Oceanography Committee

ICES CM 1999/C:3
Ref.: ACME

REPORT OF THE

WORKING GROUP ON PHYTOPLANKTON ECOLOGY

Lowestoft, United Kingdom
14–17 April 1999

This report is not to be quoted without prior consultation with the General Secretary. The document is a report of an expert group under the auspices of the International Council for the Exploration of the Sea and does not necessarily represent the views of the Council.

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

TABLE OF CONTENTS

Section		Page
1	OPENING OF THE MEETING	1
2	TERMS OF REFERENCES, AGENDA AND AVAILABLE PAPERS	1
3	DISCUSSION OF TERMS OF REFERENCES	1
4	ANY OTHER BUSINESS	7
5	ACTION LIST FOR NEXT YEAR	7
6	CLOSE OF THE MEETING	8
ANNEX 1 – AGENDA OF THE MEETING		9
ANNEX 2 – TERMS OF REFERENCE		10
ANNEX 3 – LIST OF PARTICIPANTS		11
ANNEX 4 – A PROPOSAL FOR A STANDARD PROCEDURE FOR THE MEASUREMENT OF CHLOROPHYLL *A* IN SEAWATER SAMPLES TO BE USED IN THE ICES COMMUNITY.		12
ANNEX 5 – STATE OF THE ART OF SATURATION PULSE FLUORESCENCE TECHNIQUES		17
ANNEX 6 – ADVANCED PLANKTON MONITORING – UPDATING THE CONTINUOUS PLANKTON RECORDER		34
ANNEX 7 – NEW DEVELOPMENTS IN INTEGRATED ECOSYSTEM MONITORING – UPDATING THE CONTINUOUS PLANKTON RECORDER		44
ANNEX 8 – THE CONTEXT OF ENVIRONMENTAL CHANGE ON THE EUROPEAN SHELF		46
ANNEX 9 – BIOSENSORS FOR ENVIRONMENTAL MONITORING AND THEIR PARTICULAR RELEVANCE TO HARMFUL ALGAL–BLOOM DIRECTION		47

1 OPENING OF THE MEETING

The Chair Dr. Dave K. Mills opened the meeting at 9 am on the 14 April. He welcomed the participants to the Working Group Meeting, being held at CEFAS in Lowestoft, United Kingdom. The Chair started the meeting with a series of announcements, mainly regarding practical details.

Nine members attended the meeting (Annex 1). A few members were unable to attend and had informed the chair. There was no participation from Denmark, the Baltic countries, Poland, Russia, the Netherlands, Belgium, France, Ireland and Portugal and Canada. Efforts should still be given to try to involve more scientists in the work of the Working Group on Phytoplankton Ecology, as many interesting scientific and applied problems are discussed within the group (eutrophication, growth rates, food web structures, global change, operational monitoring strategies, global change etc.). Dr. Lars Edler was asked to be rapporteur.

2 TERMS OF REFERENCES, AGENDA AND AVAILABLE PAPERS

The Terms of Reference (Annex 2) were considered and the Chair presented the Agenda for the meeting (Annex 3).

The Chair informed that the following papers were available for discussions:

A proposal to a standard guideline for the measurement of chlorophyll *a* in seawater samples to be used in the ICES community. Francisco Rey (Annex 4)

State of the art of saturation pulse fluorescence techniques (PAM:Pulse amplitude modulated fluorescence, P&P: pump and probe fluorescence, FFR: Fast repetition rate fluorescence. Peter Hartig, Sebastian Lippemeier and Franciscus Colijn (Annex 5)

Advanced Plankton Monitoring – Updating the Continuous Plankton Recorder. D.K. Mills, A.W. Walne, S.I. Heaney, K. Embleton, M. B. Rawlinson and K. B. Cook (Annex 6)

Smart Moorings – The Development of Enabling Technology. D.K Mills, S. Malcolm and M. B. Rawlinson (Annex 7)

The context of environmental change on the European Shelf. Bob Dickson [Abstract] (Annex 8)

Biosensors for environmental monitoring and their particular relevance to harmful algal-bloom detection. Dr. Silke Kroeger, CEFAS (Annex 9).

3 DISCUSSION OF TERMS OF REFERENCES

ToR a Fluorescence techniques for measuring phytoplankton production and growth

Sebastian Lippemeier gave an overview of the use of variable fluorescence techniques for the measurements of phytoplankton production, growth and physiological state. These techniques include PAM (Pulse-amplitude-modulation-fluorescence), P&P (the pump and probe technique) and FRRF (the fast repetition rate fluorometer). These fluorescence methods offers a number of advantages compared to the traditional radiocarbon or oxygen incubation methods by being non-radioactive, non-invasive and without long incubation times. Possible applications of the methods are the assessment of nutrient status and in particular detection of nutrient limitation and the potential to measure gross primary production. His talk resulted in a lively discussion on the applications possible for these kind of measurements. (Annex 5)

ToR b Review knowledge on the growth of phytoplankton

Prof. Ted Smayda gave a review on the growth of phytoplankton, focusing on the difference between cellular, population and community growth.

Growth is in essence a transfer of energy. There has been much effort to determine rates of growth and the mechanisms by which growth occurs. This review attempts to give an overview of some of the issues.

The terms used to describe growth may be both relative and absolute. Different kinds of growth occur cellular, population and community and we employ different techniques to measure these.

Cellular growth is the basic growth unit and the outcome of coupled physiological processes under genetic (determining maximum growth rate) and multi-factorial control, particularly irradiance and nutrients. Other important factors are temperature and toxins. It can be measured in the lab but not in the field.

Population growth includes losses. It is the environmentally modified outcome of cellular growth; it is a recruitment term and is therefore the bloom unit. Although dependent on cellular growth the factors regulating population growth are not the same as for cellular growth. It is always less than cellular growth rate. The loss terms include advection, swimming, mortality (grazing) and sinking.

A phytoplankton community is an assemblage of multiple, concurrent blooms, equal to the total number of taxa present; each in different bloom cycle stages and each regulated by different combinations of growth factors. There is always a bloom of some type taking place. Different classes may exhibit different strategies. For example dinoflagellates have a swim strategy and diatoms have a sinking strategy. Dinoflagellates have a high compensation irradiance and diatoms a low one. At any one time it is only a certain part of the population that is actively growing.

ToR c Standard Chlorophyll-*a* technique

Mr. Francisco Rey presented his paper on chlorophyll methodology. The background to this ToR is the requirement of ICES to identify a standard method in order to have a more useful and comparable database on chlorophyll. This paper has already been discussed in the MCWG. The meeting was positive to the paper and only wanted to add some minor comments.

It is a requirement that a monitoring method such as this is practical and easy to conduct. At the same time it is important to be aware of health risks (extraction solvent) and costs. The meeting was of the opinion that other methods may be used, but in that case it has to be shown that the other method agrees with the standard method and that any discrepancies must be identified and justified. It is also of importance that suggestions for mandatory international inter-calibrations are included. The meeting propose to adopt this paper as a Standard Procedure (not a guideline) for the Measurement of Chlorophyll-*a* in the ICES area. (Annex 4)

ToR d Develop a proposal for a Mesocosm Experiment

The proposal written at the 1998 meeting was discussed. There is considerable interest among the group members to conduct the experiment. Good planning is absolutely necessary and it is important that there is a follow up with workshops afterwards.

Discussion on a suitable location took place. Of the Large Scale Marine Facilities, which are free of charge, Bergen appears to meet our needs best. Applications need to be prepared by September and submitted before December. Further details of mesocosm facilities at Bergen will be sought by Mills.

Specific questions raised by the working group included the nature of the light field and depth of the mesocosm, regarded as important where vertical structure in the water column is desirable.

Further planning will be carried out by Smayda and Mills to include identification of needs and the personnel. A maximum of 10 – 12 participants was suggested. Later we need to specify needs/people A possible date in autumn was identified in September 2000 for the experiment. At this time of year the dinoflagellate species *Ceratium* was believed to be an important component of the phytoplankton community in the Bergen region.

The working group agreed that Ted Smayda and Dave Mills should communicate by Email in order to continue the work of this ToR. In particular it was agreed that a full proposal for a mesocosm experiment be written dependant upon identification of appropriate mesocosm facilities and sufficient participants to carry out the work.

ToR e Review of the work of SGPHYT/IOC

The meeting was informed that the planned meeting of the Study Group on an ICES/IOC Checklist of Phytoplankton, under the Chairship of Prof. Ö. Moestrup did not take place in January 1999 as planned. Prof. F. Colijn had discussed

the situation with Prof. Moestrup and they suggest that the work should be done by E-mail in smaller groups covering different taxonomic groups of algae. The job of the groups would be to revise current lists or to set up new ones.

The working group discussed the situation. It was agreed that the initial work was actually not taxonomic but more one of compilation and that only in a later stage will there be a need for taxonomic control of the list. It was suggested that Dr. Lars Edler should take the lead in the initial step and that the EU project on benthos should be consulted. The WG identified the importance of a clearly formulated criteria for inclusion of a species. A first proposal for criteria will be formulated by Lars Edler and circulated for comments.

ToR f Prepare the case for a joint meeting with WGZE

The WG considered a range of potential topics for discussion with WGZE. The meeting agreed that the following topics are of particular interest for a joint meeting with WGZE.

- the role of phytoplankton-zooplankton interaction in determining the fate of carbon in nutrient enriched environments
- the limits to modelling phytoplankton - zooplankton interaction
- species - species interaction for example in terms of selective grazing
- can a collapse in grazing pressure lead to symptoms of eutrophication
- the role of physical forcing in determining the fate of phytoplankton carbon

The possibility of a joint meeting to be held in Bergen had previously been discussed by chairs of the two working groups. The members of the WGPE were agreeable to such a meeting which then depended on the outcome of the WGZE meeting to be held subsequently.

Tor g Propose tactics, activities and products in support of the Oceanography Committee Five-year Plan Objectives

In this section each relevant objective is presented together with background. The outcome of discussion by the working group is summarised for each objective in terms of general comments, tactics, activities and products (where identified). The working group had access to an earlier document which gave examples of tactics and activities for each objective. Some of these examples are included where the WG felt they were relevant and some amendments incorporated.

Objective o1:

Describe, understand and quantify the variability and state of the marine environment in terms of its biological, physical and chemical components.

Background

Ongoing knowledge of the environmental and ecosystem conditions in the ICES area is of fundamental importance to accomplishing the ICES mission. This objective will document relevant environmental and ecosystem conditions by monitoring important environmental and ecosystem parameters through time. This information will be used both in achieving an increased understanding of ecosystem processes and for initiating, evaluating and applying models forecasting ecosystem conditions. The capability to forecast the effect of natural environmental variability on the physical conditions within the ocean will require an understanding of how variability in the driving forces influences critical physical processes within the ocean. This objective seeks to develop a process based understanding of the connections between variability in climate and other driving forces with important marine physical processes.

General comments

We need to define the term "marine environment". Do we include coastal regions including for example the intertidal zone. With regard to "state" we also felt a need to better define the term – do we mean state of knowledge. The question arose as to the possibility of overlap with other programmes, e.g. production of regional QSR's and the possibility of duplication of effort. This also raises the question of overlap with regional conventions such as OSPAR/HELCOM. Problems are envisaged in terms of an increase in workload for the working groups. Not all ICES countries are represented in all Working Group and we envisage problems in achieving this objective in for all the ICES area. Points arising during discussion included the role of plankton as one of the most sensitive indicators of ecosystem change. Whilst there will be an important role for modelling in meeting the aims of this objective there is a need for appropriate

data sets for validation and testing. The lack of data at appropriate spatial and temporal resolution is a problem limiting the proper evaluation of model performance. A further point of clarification regarded as important by the working group was to better define scales of variability. Are we interested in all scales of variability e.g. from molecular to gyre circulation or is it more limited than this?

Tactics
- compilation of checklists
- regularly updated lists and evaluation of environmental sensor and sampling system
- reports on recurrent algal blooms
- provide support for maintenance of long term time series of plankton

Activities
- participate in GOOS and other relevant programmes e.g. GEOHAB/EUROHAB
- maintain long term time-series of plankton
- identify existing long-term monitoring programmes in the ICES area for hydrographic, chemical and biological variables to enable retrospective analysis
- identification of datasets for validation and testing of models

Products
- checklists
- sensor and sampling systems database
- recurrent algal bloom reports
- databases of long-term monitoring programmes
- technical papers on the "state of the art" techniques for monitoring

Objective o3:

To understand and quantify the impacts of climatic variability on the dynamics of marine ecosystems

Background
The capability to forecast the effect of natural environmental variability on ecosystem conditions will require an understanding of how climate variability influences critical ecosystem processes. This objective seeks to develop a process-based understanding of ecosystem processes as influenced by climatic forcing. This understanding will be the basis for developing management tools under Objective o6. The application of the knowledge gained through related objectives will, in many cases, require incorporating that knowledge into forecasting models. Determining what climatic and ecosystem characteristics can be forecast and the limitations and reliability of that forecasting ability is critical for the appropriate

General comments
The WG identified some specific activities related to the critical role of physical forcing in the light of recent work on the NAO and apparent increases in winter storms in the North Atlantic region and possible impacts on the timing of the spring bloom.

Tactics and Activities
- to consider the role of physical forcing in determining the timing and duration of the spring bloom
- to consider the role of physical forcing in determining the fate of production
- to consider the role of physical forcing in relation to phytoplankton – zooplankton interaction

Objective o4:

To understand and quantify the impact of human activities on marine ecosystems, in relation to natural variability

General comments
Although we did not discuss the question of whether it should stand alone rather than be merged with mh5 we did discuss the objective. A fundamental problem present in all questions of environmental change is identifying the cause and in particular distinguishing natural from anthropogenic causes.

Activities and Tactics
The WG pointed out the existence of related programmes of relevance to the aims of this objective including COMWEB which is concerned with increasing the fertility of the sea. Any activities investigating the effects of transport of contaminants on ecosystem dynamics should also consider transport via the food chain. Studies of nutrient effects should embrace other components of the plankton not just phytoplankton.

Examples of specific activities include

- to identify the effects of eutrophication on ecosystem carbon flow
- to study the effects of silicate on phytoplankton community structure
- to study the effect of nutrient ratios (e.g. N:Si; N:P) on community structure and sedimentation

Objective o6:

To promote the development of tools for the incorporation of environmental information into fisheries and ecosystem management.

Background
The impact of environmental variations on marine organisms is undeniable yet that knowledge is rarely incorporated into the interpretation of assessments or in the forecast of future abundance. Incorporating this knowledge into population assessments and management strategies represents one of the key elements in improving the understanding of long term sustainability.

Tactics and Activities

- to apply quantitative approaches of scientific method
- to evaluate the use of size structured models

Tor h Consider the case for establishing a database on phytoplankton and assess resource implications

The working group has for some time maintained an active interest in long term time-series with particular concerns over their protection and in the re-use of such datasets to address a range of questions concerning environmental change. The case for protection and maintenance within ICES has been strengthened lately especially with the Marine Science Symposium *The Temporal Variability of Plankton and Their Physico-Chemical Environment* in Kiel 1997. This meeting clearly identified the importance of such datasets and the need for their maintenance. Recent published work has for the first time identified changes in phytoplankton growth attributable climate change in the North Atlantic. This work was based on the long term time-series collected over several decades by the CPR and gives a clear example the value of long term time-series in detecting change and identifying cause.

There was sympathy in the WG to a suggestion of a further symposium possibly in 2002 to review new information derived from long term time-series. This Working Group is of the opinion that it is of crucial interest to continue existing series. It is, however, important to identify on-going long time monitoring series. In order to proceed it is suggested that we need to identify all long term time series of phytoplankton and that it may be beneficial to evaluate and give some sort of status to a particular time series (e.g. very important, important etc). Where a long term time-series of a particular status is under threat ICES may be able to participate in any moves to protect it. Also discussed was the suggestion to set up a global network of monitoring stations that continue the long time series that may contribute to regional and international time series.

The Working Group took note of the review of datasets on chemical, physical and biological variables that are available for the North Sea, taking place at the Research and Technology Center Westcoast of Kiel University in Büsum. This work is funded by the German government from a research and technology budget. The objective of this work is to evaluate the information and usefulness of longterm datasets. The results of this work will be summarised in a final report and will be accessible to ICES. It will provide a basis for assessing how assembly of databases should be done based on information derived from this review.

We noted the possible role of ICES in setting up databases for HELCOM and OSPAR and we also noted note that a Draft of Biological Reporting Format to ICES now has been presented and that it will be finalised during the summer 1999. Resource implications are that all the species names should probably be given to ICES so that they can be included in the system. As long as the checklist with proper names are not available the set-up of the database is questionable. It is important to recognise that setting up databases is only justified are when data is contributed. An

important potential use of such a database is as a source of data for detecting trends in ecosystem change by using appropriate analysis techniques and identifying cause of change; natural, climatic or anthropogenic.

In order to contribute to the task of identifying long term time series in the ICES region the Working Group decided that each member should prepare a list of known long time series and related variables and send to the Chair of the Group.

ToR i **Compile a small dataset of phytoplankton data, including relevant documentation, for analysis by WGSAEM**

The Working Group will explore the possibility of delivering a set of data including phytoplankton species composition collected in Dutch coastal waters to WGSAEM.

ToR j **Revise the report prepared for ACME in 1998 on the effects of anthropogenic nutrient inputs on the phytoplankton community**

The following text was prepared after discussion of this ToR by the working group.

The effects of anthropogenic nutrient input into selected ICES regions were summarised in the 1998 report of the WGPE. The common primary features of nutrification in these regions were to stimulate increased biomass and primary production. Other responses include enhanced growth of green algae (more characteristic of enclosed bays) and shifts in phytoplankton species composition. Secondary effects may also arise due to decay of organic matter leading to anoxia and mortality of benthos and fish.

This primary stimulation follows yield-nutrient dose principles and further reflects the common, well recognised situation that coastal marine ecosystems are nitrogen-limited. It is not possible to distinguish the threshold of elevated nutrients below which nutrification is a positive stimulus on ecosystem functioning, or above which the nutrient environment becomes degraded leading to negative ecosystem consequences. There is evidence that harmful algal blooms are associated with eutrophication, but a quantitative relationship has yet to be established, and the mechanisms and bloom-species selection identified, i.e. whether changes in nutrient ratios or bulk nutrient levels, for example, are responsible. Efforts to apply nutrient resources competition theory to the eutrophication – phytoplankton linkage have been compromised by the difficulty in sorting out the rate constants of remineralisation, nutrient delivery rates via riverine systems, from chronic build up effects and the consequences of nutrient storage in different trophic compartments.

There is a general correlation between reduced biotic diversity and phytoplankton communities and eutrophication. Often associated with such biotic shifts are blooms of unusual species or those which become dominant (*Phaeocystis pouchetii*) in the annual successional cycles.

Watershed management practices, agricultural use of fertiliser and domestic and industrial waste discharge contribute to the input of nutrients delivered into coastal systems. Efforts to quantify the exact levels of riverine nutrient delivery, into the ICES regions lie beyond the scope of the WGPE. Hydrologists, Chemists and coastal processes experts are more appropriately engaged in collating, analysing and establishing the nutrient budgets for representative rivers discharging into the coastal waters of the ICES region. This prodigious effort would not necessarily lead to improved quantification of the linkage between nutrient enrichment and the detected biomass, primary production, bloom species selection and biodiversity suggested by the regional data.

In view of the complexity of the issue and lack of consensus on the effects of anthropogenic nutrient input in coastal waters we propose to hold workshop or symposium to address the critical issues.

ToR k **Take note of the reports of SGQAB and SGQAE**

Lars Edler attended the SGQAB and SGQAE meeting and summarised their findings for the WG. Details are included here:

SGQAB: At the SGQAB meeting in February 1999

- a report from the HELCOM Environment Committee was presented. The most important item was that HELCOM now has accepted the new COMBINE Monitoring Programme and that the Manual for Combine is available at the HELCOM website: http://www.helcomfi/manual2/contents.htm/

- the Chlorophyll and Primary Production manuals were discussed
- the status of the ICES Biological Reporting Format was presented.

SGQAE: At the SGQAE meeting in February 1999

- reviewed relevant biological studies and related QA activities by countries and by discipline
- discussed criteria for evaluating the acceptability of data

The joint session between SGQAB and SGQAE presented areas of mutual interest, which include Manuals for Zooplankton, Primary Production and Chlorophyll.

4 ANY OTHER BUSINESS

A brief discussion of a possible Framework V proposal "Ferry Box" took place.

The Working Group on Phytoplankton Ecology had a lunchtime seminar presented by Dr. Bob Dickson, CEFAS, who gave a seminar on The context of environmental change on the European Shelf (Annex 8).

Dr. Silke Kroeger, CEFAS, also gave a seminar on Biosensors for environmental monitoring and their particular relevance to harmful algal-bloom detection (Annex 9).

5 ACTION LIST FOR NEXT YEAR

- To develop a full proposal for a joint mesocosm experiment dependant on identification of appropriate mesocosm facilities, (David Mills, Ted Smayda)
- To consider a report of the SGPHYT/IOC
- To organise a joint meeting with the Working Group on Zooplankton Ecology in 2000 in Bergen with 1 or 2 days overlap; alternatively to organise an independent meeting at the University of Rhode Island if WGZE have other plans.
- To prepare a case for a joint meeting with the Working Group on Shelf Seas Oceanography in 2001
- Members of the Working Group to prepare a list of long term time-series of plankton and associated environmental variables.
- To take note of reports of SGQAB/SGQAE
- To draft a proposal on criteria for inclusion of species in the Phytoplankton checklist and to circulate it for comments (Lars Edler).

5 RECOMMENDATIONS OF THE WORKING GROUP OF PHYTOPLANKTON ECOLOGY

The Working Group on Phytoplankton Ecology recommends that:

ICES identifies long term series of plankton and initiates the development of a database on long time series in the ICES countries on plankton and associated environmental variables.

In view of a lack of consensus on the effects of anthropogenic nutrient input on planktonic ecosystem change in response to anthropogenic forcing to hold a symposium/workshop in spring 2001.

Justification:
The importance of long term time-series for detection of trends and in identifying causes of environmental change has been established. Plankton are one of the most sensitive indicators of ecosystem change and therefore information on the source, content and availability of such data sets will aid the oceanography committee in meeting its objectives as well as serving the wider interests of ICES. Work in progress in Germany and potentially available ICES will assist with the process of identifying and evaluating datasets.

The lack of consensus on the response of the ecosystem to anthropogenic nutrient enrichment probably reflects the complex nature of the problem. Much work is being carried out in the ICES region on the effects of nutrient enrichment in the field and in the laboratory. A symposium or workshop to bring together workers in this field would be timely and provide ICES with an effective means of gauging scientific opinion on the nutrient status and ecosystem response to anthropogenic nutrient input within the ICES area.

The Working Group on Phytoplankton Ecology [WGPE] (Chair Dr. D.K. Mills) will meet (April/May 2000) either in Bergen, if a joint meeting with WGZE is possible, or at the University of Rode Island USA if not, to:

- To develop a full proposal for a joint mesocosm experiment dependant on identification of appropriate mesocosm facilities
- To consider a report of the SGPHYT/IOC
- To organise a joint meeting with the Working Group on Zooplankton Ecology in 2000 in Bergen with 1 or 2 days overlap; alternatively to organise an independent meeting at the University of Rhode Island if WGZE have other plans.
- To prepare a case for a joint meeting with the Working Group on Shelf Seas Oceanography in 2001
- Members of the Working Group to prepare a list of long term time-series of plankton and associated environmental variables.
- To take note of reports of SGQAB/SGQAE
- To draft a proposal on criteria for inclusion of species in the Phytoplankton checklist and to circulate it for comments.

6 CLOSE OF THE MEETING

The meeting of the Working Group on Phytoplankton Ecology closed at 1730 hours on 16April 1999.

ANNEX 1

AGENDA OF THE MEETING

1. Opening of the meeting
2. Terms of reference
3. Any other business
4. Action list for next year
5. Recommendations referring to new TOR's
6. Adoption of the WG report
7. Closing of the meeting

ANNEX 2

TERMS OF REFERENCE

2:11 The **Working Group on Phytoplankton Ecology** [WGPE] (Chair: Dr D Mills, UK) will meet in Lowestoft, UK from 13-17 April 1999 to:

a) assess the state of the art on the use of flourescence techniques for measuring phytoplankton production and growth;

b) review knowledge on the growth of phytoplankton;

c) finalise the discussion on a standard chlorophyll *a* technique for use within ICES programmes, and prepare a report for ACME, in collaboration with MCWG, on the quality assurance of chlorophyll *a*;

d) develop a proposal for a mesocosm experiment;

e) review the work of SGPHYT/IOC;

f) prepare the case for a joint meeting with WZE;

g) propose tactics, activities and products in support of the Oceanography Committee Five-year Plan Objectives;

h) consider the case for establishing a database on phytoplankton, and assess resource implications;

i) compile a small data set of phytoplankton data, including relevant documentation, for analysis by WGSAEM;

j) revise the report prepared for ACME in 1998 on the effects of anthropogenic nutrient inputs on the phytoplankton community;

k) take note of the reports of SGQAB and SGQAE.

WGPE will report to ACME before its May/June 1999 meeting and to the Oceanography Committee at the 1999 Annual Science Conference.

ANNEX 3

LIST OF PARTICIPANTS

NAME	ADDRESS	TELEPHONE	FAX	EMAIL
David Mills	CEFAS Lowestoft Lab Pakefield Road Lowestoft NR33 OHT United Kingdom.	(01502) 524253	+ 44 (0) 1502 513865	d.mills@cefas.co.uk
Lars Edler	SMHI, Ocean Lab. Doktorsg. 9D S-262 52 Angelholm Sweden.	+46 431 80854	+46 431 83167	lars.edler@smhi.se
Kristinn Gudmundsson	Marine Research Institute Skulagata 4 P.O. Box 1390 121 Reykjavik Iceland.	+354 5520 240	+354 5623 790	kristinn@hafro.is
Silke Kroeger	CEFAS Lowestoft Lab Pakefield Road Lowestoft NR33 OHT United Kingdom.	(01502) 524425	+44 (0) 1502 524546	s.kroeger@cefas.co.uk
Francisco Rey	Institute of Marine Research P.O. Box 1870 Nordnes N-5817 Bergen Norway	+47 55 23 8499	+47 55 23 8584	francisco.rey@imr.no
Sebastian Lippemeier (on behalf of F. Colijn)	Research and Technology Center Westcoast Hafentörn 25761 Büsum Germany	+49 (0)4834 604 209	49 (0)4834 604 299	Lippem@FTz-west.uni-kiel.de
Juha-Markku Leppänen	Finnish Institute of Marine Research and Technology P.O. Box 33 FIN-00931 Finland	+358 961 3941	+358 961 3944	jukkis@fimr.fi
Ted Smayda	Graduate School of Oceanography, University of Rhode Island Kingston, RI USA 02881	1 - 401 874 -6171	1 - 401 - 6682	Asmayda@gsosun1. gso.uri.edu
Harri Kousa	Finnish Institute of Marine Research and Technology P.O. Box 33 FIN-00931 Helsinki	+358 9 613 941	+358 9 613 94 494	arnold@fimr.fi
Antonio Bode	Instituto Espanol de Oceanografia (IEO) Centro Oceanografico de A Coruna Muelle de Animas s/n Apdo 130 E-15080 A Coruna Spain	+34 (9) 81 205 362	+34 (9) 81 229 077	antonio.bode@co.ieo.es

ANNEX 4

A PROPOSAL FOR A STANDARD PROCEDURE FOR THE MEASUREMENT OF CHLOROPHYLL *A* IN SEAWATER SAMPLES TO BE USED IN THE ICES COMMUNITY.

1. Introduction

The need of a standard method for the measurement of chlorophyll *a* in seawater to be applied to the different routine field programs within the ICES community led ICES to ask their Working Groups on Phytoplankton Ecology (WGPE) and Marine Chemistry (MCWG) to carry out a review of the actual state of the methodology with the aim of proposing a standard method. During the last two years both groups have discussed the different approaches being in use today and agreed upon the proposal presented here. Both groups have strongly based their discussion around a newly published reference work on the matter (Jeffrey *et al*, 1997) This publication should be checked for further details and recommendations.

A previous version of the present document was prepared ahead of the meetings of both working groups in 1999 and then thoroughly discussed and improved during these meetings. This work was led by Alain Aminot (MCWG) and Francisco Rey (WGPE). As such, the present document represents the consensus between the two groups on what is possible to agree upon at this time. Presently it is not possible or desirable to recommend a single method as a standard for the measurement of chlorophyll *a* in seawater samples. Instead, a standard procedure incorporating three spectroscopic analytical methods is proposed. Apart of these analytical alternatives , all other steps in the procedure are similar.

2. General considerations

Chlorophyll *a* is the principal pigment of plants and algae. This document will focus on its determination, as a biomass marker for phytoplankton, in discrete samples, for routine analysis and monitoring. Consequently, only non-separative procedures will be described here, since they are the most convenient for the purpose. Chlorophyll *a* is contained in the cells, i.e. in the particulate matter, and therefore it must be extracted in order to be measured after the cells have been collected by filtration. The measurement itself relies on the spectroscopic characteristics of chlorophyll *a*: light absorption or fluorescence. As the three identified types of chlorophyll (named *a*, *b*, *c*) and degradations products (mainly pheopigments) are often present and interfere, various methods have been produced for correction of these interference's. The so-called spectrophotometric tri-chromatic equations are used to measure the three main types of chlorophyll, while the acidification methods (spectrophotometric or fluorometric) allows correction for the presence of pheopigments. It must be noted that a lesser sample volume is usually required when measurements are done by fluorometry rather than by spectrophotometry. However, modern spectrophotometers with a resolution ten times better than that of the old generation (0.0001 AU) enable analysing volumes comparable to those used with fluorometry.

General precautionary note

> *As pigments are photo- and heat-sensitive, care to protect them from direct sunlight and from warming must be taken at any step of the procedure.*

3. Sampling

Seawater samples can be collected using non-toxic water samplers or pumps. Sub-samples for the chlorophyll analyses are collected in opaque plastic bottles of known volume, protected from warmth and light.

The sampling volume will depend upon the spectroscopic method chosen for the measurement. For spectrophotometric measurements at least one litre samples are generally needed. For fluorometry about 0.25 litre samples are suitable for most purposes. In turbid coastal regions filtration of one sample may take long time. In such cases it is preferable to use smaller volumes on different filters and then extract the filters together.

Precautionary notes:

- *Rinse the sample bottle with the sample before sub-sampling.*
- *The sampling volume of the opaque bottles should be checked before use. Mark each sample bottle to the desired volume. Another common procedure is to random select a number of bottles from the batch routinely used for collecting the samples and carefully measure their whole volume. The volume of seawater used for the*

measurements is then expressed as the measured average volume of the sampling bottles with the corresponding standard deviation.

4. Filtration

The seawater samples are gently mixed and filtered after collection (not more than one hour's delay) through glassfiber filters (GF/F type, 25 or 47 mm diameter) under subdued light and at a residual pressure of 0.7 bar (maximum vacuum of 0.3 bar). The last part of the water sample is gently sucked through the filter. Magnesium carbonate should not be used as a filter aid.

Any visible zooplankton is carefully removed from the filter with forceps. The filter is then removed, folded once, with the algae inside, blotted with absorbent paper to remove most of the water and placed in a proper labelled clean container. If the extraction is not to be carried out right after filtration the filters should be immediately frozen at least -20 °C.

***Precautionary notes*:**

- *All handling of the filters should be done using forceps.*
- *If the samples are not filtered immediately after collection they can be kept for a few hours in the dark and cold in a refrigerator or an ice bath.*
- *The filtration time should be kept as short as possible. Clogging of the filters should be avoided.*
- *Pre-filtration of the samples for removal of large zooplankters is not recommended without knowing the phytoplankton species composition of the samples, since large phytoplankton cells or chain-forming species could also be removed.*

5. Storage of the filters

Storage time should be kept as short as possible. Filters frozen at -20 °C can be kept for up to a 3-4 weeks period without significant decrease in chlorophyll *a*. For longer periods, colder temperatures (-70 ° C) should be used.

***Precautionary notes*:**

- *Every laboratory should check the freezing conditions by randomly running from time to time, within their normal runs, a few duplicate samples against unstored samples.*

6. Pigment extraction

Extraction is carried out by grinding the filters in a few millilitre of 90% acetone in a glass homogeniser with a motor-driven Teflon pestle for 1 minute in an ice-bath and under subdued light. After grinding the extract is carefully transferred to a stoppered and graduated centrifuge tube, the glass homogeniser and the pestle washed properly and the extract volume in the centrifuge tube made up to exactly 10 ml 90% acetone.

Soaking of the filters overnight is not recommended unless the extraction efficiency of this procedure is thoroughly checked against grinding for the actual working conditions.

***Precautionary note*:**

- *If the extracts are not measured immediately after grinding, for instance if the measurements are done in batches, they can be kept tightly stoppered dark and cold for up to one hour.*

7. Centrifugation

Immediately before measurement the extracts are thoroughly mixed and centrifuged for 10 minutes at 500 *g*. where *g* is the gravitational acceleration. Assuming *g* to be 9.81 m s^{-2}, then the centrifugation velocity (rpm) for a particular centrifuge can be estimated by $668.8/R^{0.5}$ where R is the radius, the distance (in meter units) between the axis of the centrifuge head and the mid-point of the centrifuge tube.

Precautionary note.

- *When working with glass fibre filters, centrifugation is critical since no fibre must be transferred into the optical cuvette. The blank (at 750 nm) should be checked for stability over the time required for measuring the sample. Decreasing blanks denote the presence of particles.*

8. Spectroscopic measurement

When only small sample volumes are available, fluorometry is generally the only satisfactory method, unless a highly performing (very sensitive) spectrophotometer is used. Tri-chromatic spectrophotometry should not be used when significant pheopigment concentrations are suspected. Within their respective application fields, all three methods are equally satisfactory.

8.1 Spectrophotometry

Spectrophotometric methods are usually preferred when a large enough water sample volume is available. As a rule, about one litre of water is needed for a single measurement.

A good quality spectrophotometer with a bandwidth of 2 nm and equipped with cuvettes with path length up to 5 cm is suitable as such path length is required in most instances for satisfactory measurements. The sample extracts are transferred from the centrifuge tubes to the cuvette by careful pipeting. Pouring from the tube into the cuvette is not advisable since it can transfer glass fibres.

a) **Tri-chromatic method:** This method is recommended for seawater samples containing chl *a*, *b*, and *c* as the major pigments and where chlorophyll degradation products are absent. The extinction (absorption) of the sample extract is measured at 750, 664, 647 and 630 nm against a 90% acetone blank.

The concentration of chlorophyll *a*, *b* and *c* are calculated according to the equations of Jeffrey and Humphrey (1975).Units are in mg m^{-3}.

Chlorophyll $a = (11.85*(E_{664}-E_{750}) - 1.54*(E_{647}-E_{750}) - 0.08(E_{630}-E_{750}))*V_e/L*V_f$

Chlorophyll $b = (-5.43*(E_{664}-E_{750}) + 21.03*(E_{647}-E_{750}) - 2.66(E_{630}-E_{750}))*V_e/L*V_f$

Chlorophyll $c = (-1.67*(E_{664}-E_{750}) - 7.60*(E_{647}-E_{750}) + 24.52(E_{630}-E_{750}))*V_e/L*V_f$

Where: L = Cuvette ligth-path in centimetre.
V_e = Extraction volume in millilitre.
V_f = Filtered volume in litre.

b) **Mono-chromatic method with acidification:** This method is recommended for seawater samples containing significant amounts of degradation products. The absorbency of the sample extract is measured at 665 nm both before and after acidification with 0.2 ml 1% v/v hydrochloric acid against a 90% acetone blank. The concentration of chlorophyll *a* and pheophytin *a* are calculated according to Lorenzen (1967). Units are in mg m^{-3}.

Chlorophyll $a = 11.0*K*(E_{665o} - E_{665a})*V_e/L*V_f$

Pheophytin $a = 11.0*K*((R*E_{665a}) - E_{665o})*V_e/L*V_f$

Where: L = Cuvette light-path in centimetre.
V_e = Extraction volume in millilitre.
V_f = Filtered volume in litre.
R = Maximum absorbency ratio of $665_o/665_a$ in the absence of pheopigments=1.7
K = R/(R-1)=2.43

***Precautionary notes*:**

- *The use of closed cuvettes reduce cooling due to evaporation and hence absorbency variability due to the schlierren effect.*

- *A very important point in the acidification method is to ensure that any acid residue has been thoroughly removed out of the optical cuvette before the next sample is transferred. Otherwise chlorophyll* a *would be degraded and underestimated. Suspect excess of pheopigments should alert such eventuality.*

8.2 Fluorometry

This method is recommended for seawater samples containing significant amounts of degradation products and where sample volume is a critical factor. The sample extract is measured in a fluorometer equipped with a red-sensitive photomultiplier (R-446), a blue light source (F4T5BL), an excitation filter 430-450 nm (i.e. Corning CS 5-60) and a sharp-cut emission filter > 650 nm (i.e. Corning CS 2-64). The fluorometer must previously have been calibrated against a commercial solution of pure chlorophyll *a*. the concentration of which have been previously measured by the spectrophotometric method. The calibration should be carried out with different chlorophyll *a* concentrations covering all the linear range for the relationship between chlorophyll concentration and instrument output. Also the maximum acid ratio must be determined by measuring the fluorescence of the standard before and after acidification. Ready to use primary chlorophyll *a* standards are now available from Denmark (DKI, Copenhagen) and USA (Turner Designs Inc.).

Spectrofluorometers can also be used instead of filter fluorometers. Excitation wavelength should be 430 nm (10 nm bandwidth) and the emission wavelength 680 nm (10 nm bandwidth).

The samples are measured both before and after acidification with 0.2 ml 1% v/v hydrochloric acid against a 90% acetone blank. The concentration of chlorophyll *a* and pheopigments are calculated according to the equations of Holm-Hansen *et al.* (1965). Units are in mg m^{-3}.

Chlorophyll *a* $= K*(F_m/(F_m-1))* V_e *(F_o-F_a) / V_f$

Pheopigments $= K*(F_m/(F_m-1))* V_e *((F_m*F_a)-F_o) / V_f$

Where:

K = Calibration coefficient = µg Chl *a* per ml 90% acetone per instrument fluorescence units.
F_m = maximum acid ratio (F_o/F_a) of pure chlorophyll *a* standard.
F_o = sample fluorescence before acidification.
F_a = sample fluorescence after acidification.
V_e = extraction volume in millilitre.
V_f = filtered volume in litre.

Precautionary notes:

- *Using fluorometry, the presence of chlorophyll* b *may result, if a standard lamp is used, in significant overestimation of pheopigments and hence an underestimation of chlorophyll a. In order to validate data obtained with pheopigment- correcting methods, occasional control of the presence of chlorophyll* b *using the trichromatic spectrophotometric equations should be performed.*

- *Any new setting or change of the optical design of the fluorometer implies re-calibrating the instrument. The same applies when moving the instrument.*

- *In fluorometry it is very important to keep the same temperature for both calibration and measurement of the samples. The use of a water bath covered from direct light is recommended for this purpose. The extracts should also be measured at a fixed time after being introduced in the fluorometer. If not, the heat in the instrument will cause the sample temperature to increase thus decreasing the fluorescence.*

- *The cuvettes should always be placed in the same position in the fluorometer to avoid problem with scratching of the cuvette wall that could affect the readings*

- *When calibrating the fluorometer care has to be taken in checking the chlorophyll solution for the presence of degradation products. This can be easily done by scanning the solution with a scanning spectrophotometer or by checking the absorbency of the solution at 665 nm both before and after adding hydrochloric acid. The ratio between the two measurements should be about 1.7.*

- *A secondary standard should be measured at regular intervals during each measurement run in order to keep track of the fluorometer stability. Any statistical significant change in the secondary standard should require a re-*

calibration of the fluorometer. Turner Designs Inc. (USA) can nowadays provide a solid secondary standard that simplify this task.

- *A very important point in the acidification method is to ensure that any acid residue has been thoroughly removed out of the cuvette before the next sample is transferred. Otherwise chlorophyll a would be degraded and underestimated. Suspect excess of pheopigments should alert such eventuality.*

9. Quality Control

As a stable reference material is not available, the use of replicated samples may be used to collect information on the repeatability of the procedure. Control chart can be constructed using these data, by plotting the difference between two double samples; with zero as the expected mean. Such a control chart gives information on measurement uncertainty, and also on the validity of the sampling procedure.

When the trichromatic method is used, the presence of pheopigments should be checked from time to time, or on selected samples, in order to validate the data.

Tests for the presence of chlorophyll *b* may also be useful for validation of the data obtained by the fluorometric method if the combination of lamp and filters is not optimised.

During the spring bloom in open areas, or in algal cultures in exponential growth phase, pheopigments should be at very low levels. Excessive concentrations relative to chlorophyll should alert on potential procedural errors (note that storage by freezing does not generate pheopigments).

Participation in intercomparison exercises is strongly recommended. For this purpose the tri chromatic methods is recommended as the reference method, unless significant pheopigment concentrations in the samples are suspected. It is also highly recommended that each laboratory performs occasional checking of their adapted procedure against the tri chromatic method (if another method is chosen) or even better against HPLC pigment analysis (Jeffrey *et al.*, 1997) if available.

It is recommended that every laboratory develops their own QA routines for the particular form by which the present proposed procedure is employed for measuring chlorophyll *a*. These QA routines should include all steps of the procedure, from sampling to the final result. Laboratories that use procedures deviating from the present proposal should indicate the nature of these differences and provide comparability of their method against this proposed procedure.

10. References

Jeffrey, S. W. and G. F. Humphrey (1975) New spectrophotometric equations for determining chlorophylls *a*, *b*. c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen, 167: 191-194

Jeffrey, S. W., R. F. C. Mantoura and S. W Wrigth (eds.)(1997) Phytoplankton pigments in oceanography: guidelines to modern methods. Monographs on oceanographic methodology 10, UNESCO, Paris.

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

ANNEX 5

STATE OF THE ART OF SATURATION PULSE FLUORESCENCE TECHNIQUES (PAM:PULSE AMPLITUDE MODULATED FLUORESCENCE, P&P: PUMP AND PROBE FLUORESCENCE, FRR: FAST REPETITION RATE FLUORESCENCE)

by

Peter Hartig, Sebastian Lippemeier and Franciscus Colijn

Research and Technology Center Westcoast, University Kiel, Hafentoern,
25761 Buesum, GERMANY
Phone: 0049-(0)4834-604209
Fax: 0049-(0)4834-604299
e-mail: hartig@ftz-west.uni-kiel.de

Why use saturation pulse fluorescence measurements ?

Compared to the traditionally used radiocarbon method for measuring primary production (Steemann Nielsen 1952) or the detection of released oxygen the introduction of the so-called saturation pulse fluorescence methods (PAM, P&P, FRR) offers many advantages. They can be done non-invasive, continuously and rapidly without a long incubation time, without any bottle-effects and without radioactivity. Therefore saturation pulse fluorescence techniques generally offer the potential to carry out quick measurements and to get information on a high (spatial and) temporal scale. Thus it is desirable to evaluate the measurements of the fluorescence yield as an alternative method to calculate phytoplankton production rates. However, it remains open if these fluorescence measurements really reflect the phytoplankton primary production.

Principles of saturation pulse fluorescence measurements

Normally fluorescence induction kinetics are performed after a dark adaptation of the sample. In the dark adapted state all photosystem II reaction centers are oxidized and in an "open" state. With the application of a very weak measuring light the minimal fluorescence level F_o is induced and it is assumed that under this condition the photosystem II reaction centers are still in the open state and the primary electron acceptor Q_A is still entirely oxidized (Fig.1).

By applying a saturating light flash (about 600 milliseconds, PAM; about 2 µs, P&P) or a series of repetitive subsaturating light flashes (about 1 µs, FRR) that induces saturation by a cumulative process (FRR) a stable charge separation occurs and all reaction centers are closed (Q_A is completely reduced). Under a complete closure of all photosynthetic reaction centers the fluorescence yield is at maximum (F_m). The difference between the maximum and the minimum fluorescence reveals the maximum variable fluorescence (F_v), which is a measure for the maximum photosynthetic efficiency of the sample (Fig.1).

Under ambient light, photosynthesis is working at a certain rate and the electron transport within the thylakoid membrane is active. The overlap of the continuous actinic light with short, repetitive, non-saturating light flashes allows the estimation of the maximum variable fluorescence at any time of the fluorescence induction kinetic. The fluorescence emission decreases with increasing illumination time and reaches a steady state in a few minutes equivalent to the intensity of the actinic light (Fig.1).

Further explanations and an illustration of a typical PAM fluorescence induction kinetic are given in Schreiber et al. (1986; 1993), who developed the system.

It has to be mentioned that the exact measurement of the F_o-level is of essential importance for obtaining the real variable fluorescence signal. In most conventional fluorometers the modulated light beam is strong enough to drive photochemical reactions, so the estimation of F_o remains critical.

Which saturation pulse fluorescence methods are used presently for phytoplankton research ?

In principal three methods exist: **the pump and probe-technique (P&P)** developed by Kolber and Falkowski (1993), the **PAM-technique (PAM)** developed by Schreiber (1983; 1986) and **the fast repetition rate technique (FRR)** developed by Kolber et al. (1998).

The pump and probe fluorometer (P&P) is based on the progressive closure of photosystem II reaction centers by a strong saturating (pump) flash (1µs), and subsequent detection of fluorescence by a weak (probe) flash. Both excitation flashes are usually generated by xenon flash lamps. This technique provides the estimation of the chlorophyll concentration, the fraction of open reaction centers, the maximum change of quantum yield of fluorescence (Kolber &

Falkowski 1993). If the intensity of the pump flash is increased in a number of subsequent pump and probe cycles, the effective absorption cross section of photosystem II can be calculated from the characteristics and evolution of the fluorescence yield during this series of flashes.

The PAM-instrument is a highly selective modulation fluorometer offering the potential to measure fluorescence yields in full sunlight. As a result of intensive research in a number of laboratories, methods have been developed to quantitatively analyse and evaluate the fluorescence information for review see (Bolhar-Nordenkampf et al. 1989; Demmig-Adams 1990; Foyer et al. 1990; Walker 1992; Edwards & Baker 1993; Schreiber & Bilger 1993). Measurements of quantum yield and relative electron flow rates can thereby be obtained (Schreiber & Bilger 1993; Hofstraat et al. 1994). Most of these works have been carried out on higher plant leaves or on isolated chloroplasts; only a few researchers have applied the PAM-technique to study unicellular algae and phytoplankton (Kroon et al. 1993; Hofstraat et al. 1994; Schreiber et al. 1995). As has been pointed out by Ting and Owens (1992) and Büchel and Wilhelm (1993), there have been considerable limitations in the performance of the available instrumentation for quenching analysis using dilute samples of unicellular algae with different antenna organization.

Some of them were overcome by Schreiber et al. (Schreiber et al. 1993) and Schreiber (1994), because they succeeded to measure even at low chlorophyll concentrations (10 $\mu g\ l^{-1}$). Generally the PAM-system has been used only since a couple of years for phytoplankton research, publications are therefore rare and there is a strong need to obtain further information for what purpose the PAM-techique can be used.

The fast repetition rate technique (FRR) measures fluorescence transients induced by a series of brief subsaturating excitation pulses, where the intensity, duration and interval between them is independently controlled. In contrast to the pump and probe technique the FRR-technique uses LEDs to induce fluorescence signals. This allows even the detection of the functional cross section of PSII ($\square_{PSII}$) within 100 microseconds. The method is completely described in Kolber et al. (1998). An instrument based on this technique is available and manufactured by Chelsea Instruments, U.K..

The **great advantage of the pump and probe and the fast repetition rate technique** is that these fluorometers can be attached to a CTD and thus provide vertical profiles of photosynthetic parameters along with temperature and salinity. In that case it is possible to study the response of photosynthesis to physical forcing at short time scales instead of hours when using the radioactive tracer incubation method.

Comparison of the three saturation pulse fuorescence techniques

A comparison of the P&P - and PAM-technique was carried out by Schreiber et al. (1995). Though the P&P-technique in principle is comparable to the PAM-devices, there is a significant difference in the measurement of F_m. Due to the very short duration (2-5 µs) of the saturating single turnover pulses used in the pump and probe protocol, the F_m values determined with this technique are slightly lower than those determined with the PAM-technique in which saturating pulses of 500-700 ms are used. The different time scales are caused by using halogen lamps in the PAM-technique and xenon flash lamps in the P&P-technique. However, as was elicited by Schreiber et al. (1995), both techniques are equally appropriate for the assessment of the photosynthetic performance of phytoplankton. A comparison of all three mentioned instruments was done recently by Kolber et al. 1998. Unlike to the PAM-technique, the P&P and the FRR-technique offer the additional possibility to measure the functional (or effective) absorption cross section of PSII. This parameter allows the quantification of the fraction of the incident light that is allocated to photosynthesis and thus is essential for the calculation of the overall primary production rate (Kolber & Falkowski 1993).

Terminolgy and calculations

Many different descriptions of chlorophyll fluorescence parameters measured with the so-called saturation pulse method have caused unnecessary confusions till 1989. At the ocassion of the workshop "The Use of Chlorophyll Fluorescence and other Non-invasive Spectroscopic Techniques in Plant Stress Physiology" held in Doorwerth in the Netherlands the participants agreed to standardize some of the used nomenclature (see Tab.1). These standardizations have been published by van Kooten and Snel (1990) and became generally accepted.

Table 1: Definition of chlorophyll fluorescence nomenclature after (Kooten & Snel 1990)

Fluorescence intensity indicators		
F	fluorescence intensity	actual fluorescence intensity at any time
F_0	minimal fluorescence (dark)	fluorescence intensity with all PSII reaction centers open while the photosynthetic membrane is in the non-energized state, i.e. dark or low light adapted $q_p = 1$ and $q_N = 0$.
F_s	fluorescence in steady state	
F_m	maximal fluorescence (dark)	fluorescence intensity with all PS II reaction centers closed (i.e., $q_P = 0$) all non-photochemical quenching processes are at a minimum (i.e., $q_N = 0$). This is the classical maximum fluorescence level after dark adaptation or in a low light adapted state.
F'_m	maximal fluorescence (light)	fluorescence intensity with all PS II reaction centers closed in any light adapted state, i.e., $q_P = 0$ and $q_N \geq 0$
F'_0	minimal fluorescence (light)	fluorescence intensity with all PS II reaction centers open in any light adapted state, i.e., $q_P = 1$ and $q_N \geq 0$
F_v	variable fluorescence (dark)	maximum variable fluorescence in the state when all non-photochemical processes are at a minimum, i.e. (F_m-F_0)
F'_v	variable fluorescence (light)	maximum variable fluorescence in the state in any light adapted state, i.e. $(F'_m-F'_0)$
Fluorescence quenching parameters		
q_P	photochemical quenching	$(F'_m-F)/(F'_m-F'_0)$
q_N	non-photochemical quenching	$1-(F'_m-F'_0)/(F_m-F_0)$

From the minimal (F_0) and maximal (F_m) fluorescence yields in the dark the ratio between the variable (F_m-F_o) and the maximal (F_m) fluorescence can be calculated according to:

$$\Phi_{Po} = (F_m-F_o)/F_m = F_v/F_m \qquad \text{where } F_v = F_m-F_o \qquad (\textit{equation 1})$$

Equation 1 describes the potential photochemical efficiency (Φ_{Po}) of the open reaction centers of PS II.
From the minimal (F) and maximal (F'_m) fluorescence yields in the light (or ambient light) the actual photochemical efficiency of PS II (Φ_P) can be calculated according to:

$$\Phi_P = (F_m'-F)/F_m'. \qquad (\textit{equation 2})$$

By multiplying (F_m'-F)/F_m' with the irradiance (E) the relative electron flow Φ_e can be calculated:

$$\Phi_e = (F_m'-F)/F_m' \bullet E \qquad (\textit{equation 3})$$

which is proportional to the relative photosynthetic production rate (Hofstraat et al. 1994).
By plotting the relative electron flow against incident irradiance P-E curves can be constructed.

Calculation of the absolute electron flow is possible, when the mean specific absorption coefficent (a*) of the algae is known. a* describes the overall absorption of light by the algae. After this equation Hartig et al. (1998) were able to get an index of primary productivity:

$$PPR_{fluorescence} = (F_m'-F)/F_m' \bullet E \bullet a^* \qquad (\textit{equation 4})$$

Calculation of primary productivity with the P&P-fluorometer is possible after a similar equation which was introduced by Kolber and Falkowski (1993) . The authors substituted a* by the functional absorption cross section of PSII (σ_{PSII}).

Applications of the saturation pulse fluorescence techniques in phytoplankton research

Since their introduction all mentioned saturation pulse fluorescence techniques found a wide application in phytoplankton research. Two main objectives were pursued: 1. comparision of saturation pulse fluorescence measurements with traditional methods (O_2 and ^{14}C) and 2. effects of nutrient limitation on fluorescence signals. The results of both main objectives will be described subsequently.

Comparison of saturation pulse fluorescence measurements with traditional methods (O_2 and ^{14}C)

When the steps of the photosynthetic process are considered it becomes clear that fluorescence-emission and O_2-release are closely connected at the reaction center of photosystem II (RCII), whereas the CO_2-fixation takes place at a subsequent step in the Calvin cycle. This already implies that a better correlation of O_2 than of ^{14}C with the fluorescence-measurements can be expected. The most important differences between O_2-release and carbon-fixation arise from the fact that not all electrons evolved in the watersplitting process at RCII are strictly used for C-fixation. Alternative electron sinks are e.g. NO_3^- - reduction, photorespiration, Mehler reaction and reactions to repair damages caused by high irradiances. Since the PAM-and the P&P-methods principally detect electron flow rates in RCII

(Hofstraat et al. 1994; Hartig & Colijn 1996; Hartig et al. 1998) one can only expect a close correlation between the fluorescence- and the ^{14}C- based measurements if all electrons are used for C-fixation and not for other processes.

However, fluorescence itself is highly variable due to different quenching mechanisms, which are not strictly connected with C-fixation or O_2-release. Without going into detail quenching includes all processes which lead to a reduction of fluorescence yields resulting in an inequality between fluorescence signals and O_2-release respectively C-fixation.

Relationships between classical- and saturation pulse fluorescence-based methods of photosynthetic measurements conducted with higher plants and algae in different studies are presented in Tab. 2. It should be noted that this overview does not claim to be complete. Most of the studies that are intended to provide a comparison between the different methods of photosynthetic measurements have been carried out on higher plant leaves or on isolated chloroplasts. Only a few researchers applied PAM fluorescence techniques on unicellular algae and phytoplankton for this purpose (Kroon et al. 1993; Hartig & Colijn 1996; Geel et al. 1997; Hartig et al. 1998). Because P&P found a wide application in phytoplankton research we also present the relationship to the classical methods obtained with this technique. The results of the different studies on the relationship between PSII electron transport rates and carbon fixation respectively oxygen release in higher plants and algae showed different results (Tab. 2). Even conducted under a variety of experimental conditions, most of them show a linear correlation under moderate irradiances, whereas non-linear correlation was found under low light intensities and in some cases under high light intensities. Therefore irradiance seems to be an important factor which influences the assessment of photosynthesis based on fluorescence measurements.

For the observed deviations under low and high light conditions the authors offered possible explanations similar to those we already mentioned above and some additional reasons for non-linearity like e.g. spectral differences of light sources, oxygen consumption and cyclic electron flow around PSII.

Most of the studies who revealed a non-linear relationship between the classical methods and the saturation pulse fluorescence methods where carried out with algae. Apart from the already given explanations for the observed non-linearity another important factor is the higher plasticity of the photosynthetic machinery of phytoplankton than that of higher plants. This plasticity is mainly reflected by a different pigment composition and the intracellular arrangement of the pigments, which are furthermore highly variable under different environmental conditions (Cleveland & Perry 1987; Herzig & Falkowski 1989). Therefore light absorption of phytoplankton is different to higher plants, where it is considered to be very unvariable. For calculation of fixed carbon or released oxygen based on fluorescence measurements a definite knowledge about the light absorption of phytoplankton is needed (Hartig et al. 1998). This is possible with the determination of the functional absorption cross section of PSII (σ_{PSII}).

It has been shown that the final conversion of irradiance into fixed C by phytoplankton is impaired due to different effects (e.g. different electron sinks, variable light absorption etc.). This complicates the comparision of photosynthetic measurements based on PAM-, O_2- and ^{14}C – methods. However in some studies carried out with phytoplankton a good correlation between O_2- or ^{14}C-based methods and the saturation pulse fluorescence based methods (PAM and P&P) was found (Tab. 2). See also Fig. 2, 3 and 4. Therefore we assume that the saturation pulse fluorescence methods offer the potential for rapid estimation of phytoplankton photosynthesis with high (spatial and) temporal resolution, which is

needed particularily for phytoplankton communities due to their patchy distribution and to enable studies on short-term changes in growth rates and effects of different limitations (irradiance, nutrients).

Table 2: Relationships between classical- and saturation pulse fluorescence-based methods of photosynthetic measurements conducted with higher plants and algae in different studies.

Author (Year)	Methods	Organisms	Relationships	Reasons for non-linearity
Boyd et al. (1997)	P&P, ^{14}C	Phytoplankton	Linear (for I_k, P_m); Non-linear (for □□	Spectral differences of actinic light sources
Falkowski et al.(1986)	P&P, O_2	Algae (cultures)	Linear (intermediate light); Non-linear (low and high light)	High light: cyclic electron flow around PSII Low light: unknown quenching components
Geel et al. (1997)	PAM 101, Xenon-PAM, O_2	Algae (cultures)	Linear (low and intermediate light); Non-linear (high light)	Oxygen consumption (not photorespiration)
Genty et al. (1989))	PAM 101, CO_2 (gas exchange)	Higher plants (C3, C4)	Linear	
Genty et al. (1990)	PAM 101, $^{16}O_2/^{18}O2$ (mass spectrometry)	Higher plants (C3)	Linear	
Harbinson et al. (1990)	PAM 101, O_2	Higher plants (C3)	Linear; Non-linear (at 20% O_2)	Photorespiration
Hartig & Colijn (1996)	PAM 101 (PM), ^{14}C	Algae (cultures and microphytobenthos)	Linear	species dependent with different slopes
Hartig et al. (1998)	PAM 101 (PM), ^{14}C	Algae (microphytobenthos)	Linear (intermediate light); Non-linear (low and high light)	Low-light: Spectral differences of actinic light sources High light: electron sinks
Hofstraat et al. (1994)	PAM 101, growth rates	Algae (cultures)	Linear	
Horton et al. (1989)		Higher plants	Linear	
Keiller & Walker (1990)		Higher plants	Linear	
Kolber & Falkowski (1993)	P&P, ^{14}C	Phytoplankton	Linear (r 0.86)	
Krall & Edwards (1991)	PAM 101, O_2	Higher plants (C4)	Linear	
Kroon et al. (1993)	PAM 101, O_2	Algae	Linear ; Non-linear (high light)	Phenomenon of adaptation (Baumert 1996)
Oberhuber et al. (1993)	PAM 101, CO_2 (IRGA)	Higher plants (C3,C4)	Linear; Non-linear (low light)	Respiration or presence of inactive PSII centers
Rees et al. (1992)	PAM 101, O_2	Algae (cultures)	Non-linear	Non-assimilatory electron flow
Seaton & Walker (1990)	PAM 101, O_2	Higher plants (C3,C4)	Linear; Non-linear (low light)	

Abbreviations:
PAM: Pulse Amplitude Modulated Fluorometer
P&P: Pump and Probe Fluorometer
PM: Photomultiplier
IRGA: Infra Red Gas Analyser

Effects of different nutrient limitations on fluorescence signals

An increasing number of papers are reporting the effects of different nutrient limitations on the photosynthetic capacity of phytoplankton assessed by fluorescence measurements. Almost all of these studies focus on the effects of nitrogen, phosphate or iron limitation. Whereas only a few studies deal with phytoplankton cultures (Geider et al. 1993a; Berges et al. 1996), most studies examined the effects of different nutrient conditions on the fluorescence characteristics of natural phytoplankton communities (Kolber et al. 1990; Geider et al. 1993b; Falkowski & Kolber 1995; Behrenfeld et al. 1996; Olaizola et al. 1996; Hartig et al. 1997, Fig.5). Until now only one study was performed to investigate the effects of silicate limitation on the fluorescence characteristics of diatoms (Lippemeier et al. 1999, Fig.6).

In principle all investigated nutrient limitations are detectable with the fluorescence techniques. The parameter ϕ_{PO} decreases in nitrogen-, phosphate- and iron-limited *Phaeodactylum tricornutum* (Geider et al. 1993a). Similar results were reported for nitrogen- (Berges et al. 1996) and silicate-limited *Thalassiosira weissflogii* (Lippemeier et al. 1999).
In contrast, interesting differences between the limiting nutrients can be seen in the ratios of F_0/Chl-a and F_m/Chl-a. Both ratios increase in nitrate- and iron-limited *Phaeodactylum tricornutum*, whereas they remain unaffected by phosphate limitation (Geider et al. 1993a) and decrease in silicate-limited *Thalassiosira weissflogii* (Lippemeier et al. 1999).

Conclusions and Perspectives

- As **alternatives** to classical O_2 and ^{14}C incubations, saturation pulse fluorescence measurements offer the **great potential** to estimate photosynthetic rates of phytoplankton. The measurements can be done non-invasive, continuously and rapidly without a long incubation time, without any bottle-effects and without radioactivity.

- The PAM, P&P - and FRR - techniques are **equally appropriate** for the assessment of the photosynthetic performance of phytoplankton. But unlike to the PAM-technique, the P&P and the FRR-technique offer the additional possibility to measure the **functional (or effective) absorption cross section of PSII** ($\square_{PSII}$), which is essential for the calculation of the overall primary production. Furthermore the latter instruments can be used for **submersible measurements *in situ*.**

- Most of the studies show a **strong correlation between classical - and fluorescence-based measurements** of photosynthetic rates under moderate irradiances, whereas a non-linear correlation was found under low light intensities and in some cases under high light intensities.

- **In principle all investigated** nutrient limitations **(mainly nitrogen, phosphate, silicate and iron) are detectable with the fluorescence techniques. However there are some significant differences in the connections between fluorescence signals and each single nutrient.**

- In general it remains open to what extent the **fluorescence parameters** really reflect the phytoplankton **primary production**. Interpretation is still difficult due to **alternative electron sinks** other than cell growth (e.g. NO_3^- - reduction, photorespiration, Mehler reaction and reactions to repair damages caused by high irradiances).
  For a most reliable assessment of **fluorescence-based primary production**, calculations should include the **functional absorption cross section of PSII** ($\square_{PSII}$) (detectable with the P&P- and the FRR-technique).

Literature

Behrenfeld MJ, Bale AJ, Kolber ZS, Aiken J, Falkowski PG (1996) Confirmation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. Nature 383: 508-511

Berges JA, Charlebois DO, Mauzerall DC, Falkowski PG (1996) Differential effects of nitrogen limitation on photosynthetic efficiency of photosystems I and II in microalgae. Plant Physiol 110: 689-696

Bolhar-Nordenkampf HR, Long SP, Baker NR, Schreiber U, Lechner EG (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct. Ecol. 3: 497-514

Boyd PW, Aiken J, Kolber Z (1997) Comparison of radiocarbon and fluorescence based (pump and probe) measurements of phytoplankton photosynthetic characteristics in the northeast Atlantic Ocean. Mar Ecol Prog Ser 149: 215-226

Büchel C, Wilhelm C (1993) In vivo analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. Photochem Photobiol 58: PAM, Fluorescence, method, review, problems

Cleveland JS, Perry MJ (1987) Quantum yield, relative specific absorption and fluorescence in nitogen-limited Chaetoceros gracili. Mar Biol 94: 489-497

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta 1020: 1-24

Edwards GE, Baker NR (1993) Can carbon dioxide assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynthesis Research 37: 89-102

Falkowski PG, Kolber Z (1995) Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans. Australian Journal of Plant Physiology 22: 341-355

Falkowski PG, Wyman K, Ley AC, D.C.Mauzerall (1986) Relationship of steady-state photosynthesis to fluorescence in eucaryotic algae. Biochim. Biophys. Acta 849: 183-192

Foyer C, Furbank R, Harbinson J, Horton P (1990) The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. Photosynth Res 25: 83-100

Geel C, Versluis W, Snel JFH (1997) Estimation of oxygen evolution by marine phytoplankton from measurement of the efficiency of photosystem II electron flow. Photosynthesis Research 51: 61-70

Geider RJ, Greene RM, Kolber Z, Macintyre HL, Falkowski PG (1993b) Fluorescence assessment of the maximum quantum efficiency of photosynthesis in the western North Atlantic. Deep Sea Research Part I Oceanographic Research Papers 40: 1205-1224

Geider RJ, La Roche J, Greene RM, Olaizola M (1993a) Response of the photosynthetic apparatus of Phaeodactylum tricornutum (Bacillariophyceae) to nitrate, phosphate, or iron starvation. J Phycol 29: 755-766

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87-92

Genty B, Harbinson J, Briantais JM, Baker NR (1990) The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 photochemistry in leaves. Photosynthesis Research 25: 249-258

Harbinson J, Genty B, Baker NR (1990) The relationship between carbon dioxide assimilation and electron transport in leaves. Photosynthesis Research 25: 213-224

Hartig P, Colijn F (1996) Pulse-amplitude-modulation fluorescence (PAM). A tool for fast assessment of primary productivity in the sea? In: Baeyens J, Dehairs F, Goeyens L (eds) Integrated Marine System Analysis. VUB Bruessel, Bruessel, p 103-113

Hartig P, Lippemeier S, Colijn F (1997) Direct, instantaneous measurements of photosynthesis by PAM-fluorometry: New developments. In: Baeyens J, Dehairs F, Goeyens L (eds) Integrated Marine System Analysis. VUB Bruessel, Bruessel, p 29-49

Hartig P, Wolfstein K, Lippemeier S, Colijn F (1998) Photosynthetic activity of natural microphytobenthos populations measured by fluorescence (PAM) and ^{14}C-tracer methods: a comparsion. Mar. Ecol. Progr. Ser. 166: 53-62

Herzig R, Falkowski PG (1989) Nitrogen limitation in Isochrysis galbana (Haptophyceae): I. Photosynthetic energy conversion and growth efficiencies. J Phycol 25: 462-471

Hofstraat JW, Peeters JCH, Snel JFH, Geel C (1994) Simple determination of photosynthetic efficiency and photoinhibition of Dunaliella tertiolecta by saturating pulse fluorescence measurements. Mar Ecol Prog Ser 103: 187-196

Horton P (1989) Interactions between electron transport and carbon assimilation: regulation of light harvesting and photochemistry. In: Briggs WR (ed) Photosynthesis. Allen R Liss Inc, New York, p 393-406

Keiller DR, Walker DA (1990) The use of chlorophyll fluorescence to predict carbon dioxide fixation during photosynthetic oscillations. Proceedings of the Royal Society of London Series B Biological Sciences 241: 59-64

Kolber Z, Falkowski PG (1993) Use of active fluorescence to estimate phytoplankton photosynthesis in situ. Limnol Oceanogr 38: 1646-1665

Kolber Z, Wyman KD, Falkowski PG (1990) Natural variability in photosynthetic energy conversion efficiency: A field study in the Gulf of Maine (USA). Limnol Oceanogr 35: 72-79

Kolber ZS, Prasil O, Falkowski PG (1998) Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochim. Biophys. Acta 1367: 88-106

Kooten Ov, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. 25: 147-150

Krall JP, Edwards GE (1991) Environmental effects on the relationship between the quantum yields of carbon assimilation and in vivo PSII electron transport in maize. Australian Journal of Plant Physiology 18: 267-278

Kroon B, Prézelin BB, Schofield O (1993) Chromatic regulation of quantum yields for photosystem II charge separation, oxygen evolution, and carbon fixation in Heterocapsa pygmaea (Pyrrophyta). J Phycol 29: 453-462

Lippemeier S, Hartig P, Colijn FC (1999) Direct impact of silicate on the photosynthetic performance of the diatom Thalassiosira weissflogii assessed by on- and off-line PAM fluorescence measurements. J. Plankt. Res. 21: 269-283

Oberhuber W, Dai ZY, Edwards GE (1993) Light dependence of quantum yields of photosystem II and carbon dioxide fixation in C-3 and C-4 plants. Photosynthesis Research 35: 265-274

Olaizola M, Geider RJ, Harrison WG, Graziano LM, Ferrari GM, Schlittenhardt PM (1996) Synoptic study of variations in the fluorescence-based maximum quantum efficiency of photosynthesis across the North Atlantic Ocean. Limnol Oceanogr 41: 755-765

Rees D, Lee CB, Gilmour DJ, Horton P (1992) Mechanisms for controlling balance between light input and utilisation in the salt tolerant alga Dunaliella C9AA. Photosynthesis Research 32: 181-191

Schreiber U (1983) Chlorophyll fluorescence yield changes as a tool in plant physiology 1. Tha measurimg system. Photosynth. Res. 4: 361-373

Schreiber U (1986) Detection of rapid induction kinetics with a new type of of high-frequency modulated chlorophyll fluorometer. Photosynth. Res. 9: 261-272

Schreiber U (1994) New emitter-detector-cuvette assembly for measuring modulated chlorophyll fluorescence of highly diluted suspensions in conjunction with the standard PAM fluorometer. Zeitschrift fuer Naturforschung Section C Biosciences 49: 646-656

Schreiber U, Bilger W (1993) Progress in chlorophyll fluorescence research: major developments during the past ten years in retrospect. In: Progress in Botany. Springer Press Berlin, Heidelberg, p 151-173

Schreiber U, Hormann H, Neubauer C, Klughammer C (1995) Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. Australian Journal of Plant Physiology 22: 209-220

Schreiber U, Neubauer C, Schliwa U (1993) PAM fluorometer based on medium-frequency pulsed Xe-flash measuring light: A highly sensitive new tool in basic and applied photosynthesis. Photosynth. Res. 36:

Seaton GGR, Walker DA (1990) Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. Proc. R. Soc. Lond. B. 242: 29-35

Steemann Nielsen E (1952) The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. Cons. perm. int. Explor. Mer. 18: 117-140

Ting CS, Owens TG (1992) Limitations of the pulse-modulated technique for measuring the fluorescence characteristics of algae. Plant Physiol 100: 367-373

Walker DA (1992) Excited leaves. New Phytologist 121: 325-345

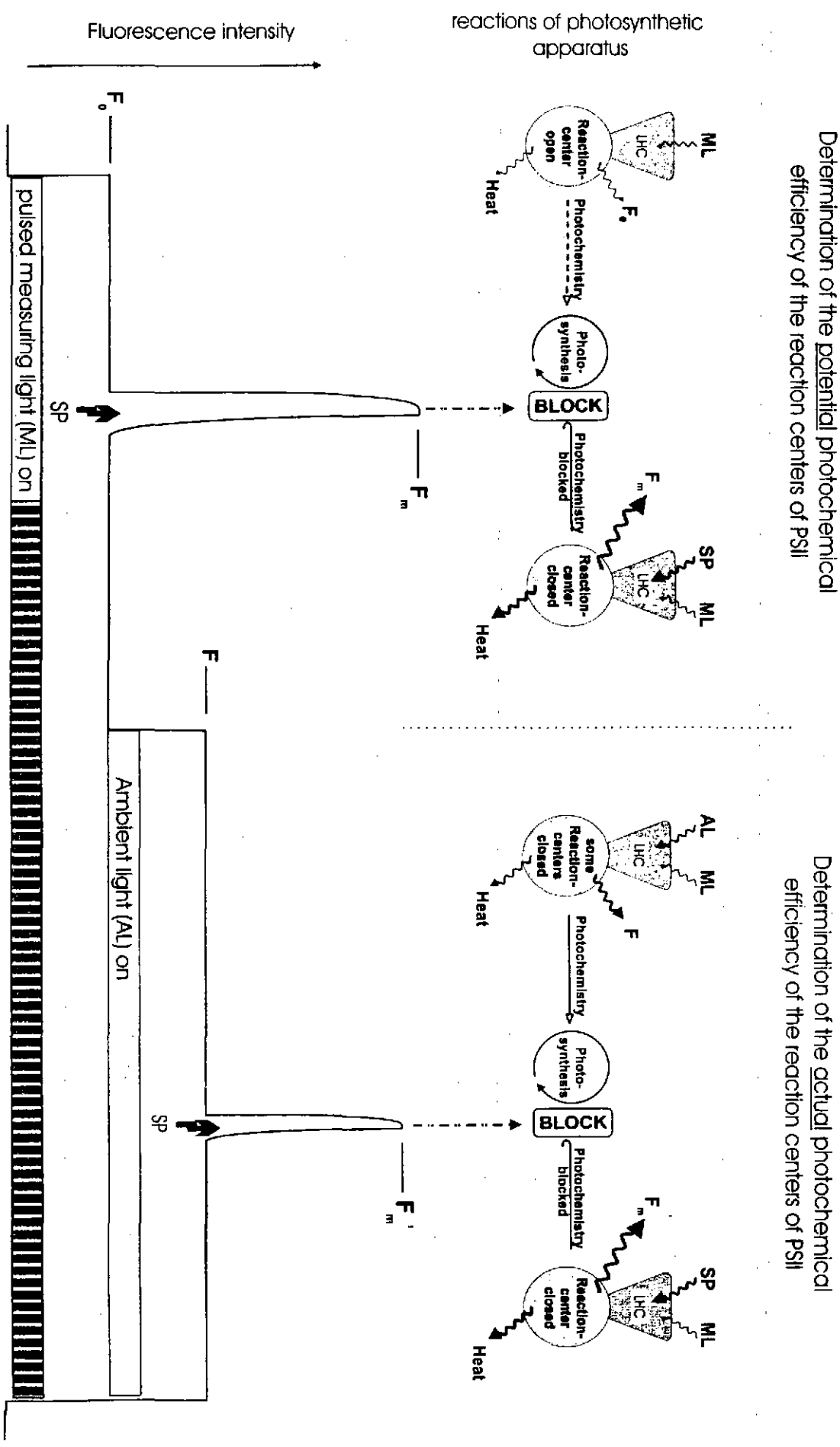


Fig. 1: Connections between excitation lights and fluorescence signals

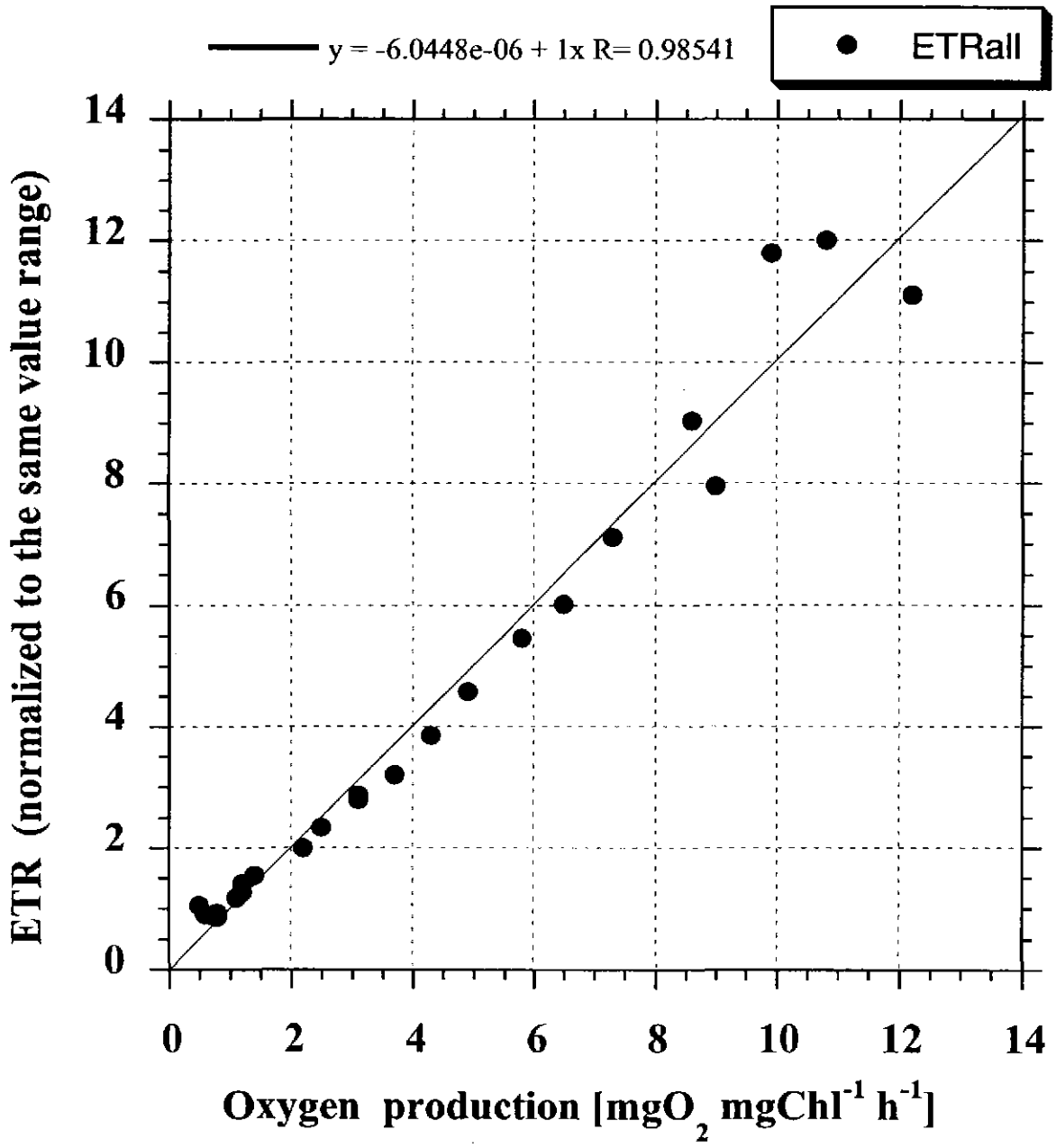


Fig. 2: Correlation between electron transport rates (ETR) (normalized to the same value range) and O_2-production in a mesocosm experiment. Samples were taken at three different times.

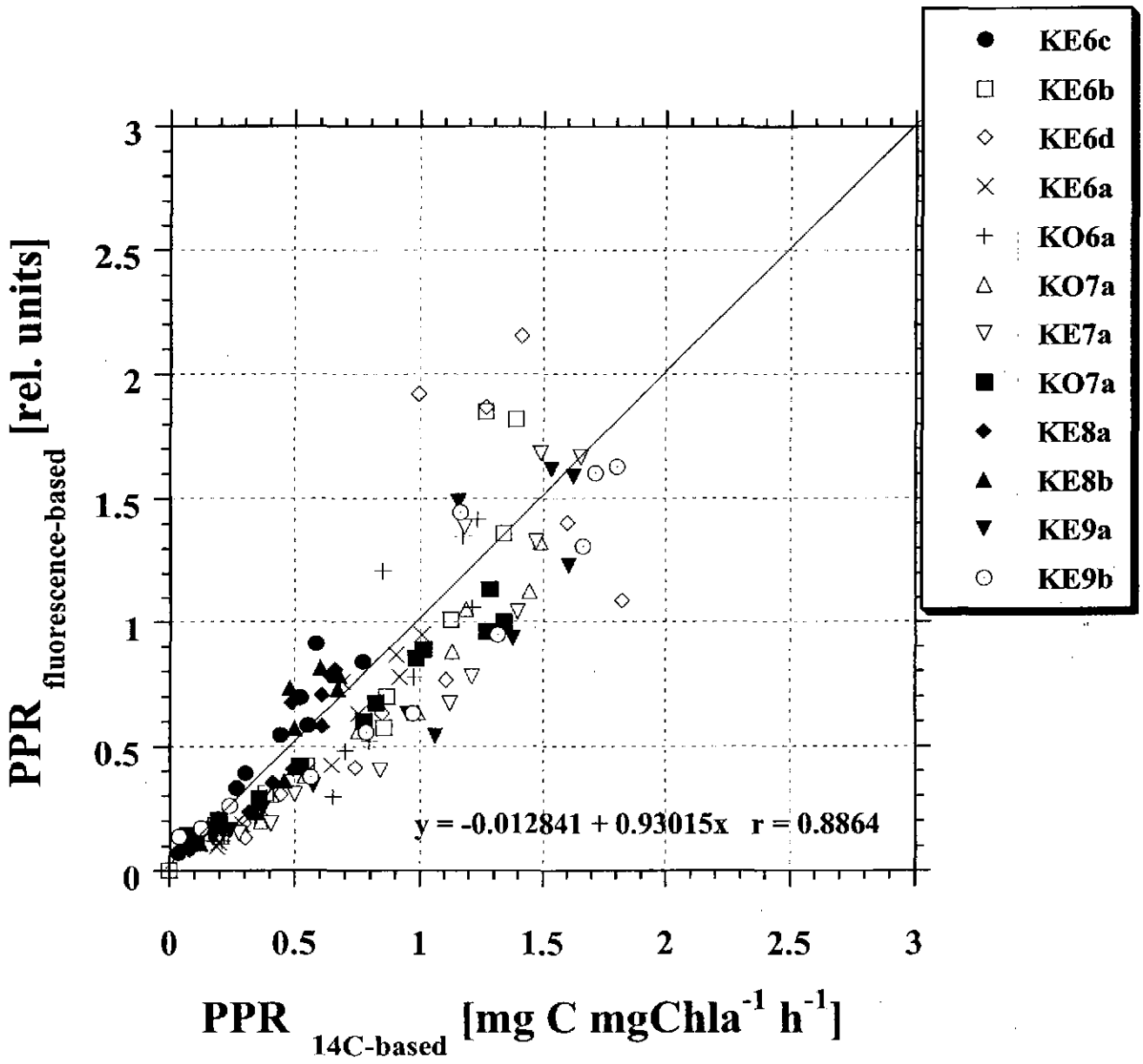


Fig. 3: Correlation between ^{14}C-based and fluorescence-based production rates [$(F_m'-F)/F_m' \bullet E \bullet a^*$] for all performed experiments. Sample abbreviations indicate the sampling location (KE: Keitum; KO: Königshafen, Sylt, Germany) with the sampling day (number: day in June 1996; letters: replicates)

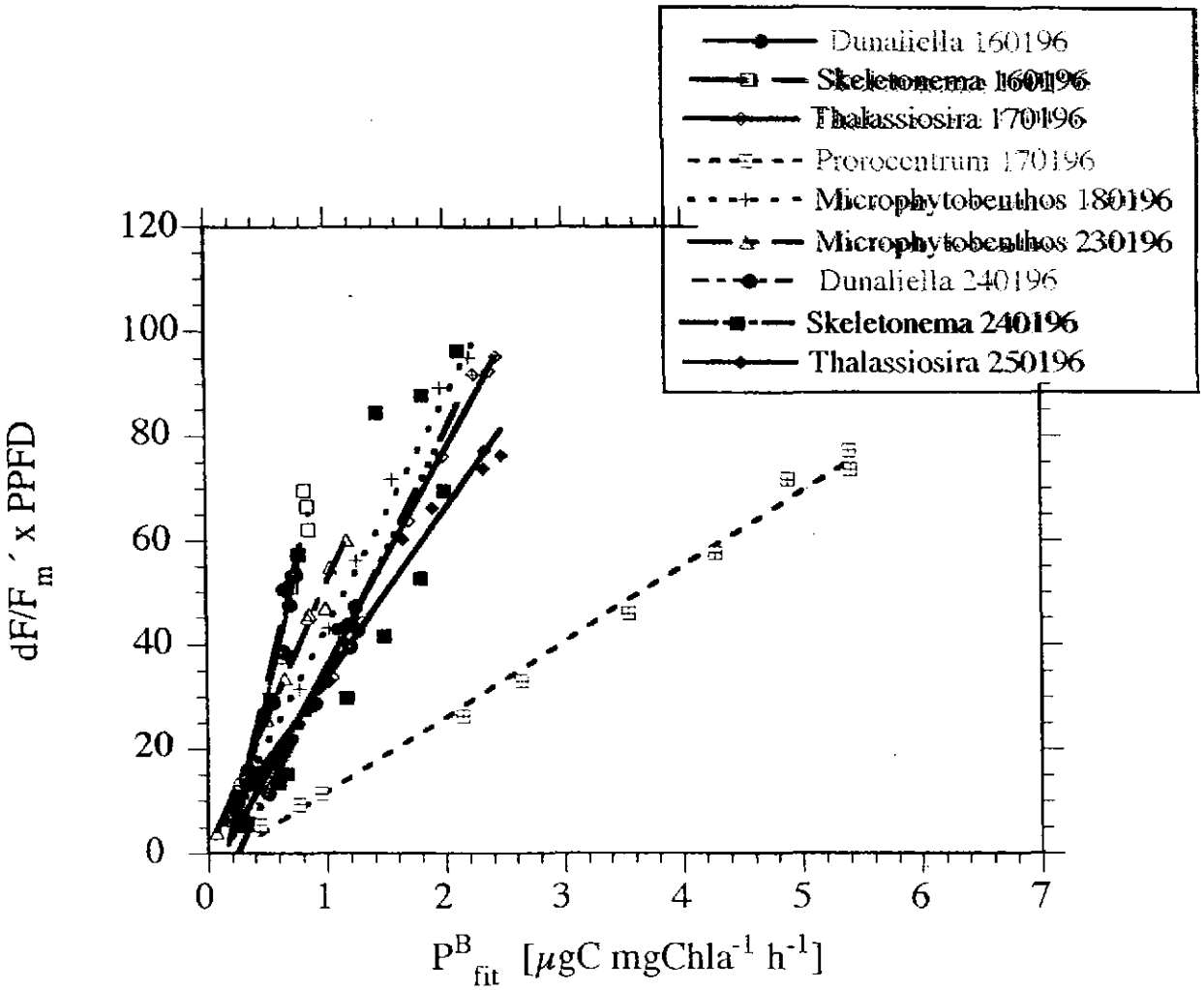


Fig. 4: Relative PSII electron flow rates versus specific carbon fixation for different algal species and microphytobenthos communities

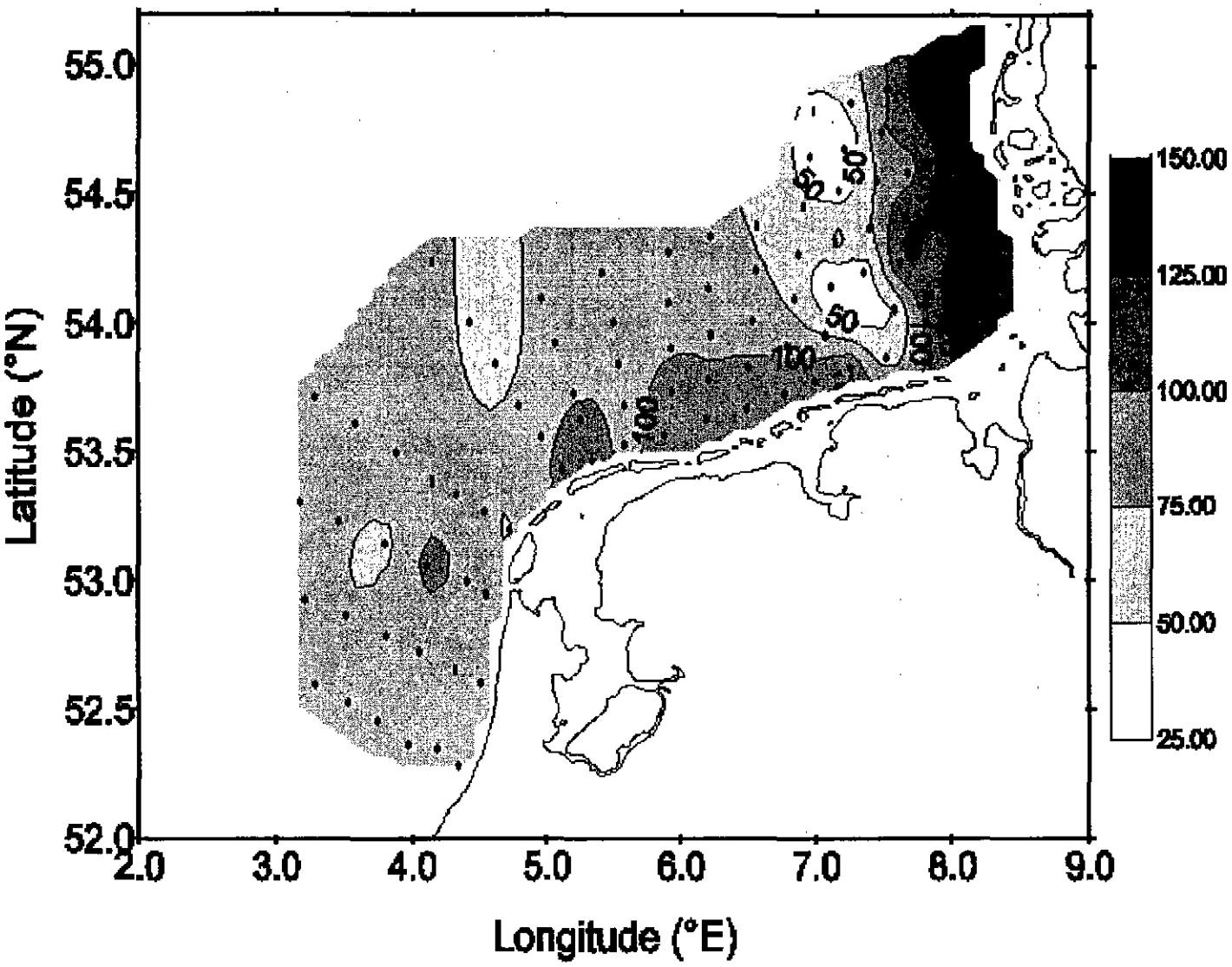


Fig. 5: Surface distribution of saturating relative electron flow rate (ϕ_c) (calculated after equation 3) during a synoptic survey conducted with on board PAM-measurements during spring in the Dutch and German coastal zone.

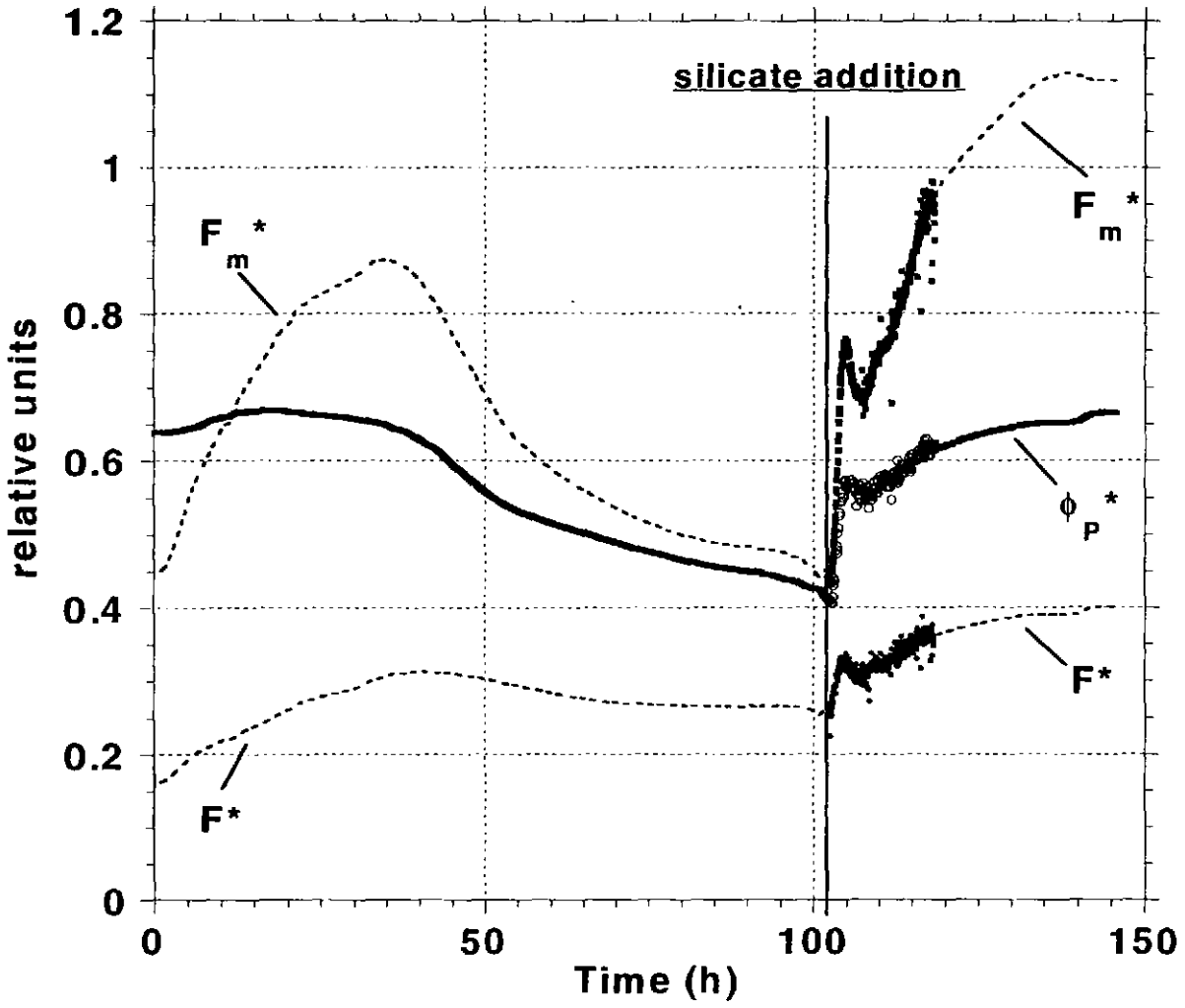


Fig. 6: Time course of the fluorescence parameters F*, F_m* and ϕ_P*. The index (*) indicates, that the values were determined online i.e. without adaptation. All measurements for a batch culture of *Thalassiosira weissflogii*. After 102.5 h the silicate concentration in the culture vessel was raised artificially by adding Na_2SiF_6.

ANNEX 6

Advanced Plankton Monitoring - Updating the Continuous Plankton Recorder

D.K. Mills[1], A.W. Walne[2], S.I. Heaney[3], K. Embleton[3], M.B. Rawlinson[4] and K.B. Cook[5]

[1]*Centre for Environment, Fisheries and Aquaculture Science (CEFAS),*
Lowestoft Laboratory, Lowestoft, Suffolk NR33 0HT
[2]*Sir Alister Hardy Foundation for Ocean Science (SAHFOS)*
The Laboratory, Citadel Hill, Plymouth PL1 2PB
[3]*Agricultural and Environmental Science Division*
Department of Agriculture (DANI), Belfast BT9 5PX
[4]*W.S. Ocean Systems Ltd., Omega Park,*
Alton, Hants, GU34 2QD.
[5]*School of Biological Sciences*
University of Wales, Swansea
Singleton Park, Swansea, SA2 3PP

Abstract

The Continuous Plankton Recorder survey has been measuring environmental change in the North Atlantic for over 60 years. As such, it forms possibly the longest time-series of biological measurements in the world and provides a yardstick against which natural and anthropogenic influences on the marine environment can be assessed. It has recently provided the first evidence of a response in the surface marine phytoplankton community to apparent climatic forcing. In order to meet future needs it needs to be updated to obtain improved information on the vertical distribution and abundance of plankton, the physico-chemical environment and on the microplankton. To meet these needs a new towed body with a increased payload capacity and the ability to undulate has been developed. A suite of environmental sensors has been identified, a microplankton sampler developed together with a conditional sampling protocol. Initial trials of a fully equipped towed body have been carried out in the North and Irish Seas. The distribution and abundance of plankton in relation to hydrography are described and the performance of the new system is assessed.

Introduction

Since its introduction over 60 years ago the Continuous Plankton Recorder (CPR) survey has provided the international community with a unique ocean basin scale monitoring programme (Gamble, 1994). The survey has provided a yardstick against which changes in the planktonic ecosystem, a most sensitive indicator of marine environmental change, can be assessed (Aebischer et al., 1990). Reid et al., (1998) recently published observations which show the first evidence of a climate induced response in surface marine plant communities with implications for CO_2 fluxes and productivity in the North Atlantic. The CPR comprises a brass bodied underwater vehicle (Figure 1a) which is towed in

horizontal flight at about 10 m depth behind Merchant ships on their normal routes of passage. Each CPR includes a plankton sampling mechanism (Figure 1b) incorporating a continuously advancing silk gauze (270 µm mesh size) which filters plankton from the seawater and preserves it for later examination. Although soft-bodied or vertically migrating plankton may not be sampled effectively the CPR obtains good samples of most medium sized zooplankton and large phytoplankton.

One of the strengths of the survey is that it has changed little since Sir Alister Hardy introduced it in 1931. However, despite its success there is a need to modernise in order to meet the needs of future monitoring programmes and to take advantage of new sampling and sensor developments. There are a number of initiatives underway, described by Mills et al. (1998), to update the programme and also develop an integrated monitoring programme combining the CPR survey and smart moorings. This paper describes the performance of a newly developed towed body (U-Tow), its sensors and sampling systems during trials carried out in the central Irish Sea in June 1998.

Methods

In order to collect samples over a wider depth range a new undulating towed body (Figure 2; U-Tow, W.S. Ocean Systems Ltd.,) has been developed with increased payload capacity. An 'intelligent' water sampler (Figure 3a; Aqua Monitor, W.S. Ocean Systems Ltd.,) has been developed in order to quantitatively sample small plankton (< 270 µm). The increased payload allows U-Tow to carry a range of environmental sensors (Table 1) together with Aqua Monitor alongside a Plankton Sampling Mechanism (PSM; Figure 3b).

Table 1. U-Tow sensor and sampler payload and the measurements derived from them.

Sensor/sampler	*Measurement*
Plankton Sampling Mechanism (PSM)	zooplankton biomass and species composition
Aqua Monitor	phytoplankton biomass and species composition
	plant nutrient (N,P, Si) concentration
	extracted chlorophyll concentration
CTD	temperature, salinity, density
Fluorometer	chlorophyll concentration
Optical baskscatter sensor	suspended load
Li-Cor PAR sensor	downwelling irradiance, vertical attenuation coefficient

Simultaneous observations of hydrography and plankton distributions were made on board the R.V. Lough Foyle by towing U-Tow at approximately 8 knots along a transect in the central Irish Sea in June 1998 (Figure 4). Observations of conductivity, temperature, pressure were obtained using a CTD while observations of fluorescence and turbidity were made with a Chelsea Instruments Minitracka fluorometer and Seapoint Optical Backscatter sensor. Water samples were collected on board U-Tow for determination of phytoplankton numbers, species composition, measurement of chlorophyll concentration and determination of plant nutrient concentrations. Chlorophyll and plant nutrient concentrations were determined using standard methods. Zooplankton were collected using the PSM

which was loaded with nylon net rolls (200 μm mesh size) prior to deployment. On retrieval of the PSM the mesh was cut to separate the discrete samples. The plankton was washed from the mesh and preserved in 4% buffered formaldehyde. For analysis, each sample was made up to a volume of either 50 ml, 100 ml or 200 ml, using filtered seawater, depending on the density of plankton. A 10 ml subsample was analysed under a binocular microscope to determine abundance and species composition. However, if less than 100 individuals were counted a further 5 ml subsample was analysed.

Aqua Monitor and the PSM were operated in two modes. Samples were collected according to a pre-set time interval whilst undulating or manually triggered from the surface in regions of interest determined from the real time displays of the vertical distribution of temperature, salinity and chlorophyll fluorescence. In the latter sampling mode U-Tow was held in level flight for the duration of sampling by Aqua Monitor (ca. 2 minutes). The range of measurements obtained in this configuration are shown in Table 1.

A conditional sampling protocol is under development to allow automated plankton sampling dependent upon external environmental conditions. Our approach has been to develop an algorithm which will identify regions of particular interest, for example peaks in phytoplankton biomass or specific hydrographic features, along a section based on real time sensor output. The algorithm has been tested in the laboratory with data sets collected on earlier U-Tow trials.

Results

Sections showing temperature and chlorophyll concentration along the transect are shown in Figure 5a and b. Marked on Figure 5a are a subset (1 in 5) of the data points used to plot the section. The location of these points describes the flight path of the U-Tow along the section. The position of water samples obtained on board U-Tow using Aqua Monitor are marked on Figure 5b. Data on zooplankton abundance and species composition are given in Table 2 with Figure 6 showing the abundance of total zooplankton along the section. The cross hairs superimposed on the ships track (Figure 4) show the start and finish of consecutive U-Tow zooplankton samples starting at the east end of the section.

Although conditional sampling has not been implemented in our field trials Figure 7 shows an example of the application of the conditional sampling algorithm to a data set of chlorophyll fluorescence.

Discussion

The primary aim of this trial work was to assess the ability of U-Tow to make measurements of plankton distribution in relation to hydrography. In particular, there was a need to determine if the new suite of sensors and water sampler functioned alongside the plankton sampling mechanism. The results

confirm that we have successfully achieved our main objective in developing a new towed undulating plankton sampler which simultaneously measures environmental conditions.

The field studies have demonstrated the potential of this unique sampling device for improving our understanding of the relationship between plankton distribution and water column physics. The section studied traversed three different hydrographic regions in the Irish Sea as is apparent in Figure 5a. An initial 40 nautical mile (nm) section was thermally stratified, followed by a region of mixed waters (ca. 40 nm) with a final 20 nm section displaying limited stratification running into Liverpool Bay. The distribution of plankton biomass along the section appears to reflect these physical differences. Highest chlorophyll concentrations (Figure 5b) were found in the central mixed regions with lower concentrations in the eastern stratified region and Liverpool Bay. Total zooplankton abundance (Figure 6) showed an opposing trend with higher levels in the eastern stratified section and Liverpool Bay. Highest concentrations were found in Liverpool Bay which is known to be nutrient enriched and has the highest levels of primary production in the Irish Sea. The apparent inverse relationship between phytoplankton and zooplankton biomass is likely to reflect grazing pressure.

In order to progress we need to ensure comparability of data sets generated by the standard CPR and U-Tow. This will determine whether we can easily we can replace the CPR with U-Tow without compromising the integrity of the existing database. We also need to trial a fully equipped U-Tow on ships of opportunity to ensure that increased complexity has not lead to a loss of robustness. Finally we need to continue an associated programme developing an image analysis system to automate and reduce the potential extra cost to the survey associated with analysis of microplankton.

Conclusion

We have successfully developed a unique tool (U-Tow) for the measurement of plankton distribution and associated environmental conditions. Preliminary result demonstrate the potential of the system to provide new insights into plankton community structure in relation to hydrography. Future work will determine if U-Tow gives comparable results to the standard CPR with a view to its eventual replacement.

Acknowledgements

The authors wish to thank the officers and crew of the R.V. Lough Foyle. This work was supported by the UK Department of Environment, Transport and the Regions co-ordinated programme of marine research for the north-east Atlantic and by the Ministry of Agriculture, Fisheries and Food.

References

Aebischer, N.J., Coulson., and Colebrook, J.M., 1990. Parallel long term trends across four marine trophic levels and weather. Nature, 347, 753-755.

Gamble, J.C., 1994. Long term planktonic time series as monitors of marine environmental change. In Long-term experiments in agriculture and ecological sciences, Ed., R.A. Leigh and E. Johnston. Wallingford, CAB International, pp 365-386.

Mills, D.K., Walne, A., Reid P.C., and S.I. Heaney in press. Updating the Continuous Plankton Recorder - An Improved Tool for Integrated Plankton Monitoring. ICES J. Mar. Sci.

Reid, P.C., Edwards, J., Hunt, H. and Warner, A.J., 1998. Phytoplankton change in the North Atlantic. Nature, 391, p546.

Warner, A.J. and Hays, G.C., 1994. Sampling by the Continuous Plankton Recorder Survey. *Prog. in Oceanogr.* 34, 237-256.

Table 2. The abundance (numbers m^{-3}) and species composition of the zooplankton along a section in the Irish Sea in 1998. The zooplankton were sampled with the Plankton Sampling Mechanism on board U-Tow fitted with a nylon filter (200 µm mesh size). The start and end position of sampling along the section is given in nautical miles.

Sample start	0	1.93	3.85	7.7	11.6	17.3	19.25	21.5	33.6	41.3	43.00	49.1	55.25	60.5	71	75.9	88.23	98.08
Sample end	1.93	3.85	7.7	11.6	17.3	19.3	21.45	25.9	39.1	43	49.13	55.3	60.50	65.8	73.5	82.1	95.62	100.5
COPEPODS																		
Centropages	0.09			0.21	0.12	0.09	0.47		0.50	0.07	0.09	0.08	0.04	0.04	0.11	0.21	0.42	1.67
Temora	0.31		0.09	0.27	0.65	0.58	1.33	0.16	0.44	0.11	0.16	0.13	0.06	0.11	0.15	0.10	0.33	2.79
Acartia	0.80	0.89	2.05	2.26	3.33	3.88	5.93	0.78	2.62	0.24	0.25	0.12	0.09	0.04	0.18	0.59	1.95	4.68
Para/ Pseudocalanus	0.04	0.18	0.27	0.69	1.55	0.98	3.67	0.28	0.62	0.07	0.09	0.10	0.06	0.06	0.20	0.35	0.28	0.84
Calanus			0.09	0.14	0.24	0.13	0.47	0.03		0.02	0.01	0.01						
Oithona	0.45	0.36	0.62	1.10	1.67	0.71	2.03	0.47	1.12	0.08	0.02	0.01	0.01	0.01	0.03	0.13	0.28	1.05
Copepod nauplii	0.36	0.09	0.18	0.48	0.54	0.62	2.11	0.53	0.25	0.06	0.03	0.04	0.02	0.04	0 01	0.04	0.51	1.05
Total copepods	2.10	1 52	3.57	5.15	8.09	7.01	16.00	2.25	5.56	0.66	0.66	0.52	0.32	0.35	0.69	1.41	3.91	12.14
OTHER ZOOPLANKTON																		
Larvacean				0.34	0.30	0.67	1.41	1.19	1.06	0.04	0.04	0.10	0.01	0.02	0.01	0.03		2.58
Echinoderm larvae	8.61	10.62	11.06	0.82	0.36	0.13	0.23		0.25	0.03	0.02	0.01	0.02		0.01	0.56	0.28	1.54
Cladocerans	0.04			0.07	0.24	0.36	0.16	0.03		0.01	0.01				0.24	0.74	6.05	11.44
Total other zooplankton	10.58	11.78	13.11	3.02	1.78	1.61	3.36	1.56	2.25	0.18	0.46	0.32	0.31	0.36	0.67	1.87	7.26	18.77
TOTAL ZOOPLANKTON	12.67	13.29	16.68	8.17	9.88	8.61	19.36	3.81	7.81	0.83	1.12	0.83	0.62	0.71	1.36	3.28	11.16	30.91

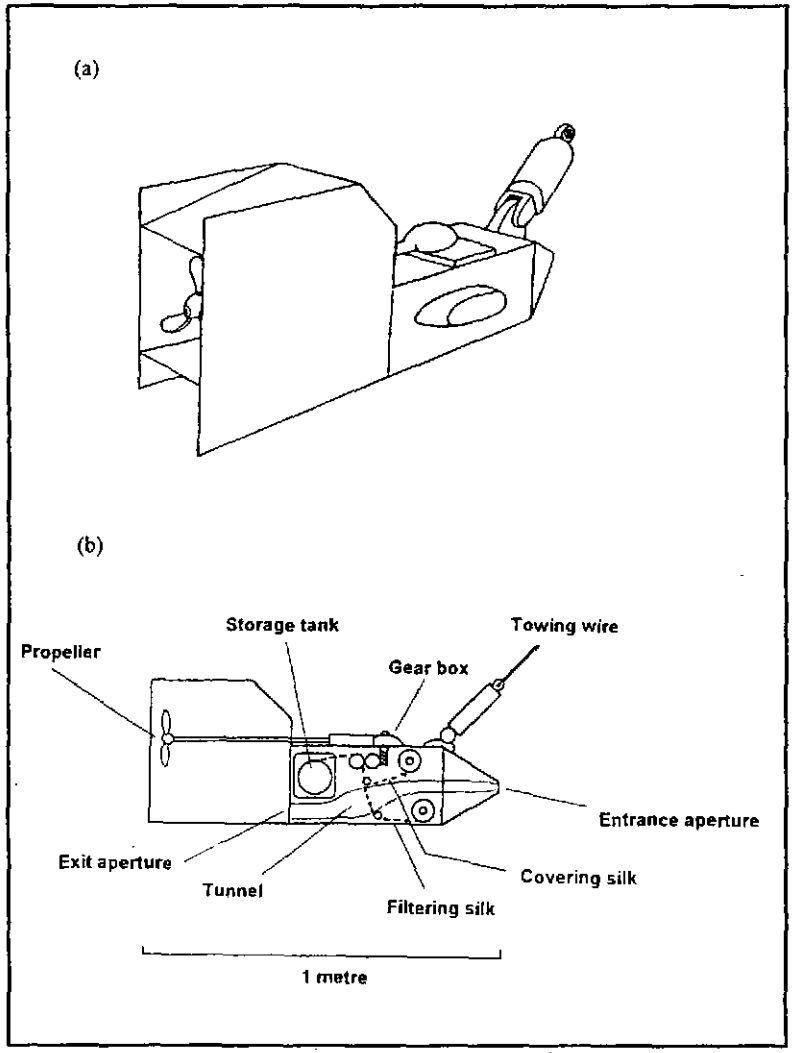


Figure 1. The Continuous Plankton Recorder comprising of (a) a metal towed body capable of use on ships of opportunity and (b) the plankton sampling mechanism. After Warner and Hays, 1994.

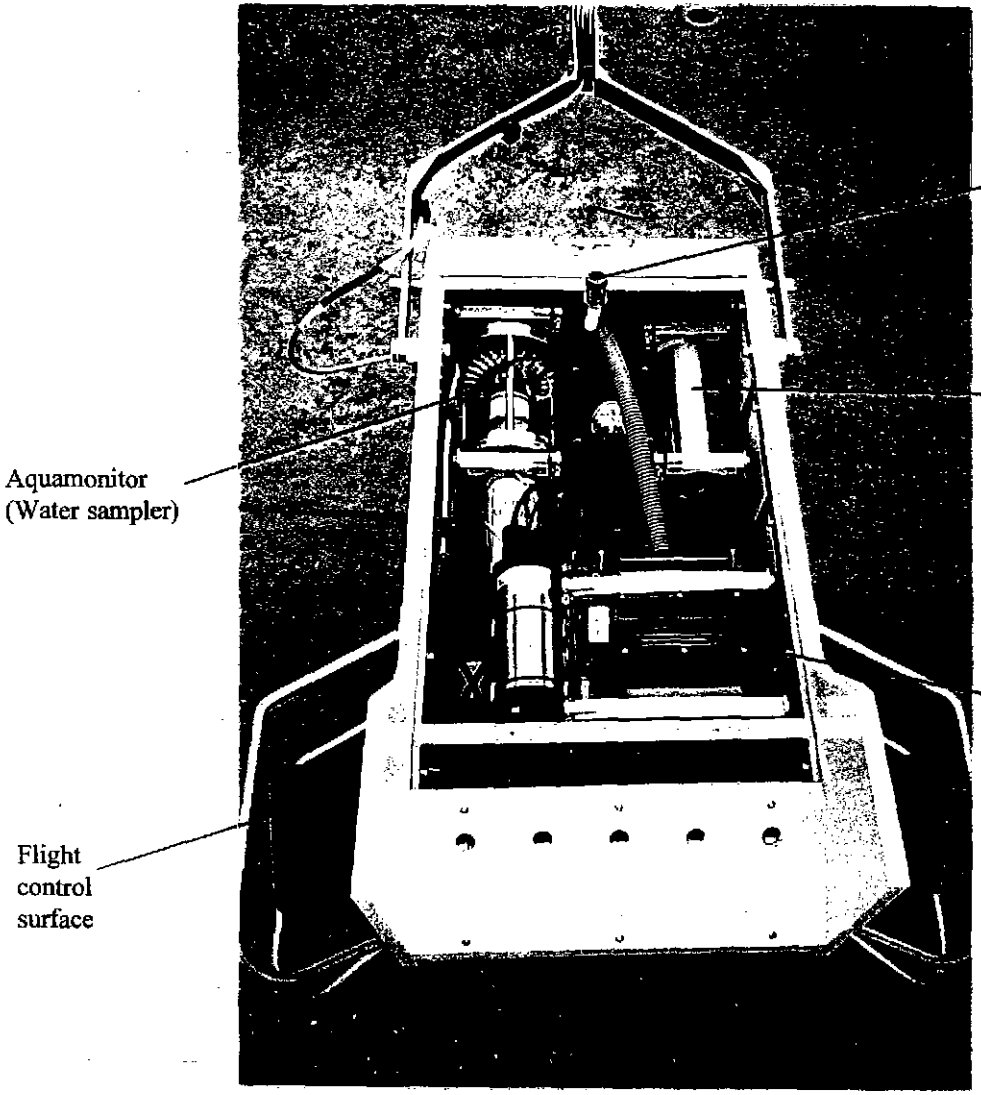


Figure 2. U-Tow prior to deployment (a) and with upper panel removed (b) exposing payload. Instrumentation includes undulation control and data acquisition systems, Plankton Sampling Mechanism, Aqua Monitor, CTD, fluorometer, optical backscatter sensor (turbidity) and a Li-Cor PAR sensor.

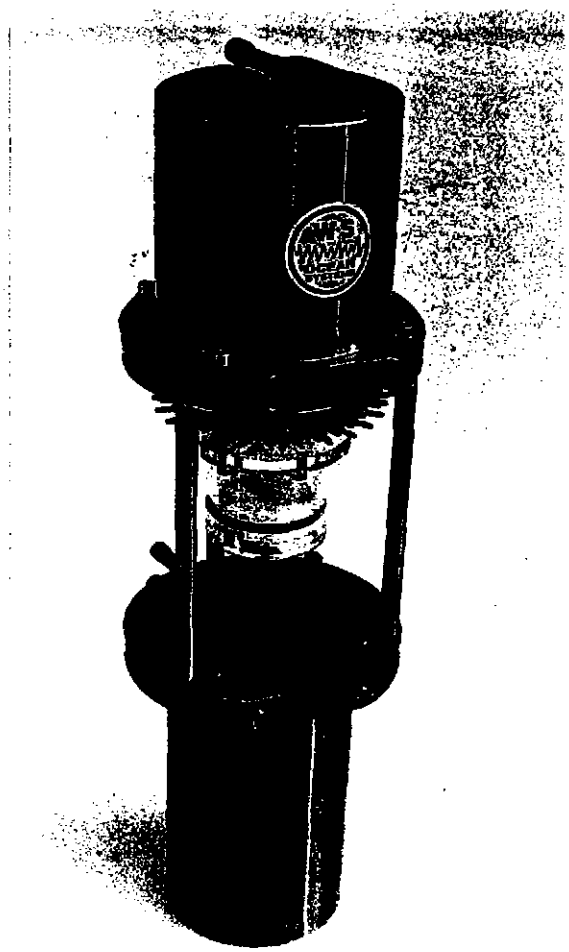


Figure 3a. Aqua Monitor - an 'Intelligent' water sampler capable of collecting up to 50 water samples of 150 ml each.

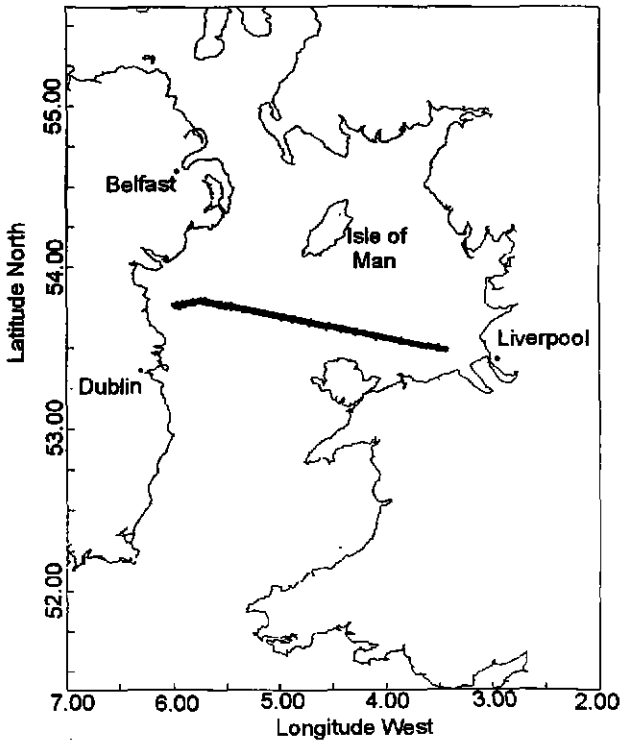


Figure 4. Map of the Irish Sea showing the location of the U-Tow section carried out on 18 June 1998.

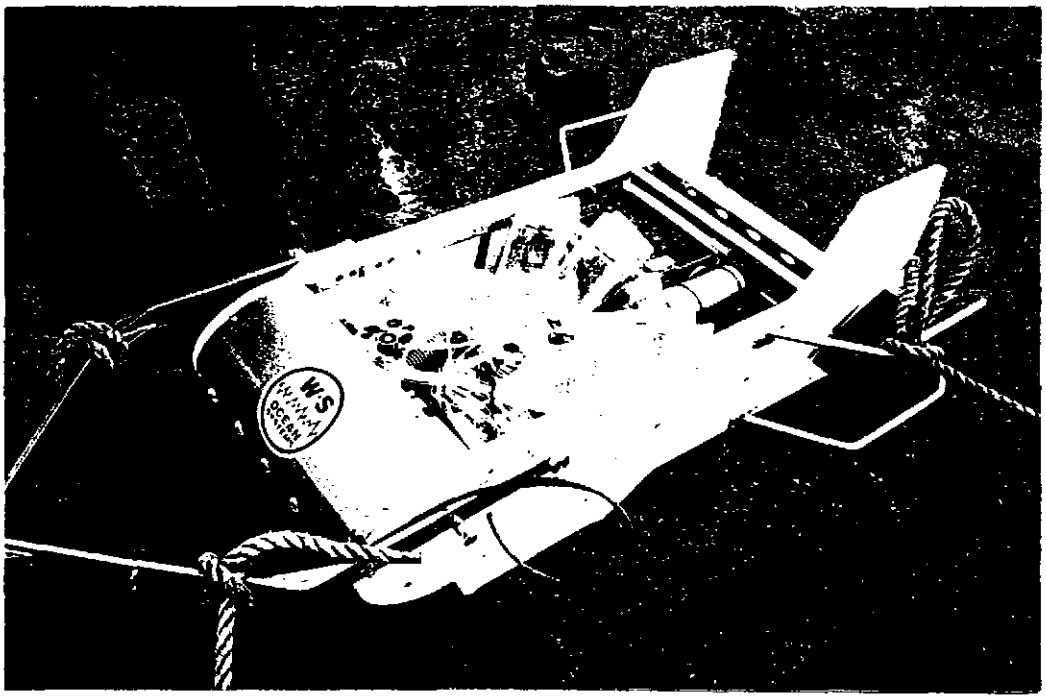


Figure 3b. Filled sample bags shown after recovery of U-Tow. Sample containers are 250 ml capacity blood transfusion bags which are packed into the U-Tow instrument bay. The bags may be loaded with a suitable preservative for storage of samples on longer tows.

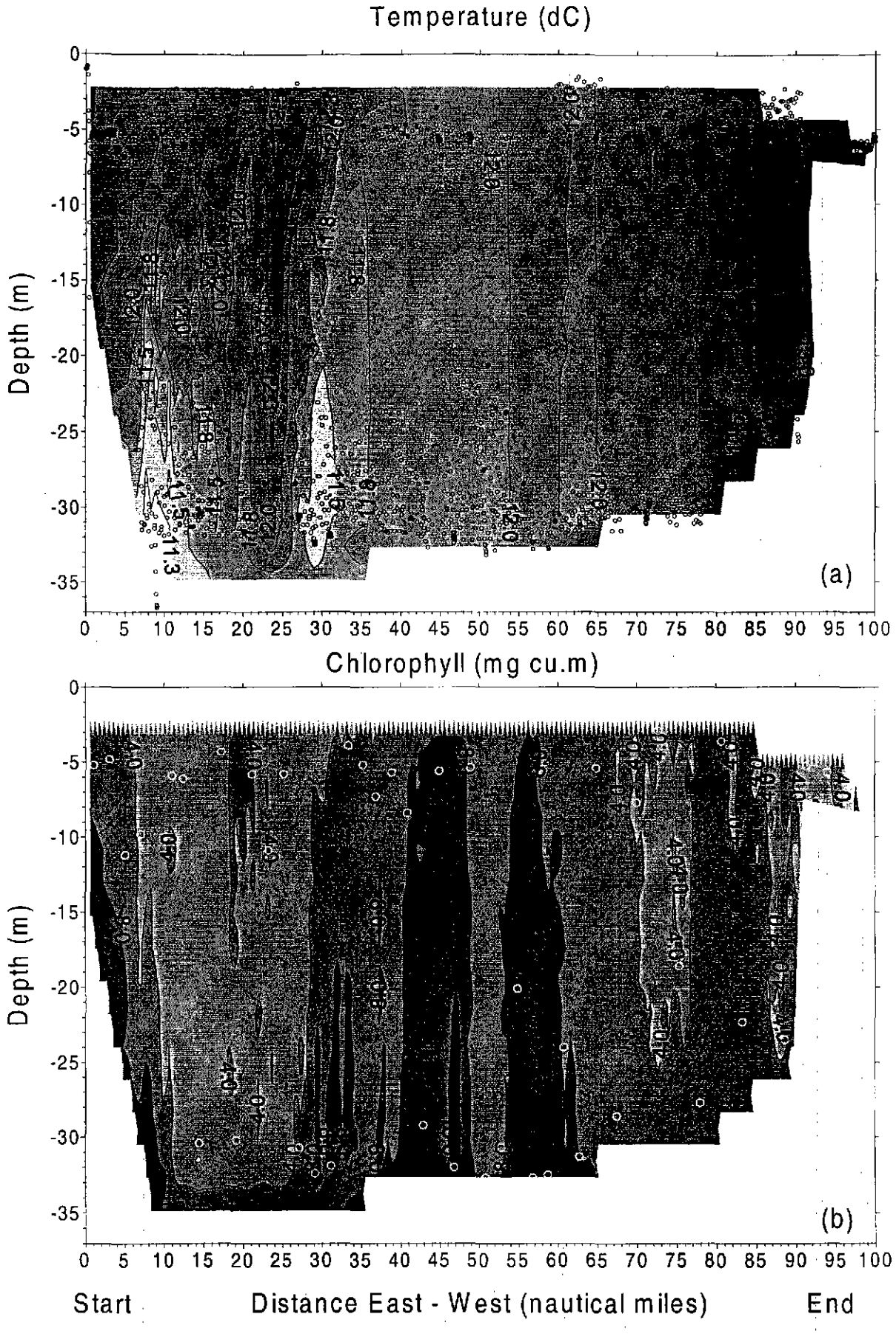


Figure 5. Section showing (a) the distribution of temperature (dC) with the open circles indicating a subset (1 in 5) of the data points and (b) the distribution of phytoplankton biomass (chlorophyll mg cu. m) on 18 June 1998. The chlorophyll concentration was derived from in vivo measurements of fluorescence after calibration of the fluorometer against known concentrations of chlorophyll extracted from seawater samples collected on board ship. The location of Aqua Monitor water samples are shown as white filled circles.

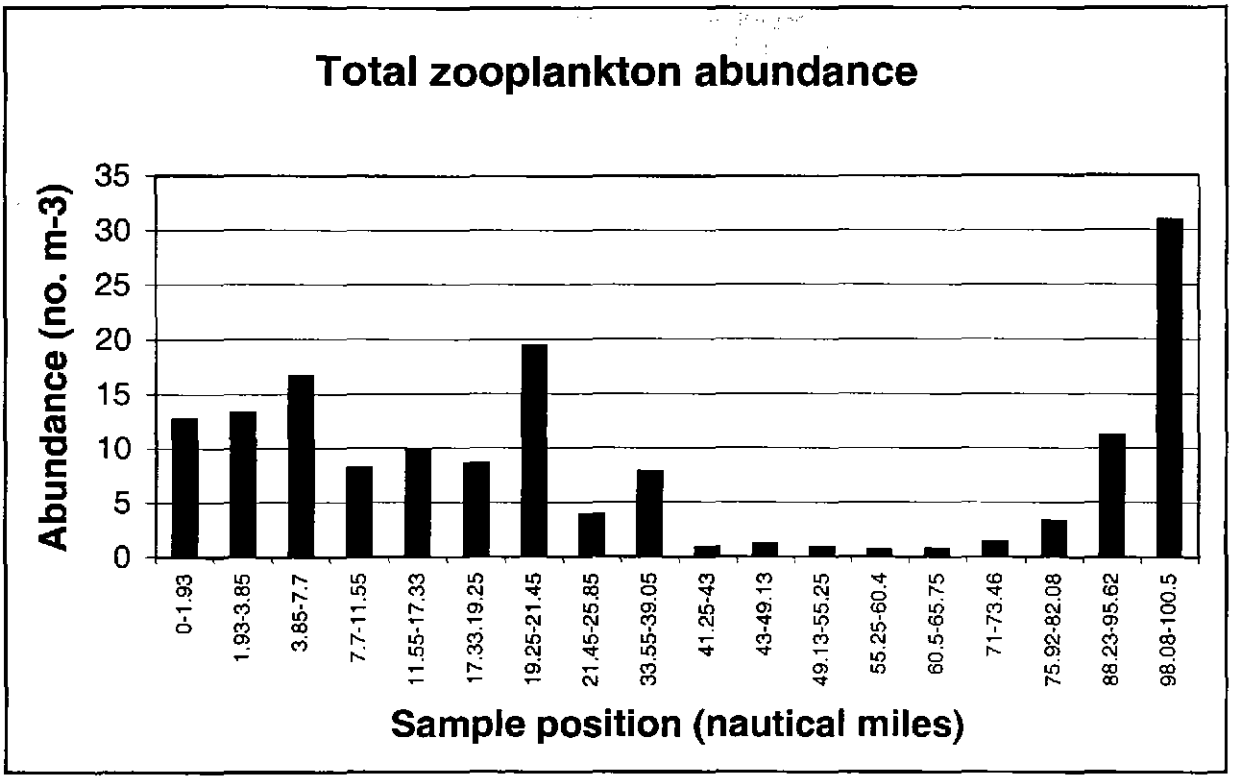


Figure 6. The distribution of total zooplankton biomass along the section. Each observation is based upon measurements derived from the Plankton Sampling Mechanism with the start and finish position along the section, in nautical miles, indicated on the x-axis. The location of each consecutive tow is indicated on Figure 4.

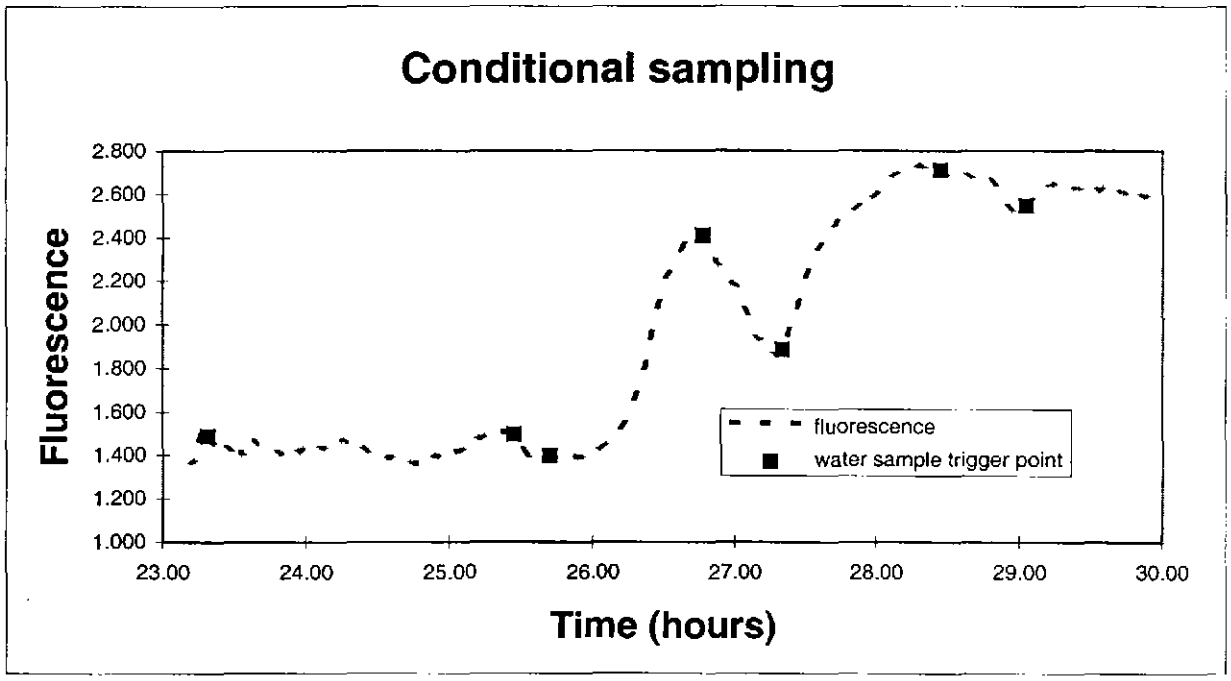


Figure 7. A record of chlorophyll fluorescence obtained during a U-Tow deployment is used to demonstrate the location of Aqua Monitor samples (shown in squares) that would have been collected by employing the conditional sampling algorithm (see text). Successful implementation of this protocol will allow automated sample collection during unattended U-Tow deployments, for example on ships of opportunity.

ANNEX 7

NEW DEVELOPMENTS IN INTEGRATED ECOSYSTEM MONITORING – UPDATING THE CONTINUOUS PLANKTON RECORDER

New Developments in Integrated Ecosystem Monitoring - Updating the Continuous Plankton Recorder

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

Since its introduction over 60 years ago the Continuous Plankton Recorder (CPR) survey has provided the international community with a unique ocean basin scale monitoring programme (Gamble, 1994). The survey has provided a yardstick against which changes in the planktonic ecosystem, a most sensitive indicator of marine environmental change, can be assessed (Aebischer *et al.*, 1990). Reid *et al.*, (1998) recently published observations which show the first evidence of a climate induced response in surface marine plant communities with implications for CO_2 fluxes and productivity in the North Atlantic. One of the strengths of the survey is that it has changed little since Sir Alister Hardy introduced it in 1931. However, despite its success there is a need to modernise in order to meet the needs of future monitoring programmes and to take advantage of new sampling and sensor developments. There are a number of initiatives currently underway described by Mills *et al.* (1998). To update the programme and also develop an integrated monitoring programme combining the CPR survey and smart moorings.

The CPR comprises a brass bodied underwater vehicle (Figure 1a) which is towed in horizontal flight at about 10 m depth behind Merchant ships on their normal routes of passage. Each CPR includes a plankton sampling mechanism (Figure 1b) incorporating a continuously advancing silk gauze (270 µm mesh size) which filters plankton from the seawater and preserves it for later examination. Although soft-bodied or vertically migrating plankton may not be sampled effectively the CPR obtains good samples of most medium sized zooplankton and large phytoplankton.

Figure 1. *The Continuous Plankton Recorder comprising of (a) a metal towed body capable of use on ships of opportunity and (b) the plankton sampling mechanism. After Warner and Hays, 1994.*

A joint SAHFOS, CEFAS and DANI programme has undertaken the development of a new system. In order to collect samples over a wider depth range a new undulating towed body (Figure 2; U-Tow, W.S. Ocean Systems Ltd.) that been developed with increased payload capacity. An 'intelligent' water sampler (Figure 3a; Aqua Monitor, W.S. Ocean Systems Ltd.) has been developed in order to quantitatively sample small plankton (< 270 µm). The increased payload allows U-Tow to carry a range of environmental sensors (Table 1) together with Aqua Monitor alongside a Plankton Sampling Mechanism (PSM; Figure 2).

Figure 2. *U-Tow prior to deployment with upper panel removed exposing payload. Instrumentation includes undulation control and data acquisition systems, Plankton Sampling Mechanism, Aqua Monitor, CTD, fluorometer, optical backscatter sensor (turbidity) and a Li-Cor PAR sensor.*

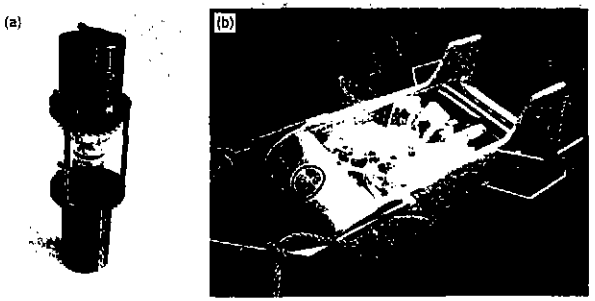


Figure 3. *Aqua Monitor (a) an 'intelligent' water sampler capable of collecting up to 50 water samples of 150 ml each. Filled sample bags shown after recovery of U-Tow (b). Sample containers are 250 ml capacity blood transfusion bags which are packed into the U-Tow instrument bay. The bags may be loaded with a suitable preservative for storage of samples on longer tows.*

Initial trials of U-Tow in this configuration have been undertaken in the central Irish Sea. Simultaneous observations of hydrography and plankton distributions were made on board the R.V. Lough Foyle along a transect from Dundalk Bay in the western Irish Sea to Liverpool Bay in June 1998 (Figure 4). Some results are shown in Figure 5. Water samples were collected on board U-Tow with Aqua Monitor (Figure 3b) for determination of phytoplankton numbers and species composition, measurement of chlorophyll concentration and determination of plant nutrient concentrations. Aqua Monitor and the PSM were operated in two modes. Samples were collected according to a pre-set time interval whilst undulating or manually triggered from the surface in regions of interest determined from the real time displays of vertical distribution of temperature, salinity and chlorophyll fluorescence. In the latter sampling mode U-Tow was held in level flight for the duration of Aqua Monitor sampling. The range of measurements obtained in this configuration are shown in Table II.

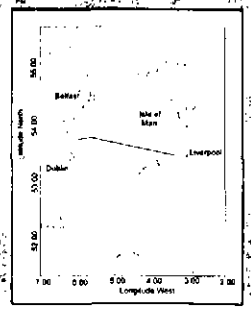
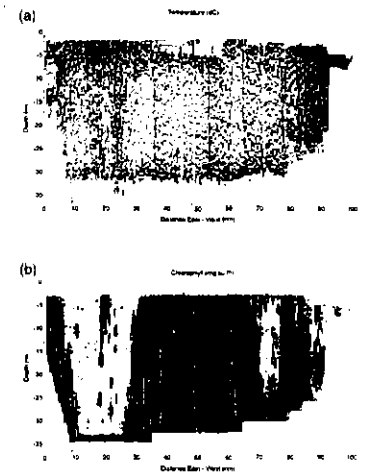


Figure 4. *Map of the Irish Sea showing the location of the U-Tow section carried out on 18 June 1998.*

Sensor/sampler	Measurement
Plankton Sampling Mechanism (PSM)	zooplankton biomass and species composition
Aqua Monitor	phytoplankton biomass and species composition
	plant nutrient (N,P, Si) concentration
	extracted chlorophyll concentration
CTD	temperature, salinity, density
Fluorometer	chlorophyll concentration
Optical backscatter sensor	suspended load
LI-Cor PAR sensor	downwelling irradiance, vertical attenuation coefficient

Table 1. *U-Tow sensor and sampler payload and the measurements derived from them.*

Figure 5. *A section showing (a) the distribution of temperature (°C) with the open blue circles indicating a subset of the data points and (b) the distribution of phytoplankton biomass (chlorophyll mg m⁻³) on 18 June 1998. The chlorophyll concentration was derived from in vivo measurements of fluorescence after calibration of the fluorometer against known concentrations of chlorophyll extracted from seawater samples collected on board ship. The location of water samples obtained with Aqua Monitor are shown as filled red circles.*



A novel component of this programme will be the introduction of conditional sampling for operation of Aqua Monitor. An algorithm has been developed which allows the detection of regions of interest, for example peaks in chlorophyll concentration (Figure 6) or hydrographic features, determined from environmental data collected in real time. Consequently, it will be possible to automatically collect water samples dependent upon sets of predefined conditions.

In these trials, we have successfully demonstrated the operation of a unique and powerful new tool for mapping the distribution of plankton in relation to environmental conditions. It combines the strengths of the CPR approach proven over many decades, with advances in sampling and sensor technology to provide the ideal system for measuring and monitoring the health of the oceans.

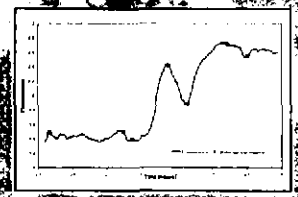


Figure 6. *[illegible] of chlorophyll fluorescence [illegible] U-Tow deployment. A [illegible] the detection of [illegible] Aqua Monitor samples (filled circles) were collected [illegible] conditional sampling [illegible] Successful implementation of this procedure will allow [illegible] sample collection during [illegible] U-Tow deployments, for [illegible] or biodiversity.*

Acknowledgements

The authors would like to thank the officers and crew of the RV Lough Foyle. This work was supported by the UK Department of Environment, Transport and the Regions co-ordinated programme of marine research for the northeast Atlantic and the Ministry of Agriculture, Fisheries and Food (A1108).

References

Aebischer, N.J., Coulson, and Colebrook, J.M. 1990. Parallel long-term trends across four marine trophic levels and weather. Nature, 347, 753-755.

Gamble, J.C. 1994. Long term planktonic time series as monitors of marine environmental change. In Long-term experiments in agricultural and ecological sciences. Ed., R.A. Leigh and E.J. Johnston. Wallingford, CAB International. pp 365-386.

Mills, D.K., Walne, A., Reid, P.C., and S.I. Heaney, In press. Updating the Continuous Plankton Recorder - An Improved Tool for Integrated Plankton Monitoring. ICES J. Mar. Sci. 55.

Reid, P.C., Edwards, J., Hunt, H. and Warner, A.J. 1998. Phytoplankton change in the North Atlantic. Nature, 391, p546.

Warner, A.J., and Hays, G.C., 1994. Sampling by the Continuous Plankton Recorder survey. Prog. in Oceanogr. 34, 237-256.

[1]*Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft Laboratory, Lowestoft, Suffolk NR33 0HT UK*
[2]*Sir Alister Hardy Foundation for Ocean Science (SAHFOS) The Laboratory, Citadel Hill, Plymouth PL1 2PB*
[3]*Agricultural and Environmental Science Division Department of Agriculture (DANI), Belfast BT9 5PX*
[4]*W.S. Ocean Systems Ltd., Omega Park, Alton, Hants, GU34 2QD.*

CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

ANNEX 8

THE CONTEXT OF ENVIRONMENTAL CHANGE ON THE EUROPEAN SHELF

R. R. Dickson, CEFAS, Lowestoft

To establish whether their findings are representative in space and time, it is clear that short-term assessments of environmental or ecological conditions must be set into the context of longer-term environmental variability. While this would be true of most times and locations, it is particularly important in the case of environmental changes of the Atlantic Sector over the last three or four decades, where the behaviour of the main atmospheric forcing has amplified to exceed the range of past experience in a Century-long record. Many of the effects of this climatic shift are focused particularly on the northeast Atlantic and European shelf.

The North Atlantic Oscillation (NAO) is the dominant recurrent mode of atmospheric behaviour in the North Atlantic Sector and one of the most robust on Earth. Over the period of the instrumental record, (since 1865), the NAO has exhibited considerable long-term variability which appears to be amplifying with time. Thus the 1960s experienced the most extreme and protracted negative phase of the NAO Index and the late 1980s/ early 1990s experienced its most prolonged and extreme positive phase.

This talk focused on aspects of the Ocean's response which appear to be of particular relevance to ecosystem of the European shelf. It examined first the effect of the amplifying NAO on the track, number and intensity of winter storms and thus on the winter wave climate, drawing implications for the timing of marine phytoplankton production. It described the changing pattern of Atlantic heat exchange and the resulting changes in sea-surface temperature, with possible implications for the recruitment to local commercial fish stocks. It described direct and indirect effects on ocean currents, both at the pan-Atlantic scale, where the main Atlantic gyre circulation appears to have strengthened by 30% in parallel with the NAO, and at the regional scale where the transport through the Faroe-Shetland Channel also increased to a record level. Finally, it described the effect of the amplifying NAO on the distribution and amount of winter rain both at the Atlantic scale and at the scale of individual UK regions, underlining the fact that the maximum contrast in precipitation anomaly between NW Scotland and SE England is as high as ±~30% of normal.

The talk lastly described the case for associating the NAO and its decadal variability with Global Change. The link is justified by the fact that NAO variability explains around 34% of the variance in Northern Hemisphere extra-tropical temperatures since 1935. However we cannot assume that this contribution has been constant with time or that the recent amplification of the NAO is anthropogenic. In fact, a recently-derived long-term proxy of winter NAO variability suggests that similar long period shifts in the NAO have been a regular if rare feature of the Atlantic climate over the past 500-600 years.

ANNEX 9

BIOSENSORS FOR ENVIRONMENTAL MONITORING AND THEIR PARTICULAR RELEVANCE TO HARMFUL ALGAL–BLOOM DIRECTION

Slike Kroeger, CEFAS, Lowestoft

A biosensor is an analytical device incorporating a biological or biologically derived sensing element either intimately connected to or incorporated within a physicochemical transducer or transducing microsystem.

A wide range of components can be selected from a list of sensing elements, for example cells, enzymes, receptors, antibodies and nucleic acids, and transducers, such as optical, electrochemical, acoustic, thermometric and magnetic devices, to develop the system most appropriate for a particular application. Generally biosensors can be divided into metabolic, inhibition and affinity sensors depending on the type of analyte/sensing element interaction they target. The format of the biosensor can range from disposable dipstick for single measurements to complex instrumentation with integrated liquid handling and data processing for continuous monitoring.

Biosensors have numerous advantageous features for example the high selectivity and sensitivity of biological reactions, the use of the relevant target (biology) in pollution studies prior to a more detailed chemical analysis and the potential for automation and miniaturisation. They can afford reduced sample handling leading to high sample throughput at lower cost. In order to be routinely applicable a number of issues will have to be addressed further such as the long-term stability of the sensing element, (bio)-fouling of the transducer, robustness of instrumentation, reproducibility and in some cases the deconvolution of complex signals.

Numerous sensors have been developed for environmentally relevant analytes and some EC funded projects are currently striving to develop sensors for nutrients, algal toxins and toxic algae that could be of future use for marine HAB monitoring (for example "Algaetox" and "Aquasense" in the Climate and Environment programme).

At CEFAS an attempt is made to convert the observations made by Chris Scholin *et al.* (J. Phycol, 30, 1994) concerning a correlation between phylogenetic lineage and observed toxicity in *Alexandrium sp.* into an initially lab-based assay for the detection of toxic algae in shellfish. The procedure relies on DNA extraction and specific amplification using nested PCR. It is envisaged that by detection of specific nucleic acid hybridisation progress can be made towards a more automated system for use at sea.

Such a device would be useful as an early warning system for the presence of potentially toxic algae, but would not allow the quantification of the algal toxin. It therefore appears desirable to integrate toxic alga detectors with algal toxin measurements to maximise the information obtained. Sensor formats under consideration for the detection and/or quantification of toxins are for example immunosensors or sensors based on immobilised receptors from the nervous membrane which are the natural targets of PSP toxins such as saxitoxin.