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REPORT OF THE

WORKING GROUP ON PHYTOPLANKTON ECOLOGY

Büsum, Germany
24–26 March 1997

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 Opening of the meeting

The meeting was opened by the Chairman, Prof. Franciscus Colijn at 9.00 hours on 24 March 1997. The chairman welcomed the participants of the Working Group Meeting to his institute and as the Director of the Institute gave a brief overview on the tasks of the Institute. The meeting was attended by 8 scientists representing 6 countries. Although this amount of scientists for a working group meeting is disappointing, no long discussion was held to see whether there are specific reasons for the low attendance. One reason is probably that currently more interest is given by member countries (or scientists) to attend the WG HABD, because it covers more politically interesting topics (see below). However, the chairman after a short discussion, promised to write letters to some national delegates to ask for attendance and collaboration in the WGPE. A list of participants is given in Annex 2. The chairman presented the agenda, which was left unchanged by the Working Group.

The following members were absent with notice: Bert Wetsteijn (the Netherlands), Stephen Bates (Canada). The chairman made the following announcements:

- The Working Group on Harmful Algal Bloom (WGHABD) will meet in La Roche Canillac, France, from 22-26 April. Regarding the functioning of both WG's, the WGPE is of the opinion that a future collaboration of both WG's is needed to avoid overlap and strengthen the further development of new techniques to study the ecology of phytoplankton in general and of harmful algal species in particular. This collaboration could be improved by having the next WG-meetings at the same place and in an overlapping time-frame, however without the *a priori* intention to merge the groups.

The chairman will contact the WG HABD chairman (P. Gentien) before their meeting, so that the item can be discussed during their meeting in La Roche.

- ICES Symposium on Temporal Variability of Plankton and Their Physico-Chemical Environment in Kiel from 19-21 March 1997

The symposium was attended by about 120 scientists from about 16 countries. Altogether 42 oral presentations were held and 22 posters presented.

The great success of the Symposium is evident at several levels. At the scientific level, it was clearly shown that variability is an inherent property of planktonic systems, both phytoplankton and zooplankton and in the physical-chemical properties of their habitats. That is to say, variability is just as much an intrinsic property of the phytoplankton, as is their requirement for nutrients and irradiance for photosynthesis. As such, variability should be measured on a routine basis. This major scientific result of the symposium supersedes the long held notion that variability is primarily a feature of fish stocks, while that at the planktonic level is relatively trivial and not particularly relevant to fisheries. It also indicates that the justification for plankton time series' analyses is not simply a practical one, e.g. to help understand fisheries stock variability, but should be incorporated into the basic methodological and conceptual approaches of Biological Oceanography.

The symposium revealed that there is now a critical mass of long term time series data sets and researchers interested in variability, trends, cycles and changes in planktonic systems. The presentation of their results at the symposium provided much needed collation, synthesis and identification of time series results. The published proceedings will be a benchmark, stimulating the integration of such results into contemporary biological oceanography issues. It should also stimulate similar research and the continuance of ongoing time series.

Symposium participants in sharing experiences with regard to being able to maintain their time series, identified common problems endangering the continuance of their time series. Many participants stated that the discontinuance of their time series was imminent for a variety of reasons, principal ones being the ending of their funding and not replacing persons responsible for the time series. There is the associated problem that long term time series are in need of rescue. That is, data are filed away in the desks of senior scientists preparing for retirement. This carries the danger, that the time series data will be discarded or lost. There is, thus, the need to identify, preserve and continue long term time series. This paradoxical situation comes exactly at that time when the need for time series observations are essential to resolve global change issues and distinction between anthropogenic effects and those attributable to natural variance (plus the biodiversity issue).

Finally the symposium was extremely valuable in allowing participants to establish communication and collaborative networks. Arrangements were made among various scientists to prepare joint publications, to exchange data, and to teach each other new techniques of data and trend analysis.

In summary, the symposium was successful at multiple levels. So much so, that the Working Group on Phytoplankton Ecology will recommend that a symposium be convened in five years (2002) to revisit this issue and variability in planktonic systems, and moreover to set up a Study Group to deal with the item of long term variability.

Apart from this generalised picture of the symposium highlights will be presented in a summarised form during the Annual Science Conference in Baltimore. These highlights pinpointing to the main issues of the Symposium, together with recommendations will be sent also by letter to the International Organisations concerned with monitoring issues of which some were sponsoring the symposium (ICES, OSPARCOM, HELCOM). One of the recommendations will be to set up or continue a network of long term observations in the marine area.

Regarding resolution 1:7 of the 84th Statutory Meeting, the chairman apologises for the delay of the finalisation of the document 'Working Manual and supporting papers on the use of a standardised incubator in primary production measurements', edited by F. Colijn, L. Wetsteijn, L. Edler and O. Lindahl, to be published in the ICES Techniques in Marine Environmental Sciences series. The Manual and papers will be submitted to ICES not before this summer, due to the large workload for the organisation of the Symposium in Kiel. In the meantime ways to produce this standard incubator are further developed. Hydrobios in Kiel (Germany) is willing to produce the incubator and will shortly announce prices and delivery conditions. The preparation of series of bottles with different irradiance levels is still subject to negotiations. At present the calibrated bottles can be obtained from a Dutch firm (details and address available on request from the chairman of the WGPE).

2 Terms of reference

The chairman informed the Working Group on Phytoplankton Ecology regarding the Council Resolution 1996/2:51, which states:

The Working Group on Phytoplankton Ecology (WGPE)(Chairman: Prof. F. Colijn, Germany) will meet at Büsum, Germany from 24 - 26 March 1997 to:

a) propose new pigment procedures for measurement of chlorophyll *a* , taking into account recommendations contained in a new SCOR report on phytoplankton pigments;
b) investigate new approaches in phytoplankton ecology on the basis of organismal functioning of the planktonic system;
c) examine the results of mesocosm experiments to study the direct effects of nutrient inputs (enhanced and reduced) on phytoplankton composition, primary production and biomass, and structure of the lower trophic level (microbial loop);
d) continue the evaluation of new techniques for the measurement of primary production and biomes with the aim of producing a systematic review of relevant instrumentation, particularly biosensors;
e) discuss, in consultation with WGSSO, the physical, chemical and biological description of the response of the marine environment to anthropogenic nutrient inflows in some example areas;
f) evaluate, in consultation with WGHABD, technical details of measurement of phytoplankton growth processes, biomass estimates including counting cells and relations with nutrients;
g) review the status of development of taxonomic coding systems with a view to recommending the adoption of a single coding system for use in ICES;
h) prepare plans for a joint meeting with WGHABD in 1998;
i) advise ICES/OSPAR SGQAE on the development of quality assurance procedures for phytoplankton measurements adopted for JAMP (OSPAR 1997/2:1);
j) review the quality assurance associated with primary production measurements carried out using a standardised method, including a standard incubator, and report to ACME before its June 1997 meeting.

The Working Group will report to the 1997 Annual Science Conference (Biological Oceanography Committee).

The chairman distributed recent additional information on questions regarding TOR's g), i) and j) about QA and standardisation procedures for the measurement of chlorophyll a and phytoplankton species composition

obtained from ICES, and/or through the chairman Dr. H. L. Rees of the ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication (SGQABM). A paper distributed by Dr. H. Dooley (ICES) on taxonomic coding systems was distributed as well. For the discussion on TOR e) the chairman distributed parts of the report of the ASMO Modelling Workshop on Eutrophication Issues held in the Netherlands from 5-8 November 1996, which were kindly made available through one of the editors of the report, Mr. Ies de Vries of the RIKZ in the Hague.

3 General Discussion of Terms of reference

a). propose new pigment procedures for measurement of chlorophyll a taking into account recommendations contained in a new SCOR report on phytoplankton pigments.

This term of reference has been in the WGPE agenda during the last two meetings, but has not been dealt with due to a delay in the publication of the SCOR Working Group 78 report. The report has now been recently published in January 1997 by UNESCO („Phytoplankton pigments in oceanography", Eds., S. W. Jeffrey, R. F. C. Mantoura and S. W. Wright, Monographs on oceanographic methodology nr. 10, UNESCO Publishing 1997, ISBN 92–3–103275–5) and has been in the hands of the WGPE only a few days before the meeting.

The WGPE is very pleased by the completion of the book that can be considered as a „state of the art" review of the complex field of measuring phytoplankton pigments and a reference source for many years to come. We would like to compliment the editors and all the contributors that have made this book a reality.

A first brief browsing through the different chapters of the book reinforces once more the general feeling about the impossibility of defining one standard procedure that satisfies all aspects in pigment determination. The choice of a method depends, as stated by the authors of the book, on the type of data required, the type and number of samples and the equipment and time available. The term of reference for the WPGE calls for a proposal for new procedures for the measurement of chlorophyll *a* and therefore the first task of the WGPE has been to define in which context this should be done.

In the opinion of the WGPE, the most widespread and important use of chlorophyll *a* has been in an ecological context as an index of the total phytoplankton biomass that also, being chlorophyll *a* the main link between light energy and the photosynthetic process of inorganic carbon fixation, can be used in connection with primary production studies in order to estimate the photosynthetic efficiency of a phytoplankton community. This application of chlorophyll *a* measurements will most probably continue in the foreseen future. This does not, of course, exclude the application of other better suited methodologies by ICES members to give answer to others aspects in the field of phytoplankton ecology.

In preparing a proposal for new procedures for the measurement of chlorophyll *a* , the following recommendations from the SCOR Working Group 78 were taken into consideration:

Sampling and preservation

Most of the aspects referring to water sample collection are of a general character and should be followed. However, the WGPE would like to emphasise the following:

Glassfiber filters should be used for the collection of samples. The Whatman GF/F type (0.7 µm, nominal pore size) or equivalent is recommended, but in cases with large phytoplankton concentrations, i.e. blooms, coarser filters (i.e. GF/C, 1.2 µm nominal pore size) can be an alternative if filtration takes too long time. For most purposes filters of 25 mm in diameter are suitable.

Magnesium carbonate should not be used as a filter aid.

Removal of any large zooplankton from the filter with fine forceps is recommended.

After filtration the filters should be folded once with the material inside and gently sucked dry or eventually blotted between several layers of absorbent paper and frozen immediately in a suitable container if analysis is not going to be done within a short period of time (about one hour).

The freezing procedure will depend on the expected storage time:

For samples to be analysed within a few days (up to a week) packing in thin plastic bags and freezing in a conventional freezer (- 20 °C) should be adequate.

If storage of samples up to about 2 months is required then the filters should be frozen in a ultra-cold freezer (- 90 °C). Thin plastic bags are also suitable for this purpose.

If storage of samples for periods longer than 2 months is needed, then freezing and storage in liquid nitrogen (- 196 °C) is required. Care has to be taken in using appropriate containers (cryotubes)

Extraction

The SCOR WG 78 reviewed and tested thoroughly the most common solvents and extraction procedures being used at present for extraction of phytoplankton pigments. The tests were done by extracting pigments from phytoplankton species widely known as „recalcitrant" species. Sonication in dimethyl formamide (DMF) was found to be the best combination according to five of six previously established criteria and it is recommended by the group as the „reference" extraction procedure. DMF showed a 100% recovery for chlorophyll *a* from the test algae. However, the high toxicity of DMF to humans made the group reluctant to recommend it for field work. The second choice was sonication in methanol, that although it produced about 15% underestimation of chlorophyll *a*, can be recommended for marine work where the test species seldom dominate the phytoplankton community. Grinding in 90% acetone resulted in about 75% recovery of chlorophyll *a* while soaking in 90% acetone produced only about 55% recovery. Although extinction coefficients for most pigments are already available for methanol and therefore this solvent can be used for HPLC work, spectrophotometric equations to correct for absorption by chlorophylls *b* and *c* have not yet been developed as they have been for 90% acetone. Work in this respect is being carried out presently. Until this is completed the SCOR WG 78 recommends extraction in 90% acetone with grinding. Soaking overnight in 90% acetone alone(or any other solvent), a common method for pigment extraction, is not recommended since pigment recovery is low, variable and always accompanied by degradation products. Soaking preceding grinding can improve slightly pigment extractability, but again at the expense of pigment degradation.

Based on the above facts the WGPE supports the recommendation by the SCOR WG 78 that extraction in 90% acetone with grinding should be used until new spectrophotometric equations for methanol based extraction are developed. This will permit a direct comparison of both solvents and provide a better background for proposing a standard extraction procedure. The WGPE agreed upon sending a request to the SCOR WG 78 in giving high priority to this work.

Measurement method

Separation techniques (i.e. TLC, HPTLC, isocratic HPLC and reversed-phase HPLC) were found to be, not surprisingly, the best way of measuring chlorophyll *a* along with a large spectra of other pigments and their derivatives. Unfortunately, these techniques are usually time consuming and require expensive equipment something not always available on a widespread basis. Besides, most ecological researches projects or monitoring programs demands the incorporation of far more parameters than phytoplankton biomass leading to the need of a simpler and more rapid methodology for measuring chlorophyll *a*.

Spectrophotometric and fluorometric methods offer a good alternative to the separation techniques, but they also have their limitations. According to SCOR WG 78, spectrophotometry (Jeffrey and Humphrey, 1975) accurately measure chlorophyll *a* (1-3% error) if samples are free of degradation products. This is unfortunately seldom the case in field samples, so its use should be carefully evaluated according to the real field conditions, for instance running a few HPLC analyses when possible. The acid-spectrophotometric method of Lorenzen (1967) reduces, but does not eliminate the interference from degradations products. The precision of this method is also variable depending on the composition of the degradation products of chlorophyll *a*. Spectrophotometry requires, in addition, fairly large water sample volumes incorporating a time factor that many researchers consider as critical during field work. Fluorometry (Holm-Hansen *et al.*, 1965) became about 30 years ago a very popular method among ecologists by increasing sensitivity and simplifying somewhat the measurement of chlorophyll *a*. This method provides also an accurate measurement of Chl *a*, according to SCOR WG 78. However, this method in its most common acidification form in order to correct for phaeopigments leads to an underestimation of chlorophyll

a in the presence of chlorophyll *b*, and should be applied with care in areas where organisms containing this chlorophyll are present in significant amounts. An improvement of this method (Welschmeyer, 1994) seems to solve this problem, although it was not tested by SCOR WG 78. The main disadvantage of this last modification is that it does not give an indication of the amount of chlorophyll degradation products present in the sample. For many ecologists this is valuable information.

The ICES WGPE agrees upon that the modification to the fluorometric method proposed by Welschmeyer (1994) seems to have a large potential for the accurate measurement of chlorophyll *a* alone in all kinds of natural waters but it should be thoroughly tested, specially with a diversity of field samples, before it can be recommended. This method combined with extraction based on 90% acetone for field samples dominated by diatoms, unarmoured dinoflagellates and prymnesiophytes, or on sonication in methanol for all kind of samples, seems to offer a major improvement in the measurement of chlorophyll *a* without incurring in large investments in new equipment. The accuracy of the method is about 6% as estimated by an evaluation carried out by the US Environmental Protection Agency (Arar, 1994). In those cases where an estimation of the amount of degradation products is needed, the fluorometric method of Holm-Hansen *et al.* with extraction in 90% acetone will provide it, together with an accurate measurement of chlorophyll *a,* as long as chlorophyll *b* is not present in significant amounts. The precision of this method is also variable depending on the kind of chlorophyll *a* degradation products. The WGPE wants also to emphasize that independently of the choice, any fluorometric method will always have to be dependent on accurate spectrophotometric absorbance measurements for proper calibration. The use of commercially available chlorophyll *a* is recommended for this purpose. (a list of purchasers is given in the SCOR Publication).

The ICES WGPE agreed in continuing this work during 1997 with the aim of preparing a concrete proposal for a standard method for the measurement of chlorophyll *a*. To achieve this it is proposed to establish a closer contact with the SCOR WG 78 in order to be rapidly updated regarding newest development, especially of trichromatic equations for methanol

To support the main conclusions a choice of relevant information was selected from the SCOR Publication. The figures and Tables are given in Annex 4.

b). investigate new approaches in phytoplankton ecology on the basis of organismal functioning of the planktonic system

The Working Group on Phytoplankton Ecology considered the issue of what new approaches in phytoplankton ecology based on organismal ecology are needed, but which are not considered in the traditional investigative approaches based on whole community biomass and mass balance dynamics. Among those organismally-based issues identified, the following were considered as particularly important: bloom species selection, the variation of phytoplankton growth, community organisation and species abundance, species successional rates and associated processes (cf. Smayda, 1996).

Bloom species selection:

The selection of bloom species has become an increasingly important issue, such as in the selection of harmful algal species. There are several published relations which show that biomass (chlorophyll) increases with nutrient levels. It is well-established that bloom species have dissimilar nutritional value and nuisance/toxic potentials, however. This yield-dose relationships revealed by the biomass-nutrient concentration correlation's hides such interspecific differences.

Phytoplankton growth types:

Classically, phytoplankton growth is assessed as a whole community response, usually based on differences in chlorophyll (biomass) and primary production rates. However, one must distinguish between cellular, population and community growth. The factors regulating these different growth modes are not the same, and cannot be investigated based on total community chlorophyll. Investigation of these growth modes requires investigation of the cellular and population growth of a given species, and the growth of the community and species present.

Community organisation and species abundance:

The biodiversity of phytoplankton communities and the abundance of the individual species based on their numerical and biomass abundance need to be investigated. Biodiversity influences the resiliency of the community to withstand induced change and stress in response to anthropogenic disturbance. Abundance of individual species based on cell numbers vs. biomass-based estimates are different indices of abundance. This has yet to be adequately assessed, and to establish whether the traditional whole community approach based on chlorophyll is an adequate measure of the dominant and most vigorous species within the community.
Successional rates and associated processes:

The succession of species is recognised as a central characteristic of phytoplankton communities of fundamental importance to bloom events and energy flow. However, succession remains to be quantified, and cannot be examined from whole community, biomass-based approaches.

A mesocosm study of growth:

Investigation of concurrent cellular, population and community growth rates in mesocosms is proposed. Individual species abundance's will be established based on both numerical and biomass abundance. Growth rates of individual, probably dominant cells will be established using different techniques (cf. TOR c and d). Such experiments should solve or at least give an impression of the ecological principles behind growth of phytoplankton cells under (semi-) natural conditions. In such experiments several growth rate determining mechanisms could be experimentally tested: effects of organic molecules as growth rate stimulators, effects of organic nutrients, parasitism, nutrient ratio's, etc.

A suitable area to do these experiments, if possible in cooperation with the WGHABD, could be the Baltic, because the general impression was that these systems are somewhat less complex than other areas (reduced grazer impact). Also there is considerable knowledge on natural conditions available. The WGPE stressed that there are no principal differences between freshwater and marine communities. The details of such an experiment should be discussed during the combined WGPE and WGHABD meeting in 1998. Apart from facilities in Finland also the LSF in Bergen could probably be used for such studies with financial support from the EU.

Recommendation: see conclusions on TOR 's f) and h)

c) examine the results of mesocosm experiments to study the direct effects of nutrient inputs (enhanced and reduced) on phytoplankton composition, primary production and biomass, and structure of the lower trophic level (microbial loop);

The WGPE decided not to try to cover this TOR by discussing all papers dealing with this item. Moreover a Mini-Symposium entitled 'Can results from mesocosm studies help to understand eutrophication effects' with co-conveners Prof. Y. Olsen (Norway) and Dr. A. C. Smaal (Netherlands) will be held during the 1998 Annual Science Conference in Lisbon. Therefore a literature overview based on the expertise of the WGPE members has been compiled and is listed as Annex 5. Only a part of the literature quoted is directly related to eutrophication as such, but most have strong links to nutrients. The information on this TOR was partly received after the meeting and compiled by the chairman.

During the meeting a presentation was given by Dr. Urban Tillmann working as a postdoc at the FTZ-Westcoast, on results of mesocosm experiments performed during a one-month enrichment study with silicate in Bergen in September 1996 (cf. Egge and Aksnes, 1992).

d) continue the evaluation of new techniques for the measurement of primary production and biomass with the aim of producing a systematic review of relevant instrumentation, particularly biosensors;

Based on the discussion in the WGPE on this TOR it was decided to present next year a written review on the following new techniques to study phytoplankton dynamics which are presently in a state of development: on LIDAR techniques; the chairman will contact the group at ENEA in Italy to present such a review; on PAM fluorescence (Schreiber, 1986; Schreiber *et al.*, 1993; Schreiber, 1994); it was decided to ask Peter Hartig to organize this review, with the suggestions to ask for contributions by Bert Kroon (AWI, Bremerhaven), Egil Sakshaug (Norway), Jan Snel (the Netherlands), Ulrich Schreiber (Germany); on moored fluorescence sensors and possible new equipment installed in the CPR to ask Dave Mills (MAFF, UK); to ask George Dubelaar to present the state of the art on flow cytometry (already promised); to ask Engel Vrieling to present a contribution on immuno-labeling of algae; and to ask Juhu Leppänen and Nick Flemming to give information on Ferry-Box

systems. All contributions should be ready before the 1. of November to be able to compile them in a report for ICES. The chairman will invite the suggested authors to deliver the information. The present state of development is given in the following contribution based on a review paper by Odd Lindahl on Harmful Algae, from which we have selected the sections dealing with monitoring techniques relevant to this TOR. Although the paper emphasises harmful algae most technical aspects hold for other phytoplankton species as well.

Management programmes, monitoring the occurrence of harmful algae, are carried out in all kinds of habitats; from small ponds and estuaries to offshore conditions and in waters with phytoplankton abundances from some hundreds of cells per litre sea water to several millions. Some of the harmful species accumulate at the sea surface while others are found as subsurface populations and a third category is more homogeneously distributed throughout the water column. Given this variety, there is no general method or technique available which can be used all over for monitoring the harmful algae. In fact there are several different techniques used for the monitoring. The methods can roughly be divided into two different kinds: i) methods where the phytoplankton cells are sampled and identified by microscopic examination and ii) methods which use remote sensing techniques or chemical/biological probes. In the first case a main problem is to take representative plankton samples in time and space, in the second case an additional problem can be to identify a species from a distance. Large efforts have been made to try to automate the monitoring of phytoplankton occurrences in different ways.

It has been suggested that the observed increase in frequency in blooms as well as the number of species identified as causing harm is basically a result of eutrophication of some coastal areas (Smayda, 1990). The eutrophication in many areas has involved not only increased nutrients concentrations but also, for example in NW European waters, an increase in the nitrogen to phosphorus ratio and a decrease in the silicate to the phosphorus and nitrogen ratios during the last decades (Hickel *et al.*, 1995; Andersson, 1996). By this development, blooms of flagellates may have become enhanced (Schöllhorn and Granéli, 1993; Hickel *et al.*, 1995) as well as the toxin production of some species (Granéli *et al.*, 1993, Johansson and Granéli, 1996). The increase of humic acid and trace metal leakage to the sea, caused by acid rain, may be another anthropogenic influence which may have increased the development of harmful algal blooms (Granéli *et al.*, 1989). Furthermore, the intensified world wide traffic of ships carrying large quantities of ballast water may also be responsible for an increase of harmful algal bloom events (Hallegraeff *et al.*, 1990; Rigby *et al.*, 1993). Finally, a scenario has been presented in which the incidence of blooms of the toxic dinoflagellate *Gymnodinium catenatum* along the coast of Galicia (NW Spain) could increase due to global climate change (Fraga and Bakun, 1993). A change in the distribution pattern of *Gymnodinium breve* along the Florida - North Carolina coast may also be a result of global warming (Tester *et al.*, 1993). It can thus be concluded that anthropogenic influences most likely have promoted the spatial and temporal increase of harmful algal events.

A prerequisite for a successful monitoring of (harmful) algae is that all work, from the sampling to the final report, should be quality assured. The author (O.L.) can refer to more than two years positive experience as an accredited performer of a monitoring programme on hydrography and nutrients, which also contains a quality assurance programme on phytoplankton.

Since the number of proven harmful species is apparently increasing, an ideal programme would be one for which phytoplankton species composition is determined throughout the year for commercially and recreationally important areas. However, this may not be feasible for many governments or organisations, in which case monitoring should be restricted to suspected or known harmful species. By itself, phytoplankton monitoring does not provide sufficient protection from public health. It may, however, lead to an early warning on which intensive monitoring of fisheries products can be based. For finfish farming, monitoring phytoplankton is often the only way of providing a warning of the presence of harmful algae. Given the need to minimise health risks to human consumers and economic losses caused by these outbreaks, two monitoring strategies are used: i) screening coastal, and in some areas also offshore, waters for the occurrence of harmful phytoplankton species, and ii) testing for toxicity in fish and shellfish.

The design of a monitoring programme.

The design of a monitoring programme on harmful algae should start by defining the kind of information which is needed to protect the specific resource. The user demand of a monitoring programme in relation to fishery and mariculture would typically be an early warning that a bloom of harmful algae is under development. An early warning allows the fish farmer to put specific contingency plans in action and farmers of shellfish and fishermen to avoid harvesting and catching seafood which may be toxic.

As already mentioned, physical, chemical and biological processes in the sea are important for the occurrence and distribution pattern of harmful algae. Thus, a monitoring programme must, in part, rely on an understanding of the hydrography and plankton ecology of the area to be monitored. In locations where a lack of background information may hamper the development of an adequate programme, there is no alternative but to initiate extensive monitoring which should subsequently be adjusted as information becomes available. In this respect, basic hydrography and phytoplankton ecology are important considerations in the development of suitable monitoring programmes. Knowledge of the temporal and geographic distribution of inorganic nutrients and their sources, as well as other phytoplankton growth factors, will also be important when planning and operating a monitoring programme. An optimum suite of variables to monitor would also include light penetration, meteorological observations and the occurrence of grazers.

In areas where harmful phytoplankton species may produce cysts or dormant cells as a part of their life cycle, knowledge of the cyst dynamics will much improve the possibility of monitoring a bloom development. Cysts generally accumulate in basins with other fine particulate material and are buried below the sediment surface so that the majority are found in anoxic sediments which can inhibit germination. It is likely that many blooms originate from benthic cyst populations, although the importance of the initial inoculum varies significantly between hydrographic regions. For example, in shallow, enclosed embayments there is close temporal and spatial connection between cysts and the subsequent blooms of *Alexandrium*, whereas the germination of cysts in deeper coastal water of the Gulf of Maine is more gradual and, thus , has less impact on the bloom dynamics in those waters (Andersson and Keafer, 1985).

Sampling of algae

A vertical tow with a plankton net of 20 µm mesh size is a non-quantitative method for detecting some species when they are present in low cell numbers, which is less than 1 000 cells per litre. This method is simple and provides the earliest possible indication of the presence of harmful species in the water column. However, fragile or small species cannot be reliably collected in this manner. Since not all harmful species are collected by net tows and quantitative data are necessary, most monitoring programmes recommend that discrete water samples are taken. To determine maximum cell densities, water samples from discrete depths are needed. Since the horizontal and vertical distribution of harmful algae seldom is homogeneous, one single water sample may not be sufficient to determine the concentration of the algae of an area. Thus, in an area to be monitored, a number of samples taken over the depth range of interest, is necessary in order to receive a representative distribution pattern of the harmful algae.

Prior to the sampling a vertical profile of the *in vivo* fluorescense would provide useful information on the vertical distribution of phytoplankton. However, it must be pointed out that the *in vivo* fluorescense profile will only be of help for the sampling of a harmful algae when the algae is dominating the phytoplankton community. In many cases it is time consuming to analyse the plankton samples and the number of samples to be counted must be reduced. One way to do this is to pool some or all the samples and count it as one sample representative of a part or the whole water column. Another way is to use a hose sampling system or to use a pump which will sample a vertical profile of the water column. However, the detailed information of the cell concentrations over depth will then be reduced.

If the harmful algae occurs in low abundance it may be necessary to concentrate the samples before counting the cells in the microscope. Samples can be concentrated by a factor of 10 to 100 by sedimentation. In most cases it is preferable that the algal samples are fixed or preserved before the concentration procedure is started. However, typical phytoplankton analyses from gravimetrically settled samples may not detect blooms of harmful algae occurring at concentrations of 10 to 1 000 cells per litre, because aliquots may be dominated by more abundant diatoms and microflagellates. It has been suggested (Turner *et al.*, 1995) that less-abundant, typically large dinoflagellates like e.g. *Dinophysis* are more frequently detected and precisely quantified if they are sampled as „microzooplankton", where several litres are concentrated by screening through 20 µm-mesh.

Sampling frequency should vary according to the seasonal abundance of the harmful species of interest and, if possible, should take into account the growth rate and/or accumulation of the species being monitored. In most areas at least weekly sampling will be necessary in order to keep track of the situation during periods when harmful algae occurs. However, in relation to the occurrence of a harmful event that results from the accumulation of cells caused by a change in meteorological or hydrographic conditions, it will not be possible to design a monitoring programme which covers all eventualities.

Methods for identification of algae

Identification by microscopy.

The only unambiguous method of determining the presence of a species is by microscopic analysis of water samples. Where possible, live samples should be used for species identification, while fixed samples can be used for species enumeration. Routine monitoring for species is easiest conducted by light microscopy. However, for many microflagellates and picoplankton, confirmation of identification requires electron microscopy. It should be pointed out that skilled cooperation with taxonomic expertise is a prerequisite for a successful monitoring programme on (harmful) algae.

A method which allows rapid and precise identification and counting of thecate dinoflagellates, e.g. *Dinophysis* spp, using epifluorescense microscopy has recently been developed for monitoring purposes (Andersen and Kristensen, 1995). This method allows for rapid handling of large volumes of sample in the presence of large biomasses of e.g. diatoms without interference of the diatoms with the identification and counting of the thecate dinoflagellates.

Methods using molecular probes.

Identification of harmful algal species using molecular probes has become an emerging perspective. Whether the problem is to distinguish between closely-related strains or to enumerate a single species in large numbers of samples, the need for species- or strain-specific „probes" is clear. The probes shall label only the cells of interest so they can be detected visually, electronically or chemically. Two such technologies targeted at identification of particular species: antibody probes and nucleic acid probes have recently been reviewed (Anderson, 1995). The antibody method involves the use of antibodies that bind specifically to proteins in the cell wall of the algal species of interest. Most immunological assay methods for cell identification use indirect immunofluorescense for visualisation or detection of the label. The nucleotide probe technology targets particular genes or gene products inside cells using short, synthetic DNA segments (oligonucleotides) which bind selectively to DNA or RNA sequences specific for a particular organism. Work is in its early stages on harmful phytoplankton species and little or no sequence information is at present available of these species. However, it is concluded that the molecular probe techniques have a great potential in quick, qualitative assays that indicate the presence or absence of a target organism, and they should assist and help in the identification of harmful species (Anderson, 1995).

Promising attempts on the identification of harmful algae on species level have been made by combining a very selective immunochemical detection technique and fast automated flow cytometric identification of labelled cells (Vrieling *et al.*, 1993). This combination of methods may not only be less time consuming and in the long run less expensive, but can also provide identification of single species within genera. The goal of this development is to allow detection of toxic marine microalgal species in mixed natural phytoplankton populations, even at pre-bloom stages, providing an early warning system (Vrieling *et al.*, 1995).

Remote sensing methods.

Remote sensing is a technique for studying the distribution of red tide organisms over larger spatial and shorter time scales than is possible with ship-based sampling (Yentsch, 1989). Multispectral scanners (e.g. Coastal Zone Colour Scanner; CZCS) have been used to detect the reflectance of chlorophyll *a* and other pigments, but these efforts have been constrained by the inability of the sensors to discriminate phytoplankton populations at the species level and the fact that only the top meters of the water-column are sensed. More progress has been made by first linking specific water masses to organisms and then identifying that water mass with an appropriate remote sensing technique. In particular, remotely-sensed sea surface temperatures have been used to locate and track the movement of water masses like plumes and coastal currents containing harmful algae or fronts and other physical features where phytoplankton accumulate. The advection of *Gymnodinium breve* from Florida into nearshore waters of North Carolina via the Gulf Stream has been followed with this approach (Tester *et al.*, 1989). Another example is the studies of the bloom dynamics and distribution of *Alexandrium tamarense* in the Gulf of Maine in relation to the short-term oceanographic processes responsible for the development and behaviour of the coastal current plume (Keafer and Anderson, 1993).

One obvious limitation using satellites is that clouds and fog can reduce the number of usable images for too long periods. Another limitation may be that the resolution is too small. Air-borne scanners may overcome some of

these difficulties and have been used successfully in the surveillance of algal blooms in the Dutch part of the North Sea (Zevenboom *et al.*, 1989). Surveillance without any instrumentation by Coast-guard pilots was carried out during the *C. polylepis* bloom in the Kattegatt/Skagerrak area in 1988 (Lindahl, unpubl.). This bloom occurred in the coastal current and was directly visible by the human eye thanks to a different water colour compared to offshore and inshore waters. The pilots could thus on-line monitor the distribution of the bloom and provide fish-farmers as well as research vessels with operative advice. However, it should be pointed out that most, if not all, remote sensing operations of harmful algae must at some point be checked against „ground truth" and that only the top meters of the water column can be investigated.

In the Baltic a method to record chlorophyll *a* fluorescence, temperature and salinity in the surface water unattended on board merchant ships has been successfully used (Leppänen and Rantajärvi, 1995). Supplemented with automated water sampling phytoplankton species composition and nutrients were also analysed. The system has proved to be an effective early warning method for exceptional and eventually harmful algal blooms. The unattended flow-through measurements served as reference data for satellite images and the images could extend the shipborne measurements basin wide.

Both in local and remote applications, laser induced fluorescence (LIF) techniques are commonly used to investigate vegetation targets, since ultra-violet lasers are suitable to excite chromophores, especially chlorophyll and cartenoids, in the living tissues of algae. A classification of algae in major groups, according to their characteristic pigments contents as reflected by suitable spectral ratios has been previously accomplished in laboratory measurements of LIF spectra (Barbini *et al.*, 1994). Main advantages of using laser remote sensing systems to monitor phytoplankton fluorescence rely upon their field applicability over large areas in contrast to *in situ* determinations performed by conventional fluorometers. A LIDAR fluorosensor system (LIght Detection And Ranging) is under development and uses *in vivo* induced fluorescence emission, in order to retrieve information of the phytoplankton species growing in the investigated water (Barbini *et al.*, 1995). A marine LIDAR fluorosensor apparatus relies on the use of a double pulse laser excitation technique and can also provide information on the growth rate of the algae.

Another approach for unattended measurements to monitor harmful algae is to use buoys which store data or transmit data on-line. Relatively simple passive optical sensors, deployed *in situ*, can be used to detect algal blooms in coastal waters (Cullen *et al.*, 1995). A tethered spectral radiometer buoy measures upwelling radiance in seven wavebands, as well as downwelling irradiance, to yield estimates of spectral reflectance. Blooms of dinoflagellates and diatoms have been detected by this type of measurements, not only because of characteristic shifts in ocean colour, but also because of the prominent signal from solar-stimulated fluorescence of chlorophyll. Passive optical sensors are unlikely to distinguish toxic from non-toxic blooms, but they are very well suited as early warning systems (Cullen *et al.*, 1995). Radiometer buoys, possibly in combination with the LIDAR-technique may be a future tool to initiate sampling in an early stage of a harmful bloom development.

The ICES Cooperative Research Report No. 181 "Effects of Harmful Algal Blooms on Mariculture and Marine Fisheries" and the draft of IOC technical series no. 44 "Design and Implementation of Harmful Algal Monitoring Systems" have been used and cited for the general information on the occurrence and monitoring of harmful algae in this section.

e) discuss, in consultation with WGSSO, the physical, chemical and biological description of the response of the marine environment to anthropogenic nutrient inflows in some example areas;

There are many examples of altered phytoplankton dynamics in global coastal waters which have accompanied nutrient enrichment. These are of two main types: changes in species composition and increased biomass. Examples include Asian coastal waters (Japan, China, Korea), Black Sea, inner Adriatic sea, North Sea, and embayments within the Skagerrak, Kattegat and Baltic Sea. The specific processes involved are still being elucidated, but both site-specific and basic ecophysiological processes influence the outcome of the nutrient stimulated responses of the phytoplankton community. One response common to several regions (e.g. Kattegat, German Bight, New York Bight) is the development of *Ceratium* blooms, which are largely ungrazed events, and which eventually become nutrient limited, sink to bottom sediments, and decompose leading to anoxia and hypoxia. Wide-spread die-offs of benthic and pelagic populations often result. A complex series of interactive biological and physical (such as watermass mixing characteristics) processes influence both the occurrences and consequences of such *Ceratium* blooms.

The Baltic Sea

In the Baltic Sea, the increase in inputs of nutrients has increased phytoplankton production and biomass during the last decades; especially plankton blooms have intensified. Enhanced sedimentation of organic matter has caused anoxia in the bottom water layers also in shallow areas. Nitrogen or nitrogen and phosphorus together are proved to be the limiting nutrients for primary production. Silicate limitation is also reported. This is expected to be the main reason for the observed dominance of dinoflagellates in spring. Low N:P ratios are promoting the development of N2-fixing cyanobacterial blooms. These blooms are not directly related to eutrophication, but the increase inputs of phosphorus are supposed to have intensified these blooms, too.

The model calculations made for the Gulf of Finland indicate that only when both nitrogen and phosphorus inputs are reduced all around the sea area, the phytoplankton biomass concentrations will be reduced by 10-40 % in few years.

The German Bight

An about threefold increase in phytoplankton biomass could be attributed to an increase in flagellates (which in this case are all non-diatoms). Within the flagellates, only nanoplankton <20 µm was responsible for major changes, as this group of mainly minute flagellates showed a sudden increase at the end of the seventies. The reasons for this are still unclear. A correlation exists with a sudden increase in nitrate concentrations, which in turn was negatively correlated with the salinity in the German Bight and hence with river water influence. Partial correlation between nanoplankton, salinity and nitrate , however, showed that the correlation was not due to nitrate but rather due to salinity resp. some other factor associated with river water.

The flagellates without the nanoplankton component, mainly consisting of larger dinoflagellates, did no longer show a clear trend. Large interannual variations in dinoflagellate biomass (about one order of magnitude) tend to mask potential smaller trends e.g. due to eutrophication. Those dinoflagellates grow mainly in summer and prefer vertically stratified water, where nutrient concentrations are reduced and motile flagellates have an advantage over diatoms. Such stratified water masses are regularly found within the convergence zone between the continental coastal and North Sea waters. This zone extends in the outer German Bight in a northwestern direction; its changing distance to the Helgoland monitoring station seems to be a major source of interannual variation in dinoflagellate populations measured there. Obviously, the hydrographical patterns such as the extension and duration of vertically stratified areas as influenced by weather and climate override the potential influence of eutrophication on phytoplankton increase in the German Bight.

This makes a direct prove of the (expected) positive effect of eutrophication on phytoplankton production and biomass in the German Bight difficult from the Helgoland data. Indirect evidence, however, indicates such influence in the outer German Bight, beyond Helgoland. This includes large areas of oxygen deficiency in the bottom waters, obviously related to sedimentation of large more frequent plankton blooms escaped to the regular observations at Helgoland.

The Kattegat region

In the autumn of 1984 Diarrhetic Shellfish Toxin (DST) suddenly became a threat to the growing Swedish mussel industry and harvest was prohibited during the whole winter due to high values of DST. This was not the first time Swedes became ill by eating blue mussels (*Mytilus edulis*), but the problem was relatively unknown and very little was known about the mechanisms behind the intoxication. This situation was not unique for Sweden and a similar pattern was observed in a number of countries in Europe during the 1990´s. Today we know that the *Dinophysis* species or *Prorocentrum lima* may contain DST and that DST-toxic mussels have become a more or less annual problem.

The apparent increase in DST-occurrence took place more or less at the same time as large dinoflagellate blooms and oxygen deficiencies in bottom water were recorded in many areas, e.g. The German Bight, The Kattegat and Scandinavian coastal waters. There is a general conviction among many scientists that the blooms and the oxygen problems were a result of eutrophication, which besides increased macro-nutrient inputs also involved an increased nitrogen to phosphorus (N/P) ratio in many areas. Laboratory experiments have shown that many dinoflagellates, which have a potential to become toxic, may increase their toxicity when grown under nutrient deficient conditions or under unbalanced nutrient conditions. In addition to the effects of eutrophication, changes in the micro-nutrient composition of the runoff in areas affected by acidification may also have influenced the

development of toxic *Dinophysis*. Thus, it can be concluded that the increase of DST in blue mussels during the 1990´s in European coastal waters followed by the recurrent closure of harvest of blue mussels may serve as an example of effects of antrophogenic inputs to the marine ecosystem.

The Oslofjord (based on a compilation of studies in the Oslofjord, Anon. 1996)

1. There are clear signs of eutrophic influences in the Outer Oslofjord both from regional and local sources. The degree of eutrophication is from low to moderate. Since the Outer Oslofjord is a relatively open system with good recipient capacity, the conditions are reasonable good and the actual situation is not acute. However, there is a need to clarify the dimensions of environmental effects further and to follow up the development in the area.
2. The central parts of the Outer Oslofjord is in open contact with the watermasses in the Skagerrak. The coastal watermasses in the inner Skagerrak and Outer Oslofjord shows signs of regional eutrophic influences mainly due to the large inputs of nutrients upstream from the southern North Sea and Kattegat. These influences are indicated by the high nutrient concentrations and distorted nutrient composition during spring and early summer, high concentrations of particulate organic matter in the upper layer, reduced oxygen concentrations in the watermasses during autumn, large input of organic material to the basins of the Outer Oslofjord and increased biomass of the bottom fauna.
3. Based on measurements carried out in the summer of 1995, the organic input and oxygen consumption in the basins of the Outer Oslofjord have been calculated to be twice as high as in similar fjord basins on the Norwegian Westcoast. There is still good capacity and the inputs to the basins of Breidangen and Rauøy must be increased by a factor of 2-3 before the average oxygen concentration in the basins at the end of the stagnation period reaches the lower limits for good oxygen conditions. For the Drøbak basin, that also directly receives inputs from the Inner Oslofjord, the capacity is smaller and the inputs can only be increased by 50% before the average oxygen concentrations in the basin periodically become less good.
4. In the inner parts of the Outer Oslofjord and in the area influenced by the Glomma river runoff, the Norwegian nutrient inputs affect the conditions in the brackish layer. In this area they contribute significantly to the total nutrient concentrations and lead to an increased algal growth and reduced water visibility. The effect is moderate with a calculated doubling in the plankton biomass and about 20% reduction in water visibility for the areas inside Fulehuk-Missingen. Due to the short residence time this biomass will be transported outside of the fjord and rapidly diluted so that it will not in significantly affect the deeper layers by sinking. The contribution of the Norwegian inputs to the open outer parts of the Outer Oslofjord is small.
5. The occurrence of phytoplankton in the Outer Oslofjord shows characteristic geographical patterns that reflects the local growth conditions. The largest occurrences are found inshore in the Østfold region where a potentially harmful species is commonly present. The level of phytoplankton production in the outer parts of the Outer Oslofjord seems not to be significantly higher than in unaffected areas on the Norwegian Westcoast.
6. There has been a reduction in the lower growth limits for macroalgae between 1950 and 1980. This reflects the reduced water visibility. The reason is probably a combination of regional eutrophication, the effect of local nutrient inputs and a changed particle transport by runoff from land.
7. Phosphorus (P) periodically seems to be the limiting nutrient for phytoplankton growth in the Outer Oslofjord. This is indicated by high N/P ratio in local inputs and far transported nutrients during spring and early summer and by increased C/P ratios in the particulate material in the outer parts of the Outer Oslofjord. A consequence of this is that a surplus of unused nitrogen can be exported from the Outer Oslofjord. The degree of phosphorus limitation seems to be low and the dimensions of such nitrogen surplus is not clear.
8. Reduction of the Norwegian nutrient input will lead to a reduction of the algal biomass and an improvement of the visibility conditions in the brackish layer of the Outer Oslofjord. Better visibility will result in better light conditions in the water column and a displacement of production to larger depths. This will partly counteract the reduced production in the upper layer and the total production will be reduced only to a limited degree.
9. Reduction of the far transported nutrient inputs from the large upstream sources will lead to an improvement of the situation in the coastal watermasses in the inner Skagerrak and Outer Oslofjord. This will, among other effects, result in a reduced organic load and improvement of the oxygen conditions in the adjacent basins. The situation is at present characterized by a nitrogen surplus during spring and early summer in the watermasses from the southern North Sea.
10. Although in the last years there have been some reductions in the input of nutrients according to the North Sea Agreement, the unbalance between nitrogen and phosphorus is expected to be maintained due to the fact that the reduction in nitrogen inputs has been small compared to the reduction in phosphorus.

The effect of the reductions in nitrogen from Norwegian inputs depends on the degree of phosphorus deficiency in relation to nitrogen. In the areas and periods where phosphorus is in clear deficiency the input of this element will primarily control the plankton production and biomass. Changes in the composition of the nutrient inputs with surplus of nitrogen and high N/P ratios can result in changes in the phytoplankton species composition. This can imply an increased risk for growth of potentially harmful species, although the relationship here is uncertain. In the evaluation of initiatives emphasis should be put towards attaining a balance with the natural composition of nutrients in the marine areas. This is valid both for long transported as well as Norwegian inputs.

The Dutch and Belgian coast

In a recent series of papers several aspects of the eutrophication status along the Dutch and Belgian coasts are described. These papers are the results of the EU-MAST Project NOWESP (North West European Shelf Programme) and will be published in the German Journal of Hydrography. The long term variation in nutrient and chlorophyll concentrations, the seasonal cycle of nutrients and chlorophyll and transport of water masses are the main topics of these papers (e.g. Bot *et al.,* Long term trends in the seasonal cycles of chlorophyll, zooplankton and nutrients on the North-West European Shelf; Laane *et al.,* Variability in the long-term advective nutrient fluxes (N, P, Si) to the North sea (1976-1995); Visser *et al.,* Time series analysis of monthly mean data in the NOWESP area, etc.). They show the importance of long term data sets for the analysis of long-term variation even on a decadal scale (cf. van Leussen *et al.*, 1996. ICES J. of Mar. Sci. 53: 926-932).

In conclusion:

Nutrient levels on a global scale have been increasing in many marine coastal waters. Examples include: in US coastal waters along the Northeast Atlantic coast, NO3 delivery increased 32% during 1974 - 1981; along the Gulf Coast and Pacific Northwest, PO4 loadings increased between 34 and 55% (Smith *et al.,* 1987). Within the Kattegat, between 1971 - 1982 winter (= pre-bloom) inorganic N and P levels increased by 2- and 1.2-fold, respectively (Andersson and Rydberg, 1988). PO4 concentrations increased 9-fold since 1940 in the Øresund (Lindahl and Hernroth, 1983).

In the North Sea near Helgoland, between 1962 - 1984 PO4 levels have approximately doubled and inorganic N levels trebled in watermasses influenced by Elbe River discharge (Berg and Radach, 1985). Changes in the ionic ratios of essential nutrients, such as in N:P, N:Si and Si:P, also accompany nutrient loading. For example, the long-term trend (1955 -1975) in the mean chemical composition of Rhine River water shows that the N:P and Si:P ratios decreased by about 6-fold over a 20-year period (van Bennekom and Salomons, 1981). The significance of such changing ratios is that riverine delivery of nutrients not only influences yield-dose responses. Redfield Ratio and resource-competition effects also accompany increased nutrification of rivers and discharge into coastal waters. Thispattern of long-term increases in nutrient loading has been accompanied by increases in phytoplankton biomass and primary production and occurrences of novel, unusual and/or toxic phytoplankton blooms. In each of the regions cited above, blooms of the dinoflagellate *Ceratium* have become common occurrences leading to anoxia and significant dieoffs of the affected benthic communities. This is but one example of the common and spreading coastal water syndrome that significant changes in phytoplankton biomass and productivity, novel species occurrences, exceptional or unusual blooms and phylogenetic shifts in predominance are occurring globally. Collectively, these events suggest that increased novel and harmful phytoplankton blooms in the sea are linked to, and a consequence of increased primary productivity accompanying long-term increases in coastal nutrient levels, and that this nutrification is a consequence of anthropogenic activities. In fact, a regional disequilibrium in phytoplankton community structure may have resulted (Smayda, 1989). These hypotheses need to be tested.

The results of the ASMO Modelling Workshop on Eutrophication Issues were only shortly discussed, because no participants of the workshop were among the members of the WG. The chairman explained the type of work done during the workshop, which was mainly towards presenting and comparing eutrophication models for different regions of the North Sea, including some smaller estuaries or embayments. The models have in common that they use nutrient input data and have a parameter related to phytoplankton as output (chlorophyll, rough species composition) and try to predict the effects of nutrient reductions on the phytoplankton biomass. The progress in these models is good and scenario calculations seem more and more realistic if compared to field data. Limitations of the models clearly are successional mechanisms and species interactions, because data to model such mechanisms are not existing. The WGPE recommends to continue these workshops in the future and

to install a mechanism of mutual exchange between the groups, so that the WGPE might help in supporting with useful information to the modeller groups.

f). evaluate, in consultation with WGHABD, technical details of measurement of phytoplankton growth processes, biomass estimates including counting cells and relations with nutrients;

This TOR is discussed under h). All relevant information is given below.

g) review the status of development of taxonomic coding systems with a view to recommending the adoption of a single coding system for use in ICES;

1. The WGPE supports the proposal that ICES should adopt a single standard taxonomic coding system and advise that the NODC system is the first choice for plankton studies.
2. The WGPE recommends that a comprehensive phytoplankton species checklist be prepared with species names and synonyms. It is recommended that a special group of taxonomists is formed to carry out this work. This workload is of such magnitude that ICES must provide funds to finance the work. The checklist should be updated at regular intervals by taxonomic experts.

The WGPE is aware of the existence of several regional phytoplankton checklists (Table and provisional list of relevant checklists). Some of these are old and need an update by taxonomic experts. As a second step the checklists should be merged to one, at least for larger areas. Each species should also have an area coding indicating all regions where it has been observed.

Table. Checklists of phytoplankton in the ICES area (incomplete).

AUTHORS	AREA	YEAR
Lange and Hasle	Skagerrak	1994
Throndsen, Heimdal, Hasle	Skagerrak	1972
Varela	Northwest Spain	?
Braarud, Gaarder, Gröntved	Northeast Atlantic	1953
Cleve-Euler	Öresund	1951-1955
Edler, Hällfors, Niemi	Baltic, Kattegat	1984
(Hargraves)	USA	?

Relevant available checklists or geographical taxonomic information:

Braarud, T., K.R. Gaarder & J. Groentved. 1963. The phytoplankton of the **North Sea** and adjacent waters in May 1948. Rapp. Proc. Verb. Reun. Cons. Perm. Explor. Mer 133: 1-87.

Chretiennot-Dinet, M-J., 1990. Atlas du Phytoplancton Marin, vol. 3. Chlorarachniophycees, Chlorophycees, Chrysophycees, Cryptophycees, Euglenophycees, Eustigmatophycees, Prasinophycees, Prymnesiophycees, Rhodophycees et Tribophycees. Editions du Centre National de la Recherche Scientifique, Paris, 261 pp. (**France Atlantic**)

Drebes, G., 1974. Marines Phytoplankton. G. Thieme Verlag Stutgart.(**North Sea**)

Drebes, G. and M. Elbrächter, 1976. Checklist of planktonic diatoms and dinoflagellates from Helgoland and List /(Sylt), German Bight. Botanica Marina 19; 75-83 (**North Sea, Wadden Sea**)

Dodge, J.D., 1982. Marine Dinoflagellates of the **British Isles**. Her Majesty's Stationery Office, London, 304 pp.

Elbrächter, M., in prep. (updated and extended Checklist of unicellular Algae of the Nordsylter **Wadden Sea** with about 75-80 more species than previous, planned)

Hartley, B., 1986. A check-list of the freshwater, brackish and marine diatoms of the **British Isles** and adjoining **coastal waters**. J. mar. biol. Ass. U.K., 66: 531-610.

Heimdal, B.R., Hasle, G.R. and Throndsen, J., 1973. An annotated Check-list of plankton algae from the **Oslofjord**, Norway (1951-1972). Norwegian Journal of Botany, 20: 13-19.

Hendey, N.I.,1974. A revised check-list of **British** marine diatoms. J. mar. biol. Ass. U.K., 54: 277-300.

Parke, M. and Dixon, P.S., 1976. Check-list of **British** marine algae-Third revision. J. mar. biol. Ass. U.K., 56: 527-594.

Ricard, M., 1987. Atlas du Phytoplancton Marin, vol. 2. Diatomophycees. Editions du Centre National de la Recherche Scientifique, Paris, 297 pp. (**France, Atlantic**)

Sournia, A., 1986. Atlas du Phytoplancton Marin, vol. 1. Cyanophycees, Dictyophycees, Dinophycees, Raphidophycees. Editions du Centre National de la Recherche Scientifique, Paris, 219 pp.(**France, Atlantic**)

Thomsen, H.A.(Ed.),1992. Plankton in the Danish coastal waters. Analysis of occurrence of algae and heterotrophs protist (excl. ciliates) in the **Kattegat**. Havforsking fra Miljøstyrelsen, nr. 11, Miljøministeriet. (In Danish).

Tomas, C.R. (Ed.) ,1993. Marine Phytoplankton. a Guide to Naked Flagellates and Coccolithophorides. Academic Press, London, 263 pp.(**Atlantic**)

Tomas, C.R.(Ed.) ,1996. Identifying Marine Diatoms and Dinoflagellates. Academic Press, London, 598 pp.(**Atlantic**)

TRIPOS ,1995. Biomonitoring van fytoplankton in de **Nederlandse** zoute en brakke wateren 1994. In opdracht van RIKZ, Rijkswaterstaat. Rappoirt 95003.1 (in Dutch, with annotated species list)

Lange, C., G.R. Hasle & E.E. Syvertsen. 1992. Seasonal cycle of diatoms in the **Skagerrak**, North Atlantic, with emphasis on the period 1980-1990. Sarsia 77:173-1876.

The WGPE suggests to take this information as a starting point and complete this list together with the WGHABD during the combined meeting in 1998. Once more attention was focused on the possible role of the European Taxonomic Institute in Amsterdam, which had started compiling taxonomic information on a CD-ROM with several groups of algae. Such initiatives need a follow-up to get a more complete set of taxonomic information.

h). prepare plans for a joint meeting with the WGHABD in 1998;

A joint meeting of the WGPE and the WGHABD has been suggested for several years by the WGPE, since the WGPE has felt that some of the terms of references has been more or less overlapping between the groups and that a faster progress within several subjects of common interest can be made by joint meetings. However, it is important that the co-operation shall benefit both groups, but also that the two groups continue as two individual groups with different areas of interests, responsibility and integrity.

A joint meeting in 1998 can be expected to host at least 30 participants, thus a rather large meeting room must be available, as well as a smaller one at the place of the venue. The length of the joint meeting is suggested to be at minimum one day and at maximum two days. Since there will be participants taking part in both groups, the separate meetings of the groups should not be held in parallel. The logical order is probably that the WGHABD will meet before the joint meeting and the WGPE after, however, this schedule has to be discussed further.

The following list of subjects are suggested from the WGPE to be discussed in a joint meeting with the WPHABD: (before finalising the report this list has been made available to the WGHABD for their meeting in France)

A. A practical check-list of all phytoplankton occurring in the entire ICES-area, with special empathise on toxic species or species known to cause harm. The complete check-list could eventually be divided into sub-areas in order to make it more accessible. (see our TOR g)

B. A discussion on a suggested mesocosm experiment carried out by the two groups together. The aim of such an experiment should be to investigate new approaches in phytoplankton ecology on the basis of organismal functioning of the planktonic system as well as evaluate technical details of measurements of phytoplankton growth processes, biomass estimates including counting of cells and relations with nutrients. Further subjects included in such an experiment could be: grazing, the development of the phytoplankton community due to different nutrient ratios (N:P:Si), regeneration processes of nutrients (new/regenerated production) and the effect of turbulence.

C. To identify and discuss methods for identification of phytoplankton by various techniques and with a special emphasis on new remote or automated techniques in relation to traditional microscopy. The need for a workshop organised by the two groups dealing with only identification techniques of phytoplankton could be also discussed.

D. To discuss and exemplify effects of antrophogenic inputs of nutrients including changed nutrient ratios over time on the phytoplankton community, with special empathise on phytoplankton bloom development and phytoplankton community changes.

E. To discuss future representation of and co-operation between the two groups and coming terms of reference.

F. To assess monitoring strategies of the pelagic ecosystem and their practical outcome in monitoring programmes within the ICES area.

Presupposed that the idea of a joint mesocosm experiment is taken up positively by the WGHABD at its meeting in April 1997, further plans on when and where an experiment can be carried out as well as the main subjects to be investigated can be made through correspondence.

i). advise ICES/OSPAR SGQAE on the development of quality assurance procedures for phytoplankton measurements adopted for JAMP (OSPAR 1997/2:1);

The WG considered the Report of the ICES/OSPAR SGQABM related to Eutrophication Effects Meeting, ICES CM 1997/Env:7, Ref.: E+L, February 1997. It was found that there is an inconsistency between the content of table 2 of Annex 3 and the more detailed Guidelines from the SIME Meeting in January 1996, concerning the variables chlorophyll and phytoplankton species composition as well as the frequency of measurements.

The inconsistency makes it difficult to develop quality assurance procedures. However, the WG recommends that SIME develops protocols of Quality Assurance for the variables. The protocols should include all necessary steps of the sampling and analytical method, in order to be able to trace possible errors in the analysis and guarantee a certain precision of the measurement.

The QA of phytoplankton analysis can be considerably enhanced by producing "complete" and "practical" checklists of species and lists of proper identification literature. Regular intercalibrations and training courses is an important part of keeping a high quality of the phytoplankton analysis. The WGPE was unable to give a more refined answer to these questions because they arrived the chairman just before the meeting.
The WGPE would like to refer to the sections on chlorophyll *a* measurements as discussed under TOR a) and the section on quality assurance of primary production measurements under TOR j) (below). During the ICES/HELCOM Workshop in Warnemünde last year several aspects of the QA of primary production measuremnts were discussed, which finally resulted in a set of guidelines (see Report).

j) review the quality assurance associated with primary production measurements carried out using a standardised method, including a standard incubator, and report to ACME before its June 1997 meeting.

The standardised incubator and the procedures to measure Pmax and P-I curve with the 14C technique are described in details by Colijn et al (in prep.) and Wetsteyn et al. (in prep.). The papers include results of intercomparisons, intercalibrations and test results describing the accuracy and precision f the method. A specific emphasis is made on the quality assurance procedures in determining the optimal light intensities in the incubator. Recommendations for sampling, incubation, 14C activity determinations, and TCO2 determinations are made. Also examples to calculate daily primary production with several equations are presented.

The papers are a good basis for the preparation of the in-house QA manuals by the various laboratories. A contribution to the topic of QA for primary production measurements using the 14C technique was made by the chairman during a meeting of the ICES/HELCOM workshop on Quality Assurance of pelagic biological measurements in the Baltic Sea (ICES CM 1997/E:5) in Warnemünde from 15-19 October 1996. A protocol including QA was developed during the meeting.

4 Any other business

A discussion was held on a possible venue next year. However, no decisions were taken because of the opportunity to have a combined meeting with the WGHABD. During the final preparation of the WG report, a message was obtained from the meeting of the WGHABD that they agreed to have a combined meeting next year, and that this meeting will be held in Lisbon in March. The final date has to be agreed upon but no problems are expected in the organisation of the meeting. On behalf of the members of the WG Lars Edler thanked the host institute and the chairman for the organisation of the meeting.

5 Action list for next year

The action list of next year contains the following points:

- prepare a more complete checklist of phytoplankton species for different parts of the ICES area;
- prepare an overview paper on the state of the art of new techniques available for the study of phytoplankton;

- support the co-conveners of the Mini-Symposium on Mesocosm Studies to understand eutrophication effects during the Annual Science Conference in Lisbon with suggestions for speakers and contributions;
- prepare or support the preparation of quality assurance procedures for phytoplankton measurements (chlorophyll a, species composition, primary production measurements);
- design a joint experiment with the WGHABD on the measurement of growth rate of natural phytoplankton communities under different nutrient regimes;

6 Recommendations referring to new TOR's

The **Working Group on Phytoplankton Ecology** (Chairman: Prof F.Colijn, Germany) will meet in Lisbon, Portugal from 24 - 28 March 1998 to:

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 or species known to cause harm;

b) propose a mesocosm experiment to investigate new approaches in phytoplankton ecology, in a joint meeting with the Working Group of the Harmful Effects Of Algal Blooms;

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

d) discuss and exemplify effects of antrophogenic inputs of nutrients including changed nutrient ratios over time on the phytoplankton community, with special empathsis 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 WGHAB, the results of the Workshop on development of *in situ* Growth Rate Measurements of dinoflagellates held in Kristineberg, 1996;

g) review, in a joint session with WGHAB, the status of taxonomic coding systems with a view to recommend the adoption of a single coding system for use in ICES.

Justification

a) There are several regional phytoplankton checklists (Table and provisional list of relevant checklists). Some of these are old and need an update by taxonomic experts. As a second step the checklists should be merged to one, at least for larger areas. Each species should also have an area coding indicating all regions where it has been observed. This work will be commenced inter-sessionally by a few taxonomic experts who have direct access to the taxonomic literature.

b) The aim of such an experiment should be to investigate new approaches in phytoplankton ecology on the basis of organismal functioning of the planktonic system as well as evaluate technical details of measurements of phytoplankton growth processes, biomass estimates including counting of cells and relations with nutrients. Further subjects included in such an experiment could be: grazing, the development of the phytoplankton community due to different nutrient ratios (N:P:Si), regeneration processes of nutrients (new/regenerated production) and the effect of turbulence. This presupposes that the idea of a joint mesocosm experiment is taken up positively by the WGHABD at its meeting in April 1997, further plans on when and where an experiment can be carried out as well as the main subjects to be investigated can be made through correspondance.

c) At its 1997 meeting, the WG discussed advances in methods to study phytoplankton biomass, production and growth rates, including techniques to identify groups of algae by flow-cytometry. Because of the pace of technological developments, the WG wishes to keep these advances under close scrutiny. The group will consider the need for a workshop organised by them and WGHABD dealing with introduction of such techniques for the improvement of *in situ* rate measurements and identification of phytoplankton species.

d) various events suggest that increased novel and harmful phytoplankton blooms in the sea are linked to, and a consequence of, increased primary productivity accompanying long-term increases in coastal nutrient levels, and that this nutrification is a consequence of anthropogenic activities. In fact changes in species composition may have resulted. These hypotheses need to be tested.

e) the various regulatory commissions are developing programmes for the monitoring of eutrophication effects on pelagic ecosystems. Such programmes have many inherent problems arising from poor understanding of the relevant spatial and temporal scales, and of weak understandings of the flux of nutrients through the pelagic web. However, currently, rapid advances in ecosystem modelling techniques, and in measurement capabilities, are helping to address these difficulties and will be reviewed by the Working Group.

f) The Kristineberg workshop which was designed and developed by the Working Group of HABD, was held in late 1996 in Kristineberg, Sweden. the final report will be produced in early spring of 1998 and evaluated together with the WGHAB

g) The WGPE and WGHABD recognizes the need to develop a single taxonomic coding system for phytoplankton. Nevertheless, the taxonomic expertise in the WGPE is not broad enough for the Group to address the question on its own. In order to draw on a broader expertise, it is therefore recommended that any decisions on adopting an existing system or developing a new one be carried out in association with the ICES Working Group on Marine Data Management, the Working Group on Phytoplankton Ecology and the WGHABD. It is recommended that the deliberations of the workshop on taxonomic nomenclature at the 8th International Conference on Harmful Algae, Vigo, Spain, June 1997, be taken into consideration

7 Adoption of the WG Report

Only part of sections of the report were available at the end of the meeting for inspection by the WG members. These were adopted by the meeting. Most other parts were available in a draft form on diskette and have later been compiled and edited by the chairman.

8 Closing of the meeting

The meeting was closed by the chairman at 13.00 hrs. on Wednesday 26 March 1997.

ANNEX 1: Agenda of the meeting

1. Opening of the meeting, announcements of the chairman, adoption of the agenda, appointment of rapporteur

2. Terms of reference

3. General discussion of terms of reference

4. Any other business

5. Action list for next year

6. Recommendations referring new TOR's

7. Adoption of the WG report

8. Closing of the meeting

ANNEX 2: List of participants

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ANNEX 3: References (in main text)

Andersen, P. and Kristensen, H.S. (1995). Rapid and precise identification and counting of thecate dinoflagellates using epifluorescense microscopy. In: Harmful marine algal blooms. Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. and Marcaillou-Le Baut, C. (Eds.), pp. 713-718. Intercept Ltd.

Anderson, D.M. (1995). Identification of harmful algal species using molecular probes: An emerging perspective. In: Harmful marine algal blooms. Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. and Marcaillou-Le Baut, C. (Eds.), pp. 3-14. Intercept Ltd.

Anderson, D.M. and Keafer, B.A. (1985). Dinoflagellate cyst dynamics in coastal and estuarine waters. In: Toxic Dinoflagellates. Anderson, D.M., White, A.W. and Baden D.G. (Eds.), pp. 219-224. Elsevier.

Andersson, L. and L. Rydberg (1988). Trends in nutrient and oxygen conditions within the Kattegat: effects of local nutrient supply. Est. Coast. Shelf Sci. 26:559-579.

Andersson, L. (1996). Trends in nutrient and oxygen concentrations in the Skagerrak-Kattegatt area. J. Sea. Res. 35, pp. 67-71.

Anon. (1996). Outer Oslofjord. Eutrophic situation, development and expected effects of reduced input of nutrients. Report from an Expert Group for evaluation of the eutrophic situation in fjords and coastal waters. (In Norwegian). Norwegian Institute of Water Research, 147 pp., Oslo, Norway.(ISBN 82-577-2945-0).

Arar, E.J. (1994) Evaluation of a new fluorometric technique that uses highly selective interference filters for measuring chlorophyll a in the presence of chlorophyll b and phaeopigments. Summary Report, Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, Ohio.

Barbini, R., Colao, F., Fantoni, R., Palucci, A., Ribezzo, S., Di Marco, G.Massacci, A. and Loreto, F. (1994). Photosynthetic activity and electron transport measurements using laser pump and probe technique. ENEA Report RT/INN/94/34, (Italy). (To be publ. In Reviews Physical measurements and Signatures in Remote Sensing).

Barbini, R., Colao, F., Fantoni, R., Guarini, R. Palucci, A., Ribezzo, S., Carlozzi, P., Torzilla, G., and Pelosi, E. (1995). Induced modifications on algae photosynthetic activity monitored by two laser technique. ENEA Report RT/INN/95/19, (Italy).

Berg, J. and G. Radach,(1985). Trends in nutrient and phytoplankton concentrations at Helgoland Reede (German Bight) since 1962. ICES C.M.1985/L:2.

Cullen, J.J., Ciotti, A.M., McLean, S.D. and Lewis, M.R. (1995). Detecting algal blooms with passive optical sensors. Abstract Proc. 7th Int. Conf. On Toxic Phytoplankton, Sendai, Japan, no. 143. Fraga, S. and Bakun, A. (1993). Global climate change and harmfulalgal blooms: The example of *Gymnodinium catenatum* on the Galician coast. In: Toxic Phytoplankton Blooms in the Sea.

Granéli. E., Carlsson, P., Olsson, P., Sundström, B., Granéli, W. and Lindahl, O. (1989). From anoxia to fish poisoning: The last ten years of phytoplankton blooms in Swedish marine waters. In: Novel Phytoplankton Blooms. Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (Eds.), pp. 407-428. Springer-Verlag.

Granéli, E., Paasche, E. and Maestrini, S.Y. (1993). Three years after the *Chrysochromulina polylepis* bloom in Scandinavian waters in 1988: Some conclusions of recent research and monitoring. In: Toxic Phytoplankton Blooms in the Sea. Smayda, T.J. and Shimizu, Y. (Eds.), pp. 23-32. Elsevier.

Hallegraeff, G.M., Bolch, C.J., Bryan, J. and Koerbin, B. (1990). Microalgal spores in ship´s ballast water: A danger to aquaculture. In: Toxic Marine Phytoplankton. Granéli, E., Sundström, B., Edler, L. and Anderson, D.M. (Eds.), pp.475-480. Elsevier.

Hickel, W., Eickoff, M. and Spindler, H. (1995). Langzeit-Untersuchungen von Nährstoffen und Phytoplankton in der Deutschen Bucht. Dt. Hydrgr. 7, suppl. 5, pp. 197-211.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W. and Strickland, J.D.H. (1965) Fluorometric determination of chlorophyll. J. Cons. Perm. Int. Explor. Mer, 30:3-15.

Jeffrey, S.W. and Humphrey, G.F.(1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytolankton. Biochem. Physiol. Pflanzen, 167 : 191-194.

Johansson, N. and Granéli, E. (1996). Effects of nutrient concentrations on the growth and toxicity of *Prymnesium parvum*. Abstract Book IX_{th} Int. IUPAC Symp. on Mycotoxins and Phycotoxins. Rome, Italy, p. 272.

Keafer, B.A. and Anderson, D.M. (1993). Use of remotely-sensed sea surface temperatures in studies of *Alexandrium tamarense* bloom dynamics. In: Toxic Phytoplankton Blooms in the Sea. Smayda, T.J. and Shimizu, Y. (Eds.), pp. 763-768. Elsevier.

Leppänen, J.-M., and Rantajärvi, E. (1995). Unattended recording of phytoplankton and supplemental parameters on board merchant ships - an alternative to the conventional algal monitoring programmes in the Baltic. In: Harmful marine algal blooms. Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. and Marcaillou-Le Baut, C. (Eds.), pp. 719-730. Intercept Ltd.

Lindahl, O. and L. Hernroth,(1983). Phyto-zooplankton community in coastal waters of western Sweden - An ecosystem off balance? Mar. Ecol. Prog. Ser. 10:119-126.

Lorenzen, C.J.(1967) Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol.Oceanogr.,12:343-346.

Rigby, G.R., Steverson, I.G., Bolch, C.J. and Hallegraeff, G.M. (1993). The transfer and treatment of shipping ballast waters to reduce the dispersal of toxic marine dinoflagellates. In: Toxic Phytoplankton Blooms in the Sea. Smayda, T.J. and Shimizu, Y. (Eds.), pp. 169-176. Elsevier.

Schöllhorn, E. and Granéli, E. (1993). Is the increase of flagellates in coastal waters caused by changes in ratios of N, P and Si? In: Toxic Phytoplankton Blooms in the Sea. Smayda, T.J. and Shimizu, Y. (Eds.), pp. 811-817. Elsevier.

Schreiber, U. (1986). Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer. Photosynthesis Research 9: 261-271

Schreiber, U., C. Neubauer & U. Schliwa (1993). PAM fluorometer based on medium-frequency pulsed Xe-flash measuring light: a highly sensitive new tool in basic and applied photosynthesis research. Photosynthesis Research 36: 65-72

Schreiber, U. (1994). New-Emitter-Detector-Cuvette assembly for measuring modulated chlorophyll fluorescence on highly diluted suspensions in conjunction with the standard PAM Fluorometer. Z. Naturforsch. 49c, 646-656

Smayda, T.J. (1997). Antagonistic phytoplankton blooms: some aspects of their population growth and community dynamics. Limnol. Oceanogr. (in review)

Smayda, T.J. (1990). Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Toxic Marine Phytoplankton. Granéli, E., Sundström, B., Edler, L. and Anderson, D.M. (Eds.), pp. 29-40. Elsevier.

Smayda, T.J. (1989). Primary production and the global epidemic of phytoplankton blooms in the sea: a linkage? In, E.M. Cosper, E.J. Carpenter & V.M. Bricelj (Editors): Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms. Lecture Notes on Coastal and Estuarine Studies. Springer-Verlag, Berlin.

Smith, R.A., R.B. Alexander and M.G.Wolman (1987). Water-quality trends in the national US rivers. Science 235:1607-1615.

Tester, P.A., Fowler, P.K. and Turner, J.T. (1989). Gulf Stream transport of the toxic red tide dinoflagellate *Ptychodiscus brevis* from Florida to North Carolina. In: Novel Phytoplankton Blooms. Cosper, E.M., Bricelj, V.M. and Carpenter, E.J. (Eds.), pp. 349-358. Spinger-Verlag.

Tester, P.A., Geesey, M.E. and Vukovich, F.M. (1993). *Gymnodinium breve* and global warming: What are the possibilities? In: Toxic Phytoplankton Blooms in the Sea. Smayda, T.J. and Shimizu, Y. (Eds.), pp. 67-72. Elsevier.

Turner, J.T., Borkman, D.G. and Pierce, R.W. (1995). Should red tide dinoflagellates be sampled using techniques for microzooplankton rather than phytoplankton? In: Harmful marine algal blooms. Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. and Marcaillou-Le Baut, C. (Eds.), pp. 737-742. Intercept Ltd.

van Bennekom, A.J. and W. Salomons,(1981). Pathway of nutrients and organic matter from land to ocean through rivers. pp. 33-51. In, River Inputs to Ocean Systems. UNEP/UNESCO, 1981.

Vrieling, E.G., Gieskes, W.W.C., Colijn, F., Hofstraat, J.W., Peperzak, L. and Veenhuis, M. (1993). Immunochemical identification of toxic marine algae: First results with *Prorocentrum micans* as a model organism. In: Toxic Phytoplankton Blooms in the Sea. Smayda, T.J. and Shimizu, Y. (Eds.), pp. 925-931. Elsevier.

Vrieling, E.G., Gieskes, W.W.C., Rademaker, T.W.M., Vriezekolk, G., Peperzak, L. and Veenhuis, M. (1995). Flow cytometric identification of the ichthyoytoxic dinoflagellate *Gyrodinium aureolum* in the central North Sea. In: Harmful marine algal blooms. Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P. and Marcaillou-Le Baut, C. (Eds.), pp.743-748. Intercept Ltd.

Welschmeyer, N.A.(1994)Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and phaeopigments. Limnol. Oceanogr., 39:1985-1992.

Yasumoto, T. (1990). Marine microorganisms toxins - An overview. In: Toxic Marine Phytoplankton. Granéli, E., Sundström, B., Edler, L. and Anderson, D.M. (Eds.), pp. 3-8. Elsevier.

Yentsch, C.S. (1989). Monitoring algal blooms, the use of Satellites and other remote sensing devices. in: Red tides. Okaichi, T., Anderson, D.M. and Nemoto, T. (Eds.), pp.181-184. Elsevier.

Zevenboom, W., Rademaker, M., Backus, J.E., Martin, J.B. and Orth, R.G. (1989). Airborne surveillance of surface algal blooms in the Dutch part of the North Sea, 1979-1989. Nota nr: NZ-N-89. 12, (The Netherlands).

ANNEX 4: Legends to Tables and Figures regarding TOR a)

(From: („Phytoplankton pigments in oceanography", Eds., S. W. Jeffrey, R. F. C. Mantoura and S. W. Wright, Monographs on oceanographic methodology nr. 10, UNESCO Publishing 1997, ISBN 92–3–103275–5).

Table I. Suitability of different filter types to collect phytoplankton pigments

Table II. Effect of temperature during storage on pigment recovery

Table III. Summary of different techniques for pigment analysis

Table IV. Different solvents for extraction and their characteristics

Table V. Pigment yield in dependence of extraction treatment

Table VI. Summary of different methods tested by SCOR WG 78

Figure 1. Summary of protocols for collection and analysis

Figure 2. Recovery of different pigments by HPLC

Figure 3. Comparison of chlorophyll *a* concentrations measured by HPLC and by spectrophoto- or fluorometric methods

Figure 4. As Fig.3 but for pheopigments, chlor. *b* and *c*

TABLE 10.1 Survey of filters used for sampling phytoplankton pigments in oceanography[a]

Filter type	Commercial suppliers	Filter usage (%)					Pore size (μm)	Advantages	Disadvantages
		HPLC	TLC	SP	SF	Total			
Glass-fibre									
GF/F	Whatman	42	0	2	3	47	0.7	High filtration capacity and rates. Insoluble in pigment-extracting solvents. GF/F retains >94% of picoplankton[b], *Synechococcus* sp.[c], *Prochlorococcus* sp.[d], and picoeukaryots[e]. Aids cell disruption. Less expensive than membrane filters. Compatible with HPLC, TLC and elemental (C, N, H) analyses.	Broad size cut-off. Unsuitable for size-fractionation.
GF/C	Whatman	12	8	9	6	35	1.2		
Other	Sartorius, Gelman, Schleicher and Schüll	2	0	3	1	6	0.7-5.0		
Total		56	8	14	10	88			
Membrane									
Cellulose esters	Millipore (HA), Sartorius	0	0	0	5	5	0.02-8.0	Available in wide range of narrow pore sizes (0.01–10 μm). Suitable for size-fractionation of phytobiomass and production[f]. Compatible with SP, SF, fluorometry and gravimetry[g].	Low filtration capacity and rates. Cellulose-ester filters dissolve in pigment-extracting solvents; therefore unsuitable for HPLC, TLC. Nuclepore polycarbonate filters release dyes that interfere with HPLC[h]. More expensive than glass-fibre filters.
Polyester	Nuclepore	4	0	0	0	4	0.4		
Other	Gelman, Anapore, Millipore	1	0	0	2	3	0.02-10.0		
Total		5	0	0	7	12			
Filter total		61	8	14	17	100			
$MgCO_3$ addition (%)		4	4	5	4	17		Buffers pH, improves particle retention[g].	Adsorbs Chlides and Phides[i]. Retards filtration.

a. Survey of 104 publications (1980–1993) in the refereed literature that used high performance liquid chromatography (HPLC) thin-layer chromatography (TLC) spectrophotometry (SP) and spectrofluorometry (SF).
b. Li *et al.* (1983), Phinney and Yentsch (1985), Taguchi and Laws (1988).
c. Guillard *et al.* (1985), Li (1986), Kana *et al.* (1988).
d. Goericke and Repeta (1992, 1993), Partensky *et al.* (1993).
e. Hooks *et al.* (1988), Vaulot *et al.* (1990).
f. Joint *et al.* (1993), Li *et al.* (1983).
g. Strickland and Parsons (1968).
h. Mantoura (unpublished results).
i. Daly *et al.* (1973).

TABLE 10.3 Pigment recoveries (%) from filtered microalgae stored for 328 days

Storage (Pretreatment)	−196°C (None)					−20°C (None)				
Microalga	*Emiliania*	*Dunaliella*	*Phaeodactylum*	*Phaeocystis*	Average[a]	*Emiliania*	*Dunaliella*	*Phaeodactylum*	*Phaeocystis*	Average[a]
Pigment										
Chlorophyll *a*	80	85	86	81	83	83	58	41	46	57
Chlorophyll *b*		104			104		83			83
Chlorophylls c_{1+2}	104		90	89	94	104		87	53	81
Chlorophyll c_3	118			91	105	104			87	96
Fucoxanthin			93	95	94			72	32	52
Diadinoxanthin	104		91	83	93	67		25	18	37
19'-hexanoyloxyfucoxanthin	111			99	105	114			44	79
Lutein		99			99		104			104
Violaxanthin		103			103		107			107
ß,ß–carotene	92	84	76	67	80		76	41	33	50
					96					75
Average[b]	102	95	87	86	93[c]	94	86	53	45	69[c]

Storage (Pretreatment)	−20°C (Snap–Freeze in Liquid Nitrogen)					+22°C (Freeze–Dry)				
Microalga	*Emiliania*	*Dunaliella*	*Phaeodactylum*	*Phaeocystis*	Average[a]	*Emiliania*	*Dunaliella*	*Phaeodactylum*	*Phaeocystis*	Average[a]
Pigment										
Chlorophyll *a*	67	59	50	38	54	25	43	19	12	25
Chlorophyll *b*		77			77		70			70
Chlorophylls c_{1+2}	81		85	46	71	33		41	11	28
Chlorophyll c_3	90			85	88	54			93	74
Fucoxanthin			71	34	53			39	17	28
Diadinoxanthin	56		29	18	34	6		30	12	16
19'-hexanoyloxyfucoxanthin	90			61	76	28			26	27
Lutein		95			95		35			35
Violaxanthin		95			95		13			13
ß,ß–carotene	50	70	41	17	45	0	1	2	0	1
					69					32
Average[b]	72	79	55	43	62[c]	24	32	26	24	27[c]

a. Average recovery of individual pigments from all algae.
b. Average recovery of all pigments from individual algae.
c. Global average of all pigments from all algae.

Table 17.1. Suitability of various techniques for pigment analysis in phytoplankton ecology (see also Chapter 4).

Technique	Data obtained	Analysis time[a] (min)	Volume required[b] (ml)	Advantages	Disadvantages
In vivo fluorometry	Approximate Chl *a* only	<1	15	Immediate data; sensitive.	Not quantitative; fluorescence signal depends not only on chlorophyll concentration but also on species composition, time of day, accessory pigments, physiological status, fluorescence quenching[c].
Extracted fluorometry	Accurate Chl *a*[d,e]	10	20	Very sensitive; accurate if no Chl *b* present; inexpensive	Interference from chlorophyll derivatives[d,e]
Spectrofluorometry	Accurate Chl *a*, *b*, *c*[d]	15	50	Very sensitive	Interference from chlorophyll derivatives; needs continual calibration[d]
Spectrophotometry	Accurate Chl *a*, *b*, *c*[d]	10	500	Accurate if no Chl derivatives present	Interference from chlorophyll derivatives[d]
Thin-layer chromatography	Chl *a*, *b*, *c*, carotenoids, degradation products	60	30000	2-dimensional TLC gives very good resolution; inexpensive; excellent for pigment purification.	Not suitable for routine analysis of oceanographic samples.
Isocratic HPLC	Accurate Chl *a*, *b*, *c*, some carotenoids, degradation products	25	1000[f]	Good for major chlorophylls[g]; simpler and faster than gradient HPLC; suitable for shipboard use.	Medium resolution permits analysis of simple samples only (e.g. cultures or chlorophylls only in field samples)
Gradient HPLC	Accurate Chl *a*, *b*, *c*, carotenoids, degradation products	40	1000[f]	Excellent resolution[h] and quantitation; very sensitive for chlorophylls and derivatives with fluorescence detection; suitable for shipboard use.	Expensive to set up; time-consuming for sample preparation, analysis, and data workup (not shown in column 3).

a. Time for analysis, including extraction time (excluding filtration time).
b. Approximate volume of oligotrophic seawater (0.1 µg Chl *a* l^{-1}) required for analysis.
c. Reviewed by Falkowski and Kiefer (1985).
d. Not accurate if samples contain Chl degradation products (see Lorenzen and Jeffrey, 1980; Appendix F and G; Chapter 14).
e. Not accurate if Chl *b* present (see Lorenzen and Jeffrey, 1980).
f. If using fluorescence detection of chlorophylls only, 200 ml can be used.
g. See Chapter 11.
h. Over 50 pigments can be resolved in a 30 min run (see Chapter 12).

TABLE 9.1. Physical characteristics and safety considerations for seven extraction solvents.

Solvent	MP (°C)	BP (°C)	FP (°C)	LD_{50}	Safety considerations
Acetone[a]	-94	56	-17	5.8	Extremely flammable; severe eye irritant; skin irritant.
Chloroform[a]	-63	61	–	0.91	Carcinogen; irritant; highly toxic; damage to liver, kidneys, heart; toxicity increased by alcohol; toxic decomposition products; usually contains HCl.
Dimethyl acetamide[a]	-20	165	70	4.9	Harmful; irritant; may cause birth defects; damage to liver; reacts with highly halogenated compounds.
Dimethyl formamide[a]	-61	153	57	2.8	Harmful; irritant; intolerance for ingested alcohol for up to 4 days after exposure; potent liver toxin; readily absorbed through skin.
Dimethyl sulfoxide[b][d]	18.6	189	88	>15	Irritant; readily absorbed through skin; enhances skin absorption of other toxins; not compatible with contact lenses; moderately flammable, produces toxic fumes.
Ethanol[b]	-130	78	13	13.7	Eye and respiratory irritant, mild skin irritant; highly flammable
Methanol[b][c]	-98	65	12	5-15	Highly flammable; highly toxic; vapours harmful to respiratory tract.

Abbreviations: MP: melting point, BP: boiling point, FP: flash point at atmospheric pressure, LD_{50}: lethal dose for 50% of cases (oral, rat, g/kg body weight).

Sources: (a) Lenga (1988) (b) Chemwatch (1993) (c) Weiss (1986) (d) De Renzo (1986)

TABLE 9.2. Preliminary screening extraction experiment. A. Relative pigment extraction. Figures as percentage of best value (summed for all pigments per species). B. Percentage (w/w) of major degradation products in each treatment.

	100% Acetone			90% Acetone			Methanol			Range
	Sonic	Grind	Soak/G	Sonic	Grind	Soak/G	Sonic	Grind	Soak/G	
A. Percentage extractibility (all pigments)										
Nannochloris atomus										
Fresh	15	68	85	19	61	46	99	87	89	15–99
Frozen	12	60	74	19	49	60	75	84	72	12–84
Tetraselmis suecica										
Fresh	64	81	84	65	60	75	98	65	79	60–98
Frozen	80	80	69	79	68	83	100	81	73	68–100
Phaeodactylum tricornutum										
Fresh	66	84	87	64	60	69	88	80	95	60–95
Frozen	71	71	72	64	67	75	84	84	84	71–84
B. Percentage degradation										
Chlorophyll *a* degradation (% of total chlorophyll *a*)										
Nannochloris atomus										
Fresh	0	1	2	0	1	45	5	3	13	0–45
Frozen	5	1	7	0	1	9	6	5	7	0–9
Tetraselmis suecica										
Fresh	1	2	1	2	1	1	6	2	4	1–6
Frozen	5	0	2	1	1	3	3	2	9	0–9
Phaeodactylum tricornutum										
Fresh	17	2	11	14	4	42	13	2	15	2–42
Frozen	12	21	6	18	21	29	8	22	4	4–29
Carotenoid isomers (% of total carotenoids)										
Tetraselmis suecica										
Fresh	56	42	40	39	47	25	21	29	22	21–56
Frozen	30	30	60	27	24	49	13	18	45	13–60

TABLE 14.2. Spectrophotometric, fluorometric and HPLC methods tested by SCOR WG 78

Method	Pigments analysed	Comments and recommended application by authors[b]
Spectrophotometric		
• Jeffrey and Humphrey (1975)	Chls *a*, *b*, $c_1 + c_2$	Designed for mixtures of pure chlorophyll pigments; no terms to correct for degradation products; recommended for use with algal cultures, 'natural phytoplankton' (Jeffrey and Humphrey, 1975) and for 'surface euphotic zone' (Lorenzen and Jeffrey, 1980).
• Lorenzen (1967)	Chl *a* and 'pheopigments'	Designed for chlorophyll *a* and the sum of pheophytin *a* and pheophorbide *a*; recommended for fresh, estuarine and coastal waters (Lorenzen, 1967).
• Porra *et al.* (1989)	Chls *a*, *b*	Designed for higher plants extracted with methanol; no terms for chl *c* or degradation products.
Fluorometric		
• Holm-Hansen *et al.* (1965)	Chl *a* and 'pheophytins'	Designed for chlorophyll *a* and pheophytin *a*; pheophorbide *a* is also detected. Chl *b* lowers chl *a* estimation and increases 'pheophytin' estimates. Recommended for surface to deep open ocean samples (Holm-Hansen *et al.* 1965).
HPLC		
• Mantoura and Llewellyn (1983)[a]	Chls *a*, *b*, chlides *a*, *b*, phytins *a*, *b*, *c*, phides *a*, *b*, pyro- and allomeric derivatives	Major and minor chlorophylls and derivatives separated (not chl c_1 from c_2, or DV Chl *a* from Chl *a*). Recommended for phytoplankton and detrital pigments in all natural waters.

a. The recommended Wright *et al.* (1991) HPLC system was not fully developed at the time of the SCOR WG 78 workshops.
b. Our recommendations may differ from those of the original authors as a result of the present tests (see section 14.5).

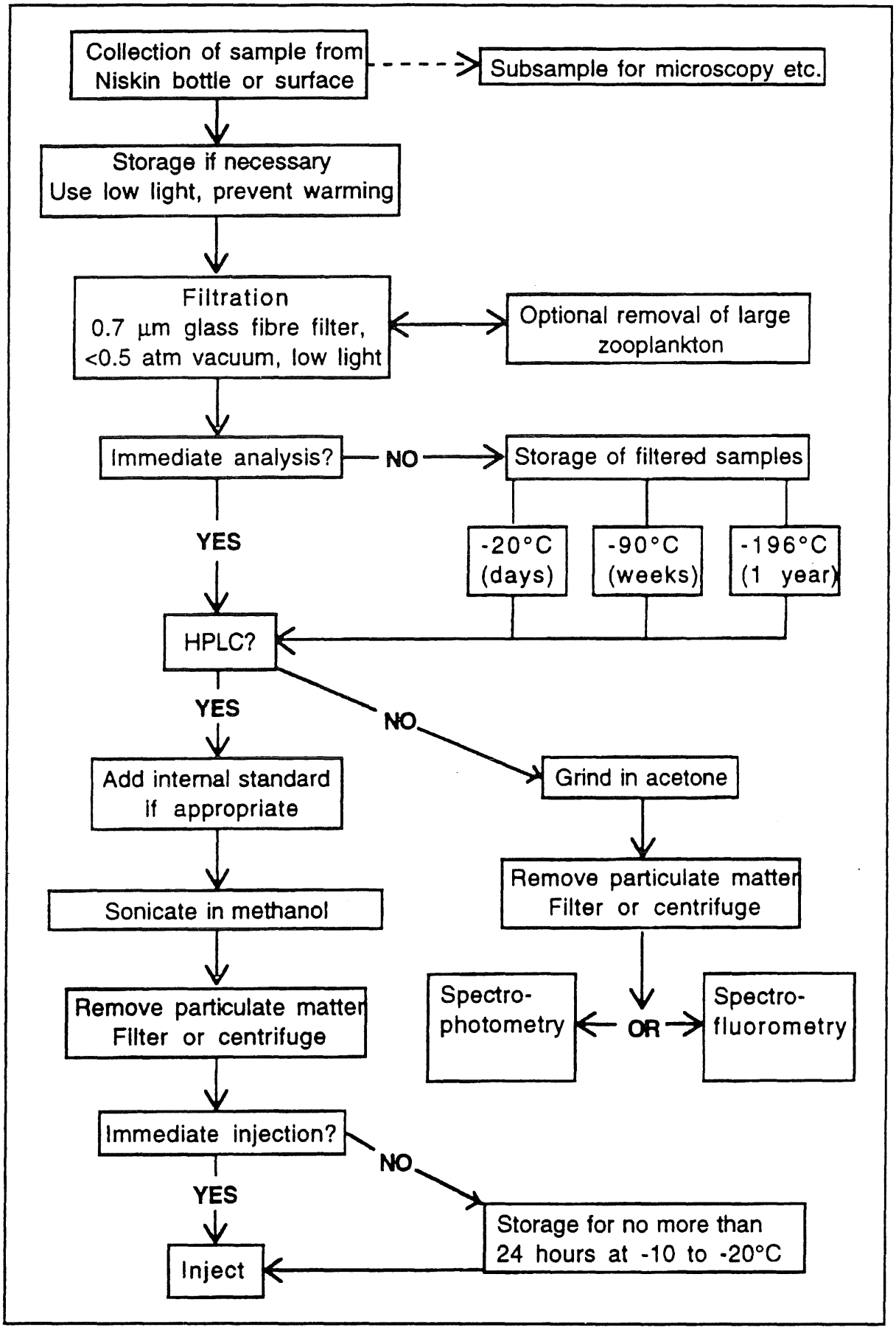

Figure 17.2
Summary of protocols for collection and analysis of phytoplankton pigments.

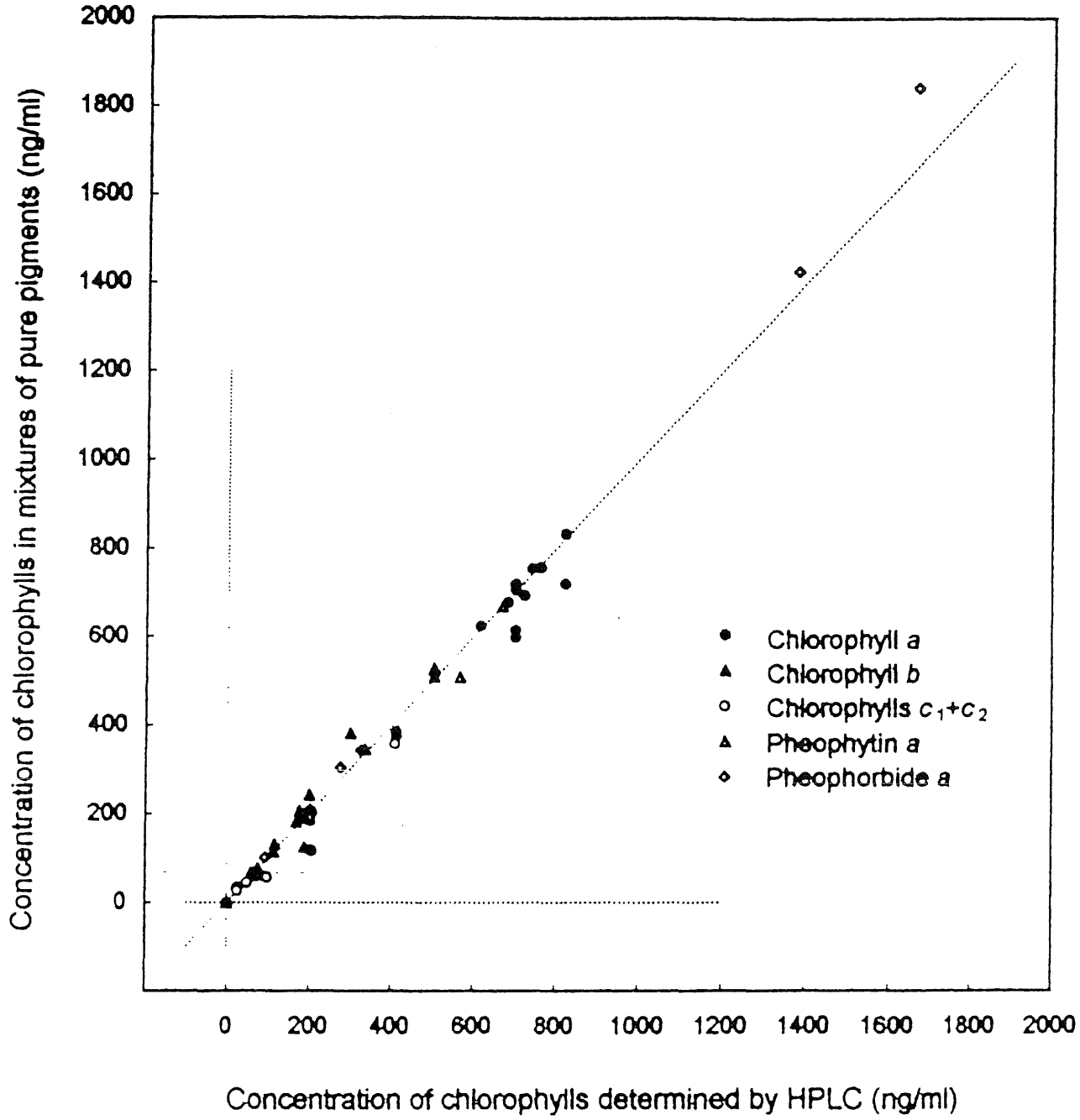

Figure 14.1
The concentrations of chlorophylls *a*, *b*, c_1+c_2, pheophytin *a* and pheophorbide *a* determined by HPLC compared to their actual concentration in the original mixture (r^2 = 0.998; slope = 1.019).

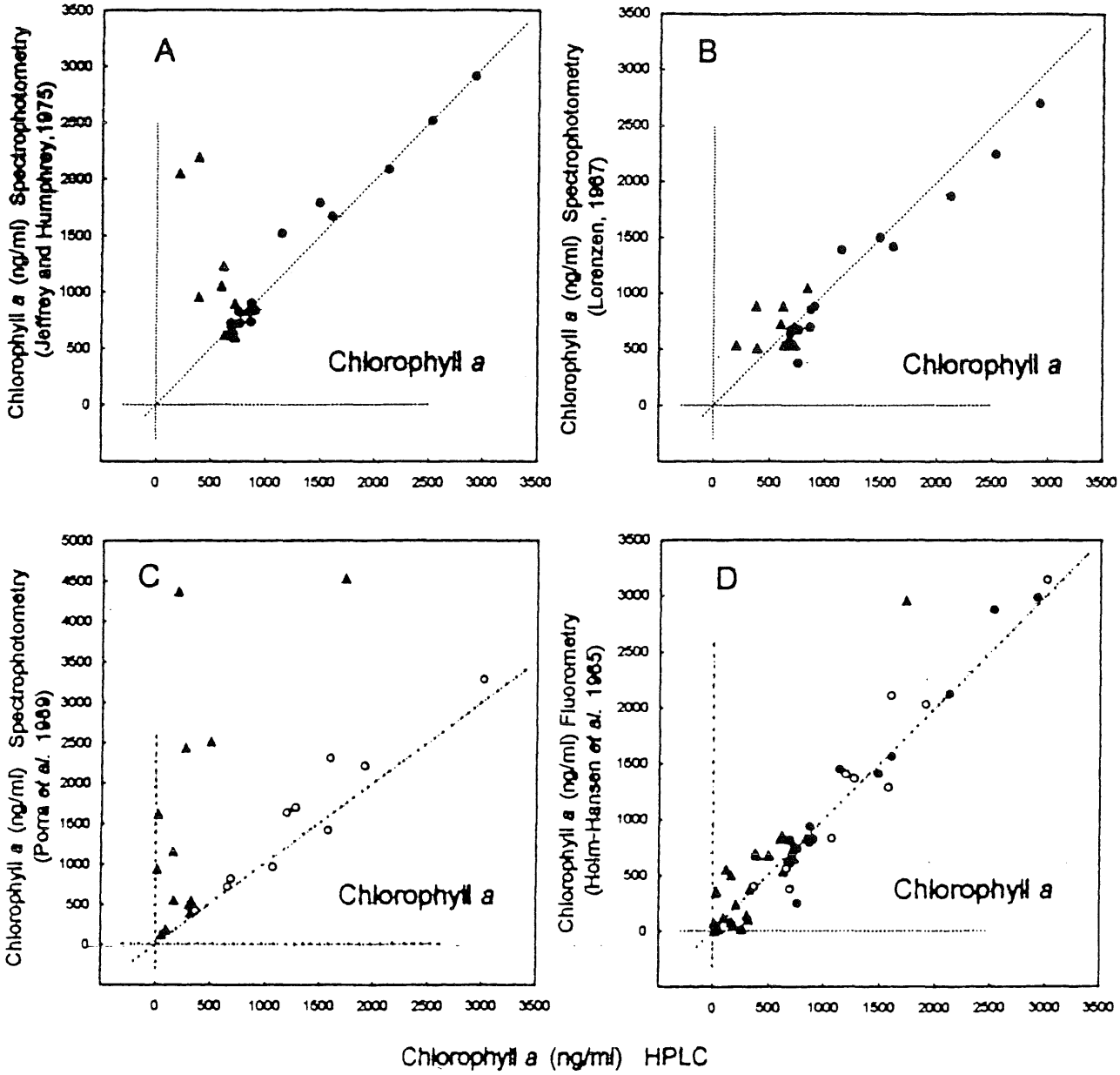

Figure 14.2
Comparisons of chlorophyll *a* concentrations determined by HPLC and the spectrophotometric methods of A, Jeffrey and Humphrey (1975); B, Lorenzen (1967); C, Porra *et al.* (1989); D, the fluorometric method of Holm-Hansen *et al.* (1965). Diagonal lines indicate a 1:1 agreement between methods. The data symbols correspond to: (▲) mixed standards of chlorophylls and degradation products prepared in acetone (sample number 1–14 see Table 14.4); (●) microalgal chlorophylls extracted in acetone (samples 15–30); (○) microalgal chlorophylls extracted in methanol (samples 31–40); (△) seawater, estuarine water, detrital and faecal samples extracted in methanol (samples 41–54).

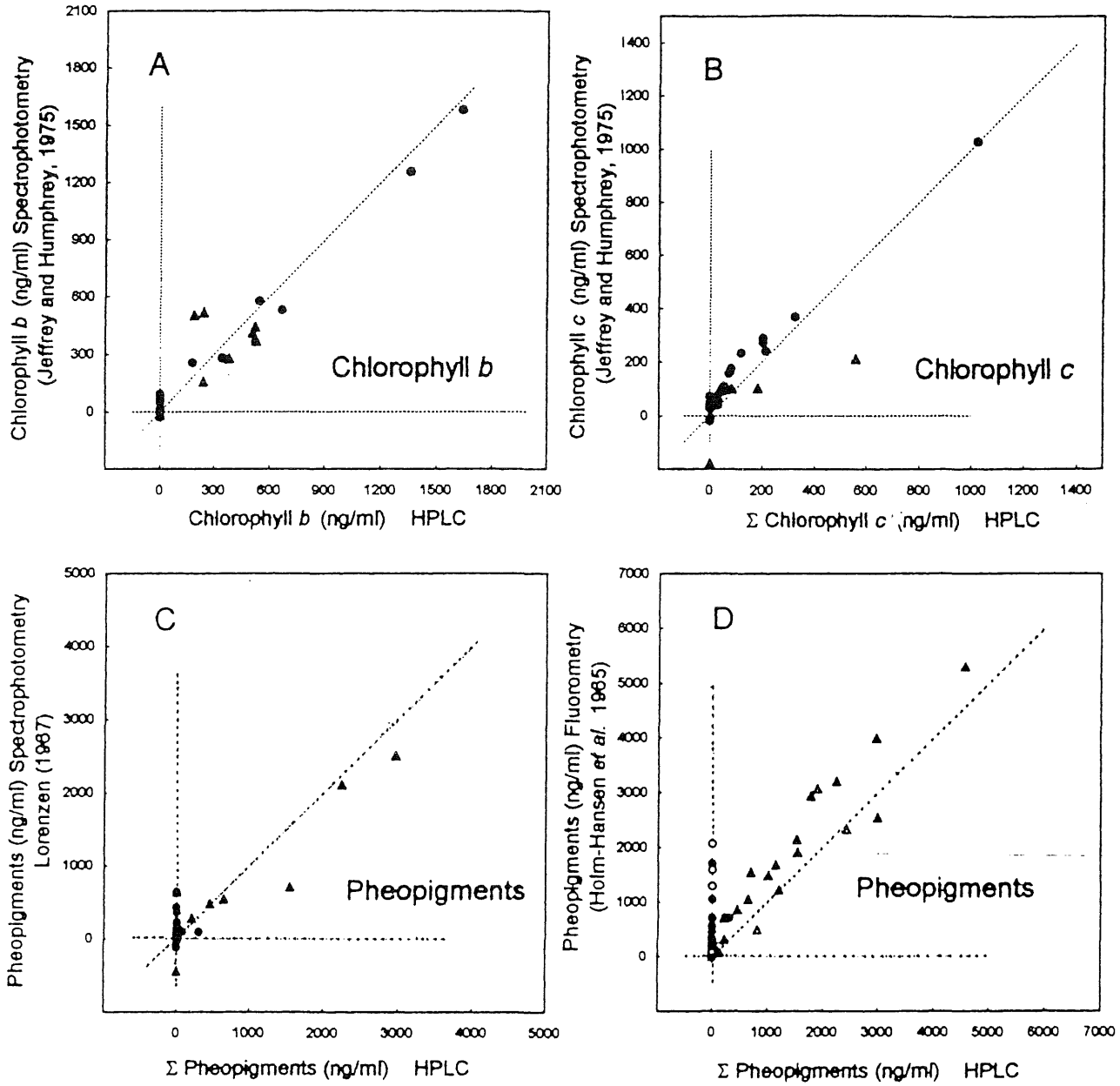

Figure 14.3
Comparisons are shown between chlorophyll *b* (A) and chlorophyll *c* (B) determined by HPLC and the spectrophotometric method of Jeffrey and Humphrey (1975) in mixed standards and microalgal extracts; and between pheopigments determined by HPLC and Lorenzen's (1967) spectrophotometric method (C) and Holm-Hansen's *et al.* (1965) fluorometric method (D). Data symbols correspond to those of Fig. 14.2.

ANNEX 5: Compilation of literature on effects of nutrients in mesocosms (selection)

Abreu, P.C., E. Granéli, C. Odebrecht, D. Kitzmann, L. A. Proença and C. Resgalla Jr., 1994. Effect of fish and mesozooplankton manipulation on phytoplankton community in the Patos Lagoon Estuary, Southern Brazil. Estuaries 17: 575-584.

Aksnes, D.L., T. Magnesen and U. Lie, 1985. Nutrient enrichment experiments in plastic cylinders and the implications of enhanced primary production in Lindaspollene, Western Norway. Sarsia 70: 45-58.

Bleijswijk, J.D.L. van,. E.S. Kempers, P. van der Wal, P. Westbroek, J.K. Egge & T. Lukk. 1994. Standing stocks of PIC, POC, PON and Emiliania huxleyi coccospheres and liths in seawater enclosures with different phosphate loadings. Sarsia 79: 307-318.

Carlsson, P. and E. Granola, 1993. Availability of humic bound nitrogen for coastal phytoplankton. Estuar. Coastal and Shellf Sci. 36: 433-447.

Carlsson, P., A. Z. Segatto and E. Granéli, 1993. Nitrogen bound to humic matter of terrestrial origin - a nitrogen pool for coastal phytoplankton?- Mar. Ecol. Progr, Ser., 97: 105-116.

Carlsson, P., E. Granéli, P. Tester and L. Boni, 1995 . Influences of river transported humic substances and copepod grazing on a coastal plankton community. Mar. Ecol. Prog. Ser. 127: 213-221.

Conte, M., A. Thompson & G. Eglinton. 1994. Primary production of lipid biomarker compounds by Emiliania huxleyii. results from an experimental mesocosm study in fjords of southwestern Norway. Sarsia 79: 319-3332.

Egge, J.K. and D.L. Aksnes, 1992. Silicate as regulating nutrient in phytoplankton competition. Marine Ecol. Progr. Ser. 83: 281-289

Eppley, R.W., P. Koeller, and T.W. Wallace Jr., 1978. Stirring influences the phytoplankton species composition wothin enclosed columns of coastal water. J. exp. mar. Biol. Ecol. 32: 219-239.

Escaravage, V., T.C. Prins, C. Nijdam, A.C. Smaal and J.C.H. Peeters, 1995. The response of phytoplankton communities to N-input reduction: results from mesocosm experiments. ICES C.M. 1995/T:10, 16 pp

Fagerbakke, K.M., M. Heldal, S. Norland, B.R. Heimdal & H. Baatvik. 1994. Emiliania huxleyii. Chemical composition and size of coccoliths fromenclosure experiments and a Norwegian fjord. Sarsia 79: 349-356.

Granéli, E., 1981. Experimental investigations of limiting nutrients for phytoplankton production in the brachish-water Öresund, SW Sweden. PhD Thesis, University of Lund, 207 pp.

Granéli, E., 1981. Bioassay experiments in the Falsterbo Channel.- Nutrients added daily.- Kieler Meeresforchung Sonderh. 5: 82-90.

Granéli, E. and K. Sundbäck, 1985. The response of planktonic and microbenthic algal assemblages to nutrient enrichment in shallow coastal waters, Southwest Sweden.- J. Exp. Mar. Biol. Ecol., 85: 253-268.

Granéli, E., W. Granéli and L. Rydberg, 1986. Nutrient limitation at the ecosystem and the phytoplankton community level in the Laholm Bay, South-East Kattegat.- Ophelia, 26: 181-194.

Granéli, E., P. Olsson, B. Sundström and L. Edler, 1989. In situ studies of the effects of humic acids on dinoflagellates and diatoms. In: T. Okaichi, D. M. Anderson and T. Nemoto (Eds.)- Red Tides - Biology, Environmental Science, and Toxicology. Elsevier Science Publishing Co., Inc., New York, 209-212.

Granéli,E., P. Carlsson, P. Olsson, B. Sundström, W. Granéli and O. Lindahl, 1989. From anoxia to fish poisoning: the last ten years of phytoplankton blooms in Swedish marine waters. In: E. M. Cosper, E.

Carpenter and M. Bricelj (Eds.). Novel phytoplankton blooms: Causes and impacts of recurrent brown tides and other unusual blooms. Lecture Notes on Coastal and Estuarine Studies, Springer Verlag, pp. 407-427.

Granéli, E., K. Wallström, U. Larsson, W. Granéli and R. Elmgren, 1990. Nutrient limitation of primary production in the Baltic Sea area. Ambio, 19: 142-151.

Granéli, E., P. Olsson, P. Carlsson, W. Granéli and C. Nylander, 1993. Weak „top-down" control of dinoflagellate growth in the coastal Skagerrak. J. Plankton Res. 15: 213-237.

Heiskanen, A.-S., T. Tamminen and K. Gundersen, 1996. Impact of planktonic food web structure on nutrient retention and loss from a late summer pelagic system in the coastal northern Baltic Sea.

Jak, R.G. and M.C.T. Scholten, 1993. Mesocosm experiments at TNO Den Helder: Indications for the development of eutrophication effects (in Dutch). BEON Reports 1993-4, 36 pp.

Kivi, K., H. Kuosa and S. Tanskanen, 1996. An experimental study on the role of crustacean and microprotozoan grazers in the planktonic food web. Mar. Ecol. Progr. Ser. 136: 59-68.

Kononen, K., E.O. Lahdes and L. Grönlund, 1993. Physiological and community responses of summer plankton to different manipulations in the Gulf of Finland (Baltic sea) with special reference to phosphorus. Sarsia 78: 243-253.

Kuosa, H., B. Norrman, K Kivi and F. Brandini, 1992. Seeding impact of Weddell Sea (Antarctica) sea ice microbial communities as studied in aquaria experiments. Polar biol. 12: 333-339.

Kuosa, H., R. Autio, R. Kuuppo, O. Setälä and S. Taskanen (in press). Nitrogen, silicon and zooplankton controlling the Baltic spring bloom. Nutrients and phytopalnkton. Est. Coast. Shelf. Sci.

Kuuppo-Leinikki, P.T., R. Autio, S. Hällfors, H. Kuosa, J. Kuparinen and R. Pajuniemi, 1994. Trophic interactions and carbon flow between picoplankton and protozoa in pelagial enclosures manipulated by nutrients and a top-predator. Mar. Ecol. Progr. Ser. 107: 89-102.

Lignell, R., S. Kaitala and H. Kuosa, 1992. Factors controlling phyto-and bacterioplankton in late spring on a salinity gradient in the northern Baltic. Mar. Ecol. Progr. Ser. 84: 121-131.

Nejstgaard, J.C. , H.J. Witte, P. van der Wal & A. Jacobsen. 1994. Copepod grazing during an Emiliania huxleyii bloom. Sarsia 79: 369-378.

Olsson, P., E. Granéli, P. Carlsson and P. Abreu, 1992. Structuring of a post-spring phytoplankton community by manipulation of trophic interactions. J. Exp. Mar. Biol. Ecol. 158: 249-266.

Oviatt, C., P. Doering, B. Nowicki, L. Reed, J. Cole and J. Frithsen, 1995. An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. Mar. Ecol. Progr., ser. 116: 171-179.

Peeters, J.C.H., A.C. Smaal, H.A. Haas and C.H.R. Heip, 1994. The impact of marine eutrophication on phytoplankton and benthic suspension feeders. Progress report II: results of mesocosm experiments with reduced N-load and increased grazing pressure, Report RIKZ 95.048, 174 pp.

Prins, T.C., V. Escaravage, A.C. Smaal and J.C.H. Peeters, 1995. The effects of top-down control on nutrient-phytoplankton relations results from mesocosm experiments. ICES CM 1995/T:9. 16 pp

Prins, T.C., V. Escaravage, A.C. Smaal and J.C.H. Peeters, 1995. Nutrient cycling and phytoplankton dynamics in relation to mussel grazing in a mesocosm experiment. Ophelia 41: 289-315.

Schöllhorn, E. and E. Granéli, 1993. Is the increase of flagellates in coastal waters caused by changes in the N and P to Si ratios? In: Toxic Phytoplankton Blooms in the Sea. T. J. Smayda and Y. Shimizu (eds.), Elsevier, Amsterdam, pp.811-817.

Schöllhorn, E. and E. Granéli, 1996. Influence of different nitrogen to silica ratios and artificial mixing on the structure of a summer phytoplankton community from the Swedish West Coast (Gullmar Fjord). Journal of Sea Research, 35: 159-167

Sommer, U., 1995. Eutrophication related changes in phytoplankton species composition: is there a role of nutrient competition? ICES CM 1995/T:7, 6 pp.

Smaal, A.C., J.C.H. Peeters, H.A. Haas and C.H.R. Heip, 1994. The impact of marine eutrophication on phytoplankton and benthic suspension feeders. Progress report I: results of mesocosm experiments with reduced P-load and increased grazing pressure. Report RIKZ 94.035, 161 pp.

Turner, J.T: and E. Granéli, 1992. Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms from the west coast of Sweden. J. Exp. Mar. Biol. Ecol. 157: 19-31.

Wal, P. van der, J.D.L. van Bleijswijk & J.K. Egge. 1994. Primary production and calcification rate in blooms of the coccolithophorid Emiliania huxleyii. Sarsia 79: 401-408.

Wal, P. van der, G.W. Kraay & J. van der Meer. 1994. Community structure and nutritional state of phytoplankton growing in mesocosms with different N:P ratios studied with high performance liquid chromatography. Sarsia 79: 409-416.