients. The chlorophyll *a* data is used to convert the fluorescence readings to chlorophyll *a* concentrations. Recordings are carried out on several ferries and the routes are covering most of the Baltic Sea basins. At present, the phytoplankton species composition is determined in ca. 300 samples annually.

Zooplankton is sampled only during late summer at fixed sampling stations covering the maritime regions around Finland. In 1998, a pilot project using the CPR technique, will be started to collect zooplankton samples in a transect between Finland and the south-western Baltic Sea.

The mandatory programme includes also the mapping of the winter reserves of nutrients, monitoring the hydrographic variation of the various basins and annual mapping of the macrozoobenthos species composition and biomass.

Monitoring of coastal waters

The coastal monitoring programme is carried out by the Finnish Environment Institute with the Regional Environment Centers. The programme covers the whole Finnish coastline. The sampling is carried out at 12 high frequency stations, at ca. 100 mapping stations and in connection with the statutory monitoring at ca. 400 stations.

The high-frequent stations (sampled ca. 20 times a year) are located in the outer archipelago or off the archipelago along the whole Finnish coast. This programme is able to serve for the annual cycle of nutrient and phytoplankton dynamics. The variables are temperature, salinity, and oxygen. pH, turbidity, water colour, total nitrogen, NO_2+NO_3-N, NH_4-N, Total phosphorus, PO_4-P, SiO_2-Si, total organic carbon, iron and chlorophyll *a*. Phytoplankton species composition is determined at five stations.

Nutrient reserves and oxygen content in water in the coastal areas are mapped twice a year in March and in July-August at an extended station network comprising ca. 100 stations. In July-August, chlorophyll *a* concentrations are recorded as well.

The pollution control system based on the Water Act provides an extension for the Baltic Sea Monitoring Programme towards the coast. Programmes for regions receiving waste discharges from one or several sources are planned individually according to the local environment conditions and the quality of the waste load. The sampling frequency varies from 1 to 14 times per year and is mostly 4-6 times. The parameters determined vary according to the programme but some variables, e.g. total phosphorus and nitrogen, chlorophyll *a* and oxygen are measured annually in every programme. Every 3rd-5th year a more comprehensive programme including biological studies is performed.

Major part of the data collected in the open sea is delivered annually to the HELCOM database, while the ship-of-opportunity data and most of the coastal data is available for the assessment purposes in the national databases.

Conclusions

The WG concludes that a tremendous effort is given to monitor pelagic parameters for a series of objectives, from purely scientific till commercial/user oriented. However, there are still very few attempts to coordinate such national monitoring programmes so that a direct comparison between national data is possible on an international level. Only parameters which are part of the JAMP under OSPARCOM are subject to intercalibration and standardisation, up to attempts to introduce quality control. For some parameters this will shortly be or has been arrived, for others (phytoplankton species composition) we are still far away from reaching such goals. Here support from international bodies (ICES, OSPARCOM, EC, EEA) are needed to improve the present and future quality of data. In a just accepted EU project (BEQUALM) parts of these goals for QA will be considered.

The Working Group on Phytoplankton Ecology recommends that the high value of long-term ecological monitoring data necessitates a proper analysis of the data. This process should have more attention to be sure that no important expensive information is lost. A selected group of members of the Working Group on Phytoplankton Ecology should take care of existing series.

f) review in a joint session with ICES/IOC Working Group on Harmful Algal Bloom Dynamics on 24 March the results of the Workshop on Development of in situ growth rate measurements of Dinoflagellates held in Kristineberg;

The reader is referred to the Section under 4.

g) review in a joint session with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics on 24 March the status of taxonomic coding systems with a view to recommend the adoption of a single coding system for use in ICES;

The reader is referred to the appropriate Section under 4.

h) consider the future work programme in relation to the remit of the Oceanography Committee and the development of the ICES five year plan, including cooperation with other working groups.

In a discussion on the 5-year plan, a list of possible future Working Group on Phytoplankton Ecology Themes was discussed. It encompasses questions like:

1. What is the influence of diurnal variations on habitat parameters and diurnal rhythms in planktonic behaviour on phytoplankton dynamics? (with ICES/IOC Working Group on Harmful Algal Bloom Dynamics, Working Group on Zooplankton Ecology)

2. Do whole community measurements of biomass, primary production and growth rates represent the behaviour of the dominant species?

3. Is the effect of eutrophication on phytoplankton dynamics primarily a direct nutrient effect, or a secondary indirect response resulting from eutrophic modifications of grazer dynamics? (Working Group on Zooplankton Ecology, Working Group on Shelf Seas Oceanography, Working Group on Recruitment Processes)

4. Do blooms result primarily from a collapse (relaxation) of grazing pressure once light is no longer limiting? (Working Group on Zooplankton Ecology, Working Group on Recruitment Processes)

5. What are the dynamics of formation, the habitat properties of and the biotic processes in thin-layer phytoplankton communities and what is the influence of this assemblage on overall (i.e., water column) community dynamics? (ICES/IOC Working Group on Harmful Algal Bloom Dynamics) Which of these parameters can be used for pelagic monitoring?

The above themes are proposed for joint sessions with the ICES-Working Groups on Zooplankton Ecology, HABD, marine chemistry and shelf seas oceanography.

Subsets of these themes appropriate for Working Group on Phytoplankton Ecology internal discussion include:

1. is the compensation depth determined for the whole community representative of that for the individual species present?

2. are the phytoplankton growth rates based on changes in cell abundance ecologically comparable to those based on carbon, nitrogen or other cellular constituents?

3. what is the evidence that Fe may be limiting in coastal waters?

4. how widespread and important is mixotrophy among the phytoplankton?

5. how significant are allelochemical and allelopathic processes in phytoplankton dynamics?

6. what have GLOBEC and Fe-enrichment studies demonstrated relevant to the Working Group on Phytoplankton Ecology mission?

The above themes are not ranked, but among the last six themes items 1, 2 and 4 would have higher priority, in our view, than the others.

The 5 themes suggested for joint consideration with other ICES Working Groups would appear to be relevant to other Working Groups as well as provide ample opportunity for discussion within the Working Group on Phytoplankton Ecology on specialised topics beyond those listed above.

The Working Group on Phytoplankton Ecology will bring the general themes to the Annual Science Meeting and discuss them in the Oceanography Committee.

i) (new/added) discuss the role of ICES in (EURO)GOOS and report suggestions to the chairman of Steering Group on the Global Ocean Observing System, R. Saetre.

The chairman gave information to the members on the meaning of (EURO)GOOS. Of particular interest for the Working Group on Phytoplankton Ecology are the modules Health of the Ocean and Living Resources. The role of a ferry box in surface mapping was pointed out with specific operational interests. Examples of using these techniques are available and discussed several times in the Working Group on Phytoplankton Ecology.

Some general concern was expressed at the over interest in technology at the expense of science. However there should not be a conflict between these two viewpoints, because data acquisition is of great help in e.g., modelling studies, and interaction between long term climatic changes and biological parameters. Simply the possibilities now appearing should be used as much as possible. A short description of SEANET and its role in dealing with fixed point monitoring network was given.

In general it was concluded that the expertise available in the Working Group on Phytoplankton Ecology should be used to inform ICES about the possibilities to measure in an operational way pelagic parameters (e.g., TOR on Monitoring Networks). Moreover the development of new techniques should be stressed, a topic long dealt with in the Working Group on Phytoplankton Ecology. These include new methods to measure primary production based on PAM fluorescence, species composition/groups by flowcytometry and the cooperation in developing blue-box like instrumentation, including updated versions of CPR's with newly added instrumentation to measure abiotic parameters.

Regarding the structure of the ICES-GOOS cooperation no clear views were expressed. The importance of GOOS as an instrument for long term observations of pelagic parameters however is well taken and strongly supported. Quality of data however must be secured, and comparability needs to be guaranteed.

The chairman will give the suggestions to the Chairman of WGGOOS, R. Saetre.

4 JOINT MEETING WITH THE ICES/IOC WORKING GROUP ON HARMFUL ALGAL BLOOM DYNAMICS

The agenda of the joint Meeting with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics on March 24, is given in Annex 12. Three joint TOR's were formulated, including one on the use of mesocosm experiments, discussion on the results of the Kristineberg workshop, and the questions regarding the status of taxonomic coding systems. After consultation with the chairman of the ICES/IOC Working Group on Harmful Algal Bloom Dynamics Patrick Gentien a fourth item was presented by the WGPE: the development of a five-year plan within the Oceanography Committee. The meeting was opened by Prof. Mario Ruivo, former chairman of IOC. He welcomed especially the members of the ICES/IOC Working Group on Harmful Algal Bloom Dynamics, who had their first meeting day in Lisbon. The chairmen of both WG's decided to take over as rapporteurs for this joint meeting. The meeting was chaired by F. Colijn and P. Gentien.

The agenda was adopted as presented to the members, but the order of the discussion has been changed.

TOR 1. Odd Lindahl presented the results of the ICES/IOC Workshop on the Intercomparison of the in situ growth rate measurements (Dinoflagellates) held at Kristineberg Marine Research Station, Sweden from 9–15 September 1996. Although the report has been discussed already during last years meeting of the ICES/IOC Working Group on Harmful Algal Bloom Dynamics it still could not be finished due to some missing parts and because some information needed further elaboration. A shortage of the Workshop certainly was that some techniques were not yet established and were used for the first time. This includes risks which might be reduced with a more careful choice of available techniques.

It was decided to discuss the main shortcomings and missing parts in a smaller group of interested people of which many were attending in direct connection with the session (Anderson, Lindahl, Colijn, Gentien, Edler, Dahl and Sampayo). The main point which should be covered is to get calculations of growth rates even from those measurements which were only intended to give estimates. Also decisions were taken to finish the report within short time after the meeting. Information not available at that time will not be included.

TOR 3. Mesocosm experiments. The Working Group on Phytoplankton Ecology had already discussed this TOR in their meeting and came up with a series of ideas for a joint mesocosm project. A possible mesocosm experiment was proposed by Smayda and encompasses a study on the diurnal variation in flagellates growth (see appropriate section of the Working Group on Phytoplankton Ecology report). This topic should be interesting for both groups. The experiment was criticised at several points: which hypotheses were to be tested in the experiment? which ideas were available on the planning of such an experiment? should it be executed with a natural community or with a (particular) bloom species? Also a timetable for the experimental planning was needed and a possible location. Because of all these questions, it was suggested not to rush, especially because the Kristineberg report was not yet available. Based on the outcome of the Kristineberg workshop and the difficulties in establishing thin layers in mesocosm systems, at this moment there was no basis for a new experiment. However the Working Group on Phytoplankton Ecology was encouraged to proceed ahead, possibly with species easier to handle. Therefore there is no immediate need to discuss this matter once more in the ICES/IOC Working Group on Harmful Algal Bloom Dynamics. A more definitive planning for an experiment to be executed in 1999 should be arranged within the Working Group on Phytoplankton Ecology.

TOR 2. The status of taxonomic coding systems with a view to recommend the adoption of a single coding system for use in ICES was introduced by Catherine Belin. She presented the common problems dealing with taxonomic coding (synonyms, new names, etc.). She also emphasised that computer codes can be used as hidden codes and that there is no need to have complex numerical codes. It proves to be far more easy to use letter codes (acronyms) which resemble the species names. In France therefore the data base Quadrige is used.

A checklist per area should be made, which should be updated every 5-year. Then Jorge Diogene presented his contribution on the development of a computer data base on harmful algal occurrences world-wide (HABDAT). It will encompass the former algal bloom reports but it should be expanded to contain relevant information on abiotic parameters during the bloom. He stressed the need for an operational list. More information can be taken from the ICES/IOC Working Group on Harmful Algal Bloom Dynamics report where additional information on the present state of the data bank was presented.

Finally the contribution of the Working Group on Phytoplankton Ecology to the 5-year plan for the Oceanography Committee was presented in plenary. This presentation proved to be helpful to the later discussion of the ICES/IOC Working Group on Harmful Algal Bloom Dynamics on this point.

There was no any other business to discuss and therefore the meeting was closed by the chairmen at 17.00 hrs.

5 ANY OTHER BUSINESS

The Working Group on Phytoplankton Ecology decided to write a supportive letter to H.L. Rees (Chairman ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements Related to Eutrophication Parameters: Steering Group on quality assurance of biological measurements related to eutrophication effects) about the cooperation of the Working Group on Phytoplankton Ecology for setting up QA procedures for the following aspects of phytoplankton measurements: biomass measurement based on chlorophyll a; primary production with a standard ICES incubator including a protocol; and help with developing procedures to increase QA for species identifications in monitoring studies.

The chairman then announced that he would withdraw as chairman of the WG, and therefore that election of a new chairman should take place. As a candidate he suggested Dave Mills from the UK. Dave Mills was invited to give his ideas o about the future development of the WG. After heaving heard his ideas, he was elected unanimously. (note: this election needs approval from the Delegates in September).

6 ACTION LIST FOR NEXT YEAR

To present a state of the art paper on the use of fluorescence techniques for measuring phytoplankton production (and growth): Colijn et al.; To present a paper on the growth of phytoplankton (species, community, growth rate estimates, factors involved in growth rate regulation: Smayda);to finalise the discussion on a standard chlorophyll-a technique for use within ICES programs (Rey); to discuss the further possibilities for a (joint) mesocosm experiment in 1999 (Mills); to support intersessionally the workshop of experts to set up a checklist of phytoplankton for the ICES area (Edler); to continue the efforts to set-up joint sessions with other relevant working groups within the Oceanography Committee; to organise next years meeting in Lowestoft from... to March/April with a one day/two days joint meeting together with the Working Group of Zooplankton Ecology to discuss problems of interaction between these two trophic levels and consideration of food web relations.

7 RECOMMENDATIONS OF THE WORKING GROUP ON PHYTOPLANKTON ECOLOGY

The Working Group on Phytoplankton Ecology recommends that

The document **Flow cytometry as a tool for counting and identification of phytoplankton (groups) and other applications** by G. Dubelaar (Netherlands)and R. Jonker (Netherlands) as reviewed and accepted by the Chairman of the Oceanography Committee will be published in the ICES *Techniques in Marine Environmental Sciences* series. The estimated number of pages is 21

A Study Group on an **ICES/IOC Checklist of Phytoplankton** will be established under the chairmanship of Dr. O. Moestrup (Denmark) and will meet at ICES Headquarters from 11 to 13 January 1999 to:
a) commence the compilation of an ICES phytoplankton checklist, including synonyms, authors, distribution and ,if available, ecophysiological information . This checklist should be based on available local checklists for different ICES regions.
b) discuss the relevance of a complete ICES checklist or the propagation of regional checklists

Justification:

Setting up checklists and moreover control them, is a task for experienced taxonomists to have a long term value. Therefore a group of taxonomists dealing with phytoplankton species should gather to discuss the existing checklists for regional areas, or try to get them compiled if they do not yet exist. Preliminary work by WGPE and WGHABD can be used to have a first estimate of the number of species. For local regions already checklists are avialbale but a critical look at these lists is needed. A series of names has been suggested to attend this meeting, which might be co-sponsored by IOC (Henrik Enevoldsen), because of the general interest of IOC for toxic species.

These names are: Sournia and Belin (France), Crawford and Elbrächter (Germany), Throndsen (Norway), Edler (Sweden), Hällfors (Finland), Thomsen (Denmark), Sampayo (Portugal), .Marshal and Taylor (USA), Santiago de Fraga (Spain), etc.

The **Working on Phytoplankton Ecology** [WGPE] (Chairman*: Dr D Mills,UK) will meet at Lowestoft (UK) from March/April 1999 to:

a) assess the state of the art on the use of fluorescence techniques for measuring phytoplankton production and growth;
b) review knowledge on the growth of phytoplankton
c) finalise the discussion on a standard chlorophyll *a* technique for use within ICES programmes;
d) develop a proposal for a joint mesocosm experiment;
e) review and support the work of the Study Group on "An ICES Checklist of Phytoplankton";
f) prepare for a joint meeting with the Working Group on Zooplankton Ecology in 2000;
g) contribute to the five-year plan of the Oceanography Committee

8 CLOSING OF THE SESSION

The meeting of the Working Group on Phytoplankton Ecology was closed on Monday 24. March at 17.00 Hrs

ANNEX 1 AGENDA OF THE MEETING

1. Opening of the meeting
2. Terms of reference
3. General discussions of Terms of reference
4. Joint meeting with the ICES/IOC Working Group on Harmful Algal Bloom Dynamics
5. Any other business
6. Action list for next year
7. Recommendations referring to new TOR's
8. Adoption of the WG Report
9. Closing of the meeting

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ANNEX 3 REFERENCES (TO BE SUPPLEMENTED)

Burkholder, J.M. & H.B. Glasgow, 1997. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflaggelates: Behaviour, impacts, and environmental controls. Limnol. Oceanogr. 42(5), 1052–1075

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ANNEX 4 CHECK LIST OF PHYTOPLANKTON SPECIES OF THE NORTHWESTERN IBERIAN ATLANTIC (1948–1996) VARELA, BODE AND LORENZO)

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Abstract

This review includes an extensive collection of citations of phytoplankton species along the Northwestern Iberian Atlantic from 1948 to 1996. The citations come from mostly published quantitative phytoplankton studies from a total of 313 stations. A total of 589 species were identified: 294 Dinoflagellates, 276 Diatoms, 2 Euglenophyceae, 2 Dictyochophyceae, 5 Chrysophyceae, 3 Prymnesiophyceae, 4 Raphidophyceae, 1 Prasinophyceae, 1 photosynthetic Protozoa (*Mesodinium rubrum*) and unidentified Criptomonadineae.

Method

The list of studies reviewed and sampling periods appear in Table 1. The study area was divided in four zones according to the number of data available. Galicia rias includes the estuarine-like rias of Northwestern Spain. The A Coruña zone includes samples collected from the coast to the shelf break off A Coruña from 1989 to 1996 at monthly intervals. Galicia shelf includes all phytoplankton studies made outside the rias generally over the continental shelf but also up to the oceanic side of the shelf break, from the Miño River to Cape Ortegal. The Cantabrian zone includes samples collected in the Northern coast of Spain: Cantabrian Sea (from Cape Ortegal to the French border). Samples were generally collected with oceanographic bottles and also with plankton nets. Phytoplankton was preserved with either Lugol's iodine or formalin and observed under an inverted microscope by the Uthermohl's technique (eg. Casas 1995). Generally, several depths (3 to 7) were sampled within the euphotic zone. Species names were given following Schiller (1937) and Dodge (1982) for dinoflagellates, Peragallo and Peragallo (1908), Hustedt (1959) and Hendey (1964) for diatoms, and Tomas (1993) for naked flagellates.

References

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Table 1. List of references with phytoplankton species data of the NW Iberian region used in this review

Zone	Reference	Year	Remarks
Galicia rias	Margalef 1952	1948-50	Annual cycle, 3 stations
Galicia rias	Margalef 1956	1955	August-September, 5 stations
Galicia rias	Vives & López-Benito 1957	1955-56	Annual cycle, 6 stations
Galicia rias	Campos & Mariño 1984	1978-79	Annual cycle, 3 stations
Galicia rias	Blanco 1985	1984	April-June, 1 station
Galicia rias	Mariño et al. 1985	1978	Annual cycle, 1 station
Galicia rias	Figueiras & Niell 1987	1980-81	Annual cycle, 15 stations
Galicia rias	Figueiras & Pazos 1991	1984	July-August, 25 stations
Galicia rias	Fraga et al. 1995	1992	July, 1 station
A Coruña	Casas 1995	1989-92	Annual cycle, 2 stations
A Coruña	Varela et al. 1997	1994	Annual cycle, 15 stations
A Coruña	Varela (unpublished) (1)	1995-96	Annual cycle, 2 stations
Galicia shelf	Varela 1982	1978	September, 25 stations
Galicia shelf	Estrada 1984	1977	October, 80 stations
Galicia shelf	Varela et al. 1987a	1984	September-October, 18 stations
Galicia shelf	Varela et al. 1987b	1984	June, 19 stations
Galicia	Varela 1991	1952-87	Review (diatoms only)
Cantabrian	Estrada 1982	1976	Annual cycle, 6 stations
Cantabrian	Fernández 1990	1987	Annual cycle, 10 stations
Cantabrian and Galicia	Varela (unpublished) (2)	1991-92	March-April, 76 stations

(1) Samples from the permanent transect sampled at least monthly at A Coruña (Casas 1995).

(2) Samples from shelf wide cruises ECOSARP-491, PROSAR-392 and PROSARP-492 (López-Jamar et al. 1991, Fernández de Puelles et al. 1996).

Table 2. List of phytoplankton species identified in the study area. Their presence in different zones is noted by x.

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
DINOPHYCEAE				
Alexandrium kutnerae Balech		x		
Alexandrium lusitanicum Balech		x		
Amphidinium acutissimum Schiller	x	x	x	x
Amphidinium acutum Lohmann	x	x		
Amphidinium amphidinioides (Geidler) Schiller	x			
Amphidinium crassum Lohmann	x	x	x	x
Amphidinium cucurbitella Kofoid & Swezy	x			
Amphidinium curvatum Schiller	x		x	
Amphidinium extensum Wulff	x			
Amphidinium flagellans Schiller	x			
Amphidinium klebsii Kofoid & Swezy	x			
Amphidinium operculatum Clapparede & Lachmann	x			
Amphidinium ovoideum Lemmermann	x			
Amphidinium spp.	x			x
Amphidinium sphenoides Wulff	x	x	x	x
Amphidinium stigmatum Schiller	x			
Amphinium turbo Kofoid & Swezy	x			
Amphidoma acuminata Stein				x
Amphidoma caudata Halldal		x	x	x
Amphisolenia globigera Stein				x
Brachidinium capitatum Taylor				x
Cachonina hallii Freudentahl & Lee	x	x	x	x
Centrodinium intermedium Pavillard	x			
Ceratium arietinum Cleve	x	x	x	x
Ceratium arietinum var. *bucephalum* Cleve (Sournia)				x
Ceratium arietinum var. *detortum* Jörgensen	x			
Ceratium azoricum Cleve	x			x
Ceratium belone Jörgensen	x			
Ceratium breve Böhm		x		
Ceratium bucerus Zacharias				x
Ceratium candelabrum (Ehrenberg) Stein	x	x	x	x
Ceratium carriense Gourret	x			
Ceratium compressum Gran				x
Ceratium declinatum (Karsten) Jörgensen				x
Ceratium dens Ostenfeld & Schmidt	x		x	
Ceratium extensum (Gourret) Cleve	x			x
Ceratium falcatum (Kofoid) Jörgensen	x			x
Ceratium furca (Ehrenberg) Claparede & Lachmann	x	x	x	x
Ceratium furca f. *eugrammum* Jörgensen	x			
Ceratium furca f. *magnipes* Jörgensen	x			
Ceratium fusus (Ehrenberg) Dujardin	x	x	x	x
Ceratium gibberum Gourret				x
Ceratium gibberum var. *subaequale* (Gourret) Jörgensen	x			
Ceratium hexacanthum Gourret			x	x
Ceratium horridum (Cleve) Gran	x	x	x	x
Ceratium horridum f. *buceros tenue* (Ostenfeld & Schmidt) Jörgensen	x			
Ceratium inflatum (Kofoid) Jörgensen	x			x
Ceratium karsteni Pavillard	x			
Ceratium kofoidii Jörgensen				
Ceratium lineatum (Ehrenberg) Cleve	x	x	x	x
Ceratium longipes (Bailey) Gran			x	
Ceratium longirostrum Gourret	x			x
Ceratium macroceros (Ehrenberg) Vänhoffen	x	x	x	x
Ceratium massiliense (Gourret) Jörgensen	x			x

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Ceratium massiliense f *protuberans*		x		
Ceratium minutum Jörgensen	x	x	x	x
Ceratium pavillardi Jörgensen				x
Ceratium pentagonum Gourret	x	x	x	
Ceratium platycorne Daday	x			x
Ceratium porrectum Karsten		x	x	
Ceratium pulchellum Schröder		x	x	
Ceratium setaceum Jörgensen	x			x
Ceratium spp.	x			x
Ceratium strictum Kofoid	x			
Ceratium symmetricum Pavillard	x			
Ceratium teres Kofoid	x			
Ceratium trichoceros (Ehrenberg) Kofoid				x
Ceratium tripos (O.F. Müller) Nitzsch	x	x	x	x
Ceratocorys armata (Schütt) Nitzsch				x
Ceratocorys horrida Stein	x			x
Cochlodinium achromaticum Lebour		x		
Cochlodinium brandtii Wulff	x			x
Cochlodinium helix (Pouchet) Lemmermann	x	x		x
Cochlodinium pulchellum Lebour		x		x
Cochlodinium pupa Lebour	x			
Cochlodinium spp.	x	x	x	x
Cystodinium spp.				
Cystodinium steinii Klebs	x			
Dinophysis acuminata Claparede & Lachmann	x	x	x	x
Dinophysis acuta Ehrenberg	x	x	x	x
Dinophysis acutoides Ehrenberg				x
Dinophysis amandula Sournia				x
Dinophysis caudata Saville Kent	x	x	x	x
Dinophysis caudata abbreviata Jörgensen	x			
Dinophysis dens Pavillard	x			
Dinophysis *diegensis* Kofoid	x			x
Dinophysis doryphorum (Stein) Abé vel Balech				x
Dinophysis forthii Pavillard		x		x
Dinophysis hastata Stein	x			x
Dinophysis infundibulus Schiller				x
Dinophysis intermedia Pavillard	x			
Dinophysis micropterygia Dangeard	x			
Dinophysis nasutum (Stein) Parke & Dixon	x			
Dinophysis norvegica Clapparede & Lachman	x			
Dinophysis operculoides Schütt	x			
Dinophysis ovata Claparede & Lachman	x			x
Dinophysis ovum Schutt	x		x	x
Dinophysis parvula (Schütt) Balech	x			x
Dinophysis pulchella (Lebour) Balech				x
Dinophysis punctata Jörgensen	x	x	x	x
Dinophysis rapa Stein				x
Dinophysis rotundata Claparede & Lachmann	x	x	x	x
Dinophysis sacculus Stein	x			x
Dinophysis spp.	x	x	x	x
Dinophysis tripos Gourret	x			x
Dinophysis truncata Cleve	x			
Dinophysis uracantha Stein		x		
Diplopsalis asymmetrica Claparede & Lachman			x	
Diplopsalis lenticula Bergh	x		x	
Diplopsalis spp.	x	x	x	x
Dissodium asymmetricum (Mag.) Loeblich	x			
Dissodinium pseudolunula Swift		x	x	x
Dissodinium lenticulum (Bergh) Loeblich	x			x

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Eritropsis cornuta (Schütt) Kofoid & Swezy	x			
Eritropsis pavillardi Kofoid & Swezy	x			
Eritropsis spp.				
Glenodinium dinobryonis (Wolosz.) Schiller	x			
Glenodinium foliaceum Stein				x
Glenodinium monensis Herdman				x
Glenodinium spp.	x			x
Gonyaulax buxus Balech	x			
Gonyaulax diacantha (Meunier) Schiller	x			
Gonyaulax diegensis Kofoid	x		x	x
Gonyaulax digitale (Pouchet) Kofoid	x	x	x	x
Gonyaulax cf. *excavata* Balech				x
Gonyaulax grindleyi Reinecke				x
Gonyaulax monocantha Pavillard	x			x
Gonyaulax monospina Rampi				x
Gonyaulax polyedra Stein	x	x	x	
Gonyaulax polygramma Stein	x	x	x	x
Gonyaulax spp.	x		x	x
Gonyaulax spinifera (Claparede & Lachmann) Diesing	x	x	x	x
Gonyaulax spinifera f. *stelae* Margalef	x			
Gonyaulax tamarensis Lebour	x	x		
Gonyaulax unicornis Lebour	x			
Gonyodoma polyedricum (Pouchet) Jörgensen	x			x
Gonyodoma sphaericum Murray & Whitting	x			x
Gymnodinium agile Kofoid & Swezy	x			
Gymnodinium agiliforme Schiller	x			
Gymnodinium adriaticum (Schmarda) Kofoid & Swezi	x			
Gymnodinium attenuatum Kofoid & Swezy	x			
Gymnodinium breve Davis	x			
Gymnodinium catenatum Graham		x	x	x
Gymnodinium conicum Kofoid & Swezi	x			x
Gymnodinium cruciatum Massart	x			
Gymnodinium cucumis Schütt		x		
Gymnodinium dogieli Kofoid & Swezy	x			
Gymnodinium filum Lebour		x		x
Gymnodinium fuscum (Ehrenberg) Stein	x	x		
Gymnodinium fusus Shütt	x			x
Gymnodinium gelbum Kofoid	x			
Gymnodinium grammaticum (Pouchet) Kofoid & Swezy	x			
Gymnodinium heterostriatum Kofoid & Swezy		x		
Gymnodinium minus Lebour	x			
Gymnodinium paulseni Schiller	x			
Gymnodinium rhomboides Lebour	x			
Gymnodinium rotundatum Klebs	x		x	
Gymnodinium simplex (Lohmann) Kofoid & Swezy	x	x		x
Gymnodinium splendens Lebour	x	x		
Gymnodinium spp.	x	x	x	x
Gymnodinium viridescens Kofoid	x			
Gyrodinium aureolum Hulburt		x	x	
Gyrodinium britannicum Kofoid & Swezy	x	x	x	x
Gyrodinium fusiforme Kofoid & Swezy		x		x
Gyrodinium glaucum (Lebour) Kofoid & Swezy		x	x	x
Gyrodinium impudicum Fraga & Bravo	x			
Gyrodinium lachrima (Meunier) Kofoid & Swezi	x			x
Gyrodinium nasutum (Wulff) Schiller	x			
Gyrodinium pingue (Schütt) Kofoid & Swezy	x			
Gyrodinium spirale (Bergh) Kofoid & Swezy	x	x	x	x
Gyrodinium spp.	x	x	x	x
Gyrodinium varians (Wulff) Schiller	x			
Heterocapsa triquetra (Ehrenberg) Stein		x		

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Kofoidinium velelloides Pavillard				x
Massartia rotundata (Löhman) Schiller	x			
Massartia spp.	x	x	x	x
Mesoporos adriaticus (Schiller) Lillick				x
Mesoporos globulus (Schiller) Lillick				x
Mesoporos perforatus (Gran) Lillick		x		
Micracanthodinium spp.		x	x	x
Nematodinium armatum (Dogiel) Kofoid & Swezi				x
Nematodinium torpedi Kofoid & Swezy	x			
Nematopsides spp.				x
Noctiluca miliaris Suriray	x			
Noctiluca scintillans (McCartney) Ehrenberg	x			x
Oblea spp.				x
Oxyrrhis marina Dujardin	x			
Oxytoxum adriaticum Schiller	x			x
Oxytoxum areolatum Rampi				x
Oxytoxum belgicae Meunier		x	x	
Oxytoxum caudatum Schiller				x
Oxytoxum constrictum (Stein) Bütschli			x	x
Oxytoxum gladiolus Stein	x			
Oxytoxum gracile Schiller		x	x	
Oxytoxum laticeps Schiller				x
Oxytoxum longiceps Schiller	x			x
Oxytoxum margalefi Rampi				x
Oxytoxum milneri Murr. & Whitt.				x
Oxytoxum mitra Stein				x
Oxytoxum scolopax Stein	x	x	x	x
Oxytoxum spp.	x	x	x	x
Oxytoxum sphaeroideum Stein+A18	x	x	x	
Oxytoxum tonoilii Rampi				x
Oxytoxum variabile Schiller			x	x
Palaephalachroma spp.				x
Peridiniopsis asymmetrica Mangin	x			
Peridinium murrayi Kofoid				
Podolampas bipes Stein	x			x
Podolampas palmipes Stein	x		x	x
Podolampas spinifer Okamura				x
Polykrykos schwarzi Bütschli	x			
Polykrykos spp.			x	
Pronoctiluca acuta (Lohmann) Schiller		x		
Pronoctiluca pelagica Fabre-Domergué	x			
Pronoctiluca spp.		x	x	x
Prorocentrum aporum (Lochman) Loeblich				x
Prorocentrum balticum (Lohmann) Loeblich	x	x	x	x
Prorocentrum compressum (Bailey) Abbé	x		x	x
Prorocentrum cornutum Schiller	x			
Prorocentrum dentatum Stein			x	x
Prorocentrum gracile Schütt	x	x		x
Prorocentrum lima (Ehrenberg) Dodge			x	x
Prorocentrum maximum (Gourret) Schiller				x
Prorocentrum micans Ehrenberg	x	x	x	x
Prorocentrum minimum Schiller			x	
Prorocentrum nanum Schiller			x	x
Prorocentrum pyriformis (Schiller)				x
Prorocentrum rostratum Stein	x			
Prorocentrum spp.	x		x	x
Prorocentrum triestinum Schiller		x	x	x
Protoperidinium biconicum (Dangeard) Balech	x			
Protoperidinium bipes (Paulsen) Balech	x	x	x	x
Protoperidinium brevipes Balech	x	x	x	x

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
*Protoperidinium brochii (*Kofoid & Swezy) Balech	x			x
Protoperidinium cerasus (Paulsen) Balech	x		x	x
Protoperidinium claudicans (Paulsen) Balech	x		x	
Protoperidinium conicum (Gran) Balech	x	x		x
Protoperidinium cf *crassipes* (Kofoid) Balech	x		x	x
Protoperidinium curtipes (Jörgensen) Balech	x	x	x	
Protoperidinium depressum (Bailey) Balech	x	x	x	x
Protoperidinium diabolus (Paulsen) Balech	x	x	x	x
Protoperidinium divergens (Ehrenberg) Balech	x		x	x
Protoperidinium excentricum (Paulsen) Balech	x			
Protoperidium globulum (Stein) Balech	x			x
Protoperidinium grande (Kofoid) Balech	x			
Protoperidinium granii (Ostenfeld) Balech	x			x
Protoperidinium inclinatum Balech	x			x
Protoperidinium inflatum (Okamura) Balech	x			x
Protoperidinium leonis (Pavillard)	x	x	x	x
Protoperidinium longipes (Karsten) Balech	x			
Protoperidinium marielebourae (Paulsen) Balech	x			
Protoperidinium minutum (Kofoid) Loeblich II	x			x
Protoperidinium mite (Pavillard) Balech	x			x
Protoperidinium nudum (Meunier) Balech	x			
Protoperidinium oblongum (Aurivillius) Parke & Dodge	x			
Protoperidinium obtusum (Karsten) Parke & Dodge	x			
Protoperidinum oceanicum (Vanhoffen) Balech	x			
Protoperidinium ovatum (Pouchet) Schütt	x	x	x	x
*Protoperidinium pallidum (*Ostenfeld) Balech	x			x
*Protoperidinium paulseni (*Pavillard) Balech	x			
Protoperidinium pedunculatum (Schütt) Balech				x
Protoperidinum pellucidum (Bergh) Schütt	x	x	x	x
Protoperidinium pentagonum (Gran) Balech	x	x		
Protoperidinium punctulatum (Paulsen)	x			
Protoperidinium pyriforme (Paulsen) Balech	x	x	x	x
Protoperidinium quarnerense (Schöder) Balech	x			
Protoperidinum remotum Karsten			x	
Protoperidinum saltans (Meunier) Balech	x			
Protoperidinum sphaericum Okamura	x			
Protoperidinium spp.	x	x	x	x
Protoperidinium solidicorne (Mangin) Balech	x			x
Protoperidinium steinii (Jörgensen) Balech	x	x	x	x
*Protoperidinium subinerme (*Paulsen) Loeblich	x			
*Protoperidinium subinerme punctulatum (*Paulsen) Loeblich	x			
Protoperidinium trochoideum (Stein) Lemm.	x			
Ptychodiscus noctiluca Stein	x			
Pyrocystis fusiformis Wyville-Thompson				x
Pyrocystis gerbaulti Pavillard				x
Pyrocystis hamulus Cleve	x			
Pyrocystis lunula Schütt	x		x	
Pyrocystis obtusa Pavillard		x		x
Pyrocystis robusta Kofoid				x
Pyrocystis spp.	x		x	
Pyrophacus horologium Stein	x			x
Pyrophacus steinii (Schiller) Taylor				x
Scrippsiella faeroense (Paulsen) Balech & Soares				x
Scrippsiella trochoidea (Stein) Loeblich	x	x	x	x
Torodinium robustum Kofoid & Swezy	x	x	x	x
Torodinium spp.		x	x	x
Triadinium polyedricum (Pouchet) Dodge			x	x
Triadinium sphaericum (Murray & Whitt.)				x
Warnovia polyphemus (Pouchet) Schiller	x	x		
Warnowia spp.	x			x

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Zygabikodinium lenticulatum (Paulsen) Loeblich & Loeblich	x			
DIATOMOPHYCEAE				
Achnantes brevipes Agardh	x			
Achnantes brevipes var. *minor* Peragallo & Peragallo	x			
Achnantes longipes Agardh	x			
Actinoptychus spp.		x		x
Actinoptychus undulatus (Bailey) Ralfs	x		x	
Amphiprora spp.		x		
Amphora coffeaeformis var. *acutiuscula* (Kützing) Hustedt	x			
Amphora costata Wm. Smith	x			
Amphora crassa Gregory	x			
Amphora exigua Gregory	x			
Amphora ostrearia Brébisson in Kützing	x			
Amphora ovalis (Kützing) Kützing	x			
Amphora turgida Gregory	x			
Amphora spp.		x		
Asterionella japonica Cleve y Möller	x	x	x	x
Asterionella notata (Ehrenberg) Kützing	x			
Asterionella spp.		x	x	x
Asteromphalus flabellatus (Brébisson) Greville	x			
Asteromphalus heptactis (Brébisson) Ralfs in Pritchard	x			
Asteromphalus hookeri Ehrenberg	x		x	
Asteromphalus spp.		x		
Auliscus sculptus (Wm. Smith) Raphs in Pritchard	x			
Bacillaria paradoxa Gmelin in Linnaeus	x			
Bacteriastrum delicatulum Cleve	x	x	x	x
Bacteriastrum elegans Pavillard	x			
Bacteriastrum hyalinum Lauder	x	x		x
Bacteriastrum solitarium Mangin		x		
Bacteriastrum spp.			x	
Biddulphia aurita (Lyngbye) Brébisson & Godey	x			
Biddulphia aurita var. *obtusa* (Kützing) Hustedt	x			
Biddulphia mobiliensis (Bailey) Grunow	x	x	x	x
Biddulphia pulchella Gray	x			
Biddulphia regia (Max Schultze) Ostenfeld	x	x		
Biddulphia rombus (Ehrenberg) Smith		x		
Biddulphia rombus var. *trigona* (Cleve) Hustedt	x			
Biddulphia sinensis (Greville)		x		
Biddulphia spp.				x
Caloneis amphicephala Hustedt	x			
Caloneis liber (Wm Smith) Cleve	x			
Caloneis linearis (Grunow) Boyer	x			
Caloneis subsalina (Donkin) Hendey	x			
Caloneis spp.				x
Campylosira spp.		x		
Cerataulina pelagica (Cleve) Hendey	x	x	x	x
Cerataulus turgidus (Ehrenberg) Ehrenberg	x			
Chaetoceros affinis Lauder	x	x	x	x
Chaetoceros affinis var. *willei* (Gran) Hustedt	x		x	
Chaetoceros anastomosans Grunow		x		
Chaetoceros atlanticus Cleve	x			x
Chaetoceros atlanticus var. *neapolitana* (Schröder) Hustedt		x		
Chaetoceros borealis Bailey	x			
Chaetoceros brevis Schütt	x	x	x	x
Chaetoceros ceratosporum Ostenfeld	x	x		
Chaetoceros compressus Lauder	x	x	x	x
Chaetoceros concavicornis Mangin	x	x	x	x
Chaetoceros constrictus Gran	x	x		

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Chaetoceros convolutus Castracane	x		x	x
Chaetoceros costatum Pavillard	x	x		x
Chaetoceeros crinitus Schütt	x			
Chaetoceros curvisetus Cleve	x	x	x	x
Chaetoceros danicus Cleve	x	x	x	x
Chaetoceros debilis Cleve	x	x	x	x
Chaetoceros decipiens Cleve	x	x	x	x
Chaetoceros densus Cleve	x	x	x	x
Chaetoceros diadema (Ehrenberg) Gran	x	x	x	
Chaetoceros didymus Ehrenberg	x	x	x	x
Chaetoceros didymus var. *anglica* (Grunow in Van Heurck) Gran	x			
Chaetoceros difficilis Gran	x			
Chaetoceros diversus Cleve	x			
Chaetoceros eibenii Grunow	x	x	x	x
Chaetoceros filiformis Meunier		x		
Chaetoceros fragilis Meunier	x			
Chaetoceros furcellatus Bailey	x			
Chaetoceros gracilis Schütt		x	x	x
Chaetoceros holsaticus Schütt	x			
Chaetoceros laciniousus Schütt	x	x	x	x
Chaetoceros lauderi Ralfs	x	x	x	x
Chaetoceros lorenzianus Grunow	x	x		x
Chaetoceros mitra (Bailey) Cleve	x			
Chaetoceros neogracile Van Landinghan	x	x	x	
Chaetoceros pelagicus Cleve	x	x		
Chaetoceros perpusillum Cleve	x	x	x	x
Chaetoceros peruvianus Brightwell	x	x	x	x
Chaetoceros pseudocrinitus Ostenfeld	x			
Chaetoceros pseudocurvisetus Mangin	x	x	x	
Chaetoceros radians Schütt	x	x		
Chaetoceros radicans Schütt	x		x	x
Chaetoceros seiracanthus Gran		x		
Chaetoceros septentrionalis Oestrup		x		
Chaetoceros similis Cleve	x			
Chaetoceros simplex Ostenfeld	x	x		
Chaetoceros socialis Lauder	x	x	x	x
Chaetoceros spp.		x	x	x
Chaetoceros subsecundus (Grunow) Hustedt		x	x	x
Chaetoceros teres Cleve	x	x	x	x
Chaetoceros tetrastichon Cleve	x			
Chaetoceros tortissimus	x	x	x	
Chaetoceros wighami Brightwell	x			
Cocconeis costata Gregory	x			
Cocconeis peltoides Hustedt	x			
Cocconeis pseudomarginata Gregory	x			
Cocconeis scutellum Ehrenbergh	x			
Corethron cryophilum Castracane	x			x
Corethron hystrix Cleve		x	x	x
Corethron pelagicum Brun	x	x		
Coscinodiscus alborani Pavillard	x			
Coscinodiscus centralis Ehrenbergh	x			
Coscinodiscus concinnus Wm. Smith	x	x		
Coscinodiscus excentricus Ehrenberg	x	x		
Coscinodiscus gigas Ehrenberg	x			
Coscinodiscus granii Gouch	x	x	x	
Coscinodiscus janischii Schmidt	x			
Coscinodiscus lineatus Ehrenberg	x			
Coscinodiscus nitidus Gregory	x			
Coscinodiscus oculus-iridis Ehrenberg	x			
Coscinodiscus perforatus Ehrenberg	x			

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Coscinodiscus radiatus Ehrenberg	x			
Coscinodiscus spp.		x	x	x
Coscinodiscus thorii Pavillard	x			
Coscinosira oestrupii Ostenfeld		x		x
Coscinosira polychorda Gran	x	x	x	
Coscinosira spp.		x		
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin	x		x	x
Dactyliosolen mediterraneus (Peragallo) Peragallo	x	x	x	x
Diploneis advena (Schmidt) Cleve	x			
Diploneis bombus Ehrenberg	x			
Diploneis didyma (Ehrenberg) Ehrenberg	x	x		
Diploneis fusca (Gregory) Cleve	x			
Diploneis incurvata (Gregory) Cleve	x			
Diploneis splendida (Gregory) Cleve	x			
Diploneis spp.				x
Ditylum brightwelli (West) Grunow	x	x	x	x
Eucampia spp.		x		
Eucampia zodiacus Ehrenberg	x	x	x	x
Fragilaria crotonensis Kitton				x
Fragilaria hyalina (Kützing) Grunow	x			
Fragilaria spp.		x		
Grammatophora angulosa Ehrenberg		x		
Grammatophora marina (Lyngbye) Kützing	x	x		x
Grammatophora oceanica Ehrenberg	x			
Grammatophora serpentina (Ralfs) Ehrenberg	x			
Guinardia blaviana Peragallo	x	x		
Guinardia flaccida (Castracane) H. Peragallo	x	x	x	x
Gyrosigma balticum (Ehrenberg) Rabenhorst	x			
Gyrosigma fasciola (Ehrenberg) Griffith & Henfrey	x			
Gyrosigma spp.		x		
Hantzschia amphioxys (Eherenberg) Grunow in Cleve & Grunow	x			
Hantzschia marina (Donkin) Grunow	x			
Hemiaulus hauckii Grunow	x	x	x	x
Hemiaulus membranaceus Cleve				x
Hemiaulus sinensis Greville	x	x	x	x
Hemidiscus cuneiformis Wallich	x	x	x	
Lauderia annulata Cleve	x			
Lauderia borealis Gran	x	x	x	x
Leptocylindrus danicus Cleve	x	x	x	x
Leptocylindrus danicus var. *adriaticus* (Schröder) Schiller	x			
Leptocylindrus minimus Gran		x	x	x
Licmophora abbreviata Agardh	x			
Licmophora flabellata (Carmichael) Agardh	x			
Licmophora juergensi Agardh	x			
Licmophora spp.		x		x
Lithodesmium undulatum Ehrenberg		x		
Mastogloia spp.		x		
Melosira moniliformis (O.F. Müller) Agardh	x	x		
Melosira granulata (Ehrenberg) Ralfs	x	x		
Melosira juergensi Agardh	x	x		x
Melosira nummuloides (Dillwyn) Agardh	x	x		
Melosira sulcata (Ehrenberg) Kütz	x			x
Navicula abrupta (Gregory) Donkin	x			
Navicula cancellata Donkin	x	x		
Navicula cryptocephala Kützing	x			
Navicula distans (Wm. Smith) Schmidt	x			x
Navicula forcipata Greville	x			
Navicula humerosa Brébisson in Wm. Smith	x			
Navicula maculosa Donkin	x			
Navicula membranacea Cleve	x	x	x	x

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Navicula molinifera Cleve	x			
Navicula mutica Kutzing	x			
Navicula cf *pelagica* Cleve		x		
Navicula ostrearia (Gaillon) Bory	x			
Navicula cf *salinarum* Grunow in Cleve & Möller	x	x	x	x
Navicula spp.		x	x	x
Navicula tuscula Ehrenbergh	x			
Navicula cf *wawrikae* Hustedt		x		
Nitzschia acicularis (Kützing) Wm. Smith	x			
Nitzschia acuminata (Wm. Smith) Grunow	x			
Nitzschia angularis Wm. Smith	x			
Nitzschia coarctata Grunow		x	x	
Nitzschia compressa (Bailey) Boyer	x			
Nitzschia frigida Grunow in Cleve & Grunow	x			
Nitzscia linearis Wm. Smith	x			
Nitzschia longissima (Brébisson in Kützing) Grunow	x	x	x	x
Nitzschia mediterranea Hustedt	x			x
Nitzschia obtusa Wm. Smith		x		
Nitzschia pacifica Cleve		x	x	x
Nitzschia cf. *pungens* Grunow	x	x	x	x
Nitzschia recta Hantzsch ex Rabenhorst	x			
Nitzschia sigma (Kützing) Wm. Smith	x			
Nitzschia sigma var. *rigida* (Kützing) Grunow	x			
Nitzschia spathulata Brébisson		x		
Nitzschia spp.		x	x	x
Nitzschia cf *turgidula* Hustedt		x	x	
Nitzschia vitrea Norman	x			
Paralia sulcata (Ehrenberg) Kützing		x	x	x
Phaeodactylum tricornutum Bohlin	x	x	x	
Pinnularia ambigua Cleve				x
Pinnularia subcapitata Gregory	x			
Pinnularia spp.		x		
Plagiogramma vanheurckii Grunow in Van Heurck	x	x		
Planktoniella sol (Wallich) Schütt	x	x	x	x
Pleurosigma acutum Norman		x	x	x
Pleurosigma angulatum (Quekett) Wm. Smith	x			
Pleurosigma elongatum Wm. Smith	x			
Pleurosigma formosum Wm. Smith	x			
Pleurosigma ibericum H. Peragallo			x	
Pleurosigma intermedium Wm. Smith	x			
Pleurosigma normanii Raphs in Pritchard	x			
Pleurosigma spp.		x	x	x
Podosira stelliger (Bailey) Mann	x	x		
Porosira glacialis (Grunow) Jörgensen		x	x	
Pseudonitzschia delicatissima (Cleve) Heiden	x	x	x	x
Rhabdonema adriaticum Kützing	x			
Rhizosolenia alata Brightwell	x	x	x	x
Rhizosolenia alata var. *gracillima* (Cleve) Grunow	x			x
Rhizosolenia alata var. *indica* (H. Peragallo) Hustedt				x
Rhizosolenia bergonii H. Peragallo		x		x
Rhizosolenia calcar-avis Max Schultze	x			
Rhizosolenia castracanei Peragallo	x			
Rhizosolenia delicatula Cleve	x	x	x	x
Rhizosolenia fragilissima Bergon	x	x	x	x
Rhizosolenia hebetata Gran	x			x
Rhizosolenia hebetata f. *semispina* (Hensen) Gran	x	x	x	
Rhizosolenia imbricata Brightwell	x			x
Rhizosolenia imbricata var. *shrubsolei* (Cleve) Van Heurck	x	x	x	x
Rhizosolenia robusta Norman	x			x
Rhizosolenia setigera Brightwell	x	x	x	x

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Rhizosolenia stolterfothii H. Peragallo	x	x	x	x
Rhizosolenia styliformis Brightwell	x	x	x	x
Rhizosolenia spp.				x
Schroederella delicatula (Peragallo) Pavillard	x	x	x	x
Skeletonema costatum (Greville) Cleve	x	x	x	x
Stauroneis acuta Wm. Smith	x			
Stauroneis salina Wm. Smith	x			x
Stephanopyxis palmeriana (Greville) Grünow	x	x	x	x
Stephanopyxis turris (Greville & Arnott) Ralfs	x	x	x	x
Streptotheca thamensis Shrubsole		x	x	x
Striatella unipunctata (Lyngbye) Agardh	x			
Surirella gemma Ehrenberg	x			
Synedra gaillonii (Bory) Ehrenberg	x			
Synedra spp.		x	x	x
Synedra tabulata (Agardh) Kützing	x			
Synedra undulata Bailey	x			
Thalassionema nitzschioides (Grunow) Van Heurck	x	x	x	x
Thalassionema spp.				x
Thalassionema bacillaris (Heiden) Kolbe				x
Thalassiosira aestivalis Gran & Angst		x		x
Thalassiosira condensata Cleve	x			
Thalassiosira decipiens (Grunow) Jörgensen		x	x	x
Thalassiosira fallax Meunier	x	x	x	x
Thalassiosira gravida Cleve		x		
Thalassiossira hispanica Paulsen	x			
Thalassiosira hyalina (Grunow) Gran		x		
Thalassiosira levanderi Van Goor	x	x	x	x
Thalassiosira nordenskioeldii Cleve	x			
Thalassiosira rotula Meunier	x	x	x	x
Thalassiosira spp.		x	x	x
Thalassiosira subtilis (Ostenfeld) Gran	x	x	x	
Thalassiothrix frauenfeldii Grunow	x	x	x	x
Thalassiothrix longissima Cleve & Grunow in Cleve & Möller	x	x	x	
Thalassiothrix mediterranea Pavillard	x	x	x	x
Thalassiothrix spp.		x		
Trachyneis aspera (Ehrenberg) Cleve		x		
Trachyneis spp.		x		
Triceratium alternans (Bailey)		x	x	x
Triceratium antediluvianum (Ehrenberg) Grunow	x			
Triceratium spp.				x
EUGLENOPHYCEAE				
Eutreptia spp.	x	x	x	
Eutreptiella spp.	x			
DICTYOCHOPHYCEAE				
Dictyocha fibula Ehrenberg		x	x	x
Dictyocha speculum Ehrenberg	x		x	x
CHRYSOPHYCEAE				
Dinobryon balticum (Schütt) Lemmermann	x	x		
Dinobryon cf *belgica* Meunier		x		
Dinobryon mediterraneum Pavillard	x			
Dinobryon spp.	x	x	x	x
Solenicola setigera Pavillard		x	x	x
PRYMNESIOPHYCEAE				

species	Galicia rias	A Coruña	Galicia shelf	Cantabrian
Chrysochromulina spp.		x		
Corymbellus spp.		x		x
Phaeocystis poucheti (Hariot) Lagerheim	x	x	x	x
RAPHIDOPHYCEAE				
Olisthodiscus luteus N. Carter	x	x		
Olisthodiscus spp.	x	x	x	
Oltmansiella lineata Zimmermann		x		
Tetraselmis spp.		x		
PRASINOPHYCEAE				
Halosphaera viridis Schmitz	x			
CRYPTOPHYCEAE				
Cryptomonadinae	x	x	x	x
PROTOZOA				
Mesodinium rubrum Lohmann	x	x	x	

ANNEX 5 PHYTOPLANKTON SPECIES COMPOSITION IN THE SOUTHERN IBERIAN COAST (BODE AND VARELA)

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Abstract

A review of available data on phytoplankton species composition in the Gibraltar Strait and nearby areas was made in the Southern Iberian coast covering the period between 1979 to 1994. Only a limited number of datasets on this specific subject were found. No citations were found on phytoplankton species distributions across the Gibraltar Strait. A selected reference list of papers wich contain records of phytoplankton species and/or phytoplankton cell abundance data for the study and nearby areas is provided, but only three references were considered to make a species check-list of the main phytoplanktonic groups. A total of 319 species or other taxa of phytoplankton were recorded in the study area, of which 177 were diatoms, 112 dinoflagellates and 30 belonged to other groups (cyanobacteria and various flagellates).

Method

Species records were classified according to the locations of sampling in each study. One study was made along the Mediterranean coast along SE Spain with some samples in the Alboran Sea (Delgado, 1990). The references of samples taken in Atlantic waters were from two coastal sites in SW Spain: Cádiz Bay (Establier et al., 1986) and the Ría de Huelva (Cortés and Varela, 1992). The check list provided may be considered as preliminar, given the scarce number of studies made in this area. More complete species lists of the Western Mediterranean can be consulted in Branconnot (1983), Delgado and Fortuño (1991) and Margalef (1994), while those of the Eastern Atlantic can be found in Reyssac (1979) and Ojeda (1985). Species names were given following Schiller (1937) and Dodge (1982) for dinoflagellates, Peragallo and Peragallo (1908) and Hustedt (1959) and Hendey (1964) for diatoms.

Results

A total of 319 species or other taxa of phytoplankton were recorded in the study area, of which 177 were diatoms, 112 dinoflagellates and 30 belonged to other groups (cyanobacteria and various flagellates). Eleven species were potential producers of harmful algal blooms. The detailed species list and records in each site considered are the main deliverables of this report and appeared in tables 1 to 3. As a preliminary comparison of floras, only 96 species of diatoms and 4 species of dinoflagellates were found at both sides of the Gibraltar Strait. There were more species of diatoms in the Atlantic sites (164) than in the Mediterranean coast (96). However the number of dinoflagellate species recorded was similar at both sides of the Gibraltar Strait (72 species in the Atlantic coast versus 79 species in the Mediterranean coast). As expected, phytoplankton species composition appears to be quite different at both sides of the Gibraltar Strait and can be used as an indicator of water exchange through the strait. Nevertheless, it must be taken into account that the results analysed in this report are based on only a limited number of studies and localities (some of them very close to the coast), and will need further confirmation by the proposed field studies in the Gibraltar Strait.

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Table 1. Diatom species found in the study area. HAB indicates toxic species.

Species	HAB	Mediterranean	Cadiz Bay	Huelva Coast
Achnantes brevipes Agardh		X		
Achnantes sp.			X	
Actinocyclus sp.		X		
Actinocyclus subtilis (Gregory) Ralfs			X	
Actinoptychus adriaticus Grünow			X	
Actinoptychus splendens (Shadbolt) Ralfs		X		
Actinoptychus undulatus (Bailey) Ralfs			X	
Actinoptychus sp.			X	
Amphiprora gigantea Grunow		X		
Amphiprora spp.			X	X
Amphora ostrearia Cleve			X	
Amphora sp.		X	X	
Asterionella japonica Cleve ex Möller		X	X	X
Asterionella spp.		X	X	X
Asteromphalus robustus Castracane		X		
Bacillaria paxillifer (Muller) Hendey		X	X	
Bacteriastrum delicatulum Cleve		X		
Bacteriastrum hyalinum Lauder		X		X
Bacteriastrum mediterraneum Pavillard			X	
Bacteriastrum sp.			X	X
Biddulphia aurita (Lyngbye) Brébisson ex God.			X	
Biddulphia mobiliensis (Bailey) Grunow		X	X	X
Biddulphia pulchella Gray		X	X	
Biddulphia regia (Schultze) Ostenfeld		X		X
Biddulphia tuomeyi (Bailey) Roper		X		
Biddulphia sp.			X	
Campylodiscus spp.			X	
Cerataulina bergonii Peragallo			X	X
Cerataulina pelagica (Cleve) Hendey				
Cerataulina spp.			X	
Cerataulus smithii Ralfs			X	
Chaetoceros affinis Lauder		X		
Chaetoceros borealis Bailey			X	
Chaetoceros brevis Schütt			X	X
Chaetoceros ceratosporum Ostenfeld				X
Chaetoceros constrictus Gran			X	
Chaetoceros costatum Pavillard				X
Chaetoceros curvisetus Cleve		X	X	X
Chaetoceros danicus Cleve			X	
Chaetoceros decipiens Cleve		X	X	X
Chaetoceros didymus Ehrenberg		X	X	X
Chaetoceros laciniosus Schütt			X	
Chaetoceros lorenzianus Grunow		X		
Chaetoceros peruvianus Brightwell		X	X	
Chaetoceros pseudocurvisetus Mangin		X	X	
Chaetoceros radicans Schütt		X		
Chaetoceros rostratus Lauder			X	
Chaetoceros simplex Ostenfeld				X
Chaetoceros socialis Lauder		X	X	X
Chaetoceros spp.			X	X
Cocconeis sp.		X		
Corethron criophilum Castracane				X
Corethron hystrix Cleve		X		
Coscinodiscus alboranii Pavillard		X		
Coscinodiscus centralis Ehrenberg			X	
Coscinodiscus concinnus W. Smith			X	
Coscinodiscus curvatulus Grunow			X	

Species	HAB	Mediterranean	Cadiz Bay	Huelva Coast
Coscinodiscus denarius A. Schmidt			X	
Coscinodiscus excentricus Ehrenberg		X	X	
Coscinodiscus gigas Ehrenberg		X	X	
Coscinodiscus granni Gough		X	X	
Coscinodiscus janischii A. Schmidt			X	
Coscinodiscus lineatus Ehrenbergh			X	
Coscinodiscus moelleri Schmidt		X		
Coscinodiscus obscurus A. Schmidt			X	
Coscinodiscus oculus-iridis Ehrenberg			X	
Coscinodiscus radiatus Ehrenberg		X	X	
Coscinodiscus spp.			X	X
Coscinosira mediterranea Schröder				X
Coscinosira oestrupii Ostenfeld				X
Coscinosira polychorda Gran			X	
Dactyliosolen mediterraneus Peragallo		X	X	
Diploneis bombus (Ehrenberg) Cleve		X		
Diploneis didyma Ehrenberg				X
Diploneis sp.		X		X
Ditylum brightwelli (West) Grunow		X	X	X
Eucampia zodiacus Ehrenberg		X	X	X
Eunotia arcus Ehrenberg				X
Fragilaria spp.				X
Gossleriella tropica Schutt		X		
Grammatophora oceanica var. *adriatica* Grunow		X	X	
Grammatophora undulata Ehrenberg		X		
Grammatophora sp.		X	X	
Guinardia flaccida (Castracane) Peragallo		X	X	
Guinardia sp.				X
Hantzschia vivax Wm. Smith		X		
Hemiaulus hauckii Grunow		X		
Hemiaulus sinensis Greville		X	X	X
Hemidiscus cuneiformis Wallich		X		
Lauderia borealis Gran			X	
Lauderia sp.				X
Leptocylindrus danicus Cleve		X	X	X
Leptocylindrus minimus Gran		X		X
Leptocylindrus sp.			X	
Licmophora gracilis (Ehrenberg) Grunow		X		
Licmophora spp.			X	X
Lithodesmium undulatum Ehrenbergh			X	X
Melosira distans (Ehrenberg) Kützing		X		
Melosira granulata (Ehrenberg) Ralfs		X		X
Melosira moniliformis (O.F. Müller) Agardh			X	
Melosira varians Agardh				X
Melosira sp.		X		
Navicula cf. *wawrikae* Hustedt				
Navicula digitoradiata (Gregory) Ralfs				X
Navicula spp.		X	X	X
Nitzschia closterium (Ehrenberg) W. Smith		X		X
Nitzschia delicatula Hasle		X		
Nitzschia dissipata (Kützing) Grunow		X		
Nitzschia fraudulenta Cleve		X		
Nitzschia heimii Mangin		X		
Nitzschia lanceolata W.Smith		X		
Nitzschia longissima (Brébisson ex Kützing) Ralfs in Pritch		X		X
Nitzschia longissima cf. *closterium*			X	
Nitzschia longissima var. 1 (Brébisson) Grunow (large)				X
Nitzschia marina Grunow		X		
Nitzschia obtusa Wm. Smith				X
Nitzschia punctata (Wm. Smith) Grunow				X

Species	HAB	Mediterranean	Cadiz Bay	Huelva Coast
Nitzschia pungens Cleve ex Moeller	*	X		X
Nitzschia recta Hantzsch				X
Nitzschia seriata Grunow	*		X	X
Nitzschia sigma var. *intercedens* Grunow				X
Nitzschia vermicularis (Kützing) Grunow		X		
Nitzschia spp.			X	X
Paralia sulcata (Ehrenberg) Kützing		X		X
Phaeodactylum tricornutum Bohlin			X	
Pinnularia spp.				X
Pinnularia viridis (Nitzsch) Ehrenberg				X
Pleurosigma angulatum (Quekett) Wm. Smith			X	
Pleurosigma spp.		X	X	X
Podosira stelliger (Bailey) Mann			X	X
Porosira sp.			X	
Pseudonitzschia delicatissima (Cleve) Heiden				X
Rhabdonema adriaticum Kützing		X		
Rhabdonema arcuatum (Lyngbye) Kützing			X	
Rhabdonema sp.			X	
Rhizosolenia alata Brightwell		X	X	X
Rhizosolenia alata f. gracillima (Cleve) Gran		X		
Rhizosolenia alata f. indica (Peragallo) Gran		X		X
Rhizosolenia calcaravis Schultze		X		
Rhizosolenia delicatula Cleve		X	X	X
Rhizosolenia fragilissima Bergon		X		X
Rhizosolenia hebetata f. semispina (Hensen) Gran		X		
Rhizosolenia hebetata var. *shrubsolei* (Cleve) Schroeder		X		
Rhizosolenia imbricata Brightwell		X	X	
Rhizosolenia robusta Norman in Pritch		X	X	
Rhizosolenia setigera Brightwell		X	X	X
Rhizosolenia stolterfothii Peragallo		X	X	X
Rhizosolenia styliformis Brightwell		X	X	
Schroederella delicatula (Peragallo) Pavillard		X	X	X
Scoliopleura tumida (Brébisson ex Kützing) Rabenhorst				X
Skeletonema costatum (Greville) Cleve		X	X	X
Stauroneis membranacea (Cleve) Hustedt		X		
Stephanopyxis palmeriana (Greville) Grunow			X	
Stephanopyxis turris (Greville ex Arnott) Ralfs		X		
Streptotheca tamesis Shrubsole			X	
Striatella unipunctata (Lyngbye) Agardh			X	
Surirella fastuosa Ehrenberg		X		
Surirella ovata Kützing				X
Surirella sp.			X	X
Synedra affinis Kützing				X
Synedra ulna (Nitzsch) Ehrenberg		X		
Synedra undulata (Bailey) Gregory			X	
Synedra vaucheriae Kützing		X		
Synedra spp.			X	X
Thalassionema nitzschioides (Grunow) Van Heurck		X	X	X
Thalassiosira rotula Meunier		X		X
Thalassiosira subtilis (Ostenfeld) Gran				
Thalassiosira spp.		X	X	X
Thalassiothrix delicatula Cupp		X		
Thalassiothrix frauenfeldii Grunow		X		
Thalassiothrix mediterranea Pavillard		X	X	
Thalassiothrix spp.			X	
Triceratium alternans (Bailey)		X	X	
Triceratium shadboltianum Greville		X		
Tropidoneis sp.		X		X

Table 2. Dinoflagellate species found in the study area. HAB indicates toxic species.

Species	HAB	Mediterranean	Cadiz Bay	Huelva Bay
Amphidinium sp.		X		
Amphisolenia bidentata Schroeder		X		
Amphisolenia extensa Kofoid		X		
Centrodinium elongatum Kofoid		X		
Centrodinium maximum Pavillard		X		
Ceratium arietinum Cleve		X		
Ceratium buceros (Zacharias) Schiller		X		X
Ceratium candelabrum (Ehrenberg) Stein		X		
Ceratium carriense Gourret		X	X	
Ceratium declinatum Karsten		X		
Ceratium extensum (Gourret) Cleve		X		
Ceratium furca (Ehrenberg) Claparede ex Lachmann		X	X	X
Ceratium fusus (Ehrenberg) Dujardin		X	X	X
Ceratium gravidum Gourret		X		
Ceratium horridum (Cleve) Gran		X		
Ceratium limulus Gourret		X		
Ceratium longirostrum Gourret		X	X	
Ceratium macroceros (Ehrenberg) Vänhoffen		X		X
Ceratium massiliense (Gourret) Jorgensen		X	X	
Ceratium minutum Jorgensen		X		
Ceratium pentagonum Gourret		X	X	
Ceratium praelongun (Lemn.) Kofoid		X		
Ceratium ranipes Cleve		X		
Ceratium teres Kofoid		X		
Ceratium trichoceros (Ehrenberg) Kofoid		X		
Ceratium tripos (O.F. Müller) Nitzsch		X	X	
Ceratium volans Cleve			X	
Ceratium vultur Pavillard		X		
Ceratocorys armata (Schutt) Kofoid		X		
Ceratocorys horrida Stein		X		
Dinophysis acuminata Claparede ex Lachmann	*			X
Dinophysis acuta Ehrenberg	*	X		
Dinophysis caudata Saville Kent	*	X	X	
Dinophysis lenticula Pavillard		X		
Dinophysis operculoides (Schutt) Kofoid		X		
Dinophysis ovum (Schutt)	*	X		
Dinophysis parvula (Schutt) Balech		X		
Dinophysis pulchellum (Lebour) Balech		X		
Dinophysis rotundata Claparede ex Lachmann	*		X	
Dinophysis sacculus Jorgensen ex Pavillard	*		X	X
Dinophysis schuetti Murray ex Whitting		X		
Dinophysis tripos Gourret		X		
Dinophysis spp.		X		X
Erythropsis agilis Hertwig		X		
Gonyaulax digitale (Pouchet) Kofoid			X	X
Gonyaulax polyedra Stein	*	X	X	
Gonyaulax polygramna Stein		X		X
Gonyaulax sp.			X	
Gymnodinium catenatum Graham	*	X		X
Gymnodinium simplex (Lohman) Kofoid ex Schwezy				X
Gymnodinium splendens Lebour			X	X
Gymnodinium spp.		X	X	X
Gyrodinium aureolum Hulburt				X
Gyrodinium fusiforme Kofoid ex Swezy				X

Table 2. Dinoflagellates (cont.)

Species	HAB	Mediterranean	Cadiz Bay	Huelva Bay
Gyrodinium glaucum (Lebour) Kofoid ex Swezy				X
Gyrodinium spirale (Bergh) Kofoid ex Swezy				X
Gyrodinium spp.		X		X
Massartia spp.				X
Murrayella spinosa Kofoid		X		
Noctiluca miliaris Suriray in Lamarck		X		
Noctiluca scintillans (Macartney) Ehrenberg			X	
Ornithocercus heteroporus Kofoid		X		
Ornithocercus magnificus Stein		X		
Ornithocercus thurnii (Schmidt) Kofoid ex Skoysberg		X		
Oxytoxum longiceps Schiller		X		
Oxytoxum scolopax Stein		X		
Podolampas palmipes Stein		X		
Podolampas spinifera Okamura		X		
Pronoctiluca spp.				X
Prorocentrum balticum (Lohmann) Loeblich		X		X
Prorocentrum cf. *cornutum* Schiller				X
Prorocentrum compressum (Bailey) Abe		X		
Prorocentrum gracile Schutt			X	
Prorocentrum micans Ehrenberg		X	X	X
Prorocentrum nanum Schiller				X
Prorocentrum rostratum Stein				X
Prorocentrum rotundatum Schiller				X
Prorocentrum scutellum Schroeder			X	X
Prorocentrum sphaeroideum Schiller				X
Prorocentrum triestinum Schiller				X
Prorocentrum sp.		X	X	
Protoceratium areolatum Kofoid		X		
Protoperidinium bipes (Paulsen) Balech			X	X
Protoperidinium brochii Kofoid ex Swezy		X	X	
Protoperidinium cerasus (Paulsen) Balech				X
Protoperidinium claudicans Paulsen		X		
*Protoperidinium conicum (*Gran) Balech		X		
Protoperidinium crassipes Kofoid		X		
Protoperidinium curvipes Ostenfeld		X		
Protoperidinium depressum (Bailey) Balech		X	X	
Protoperidinium diabolus (Paulsen) Balech		X	X	
Protoperidinium divergens Ehrenberg		X		X
Protoperidinium globulus Stein		X		
Protoperidinium granii Ostenfeld		X	X	
Protoperidinium latispinum Mangin		X		
Protoperidinium leonis Pavillard		X	X	
Protoperidinium marielebourae (Paulsen) Balech			X	
Protoperidinium oceanicum Vanhoffen		X	X	
Protoperidinium ovatum (Pouchet) Schutt		X		X
Protoperidinium pentagonum (Gran) Balech		X		
Protoperidinium pyriforme (Paulsen) Balech		X	X	X
Protoperidinium spp.			X	X
Pyrocystis elegans Pavillard		X		
Pyrocystis fusiformis Wyville Thomson ex Murray		X		
Pyrophacus horologium Stein			X	
Pyrophacus sp.			X	
Scrippsiella trochoidea (Stein) Lohmann			X	X
Torodinium robustum Kofoid ex Swezy				X
Triposolenia truncata Kofoid		X		

Table 2. Dinoflagellates (cont.)

Species	HAB	Mediterranean	Cadiz Bay	Huelva Bay
Warnowia polyphemus (Pouchet) Schiller				**X**
Warnowia sp.		**X**		

Table 3. Taxa of other groups of phytoplankton found in the study area.

Species	Mediterranean	Cadiz Bay	Huelva Bay
CYANOPHYCEAE			
Oscillatoria sp.		X	
EUGLENOPHYCEAE			
Eutreptia spp.			X
Eutreptiella sp.	X		
Phacus sp.			X
CHRYSOPHYCEAE			
Dictyocha fibula Ehrenberg	X	X	X
Dinobryon spp.			X
Distephanus speculum (Ehrenberg) Haeckel	X	X	
Mesocena sp.		X	
Solenicola setigera Pavillard	X		X
PRYMNESIOPHYCEAE			
Acanthoica sp.	X		
Coccolithus leptoporus Lochman	X		
Coronosphaera sp.	X		
Discosphaera tubifer (Murray ex Blackman) Ostenfeld	X		
Discosphaera sp.	X		
Emiliania huxleyi (Lohmann) Hay ex Mohler	X		
Gephyrocapsa oceanica Kamptner	X		
Phaeocystis poucheti (Hariot) Lagerheim	X		
Pontosphaera discopora Schiller	X		
Pontosphaera syracusana Lohmann	X		
Rhabdosphaera clavigera Murray ex Blackman	X		
Sciphosphaera apsteinii Lohmann	X		
Syracosphaera spp.	X		
Umbellosphaera sp.	X		
PRASINOPHICEAE			
Halosphaera viridis Schmitz	X		
CHLOROPHYCEAE			
Ankistrodesmus spp			X
Closterium sp.		X	
Pediastrum clathratum (Schroeder) Lemman			X
Pediastrum sp.		X	
Scenedesmus quadricauda (Turpin) Brébisson			X
CRYPTOPHYCEAE			
unidentified Cryptophyceae			X

ANNEX 6 WORKING MANUAL AND SUPPORTING PAPERS ON THE USE OF A STANDARDISED INCUBATOR-TECHNIQUE IN PRIMARY PRODUCTION MEASUREMENTS)

(Version 23 April 1998, filename MAN_NEW2.doc)

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Preface

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

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

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

Introduction

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

The method for estimating primary production by the "ICES Incubator" given in this manual (cf. Annex 1) is intended for monitoring purposes. Measurements should be possible from small, as well as from large vessels. Because of this, some simplifications from what could be considered to be the ideal method, have been introduced. It should be pointed out that the "ICES Incubator" method is not meant as a replacement of other "P-E techniques". It has been designed to provide a reliable measurement of primary production parameters, using a <u>simple incubator</u> and a <u>standard protocol</u>. The incubator is a rectangular perspex tank (33 x 33 x 9 cm) with a turning wheel on which a maximum of 12 experimental bottles can be clamped. It is illuminated by 10 fluorescent tubes (TLD 8W J8, no 33). The full description of the incubator is given in Annex 1. The standard protocol is presented here. The incubator is manufactured by HYDROBIOS, Kiel, Germany (adress see appendix).

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

Sampling strategy

Mixed water columns

In areas where the euphotic zone is mixed and the phytoplankton community is uniformly distributed, one representative sample, obtained with a hose (0-10 m) is sufficient (Lindahl, 1986). As an alternative, mixed discrete samples from 0 to 10 m depth can be used.

Stratified water columns

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

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

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

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

Measuring protocol (see Fig. 1)

General Preparation

1. Placement of the incubator

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

2. Incubation flasks

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

3. Irradiance levels in the incubator (for details see Annex 2)

A set of 12 incubation flasks with different transmission levels, from 0 to 100% should be used (for manufacturer of special prepared bottles see appendix). It is important that there should be enough measuring points to obtain a good measurement of α and P_{max}. With the 12 bottles this is not a problem. (After some experimentation with the incubator normally a series of 6 bottles will suffice to measure a reliable P-E relationship).The exact irradiance in each bottle should be measured, even if the transmission percentages are known. This can be done with a small sensor which can be introduced into the bottles (a manufacturer of this calibrated sensor can be found in the appendix) To obtain irradiance saturated photosynthetic rates (P_{max}) a minimal irradiance of 500 $\mu E.m^{-2}.s^{-1}$ should be available. This is achieved by using 10 fluorescent lamps (TLD 8W J8, no 33,). In case 500 $\mu E.m^{-2}.s^{-1}$ is not reached, a mirrow behind the lamps and possibly on the other side of the tank will increase the irradiance flux.

4. ^{14}C solution

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

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

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

5. Accompanying field measurements

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

6. Sampling

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

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

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

7. Total CO_2 concentration. (Fig. 1, Step 2)

Total CO_2 concentration should be calculated according to other standard methods, using titration of carbonate (Strickland and Parsons, 1972). In brackish waters, such as the Baltic Sea, the CO_2 concentration can be calculated by the formulas of Buch (1945). In both cases temperature, salinity and pH must be measured.

8. Addition of ^{14}C (Fig. 1, Step 3)

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

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

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

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

9. Incubation. (Fig.1, Step 5)

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

10. End of incubation. (Fig. 1, Step 6)

After incubation the flask contents are filtered immediately.

In case of high algal biomass or high sedimentation load it might be needed to filter a subsample. A defined portion should be taken and filtered.

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

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

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

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

11. Counting of the radioactivity.(Fig.1, Step 8)

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

12. Calculation of carbon uptake (Fig. 1, Step 9)

The total carbon uptake is calculated from the equation:

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

Where

(a) = sample activity (minus back-ground), dpm

(b) = total activity added to the sample (minus back-ground), dpm

(c) = total concentration of $^{12}CO_2$ in the sample water, µmol/L (or µM)

(d) = the atomic weight of carbon

(e) = a correction for the effect of ^{14}C discrimination

k1 = subsampling factor (e.g. sample 50 ml, subsample 10 ml: k1=subsample factor 50/10= 5)

k2 = time factor (e.g. incubaton time 125 minutes: k2= 60/125= 0.48)

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

13. Calculation of daily primary production

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

1. Vertical attenuation (extinction) coefficient, in m^{-1}. In case no attenuation has been measured, Secchi disc values can be used by conversion. The attenuation coeficient is calculated as

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

   where x is 1.7–2.3 (1.7 (Raymont, 1967), 2.3 (Aertebjerg and Bresta, 1984), 1.84 (Edler, 1997)). This factor changes with sea area. In principal it increases with decreasing salinity going into the Baltic Sea.

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

3. P_{max}, E_k, and α.

In order to transform the hourly production corrected for dark uptake into daily production which is the ultimate ecological goal, the procedure according to the protocol should be followed.

A computer program for the calculation of the daily production will be made available. The program also combines the data in a small database for the ICES data bank.

14. Quality assurance

(To be elaborated)

Validation

a) Selectivity
b) Sensitivity
c) Range
d) Limit of detection
e) Accuracy

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

(Certified reference material of ^{14}C shall be used. Internal methods shall be properly calibrated. The analysis should be subject to international calibration exercises.

15. Data delivery:

In order to have the possibility to check and recalculate daily productivity data it is important that all laboratories deliver their data in the same format and that this includes the fixation rates for every irradiance.

At present there is an ICES E-mail workshop on how to develop matrices, in order to ensure that all necessary basic data are included. As soon as a final format has been established this will be distributed and attached to this protocol.

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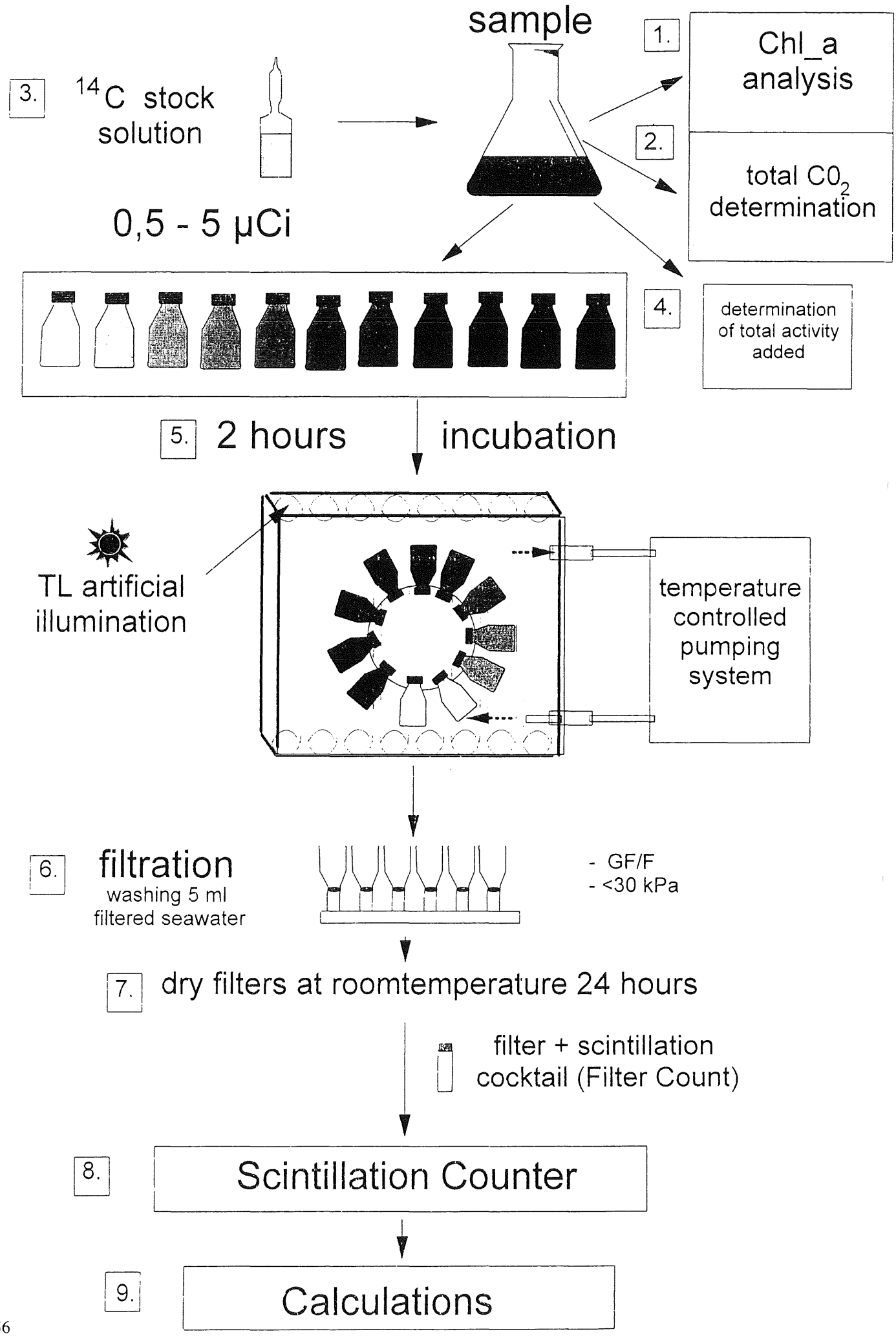
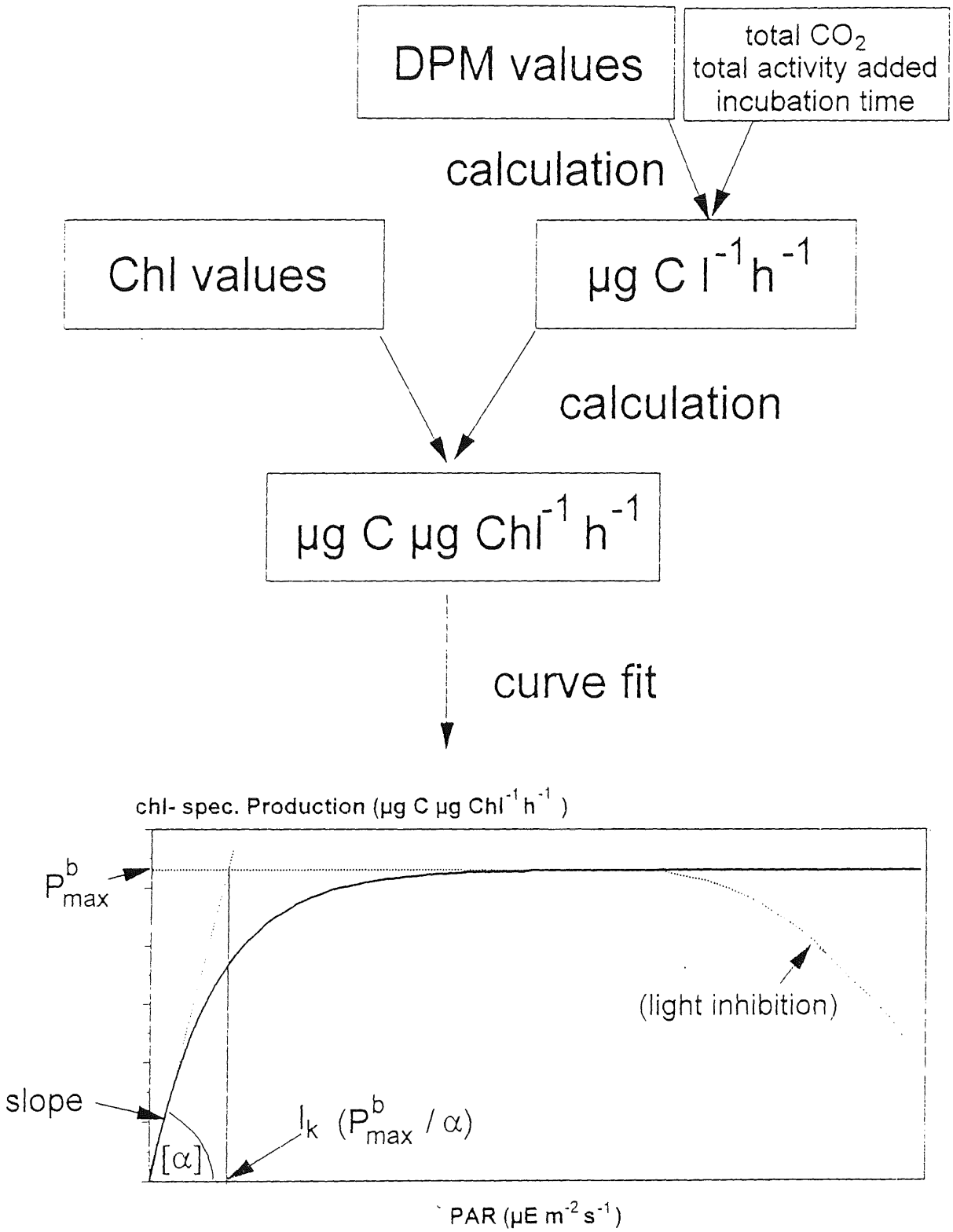
Strickland J.D.H. and Parsons T.R. 1972. A practical handbook of seawater analysis. Fish. Res. Bd. Canada. Bull. 167. Ottawa. 310 pp.

Appendix: Manufacturers

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

ZEMOKO, c/o, ing. Jan de Keyzer, Dorpsplein 40, 4371 AC Koudekerke, the Netherlands, Tel/Fax: +31-118-551182


sample
1.
Chl_a analysis
2.
total CO_2 determination
3.
^{14}C stock solution
0,5 - 5 µCi
4.
determination of total activity added
5.
2 hours
incubation
TL artificial illumination
temperature controlled pumping system
6.
filtration
washing 5 ml filtered seawater
- GF/F
- <30 kPa
7.
dry filters at roomtemperature 24 hours
filter + scintillation cocktail (Filter Count)
8.
Scintillation Counter
9.
Calculations


DPM values
total CO_2
total activity added
incubation time
calculation
Chl values
µg C l^{-1} h^{-1}
calculation
µg C µg Chl^{-1} h^{-1}
curve fit
chl- spec. Production (µg C µg Chl^{-1} h^{-1})
P^b_{max}
(light inhibition)
slope
[α]
I_k (P^b_{max} / α)
PAR (µE m^{-2} s^{-1})

ANNEX 7 FLOW CYTOMETRY AS A TOOL FOR COUNTING AND IDENTIFICATION OF PHYTOPLANKTON (GROUPS) AND OTHER APPLICATIONS BY G. DUBELAAR AND R. JONKER

Please note that an electronic copy of this file is available under: http//home.wxs.nl~dubelaar/icesrep.html

Flow cytometry as a tool for counting and identification of phytoplankton (groups) and other applications

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Foreword

A mini survey is presented on the state of the art of flow cytometry as a tool for counting and identification of phytoplankton species and groups and other analyses. Instead of an extended literature review, a questionnaire was sent out via email to 47 scientists at 43 institutes known to us as being involved in flow cytometric analysis of phytoplankton. In total 19 scientists responded and their answers are included marked as 'mini-survey' in italic print. Some basics on the analysis technique are included.

Introduction

The aquatic environment is subject to dynamic processes on widely varying time and space scales. The scale of the smallest independent biological unit, the cell, remains a key scale for interpretation and calibration of data. Traditional microscopical analysis is unsurpassed with regard to species identification power. There are drawbacks however in terms of enumeration, quantification and speed. An automated analysis technique known as flow cytometry allows fast counting and optical analysis of individual particles, with less detailed species discrimination, however. Using flow cytometry, Li (95) for instance showed that Prochlorococcus spp. comprised 78% of the cells of central North Atlantic Ocean ultraphytoplankton, representing 28% of total fluorescence (a measure of chlorophyll biomass) and about 11% of total light scatter (a measure of carbon biomass). In addition, flow cytometry yields information on the variance between individuals, important for the analysis of population and community structure. The first flow cytometric studies in aquatic sciences were published over a decade ago (Paau et al. 78 & 79, Trask et al. 82, Yentsch et al. 83a). Milestones were the special issue of Cytometry (Yentsch and Horan, 89) and the NATO Advanced Study Institute on Individual Cell and Particle Analysis in Oceanography (S. Demers, 91). Overviews were presented by Yentsch (90) and Olson et al. (91). Particles in the open oceans tend to be small, allowing oceanographers to use flow cytometers without much problems. The deployment of flow cytometers on board ship led to the discovery of an abundant oceanic flora of prochlorophytes: small (1 micron) cells which are weakly fluorescent and photobleach so quickly that they escaped detection by other optical techniques (Chisholm et al., 88). Phytoplankton populations in fresh water and coastal waters are more heterogeneous in terms of taxonomic composition, size, concentration, morphology and colony formation. This still hampers the use of flow cytometry in these areas, although some developments are ongoing.

All institutes of the mini-survey are or were using flow cytometry for the analysis of field samples. Twelve institutes say they use, used or will use flow cytometry on a routine basis. Specific answers included: phytoplankton and bacterioplankton analysis, monitoring program of the Bay of Naples, monthly analyses at the US-JGOFS Bermuda/Atlantic time series, Baltic microbial food webs and monitoring. The 9 institutes who are currently doing regular analysis of field stations have sampling strategies varying from weekly to yearly analysis, depending on water type (estuarine > shelf > oceanic), logistics (close to the lab > far away) or otherwise. The combined numbers of stations covered are: 17 stations at a weekly or biweekly frequency, 13 stations at a monthly or bimonthly interval and more than 20 stations once per season or year. Sample collection varies between 1 and 10 depths sampled per station. The total number of field samples processed annually varies from about 50 to about 1000 per lab. In addition to daily laboratory operation, 7 institutes employ the flow cytometers on research cruises on a more or less regular basis. During the cruises, typical strategies are daily analysis of depth profiles, up to sampling every few hours.

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Technology

Measuring principle

Figure 1 is a schematic presentation of the measuring principle and data processing of a flow cytometer. Flow cytometers measure light scatter and fluorescence of particles passing a zone of intense (laser) illumination, carried and centred inside a high speed water jet, in air or in a quartz flow cuvette. The cells are pumped in single file through the analysis point at typically 1,000 cells or more per second, with a practical analysis speed of 1-5 minutes per sample. The successive scattering and fluorescence signals generated by each passing particle are detected by photomultiplier tubes or photodiodes. The detection sensitivity is sufficient to analyse submicron particles. The electronics interface converts these raw signals into correlated digital data, that is stored on disc for data analysis and presentation as distributions (univariate) or multivariate scatterplots or grey/colour maps. Instruments may have a sorting device, allowing the physical separation of selected cells from the main stream during analysis.

The cells of a single population give similar results, showing as a single peak in a univariate distribution, or a close group of datapoints in a multi-dimensional scatter plot. In addition to the instrumental properties, it is the biological variance, such as differences in cell size, life cycle, pigment content etc., that generates the largest part of the variance seen as the width of the peaks or clusters (Campbell et al. 89abc). With mixed cultures or field samples, more clusters appear, more or less separated, representing groups with different optical properties. Figure 2 shows a typical bivariate plot of a sample containing several species. The datapoints from a cluster can be selected to yield distributions of physiological properties of the cells, per group (Li 90, Demers et al. 92). The discriminating power of the analysis increases if more independent optical properties of the particles are measured, or if specifically binding fluorescent probes are used. Whereas in the biomedical field, the cytometrist is faced with perhaps four to six or so cell types to differentiate, in marine waters the numbers of cell types are typically up to an order of magnitude greater. The development of procedures for automated data analysis is crucial. Recent advances achieved in neural network computing are promising (Smits et al. 92, Balfoort et al 92b, Frankel et al. 89, 96, Wilkins et al. 96).

Commercial instruments

The number of flow cytometers world wide is probably approaching the 10.000 figure. Designed for analysis and sorting of mammalian cells, by far the largest part, say about 95% of the instruments is used in biomedical applications. The rest is exploited in a variety of fields such as pharmaceutical industry, food and beverages industry, diary industry, botany, marine science, limnology and drinking water industry. Becton Dickinson and Coulter Electronics hold the major market shares with their FACS and EPICS flow cytometry systems. BioRad and Partec are smaller competitors with the arc lamp based Bryte HS flow cytometer and the PAS multiparameter laser and arc lamp flow cytometry system, respectively. Compucyte and Cytomation are 'niche' manufacturers with respectively the LSC microscope-slide based laser scanning cytometer and the MoFlo 3 laser, 12 parameter high speed research sorter. Aber is a newcomer aiming specifically for the low cost side with the small all solid state Microcyte flow cytometer. None of these instruments is optimal for marine and aquatic research. It seems practice that aquatic scientists have to explore changing the main characteristics of their standard instruments from medicine to plankton research, e.g. to find the optimum optical filters and combinations and to enhance the signal/noise-ratio as far as possible. However, even if a cheap and useful instrument would generate a boost in flow cytometer sales in the aquatic research field, the resulting market still would remain an order of magnitude smaller as compared with the biomedical market. It is not likely therefore that a dedicated instrument for the aquatic market will be released soon by these existing manufacturers.

The answers on instruments used in the mini-survey concerned in total 30 instruments more or less regularly used by the institutes for phytoplankton analyses. Most frequently used are the instruments from Becton Dickinson (14 instruments with 5 FACSort and 4 FACS Calibur instruments) and Coulter Electronics (8 instruments). Some no longer built instruments are still in use such as a Bruker instrument (related successor by Bio-Rad now). Some in-

home modified machines and completely dedicated instruments are used (Table 1). About 10 institutes operate more instruments, with a relative new instrument for routine work and cruises and an older instrument still in use for back-up, and experimental work or modifications.

Limitations and pitfalls with phytoplankton samples, dedicated instruments, methodology

Cells may be affected by fluid acceleration, electrical shock and most importantly, light shock in flow cytometers, possibly influencing subsequent analyses (Rivkin et al. 86, Haugen et al. 87). The instrument performance in turn may be impaired by the specific properties of aquatic samples. Low concentrations may require either preconcentration which deteriorates the sample composition, or pushing the sample flow rate to the max, leading to less accurate measurements.

Almost all participants of the mini-survey considered the small sample volumes processed by flow cytometers a bottleneck. With low cell concentrations in natural samples, relatively large sample volumes should be analyzed in order to get an acceptable statistical count. This is time consuming in standard machines: 2ml = around 25 minutes using the FACSCalibur. In addition, as diversity increases, the number of measured particles per sample has to increase accordingly for proper statistical analysis of the less abundant species. Typically the cells larger than 15 - 20µm, often comprising the bulk of biomass in eutrophic coastal environments are relatively rare.

Standard instruments have limited particle size ranges, with upper limits of typically 30 to 150 µm. Large(r) particles result in system clogging or 'merely' loss of data quality. Examples of the latter are selectivity against large particles owing to small orifices and tubes in the fluid system or 'dead spaces' where large particles settle out. Particles may exceed the size of the optical sensing zone of the instrument, their electronic signals may exceed the proper range, a source of artefacts such as peak height sensing with particles longer than the height of the laser focus (Peeters et al. 89) or time-of-flight analysis of filamentous species. Big particles slow down the fluid in a cuvette (about 20% for a particle half the channel diameter). Long fragile particles may break upon entering the flow channel, where velocity gradients are highest. Other limitations are insufficient sensitivity for the small aquatic microorganisms, insufficient analytical power for the wealth of cell and colony shapes. Olson et al (83) and Cunningham (90a) constructed low-cost flow cytometers for phytoplankton analysis. Frankel et al (90) developed a high-sensitivity flow cytometer for studying picoplankton. Hüller et al. (91) report on a macro flow planktometer for analysis of large marine plankton organisms (>100µm). With the optical plankton analyser (OPA), samples can be analyzed containing single cells and colonies, including aggregates and filaments with lengths over a millimetre (Balfoort et al. 92a, Dubelaar et al. 89), with fair linearity (Dubelaar and van der Reijden 95). Its compacter successor the EurOPA instrument features a photodetector array probing diffracted light, a pulse profile acquisition module (Cunningham 90b), a cytometric imaging device (FCI, Wietzorrek 94) and a sorter system. The measured data is processed by analysis software with neural network routines (Boddy and Morris 93, Boddy et al. 94ab) and multi-variate statistics (Carr et al, 94, 96). Exploration of data analysis (e.g. multivariate curve-fitting; diversity indices) was also mentioned by Li (mini-survey). The EurOPA prototype (Dubelaar et al. 95b) is currently being used in test trials. None of these instruments were commercially produced on a significant scale.

Sample handling and preprocessing

Avoiding particle selectivity and damage to fragile particles is not trivial. Each of the sampling, sub-sampling, filtration, preservation, concentration, staining, storage and transportation processes are potential sources of bias or variability. Flow cytometry requires small sample volumes, but allows more samples and less sub-sampling to obtain good statistical significance. Filtration of field samples prevents clogging of the instrument flow system; concentration reduces the sample volume. Both easily impair the composition of samples containing different and fragile species. For tenfold concentration of North Sea samples, Hofstraat et al. (90) successfully applied a combination of sedimentation and upward filtration at low suction head. Bloem et al. (86) examined filtration and centrifugation of heterotrophic nanoflagellates. Centrifugal elutriation provides an alternative cell separation and concentration technique when large numbers of cells are required (Pomponi and Cucci 89).

The mini-survey showed that the general working principle is to try to analyse the samples in as close to natural state as possible. Preconcentration was not mentioned, prefractioning once. Adding calibration beads for concentration and data quality assessment was mentioned once. Preferentially, samples are measured fresh, immediately after sampling. Logistics may make it necessary to store samples, i.e. for a few days (storage at 4° in the dark). This applies for the analyses done during cruises, and samples collected from shore or at short cruises. Small and fragile cells suffer also from these short periods of storage and may disintegrate. Light scatter properties will be influenced, as well as chlorophyll-a fluorescence. Fixatives used are based on what is used in microscopy and include formaldehyde (mentioned 4 times), paraformaldehyde (mentioned 5 times), glutaraldehyde (mentioned 6 times) and sometimes combinations. Lugol was not mentioned since it deteriorates fluorescence. Long time storage predominantly is in liquid N_2, in combination with 1% glutaraldehyde fixation after Vaulot et al. (89).

This method works well with picoplanktonic populations. Troussellier et al (95) examined effects on bacterioplankton and picophytoplankton. Larger and more fragile cells can be lost to an important extent however, and show variation of chlorophyll fluorescence. A possibility for improvement would be the addition of cryoprotectant(Lepesteur et al. (93), although both optical properties and cell numbers could not be preserved well. A protocol with 0.1% to 0.5% paraformaldehyde (methanol-free) and storage at 4° was developed at JRC-Ispra (Premazzi et al. 92). Higher concentrations of paraformaldehyde increasingly impaired the results. Staining cells requires extra steps like permeabilization, washing etc.

Measured entities

Light scatter and related parameters

The light scatter of particles is measured parallel to the laser beam: the forward or low angle scattering, and perpendicular to this: the side scattering. Simple dependence on for instance particle diameter or volume is restricted to limited classes and size ranges of particles. The highest scatter intensities are at small (low) scattering angles. Significant differences exist between small particles as for instance bacteria and larger particles as for instance ciliates. The intensity of the light scattered by the bacteria drops 3 orders of magnitude with increasing angle, whereas the light scattered by the ciliates drops 6 orders of magnitude. The measured forward light scatter depends mostly on the overall cell cross section and not so much on smaller structures, whereas the side scatter is dominated by the small internal and external structures of the particles. Internal microstructures such as light absorbing pigments and intracellular gas vacuoles may have significant effects on the forward light scatter signal however, caused by their effect on the global refractive index of the small and medium sized cells (Dubelaar et al. 87). The side scatter may yield the most straightforward relation (proportional to particle cross section) for particles of low refractive index, sized from about 1μm upward (Morel 91), but is known to be very sensitive to small cellular structures which cause large variation in the data. Whereas forward light scatter is a good measure for cell volume with very small particles (<0.6 μm^3) as shown by Koch et al. (96), the forward light scatter is linearly proportional to cellular cross section only for optically large cells (tens of microns diameter and/or highly absorbing), and shows a fluctuating behaviour at intermediate sizes. In the recently started EC MAST project AIMS (Automated Identification and Characterisation of Microbial Populations), algorithms are being developed to translate flow cytometric light scatter signals to size spectra (Rodriguez, mini-survey).

Under conditions, size and refractive index of marine particles can be measured (Ackleson and Spinrad 88, Spinrad and Brown 86). Dilution (osmosis), chemical fixation and/or staining as well as cell damage cause changes in forward light scatter signatures (Ackleson et al. 88, Navaluna et al. 89). Ratios of intensities at different angles can be used for sizing bacteria (Koch 86). With azimuthally resolved forward light scatter measurements, typical cell shape information may be obtained (Buonaccorsi and Cunningham 90, Cunningham and Buonaccorsi 92, Forrest 85, Premazzi et al. 89). Smart wiring of a 25 pixel photodiode array, reduces to only 4 measured numbers per particle for symmetry and size information (Dubelaar et al. 95b). As the laser light is linearly polarized, depolarization measurements can be implemented also relatively simply, to probe isotropic

cell structures. Olson et al. (89) used polarization properties of forward scattered light in addition to other parameters to discriminate eukaryotic phytoplankton cell types. Particularly, the coccolithophores depolarized forward scattered light. Direct measurements of particle absorption are very difficult with flow cytometers, and no such possibilities were reported. Beam attenuation (axial light loss) can be measured with flow cytometers, but this parameter is dominated by light scatter (Eisert, 79). Many flow cytometers measure the duration of the pulses, which is a good measure for particle length if scatter pulses are used. Electrical resistance sizing (Coulter volume) is optional on a few instruments.

Endogenous fluorescence

Pigment fluorescence, the major component of endogenous fluorescence is used for quantification of photosynthetic capacity, biomass and cell size (Chisholm, 92), and identification of cellular pigment composition type. Absorbed photons generate higher excitation stages in the chlorophyll antenna pigments, from which photosynthesis is driven or by-products are generated such as heat or lower energy light, fluorescence. One of the key problems in fluorescent diagnostics of photosynthetic organisms is to distinguish the contributions of constant and variable fluorescence components, because the first one contains information on the efficiency of exciton migration through the light harvesting antenna, while the second one reflects the state of PS II reaction centres and the electron-transport chain (Chekalyuk et al. 92). At low light conditions, photosynthesis competes efficiently with fluorescence and fluorescence is low (constant fluorescence). The light intensity in flow cytometers is very high, but the passage time of the particles is extremely short (a few microseconds). Depending on focus dimensions, laser power and flow speed, the total photon dose is roughly in the range of 0.1 to 10 seconds of continuous actinic light of 1000$\mu E/m^2/s$ (algae grow well at about 50-500 $\mu E/m^2/s$). The question is to what extent the variable fluorescence rises during the short passage time of the cell through the laser beam. With double or triple beam cytometers, the cell has to travel some time between the laser beams, and effects may be quite different at the down stream laser foci. Studies (Ashcroft et al.86, Neale et al. 89 and Xu et al. 90) on flow cytometric fluorescence origin did not provide coherent results. Another approach is to chemically force chlorophyll fluorescence with DCMU (Furuya and Li 92, Li 93) for evaluating photosynthetic rates of natural populations. At very high photon densities such as in focused laser beams, 'exciton annihilation' may reduce fluorescence yield (Chekalyuk et al. 92). Similarly, Van den Engh and Farmer (92) studied photo-bleaching and saturation of cytochemical dyes in flow cytometry. The fluorescence emission becomes independent of light intensity, determined only by the damaging rate and the fluorescence life time. Another question is whether the amount of absorbed quanta is (dis)proportional to the amount of intracellular pigment owing to the self shading effect, also called packaging effect (Duysens, 56). At the small size range the packaging effect is not significant; Kerker *et al.* (82) showed a linear relation between fluorescence and size of small calibration beads. Natural chlorophyll absorbs light more efficiently, and obviously phytoplankton cells may be much bigger. Sosik et al.(89) investigated fluorescence as a function of cellular chlorophyll content, and showed saturating dependencies for *Hymenomonas carterae* and *Amphidinium carteri* owing to the packaging effect. Calibration is required for accurate use of fluorescence as a size indicator (Legner, 90). Flow cytometric 'pump and probe' analysis was demonstrated by Olson and Zettler (95). For larger cells, like 30 µm diatoms, they showed that the fluorescence yield enhanced due to a pump beam. This correlated well with DCMU-enhanced bulk fluorescence. The reduced laser power required to measure the constant fluorescence part, was too low to obtain sufficient signal to noise (measurable signal) from the smaller cells. Their intensities used for pump (!) and probe were orders of magnitude smaller than used in the studies mentioned above. The conclusion seems justified that more investigation of fluorescence rise times in the microsecond area, at a range of controlled flow cytometric illumination conditions, including estimation of packaging and annihilation effects, is required for a better understanding of these phenomena.

The total amount of red chlorophyll fluorescence per volume of sample (a sum of the individual particle fluorescence) correlates well to spectrophotometrically analyzed, as shown in natural samples (Hofstraat 91, Jonker, 95). Especially for larger cells and colonies, these measurements require a dedicated optical measuring system like in the OPA as was shown for natural Microcystis colonies (Dubelaar and van der Reijden, 95a).

Based on this relationship the contributions of different groups of algae to 'biomass' can be assessed, which give more valuable information than counting of cells and colonies of different size alone. The combination of data on concentration, species and group discrimination and quantitatively measured chlorophyll fluorescence makes it possible to estimate the contribution of different species and groups to the total phytoplankton biomass. Fluorescence emission and excitation characteristics have been demonstrated as tools to classify groups such as cyanobacteria, cryptophytes, chlorophytes and prasinophytes, bacillariophytes and dinophytes based upon spectrally similar accessory pigments within these groups (Yentsch and Yentsch 79, SooHoo et al. 86, Hilton et al. 89, Hofstraat et al. 90, 91, 94). Olson et al. (89) simultaneously used Coulter-volume, intensity and polarisation of forward scatter, right-angle scatter, and fluorescence. From 26 laboratory cultures, the two cryptophytes and the rhodophyte, the coccolithophorids, and chlorophytes could be distinguished from others. Instead of fluorescencing themselves, accessory (antenna) pigments rather increase chlorophyll-a fluorescence by energy transfer, the exception being phycobilin and phycoerythrin containing species. Therefore, excitation spectra probe the spectral properties of pigments capable of energy transfer to chlorophyll a (Owens, 91). This could encourage the application of flow cytometers with three or more excitation beams. These instruments are the most complex, unfortunately, especially the electronic timing circuitry which correlates the data from one cell, coming from three lasers.
Artificial neural net analysis (Boddy et al. 94a, Wilkins *et al.*, 94, 96) showed that 20-40 species can be sufficiently discriminated based on endogenous fluorescence and light scatter alone, but also showed that discrimination by this technique is not primarily based on taxonomic group identification.

Exogeneous fluorescence

There are several options to extent analysis of phytoplankton by staining specific components of the cell. These fall apart in A: very specific but normally non-quantitative techniques in order to discriminate species and B: techniques for quantitative analysis of various constituents and physiological conditions of cells like DNA content. The dye can be fluorescent or the product of the dye and the cellular component of interest may be fluorescent. Except for the membrane binding dyes, the dyes have to enter the cell to interact with their goal substances. This can be used as a diagnostic technique for estimation of membrane integrity, otherwise the membranes have to be perforated to let the dye in (electroporation technique is described by Berglund and Starkey, 91). An example is the use of propidium iodide for live/dead cell discrimination. Cytochemical stains are used to stain protein, DNA, RNA, lipids and membranes. Hull et al. (82) presented staining techniques for nuclear DNA in algae. Schäfer et al. (96) applied three-laser flow cytometry for simultaneous measurement of photosynthesis pigments and protein content using FITC of phytoplankton populations in lakes and rivers. Edvardsen and Vaulot (96) used cell size and relative DNA content for ploidy analysis of Prymnesiophyceae spp. The sensitivity of flow cytometers allows the detection of very small quantities of fluorescence, down to a level of a few thousand fluorescent molecules. This allowed the development and application of immunochemical labelling techniques. Double staining allows the assessment of more than one property at the same time, e.g. the double staining of bacteria with a DNA and a protein stain to monitor cell volume as a function of cell cycle (Steen et al. 82).

Immuno-techniques

An early overview on quantitative immunofluorescence in flow cytometry and related staining techniques was given by Visser et al (78). Ward and Perry (80) presented an immunofluorescent assay for the marine ammonium-oxidizing bacterium *Nitrosococcus oceanus*. Clones of marine chroococcoid cyanobacteria were analyzed by Campbell et al. (83) using immunofluorescence. Antibodies to eukaryotic cells (to probe pigment types and/or cell wall composition) were presented by Shapiro et al. (89) and Campbell et al (89). Antibodies were found to various cellular molecular constituents (Yentsch 81, Yentsch et al. 88). Flow sorting gated on forward light scatter and FITC labelled anti-*Cryptosporidium* is being used in water quality analysis as a quantitative preconcentration method, which allows routinely screening of hundreds of litres of water for *Cryptosporidium* oocysts (Vesey, 94). The antibody is not 100% specific, but the highly infectious oocysts are counted microscopically from the sorted fractions much quicker than before, without enrichment. Vrieling et al. (95),

Vrieling and Anderson (96), showed that antisera against purified cell walls and against extruded trichocystal cores of the organism, allow immunofluorescent detection in flow of the dinoflagellates *Prorocentrum micans* and *Gymnodynium nagasakiense*, respectively.

DNA and RNA quantification

DNA can be fluorescently stained with many fluorochromes. Flow cytometric determination of phytoplankton DNA in cultures and field samples was reported by Yentsch et al. (83b), Bonaly et al. (87), and Boucher et al. (91) from cultured samples stained with DAPI, which can be excited with UV light. The DAPI-DNA fluorescence was related to cell DNA content over almost 4 orders of magnitude. In natural populations, the fraction of particulate DNA contained in photosynthetic picoplankton could be computed. Currently, a new series of fluorochromes are being applied for high-resolution DNA quantification by flow cytometry. Among these are ToTo-1, SYBR-Green, Picogreen, SYTOX green (Marie et al. 96, Li 95, Marie et al. 97). They have major advantages over previously used dyes like DAPI and propidium iodide. They can be excited at 488 nm, the normal laser wavelength for flow cytometry, result in green fluorescence which hardly interferes with pigment fluorescence (Chl-a), and are specific for double stranded DNA, instead of also staining RNA (Pan and Cembella 96). Veldhuis et al. (97a) established a nice data set of DNA content of individual phytoplankton species. They also showed that there is a good correlation between DNA content, as measured with these dyes, and phytoplankton biomass. This correlation is better than using Chl-a (fluorescence). The next important step would be to transfer this to field samples. The ratio between RNA and DNA can be used to discriminate actively growing cells from resting cells. The application of e.g. SYTO 13 allows assessment of bacterial abundance (Del Giorgio et al. 96); SYBER Green allows for discrimination between heterotrophic bacteria and autotrophic *Prochlorococcus* cells (Marie et al. 97).

Cell cycle analysis

Whereas the connection between cell division and the need for metabolites and photosynthates is obvious, the mechanism of light acting upon cellular DNA synthesis regulation is not so clear (Yee and Bartholomew, 88). Flow cytometric DNA analysis is a helpful tool in this respect (Chisholm et al. 86). Brzezinski et al.(90) examined the role of silicon availability on cell-cycle progression in marine diatoms. Examination of DNA histograms allowed the localization of the effect of silicon deprivation in terms of progress through G1, S, and G2+M phase. Yee and Bartholomew (88) studied light regulation of the cell cycle in Euglena gracilis bacillaris. Euglena grown under phototrophic conditions are easily synchronized to a 12 h light-12 h dark regime. By inoculating stationary phase, nondividing cells into fresh media and exposing the diluted cells to either light or darkness, it was observed that initiation of DNA synthesis for the cell division cycle is light dependent. Commitment to the cell cycle requires exposure to more than 6 h of light, supposedly to allow the accumulation of an initiating factor that will enable DNA synthesis to begin. Flow cytometry analysis showed that once cells are committed to the cell cycle, they complete the cycle in the dark, so mitosis is a light-independent step. Lefort et al. (87) used DNA flow cytometry to study cell cycle blockade of vitamin B12-starved cells. Binder and Chisholm (90) studied the relationship between DNA cycle and growth rate in *Synechococcus-sp* strain PCC 6301. This cyanobacterium was shown to contain multiple chromosome copies even at very low growing rates. Evidence was found for asynchronous initiation of DNA replication. Vaulot et al. (95) elegantly showed that DNA replication occurred in the afternoon by analyzing *Prochlorococcus* in samples from different depths using Hoechst 33342. The next step in *in situ* growth rate analysis is to combine specific detection of single species with cell cycle analysis (Reckermann: mini-survey, Pan and Cembella 98). Specific detection can be done with *in situ* hybridization or with monoclonal antibodies. Peperzak et al (98) showed that flow cytometry can be used for the analysis of the dial DNA cycle.

Identification of species and groups using ribosomal RNA-targeted nucleic acid probes

The use of ribosomal RNA probes for flow cytometric identification of both individual species and taxonomic groups is very promising. Parts of the ribosomal RNA sequences were highly conserved during evolution and the differences in rRNA sequences correlate well with evolutionary relations. Fluorescently labelled rRNA probes are

relatively small and penetrate easily in fixed cells (Amann et al. 90). There they hybridize specifically to the target sequences. This is very useful for fluorescent *in situ* hybridization (FISH), a process which can be visualized using both microscopy and flow cytometry. Recently, the application of rRNA probes for detection of phytoplankton species and groups was established (Simon et al. 95, Knauber, 96, Rice et al. 97). Lange et al. (96) have shown that target regions specific for the class Prymnesiophyceae and the genus *Phaeocystis* (Hariot) Lagerheim could be identified from 18S rRNA coding regions, and two complementary probes were designed. Detection of whole cells hybridized with these probes labeled with fluorescein isothiocyanate (FITC) was difficult using epifluorescence microscopy because autofluorescence of the chlorophylls seriously interfered with the fluorescence of the probes. In contrast, flow cytometry proved very useful to detect and quantify the fluorescence of the hybridized cells. Hybridization conditions were optimized, especially with respect to formamide concentration. Both probes were tested on a large array of both target and non-target strains. Positive and negative controls were also analyzed. Specificity was also tested by adding a competing non-labeled probe. Whereas probe PHAEO01 seems to have good specificity, probe PRYMN01 appeared less specific and must be used with stringent positive and negative controls. A large number of rRNA sequences has already been analyzed and is available through internet, i.e. GenBank at http://www.ncbi.nlm.nih.gov/Web/Search/index.html. The use of a 18S rRNA probe for detection of *Cryptosporidium* was established by Vesey (96). This allows for a very quantitative technique for analysis of *Cryptosporidium*.

Monoclonal antibodies and/or other molecular probes are used for aquatic bacteria and phytoplankton analysis by 6 of the 19 mini-survey institutes; 4 other institutes say they will in the future.

Applications

The mini-survey institutes all carry out species or (pigment or taxonomic) group identification in field samples by flow cytometry, mostly taxonomic groups based on pigment analysis, such as prochlorophytes, cyanobacteria, (sub-types of) Synechococcus (e.g. phycocyanin- and phycoerythrin-rich Synechococcus spp.), cryptophytes, dinoflagellates and diatoms. Furthermore, eukaryotic picoplankton (three types of picoeukaryotic algae), and nanoplankton can be identified. Size is used by RIKZ-NL as extra indicator to identify 6 groups in North Sea waters: Phaeocystis (only blooms), Mesodinium, Rhodomonas, Dinoflagellates and Diatoms <25 µm, 25 - 100, >100 µm. Gymnodinium mikimotoi is being identified using monoclonal antibodies. With the use of molecular probes also heterotrophic bacterial populations can be identified. Applications mentioned included

- *Phytoplankton photophysiology, ecophysiology, toxicology, metabolic activity (3 times)*
- *The relationship between phytoplankton, bacteria and detritus (4 times), bacterial sorting to discriminate active cells (2 times), size distribution of bacteria (2 times)*
- *Grazing impact studies (6 times), food selectivity of cockles*
- *Isolation of strains (e.g. gonyaulacoid cells) from natural populations by sorting (2 times), isolation of diatoms transformed with GFP gene*
- *In situ hybridization with taxon-specific rRNA probes*

Phytoplankton abundance

The basic application of flow cytometry is rapid phytoplankton counting and sizing based on the chlorophyll fluorescence, and identification of taxonomic groups and species as far as possible (Li and Wood 88, Yentsch and Yentsch 79, Yentsch and Pomponi 86, Olson et al. 89, Hofstraat et al. 90, 94, Blanchot and Rodier 96, Binder et al. 96, Li 97, Veldhuis and Kraaij 90, Wood et al. 85). Flow cytometry allows higher sampling frequencies, important for the execution of ship transects, depth profiles and also incubation experiments to study the distribution and composition of phytoplankton populations, including diurnal variations (Tarran and Burkill 93, Burkill 87, Olson et al. 85, Li 89, DuRand et al. 94). Flow cytometry is likely to prove useful for detecting the low-level occurrence of harmful species, giving early warning of the probability of bloom development. It enables monitoring of bacteria in seawater (Button and Robertson 89+90, Robertson and Button 89, Wiebenga et al. 97, Zubkov et al. 98ab). Clearly, pigment analysis using flow cytometry is limited as compared to e.g. HPLC, but

main pigments can be studied with success (Olson et al. 90, Hess et al. 96). Using the specific, immuno- and molecular probes enables the determination of intracellular substances such as lipid droplets in diatoms and toxins in dinoflagellates, and allows more specific species/group characterisation and discrimination (Bloodgood et al. 87, Simon et al. 94, 95, Subba Rao et al. 91).

Ecology and physiology

Flow cytometers can play an important role in the experimental verification of ecological models (Campbell et al 97). These models include spatial and/or temporal distributions of species and groups (Olson et al. 90, Partensky et al. 96, Shimada et al 93, 95, Tarran et al 94, Vaquer et al. 96, Vaulot et al. 90, Zubkov et al. 98ab), size distributions and population dynamics, but can also consider flow cytometrically probed status of life cycle (Binder and Chisholm 95, Brzezinski et al. 90, Van Bleijswijk et al. 94, Van Bleijswijk and Veldhuis 95) allowing growth rate determination (Veldhuis et al. 97b), and physiological properties of the cells such as metabolic activity (Dorsey et al. 89, Jellet et al. 96). The response to changes in light conditions (Armbrust et al. 89, 90, Gerath and Chisholm 89, Vaulot et al. 86), nutrient availability (Trousselier et al. 97, Vaulot et al. 87, Veldhuis and Kraaij 93, Zettler et al. 96) can be assessed. Huisman (97) elegantly used flow cytometry in competition experiments in order to validate models describing competion for light using fresh water species from eutrophicated systems. Other applications include the investigation of microbial food webs (Cowles et al. 88, Cucci et al. 85, 89, Gerritsen and Sanders 87, Lesser et al. 91, Sellner et al 94, Hansen et al. 96, Reckermann and Veldhuis 97) and the evaluation of the effect of toxic substances on algae (Gala and Giesy 90, 94), or toxic algae on zooplankton (Turner et al 98). A flow cytometric approach to assess the environmental and physiological status of phytoplankton was presented by Demers et al (89). The analysis of natural phytoplankton populations can indicate changes in water quality and environmental stress (Olson and Chisholm 86, Olson et al 86, Parpais et al. 96) and contamination by anthropogenic inputs (Berglund and Eversman 88, Bonaly-Cantarel 88, Cavender-Bares 98).

Size & biomass

Many biological processes involved in biogeochemical fluxes (production, respiration, grazing, sinking, aggregation etc.) are linked to the biomass and size distributions of bacteria (Button and Robertson 89) and phytoplankton (Rodriquez and Li 94, Gin et al. 98). For that reason the development of flow cytometric techniques for particle size determination was advocated by Legendre and Le Fevre (91). Shipboard based instruments allow fast assessment of abundance variations (Burkill 87, Borsheim et al 89). Correlation of metabolic with flow cytometric characteristics is a feasible means of investigating the heterogeneity of phytoplankton metabolic state in the marine environment.

Almost half (8) of the mini-survey participants carry out quantification of growth/production rates (basing on DNA / cell cycle analysis) of phyto-/bacterio-plankton.

The ocean colour is a prime source of information (Morel and Prieur 77). Remote sensing data may be utilized in calibration and validation of hydrodynamic and ecological models, and for instance the assessment of the spatial variability of biomass within a bloom area, or on a larger scale the photosynthetic carbon fixation in the world oceans. The retrieval of upper ocean chlorophyll concentrations from satellite ocean colour data has dramatically improved the global synoptic measurement of chlorophyll biomass. Data interpretation depends on atmospheric correction methods and bio-optical algorithms, calibrated with locally (on site) acquired entities such as extracted chlorophyll. Calibration with 'sea truth' data on the particle level is complicated (Lewis and Cullen 91) but feasible, for instance for coccolithophore blooms (Holligan and Balch 91, Balch et al. 92,93), or a bio-optical classification of sea particles (Ackleson and Robins, 90).

The main advantage of the flow cytometer is the large number of individual cells that are measured, and the straightforward evaluation of phytoplanctonic biomass within main groups. One aspect is that major changes in ocean colour are based on new production (hence large) species. If cytometers will be technically tuned towards larger organisms (probably even aggregates) and larger measuring volumes they can be useful for that purpose, in addition to the importance of flow cytometry for characterising small particles.

Although flow cytometers can measure cellular light absorption indirectly only (Perry and Porter 89), other entities such as fluorescence, forward and perpendicular light scatter are useful to assess particle optical properties and the relationship with the *in situ* light fields (Spinrad 84, Ackleson et al. 93, Cullen et al. 88, DuRand and Olson 96). The large variations in C:Chl ratios in phytoplankton prevent accurate estimates of phytoplankton carbon biomass and growth rate. Flow cytometry can be used to probe this ratio on the particle level, and to relate that measurement to photosynthetic carbon fixation.

The general idea is, although it is obviously not a trivial matter, that flow cytometry data could be related to measurements of bulk light scatter / attenuation data measured with other instruments. Scientific work is being done at Woods Hole (Sosik and Olson), and a MAST project on this topic has recently been started (AIMS). Some institutes (NIOO, NL) compare flow cytometry data with microscopic counts and optical telemetry (shipboard SpectraScan) data from specific stations.

Discussion

A high potential of the technique was acknowledged by all participants of the mini-survey. It was stated that the direct on-line estimation of phytoplankton biomass and the ability to discriminate between different phytoplankton groups allows analysis of undisturbed natural samples (growth, grazing, the spatial or seasonal evolution of populations). Also, the application of specific fluorescent stains (bound to antibodies, or as DNA probes) may provide early detection of plankton groups of special interest, e.g. toxic species. By combining auto- and acquired fluorescent properties, potential developments include monitoring, in-situ growth rates, biomass estimation of both autotrophs and heterotrophs, ecosystem processes, physiological status of microbial assemblages such as nutritional status, viability, photosynthesis, and various stress physiology. In a broader scale, we can use a similar approach to study primary productivity, and new production in the field. Flow cytometry also carries potential for the determination of small particles such as bacteria. Most bottlenecks identified were mentioned in the previous text (viz. for instance "limitations etc"). An inherent drawback of flow cytometry is that it can only differentiate particles based on their optical characteristic as seen by the PMTs; this is better than the eye for the very small cells (picoplankton), and vice versa for the larger cells. The species-resolution of field samples done by flow-cytometry is far below the Utermoehl-microscopy. The expectation that this situation can improve if the lack of specific fluorescent probes is reduced was widely supported in the mini-survey. The laborious work to establish them remains a hurdle, as well as perhaps the human factor: ecologists prefer microscopic monitoring data. With regard to reproducability it was mentioned that there is a real need for (i) standardization of flow cytometric analysis of field samples, and (ii) continuous checking against other independent methods (such as microscopy).

Clearly, applications of flow cytometry in the field of aquatic sciences are numerous and expanding. It is not possible to define general requirements for all aquatic science applications. Shipboard analysis requires robust, easily transportable equipment; oceanography requires high sensitivity analysis of small cells; coastal and fresh water applications require a large dynamic range. Many physiological experiments and species identification using molecular techniques can be done with one or two parameters, whereas species identification based on inherent optical properties requires large numbers of independent entities measured from a single particle. Broadly used dedicated commercial equipment is not expected soon, but the general instrumental developments are promising. Data acquisition and processing gets more efficient, lasers and detectors get smaller, PC's get faster and hard discs get bigger, costs decrease. Instrument size, -operation, -costs and -reliability and data handling will not remain bottlenecks for long. Developments over a longer period will allow real-time in-situ measurements. The EC MAST III project CytoBuoy pioneered herein by building a compact, single laser (Eur)-OPA type flow cytometer into a small buoy (Dubelaar et al. 98, http://home.wxs.nl/~dubelaar/cytobuoy.html). The buoy is tested for moored operation with radiotransmission of full morphological data (viz. Fig. 1) to shore. The development of autonomous underwater vehicles (AUV's) is ongoing, and payloads are large enough in principle to accommodate to these existing smallest flow cytometers. Much effort has to be devoted to standardization of sampling and preservation and of reporting and data analysis. This also requires intercalibration of different flow cytometers. Also the systematic investigation of the phenomena governing the light scatter and fluorescence of

phytoplankton cells as measured flow cytometrically are required for a better understanding of these analysis tools. This may turn out to be a prerequisite for intercalibration between flow cytometers but at least to intercalibrate data from flow cytometers with remote sensing data and other 'bulk' techniques (Dubelaar et al. 94), and also to successfully implement "pump and probe' or "fast repetition rate" capabilities in flow cytometric instrumentation that will measure various photosynthetic parameters in a single phytoplankton cell such as cellular carbon and the cellular C:Chl ratio. As soon as analysis of major functional groups within the phytoplankton and bacteria becomes routine, it will be possible to base time-series analysis of phytoplankton and bacterial population on flow cytometry. This will also make it feasible to perform these time series analyses at a much higher frequency in time and space.

Acknowledgements

Information was used from the internet sites of

Station Biologique CNRS - INSU - UPMC, Roscoff	www.sb-roscoff.fr/Phyto/cyto.html	France
J.J. MacIsaac Facility for Individual Particle Analysis	www.bigelow.org/~Sieracki/index.html	Maine USA
Institut für Meereskunde Kiel	www.ifm.uni-kiel.de/pl/flowcyto/default.html	Germany

For responding to the mini-survey we gratefully thank

Dr. P.H. Burkill,	Plymouth Marine Laboratory	United Kingdom
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Table 1: instruments and set-up used among the mini-survey group

#	manufacturer	type	illumination bands				fluoresc. detector bands					nozzle cuvette	sorting	other
			UV	blue	green	red	blue	green	yel	oran	red			
			mW	mW	mW	mW	nm	nm	nm	nm	nm	µm		
4	Becton Dickinson	FACS Calibur		15				x		x	x		x	
5	Becton Dickinson	FACSort		x				x		x	x		x	
1	Becton Dickinson	FACS Analyzer	high pressure Hg lamp						x		x			ERS
2	Becton Dickinson	FACScan		15				x	x		x			
1	Becton Dickinson	FACScan	in-home modified for high sensitivity analysis											
1	Becton Dickinson	FACS Vantage	50	150		35		x	x		x	100-400	<200 µm	
1	Bruker Spectrospin	Argus	arc lamp instrument, jet-on-open-surface instrument											
1	Coulter Electronics	Epics Elite	100	30			x	x		x	x	250	<40 µm	
1	Coulter Electronics	Epics Elite ESP	50	200										
2	Coulter Electronics	Epics XL		15										
1	Coulter Electronics	Epics 753	in-home modified for high sensitivity analysis											
1	Coulter Electronics	Epics V	or	or	or								high speed	
1	Coulter Electronics	Epics V	in-home modified for high sensitivity or high sample flow settings											
1	Coulter Electronics	Epics 541	or	or	or		2 fluorescence detectors						autosampler	
1	Ortho Diagn. Instr.	Cytoron Abs.		15				x		x	x			
1	Max-Planck Inst.	Fluvo II	mercury-lamp				2 fluorescence detectors							ERS
1	PARTEC	PAS-III			30				x	x	x			
1	PARTEC	PAS-III	lamp	150				x		x	x		optional	ERS
1	TNO - NL	OPA	Exp. instr. for large size range linear analysis									1000		
2	EC MAST-3 group	Eur-OPA	OPA type instrument with various experimental options											

total nr. of instruments in mini-survey group

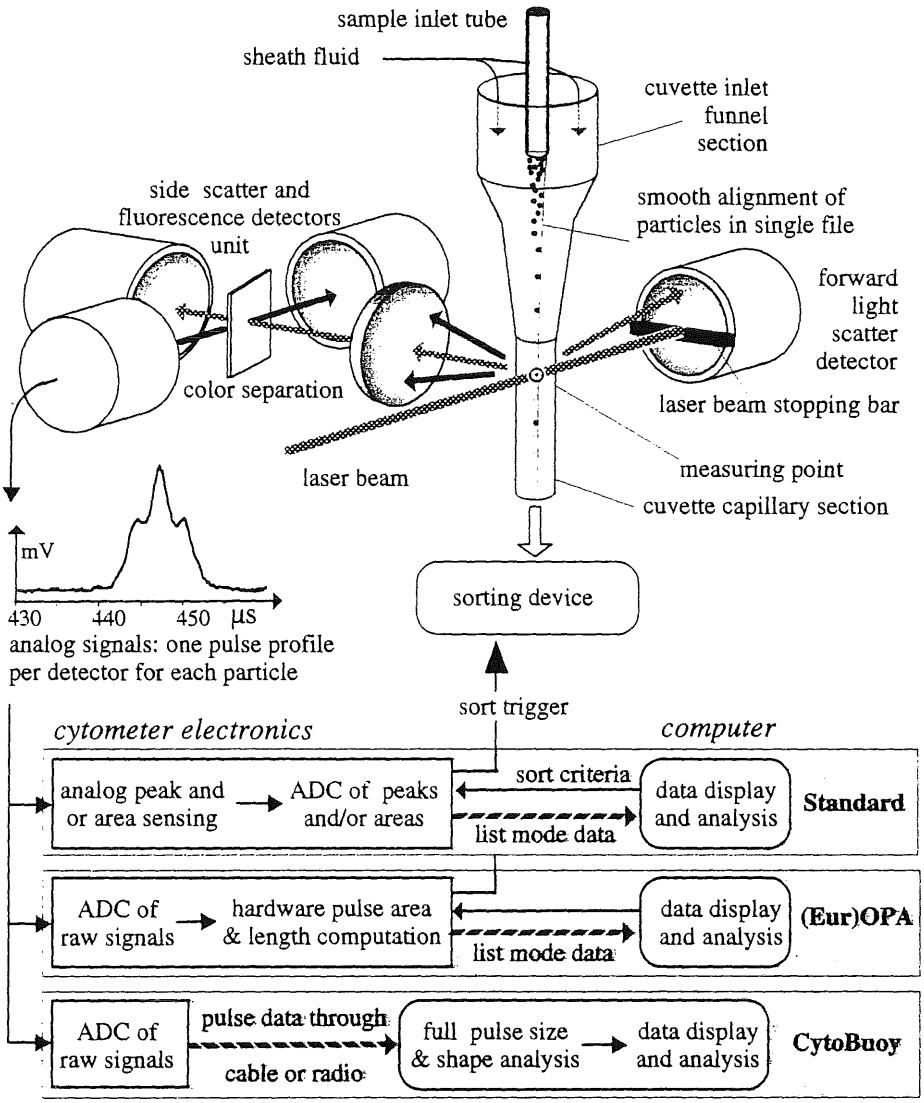


Fig. 1: Schematic drawing of typical flow cytometer operating principle and signal processing, including schemes of plankton instruments.

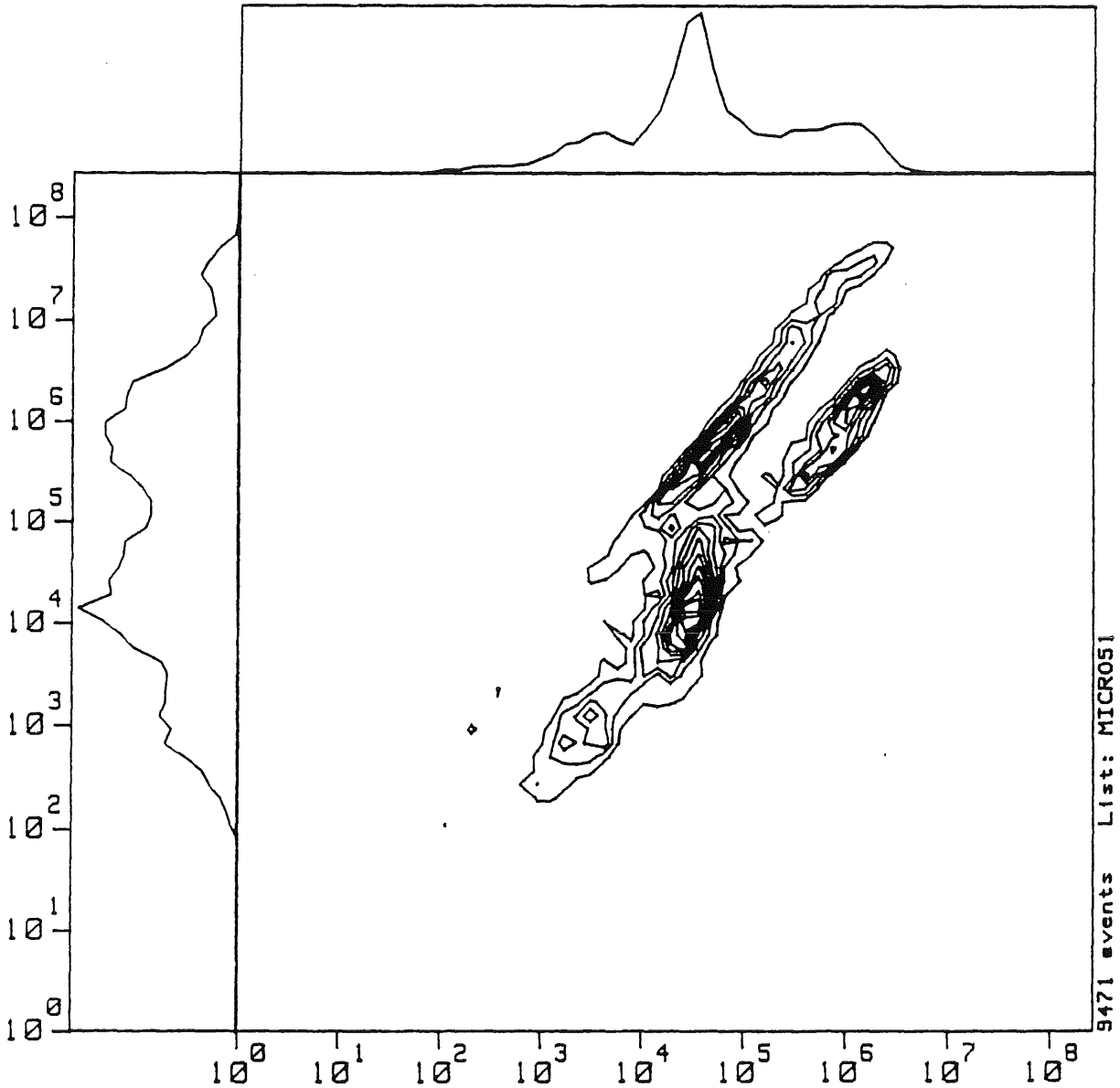


Fig. 2: Example of a bivariate diagram (contour plot: lines indicate equal particle densities), and the corresponding univariate histograms. Fresh water field sample containing some colony forming cyanobacterial species and single cells; measured with a OPA cytometer, vertical=side scatter; horizontal = forward scatter.

ANNEX 8

EXTRACT FROM THE 1998 DRAFT REPORT OF

MARINE CHEMISTRY WORKING GROUP,

Stockholm, 2 - 6 March 1998

8.3.3.d. Quality assurance aspects in the determination of chlorophyll a in sea water

At the meeting held in 1997, the Chemical Oceanography Sub-group agreed to produce a paper recommending a method for the routine determination of chlorophyll *a* as a biomass marker. A. Aminot was in charge of the preparation of this paper.

The paper was not intended to be an analytical manual, but was meant to i) summarize the background on the ecological importance of chlorophyll *a*, ii) summarize the analytical principles and the procedures for its determination, and iii) point out the critical and controversial aspects of the protocol.

In parallel, the Working Group of Phytoplankton Ecology (WGPE), at its 1997's meeting, undertook a similar task. The report of the WGPE was available this year for the CO sub-group discussion of Aminot's draft. Both documents benefited from the recent (early 1997) publication of the SCOR Working Group 78 report, under the auspices of UNESCO (« Phytoplankton pigments in oceanography » by Jeffrey et al.). This monograph served as a reference for the recommendations given in the two documents although these were produced independently.

Two additional external contributions were available: the HELCOM recommended procedure (Manual of the Baltic Monitoring Programme, 1998) and comments on Aminot's draft from Lars Edler (biological oceanographer, SMHI, Sweden).

Very similar advice and recommendations were given in the WPGE report and in the MCWG-CO draft. The main difference concerned the time of storage of the filtered samples at a temperature of - 20°C. The WGPE retained the recommendation of the SCOR group, i.e. a few days, although this applied to all the carotenoid and chlorophyll pigments tested. However, for the routine spectrophotometric or fluorometric determination of chlorophyll *a* only, table 10.4 in the UNESCO monograph showed that recovery after 60 days was 100 ± 10 % of the initial concentration, for both artificial mixtures of microalgae and natural populations. Consequently, it was considered that a storage time of up to 2 months at - 20°C could reasonably be recommended. Note that the HELCOM procedure recommends that the filters not be stored, or, if this is not possible, that they be kept in a dessicator at - 20°C for no more than 24 h.

A main point of discussion concerned the extraction solvent. Although the SCOR work showed that 90 % acetone does not completely extract chlorophyll *a* from a few specific algae, tests on natural populations gave satisfactory results when compared with the reference solvent (dimethylformamide). Therefore, given that extinction coefficients are well established in 90 % acetone, and that this solvent has a low toxicity, the SCOR group recommended it for routine spectrophotometric and fluorometric determinations. This recommendation is followed by the two ICES WG (WGPE and MCWG). The HELCOM procedure recommends the use of 96 % ethanol. However, despite potential advantages of that method, it cannot presently be recommended for the ICES community since the complete methodology using this solvent is not available in the international literature.

When the ethanol method is finally documented, the MCWG recommends that further work be undertaken to compare the relative merits of the two extraction procedures.

ANNEX 9 AVAILABLE CHECKLISTS FOR PHYTOPLANKTON IN THE ICES AREA

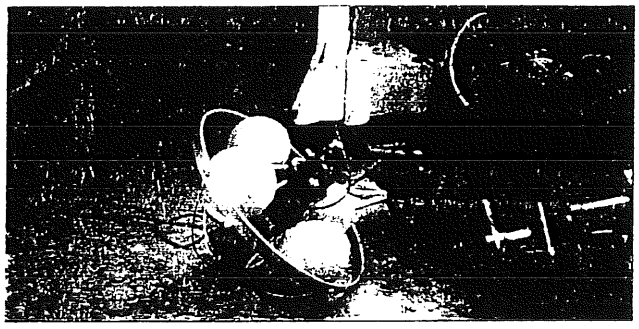
Published and/or required checklists and species lists of phytoplankton in the ICES area.

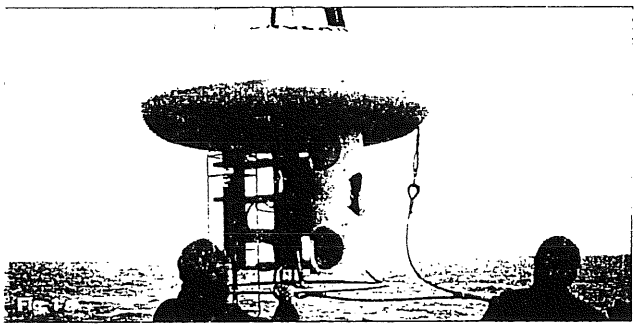
	Area	Year	
Elbrächter and Drebes	North Sea	1991 ?	update
Lange and Hasle	Skagerrak	1994	
Thomsen, Moestrup, Larsen	Danish waters	?	update
Heimdal, Hasle, Throndsen	Skagerrak	1973	
Bode, Varela	Southern Iberian Coast	?	
Varela, Bode, Lorenzo	Northwest Iberian Atlantic?		
Bérard-Therriault, Poulin, Bossé	Gulf of Saint Lawrence	1998	
Sournia	French waters	?	
Braarud, Gaarder, Gröntved	Northeast Atlantic	1963	
Cleve-Euler	Öresund	1937	
Hendey	British waters	198?	
Edler, Hällfors, Niemi	Baltic, Kattegat	1984	
Hargraves, Rines	Northeast USA	?	species list?
Marshall	Northeast USA	?	species list?
Hällfors	Baltic	in progress	
Edler	Baltic	in progress	
Sampayo, Moita	Portuguese coast		species list
Rademaker et al.	Dutch coast	in progress	

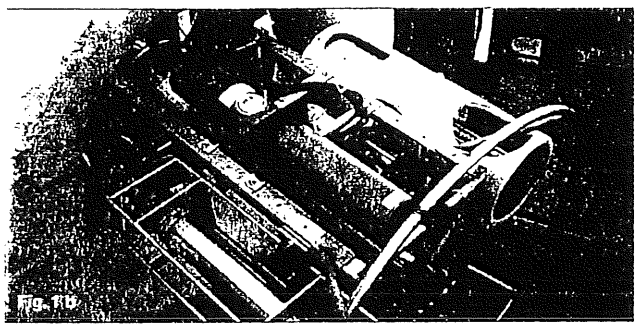
ANNEX 10 NEW METHODS FOR SMART MOORINGS AND IMPROVED CPR EQUIPMENT

This annex is only available in paper form

Smart Moorings
in the Irish Sea







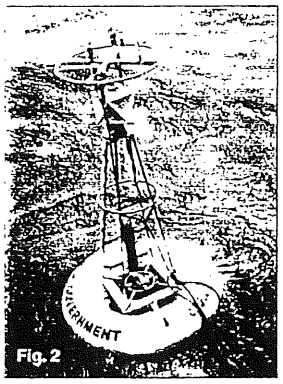
D.K. Mills[1] and M.B. Rawlinson[2]

Smart moorings are observational platforms measuring physio-chemical and biological parameters which incorporate an 'intelligent' water sampler that can collect, preserve and store up to 50 water samples. They have been developed in response to the need to provide improved systems for measuring natural and anthropogenic change in marine ecosystems. A novel component of this programme is the implementation of conditional logic which will allow water sampling dependant upon sensor input (e.g. chlorophyll fluorescence, turbidity, density).

Although the exact configuration will vary to suit purpose our prototype core systems measure surface (< 2m) nitrate concentration, chlorophyll fluorescence and turbidity together with conductivity, temperature and pressure. These sensors are logged centrally by a high specification marine data acquisition system (Marine Monitor) mounted within a cage immediately beneath a toroidal buoy (Figure 1). Telemetry (Paknet) of surface sensors is currently available together with the option of deploying meteorological sensors as also shown in figure 2.

Results from work carried out in Liverpool Bay as part of the JoNuS II mooring programme between April and July 1997 are shown in Figure 3. Surface chlorophyl a fluorescence, turbidity, temperature and salinity were measured during five minute bursts four times per hour.

Smart moorings represent another step forward in the development of technology for observing marine ecosystems. By incorporating an 'intelligent' water sampler (Aqua Monitor) into a multi-parameter sensing platform we combine the advantage of high frequency fixed point measures, with their ability to resolve episodic events (e.g. algal blooms), together with an ability to collect, preserve and store a sample for subsequent analysis. Water samples may be examined microscopically for determination of microplankton numbers and species composition or possibly for analysis of nutrients or other constituents. These systems are particularly suited for time-series monitoring purposes where they may be left unattended for (potentially) long periods, for providing the high frequency data necessary for validation and testing of coupled physical-biological models and provision of sea truth data for remote sensing.



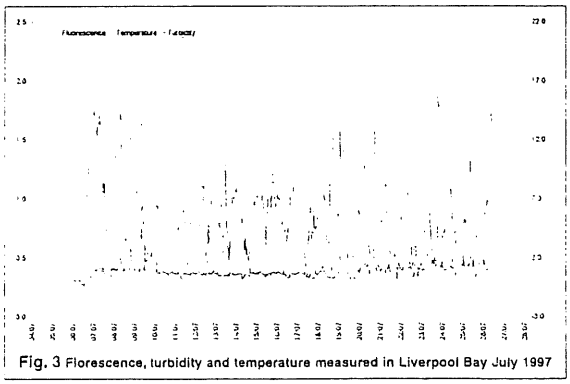
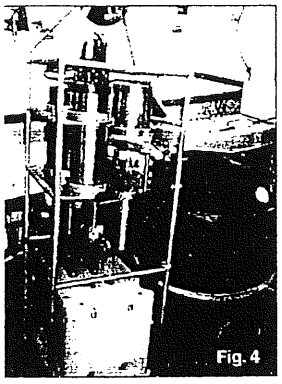


Fig. 3 Florescence, turbidity and temperature measured in Liverpool Bay July 1997



Aqua Monitor (Figure 4) was developed as part of a DETR (Dept of Environment, Transport and the Regions) funded programme, described elsewhere (Mills et al., 1998) Prototypes have recently been (February 1998) deployed for test purposes and will be incorporated into smart moorings during our next field programme in the Thames estuary this year. Other developments planned include 2-way telemetry to allow user control of logging regimes and the possibility of triggering Aqua Monitor remotely and telemetry of data from sub-surface instruments.

CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

References

Mills, D.K., Walne, A. Reid, P.C. and I. Heaney, 1998. Updating the Continuous Plankton Recorder - An Improved Tool for Integrated Plankton Monitoring. ICES Journal of Marine Science in press.

Acknowledgments

This work is funded by the Ministry of Agriculture, Fisheries and Food and the Dept. of the Environment, Transport and the Regions. Practical support was provided by the Dept. of Agriculture for Northern Ireland.

W·S OCEAN SYSTEMS

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Advanced Plankton Monitoring

Updating the Continuous Plankton Recorder

D.K. Mills[1], A.W. Walne[2], I.S. Heaney[3] and M.B. Rawlinson[4]

Figure 1 - U-Tow recovery aboard R.V.Cirolana

The Continuous Plankton Recorder (CPR) survey has provided a unique, sixty five year length dataset on the distribution of plankton in European Regional Seas and the North Atlantic. It has provided a yardstick against which changes in the planktonic ecosystem, perhaps the most sensitive indicator of environmental change, can be assessed. The CPR programme has been successful but there is a need to modernise in order to meet the needs of future monitoring studies and to take advantage of new developments in sensor and sampling technology. There are a number of initiatives currently underway to update the programme and also develop an integrated monitoring programme using moorings. These two strategies will provide effective observational platforms for monitoring the planktonic ecosystem and in particular the distribution and biodiversity of the plankton in relation to their physio-chemical environment.

The Programme - An Improved Tool for Integrated Plankton Monitoring

A 3 year collaborative venture between SAHFOS, CEFAS and DANI began in 1996 and has commissioned the development of a sampling and sensing system for an updated CPR for the determination of biomass and species composition of microplankton. This system will sample the smaller plankton which are not quantitatively sampled by the CPR standard mechanism and will be installed alongside a suite of environmental sensors. Information from such a system is not only essential for the interpretation of plant pigment data, but is also most important for the identification of temporal and spatial changes in the population composition of the tightly bound communities of auto- and heterotrophic organisms that constitute the microplankton. Only specific taxonomic analyses can give understanding of community variability, biodiversity and its role in influencing ocean metabolism.

Figure 2 - U-Tow launch

Progress to Date - Development of Enabling Technology to Meet Scientific Needs

A new and potential replacement body for the CPR survey has been developed with the ability to undulate and to carry a comprehensive payload. Other developments have been to quantify the flow through both the towed body and the sample mechanism itself using miniature electromagnetic current meters and instrumenting the towed body with a range of environmental sensors (Figure 1 and 2).

An intelligent phytoplankton sampler has been designed and a prototype built (Aqua Monitor, W. S. Ocean Systems). It is able to collect and preserve up to 50 water samples of 150 ml each dependent upon environmental conditions and be capable of operating within the confined space of U-Tow (Figure 3) The design is based on an "intelligent" programmable syringe system. A water sample is collected and stored temporarily prior to fixation and dispatch to permanent storage. This two stage process permits a choice between rejecting the initial sample in favour of a second. Such an approach allows the use of a conditional sampling protocol where sample collection and permanent storage is based upon a pre-determined set of user defined conditions. These conditions will be based upon electronically measured environmental variables such as chlorophyll fluorescence, turbidity and density. The particular advantage of our intelligent sampler is that in conditional sampling mode, for example, a sample is likely to be required at a maxima or minima in signal strength from a sensor. The peak in sensor output may be defined as a shift from a positive to a negative slope in a record. Consequently, the sensor output recorded as a water sample is taken may be compared with the following sensor readings and a decision reached as to reject or store the captured water sample. If rejected a further water sample may be immediately taken and stored or rejected subject to the conditional criteria. An algorithm has been developed to implement conditional sampling and an example of its operation is shown in figure 4.

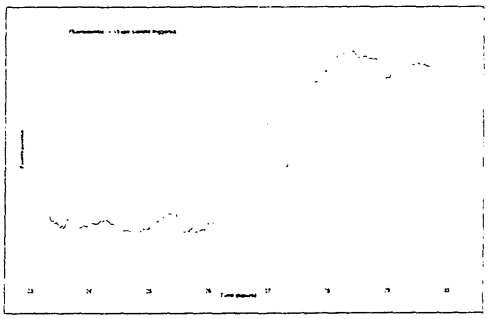

Figure 4 - U-Tow fluorescence data with sampling points predicted by the "Smart" sampling algorithm

initial sea trials of Aqua Monitor have been carried out on board R.V. Cirolana in February 1998 in the North Sea. Water samples have been successfully collected on a ship lowered rosette sampling system and also on level flight tows of U-Tow. Comparison of Niskin bottle and Aqua Monitor collected water samples showed no measurable difference in dissolved inorganic nitrogen content over a tidal cycle. Further trials are planned and will include comparison of phytoplankton counts and species composition derived from Aqua Monitor with those derived from traditional sampling techniques. Implementation of the conditional water sampling protocols will also take place this year in the laboratory and at sea.

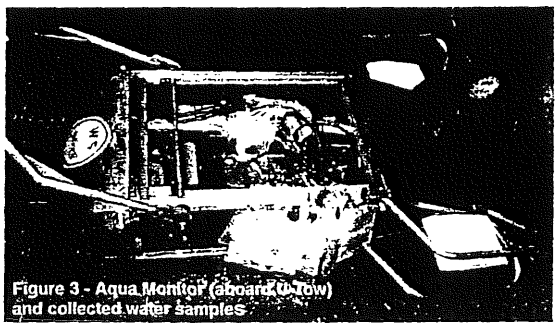

Figure 3 - Aqua Monitor (aboard U-Tow) and collected water samples

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Acknowledgements
Funding of this work is provided by the UK Department of Environment, Transport and the Regions (EPG 1/9/90) and the Ministry of Agriculture, Fisheries and Food.

DEPARTMENT OF AGRICULTURE FOR NORTHERN IRELAND

CEFAS The Centre for Environment, Fisheries & Aquaculture Science

W·S OCEAN SYSTEMS

ANNEX 11 AGENDA JOINT MEETING WORKING GROUP ON PHYTOPLANKTON ECOLOGY AND ICES/IOC WORKING GROUP ON HARMFUL ALGAL BLOOM DYNAMICS

1. Opening of the meeting by the chairman of Working Group on Phytoplankton Ecology

2. Announcements, appointment of rapporteur, agreement on agenda

3. Discussion on TORs

3.1. TOR 1: Results of the ICES/IOC Workshop on Intercomparison of the in situ growth rate measurements (Dinoflagellates), Kristineberg Marine Reseacrh Station, Sweden, 9–15 September 1996

3.2. TOR 2: Review the status of taxonomic coding systems with a view to recommend the adoption of a single coding system for use in ICES

3.3. TOR 3: To develop and propose a new mesocosm experiment to investigate new approaches in phytoplankton ecology

3.4. TOR 4: To discuss contributions of the respective Wgs to the future work programme in relation to the remit of the Oceanography Committee and the development of the ICES 5 Year Plan

4. AOB

5. Closing of the session

ANNEX 12 TABLE WITH STATIONS WHERE MESOCOSM EXPERIMENTS COULD BE PERFORMED WITH THEIR MAIN FACILITIES

place	lab facilities	B&B	mesocosm	flagellates	land-based	cultures	optimal time
Kristineberg, Sweden	+	+	-	P. micans	+	-	July - September
Helgoland, Germany	+	+	+ 2 phyto bags - external	Ceratium	+	?	June - August
Bergen Norway LSF	+	+	+ bags	Phaeocystis Emiliana	+	++	April - May
Trondheim, Norway	web site						
Arundal, Norway LSF	+ 2	+/-	2 out door basin 4m deep wide 10*20	dinoflagellates	+	- but facilities for maintaining	August - October
Algarve, Portugal	+	+/-	3 of 3m ht variable width	flagellates	+	-	June - September
Lowestoft, UK	+	+	various	phaeocystis	+	+	May - June
Kingston*, USA	+	+/-	8 off 2 x 6 m	Heterosigma akashiro Prorocentrum minimum, Scrippsiella trochoidea, Heterocapsia triguetia	+	+	June - July but unpredictable

* $10000 per week costs.

LSF = Large Scale Facility