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Project synopses

EurOCEAN 2000

The European Conference on Marine Science and Ocean Technology

Hamburg, 29 August - 2 September 2000

Vol. I: Marine processes, ecosystems and interactions

ENERGY, ENVIRONMENT
AND SUSTAINABLE DEVELOPMENT

EUR 19359

EurOCEAN 2000 — *The European Conference on Marine Science and Ocean Technology*
Vol. I: Marine processes, ecosystems and interactions

15
KI-NA-19359-EN-C

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A great deal of additional information on the European Union is available on the Internet.
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Cataloguing data can be found at the end of this publication.

Luxembourg: Office for Official Publications of the European Communities, 2000

ISBN 92-828-9713-3

Printed in Italy

PRINTED ON WHITE CHLORINE-FREE PAPER

TABLE OF CONTENTS (Volumes I - II)

Volume I

Marine Processes, Ecosystems and Interactions

I.1. Marine systems research

I.1.1. Circulation and exchange of water masses

TITLE : VARIABILITY OF EXCHANGES IN THE NORTHERN SEAS : **VEINS**

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VARIABILITY AND EXCHANGES IN THE NORTHERN SEAS (VEINS)

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INTRODUCTION

Exchanges between the North Atlantic and the Arctic Ocean result in the most dramatic water mass conversions in the World Ocean: Warm and saline Atlantic waters flowing through the Nordic Seas into the Arctic Ocean are separated by cooling and freezing into shallow fresh waters (and ice) and saline deep waters. The outflow from the Northern Seas to the south provides the initial driving of the global thermohaline circulation cell, the one to the north is of major impact to the large scale circulation of the Arctic Ocean. Measuring these fluxes is a major requirement to quantify the turnover-rates within the large circulation cells of the Arctic and the Atlantic Oceans and a basic condition to understand the role of these ocean areas in climate variability on interannual to decadal scales.
There are several national, European and international research projects focussing on processes relevant to forming the characteristics of the individual regions of the Northern Seas. However, a study on the climate control of the Northern Seas primarily requires information on integrated fluxes from the Atlantic Ocean through the Nordic Seas into the Arctic Ocean and vice versa. This can be achieved by obtaining synoptic time series of water and property transports through the key passages. In the past three years the VEINS-project has carried out a concerted effort of both sea-going and modelling activities to obtain the information needed.

OBJECTIVES

The general objective of VEINS was to measure and to model the variability of fluxes between the Arctic Ocean and the Atlantic Ocean with a view on implementing a longer term system of critical measurements needed to understand the high-latitude oceans steering role in decadal climate variability.
This objective is based on the fact that interannual to decadal climate variability has an important cause in the water mass conversion processes of the Northern Seas, the effects of which are propagated by the fluxes through the boundaries into the global ocean.
Therefor the ultimate goal is to obtain time series measurements of the relevant oceanic fluxes up to decadal period. However, such a long-term commitment can only be taken up after a conceptual model on the fluxes and their variability has been established to answer the following questions: - What are the relevant parameters? - Do we have the most efficient method of measuring them? - Do we have the modelling tools to assist in measurement interpretation? - Do we have an optimal experimental design for such a long-term activity?

This set of questions lead to the VEINS-specific objectives.

- To obtain time-series of heat, salt and water fluxes for the exchange routes through the Northern Seas, i.e. between the Atlantic Ocean and the Nordic Seas and between the Nordic Seas and the Arctic Ocean.
- To quantify the magnitude of the variability of these fluxes.
- To improve the understanding of processes responsible for the variability.

- To develop a conceptual model of exchanges and water mass alterations between the Atlantic Ocean and the Arctic Ocean. It will be used to estimate the integrated effect of the exchanges from the four measurement areas and to design an optimised measurement programme to be continued up to a decadal period.

METHODS

The general methods were:

- Repeat hydrographic sections:

Physical and chemical parameters with CTD/Rosette for 4-6 times per year along the lines Greenland/Svalbard/Norway and Greenland/Iceland/Faroes/Shetland.

- Special hydrographic cruises:

Physical parameters and tracers (CFC's, SF6, O18) with special emphasis on the Iceland-Scotland and the Denmark Strait overflows.

- Current measurements:

Deployment of long-term moored current meters across the passages, partly with additional high-quality salinity sensors. Deployment of long-term moored upward looking sensors to establish the thickness-variability of the overflows. Acoustic Doppler Current Measurements from ships operating the repeat-sections

- Modelling:

Numerical and analytical modelling of flows through the individual passages in order to provide for means of intelligent interpolation between the discrete current measurements (Regional Models). Numerical , analytical and rotating hydraulic modelling of processes controlling the flow through passages (Process Models). Numerical circulation model (including ice) with real wind-forcing to understand the controls of the transport variations through the passages (Large Scale Circulation). Numerical circulation model (including ice) with real wind-forcing to derive a conceptual model of the observed transport variability for the project synthesis and to design a monitoring scheme, for the exchanges in the Northern Seas (Conceptual Model).

RESULTS

Analysis of fluctuations will be based on long-term synoptic measurements of fluxes through all major passages between the Arctic Ocean and the North Atlantic and numerical modelling. The current meter arrays for the flux measurements are still in the water, so that the full 3 years time series are not yet available at present. However, for several locations the VEINS measurements are continuations of already existing time-series. Their analysis has revealed a first core result (Dickson et al., 1999): decadal variations in the flux of near surface Atlantic Water observed west of Spitsbergen and found to be closely correlated to the North Atlantic Oscillation (NAO), i.e. the fluctuations of North Atlantic cyclone track, were observed to have propagated down the East Greenland Current and into the deep overflow south of the Denmark Strait. The travel time of the signal was three years. This result is a first proof of a rapid and effective coupling between the variability of the poleward surface limb and the equatorward cold and deep limb of the North Atlantic overturning circulation. Model results (Gerdes, 2000) have confirmed both the path and the speed of the NAO induced signals. Based on this the ongoing analysis phase of the Project is aimed at identifying prominent signals in the VEINS time series of currents and stratifications, and by following the model prescribed propagation paths, trying to verify the spatial coherence pattern. This is expected to lead into the definition of an optimum monitoring array for a longer term monitoring of Arctic – North Atlantic exchanges.

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TITLE : TRACING THE WATER MASSES OF THE NORTH ATLANTIC AND THE MEDITERRANEAN (**TRACMASS**)

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TRACING THE THERMOHALINE CONVEYOR WITH LAGRANGIAN TRAJECTORIES IN OCEAN GENERAL CIRCULATION MODELS

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ABSTRACT

TRACMASS uses a new method that employs Lagrangian trajectories to investigate the North Atlantic and Mediterranean water mass circulation as they result from numerical simulations of the global ocean. Specifically, the major goals of the project are:
A. The origin and formation of the North Atlantic Deep Water (NADW).
B. The fate and transformation of the North Atlantic Deep Water.
C. The Mediterranean Water (MW) mass circulation.
D. Theoretical studies of the trajectory methods.

Simulations from four different general circulation models is used. Two high resolution Cox-Bryan type (OCCAM and MEDMOM), one C-grid model with depth coordinates (OPA) and one isopycnic model (GIM). They are all global except MEDMOM, which is only applied over the Mediterranean. The project is divided into 4 parallel sub-projects, which are all linked and dependent on each other.
Qualitative and quantitative studies of the formation and transformation of the NADW and its origins is performed in subprojects A and B. The NADW is probably the most important water mass the ocean produces in terms of impact on the world ocean thermohaline circulation and the climate. The NADW forms the deep part of the World Thermohaline Conveyor Belt that redistributes the heat and salt in the ocean on a global scale. The NADW is transformed into other water masses by ventilation (60%) along isopycnals in the Southern Ocean by approximately 40% by diffusion through the thermocline in the tropics and other upwelling areas.

INTRODUCTION

The density difference between the cold and salty water of the north Atlantic and the fresher and warmer waters of the Pacific and Indian Oceans is believed to drive the largest meridional cell in the ocean, known as the Conveyor Belt (Broecker 1991). The route of the North Atlantic Deep Water (NADW), which is formed by convection at high latitudes, constitutes the deeper branch of the Conveyor Belt. Changes in the movement and characteristics of the NADW is of fundamental importance for understanding our climate, its history and any possible future states. The traditional view of deep water masses is that they slowly diffuse up evenly through the thermocline at low latitudes. More recently studies of the path of the North Atlantic Deep Water have indicated that the conversion takes to a large extent place at the surface after being upwelled along the isopycnals.

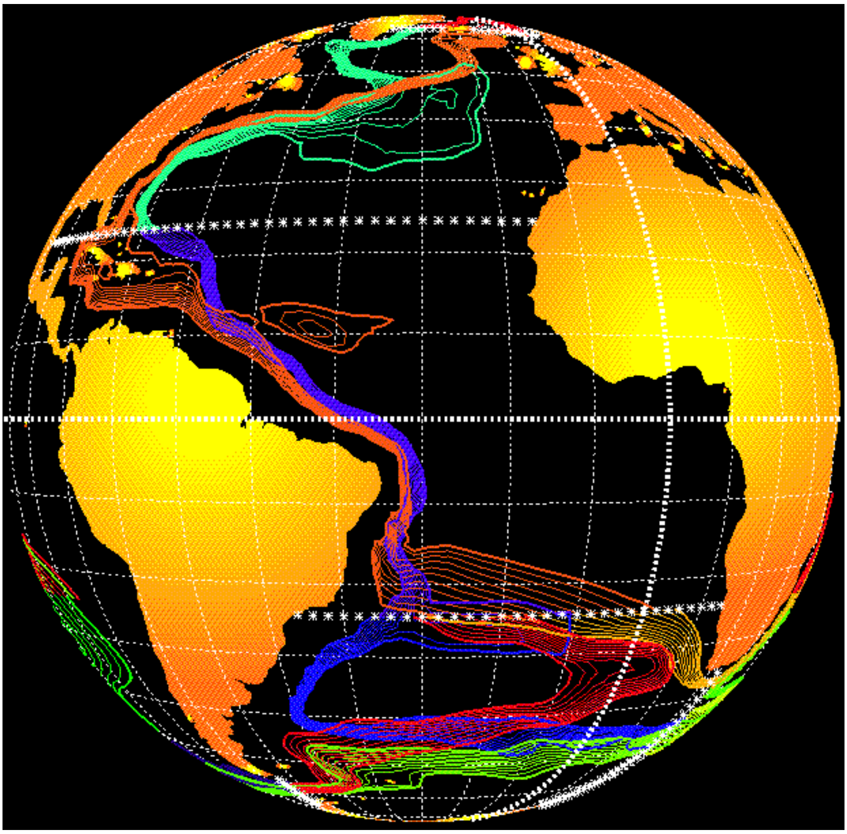

Figure 1. Schematic view of the dominant inter-basin water mass pathways in the Atlantic that are parts of the Conveyor Belt. Warm colours refer to near surface exchanges (mostly northward) and cold colours to deep transfers (mostly southward).

The upwelling behaviour of NADW and its connection to the upper branch of the Conveyor Belt have is studied in the TRACMASS project. According to common upwelling of deep water should occur via diffusion through the thermocline. This implies that upwelling is globally uniform. On the contrary, recent theoretical studies suggest that upwelling occurs southward of the Antarctic Circumpolar Current (ACC). Döös and Coward (WOCE Newsletter 27, 3-4, 1997) have shown that the major upwelling region of NADW is indeed the Southern Ocean. The NADW is characterised by a deep salinity maximum in a large part of the World Ocean, and by relatively high temperatures. The source of the high temperature and salinity is the warm and salty water of the NADW return flow in the thermocline.

The methods to calculate the Lagrangian trajectories used to trace the water masses will also be developed further and validated. The results from this will provide the tools needed for the 3 other sub-projects (A, B and C). Our Lagrangian tool has several advantages with respect to a more traditional model for transport of tracers, for which passive quantities are advected and diffused with sophisticated (and expensive) numerical schemes, and makes use of the fields (velocity, diffusivity) produced by an ocean GCM. Some of the strong points of the approach are: speed and accuracy in the computation of the trajectories, access to estimates of directional transports (for water flowing from one given oceanic section to another); and to backward integrations (for tracking water masses in the past). Various exciting studies have recently illustrated the power of the approach (Döös 1995, Blanke and Raynaud 1997).

RESULTS

The path of the Conveyor Belt in the Atlantic has been traced and is illustrated in Figure 1 in the Atlantic. The North Atlantic Deep Water can convert into shallower warm water either by isopycnic ventilation in the Southern Ocean, which is then converted into warmer water in the northward Ekman transport, or by tropical upwelling through the thermocline. For this purpose the NADW has been followed south from the Equator in the Atlantic until it is converted into a less dense water mass. The usual definition of NADW is that water which is denser than 1027.35 kg/m^3 (relative to the surface), however, for the current study, all water denser than 1027.625 kg/m^3 is defined as NADW. This, slightly denser, value was chosen by noting the turning point of the meridional overturning stream function, in isopycnic co-ordinates, at the Equator in the Atlantic. That is to say, water lighter than 1027.625 kg/m^3 has a net northwards transport at the Equator in the Atlantic, whereas denser water has a net southwards component. Thus, by tracking water until it is no longer denser than this value we are able to map out the deeper branch of the Conveyor Belt as it spreads outwards from the equatorial Atlantic to the rest of the world's oceans.

An important implication of this result is that the details of the ocean circulation in the Southern Ocean may be more important to climate studies than is traditionally considered to be the case. For example, if NADW outcrops south of the westerlies it will not be driven northwards and converted into a lighter water mass. The precise latitude at which the outcropping occurs is strongly dependent on the surface conditions. For ocean-only models, the surface conditions will rely on the quality and coverage of the observational data.
A quantification of where the NADW is converted into less dense water can be made by summing the volume transport at the isopycnal 1027.625 kg/m^3 for each horizontal model grid cell and then dividing by the area. A NADW upwelling velocity can hence be calculated (Fig. 2.). The major region of upwelling is the Southern Ocean where 9.3 Sv (59%) of the 15.8 Sv of NADW is ventilated along the isopycnals south of 50°S. Most of this is located in the southern part of the Antarctic Circumpolar Current. The second most important region of relatively high vertical velocities of the upwelling NADW is between 36°S and 38.5°S with 1.3 Sv (8%). This is the highly eddy active region of the Agulhas around South Africa where the NADW is converted by mixing with other water masses. The equatorial upwelling is clearly illustrated and is constrained to within a few degrees either side of the equator. The upwelling is only 1.1 Sv (7%) which is rather small compared to the traditional view of strong Equatorial upwelling. The rest of the water is more evenly upwelled over the ocean with stronger upwelling in regions of high eddy activity and western boundary currents. This seems contrary to the traditional view of a uniform upwelling through the thermocline and instead indicates that the upwelling of abyssal water occurs primarily near Antarctica as other recent studies indicate.

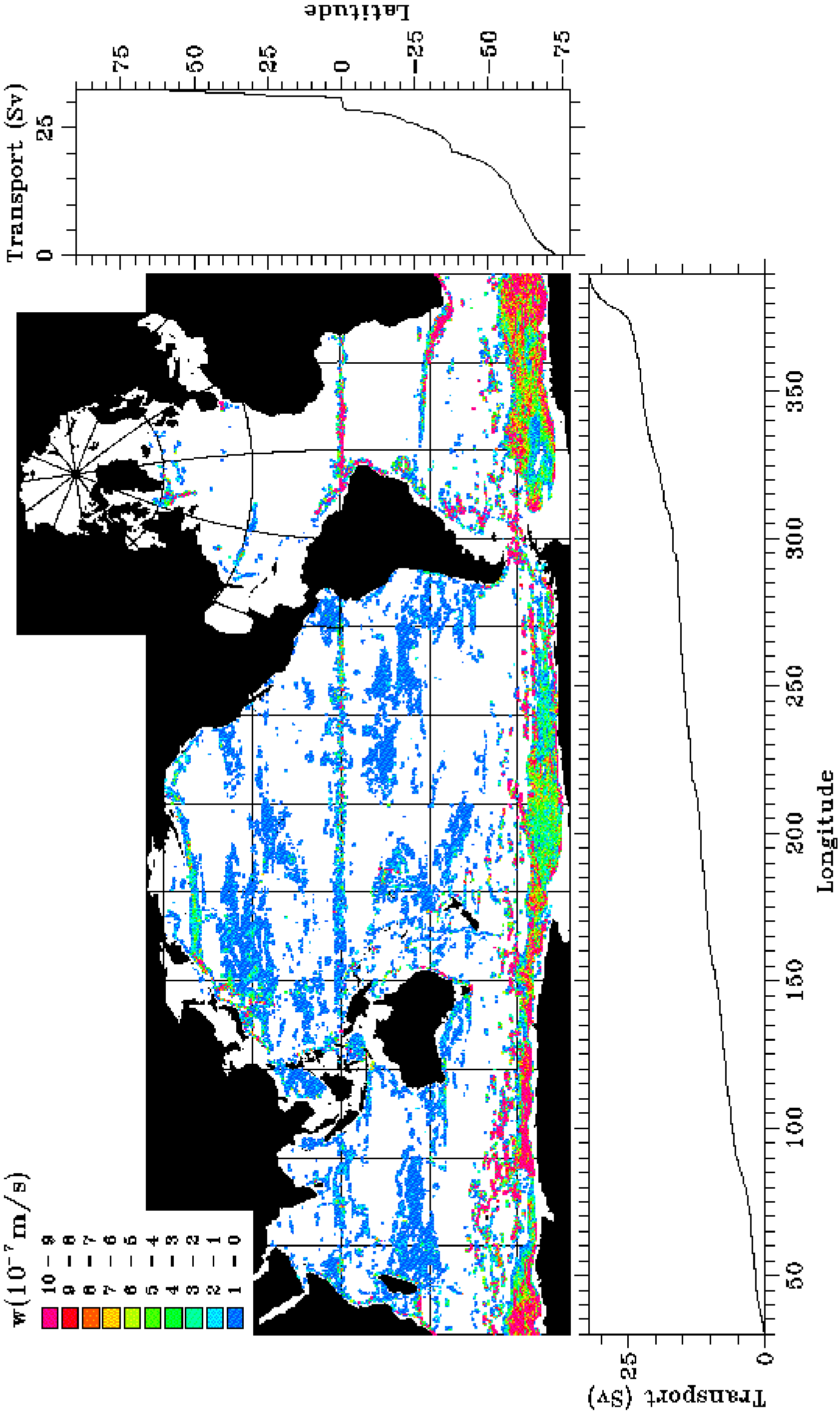

Fig. 2. The upwelling sites of NADW originally flowing southwards from the Atlantic equator are shown. Deep water is considered upwelled when its density reaches the value of 1027.625 kg/m^3 from below. Colours indicate the strength of the vertical upwelling velocity.

CONCLUSION

Simulations from four different general circulation models, is used: two high resolution "level" type models (OCCAM and MEDMOM), one model with topography following coordinates (OPA) and one isopycnic (density layers) model (GIM). They are all global except MEDMOM, which is only applied over the Mediterranean. The project is divided into 4 parallel sub projects, which are all linked and dependent on each other. Qualitative and quantitative studies of the formation and transformation of the NADW will be performed in subprojects A and B. Both the conditions of NADW formation and its deformation will be traced. At present, it is unclear whether the NADW is transformed into other water masses by ventilation along surfaces of constant density in the Southern Ocean or by diffusion through the thermocline in the tropics and other upwelling areas. The mass circulation in the Mediterranean, its time dependence and the MW influence on the formation of the North Atlantic Deep Water will be studied in subproject C. In subproject D a study of the trajectory methods themselves will be carried out.

Our Lagrangian tool for evaluating the transport of tracers has several advantages over more traditional methods, for which passive quantities are advected and diffused with sophisticated (and expensive) numerical schemes Some of the strong points of the approach are: speed and accuracy in the computation of the trajectories, access to estimates of directional transports (for water flowing from one given oceanic section to another); and to backward integrations (for tracking the history of water masses). Various exciting studies have recently illustrated the power of the approach (Döös 1995, Blanke and Raynaud 1997).

By analysing where the water carried along each trajectory changes property and ceases to be NADW a map of the "upwelling" velocity of NADW may be obtained. This effectively "maps out" the deeper branch of the Thermohaline Conveyor Belt and the results suggest an alternative to the traditional view. Rather than major upwelling and transformation sites in the equatorial Pacific the results suggest most transformation takes place in the Southern Ocean. This leads to a refinement in our concept of the Thermohaline Conveyor Belt.

The latest news on the TRACMASS project can be found on the web site:
http://www.misu.su.se/~doos/tracmass/tracmass.html

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TITLE: NORTH SEA MODEL ADVECTION DISPERSION STUDY 2: ASSESSMENTS OF MODEL VARIABILITY : **NOMADS-2**

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(NOMADS-2)

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INTRODUCTION

With numerical models being increasingly used in management decisions affecting the marine environment, quantification of the variability expected from model results, both between different models and compared to nature, is important. This project sets out a programme of study directed at a quantification of variability for three-dimensional hydrodynamic advection-dispersion models used in European Seas. The project is a follow-on from NOMADS-1 (MAS2-CT94-0105) which analysed the response of 16 different 2-dimensional and 3-dimensional models in realistic and quasi-analytic simulations. These results are reported in Proctor (1997) and Tartinville et al. (1998).

The specific objectives of the NOMADS-2 project are:

a) to establish measurements of variability in terms of cost functions;
b) to carry out sensitivity studies on models by defining input functions and measuring the response of the models to controlled variation of these functions;
c) to provide an assessment of the natural spatial and seasonal variability of the marine system;
d) to assess model spatial and seasonal variability against the natural variability;
e) to carry out a detailed assessment of model variability by direct comparison with suitable observations.

The cost functions (a)) will provide the measure of variation. The sensitivity studies (b)) will provide a measure of expected model variability. Objectives (c)) and (d)) will provide measures of the actual variability, and (e)) will provide a detailed assessment of the variability.

Within the study, the limitations applied will be:

a) salinity and temperature will be the variables assessed in (c) and (d), using data spanning at least 10 years;
b) the domain of study will be the southern North Sea (to 57N);
c) one year (1988/89) will be the focus for direct model-data intercomparison, taking advantage of the availability of UK North Sea Project data.

PROJECT METHODOLOGY

A three-step procedure is laid out to arrive at the final aim of the project, which is to provide measures (and tools) for describing natural and model variability. As such the work content can be classified in the following three steps:

Step 1:
A sensitivity study, which compares the reaction potential of different models. One specific domain is chosen. Partners run their models with their standard resolution with their standard geographical coverage (including the North Sea to 57N), however,
a) all model topography is interpolated from the same bathymetry;
b) synthetic data is prescribed for initial and forcing fields, e.g. constant windfield.
Different model runs are carried out, always varying only one specific parameter and keeping the others unchanged. This variation of input parameters should lie in the range of the natural variability of the specific parameter. Parameters that will be varied include: windspeed, river run-off, net heat flux. As the models will cover different geographical domains the model with the largest domain (including all others) is used to provide boundary conditions for all other models. The resulting reactions of each model are compared, utilizing the cost function approach described above, taking the mean of all the model results as the reference value.

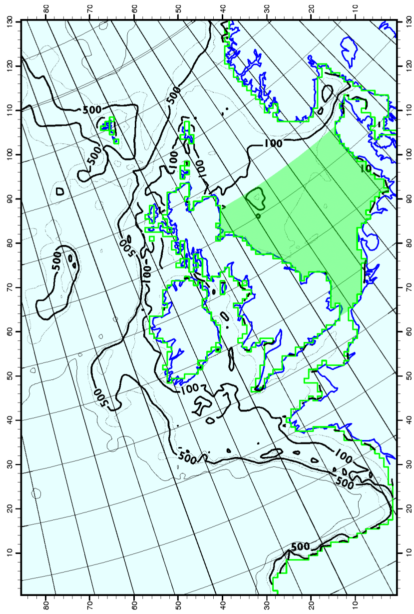
Step 2:
All models should give results that are within what is believed to be the natural variability of the system. To provide an estimate of this variability data (salinity and temperature) from the North Sea from the last 10 years will be used. The data will be split into several seasonal fields with corresponding standard deviation (one for each season). Within this average the standard deviation should give a good estimate for the natural variability of the system. The models will then be run for one specified year (1988/89), and several cost functions (time, area) computed. This exercise will give quantitative information on how the models (for that specific year) compare to a climatic field. The choice of 1988/89 is made as the focal year because of the availability of extensive observations (the UK North Sea Project and others) and forcing data for the model simulations. Other data decomposition methods (Empirical Orthogonal Functions (EOF’s), Principal Oscillation Patterns (POP’s)) will be investigated to determine their usefulness in intercomparison.

Step 3:
A final step is to take data for one specific period and to do a real 1:1 validation of the model again employing the cost function as a tool to describe the agreement. The dimensionless values obtained with the costfunction can subsequently be compared, analogously to steps 1 and 2. As in Step 1, boundary conditions for sub-area models will be provided by the largest domain model and the same initial and forcing functions will be used by all partners. EOF’s and POP’s will also be employed if their usefulness is verified under Step 2.

Models used in the study

The requirement is that all models include the common area of study within their domain. All models used are three-dimensional hydrodynamic models using the finite difference solution technique. There are no constraints on grid size or hydrodynamic formulation. To minimise variability, the model with the largest domain provides initial and boundary conditions to the other models. The common area is marked within the largest model domain in fig. 1.

Fig. 1 - Largest model domain showing common area



RESULTS

Much effort has gone into the collection of datasets for the assessment of natural variability and as input / validation data for realistic simulations, and into the modification and testing of analysis tools. Results from the project presented here are limited to preliminary results from the sensitivity study.

Sensitivity Study

Two quasi-realistic simulations representing 'spring' and 'autumn' in the North Sea are carried out. A number of model runs are performed (Table 1) in which key parameters are varied. Model forcing functions common to all models are: annual mean freshwater inflow for 7 North Sea rivers and the Baltic; common river temperatures for spring (15°C) and autumn (12°C) and for the Baltic (9°C and 12°C respectively); zero salinity for rivers, 13 for the Baltic; constant heat flluxes for spring (100 Wm^{-2}) and autumn (-100 Wm^{-2}); spatially uniform windstress with stochastic speed and direction temporal variability (as in Warrach 1998); 5 constituent tidal forcing.

Common bathymetry and initial conditions were taken from the largest domain model, which was run first to provide time varying boundary conditions for the other models. In this way, the effects of forcing on the simulations is minimised (although some variability arises through interpolation from one model grid to another) leaving the variability in model response to be accounted for through other mechanisms (e.g. grid resolution).

Table -1 Sensitivity Study runs

Run 0 = Reference run : – Typical spring conditions – SW Wind stress (8 m/s)	**Run 2** : – Typical spring conditions – NW Wind stress (8 m/s)	**Run 4** : – Typical spring conditions – SW Wind stress (8 m/s) – Fresh water input x 5
Run 1 : – Typical autumn conditions – SW Wind stress (8 m/s)	**Run 3** : – Typical spring conditions – WSW Wind stress (5 m/s)	**Run 5** : – Typical spring conditions – SW Wind stress (8 m/s) – Bottom Drag Coef. x 2

The study aims to contrast spring and autumn scenarios, and examine the sensitivity of the model results to wind strength & direction, freshwater inflow and changed bottom stress. Each model run was for one month with analysis concentrated on the final 5 days. Thus emphasis is placed on quasi-steady state response, rather than the transient response to initial adjustment.

Six output parameters have been selected to highlight the models' response to the varied forcing. Integral measures over the common area of energetics (kinetic energy and stratification potential energy) and stratification area (thermal stratification area and river plume area and width), time varying volume transport across two E-W sections in the common area, and surface and bottom temperature at three locations. These parameters have been analyzed in three different ways :

a) the common response of the models to the variations of the parameters/forcing data
b) the specific model results are examined to identify models or group of models that behave in the same way and models responding in opposite ways to changes in the parameters
c) the sensitivity of the different models.

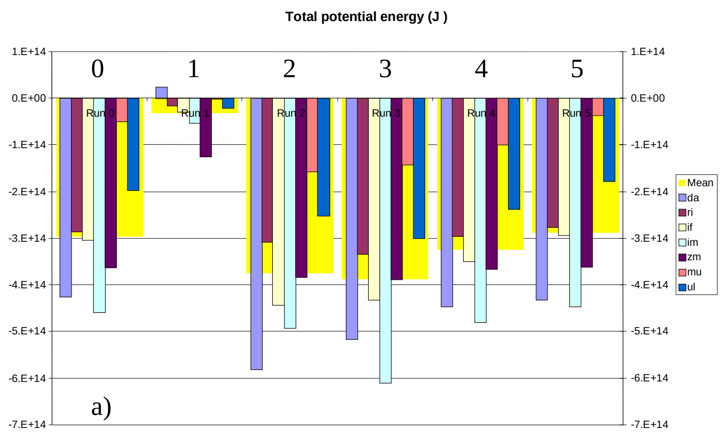
As an example consider the parameter describing stratification potential energy, V_s (in Joules). This is formulated as:

$$V_S = \sum_{i,j,k} \left(\rho_{i,j,k} - \overline{\rho}_{i,j}\right) g x_3 \Delta x \Delta y \Delta z$$

where $\overline{\rho}_{i,j}$ is the depth-mean water density at the grid point (i,j) and x_3 is the vertical co-ordinate (=0 at the surface, increasing upwards). This is zero for a well mixed water column. The following figures show a) V_s from a subset of the models, and b) V_s relative to the reference run. Both plots show the mean value for each run. The variability of the models may be ascribed to:

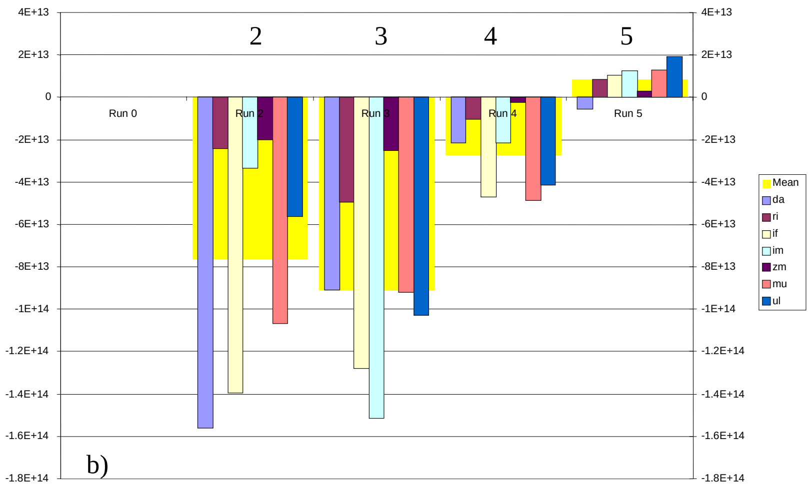
- the different turbulence closure schemes;
- the different response to the initial stratification;
- the different vertical resolutions.

The largest influences come from the wind forcing. In particular, run 2 is associated with a larger influence of the stratified northern North Sea on the common area. This is promotes the stratification. In run 3, the increased stratified area comes from the reduction of the wind stress. Minor influences of the freshwater input (increased stratification because of the



associated buoyancy flux) and of the bottom stress coefficient (increased dissipation and turbulence) can also be identified.

The next plot shows the variations with respect to the reference run. This shows that all the model respond consistently. Notice however, that different processes are involved in run 2 and 3 and hence that the specific models react in different ways to two runs (look at da, im and ul).



One can also compute statistics about the strength of the stratification by dividing the potential energy by the stratified area. One gets then

	da	**ri**	**if**	**im**	**zm**	**mu**	**ul**
/ Run 0	0.20	0.31	0.16	0.24	0.24	0.68	0.30
/ G. Std. Dev.	0.36	0.85	0.39	0.54	1.13	0.51	0.79

showing that the increased potential energy computed by model **mu** is associated with a reinforcement of the strength of the thermocline while, for instance, the variations of model **if** results are more associated with the changes in the stratified area rather than the strength of the thermocline.

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TITLE : **COMPARATIVE ANALYSIS AND RATIONALISATION OF SECOND-MOMENT TURBULENCE MODELS: CARTUM**

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COMPARATIVE ANALYSIS AND RATIONALISATION OF SECOND-MOMENT TURBULENCE MODELS

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OBJECTIVES

The principal objectives of this EC Concerted Action are a confrontation and synthesis of theoretical concepts with new field observations (microstructure measurements), laboratory measurements, model data (Large Eddy Simulation, LES, and Direct Numerical Simulation, DNS), computational and numerical considerations for the benefit of improved second-moment closure models. In particular, we work on better calculations of turbulence characteristics such as the Kolmogorov, Batchelor and Thorpe scales in the surface mixed layer. CARTUM scientists are carrying out comprehensive comparisons of Mellor-Yamada and k-, closure models with the purpose of development of one or of a group of considerably improved turbulence models for various conditions in the fluid and/or with different applications.

PROJECT TASKS

Project Partners are focusing on thematic Research Fields such as stratified shear turbulence , 2-D turbulence and flow two-dimensionalization , Large Eddy Simulation (LES) and Direct Numerical Simulation (DNS), rotation, convection , boundary conditions , internal waves, intermittency, low Reynolds numbers, Renormalization Group Theory (RNG), and computational and numerical aspects.

DELIVERABLES

There are three principal types of deliverables: data sets (field observations, model data), a bibliography on marine turbulence, and the CARTUM Book with CD-ROM (Springer Verlag).

DISSEMINATION

Distribution and advertisement of results will be done at the annual EGS Assemblies, EurOcean 2000, ICES 2000 and other scientific conferences. Furthermore, announcements and articles will be published in a project brochure, in reviewed scientific journals, un-reviewed scientific and popular science publications, on the Internet and broadcast media.

EXPLOITATION

We foresee applications and utilisation of CARTUM's research results in the areas of product improvement, product design and -development in various market segments, such as construction of offshore technology, consulting regarding risk analysis, prediction and avoidance of damages, among others also weather prediction services. Furthermore research results will prove useful for management decisions concerning coastal and oceanic resources, in governmental agencies concerned with national safety (sound propagation, turbidity zones, refraction, reflection and damping of sound), for the estimation of natural resources from local to global scales done by international organisations, e.g. prediction of marine primary production in the euphotic mixed surface layer, which is controlled by turbulence, and in

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climate research and prediction models: cycles of gashouse gases and their sequestering in the depths of the ocean through mixing processes.

SUMMARY OF CONCERTED ACTION ACTVITIES

In the first half of CARTUM's 3 years duration, i.e. until August 2000, 4 of the planned 7 international meetings will have taken place with the organisational responsibility distributed among Partner Institutions: 2 workshops of 3 and 2 days length took place in Hamburg and Marseille, respectively, while a 5-days autumn school was organised by Project Partner JRC in Ispra in October 1999, and the summer school (5 days also) takes place at Proudman Oceanographic Laboratory in Bidston (31.7. - 4.8. 2000). While research talks addressing issues from all of CARTUM's Research Fields are presented at the workshops, at the seasons schools characteristically also longer lectures are offered. For example, CARTUM's participant from Israel, S. Sukoriansky gave a comprehensive introduction into Renormalization Group Theory as a tool for the elimination of small-scale, high frequency modes and the derivation of the equations of motion for large scales. As is often the case, this technique had achieved merit in another scientific discipline, namely in the theory of critical phenomena, before being utilised in the case of fully developed turbulence. The same speaker also lectured on quasi-two-dimensional turbulent flows (stratified and rotational, for example) which widely occur in geophysics and engineering, and demonstrated relevant analogies existing with the turbulent transport properties of magnetohydrodynamic flows, which are also strongly anisotropic. Another set of lectures was authored by D. Mironov (formerly at CARTUM Partner AWI). He reported the results of a Large Eddy Simulation study of the vertical structure of turbulence and second-moment budget in convection with rotation. His illustrations of the transition from non-rotating convective boundary layers to rotating ones, i.e. the early and mature stages of the vertical mixing phase of open-ocean deep convection, included the horizontal distribution at various depths (scaled with the boundary layer depth) of fluctuations of potential temperature about its horizontal mean for the cases of a) free convection and b) convection strongly affected by background rotation. D. Mironov has also provided a LES data set suitable for further analysis to the CARTUM Data Management Committee, chaired by P. Luyten of CARTUM Partner MUMM (Belgium), and thereby fulfilled one of CARTUM's "Deliverables" items.

The spectrum of lectures and talks was enriched by contributions from turbulence experts from non-European scientific institutions. Boris Galperin of the University of South Florida, for example, gave lectures on geostrophic turbulence, i.e. strongly nonlinear, rapidly rotating and stably stratified flow. He expanded on forced and unforced problems on the ∃-plane, discussed the characteristic wavenumber scales and spectra and pointed out key references of prominent turbulence researchers. V. Canuto of the NASA Goddard Institute for Space Studies in his lectures presented improvements to one-point turbulence closure models of the Mellor-Yamada type. For example, now the critical Richardson number assumes a value closer to the one required empirically, i.e. near unity as opposed to the traditional one of 0.2 of former model versions. Furthermore, he described the encouraging results of testing new two-point closures in an ocean general circulation model, listing advantages over one-point-closures: a) no adjustable parameters are needed, b) good performance in tests on about 80 statistics representing a variety of turbulent flows, and c) a straightforward inclusion of the salinity field distinct from the temperature field is possible. A contribution to CARTUM Research Field "Boundary Conditions" were P. Craig's (CSIRO, Hobart, Tasmania) lectures on the generation of turbulence by breaking waves. Supported by measurements in oceans, lakes, and the laboratory and recent development of improved dissipation measuring devices the ocean surface layer has been found to consist of a) a constant stress layer where shear-produced turbulence is balanced by dissipation which has a maximum close to the surface and decays proportional to the inverse of the distance from it, and b) a thin sublayer immediately beneath the surface with a thickness of about one or two wavelengths where dissipation decays at a rate faster than proportional to inverse depth. This lecturer reported the results of several surface microstructure field experiments and related them to the terms of the steady equation for Turbulent Kinetic Energy and derived the algebraic expressions for the pertinent coefficients. These are important for the evaluation of empirical parameters in turbulence closure models, and the re-evaluation of the concept of surface roughness for ocean models.

At CARTUM’s first three meetings 55 research talks were presented, whose abstracts are included in CARTUM’s Annual Scientific Report(s). Here we can only highlight a few.

A theory of generalized von Karman vortex streets has been developed at AWI by Gryanik, Borth and Olbers. V. Gryanik described how zonal mean flows such as turbulent quasigeostrophic baroclinic jet streams on a two-layer ∃-plane are induced by the vortex streets. From a cooperative effort with H. Peters of the University of Miami H. Baumert (HYDROMOD) presented second-moment closures and length scales for weakly stratified turbulent shear flows. Their theory for various parameterizations involving the gradient Richardson number, the Thorpe (or Ellison) and Ozmidov scales was shown to be consistent with laboratory experiments, large eddy numerical simulations, and oceanic microstructure measurements. One outcome of their research is that the high scatter of field data is most likely a consequence of the omnipresence of internal waves in the ocean. A review talk related to this work on the transition from waves to turbulence was given by P. Müller of the University of Hawaii who pointed out the differing modelling approaches of the internal waves community and the turbulence community. While the former view internal wave breaking and its ensuing turbulence as an inherently three-dimensional process that is local in space with divergences and propagation being essential parts of the physics, the latter view stratified shear turbulence (their expression for ‘breaking internal waves’) as a homogeneous and one-dimensional process that is in local equilibrium.

Next to theory and modelling, observational techniques/microstructure data analysis is one of CARTUM’s scientific bases. At all meetings so far new instrument developments and microstructure analysis techniques were demonstrated. Some examples are:

A turbulence probe called MILD (Mixed Layer Dissipation) that was used to determine tidal and seasonal variation in turbulence levels in a stratified channel (E. Horne, Bedford Institute of Oceanography), a new free fall microstructure instrument to measure the turbulent flow field, micro-scale hydrography and chlorophyll signals (TURBMAP, H. Yamazaki, Univ. of Tokyo), a new microstructure-turbulence profiler (MSS) developed by H. Prandke (Petersdorf, Germany) was used for dissipation rate measurements in a small Danish estuary and (under convective conditions) in Italian Lago Maggiore (A. Stips, JRC, Ispra), autumn and winter microstructure-turbulence measurements in the Baltic Sea (S. Podewski, FWG, Germany), results from temperature and current-shear microstructure measurements in a shallow lake (A. Lorke, EAWAG, Switzerland), observations of strongly increasing roughness at low wind speeds indicated by surface boundary layer dissipation estimates (Wüest, EAWAG, Switzerland).

Advances in data assimilation were shown by researchers at CARTUM Partner COM in Marseille, who optimised turbulent viscosity and second order model constants for western Mediterranean regions.

Finally, an interesting application of improved turbulence models useful for coastal zone resource managers was demonstrated by B. Galperin of the University of South Florida in his talk about the results of coupled hydrodynamic-biological simulations of the oyster beds in Apalachicola Bay, Florida in response to changes in freshwater runoff.

While the presentations at CARTUM’s meetings serve to keep project participants informed on current achievements, the principal product of this Concerted Action, the CARTUM Book, will contain a comprehensive review and state-of-the art description of marine turbulence research co-authored by many of CARTUM's participating scientists. The Book will consist of 7 Parts including some 28 chapters. The Parts are: Theoretical Bases, Observational Bases, Boundary Layers, Estuaries and Lakes, Shelf and Shelf Edge, Ocean Basin Scale, CD-ROM Description (model codes, observational and model data sets).

I.1.2. Integrated ecosystem studies

TITLE: **CYCLING OF PHOSPHORUS IN THE MEDITERRANEAN**

CONTRACT No: **EVK3-CT-1999-00009 "CYCLOPS"**

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CYCLING OF PHOSPHORUS IN THE MEDITERRANEAN: a PROJECT TO DETERMINE BY DIRECT EXPERIMENT THE LIMITING NUTRIENT IN THE EASTERN MEDITERRANEAN

The Cyclops partnership

INTRODUCTION:

The problem to be solved:

The Mediterranean in general and the eastern basin in particular is the largest body of water in the world that is thought to be phosphorus limited. However all the evidence at present for this conclusion is indirect and is based on chemical measurements and a limited number of bottle experiments.

The Mediterranean is also well known to be an extremely vulnerable marine ecosystem especially as regards to nutrient inputs. As a result of its unusual anti-estuarine circulation, it exports nutrients into the North Atlantic making it highly oligotrophic. Relatively small changes in the fluxes of nutrients have a disproportionate effect on the marine ecosystem structure in the region. Although the Barcelona treaty and subsequent EC regulations severely restrict the nature and amount of waste that can be disposed of within the Mediterranean, anthropogenic inputs of nutrients continue to reach the basin in large amounts. These nutrients come via the atmosphere, river discharges, dredge spoils as well as directly via activities such as mariculture that is an expanding industry in the region. In order to reduce the impact on the marine ecosystem it is necessary to understand the P cycling in detail.

Scientific objectives:

The aim of CYCLOPS is to confirm directly that P is the growth-limiting nutrient in the Eastern Mediterranean. We will use a novel field strategy, similar to that carried out in the IRONEX project in the Eastern Pacific, that avoids artefacts of bottle experiments. We also aim to increase our understanding of the microbial loop in the region which is the basis of the marine food chain.

Our novel techniques for P, tracer and microbiological assays are sensitive enough to track these small P additions thereby ensuring our experiments are reversible, localised and have negligible ecosystem effects. This is ideal for both (1) biogeochemical budgeting of P and (2) compliance with the Barcelona convention and all other relevant EC regulations and directives regarding nutrient inputs to the Mediterranean.

EXPECTED RESULTS FROM THE PROJECT:

The scientific understanding developed during this study will be used to develop a quantitative understanding of P cycle that will be used to calibrate nutrient & ecosystem models including the Mediterranean Forecasting system, a model currently being developed to provide a real-time oceanographic predictions across the basin. The results of this project will be used to update directives and regulations to further protect the Mediterranean from ecosystem damage. New technologies will be developed to solve environmental problems. For example, the new technology of non-polluting intensive mariculture systems will be targeted to remove P from the effluent as well as the present N, if we can show that P is the critical nutrient in the region.

SCIENTIFIC APPROACH:

Year 1 - Microcosm experiments:

In May 2000, we carried out a set of microcosm experiments at the National Institute of Oceanography in Haifa, Israel. This involved the addition of P, N, and Si to a series of microcosm flasks. A series of detailed state-of-the-art measurements (biological, chemical and physical) were made to determine how small additions of phosphate change the microbial ecosystem structure and increase biomass and productivity. These experiments were designed also to refine our measurement techniques prior to the field experiments that will be carried out in this extremely oligotrophic system in 2001 and 2002 The results of these experiments are currently being processed.

Year 2 - 1st Field Addition experiment:

In year 2 we will carry out a cruise in which phosphate and an inert SF6 tracer will be dispersed as a single addition in a 4-8 km2 patch of surface water within the stable structure of the Cyprus eddy. We will follow the changes in microbial biomass and rate and in community structure caused by this addition of phosphate. We will also measure for the first time in the Eastern Mediterranean, the complete suite of dissolved and particulate nutrients and how they change during the experiment. The export productivity will be determined directly from sediment traps. The location of the eddy and the progress of the experiment will be followed by means of satellite imagery. The physical structure of the sampling location will be monitored and the horizontal and vertical mixing rate calculated.

Year 2-3: Annual cruises and 2nd addition experiment:

Between May 2001 and May 2002 we will carry out a series of short cruises to the Cyprus eddy to determine the natural state and seasonal variability of the system. The second field experiment will be carried out in May 2002. The design of this experiment will be similar to the first field experiment except we will add phosphate 3 times successively to the same patch of water. As with experiment 1 we will then determine by detailed biological and chemical measurements the change in biomass, ecosystem structure and phosphorus speciation. In all the experiments the total amount being added to this small area, which is designed to mimic natural processes, is similar to a natural dust event and less than 20% of the natural winter turnover.

Adjacent projects:

The project is part of the IMPACTS cluster together with MATBIOPOL, MEAD and SIGNAL. The aim of the IMPACTS cluster is to quantify the effects of human activities focusing on pollution sources, transport, reactivity and impact set within the biogeochemical cycles of the contrasting marine ecosystems surrounding Europe. For more information about this project visit our website at www.leeds.earth.ac.uk/CYCLOPS

TITLE : ROLE OF MICROBIAL MATS IN THE BIOREMEDIATION OF HYDROCARBON POLLUTED COASTAL ZONES : **MATBIOPOL**

CONTRACT N° : **EVK3-CT-1999-00010**

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ROLE OF MICROBIAL MATS IN THE BIOREMEDIATION OF HYDROCARBON POLLUTED COASTAL ZONES : MATBIOPOL

The primary goal of this research programme is to evaluate the potential of using microbial mats as a means of rehabilitating oil contaminated marine sites to their original pristine state without the excessive use of chemical treatment or extensive change in the character of the coastal landscape. The integrated multi-disciplinary research programme will enable a comprehensive study of selected microbial mat systems and their responses when subjected to varying levels of pollution by crude oil or selected hydrocarbons. Different types of European microbial mat systems found on the Orkney Islands (Scotland), in the Camargue (France), and in the Delta del Ebro (Spain), are investigated by all the participants who are co-operating through the agency of a common annual field-programme. In addition, a long-term study is undertaken using stabilized microbial mats in experimental mesocoms at the marine station in Eilat, Israel, to investigate hydrocarbon degradation under controlled conditions.

The specific objectives of the project are as follows:

- To understand the role of microbial mats in the bioremediation of oil-polluted coastal waters and sediments.
- To determine the response(s) and change(s) in microbial biodiversity and biogeochemistry in the selected microbial mats and experimental mesocosms when subjected to hydrocarbon pollution.
- To isolate, characterize and determine the physiology and biochemistry of the dominant microorganisms involved in hydrocarbon degradation in microbial mats.
- To follow the degradation of selected hydrocarbons artificially introduced into the experimental microbial mat systems established in the mesocosms in Eilat.

The research programme is organised into four workpackages:

Ecosystems level (Workpackage 1): Microbial mat ecosystems: fields studies and crude oil degradation.

Analysis and comparison of different microbial mats (biogeochemistry with standard and microsensor techniques; microbial diversity with microscopy and molecular biology techniques). Experiments *in situ* with selected oils: chemical analyses of hydrocarbons; biogeochemistry and microbial diversity in the mats subjected to hydrocarbon pollution.

Community level (Workpackage 2): Mesocosm studies of the biodegradation of selected hydrocarbon molecules in a controlled microbial mat.

Biogeochemical analyses of the controlled microbial mat subjected to selected hydrocarbon molecules (aromatics or alkanes). Selection of ecologically important microorganisms in the controlled mats during hydrocarbon degradation (selection by molecular biology methods). Studies of syntrophic relationships among defined consortia obtained from the controlled mat (exopolysaccharide production, bacterial activities in hydrocarbon degradation).

Organismic level (Workpackage 3): Microbial diversity and physiology of isolated bacteria from microbial mats subjected to oil pollution.

Physiology of the isolated microorganisms of ecological importance in hydrocarbon degradation in the mats (cyanobacteria, anoxygenic phototrophic bacteria, sulfate-reducing bacteria, denitrifying bacteria, aerobic, microaerophilic and fermentative bacteria). Phylogenetic analyses and genetic probe preparation for biodiversity analyses. Degradation of some selected hydrocarbons by the isolated microorganisms (chemistry of degradation, physiology).

Sub-cellular level (Workpackage 4): Biodegradation of hydrocarbons by selected bacteria and analysis of metabolic pathways of hydrocarbon degradation in selected microorganisms (biochemistry of oxygenases in aerobic metabolism, anaerobic enzymes).

TITLE : THE INFLUENCE OF UVR AND CLIMATE CONDITIONS ON FISH STOCKS: A CASE STUDY OF THE NORTHEAST ARCTIC COD (**UVAC**)

CONTRACT N° : EVK3-CT-1999-00012

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THE INFLUENCE OF UVR AND CLIMATE CONDITIONS ON FISH STOCKS: A CASE STUDY OF THE NORTHEAST ARCTIC COD (UVAC)

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INTRODUCTION

The uncertainties related to today's fishery world wide are ubiquitous and can be categorised as random fluctuations, uncertainty in parameter estimates and states of nature, and structural uncertainty that reflects a basic lack of knowledge about the nature of the fishery system. Structural uncertainty may arise from biological mechanisms, for example fish-fish interactions, or variable fishery responses or management objectives. Though the relative influences of these mechanisms have been and are still discussed, it is a fact that we today have no clear cut answer to these questions and that we need to understand the underlying physical and biological regulators involved in order to manage the fishery systems in a better way.

There is a general agreement today that especially structural uncertainties should be focused upon. The key issue in this project is the fish-environment interaction or more specifically, mortality at an early life stage of fish. In order to investigate this in a quantitative manner, a species has to be chosen which has been and still is relevant commercially, but at the same time sufficiently localised geographically so that appropriate experiments can be performed. The Northeast Arctic cod (*Gadus morhua*) is in our opinion an excellent candidate for such an in-depth study. Our intention is to investigate this species as part of an ecosystem including other marine species, under the influence of geophysical (atmospheric and marine) processes, and to develop tools that can be used for ecosystem management rather than fishery management solely.

The Northeast Arctic cod stock in the Barents Sea is one of the world's commercially most important wild fish stocks. In order to maintain this valuable natural resource at a vital level, an effective management is indispensable. The determination of quota is based on annual assessments of the year class sizes, mainly by empirical estimates derived from Barents Sea surveys. Though the methods used today to measure and predict the cod year classes are believed to be quite advanced, the latest years' experiences with relatively large discrepancies between predictions and actually measured stock stresses the importance of a deeper understanding of the factors and processes regulating the cod stocks. It is evident that if year class size is influenced by biological and physical-biological mechanisms active at an early development stage, studies of feeding conditions (phytoplankton-zooplankton-cod interactions) and impact of the geophysical environment (water temperature – solar ultra-violet radiation (UVR) – climate), both on juvenile cod and its prey, should be included in a study aimed at detecting factors that regulate year class size.

PROJECT METHODOLOGY: HISTORICAL DATA, REMOTE SENSING DATA, FIELD RESEARCH AND MODELLING

The study will be performed using data sets with three different time scales and detail levels: with a historical time scale (100 years) applying statistical methods on annually averaged data, with a medium time scale (10-15 years) using regional satellite mapping of UVR conditions with significantly increased horizontal and temporal resolution as well as more detailed biological information, and with a short time scale (2 spring seasons) providing detailed monitoring of geophysical and biological parameters, combined with laboratory experiments. Conceptually, the project will be based on the well-known match-mismatch hypothesis that states that offsets in time between cod larvae and its prey organisms influences year class strength.

The following investigations are planned:

(1) Re-analyse historical data on cod stocks from the Lofoten fishing since the beginning of the 20th century and link them, by using relevant statistical methods, to available climate/weather parameters, both of local and global character, as well as UVR conditions derived from ozone measurements in Tromsø (since 1935/39) from the same period and region.

(2) Investigate in particular a possible signature of the North-Atlantic Oscillation/Arctic Oscillation both in the geophysical data set and in the cod stock year class strengths, using external data sets combined with long-term ozone records from Tromsø.

(3) Perform a detailed correlative analysis of UVR and biological data (cod – zooplankton – phytoplankton) from the period 1985 through 1998 in order to identify biologically relevant UVR parameters (spectral range, intensity level etc.). UVR conditions in the Lofoten/Vestfjord region in spring will be reconstructed by means of an already developed algorithm that allows calculation and area-wide mapping of surface UVR by means of satellite measurements of the relevant geophysical parameters, such as cloud cover, total ozone and surface albedo. Figure 1 shows an example of a reconstructed UVR map over the region in focus in the frame of UVAC, regarding measured ozone clouds topography and albedo conditions for March 22, 1997.

(4) Monitor, by performing four cruises per year over two spring/summer seasons, distribution in time and space of environmental factors (hydrography, UVR, PAR) assumed to regulate the abundance, species composition and distribution in time of selected organisms at the lower trophic levels, i.e. phytoplankton and zooplankton, in addition to relevant cod parameters (size and quality of spawning stock, egg and larvae abundance and quality).

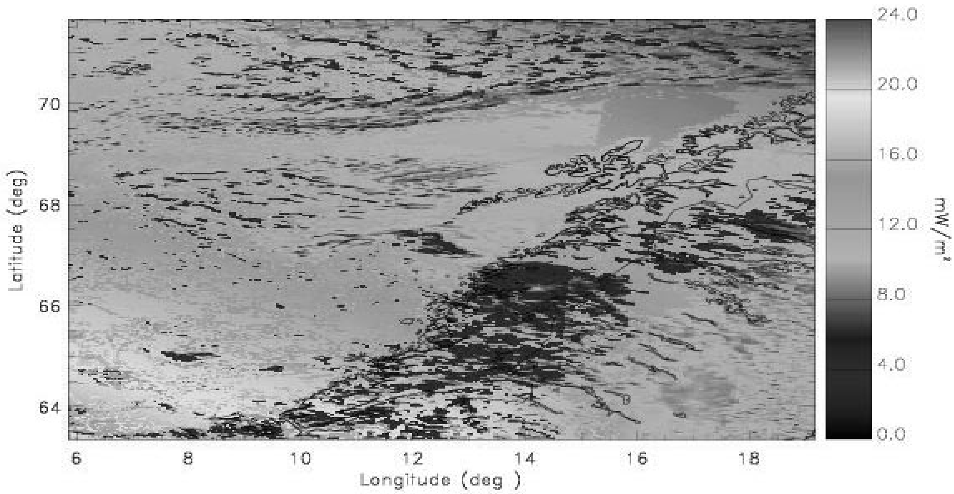

Figure 1. Erythemally weighted UV irradiance [mW/m^2] over Northern Norway on March 22, 1997, calculated with the DLR radiative transfer model, based on remote-sensing data (GOME, AVHRR).

(5) Perform laboratory and in situ experiments where phytoplankton, zooplankton and cod eggs and larvae will be exposed to gradients of UVR doses at different temperatures. Furthermore, zooplankton and cod eggs and larvae will be exposed to various concentrations of the presumably toxic phytoplankter *Phaeocystis pouchetii* in order to monitor mortality and egg production.

(6) Combine results from the above field surveys/laboratory experiments in short term process models to simulate the various scenarios that may arise from natural variations in UVR and climate, and develop modelling tools accounting for the micro-climatic/meteorological/UVR impact on cod year class strength.

RESULTS

Since the project was started only on March 1, 2000, no quality-assured results are available so far. A more detailed overview over the project as well as updated information on ongoing activities and related issues can be found at the project web page http://phaeocystis.nfh.uit.no/uvac.

TITLE: **ESTIMATION OF PRIMARY PRODUCTION FOR FISHERIES MANAGEMENT - PROOF**

CONTRACT NO: **EVK3-CT-1999-00019**

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ESTIMATION OF PRIMARY PRODUCTION FOR FISHERIES MANAGEMENT – PROOF

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SUMMARY

The CEC FP5 project PROOF (Estimation of Primary Production for Fisheries Management) started in May, 2000. PROOF aims to produce estimates of the regional and ocean basin-scale marine primary production (PP) based on the available ocean colour and temperature data from a number of earth observation satellites already in orbit or to be launched in the near future. The spatial and temporal variability of the PP will be assessed and validated against state-of-the-art ecosystem models [e.g. 4] in order to validate the methods developed. The PP estimates will be used in a publicly available marine food web model in order to assess the marine production at various levels in the food web. The higher level production, such as fish production, is essential for proper management of fisheries, implementation of national and EC Common Fisheries Policy, as well as a basis for agreements on international quotas and fish catch regulations. The project will develop a close relation with the European fisheries management authorities at national, EC and international level in order to assure user guidance in the project results.

INTRODUCTION

The UN Food and Agricultural Organisation (FAO) reports that world-wide fisheries reached peak level of production in 1994. However, during the last decades a series of fish stocks have reached, or are close to, collapse in several parts of the world. The future viability of fisheries depends upon more complete understanding and monitoring of the interrelation and dependence of ecological and socio-economic factors related to the fisheries activities and exploitation of the marine food resources. The legal basis of the EC formulation of a Common Fisheries Policy (CFP) is based on the Articles 38 to 43 in the European Treaty. The planning and regulations of the EC fisheries are performed under DG XIV – Fisheries, although also major concern to the fisheries policy is a focus of DG VIII – Development, and in particular the relation to the resources in the Developing countries. The fisheries resources are of global nature and concern, hence around 40% of the total CFP spending is related to activities in non-EC waters.

Since 1993, the EC DG VIII started a series of actions to contribute to a global sustainable management of fisheries and aquaculture. One of these actions is the ACP-EC Fisheries Research Initiative that aims to promote interdisciplinary research on fisheries and aquaculture in support to researchers and managers in African, Caribbean and Pacific (ACP) countries, with the sustainable management of living aquatic resources and conservation of aquatic bio-diversity as main goals.

As an accompanying action, the CEC started in 1997 an activity related to the development and distribution of the Ecopath-Ecosim food chain model [3] (INCO-DC funded concerted action "Placing fisheries resources in their ecosystem context: Co-operation, comparisons, and human impact"), as well as the implementation of the FishBase database. This project included model development and validation through close interaction, use and validation by a world wide group of experts from 20 nations. The Ecopath model and its simulation part Ecosim are currently widely used in the fisheries community as a tool for studying and simulating marine trophic webs and mass balance, and for ecosystem management, both within Europe and world-wide. It is routinely used for exploring consequences of alternative fishery management strategies, including optimal stocking, refuges, and changes in exploitation. The model simulates ecosystem management in response to fishing and/or environmental change, and provides a hypothesis testing framework.

Whilst significant progress has been made in modelling the biomass and abundance at high trophic level (fish), less attention has been given to the lower trophic levels, and in particular to the marine primary production, for which currently the best estimates of global primary production vary by a factor 2, and no reliable estimates exist in coastal regions. Until recently, it was commonly believed that primary production was not a limiting factor for fish stock sustainability since only 2.2 % of the world's aquatic primary production was required to sustain the fisheries [7]. Therefore, human exploitation of marine resource seemed insufficient by itself to alter on a large scale any but the target populations. However, resent analysis shows that the primary production required to sustain the world fisheries catches amounts to 8% of global aquatic primary production [7]. It was noted that the results vary by ecosystem type: the primary production required is only 2% for open ocean systems, but up to 35% in fresh water, upwelling and continental shelf ecosystems. The latter number justifies current concerns for sustainability and biodiversity, and the requirement for improved ecosystem and food chain modelling.

Therefore, an improved knowledge of ocean basin-scale and regional primary production and its temporal and spatial variability will, in the longer term, lead to a significant improvement of the management tools (in this project defined as the Ecopath model). Such knowledge should lead to better estimates of input parameters for food-chain and fish stock modelling. A direct consequence should be the production of more reliable predictive information to fisheries, with possible impact on national and international decision making and fisheries policy.

THE OBJECTIVES

The overall objectives of the PROOF project are:

1. To provide estimates of marine primary production at regional (selected European waters) and ocean basin (North Atlantic ocean) scales from satellite ocean colour and temperature data. State-of-the-art algorithms will be implemented for the retrieval of chlorophyll-a concentration and primary production in open ocean (case I) and coastal (case II) waters, and validated and improved using both archived and new data obtained during the project. A 3D-coupled physical biogeochemical model will be used to assess the spatial and temporal variability of the derived primary production, accounting for ocean dynamics.
2. To apply primary production to ecosystem modelling [4] and guarantee an efficient dissemination and exploitation of the developed products. To disseminate into the public-domain, data sets on CD-ROM, including software for integration within common food-chain and fish-stock prediction models.
3. To assess the impact of the above development in term of improved fisheries management in relation to the Common Fisheries Policy (CFP), through model simulations. Direct

interactions with the user community will gauge the usefulness and efficiency of the developed products.

The APPROACH

Marine phytoplankton have a fundamental role in global carbon cycles by taking up inorganic carbon in the upper layer of the oceans, and thus from the atmosphere, to produce organic compounds of which a fraction becomes eventually sequestered at depth and in sediments. Another role of phytoplankton is through its action on the marine food chain. The organic matter produced through photosynthesis is necessary to sustain a whole range of organisms from zooplankton to whales, and economically important fish populations. As such, the determination of phytoplankton photosynthesis or the so-called primary production in the ocean at global and regional scales remains one of the central goals in the environmental sciences. Furthermore, unless the variability in the magnitude of the primary production and its controlling factor are understood, there can be very little development in marine ecology and population modelling.

Ecosystem models must always include an input source, as such systems dissipate energy. In most cases, the input will consist of the primary production by phytoplankton. This is the case of the Ecopath food-chain model, for instance. Ecopath is a PC-based software package, developed with support from the European Commission, that performs food-chain mass-balance estimation and simulation from a variety of trophic structures that are region- and ecosystem-specific. This model is based on an improved understanding of trophic structure, population sizes and flow of matter through the food web.

Satellite remote sensing and model simulations are considered to be a promising methods for estimation of marine primary production at ocean-basin scales. However, assimilation of satellite derived measurements into numerical models is a non-trivial problem, and thus the conditions for efficient assimilation must be determined in terms of model assumptions and characteristics, and of the limitations of retrieval algorithms used for deriving biological and physical quantities from remote sensing data. Using maps of chlorophyll distribution derived from remotely sensed ocean colour data as input, algorithms for primary production have been developed, e.g. [2, 5, 8]. To apply the algorithm world-wide, a dynamic bio-geography for the main inputs of the model that are the physiological rate parameters and the biological structure of the water column, is needed.

Empirical approaches exist that are based on the direct correlation between *in situ* estimates of productivity (historically performed *in situ* with the radiocarbon technique) and satellite-derived estimates of surface chlorophyll. However, more complex semi-analytic models of primary production have been developed. These models are commonly based on a known relationship between P^B, the normalised-to-biomass photosynthetic rate and the available light I at depth z. Two main types of semi-analytical model can be distinguished: vertically resolved productivity models [1,8] and vertically integrated productivity models [2]. The first type account for the vertical distribution of chlorophyll in the euphotic layer, whereas the second type of model considers a homogeneous water column. Accounting for the depth variability can only explain up to 15 % of the variability observed in the primary production in open ocean [2]. However, this can take an increasing role in more coastal types of waters.
The data needed as input to such models are available from the ocean colour satellite sensors such as the Sea-viewing Wide Field-of-view Sensor (SeaWiFS - operational since September 1997) that provides chlorophyll concentration estimates from space. Similar earth observation data sets will be complemented by the launches of the Moderate Resolution Imaging

Spectrometer (MODIS, launched in December, 1999) and the European Medium Resolution Imaging Spectrometer (MERIS due for launch in summer 2001).

The fundamental component in the performance of an ocean colour sensor is the bio-optical algorithm used for the calculation of chlorophyll concentration (CHL) and hence the primary production. In Case I waters, such as the open ocean and many stratified shelf waters, phytoplankton chlorophyll, associated pigments and *co-varying* coloured dissolved organic matter (CDOM) are the optically active components in the water-column. Reliable relationships between atmosphere corrected water-leaving radiance and CHL have been established [9]. The commonly used bio-optical algorithms for SeaWiFS have been developed by simple empirical analysis of reflectance ratios. Despite this empirical approach these algorithms perform well in Case I waters, when the water-leaving radiance are measured with a radiometric accuracy to within ± 5%, giving chlorophyll a concentration to within ±35% over the range of 0,05-30,0 mg/m^3 water [9].

In Case II waters, near to the coast or well mixed shelf waters, suspended particulate material (SPM) and CDOM, possibly of terrestrial origin, additionally contribute to the water optical characteristics and thus to the signal measured by a remote sensor. Both these components give erroneously high values of CHL when the conventional SeaWiFS algorithms are used, while existing atmospheric correction procedures fail over case II waters. However, accurate determination of CHL in such waters is important since major European Large Marine Ecosystems (the North Sea, Baltic, and a major part of the Adriatic) contain case II waters. A novel approach is to use optical models in conjunction with the atmospheric correction to retrieve the water leaving signal [6]. Model based algorithms can also determine the inherent optical properties (IOPs) of the water constituents.

EXPECTED RESULTS

The PROOF project consists of three main phases:

1. Proof-of-Concept:
A study will be conducted to test the feasibility of integrating satellite-derived primary production into Ecopath – Ecosim, and the sensitivity of the model to variations of PP. A users' workshop will be organised to introduce the project and expected deliverables, to provide user guidance of the project and to select relevant experts to a reference group to perform a later project evaluation.

2. Development and implementation
The development part will concern methods to calculate chlorophyll, phytoplankton absorption and primary production from satellite observations.
Methods to calculate satellite retrieved chlorophyll concentration in both case I and II waters will be developed. In case II waters a combined atmospheric correction and optical model approach will be used to retrieve water-leaving radiance. Optical models will enable retrieval of inherent optical properties (IOPs). Empirical and semi-analytical models to calculate water column PP will be evaluated and implemented, where appropriate regional ocean characteristics will be used.
Simulations from a 3D-biogeochemical model will provide (i) estimates of additional input parameters for the PP algorithm, and (ii) optimal space and time interpolation and extrapolation of functions. Resulting ocean basin-scale and regional PP estimates will be validated in two European LMEs of continental shelf and coastal up-welling areas and over the ocean basin scale (North Atlantic Ocean). Model output will be validated against *in situ* data such as local

data sets from long-term oceanographic moorings (e.g., OMEX-II), individual cruise and time-series measurements to be obtained during the project.

3. Production and demonstration

The validated methods will be applied to SeaWiFS (and other ocean colour data) to produce ocean basin-scale and regional PP database at different resolutions. Results will be tested for applicability to an ecosystem (food-chain) model. Use by the community will be facilitated through CD-ROM dissemination including user-friendly software to enable browsing, selection and extraction of data subsets, and data conversion to Ecopath format. The impact of the project results in term of fisheries management and CFP will be prioritised.

ACKNOWLEDGEMENT

The PROOF project is funded under CEC contract EVK3-1999-00019 since May 2000.

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TITLE: Ocean Margin EXchange II - Phase II
(OMEX II-II)

CONTRACT NUMBER: **MAS3-CT97-0076**

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OCEAN MARGIN EXCHANGE II - PHASE II (OMEX II-II)

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SUMMARY

The main objectives of Ocean Margin EXchange II – Phase II (OMEX II-II) are to understand and to construct the carbon cycle and its associated elements along the Iberian margin. This area is dominated by wind-driven upwelling events during the summer, and by Rías inputs and downwelling during the winter. Coastal upwelling areas are among the most productive biological systems in the ocean and therefore of particular economical interest. They are also characterised by large fluxes of organic carbon, nutrients and other trace elements, which may be exported to the open ocean or rapidly deposited and buried at geological time-scales. The coastal upwelling regions are thus playing an essential role in the marine biogeochemical cycle of many elements.

Physical processes affecting horizontal and vertical transport in relation to the general circulation were investigated. Special attention was paid to the role of filaments in the exchange of material across the boundary between the coastal zone and the open ocean. Spatial and temporal variations of the inventories and fluxes of carbon and associated elements were investigated during various field surveys. Short time scale measurements of carbon and nutrients fluxes through the food web were conducted for two quasi-Lagrangian experiments during a period of strong upwelling. The vertical fluxes of particulate matter were assessed on an annual basis using sediment trap moorings.

The primary production in the coastal upwelling zones of the Iberian region is, to a large extent, rapidly deposited on the shelf. Benthic processes related to biological activity and early diagenesis were investigated in order to understand, quantify and model the flux of organic matter after deposition.

The preliminary budgets of the carbon fluxes are evaluated and presented here. The knowledge gained should improve our evaluation of the role of coastal upwelling in organic carbon production and fate in the marine system. This study will refine our understanding of the effect of coastal upwelling on the global climate control, and *vice versa* of potential effects of global warming on the upwelling processes.

INTRODUCTION

The coastal zone represents the interface between the land and the open ocean. It is an area of enhanced productivity under the strong influence of continental input, and is an important source of dissolved and particulate matter for the open ocean. On the other hand, deep ocean waters, nutrient-enriched and with high concentration of trace elements, are transferred across the shelf edge and help to sustain the high productivity of biota in the coastal zone and shelf seas. The quantification of fluxes across the ocean margin is thus a fundamental requirement for the evaluation of the carbon budget and nutrients between the continents, the coastal zone and the open ocean.

It has been widely recognised today that there is a lack of knowledge and understanding of the processes occurring at the ocean margins which is critical for the evaluation of global biogeochemical cycles. The understanding of the distinctive and often enhanced processes occurring at this boundary is, however, a prerequisite for the prediction of the consequences of climatic change and of other anthropogenic perturbations.

The Ocean Margin EXchange (OMEX) project is designed to meet the priority goals of the International Geosphere-Biosphere Programme (IGBP), and takes into account the specific features of the European marine environments and settings, and the expertise of the oceanographic community in the EU countries. It concerns essentially the study of fluxes and processes occurring along the European shelf break facing the North Atlantic Ocean. Its aim is to measure and model exchange processes at the ocean margin as a basis for the development of global models to predict the impact of environmental changes on the oceanic system and more specifically on the coastal zone.

The second phase of OMEX (hereafter, OMEX II) focuses on the upwelling region of the Iberian coast. Coastal upwelling occurs extensively at Eastern Atlantic and Pacific margins and represents the most productive marine systems. It is estimated to supply nitrate for 11% of the global new production. Because upwelling is largely wind-driven, it is complex in both space and time, making it difficult to understand of how this physically driven biogeochemical system works. Knowledge of how upwelling systems function today is essential for predicting how they may change in the future, particularly in view of the possibility of a global climate change. Such understanding can only be achieved through an interdisciplinary approach. In particular, it is necessary to gain a better knowledge of those processes that control fluxes associated with upwelling and downwelling, and exchange fluxes at the air-sea interface and sediment-water boundary. In this view, the OMEX II project is organised according to multidisciplinary Work Packages that involve the integration of physics, biology, chemistry and sedimentology disciplines:

Work Package I: Temporal Evolution of Surface Production and Fate of Organic Matter
Work Package II: Spatial and Seasonal Fluxes and Biogeochemical Processes in the Water Column
Work Package III: Fluxes and Processes in the Nepheloid Layers and Surface Sediments
Work Package IV: Integrated Margin-Exchange Product
Work Package V: Project Management and Coordination

HYDROGRAPHY

The sources and circulation of waters along the NW Iberian Margin exhibit pronounced seasonal variation because of their coupling with large-scale climatology of the northeastern Atlantic. During summer months trade winds with southerly components along the coast of western Iberia induce an equatorward slope current and an offshore upwelling of nutrient-rich water (Figure 1). By late June or July, the upwelling intensity has increased to the point that major headlands and flow instabilities in the slope current may generate several eddies and fully developed filaments which jet out far into the open Atlantic Ocean (Figure 2). Filaments have potential importance as exporters of coastal water and its contents (including large quantities of organic matter produced on the shelf) to the ocean.

The stable forcing of the upwelling system may be stopped by low-pressure passages, which alter the wind pattern and may cause periods with relaxation or even downwelling. In winter, a general northward water flow extends from the surface down to the level of intermediate water (about 1000-m depth), along the Iberian coast and into the Bay of Biscay. During the non-upwelling period in winter (Figure 3), current directions are reversed and the riverine fluxes of nutrients and terrestrial matter are at their maximum.

The intermediate waters are characterised by the poleward flow of poorly oxygenated, low-nutrient, warm and saline Mediterranean Outflow Water (MOW) on the Iberian slope. In deeper waters, there are overall trends of decreasing temperature and salinity from south to north. At depths below 2500 m, the Deep Water with high dissolved silica content and relatively low salinity is transported northwards on the deep eastern boundary.

NUTRIENTS

Surface nutrient distribution is characterised by a contrast between cold upwelled waters over the shelf and warm stratified waters in the adjacent ocean. Highest surface phosphate and nitrate concentrations were found at the coastal stations while they become rapidly depleted when moving westward from the upwelling zone.

Nutrient supply from the continent to shelf surface waters is almost negligible, except for silicate, for which it represents about 18% of the total supply. The upwelling of cold (< 13 °C) and nutrient-rich Eastern North Atlantic Water (ENAW; > 7 $\mu mol\ kg^{-1}$ nitrate, > 0.45 $\mu mol\ kg^{-1}$ phosphate and > 2 $\mu mol\ kg^{-1}$ silicate) is the main source of nutrients for the autotrophic populations on the shelf. The N/P and N/Si ratios are respectively around 16 and 3.5, indicating potential silicon limitation for a diatom population growing with a usual N/Si ratio of 1.

Under oligotrophic conditions, the concentration of urea exceeds those of nitrate and ammonium. Uptake experiments indicate that urea may become in this case an important source of nitrogen for the phytoplankton. In addition, high concentrations of dissolved organic nitrogen (DON, up to 10 µM) associated with elevated POC, PON and chlorophyll concentrations were found in coastal areas.

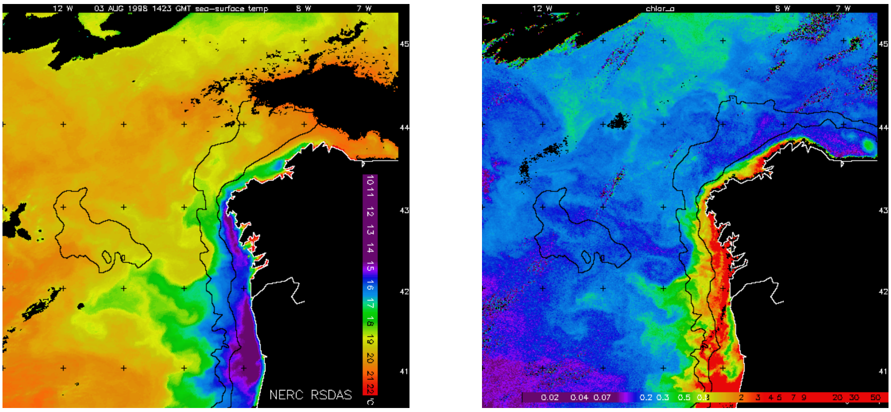

Figure 1. Sea surface temperature and SeaWiFS chlorophyll during *Charles Darwin CD114* cruise on 3 August 1998 show a strong upwelling and a high phytoplankton concentration (> 1 mgChl. m^{-3}) on shelf, with two marked filaments between 42-43°N. *Data from Remote Sensing Group, Plymouth Marine Laboratory, U.K.*

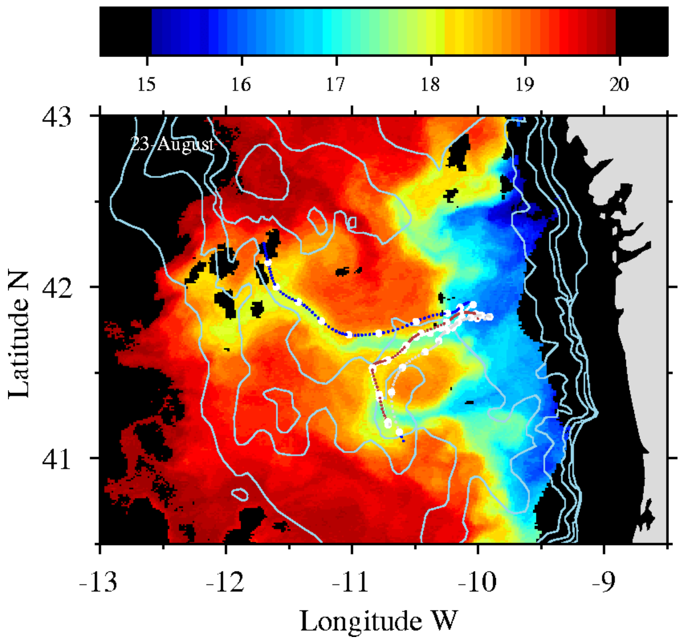

Figure 2. Sea surface temperature of 23 August 1998 and ten days of drifter data (5 days before and after the SST image). Solid dots indicate days. Deployed during a relaxation event, the drifters slowly (0.05 m s^{-1}) converged towards the filament's southern boundary for two and half days and subduction velocity based on the convergence rate was 16 m d^{-1}. During the following upwelling period, one drifter traced the offshore extent of the filament with mean velocity 0.3 ± 0.1 m s^{-1} while the others recirculated shorewards in an apparent return flow. *Data from R. Torres & D. Barton, University of Wales, Bangor, U.K.*

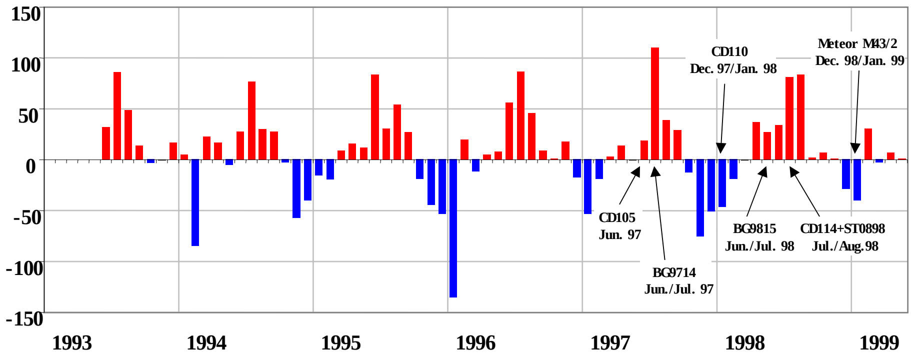

Figure 3. Monthly upwelling index for the Vigo region for the entire OMEX periods was derived from synoptic pressure analyses. Although the mean distribution is as expected, a large variability exists in all months, and a net upwelling can only be expected from June to September, whereas all other months include the possibility of a net up- or downwelling within 1 standard deviation. *Data from Remote Sensing Group, Plymouth Marine Laboratory, U.K., after FNMOC, USA data.*

PHYTOPLANKTON and PRIMARY PRODUCTION

The analysis of phytoplankton pigments using HPLC and the application of the chemotaxonomy, based on phytoplankton classes-pigment specific relationships, allow mapping of phytoplankton and inter-seasonal comparison. During summer, a clear transition in the dominant classes of phytoplankton was observed across the shelf. On the shelf, diatoms contributed for about 40% of measured chlorophyll *a*, while prokaryotes represented more than 50% of total chlorophyll *a* in offshore waters. Prymnesiophytes were especially abundant in the intermediate regions where they contributed for about 40% of the chlorophyll *a*. During winter, Prymnesiophytes, chlorophytes, cryptophytes and diatoms were equally represented (~ 20%) on the shelf and prokaryotes contributed for a maximum of about 10% of the measured chlorophyll *a*.

Carbon biomass of phytoplankton along the coast of Galicia was studied during summer and winter cruises, and the results compare fairly well with those derived from analysis of phytoplankton pigments by HPLC.

During upwelling events, nitrate was the dominant nitrogen source for phytoplankton and the > 5-µm phytoplankton was found responsible for more than 50% of the uptake. Ammonium uptake was dominated (> 65%) by the small size fraction and the recycled nitrogen source becomes increasingly more important, leading to a decrease of the *f*-ratio from 0.7 to 0.5.

In summer, under upwelling conditions, picoplankton biomass showed little change but the phytoplankton larger than 5 µm become increasingly abundant. However, the data emphasise the overall importance of the picophytoplankton contribution to the productivity of this region.

Primary production and new production were also measured daily during quasi-Lagrangian experiments (Figure 4). There were significant daily changes during the experiment in the upwelling region. Nitrate concentration declined over the first 4 days of the experiment and strong vertical gradients in nitrate concentration developed. At the beginning of the experiment, most of the chlorophyll was in the upper 15 m but by day 4, surface chlorophyll concentrations were reduced and the highest concentrations were below 20 m. Primary production showed a similar change in depth profile with a general deepening of the region of maximum production.

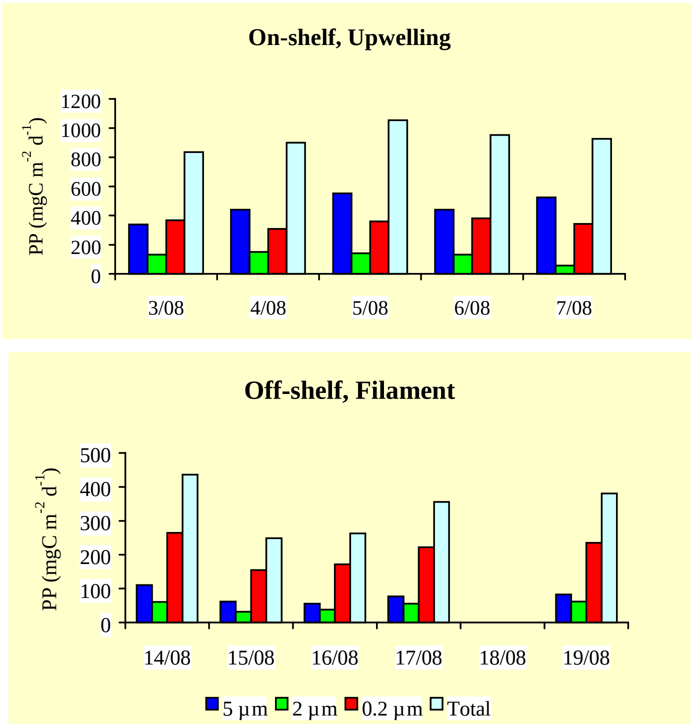

Figure 4. Size-fractionated primary production during two quasi-Lagrangian experiments. During the on-shelf upwelling experiment, the picoplankton fraction was initially more productive than the > 5-µm phytoplankton, but afterward, most of the additional production was due to this > 5-µm fraction. During the off-shelf, filament experiment, production was much lower than during the on-shelf experiment, and picoplankton was always the most productive fraction. *Data from I. Joint et al., Plymouth Marine Laboratory, U.K.*

Moreover, in order to evaluate the primary production and new production, the rates of carbon fixation and utilisation of nutrients (nitrate, ammonium, urea, phosphate) were measured under variable light conditions, either *in situ* or on deck. These experiments allowed us to determine the specific photosynthetic parameters that may be used to extrapolate carbon and nitrogen fluxes through the phytoplankton compartment, covering the highly variable temporal and spatial conditions of the region studied.

On the other hand, algorithms have been developed to estimate primary production from satellite remotely sensed chlorophyll data. Good agreement has been found between estimates of primary production from satellite data and from *in situ* determinations in both the upwelling region and the offshore filaments.

Preliminary estimates indicate that the annual primary production reaches 360 gC m^{-2} y^{-1} on the shelf and decreases to 270 gC m^{-2} y^{-1} on the slope. The export of organic carbon produced on the shelf to subsurface off-shore waters may contribute to a recycled production ($f < 0.1$) which may exceed 200 gC m^{-2} y^{-1}.

ZOOPLANKTON DISTRIBUTION AND GRAZING

Regarding the role of heterotrophic organisms as pelagic sinks, investigations of biomass distributions and activities in the OMEX area highlight differences between seasons (also related to the upwelling cycle) and between the shelf and more oceanic environments.

A Continuous Plankton Recorder (CPR) was towed monthly along the Iberian margin from May 1997 to December 1999 and also historically off the Spanish and Portuguese coast between 1958 and 1990. Copepods are the major grazers in the OMEX II region, and the review of 30-year historical CPR recordings show seasonal peaks for these organisms in April and September, and lowest numbers in December. Total zooplankton carbon distribution in the OMEX II area exhibited the same overall spatial gradient as chlorophyll in August, with high values at on-shelf stations and lower ones off the shelf. Size fractionation, however, reveals that small copepods present a maximum at the very shelf-break. Particular constraints for the fate of phytoplankton biomass may thus be expected to prevail on a band parallel to the shelf.

Microzooplankton community composition was dominated by ciliates throughout the year and had a significant 20–30% heterotrophic dinoflagellate component too. Heterotrophic nanoflagellates showed that this smaller size fraction might comprise a high proportion of total microzooplankton biomass, in particular in the offshore oceanic area.

Copepod grazing activities were investigated for the summer period. Considering the microheterotrophs as a food source, only a negligible percentage of the heterotrophic dinoflagellate stock was consumed by copepods . On average, the total copepod community ingested only about 6% day^{-1} of the chlorophyll stock. If the < 5-µm chlorophyll fraction is not assumed to be efficiently grazed upon, this average ingestion rate increases to 14% day^{-1} (but rarely exceeds 20% d^{-1} at single stations).

Microzooplankton grazing on primary production are important to consider the differences between water bodies within the summer upwelling season. In addition, it is essential to also take into account the utilisation efficiency of primary production-derived matter within the microbial food web, its transfer efficiency to higher trophic levels or its exportation rate *via* sinking particles. Microheterotrophic herbivory during summer Lagrangian experiments was shown to potentially consume all primary production on a daily basis, but pronounced changes were observed within single time–series and between stations within and outside an upwelling filament. The impact of microzooplankton on primary production during the on-shelf upwelling experiment decreased from 80% to less than 40%. Microzooplankton herbivory was much lower within the off-shelf filament but the proportion of primary production consumed in the region was still more than 40% daily (Figure 5).

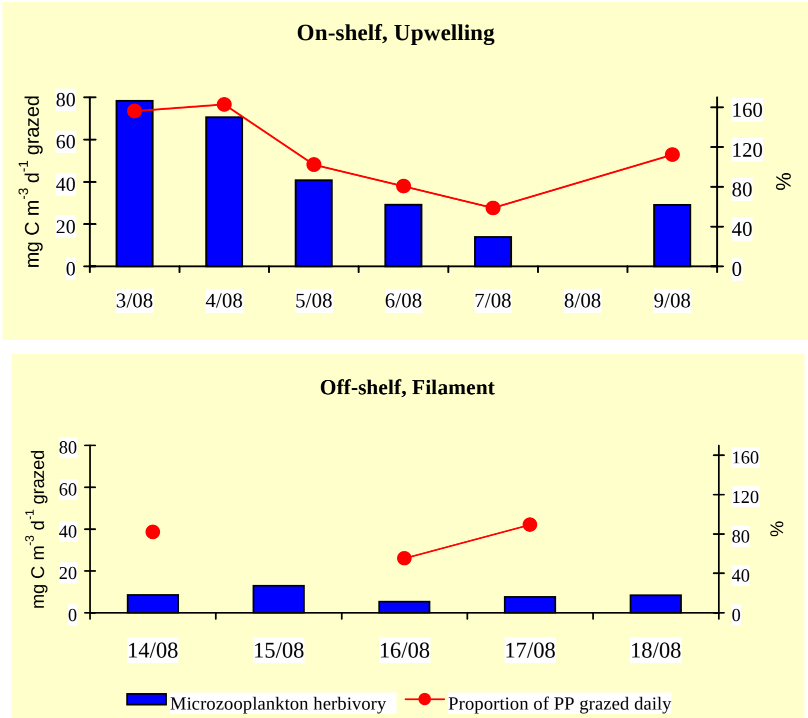

Figure 5. Trophic impact of microzooplankton grazing on phytoplankton for 2 quasi-Lagrangian experiments. *Data from E. Fileman & P. Burkill, Plymouth Marine Laboratory, U.K.*

BACTERIAL LOOP

Bacterial biomass and activity patterns were found to change seasonally and to be related to upwelling. During summer upwelling, maximal bacterial biomass amounted up to 30 µgC l^{-1} and vertical profiles observed revealed an offshore deepening of the bacterial abundance maximum from surface (nearshore) down to 50 m (offshore). During winter downwelling, bacterial abundances were generally much lower, and with reduced division rates, associated with a less stratified water column.

Lagrangian sampling along the shelf edge under relaxation conditions showed an enhancement in both bacterial abundance and specific production during the transition between mixed and stratified conditions, following an increase in primary production. These bacterial abundance and specific production contrasted with those in an upwelling filament where stratified waters yielded much lower values. During both situations, a significant correlation existed between DOC concentration and specific bacterial production. However, different slopes of the linear regressions indicated either qualitative differences in DOC between shelf edge and upwelling filament or the influence of another limiting factor for bacterial production within the upwelling filament.

Zooplankton grazing rates of bacterial biomass obtained with dilution experiments ranged from 0.29 to 1.08 d^{-1} (mean of 0.58 d^{-1}), with however no apparent trend in spatial variability.

AIR-SEA EXCHANGES

In the framework of global change investigations, the distribution of carbon dioxide pressure (pCO_2) and the net budget of CO_2 exchanges in surface seawater are key issues for identifying and quantifying oceanic sources and sinks for atmospheric CO_2. The coastal waters such as in the Iberian margin are most often neglected in global budget calculations, in spite of their intense biological activity or the coastal-related processes (*e.g.*, upwelling, river input) that can induce high CO_2 fluxes at the air-sea interface.

The role of continental shelf seas, influenced by seasonal upwelling in the global inorganic carbon cycle remains controversial because they are sites of processes that have antagonistic effects on the CO_2 flux across the air-sea interface. Upwelling brings to the shelf waters over-saturated in CO_2. These waters also have a high nutrient content, which enhances primary production that in turn reverses the air-sea gradient of pCO_2 and tends to lower the CO_2 values.

The same general pattern was observed in the seasonal distribution of subsurface pCO_2 in the OMEX II area for several summer cruises: over-saturation at Cape Finisterre, under-saturation off the Rías Baixas area and values close to saturation offshore. This pattern is imposed by the input of over-saturated water due to upwelling, primary production, outwelling from the Rías and seawater temperature variations. The situation is especially complicated since during an upwelling event, upwelled water enters the Rías where important primary production, mixing with fresh water and warming occurs. This water is then pushed to the shelf by on-going upwelling. In conclusion the water, outwelled onto the shelf from the Rías is under-saturated in pCO_2, slightly fresher and slightly warmer than surrounding shelf water. During winter, under-saturation of pCO_2 is observed in the entire OMEX II area in relation to cooling of surface seawater. The use of satellite remote sensing allows the evaluation of CO_2 fluxes at larger spatial and temporal scales. The area investigated is characterised by an important annual net flux of CO_2 to the atmosphere

PARTICLE EXPORT

The fate of pelagic primary production at the Iberian Margin is controlled by several key processes, including heterotrophic mineralisation within the microbial food web, particle aggregation and transformation by mesozooplankton or larger gazers and, further, removal from the productive surface layers by gravity sinking of particles. Pelagic food web structure and the types of grazers dominating are decisive for the composition and the amounts of biogenic matter that is available for degradation and/or export from the surface waters. Both aspects are being studied in the OMEX framework in conjunction with investigations of transports from the shelf to the adjacent deep-sea basin by advective processes.

Continuous time-series of particle fluxes recorded with moored sediment trap at two locations with a different distance to the shelf break show the net result of ambient pelagic system properties regarding export from the euphotic zone. Both mooring positions had been selected for a high probability to encounter differences as to lateral inputs of particles originating from the shelf (secondary fluxes). Mooring IM 2, thus, was positioned 10 nautical miles from the shelf break (200-m isobath) at a water depth of 1500 m, and mooring IM 3 was placed 15 nautical miles further to the west at a water depth of 2200 m. A second criterion for the site selection was that both should be under the influence of upwelling and, hence, experience vertical particles fluxes from seasonally varying export regimes. Suspended particulate matter (SPM) distribution as deduced from transmissiometer profiles during February/March 1998 shows the occurrence of a commonly observed Intermediate Nepheloid Layer (INL) at about 200 to 300-m depth. Multiple pronounced INLs were often found within a limited distance of the shelf break only, at water depths < 1500 m. It is concluded that mooring IM 2, located at this water depth, is more likely to receive lateral particle injections from such INLs than mooring IM 3 placed more offshore. The poleward current appears to form a barrier for cross-slope SPM transport below surface layers so that high particle loads are limited to areas close to the shelf break.

The sediment trap data depict the seasonality in the magnitude of fluxes, in elemental composition of intercepted particles and in plankton species exported. The magnitude of carbon fluxes, ranging from < 5 to ~ 30 mgC m^{-2} d^{-1} at 600-m depth, confirms that most of primary produced particles are recycled within the seasonally mixed layer (Figure 6). This holds true also for summer upwelling conditions which promote vertical export and during which high organic carbon fluxes are recorded together with elevated opal fluxes (up to 30 mg m^{-2} d^{-1}). Trap samples from this season contain numerous diatom frustules. Trap results in conjunction with shipboard mappings of bottom and intermediate nepheloid layers reveal that lateral advection takes place primarily close to the shelf break. Traps moored at 1100-m depth on average intercepted four times more material, and with a much higher lithogenic component, at the IM2 mooring than at the IM3 mooring.

PARTICLE DEPOSITION, BURIAL and DIAGENESIS

Particle distribution and transport mechanisms, supported by studies of short-lived isotope activities in the water column and surface sediments, and by hydrographic studies of the Iberian shelf-edge, have identified major mechanisms and fluxes of particle exchange along and across the Iberian Margin. The distribution of Iberian shelf and margin sediments shows strong relationships with sources and near-bottom hydrodynamic conditions.

The total sediment flux and organic carbon burial flux, along the transect where sediment trap moorings are installed, are shown in Figure 7. Highest values of sedimentation fluxes appear at the shelf and shelf-edge whereas considerably lower values on the middle- and lower-slopes are found. On the steep upper slope, sedimentation is presently almost negligible. As a general trend, organic carbon burial fluxes are strongly correlated with mass accumulation rate.

Organic carbon content strongly decreased with increasing distance from shore, while the sandy shelf sediments always have low carbon contents.

Mass accumulation rates and organic carbon burial fluxes on the Iberian shelf, on the slope and deeper stations are higher than those found during OMEX I on the Meriadzek Terrace and Goban Spur. The importance of the role of canyons as pathways for episodic and fast transport of particles and organic matter from the shelf edge to the abyssal plain has been well established in particular for the Nazaré canyon.

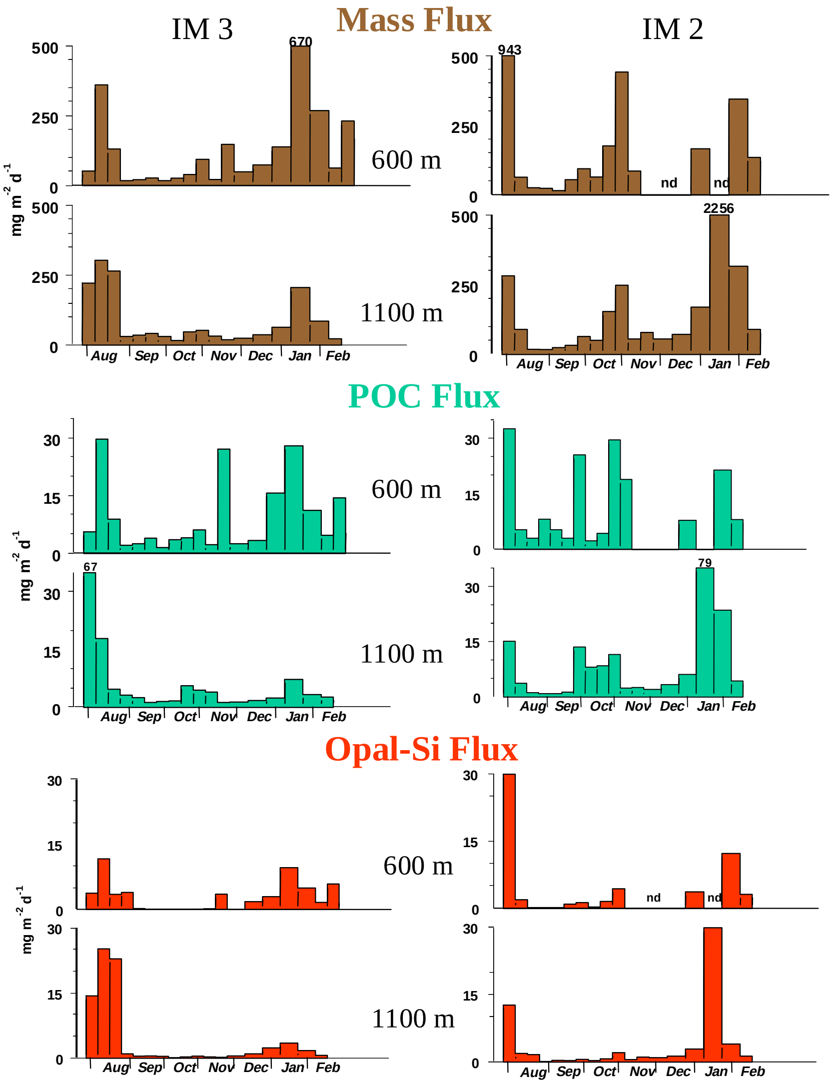

Figure 6. Mass flux, particulate organic carbon (POC) and opal-Si fluxes at the IM2 and IM3 moorings between July 1997 and February 1998. *From A. Antia & R. Peinert, Institut für Meereskunde, Kiel, Germany.*

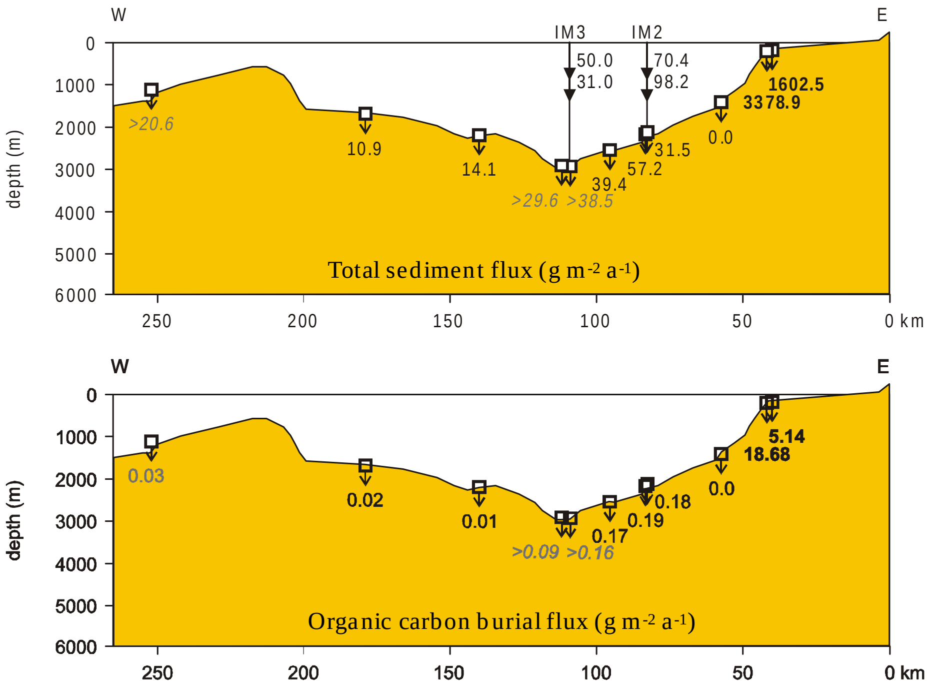

Figure 7. Total sediment and organic carbon burial fluxes. *From T. van Weering & H. de Stigter, NIOZ, Texel, The Netherlands*

Modelling of new and integrated results of pore water profiles, of redox constituents and of solid phase constituents indicate that mineralisation rates on Iberian margin and slope and at abyssal stations are relatively low compared to those derived from the Goban Spur. The carbon mineralisation rates are higher in the area of La Coruña, either caused by lateral export *via* the northern shelf break or by a stronger vertical flux and lower horizontal export flux. However, highest mineralisation rates were found in the Nazaré canyon sediments.

Results obtained up-to-date suggest that the export of organic carbon to the open ocean is higher here than at the Goban Spur area and that accumulation of carbon in sediments is strongly related to the presence of the canyons.

Carbon requirements, estimated by macrofaunal respiration, decreased with increasing water depths from ~ 6.4 gC m^{-2} y^{-1} at the shelf site to ~ 0.5 gC m^{-2} y^{-1} at the deepest station. The relative importance of the mesofauna in carbon requirements was very low (3-10%).

A comparison between the benthic community structure and activity in relation to the supply of organic carbon at the Goban Spur and off the NW Iberian Margin based on data sets obtained during OMEX I and II indicates comparable situations for the Goban Spur and Galician continental slope. However, in the Nazaré canyon rapid transfer of high quality organic matter leads to a contrasting benthic community structure compared with margin sediments at similar depth.

Assessment of long-term change and spatial variability over the Iberian Margin showed consistent patterns of slower and faster flows during glacial and interglacial conditions respectively, compared with present day conditions.

MODELLING EFFORTS

OMEX II modelling efforts include the development of several models with different approaches and aims. A general circulation model takes into account the interactions between the NW Iberian study area and the general circulation of the NE Atlantic. A nested hydrodynamic-ecological model for the water column provides the details of the local flow and its interaction with fine-resolution local bathymetry and includes several state variables describing nutrients, phytoplankton, zooplankton and their role in the formation of particles, in upwelling events and for the quasi-Lagrangian experiments. A benthic ecological-diagenetic model takes into account the benthic fauna activity and the benthic boundary layer processes (see, above). And finally a physical, benthic boundary layer model for the understanding of the physical factors controlling exchanges between the water column and the bottom sediments.

3-D Circulation Model

As a compromise between Cartesian and classical sigma models with their separate advantages and disadvantages, a double-sigma transformation model is used to simulate the circulation in OMEX II region on the Northwestern coast of Iberian Peninsula. The model is forced with the atmospheric conditions supplied by the European Centre for Medium Range Weather Forecast (ECMWF). These forcing conditions change every 12 hours and include heat fluxes; momentum fluxes and mass fluxes. The horizontal spatial step is 0.05º in both directions and the domain covers the entire west coast of Iberian Peninsula. In vertical, 22 layers are used with thickness varying between 10 m and 1000 m.

An important effort was made to obtain a good simulation of the slope current since its presence is remarkable during the entire year at intermediate levels, and also at surface levels during the winter. An accurate seawater density distribution is essential to obtain a poleward flow consistent with the observations. Indeed, results obtained with initial conditions provided by observations are in much better agreement with *in situ* observations than results obtained using Levitus climatological data. The slope current reaches 20 cm s^{-1} off Galicia during winter but is weaker in spring and summer.

During summer the surface flow is basically controlled by windstress, which is more from the North, allowing the occurrence of upwelling and associated equatorward flow. The southward jet is unstable leading to the formation of filaments in particular locations of the Iberian Coast. Offshore extension of the filaments is 100 to 200 km, and vertical signature reaches 300 m. According to model results, velocities inside the filaments can reach up to 50 cm s^{-1}. The 3-D ocean circulation model is now being coupled with a biogeochemical model covering the Iberian margin. The major goal is to estimate exchanges between the shelf and open ocean.

Fluxes of carbon and nutrients are computed in 3 boxes that encompass the whole area of study. Preliminary results show that basically there is a significant lateral export of material in the water column from the shelf to the open ocean.

Simulated Ecosystem Response due to Upwelling Events off the Galician Coast Using a Nested 3D Model

Coupled hydrodynamic and ecological models are used to simulate the response of wind events of the Galician Coast. The hydrodynamic model is nested in three levels. A basin-scale model covering the North Atlantic produces the large-scale flow pattern and necessary boundary conditions for a regional model with 10-km resolution. In order to resolve filament structures that evolve during the upwelling season, a high-resolution model with a 3.3-km resolution is used for the Galician shelf. This model takes the open boundary conditions from the 10-km model. All three model set-ups have 23 vertical layers with increasing thickness from 5 to 500 m. The modes are initialised with Levitus '99 density data and use the same atmospheric forcing.

The ecological model has 8 state variables: nitrate, silicate, flagellates, diatoms, microzooplankton, mesozooplankton, detritus and DOC. The spatial set-up is the same as for the hydrodynamic models, except for the basin scale model. Model results show upwelled nitrate (~ 2.5 mmol-N m^{-3} in the surface waters) near the coast, which are rapidly consumed by phytoplankton, leading to a primary production of around 2.5 gC m^{-2} d^{-1} near the coast and less than 0.5 gC m^{-2} d^{-1} in the offshore regions (Figure 8).

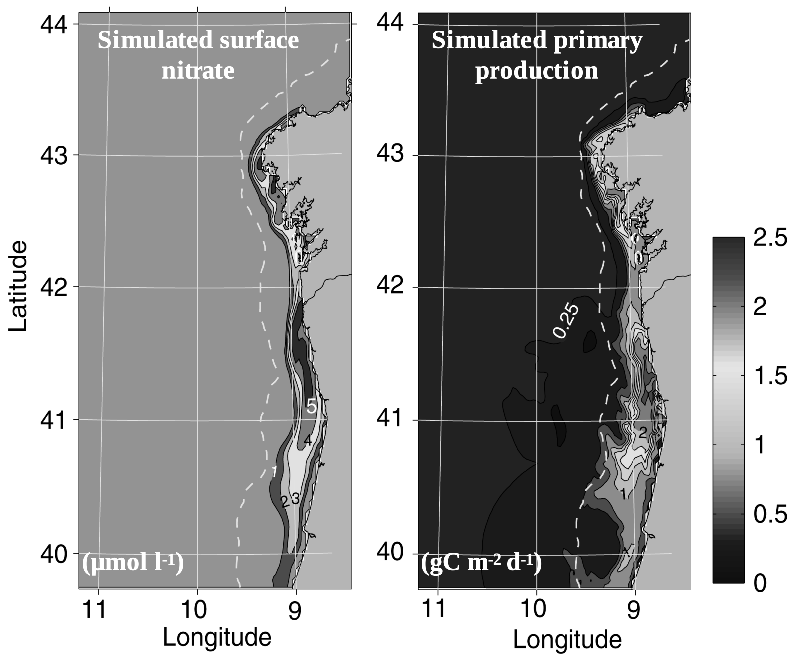

Figure 8. Model simulations showing characteristic patterns with high production near the coast due to upwelling (related to high nutrient values). *From Dag Slagstad, SINTEF, Trondheim, Norway.*

CONCLUSIONS

The results of the OMEX II - Phase II Project at the Iberian Margin, contribute to the understanding of the main processes occurring at the Northwestern European Margins, in the framework of the Global Change studies. The area of investigation is characterised by multiple forcings (tide-related processes, river inputs, wind-driven upwelling, relaxation and downwelling periods, filaments…) that support and control the enhanced production and carbon export to the open ocean.

The annual primary production was estimated to be 360 gC m^{-2} y^{-1} on the shelf and decreases to 270 gC m^{-2} y^{-1} on the slope. The export of organic carbon produced on the shelf to subsurface offshore waters may induce a recycled production ($f < 0.1$) which may exceed 200 gC m^{-2} y^{-1}.

Microzooplankton herbivory indicates that more than 100% of the daily primary production could be grazed during strong upwelling events. Copepod was estimated to ingest about 14% day^{-1} of the chlorophyll stock. Bacterial biomass and activity patterns were found to change seasonally and were related to upwelling, with lowest abundance and frequencies of dividing cells under winter downwelling conditions.

During summer, over-saturation of CO_2 with respect to the atmosphere is prevailing at Cape Finisterre, while under-saturation off the Rías Baixas area and values close to saturation offshore were often observed. This general pattern is further complicated by the input of over-saturated water due to upwelling, by primary production, by outwelling from the Rías and by seawater temperature variations. During the winter, under-saturation of pCO_2 is observed in relation to cooling of surface seawater

Sediment trap data depict the seasonality in the magnitude of fluxes, in elemental composition of intercepted particles and in plankton species exported. The magnitude of carbon fluxes, ranging from less than 5 to about 30 mgC m^{-2} d^{-1} at 600-m depth, confirms that most of primary produced particles are recycled within the mixed layer. This holds true also for summer upwelling conditions which promote vertical export and during which seasonally high organic carbon fluxes are recorded together with elevated opal fluxes (up to 30 mg Opal-Si m^{-2} d^{-1}).

Compared to the results obtained during OMEX I on the Meriadzek Terrace and Goban Spur, the organic carbon export to the Iberian upwelling area is higher, and mass accumulation rates and organic carbon burial fluxes are also higher for the slope and deeper stations. The importance of canyons has been identified, as they are pathways for rapid, episodic transport of particles and organic matter from the shelf edge to the abyssal plain.

Diagenetic modelling using results of pore water profiles, of redox constituents and of solid phase, indicates that mineralisation rates on Iberian margin and slope and at abyssal stations are relatively low compared to those derived from the Goban Spur.

Finally 3-D coupled hydrodynamic-ecological modelling within OMEX II allows the description of the main physical, chemical and biological processes. Modelling also provides prognostic tools to evaluate carbon fluxes in the Iberian margin due to physical forcing. This is particularly important in the context of Global Change studies for the prediction of the potential effects of global warming on the upwelling processes.

ACKNOWLEDGEMENTS

All OMEX II-II partners have contributed to the present work and are greatly acknowledged. We would like to express our special thanks to the Work Package leaders, Paul Wassmann (WP I), Peter Burkill (WP II), Tjeerd van Weering (WP III) and John Huthnance (WP IV). This study is supported by the EU- MAST contract number MAS3-CT97-0076.

TITLE : **BIOCOLOR** - OCEAN COLOUR FOR THE DETERMINATION OF WATER COLUMN BIOLOGICAL PROCESSES

CONTRACT NO: MAS3-CT97-0085

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OCEAN COLOUR FOR THE DETERMINATION OF WATER COLUMN BIOLOGICAL PROCESSES

THE BIOCOLOR PROJECT

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INTRODUCTION and OVERVIEW

The objective of the Biocolor project was to relate changes in the properties of the water column and associated successions in the phytoplankton with changes in optical properties and ocean colour. Thus ocean colour can be utilised to quantify and qualify biological processes in the water column. To this end, an instrument was built which is capable of making suitable in water hyperspectral measurements. This allows measurement of modifications in the underwater irradiance field (and water-leaving radiance) that would be caused by, for example, variations in the light absorbing pigments of varying phytoplankton taxa can be observed. Two study sites were chosen, in the Baltic Sea (Bay of Gdansk) and along the western Irish Shelf. These were selected (a) because they were both Case 2 waters with contrasting optical properties due to the high levels of yellow substances encountered in the Baltic Sea, and (b) the high probabilities of extensive monospecific blooms of varying taxa arising at different times of year. Thus robust algorithms could be constructed and tested whereby pigments (and phytoplankton communities) could be related to optics, and an optical model related to satellite observations would be generated to achieve the overall objective.

METHODS

In water, above water and satellite ocean colour measurements were made in field exercises which covered the diatom spring bloom in western Irish waters, blooms of the dinoflagellates *Peridiniella catenatum* in the Bay of Gdansk and *Gyrodinium aureolum* on the shelf off southwestern Ireland. Analysis of phytoplankton (light microscopy), chlorophyll and accessory pigments (HPLC) augmented the hyperspectral (SUMOSS; Weeks et al., 1999), multiple wavelength radiometers and beam attenuation measurements which were made in addition to conventional hydrographic sampling. A more compact, modular SUMoss was designed, constructed and utilised in field campaigns. Moorings containing optical instruments were deployed during the surveys, and SeaWiFS ocean colour satellite data were collected where possible.

A semi-analytical model was developed from the optical and environmental parameters allowing the calculation of inherent and apparent optical properties and subsequently the underwater light field. Additionally, a Monte Carlo model assisted in the derivation of underwater remotely sensed reflectance values for the use of remote sensing in the Baltic Sea.

RESULTS

Optical measurements were made through spring diatom blooms such as in Galway Bay in 1998 (Figure 1). Although the main collaborative fieldwork was carried out immediately prior to the bloom, a satisfactory diatom population existed which gave a suitably robust accessory pigment ratio of Chl: fucoxanthin = 1.8. This pigment ratio was significantly different from that of 4.3 observed for the dinoflagellate *Gyrodinium aureolum*, which also carries fucoxanthin as the main accessory pigment. A bloom of *Gyrodinium* was present off southwestern Ireland during a field campaign in August 1998. This bloom was observable from satellite ocean colour imagery (Figure 2). Most dinoflagellates contain peridinin as their major accessory pigment, as, for example *Peridiniella*, a bloom of which was present in Bay of Gdansk. Again, a robust chlorophyll:accessory pigment ratios with a value of 2.3.

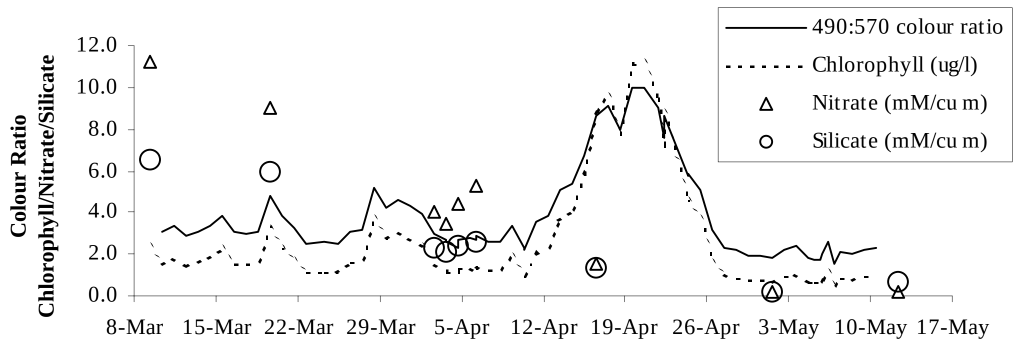

Figure 1. Upwelling irradiance ratio, chlorophyll and nutrient concentrations through the diatom spring bloom in 1998 in Galway Bay, Ireland.

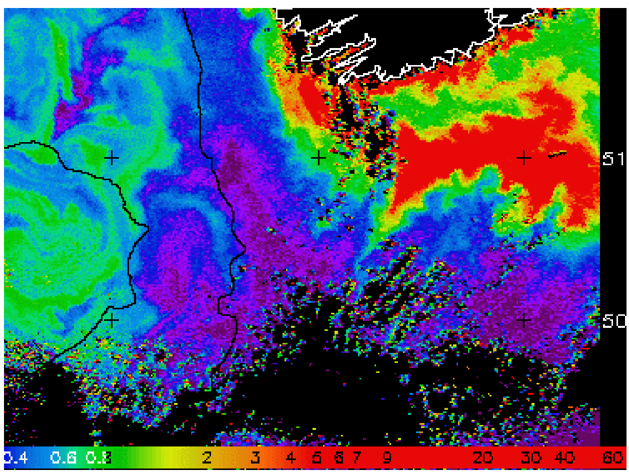

Figure 2. SeaWiFS satellite image of chlorophyll for 4th August 1998. Note the extensive area of high chlorophyll off the southern Irish coast.

Optics and modelling

Measured in situ and laboratory inherent and apparent optical properties were analysed to generate robust algorithms between them and environmental parameters such as chlorophyll, yellow substance and suspended matter concentration. Figure 3 shows comparisons between measured *in situ* and calculated remote sensing reflectance, made on the basis of chlorophyll concentration and yellow substance absorbtion. A good conformity in the overall shape and positions of the maximum were found. The differences in the proportion between reflectances for different wavelengths are important for remote sensing algorithms.

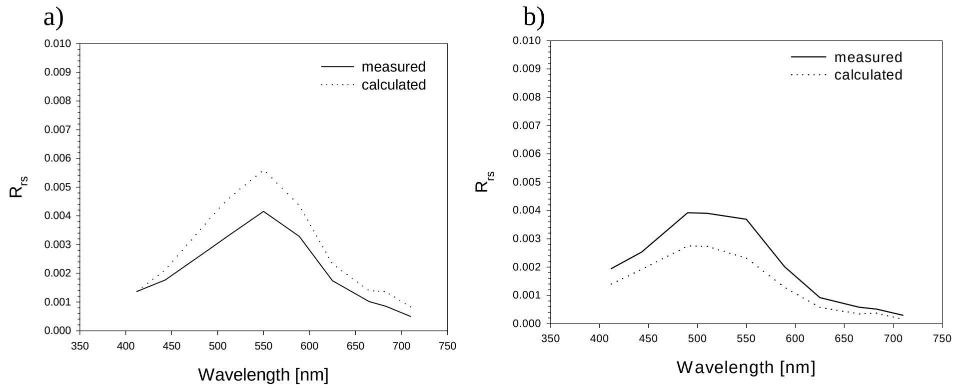

Figure 3 Measured in-situ remote sensing reflectance and that calculated from the semi-empirical model. A) Bay of Gdansk (Chl=3.8mg/m^3 and $a_{ys}(400)=0.77m^{-1}$.); b) Irish Shelf (Chla=0.59mg/m^3 and $a_{ys}(400)=0.312m^{-1}$).

Accuracy of retrieval of remote sensed data will be significantly affected by spatial variability. This was investigated by towing fluorometers and a Lightfish (Robinson et al., 1995) with a high frequency data capture during cruises. Comparison of the chlorophyll estimates resulting from statistical treatments will indicate the size of errors which may be introduced by sub-pixel variability, and re-assess the calibrations of remote sensed chlorophyll concentrations with *in situ* data (Figure 4).

Variations in the pigment distribution within the water column, pigment ratios, yellow substance concentration and suspended matter result in differing absorption spectra for different water types or season. Considerable variability in the physical conditions in the sites chosen results in contrasting phytoplankton communities dominated in turn by diatoms, dinoflagellates, cyanobacteria or microflagellates. Absorption and reflectance data were analysed, relating spectral features to the dominant pigments and phytoplankton groups, and quantifying the effects of pigment packaging. Inversion of the semi-analytical model was carried out using software involving the Marquardt-Levenburg algorithm to find the best fit between measured remote sensing reflectance and environmental data, using the algorithms already produced. Results obtained to date are exemplified in

Figure 5, where it can be seen that, although there is poor fit in the blue part of the spectrum in the case of the Baltic, for the Irish Shelf there is a good fit between the curves. Taken into consideration the natural variation in the concentration of chlorophyll a, even at this early stage of model development the results are quite acceptable. This model has been developed to include yellow substance as well as chlorophyll a and light absorbing pigments that are biomarkers of phytoplankton taxa.

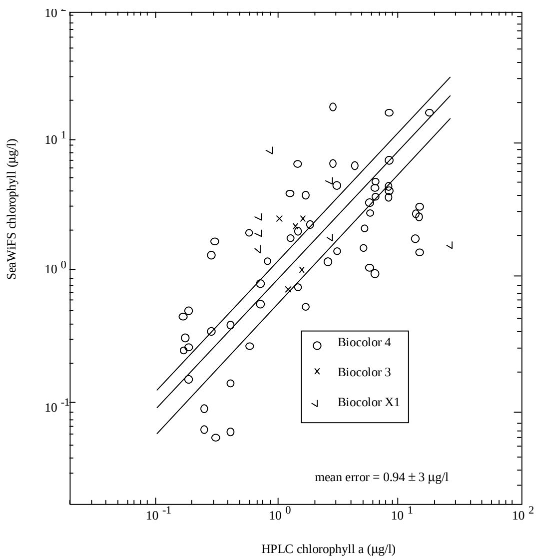

Figure 4. Comparison of chlorophyll a values derived form HPLC analysis of in situ samples and SeaWiFS data from three Biocolor cruises

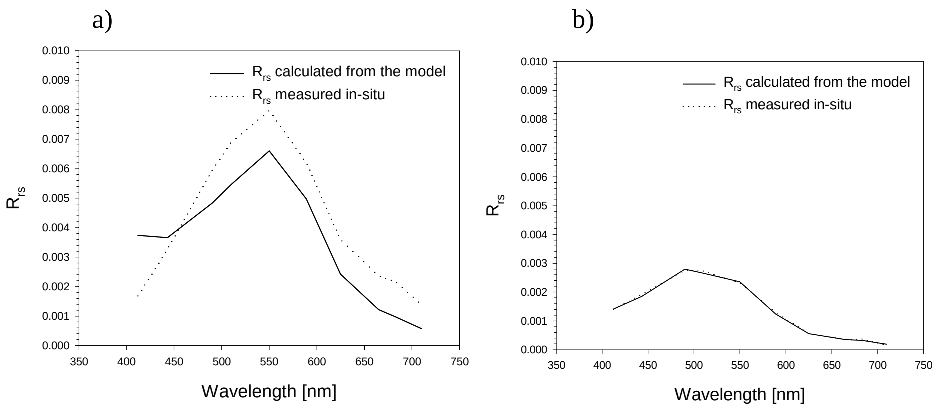

Figure 5. Remote sensing reflectance measured in the a) Bay of Gdansk, Baltic Sea and b) western Irish Shelf together with reflectance calculated from the model for parameters Chla=9.1mg/m^3 and a_{ys}(400nm)=0.22m^{-1} (Baltic) and Chla=0.15mg/m^3 and a_{ys}(400nm)=0.321m^{-1} (Irish Shelf).

CONCLUSIONS

So far in the Biocolor project a model has been produced, based on chlorophyll and yellow substance absorption, which is robust and predicts apparent optical properties with reasonable accuracy, particularly for western Irish Shelf waters. In achieving this result, the accurate measurement of in situ absorption has been of crucial importance. This is a vital goal shared by optical oceanographers, rendered difficult because of the introduction of artifacts in applying methodology. Utilising instruments such as SUMOSS has proved very effective in indicating how this goal can be achieved.

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TITLE : EFFECT OF NUTRIENT RATIOS ON HARMFUL PHYTOPLANKTON AND THEIR TOXIN PRODUCTION SEA : **NUTOX**

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INTERACTIONS BETWEEN NITROGEN: PHOSPHORUS RATIOS AND CONCENTRATIONS AND THE GROWTH AND TOXIN PRODUCTION OF HARMFUL PHYTOPLANKTON

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INTRODUCTION

During the last decades, eutrophication of coastal marine and enclosed brackish waters has led to the alteration of these ecosystems due to increasing nutrients from human activities (industrial effluents, agricultural runoff, and municipal sewage). The discharge of nitrogen and phosphorus to coastal and estuarine waters has increased dramatically during the last 50 years, although phosphorus input has been controlled in most European waters since the 1970's. In contrast, silicon concentrations have remained constant or decreased in coastal waters due to its absence in human wastewater, its exhaustion by large diatom blooms in river or estuaries, and hydraulic management. As a consequence the ratios (in relation to the Redfield ratio) and concentrations of nitrogen (N) and phosphorus (P) in relation to silica (Si) have increased all around Europe during the last decades. In addition to the increased phytoplankton biomass, the number of harmful algal blooms (HAB), often toxic ones, the economical losses from them, the types of resources affected and the kind of toxins and toxic species (mostly non-siliceous) have all increased. In many cases these increases have been related to human activities. The NUTOX project initiates a long-term research to understand the interactions between HAB and the changes of environmental conditions in the European coastal ecosystems related to human activities (EUROHAB initiative). The ultimate objective is to determine the principal causes of these HABs in order to achieve better skill at forecasting their occurrence and possibly control them.

Since 1998, the NUTOX team is focusing its research to determine if the increase of N and P in relation to Si, in the Baltic Sea and the Norwegian Sea, has favoured the occurrrence of blooms of non-siliceous HAB species (i.e. dinoflagellates, haptophytes, cyanobacteria), and to understand how the ratios between N and P affect toxin production in some of these species. There is great interest to establish if toxin production is regulated by external nutrient conditions and increases during nutrient stress regardless of the chemical structure of the toxins produced by HAB species from different taxonomic groups.

Making general recommendations for minimizing HAB toxin production is the ultimate goal of NUTOX, bringing together biologists, chemists and physicists of Northern, Western and Southern Europe. More specifically, the project addresses the following questions : Are potentially toxic flagellates and cyanobacteria outcompeting diatoms at high ratios and concentrations of N. P in relation to Si ? Is toxin production directly connected to external nutrient (N, P) conditions ? Is toxin production connected to cellular nutrient status? Is toxin production regulated at the gene level? Can toxin production be reduced by nutrient manipulation?

PROJECT METHODOLOGY :
Algal ecophysiology, physics,chemistry , molecular biology

The interactions between the nutrient (N, P, Si) ratios/concentrations and harmful algal dynamics and toxin production require integrated studies at the phytoplankton assemblages- and cell-level. The NUTOX approach was to conduct studies with natural phytoplankton communities in mesocosms, and with strains isolated from European waters in semi-continuous and batch cultures. These studies were combined with algal ecophysiology investigations, the creation of molecular probes, the use of a nuclear microprobe (elemental mapping) and bio-optical tools, the development of new analytical methods for algal toxins. The toxic phytoplankton species studied are producing ichthyotoxins *(Chrysochromulina polylepis, C. leadbeateri, Prymnesium parvum/patelliferum, Gymnodinium mikimotoi*), Paralytic Shellfish Poisoning (PSP) toxins (*Alexandrium tamarense, A. minutum*), nodularin (*Nodularia spumigena*), and Diarrhoeic Shellfish Poisoning (DSP) toxins (*Dinophysis* spp.). To determine the relationship between cell nutrient and toxin quotas in these different algal species, we established semi-continuous cultures with different levels of N and P. During steady-state, cell numbers, CNP and pigment composition and toxin quotas were determined. Samples were also collected for designing molecular probes and genetically compared various strains of *Alexandrium* isolated in European waters. Two mesocosms studies were organised in the Baltic Sea (summer 1998) and in the Trondheimfjord (summer 1999) where phytoplankton communities were exposed to a gradient of N:P:Si ratios/concentrations to determine if non-diatom species were favored due to a shortage of Si in the water. Phytoplankton succession was determined by phytoplankton identification and counts, and bio-optical tools (Johnsen et al. 1999a). Cell toxin quotas and profiles were analysed using e.g. High Pressure Liquid Chromatography, LC-mass spectrometry (Hummert et al. 1999, Hummert et al. 2000, Yu et al. 1998), and various toxicity tests e.g. *Artemia*, protein phosphatase, haemolytic assays were applied when necessary. The determination of the cell CNP quotas were analysed with standard methods in cultures (CHN analyser). However, in natural communities, where the toxic species are among hundreds of other species this is an impossible task. Therefore, for the first time in marine phytoplankton ecology, the CNP composition of single cells was successfully measured with a nuclear microprobe (i.e. proton accelerator) (Pallon et al. 1999), which is a technique applied in nuclear physics. This technique allows the analyses of several elements (atomic determination) in single cells.

RESULTS

Effect of high N:P ratios in relation to Si on the tructuration of Baltic summer phytoplankton communities and their toxin production

The manipulation of nutrient conditions (mesocosms studies) modified the quantitative and qualitative of Baltic Sea summer phytoplankton communities where large zooplankton was removed (Granéli et al. 2000). Lowest algal biomass was obtained under N-deficient conditions compared to P-deficient or N, P, Si suffcient conditions (in relation to the Redfield ratio). The chain forming diatom *Skeletonema costatum* dominated the phytoplankton assemblages including at high N:P ratios and low silicon conditions. Although the total phytoplankton biomass was slightly lowered in these conditions, the results indicate that silicon limitation does not seem to exist during summer in the coastal areas of the Baltic Sea Small flagellates were dominated by cryptophytes and potentially toxic haptophytes and reached high biomass in the N deficient treatments compared to nutrient sufficient conditions. No haemolytic activity was associated to these species. The diversity of dinoflagellates species decreased within the experiments; a mixed assemblage of *Peridienella catenata*, *Heterocapsa triquetra*, *Katodinium rotundata*, *Dinophysis norvegica*, *D. acuminata* was replaced by almost exclusively the red coloured *H. triquetra* in all treatments. However, *H. triquetra* was most abundant in N-deficient compared to nutrient suffcient conditions. This result confirms the dominance of this species in the phytoplankton assemblages observed during the last decade along the Swedish coast and part of the Finnish coast from July to September where inorganic N concentrations are below detection. This red coloured dinoflagellate is not toxic but harmful creating high biomass blooms which can alter the marine food web by e.g. creating O_2 depletion. Initially dominating the filamentous cyanobacteria, *Aphanizomenon* sp. disappeared from all treatnemnts, replaced by *Anabena* spp and mostly the toxic *Nodularia spumigena*, producing nodularin, a potent protein phosphatase inhibitor. No marked difference in the growth of *N. spumigena* ($\mu < 0.2\ d^{-1}$) was observed between the various nutrient conditions, except under the most P-deficient conditions ($\mu = 0.3\ d^{-1}$) . In addition, the nodularin quotas were higher in the cells grown under these conditions. Since phosphorus has a high turnover rate in marine systems, and that N shortage will produce less phytoplankton biomass, it is unlikely that a sole reduction of the discharge of P in the Baltic Sea will lead to lower biomass or toxin production in non-diatom species.

Effect of environmental nutrient conditions at the phytoplankton cellular level: nutrient and toxin quotas, inducible genes related to toxin production

A strong relationship between external nutrient availability, cell nutrient and toxin quotas was demonstrated for several phytoplankton species isolated from European coastal waters: *Chrysochromulina polylepis, Prymnesium* spp., *Alexandrium tamarense, A. minutum*, (Johansson & Granéli 1999ab, Legrand et al. 1999, Béchemin et al. 1999*), Gymnodinium mikimotoi, Nodularia spumigena.* All species were toxic regardless the conditions, and their toxin production was enhanced by nutrient limitation in N or P. However, the N-rich saxitoxin production decreased in N-limited *A. tamarense* cultures. Other dissolved substances in the water (e.g. polyamines). can have a synergic effect with nutrients and increase the toxicity of certain algal species (Johnsen et al. 1999b), indicating that toxin production in phytoplankton is not strictly connected to nutrient limitation but also to nutrient stress.

In NUTOX, probes for detecting the presence of stress proteins in *A. tamarense* cells have been designed (Martinez et al. 2000). These stress proteins are expressed in various degree depending upon the conditions of algal growth, and some of them are expressed concomitantly with changes of cell toxin quotas.

For the first time, using a nuclear microprobe (NMP) the cellular chemical composition (C, N, P) has been measured in individual cells of the toxic dinoflagellate *D. norvegica* isolated from natural assemblages. In the Baltic *D. norvegica* assemblages, the cellular C, N, P quotas showed a large diversity, indicating that 35 %, 50 % and 15 % of the cells were N replete (C:N < 20), moderately N-deficient (C:N = 20-40) and severely N-deficient (C:N > 40) respectively (Gisselson et al. 2000). In concentrated *D. norvegica* cells collected from the Trondheimfjord (Norway) , and incubated at different nutrient levels, toxin quotas (measured in bulk samples) increased in all treatments (Karlsson et al., unpublish. data), but most in cells with the lowest P quotas. This approach reveals that differences in nutritional requirements within the same phytoplankton species are more significant than previously thought, and thus the connection between nutrient and toxin quotas are more complex to tackle in natural assemblages than in monocultures.

Minimizing toxin production in HAB species: utopia or reality ?

First results showed that in e.g. *P. patelliferum,* since nitrogen or phosphorus stress increases the algal cellular toxin quotas or the toxicity, supplying the limiting nutrient to the water surrounding the phytoplankton lead to decreasing or minimizing toxin production (Legrand et al. in press). The decrease of cell toxin quotas can be obtained rapidly (1-4 days) depending on the specific growth rate or the chemical structure of the toxin. In case of algae producing N-rich toxins (e.g. *A. tamarense* and saxitoxins), the supply of N (i.e. pulses) can in some cases increases dramatically the toxin quotas which are then higher than in P-limited cells. At the cell level, a rapid inhibition of toxin production proves possible. However, at the ecosystem level, this approach is equivalent to "add gazoline to a fire" since the supply of either limiting nutrients will restore nutrient balance close to the Redfield ratios, but it will increase eutrophication i.e. more algal biomass and lead to problems comparable to the actual situation in polluted European coastal systems. Nevertheless, considering the socio-economical aspects of certain HAB outbreaks, this approach could be tested as a short-term solution during critical situations in small basins with a large catchment area (e.g. aquaculture ponds). Therefore, as the ultimate solution, we recommend to decrease both nutrients (N and P) loading in European coastal waters restoring low and balance nutrient conditions.

CONCLUSION

Experimental achievements already made by the NUTOX project indicate that

- Potentially toxic flagellates and cyanobacteria can outcompete diatoms at high ratios and concentrations of N: P in relation to Si. However, silica is not controlling the algal succession in summer phytoplankton communities in the Baltic Sea.
- Toxin production is directly connected to cell nutrient quotas and therefore to external nutrient (N, P) . Toxin content is usually high when the algae are under nutrient stress.
- Toxin production can be reduced on a short time scale by nutrient manipulation in controlled conditions, and this is achieved by restoring the nutrient balance between nitrogen and phosphorus. However, this approach implies the supply of the limiting nutrient in excess which would lead to increasing eutrophication. Therefore, decreasing both nitrogen and phosphorus loading to coastal systems is the ultimate solution to reduce algal toxin production

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TITLE: THE MOLECULAR ECOLOGY OF THE PHOTOSYNTHETIC PROCARYOTE *PROCHLOROCOCCUS*, A KEY ORGANISM OF OCEANIC ECOSYSTEMS : **PROMOLEC**

CONTRACT N°: **MAS3- CT97- 0128**

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DEVELOPMENT OF MOLECULAR TOOLS FOR THE STUDY OF *PROCHLOROCOCCUS*, A KEY MARINE ORGANISM

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INTRODUCTION

The study of the diversity, structure, function and dynamics of communities of autotrophic picoplankton is one of the fields in which applying a molecular approach is expected to yield the most significant advances (Falkowski and LaRoche., 1991a). These communities not only play a very important role in the biogeochemical cycling of carbon, especially in oligotrophic parts of the world oceans and seas, but also constitute an essential compartment of the microbial food web. Unresolved key questions regarding this compartment include 1) the extent of genetic diversity and its mechanisms of maintenance in the field and 2) the effect of environmental factors on the biochemical and genetic processes controlling photosynthesis, nutrient assimilation and growth in these organisms.

The biological models used nowadays by molecular biologists and biochemists have most often little if any ecological relevance. To draw solid ecological conclusions from laboratory studies and check them on natural populations, it is necessary to deal with model organisms which are ubiquitous, ecologically important (i.e. represent a significant part of the phytoplankton biomass and production), and allow easy genetic work. The marine prokaryote *Prochlorococcus* (Chisholm et al. 1988, 1992) constitutes one of the best candidates for such studies. It proliferates in oligotrophic areas of the world oceans and seas between 45°N to 45°S (Partensky et al., 1999a, b) including the Mediterranean and Red Seas (Vaulot et al., 1990; Veldhuis and Kraay, 1993; Lindell and Post, 1995). It is also the smallest (ca 0.6 µm diameter) and numerically the most abundant photosynthetic organism known to date in the oceans. Typical concentrations in the central Pacific or Atlantic are 2 to 3 10^5 *Prochlorococcus* cells per mL (Campbell and Vaulot, 1993; Partensky et al., 1996). Because of its abundance, this organism is an important contributor (20-50%) to the photosynthetic biomass and primary production in oligotrophic areas (Li et al., 1992; Goericke and Welschmeyer, 1993; Campbell et al., 1994). *Prochlorococcus* is particularly well suited for *in situ* studies since (1) natural populations are easily identifiable and can be precisely counted by flow cytometry (see e.g. Olson et al., 1990) and (2) its specific pigmentation, including pigments unique to this organism (divinyl-chlorophylls *a* and *b)*, can be identified by HPLC pigment analyses (Goericke and Repeta, 1993). *Prochlorococcus* is able to sustain growth and photosynthesis under a very wide range of irradiance from the surface (100% incident sun light) to depths of ca. 100-200 m receiving less than 0.1% of the incident light irradiance (Olson et al., 1990; Campbell and Vaulot, 1993). Moreover, *Prochlorococcus* is most abundant in subsurface waters with low to undetectable levels (< 3 nM) of inorganic nutrients (nitrogen and phosphorus). This raises the question of which biochemical mechanisms or alternative modes of nutrition (chemo- or heterotrophy) this organism may have developed to both optimize its nutrient assimilation and reduce functional and (or) structural alterations of its photosynthetic and growth apparatus expected during nutrient depletion.

For all these reasons, the PROMOLEC project focuses on *Prochlorococcus*. The interest of studying a single ecologically relevant microorganism is that it allows to precisely determine its specific function within ecosystems, i.e. how it is controlled and may in turn influence its own environment. In contrast, studies on communities are generally limited to either global or average measurements on heterogeneous populations, which hardly reflects the dynamics and particularities of the individual components.

Aims of PROMOLEC are to study intra-generic diversity and major cellular functions of *Prochlorococcus*, first *in vitro***,** under controlled conditions, then *in situ*, under natural conditions. The work content has been split into the following topics: genetic diversity, photosynthesis, nutrient assimilation and limitation and cell cycle/growth. Each topic gathers activities in the laboratory and in the field. The project combines an extensive genetic study and classical physiological (e.g. photosynthetic rates) and biochemical (e.g. pigments) measurements. One workshop gathering all partners was performed in Roscoff (France) from May 25 to June 2, 1999 and consisted at studying the physiological responses of cells to light and nutrient stresses. A second workshop will be held in Eilat (Israel) in September 2000 and will aim at determining the genetic and phenotypic diversity as well as the physiological status of cells collected during a natural bloom in the Red Sea. Beside these workshops, numerous natural samples have been or will be collected during cruises conducted in the Mediterranean Sea, Red Sea or Atlantic ocean, within the framework of independent oceanographic programmes. Thus, at the end of the project, information will be gathered on *Prochlorococcus* populations from a variety of marine ecosystems. The main novelty of the project is to relate information provided by molecular tools to that obtained with traditional oceanographic methods (e.g. HPLC pigment analyses, ^{14}C incubation, spectrophotometry).

GENETIC DIVERSITY OF *PROCHLOROCOCCUS* STRAINS AND NATURAL POPULATIONS

The isolation and characterization of different pigment types of *Prochlorococcus* with distinct irradiance optima for growth and photosynthesis (Partensky et al., 1993; Moore et al., 1995; Moore and Chisholm, 1999), as well as the recurrent observation at certain depths in the field of populations with different mean chlorophyll red fluorescence as measured by flow cytometry (Campbell and Vaulot, 1993; Partensky et al., 1996), have shown that this genus is constituted by several genetically distinct ecotypes (Moore et al., 1998). Thus, in stratified waters such as in the tropical Atlantic or Pacific oceans, there are *Prochlorococcus* populations adapted to grow in the upper layer of oceans (from surface to 60-100 m) and others adapted to grow at deeper depths, i.e. at and below the chlorophyll maximum (ca. 80-180m). These have been dubbed "high light" (HL) and "low-light" (LL) ecotypes, respectively (Urbach et al., 1998).

One major objective of PROMOLEC is to characterize the diversity of cultures and natural populations of *Prochlorococcus* using a combination of approaches (genetic, biochemical and phenotypic) and to determine the role of environmental parameters on the origin and maintenance of this diversity. Our major findings till now may be summarized as follows :

i) On a genetic basis, surface isolates from many different areas of the world ocean are relatively little divergent. Still, they can be separated between HLI and HLII types on the basis of their 16S rRNA sequences (West and Scanlan, 1999; West et al., in prep.). The biochemical (pigments) and physiological characterization (growth irradiance optima) of these two HL types did not allow to uncover phenotypic differences between them yet. It is possible that they differ by their nutrient acquisition behaviour.

ii) The genetic distance between the HL clusters and the LL strains (the latter are not gathered together on a separate cluster but are on different branches) is far wider than in-between HL strains.

iii) The distribution of *Prochlorococcus* genotypes within the water column is dependent on hydrodynamism: The community structure of *Prochlorococcus* at two stratified stations in the north-eastern Atlantic was investigated. Flow cytometry data indicated that *Prochlorococcus* comprised 93-97% of the total picophytoplankton and exhibited a sub-surface maximum at approximately 50 m. DNA was extracted then the 16S rDNA gene was amplified by PCR using *Prochlorococcus*-specific primers (West and Scanlan, 1999). Dot-blot hybridisation using specific oligonucleotide probes to different genotypes (HLI, HLII, LL and one probe specific to a well-studied LL *Prochlorococcus* strain: SARG) revealed that, in these water conditions, only HLI and LL were present, with an obvious niche-partitioning along the water column. A clear shift from the HL to the LL genotype was observed down the vertical, a transition correlated with the depth of the surface mixed layer (SML). Only the HLI genotype was found in the SML, whereas the low light genotype was distributed below the SML (West & Scanlan, 1999). More recently, a mixed station from the Sargasso Sea was also studied using these oligonucleotides. Dot-blot hybridisation experiments revealed the presence of LL and HLII genotypes only, i.e apparent absence of HLI and, again, SARG genotypes. The vertical distribution of HLII and LL genotypes down the water column correlated well with its mixed status (West & Scanlan, in prep.).

PHOTOACCLIMATION VS. PHOTOADAPTATION

"Photo*acclimation*" refers to the capacity of a cell to maximize light harvesting when photon densities are low and minimize photooxidative damage to the photosynthetic machinery at high irradiance levels (Falkowski and Laroche, 1991b). In the specific case of *Prochlorococcus,* the real extent of the physiological plasticity of cells in the field is hindered by the occurrence of genetically distinct ecotypes with pigment ratios (especially divinyl Chl *a* to Chl *b* ratio). Ecotypic differentiation of pigment content is an *evolutionary* process, that can be termed "photo*adaptation*". It is driven by long-term or permanent exposure to particular light niches in the ocean. This superimposition of *physiological* and *evolutionary* processes leads to an *apparent* high adaptability of *Prochlorococcus* populations to light.

Within the PROMOLEC project, we have looked at the effect of genetic diversity on the composition and structure of the photosynthetic apparatus and we have discovered several major differences between ecotypes: The *pcb g*ene, which encodes the major divinyl-chlorophyll *a/b* light harvesting complex (LaRoche et al., 1996), was found to be present in multiple (up to 7) copies in LL ecotypes while it was present as a single copy in four HL ecotypes (Garczarek et al., 2000 and unpubl. data). Phylogenetic analyses showed that *pcb* genes from all HL ecotypes clustered together whereas the seven *pcb* genes from the LL *Prochlorococcus* SS120 were more variable and were separated between two widely distant clusters. We suggest that *pcb* gene multiplication is associated with a differentiation in the function of some of the resulting *pcb* genes, which are all different. This probably corresponds to a major adaptation of LL strains to cope with low light levels. We checked that all 7 genes are translated and found that their level of expression significantly differ (Garczarek et al., in prep.). Whether they are differentially regulated by light remains to be checked. Although functionally less important in *Prochlorococcus*, presence of phycoerythrin in LL but not HL strains is also a major differentiation between ecotypes (Hess et al., 1996, 1999). On the genetic level, LL strains possess a gene region including 11 genes implicated in phycoerythrin synthesis and function, which is reduced to a single highly mutated gene in HL strains (Hess et al., 1999). Expression studies shows that phycoerythrin is present in low amounts in LL but not HL strains and this phycobiliprotein proved to be functional (Lokstein et al., 1999), although apparently its cell concentration is insensitive to both growth irradiance level (Hess et al., 1999) and nitrogen starvation (Steglich et al., in prep.). Several other photosynthetic genes belonging to both photosystems I and II were compared between ecotypes but did not reveal any significant differences, although they did show some specificities with regard to corresponding genes in other known organisms, including cyanobacteria (Garczarek et al., 1998; van der Staay et al., 1998; van der Staay and Partensky, 1999; van der Staay et al., in revision).

The effect of a light:dark cycle on the circadian variations of metabolic activity, including growth, cell cycle, carbon assimilation and photosynthetic gene expression was studied on an axenic HL *Prochlorococcus* strain during the lab Workshop in May-June 1999. A sophisticated illumination system allowed to simulate a very smooth light:dark cycle resembling the natural cycle of solar light, including a very high photon fluxes at noon (Bruyant et al., submitted). As in the field (Vaulot et al., 1995), these conditions allowed a tight synchronization of the cell cycle of *Prochlorococcus*, with an entry of cells in the DNA synthesis phase (S) in the early afternoon and a division starting 4 hours after the light-to-dark transition (Partensky et al., in prep.). Expression of photosynthetic genes was also tightly synchronized. Interestingly, transcript levels of the antenna gene (*pcbA*) showed maxima at the light-dark transitions whereas the expression of the *psbA* gene, encoding the photosystem II core, was tightly correlated with growth irradiance (Garczarek et al., in prep.). These data allow to better understand the strategy developed by HL *Prochlorococcus* ecotypes to cope with high light irradiances received by cells in the upper layer of oceans.

NUTRIENT UPTAKE AND LIMITATION

The question of whether natural populations are nutrient limited is particularly intriguing in the case of *Prochlorococcus* which is able to grow in environments where no mineral nutrients can be detected, such as the mixed layer of oceanic subtropical waters (Campbell and Vaulot, 1993; Lindell and Post, 1995; Partensky et al., 1996). To understand better this ability, we have begun to look at the presence of know protein components of systems involved in N (*AmtA*, *ntcA*, *glnA*, *glnB*) and P (*pstS*) uptake in selected *Prochlorococcus* strains and are searching for new ones. We also are studying the expression of the corresponding genes under nutrient stress conditions. Those proteins which are specifically induced during N or P starvation will be used as markers of nutrients limitation of *Prochlorococcus* cells in the field.

Amazingly, an axenic HL isolate *Prochlorococcus* (PCC9511) was found incapable of nitrite and nitrate assimilation (Rippka et al., in press). This finding is consistent with recent information made available by the American Department of Energy on the genome of a HL strain (MED4; http://spider.jgi-psf.org/JGI_microbial/ html/prochlorococcus_homepage.html), which indicates that most genes required for nitrate assimilation, namely nitrate reductase (*narB*), nitrite reductase (*nir*), and *nrtA* (encoding the nitrate transport protein *NrtA*), which are located next to *nrtBCD* in the cyanobacterium *Synechocystis* PCC 7942, are missing in MED4. Therefore, this HL ecotype feeds mainly on ammonium and urea. It has no specific requirement for amino acids, and in particular cannot grow on arginine or glutamine as the sole nitrogen sources. Rates of ammonium assimilation are enhanced in N-deplete cultures of *Prochlorococcus* PCC 9511 when compared to N-replete cells (Lindell and Post, unpublished). Other adaptations include the lack of effect of nitrogen starvation on glutamine synthetase activity which is very unusual and could reflect an adaptive response of *Prochlorococcus* to cope with environments limited in nitrogen (Garcia-Fernandez et al., submitted).

An important information derived from the availability of the genome sequence of *Prochlorococcus* MED4 is the absence of *nifH* gene, which implies that it is incapable of uptaking atmospheric nitrogen (N_2).

GROWTH AND CELL CYCLE

The cell cycle is loosely defined as the coordination of all cellular processes between cell birth and cell division. By homology with that of eukaryotes, *Prochlorococcus* cell cycle can be divided into 3 phases: G_1 , S and G_2. S corresponds to the replication of DNA, while G_1 and G_2 are intermediate phases more dedicated to cell growth, the latter preceding cell division (Vaulot, 1995). Recently it has been shown, by measuring the cell cycle of *Prochlorococcus* in oceanic waters with flow cytometry, that the application of the cell cycle concepts can yield invaluable information on both the nutritional status and the net growth rate of natural populations (e.g. Vaulot et al., 1995, Liu et al., 1997). These data revealed that these prokaryotes are highly synchronized to the daily light cycle with division rates in the upper euphotic zone of the order of 1 doubling per day.

Two key cell cycle genes (and their surrounding genomic region) have been sequenced in several *Prochlorococcus* strains: *dnaA*, coding for a protein required for DNA replication, and *ftsZ*, essential for cell division (Holtzendorff et al., in prep.). The organisation of these genes in the genome is similar in both *Prochlorococcus* ecotypes. Their circadian expression was studied on a synchronized axenic culture of PCC 9511 during the Roscoff lab workshop. Both genes exhibited clear diel patterns with maxima during the replication (S) phase. Thus, the transcription of genes involved in replication and division is co-ordinated in *Prochlorococcus* sp. PCC 9511 in a circadian manner and determines the timing of DNA replication and cell division (Holtzendorff et al., in prep.).

The effects of light intensity, light duration (relevant to the natural photocycle) and light modulation (i.e. progressive variation from dark to maximum irradiance) on *Prochlorococcus* cell cycle was investigated in detail in culture. Experiments performed under 12:12 L:D cycle revealed that HL and LL ecotypes have similar division patterns but differ with other picoplankters (Jacquet et al., in prep. a). Three major types of experiments and can be summarized as follows:

i) comparison between light:dark (L:D) cycles with either a fixed or a modulated light regime over the photoperiod shows that the latter conditions induce a slightly better synchronization of the cell cycle.

ii) variations in the timing of the “light on” signal on populations acclimated to a given L:D cycle revealed a sharp coupling between light onset and the initiation of DNA synthesis in *Prochlorococcus*, suggesting this signal to be a key parameter for the synchronization of cell cycle.

iii) the effect on L:D entrained populations of continuous darkness or constant light : when put in dark conditions, cells are unable to restart a division cycle, whereas when placed under constant irradiance, they continue to cycle for at least two days, suggesting the presence of an endogeneous clock (Jacquet et al., in prep. b).

CONCLUSION

The multi-disciplinary work performed within PROMOLEC should allow to have a large and unprecedented picture of the genetics, ecophysiology and diversity of the smallest and most abundant photosynthetic organism known to date on Earth: *Prochlorococcus*. As a major contributor to the photosynthetic biomass and primary production in oligotrophic parts of the world oceans, this single organism has clearly a tremendous influence on biogeochemical cycles. The information that will be drawn from the PROMOLEC project should therefore be useful for many members of the oceanographic community. To this date, work has mainly concerned cultures, but we now have basic information that can be checked on natural populations and will allow to better understand the functioning of oligotrophic ecosystems.

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TITLE : ECOLOGICAL EFFECTS OF PROTECTION IN MEDITERRANEAN MARINE RESERVES - **ECOMARE**

CONTRACT N° : MAS3-CT97-0155

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ECOLOGICAL EFFECTS OF PROTECTION IN MEDITERRANEAN MARINE RESERVES (ECOMARE)

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OBJECTIVES OF THE ECOMARE PROJECT

The overall aim of ECOMARE is to unite and coordinate the efforts of a broad group of research teams involved in assessing the effects of protection in Mediterranean littoral ecosystems. Within EU waters, Mediterranean reserves are singled out for this study, not only because of their geographical proximity, but also because they share important oceanographic and ecological features.

The general objectives of the ECOMARE project are:

1. To establish the state of knowledge of the responses of marine communities to protective measures in Mediterranean littoral ecosystems.

2. To identify the main research needs and steps forward to progress from the assessment of effects on exploited species to the assessment of the reserve effect at the ecosystem level.

3. To standardize the working methodologies and data analysis and management procedures for research in the areas identified in 2 so that in the future, investigations can be carried out in a coordinated and comparable manner.

CONCEPTUAL MODEL

The first step of ECOMARE was to design a conceptual model of the reserve effect in Mediterranean littoral ecosystems. Once built the conceptual model served as a tool for identifying priority areas for research. The conceptual model was developed around five modules:

1. Human activities affected by protection.
2. Effects of protection on recruitment: distribution-dispersion of eggs, larvae, and juveniles.
3. Demographic changes and changes in reproductive biology in protected areas.
4. Indirect effects of protection: cascade effects (trophic webs).
5. Relations with habitat and spatio-temporal distribution.

STATUS OF KNOWLEDGE OF THE "RESERVE EFFECT" IN MARINE ECOSYSTEMS

The second step in ECOMARE was to conduct a review of the status of knowledge of the "reserve effect" in marine ecosystems in the priority areas identified in the conceptual model. Given the scope of ECOMARE, Mediterranean marine reserves have been treated as a special case. That is, available studies from marine protected areas (MPAs) from around the world that may be relevant to understanding the responses of protected communities in the Mediterranean have been reviewed, along with the available data for Mediterranean MPA's. The final objective of this revision was to highlight what is known about the responses of marine communities to protection in Mediterranean littoral ecosystems, what can be inferred from knowledge acquired in other marine regions, and finally what are the most important gaps in knowledge which need to be filled to better understand the response of communities in protected marine ecosystems.

The reviews will be published in the June 2000 issue of the journal Environmental Conservation (Cambridge University Press).

ABSTRACTS OF REVIEWS

1. The need to consider cultural and socio-economic factors in establishing Mediterranean marine reserves

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Abstract

Marine reserves may not only be important for protecting the marine environment, but may have substantial socio-cultural impacts about which very little is currently known, or acknowledged. In the Mediterranean, few data are available on the socio-economic consequences of protection. The present study reviews the existing data on Mediterranean marine reserves, on four reserves in Spain, four in France, two in Italy and one in Greece and highlights the need to consider cultural and socio-economic factors in reserve establishment.

A general increase in tourist activities in Mediterranean marine reserves is evident, as are greater yields for fisheries and an increase in the abundance of larger fish species. Data to support a hypothesis that greater yields for fisheries occur when exploited species increase in biomass in marine reserves are scarce. A large increase in the number of divers and vessels

using marine reserves has already had impacts on natural benthic communities as a result of diver damage, mooring and the feeding of large fish by divers. Emphasis has been given in only a few marine reserves to promoting public awareness of these impacts.

Although the conservation of nature should be considered as the fundamental objective of marine reserves, neglecting their social, cultural and economic impacts has at times led to poor local consensus, if not hostility. We believe that planning and managing marine reserves should be conducted on a multidisciplinary basis. Nonetheless, no single model can be considered valid for the whole Mediterranean: the very variable characteristics of coastal areas, from those of small uninhabited islands to those of cities requires different weightings to be assigned for each factor in order to achieve a durable equilibrium and realise the original objectives of a marine reserve.

Only with such flexibility of management will it be possible to reach a greater understanding of the marine reserve system and create a lasting consensus in favour of conservation, a consensus which would mean an overwhelming majority of people actively avoiding damaging nature and preventing others from doing so.

Key words: Marine Reserves, socio-economic aspects, tourism, diving, fisheries, Mediterranean sea.

2. Density dependence in marine protected populations: A review

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Abstract

The cessation or reduction of fishing in marine protected areas (MPAs) ecosystem should promote an increase of abundance and the mean size and age of previously exploited populations. Density-dependent changes in life history characteristics should occur when populations are allowed to recover in MPAs. In this review we synthesise the existing information on resource limitation in marine ecosystems, density-dependent changes in life history traits of exploited populations and evidence for biomass export from MPAs. Most evidence for compensatory changes in biological variables has been derived from observations on populations depleted by high fishing mortality or on strong year classes, but these changes are more evident in juveniles than in adults and in freshwater than in marine systems. It is unclear if adults of exploited marine populations are resource limited. This may suggest that exploited populations are controlled mainly by density-independent processes, which could be a consequence of the depleted state of most exploited populations. MPAs could be a useful tool for testing these hypotheses. If we assume that resources become limiting inside MPAs, it is

plausible that, if suitable habitats exist, mobile species will search for resources outside of the MPAs, leading to export of biomass to areas that are fished. However, it is not possible to establish from the available data whether this export will be a response to resource limitation inside the MPA, the result of random movements across MPA reserve boundaries or both. We discuss the implications of this process for the use of MPAs as fisheries management tools.

Keywords: Marine reserves, Carrying capacity, Density-dependence, Spillover, Fisheries enhancement, Mediterranean.

3. Effects of marine protected areas on recruitment processes with special focus on Mediterranean littoral ecosystems

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Abstract

The present work reviews concepts and field evidence for the efficiency of marine protected areas (MPAs) in enhancing recruitment of protected species. Geographically this review focuses on assessment of the effects of protection on littoral communities in Mediterranean marine reserves. Despite this geographic focus, we review available evidence from studies on recruitment processes in general and from studies carried out in MPAs both in the Mediterranean and elsewhere. The general questions of interest are whether the increase in biomass of the protected species in a marine reserve has an effect on recruitment in the reserve or in neighbouring areas and if it affects competition and predation on new recruits inside the marine reserve. A flow diagram of the effects of MPA status on recruitment is developed and employed to identify the relevant processes. The diagram incorporates three levels of factors: 1) characteristics of the MPA (location, size, habitat type, oceanography and level of protection); 2) life stages of protected species relevant to recruitment (eggs, larvae, settlers and juveniles); and 3) fundamental processes of dispersion/movement, predation and competition. From this conceptual diagram the following main components of the recruitment process were identified and used to structure the review: A) Relationship between the ecology of the pelagic stages and the design, location and oceanographic regime of the MPA; B) Effects of protection of nursery habitats on settlement success; and C) Effects of protection on survival of settlers and juveniles (competition and predation). We found a exceptionally low number of studies specifically addressing recruitment processes in MPAs. This was particularly the case in what concerns the relationship between larval ecology and the characteristics and oceanographic regime of MPAs. The effectiveness of an MPA in promoting recruitment mainly depends on the location and on the size of the MPA in relation to the reproductive biology and the larval ecology of the species to be protected. The choice of the location and size of the MPA from the point of view of recruitment enhancement in turn depends on its objectives, whether it is to protect the entire species life cycle, the juveniles, or to increase egg production and larvae export to surrounding non-protected areas. The assessment of the relationship between the protection of nursery habitats and settlement success evidences that the magnitude of the effects of protection depends on whether the recruitment of the protected species is restricted to narrowly defined environmental situations or can occur in diverse environments, including areas out of the influence of the MPA. Thus, the location of the MPA determines the protected habitats and consequently the species for which settlement will be favoured. For Mediterranean species, the near-shore strip is identified as the area encompassing the most essential nursery habitats for protection. The assessment of available data on the effects of protection on competition and predation at the post-settlement stage of Mediterranean littoral fish showed no differences in survival of newly recruited individuals inside and outside Conversely, for older recruits mortality was found to be higher inside MPAs, probably due to the increased abundance and size of large predators in protected areas. The available information for lobster species in other marine regions suggests that recruitment success inside protected areas might also be negatively affected by MPA status because their predators would be more abundant. This study highlights the almost total absence of studies addressing even the most elemental questions of recruitment in the context of marine reserves.

Key words: Eggs and Larval Export, Habitat, Marine Protected Areas, Mortality, Mediterranean, Recruitment, Settlement

4. Trophic cascades in fisheries and protected-area management of benthic marine ecosystems

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Abstract

An important principle of environmental science is that change in single components of systems is likely to have consequences elsewhere in the same systems. In the sea, food web data are one of the only foundations for predicting such indirect effects, whether of fishery exploitation or recovery from its effects in marine protected areas (MPAs). We review the available literature on one type of indirect interaction in benthic marine ecosystems, namely trophic cascades, which involve three or more trophic levels connected by predation. Our purpose is to establish how widespread cascades might be, and infer how likely they are to affect community outcomes of marine protected area management or intensive resource exploitation. We review 35 well-substantiated cascades from 19 locations around the world, and all but two of these are from shallow systems underlain by hard substrata (kelp forest, rocky subtidal, coral reefs and rocky-intertidal). We argue that these systems are well represented because they are accessible and also amenable to the type of work which is necessary. Nineteen examples come from the central-eastern and northeastern Pacific, while no benthic cascades have been reported from the NE, CE or SW Atlantic, the Southern Oceans, E Indian Ocean or NW Pacific. The absence of examples from those zones we suggest as being due to lack of study. Sea urchins are very prominent in the subtidal, and gastropods, especially limpets, in the intertidal, examples; we suggest that this may reflect their predation by fewer specialist predators than is the case with fishes, but also their conspicuousness to investigators. The variation in ecological resolution among studies, and in intensity of study among systems and regions indicates that more cascades will be identified in due course, while broadening the concept of cascades to include pathogenic interactions would immediately increase the number of examples. The existing evidence is that cascade effects are to be expected when hard-substratum systems are subject to small-scale resource exploitation, but that the particular problems of macroalgal overgrowth on Caribbean reefs and coralline barrens in the Mediterranean rocky-sublittoral will not be readily reversed in MPAs, probably because factors other than mere predation cascades have contributed to them in the first place. More cascade effects should be found in the soft-substratum systems which are crucial to so many large-scale fisheries, when opportunities such as those of MPAs and fishing gradients are available for study in such systems, and the search is widened to less conspicuous focal organisms such as polychaetes and crustaceans.

Keywords: Marine protected areas, fishing effects, ecosystems, food webs, management

5. Evaluating the ecological effects of Mediterranean marine reserves: habitat, scale and the natural variability of ecosystems

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Abstract

Our capability to detect and predict the responses of marine populations and communities to the establishment of marine protected areas (MPAs) depends on our ability to distinguish between the influence of management and natural variability due to the effects of factors other than protection. Thus, it is important to understand and quantify the magnitude and range of this natural variability at each scale of observation. Here we review the scale of responses of target populations and communities to protection within Mediterranean MPAs, against their 'normal' spatio-temporal heterogeneity, and compare those with documented cases from other temperate and tropical marine ecosystems. Additionally, we approach the problem of the relative importance of habitat structure, considered as a set of biological and physical elements of the seascape hierarchically arranged in space at multiple scales, to drive natural variability. We conclude that much more effort has to be done to characterise heterogeneity in relation to Mediterranean MPAs, and to quantify (and explain) the relationship between target species and their habitat as a source of such variability. These studies should be based on sound sampling designs, which (1) generate long-term data sets, (2) would ideally be based on a Mediterranean-wide comparison of a number of protected and unprotected localities, (3) be from a multi-scaled perspective, and (4) control for factors other than protection, in order to avoid their confounding effects. The need for appropriate spatial and temporal replication, nested designs and power analysis is advocated.

Keywords: marine protected areas, Mediterranean, heterogeneity, scale, habitat structure, sampling designs, target species, fishes.

BOOKLET OF METHODS

The last objective of ECOMARE was to produce a handbook of methods for research of interest in the context of Mediterranean marine reserves. Three research priorities were identified from the review work carried out before: 1) methods to assess biomass export from marine reserves; 2) methods for assessing the impacts of human frequentation on marine protected areas; and 3) methods to evaluate indirect effects of protection mediated by trophic interactions, in particular the fish-sea urchin–algae interaction.

The handbook of methods will serve as an introductory guide to the researchers interested in tackling this type of questions in marine reserves. The Booklet of Methods will be published by the Group d'Interet Scientifique (GIS, Marseille, France) during the third quarter of 2000.

TITLE: PROCESSES OF VERTICAL EXCHANGE IN SHELF SEAS : **PROVESS**

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PROCESSES OF VERTICAL EXCHANGE IN SHELF SEAS - PROVESS

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SUMMARY

PROVESS is a three year MAST III project which started on 1 March 1998 studying vertical exchanges in shelf seas. The main aims of the project are described and progress outlined.

INTRODUCTION

Vertical exchanges are central to many shelf sea processes, for instance the development and erosion of the pycnocline; the onset and decline of phytoplankton blooms; particle sedimentation and the remineralisation of particulate matter in the benthic boundary layer. Vertical exchanges are controlled principally by the turbulence characteristics of the water column. Turbulence is generated both at the surface and at the sea bed, whilst at the pycnocline turbulence levels are reduced and vertical fluxes can be inhibited.

Reliance is placed on turbulence closure schemes to quantify fluxes in shelf sea environmental models - a crucial example is the entrainment of nutrients into the photic zone. Studies have shown that accurate modelling of physical processes in the water column, especially of vertical advection and turbulent mixing, is critical for predicting plankton dynamics (Sharples & Tett, 1994). In present models the entrainment of nutrients across the thermocline appears to be incorrectly estimated, so that predictions of autumn blooms and midwater chlorophyll maxima are in error. The failure stems from a lack of understanding in two areas - firstly the quantification of vertical fluxes in the vicinity of the thermocline and secondly which processes control nutrient recycling in the benthic boundary layer. PROVESS (Howarth, 1998) is studying the physical-biological coupling involved in recycling by measuring and modelling both the agglomeration - sedimentation - deposition - resuspension of particulates, and the mineralisation of nutrients, in relation to bed stress and turbulence regimes in the bottom mixed layer at sites with contrasting physical forcing. A key aspect is to distinguish processes occurring in the water column from those in the 'fluff layer' formed by freshly deposited particulates and those in the compacted sediment.

OBJECTIVES

PROVESS is an interdisciplinary study of the vertical fluxes of properties through the water column and the surface and bottom boundaries based on the integrated application of new measuring techniques, new advances in turbulence theory and new models. Its objectives are to -

Improve understanding and quantification of vertical exchange processes in the water column, in the surface and benthic boundary layers and across the pycnocline.

Explore mechanisms of physical-biological coupling in which vertical exchanges and turbulence significantly affect the environmental conditions experienced by biota.

Apply innovative technologies to the measurement of physical microstructure features and phenomena, in particular turbulence properties in the water column.
Provide a new, comprehensive and synoptic data set for validation of vertical fluxes of energy and matter calculated by physical and biological models.
Develop 1-D physical models for the computation of statistical moments of microstructure phenomena and integrated biological-physical models of the water column, including fluxes from the surface and the sediment.
Test and validate the models against measurements of mean and fluctuating properties in the water column.
Provide modules for vertical exchanges which can be implemented in state-of-the-art 3-D water quality and ecological models.

RESULTS

The project is founded on the synthesis of experimental, theoretical and modelling studies aimed at improving understanding of small-scale features of coastal seas and their effects on vertical exchange processes. Measurements of turbulence properties in continental shelf seas (dissipation rate throughout the water column and intensity over a wide frequency range) are the heart of the project. These, together with biological and nutrient recycling measurements concentrating on fluxes near the sea bed, have been made at two contrasting sites – in a shallow, tidally dominated, particle active region (the southern North Sea) and in a lower energy region where the surface mixed layer and the benthic boundary layer are separated (the northern North Sea). Since turbulence also directly affects the environment perceived by particles, including living biota, detritus and suspended sediment, aggregation, flocculation and sedimentation of particulate organic and inorganic matter is being studied. New hypotheses about turbulence effects on zooplankton grazing rates, diet selectivity, vertical distribution and patchiness are being tested against field and laboratory measurements. The project contributes to the long term goal of developing robust water column plankton models applicable over the range of turbulence environments found in shelf seas.

Turbulence measurement innovations

The major aim is to make measurements of accessible turbulence parameters, with an ultimate goal of determining all the main terms in the turbulent kinetic energy (TKE) equation - (i) production, (ii) dissipation, (iii) work against buoyancy forces, (iv) time derivative of TKE and (v) its vertical diffusion. Until recently measurement of any of the parameters has not been possible, except in restricted cases in the bottom boundary layer. Since vertical fluxes of particles are also of interest, new methods will be applied to provide improved profiles of particle concentration and size.

Turbulent dissipation measurements have been obtained with two proven microstructure probe systems - FLY 4 and MICSOS. The FLY 4 profiler has recently made one of the first series of vertical profile measurements of dissipation in shelf seas, in the Irish Sea (Simpson *et al.*, 1996). Measurement of dissipation are made to within 0.15 m of the sea bed; near surface coverage is limited by the influence of the ship's wake and by the distance taken for the probe to achieve a uniform fall velocity - a few metres. Turbulent velocity fluctuations can be sensed on length scales down to 6 mm which allows direct determination of most of the dissipation spectrum, except in the most energetic shelf sea regions. The MICSOS system is being developed within the EUREKA project EUROMAR -MICSOS (Prandke *et al.*, 1995). Data are acquired by a free-falling probe with a sinking speed of about 0.5 m s^{-1} and a sample rate of

1 kHz. Turbulence velocity fluctuations can be sensed on length scales down to 3 mm; dissipation levels down to 4×10^{-8} W m^{-3}.

Most particles in marine environments are aggregates whose effective *in situ* size bears little relation to the absolute size of the primary constituents. Since many aggregates are fragile and easily disrupted by conventional sampling, size measurement demands *in situ* techniques. A new laser sizing instrument using a single window backscatter system has been adapted for *in situ* deployment. It is capable of high resolution sampling in space (as a vertical profiler) and time.

Northern and Southern North Sea Experiments

The objective of the northern North Sea experiment was to study turbulence regimes during the autumnal breakdown of stratification in an area of weak tidal currents, including the mixing of nutrient rich near bed water into the photic zone creating conditions for an autumn bloom. Of major importance were exchanges across the thermocline and the processes leading to its deepening, including overturning, surface mixing and cooling.

The experiment took place in September and October, 1998. At the start the pycnocline was at 35 m below the surface and 5.5°C in magnitude and by the end it had deepened to 60 m below the surface and weakened to 1.3°C, in water 100 m deep. In contrast to measurements made in the area in autumn 1991 (Howarth & Glorioso, 1995), there was significant salinity stratification, initially about 0.5. During the two months there was no vertical exchange of heat or fresh water downward into the near bed layer. First analyses showed that the nutrient situation was favourable for an autumn bloom of diatoms. In both 1991 and 1998 sea-bed photographs showed that wave activity was sufficient to rework the bed, changing it from one dominated by the effects of benthic biology in September to one of rippled sand in November, which the multicorer found difficult to penetrate.

The research vessels Valdivia, Dana, Pelagia and Challenger participated. Measurements were concentrated at the central position, 59° 20′ N 1° E, with additional measurements being made to estimate horizontal gradients and transports. An array of 18 moorings and bottom frames measuring current and temperature profiles, pressures, meteorology, suspended sediments, nutrients and chlorophyll was deployed for the two months. Bursts of inertial activity were recorded throughout the two months, with one noteworthy period from 20 to 27 October.

Turbulence dissipation measurements, over tidal cycles, were recorded on three of the cruises, two of which overlapped to allow comparison between the two different instrument systems (FLY and MICSOS). CTD profiles were recorded on all cruises. Optical properties and photosynthetic characteristics of the phytoplankton community were studied. Zooplankton and particle measurements were made on Dana and particle and benthic flux measurements on the Challenger.

Observations of turbulence, dissolved inorganic nitrogen, silicate, phytoplankton and micro-heterotrophs (amount and dominant species) and organic detritus in the surface mixed layer and upper thermocline are needed to test predictions of the dynamics of plankton blooms that follow the entrainment of nutrients into the surface mixed layer. The key measurements for developing parameterisation and testing of the benthic-pelagic model include: near-bed current shear; oxygen demand and nitrate+nitrite, ammonium and silicate fluxes at the sediment-water interface (including the fluff layer), and of oxygen demand of water samples from the bottom mixed layer; sediment and fluff layer organic content; concentrations of dissolved nutrients, oxygen, and particulate organics in the bottom mixed layer. Denitrification rates will be estimated from the model once this has been validated against other measurements of nitrogen flux.

In contrast the objectives of the southern North Sea experiment were to study vertical exchange processes in a high tidal energy environment: flocculation and sedimentation of (in)organic material, the development of high concentration near-bed (benthic fluff) layers, and remineralisation of nutrients in flocs and benthic layer. The near-bottom current structure, sediment concentration and the possible formation of high concentration benthic layers were of special interest.

The experiment took place from 29 March to 21 May, 1999, to coincide with the end of the spring bloom and involved the research vessels Pelagia, Mitra and Belgica. The experiment's design was similar to that in the northern North Sea. The site, chosen after a preliminary cruise in 1998, was centred at 52° 18' 22.0" N 4° 18' 01.3" E, water depth 20 m. On the first cruise, ten moorings were deployed and turbulence dissipation measurements made with a FLY system. The moorings were in two lines, one parallel to and the other perpendicular to the shore and included two temperature / conductivity chains. The near surface M_2 amplitude was 0.65 m s^{-1}. During the second cruise, turbulent dissipation measurements were made from the Noordwijk Platform, about 3 km from the main site, with a MICSOS system, while benthic exchange measurements were made from the ship using multicores and benthic chambers placed on the sea bed by divers. The moorings were recovered and turbulence dissipation measurements made with a MICSOS system on the last cruise.

Particle size, CTD profiles and water samples were obtained on all three cruises. Chlorophyll concentrations in water samples were measured on the Pelagia and samples preserved for analysis. Nutrients and dissolved oxygen were measured on the Mitra. The water column was generally well mixed, warming up from 7.2°C to 12.8°C. Stratification only occurred on about four occasions when the Rhine plume crossed the site, and surface salinity decreased to below 26, compared with a mean of about 31.2. The fluorometer records showed the bloom started in the second week of April and peaked on 4 May, with chlorophyll concentrations of about 30 :g m^{-3}. The silicate analyser provided the first useable data from such an instrument.

Plankton-turbulence coupling

Two hypotheses concerning variations in zooplankton feeding rates and diet are being tested with respect to quantified levels of prey and turbulent dissipation:
a) the composition of zooplankton diets varies with turbulent dissipation rates and feeding strategy (ambush predator v. filter-feeder).
b) estimates of plankton feeding rates can be improved if turbulence effects on diet composition, encounter rate, and capture success and prey patchiness are considered.

Variations in zooplankton vertical distributions and patchiness will be evaluated with respect to vertical profiles of turbulent dissipation, food, light and water column structure. For an effective comparison of vertical profiles of turbulence and zooplankton distributions, the zooplankton sampling (pumped zooplankton samples and optically-derived particle counts) were conducted on the same vessel as that being used to collect turbulence data – the northern North Sea Dana cruise. Two hypotheses are being tested:

a) zooplankton occupy depths which minimize turbulence-induced stress, even though such behaviour could result in exposure to less food.
b) zoo- and phyto-plankton patch size (m - 10 m scale) depends on dissipation rate and on body size, which via swimming ability, is a likely to correlate with ability to resist turbulent dispersion.

Physical and integrated biological-physical modelling

To achieve credible biological simulations with a water column model the best possible physical model is essential. The following physical processes are being addressed – the interaction between turbulence and tidal and wind-driven currents; diurnal and seasonal cycles of heating and cooling; the interaction between waves and currents at the sea surface and at the sea bed; sediment damping of turbulence; the influence of internal wave activity on turbulence in the thermocline; the role of horizontal and vertical advection.

Various turbulence schemes are envisaged, primarily within the GOTM framework, from simple models with a flow and/or Richardson number dependent viscosity and diffusivity to Mellor-Yamada level 2 and k-ε type formulations. Theoretical analysis has shown that the Mellor-Yamada model is in principle not capable of simulating the Thorpe scale in stably stratified flows whilst the k-ε model is able to do that if the parameter c3 is chosen according to Burchard & Baumert (1995). Therefore, although both models will be used since both are widespread in the oceanographic community, the k-ε model will be preferred.

Since horizontal and vertical advection can be assumed to be small, 1-D simulations with realistic forcing parameters are being concentrated on. Model results will be compared with existing publicly available data sets, followed by a more profound comparison with the PROVESS measurements of turbulence dissipation rate; turbulence kinetic energy; fluxes of momentum, temperature, salinity; bottom stress. The result will be a validated unified 1-D physical model, which can be applied to the study of different physical processes.

3-D hydrodynamic models are, however, needed to describe some physical processes. The unified 1-D model is being integrated into the existing 3-D COHERENS model. This is being applied to the northern North Sea site to evaluate the role of advection there and estimate the accuracy that may be expected with 1-D turbulence modelling.

The development of the physical and the integrated biological-physical water column process models is being performed in close interrelation. The aim is continued development of a 1-D water column model with dynamic coupling between physical and microbiological processes, as a platform for testing hypotheses about the relationship between production and mineralisation processes and the turbulence regime. The starting point is a 1-D model with four components:-
a) turbulence closure, for computing seasonal cycles of turbulent diffusivities from tidal and meteorological forcing at defined sites on the continental shelf (Sharples & Tett, 1994).
b) resuspension and deposition of particulate material from a 'fluff' layer overlying the sea-bed (Jones *et al.*, 1995).
c) autotrophic and heterotrophic microbial processes in the water column, involving microplankton and detrital compartments, and carbon, nitrogen and oxygen, where the key principle is the paramet-erisation as a single compartment of all processes with similar time-scales: thus the microplankton compartment includes phytoplankton and the bacteria and

pelagic protozoa that exploit primary production and which can reproduce at the same rate as the autotrophs (Tett & Walne, 1995).
d) a diagenetic model for the mineralisation of carbon and nitrogen (with consequent oxygen demand) in sediments (Soetaert *et al.* 1996).

The main tasks are (i) to study the influence of the improved turbulence schemes on biological fluxes; (ii) to include the effects of lateral gradients of stability and nutrients; (iii) to improve the coupling between the benthic 'fluff' and diagenetic models; (iv) to include descriptions of turbulence-related processes influencing particle size and sinking rate; (v) to include cycling of biogenic silicon; and (vi) to represent seasonal changes in the properties of the plankton that are related to sedimentation. Although the last task could be done by increasing the complexity of the microbial ecosystem representation, given the complexity of the interactions amongst the main components of the complete model, simple and robust submodels will be sought. The extent to which submodel complexity is necessary will be examined and a solution sought which involves minimum additions to the existing micro-plankton-detritus model. The remaining problem is how to supply the grazing pressure exerted by mesozooplankton on the microplankton. Although this could be done with an explicit mesozooplankton model our initial preference is to impose the pressure on the basis of observations at the experimental sites

CONCLUSIONS

The project is founded on the integration of experimental, theoretical and modelling studies of vertical exchanges in continental shelf seas, including the joint analysis and interpretation of measurements and model calculations. Innovative measurements of turbulence properties (dissipation rate throughout the water column and intensity over a wide frequency range) are the heart of the proposal. These, together with biological measurements concentrating on fluxes near the sea bed, have been succesfully made at two contrasting sites in the North Sea - one shallow, high energy, the other deeper, low energy. Since turbulence directly affects the environment perceived by particles, including living biota, detritus and suspended sediment, studies are being made of aggregation, flocculation and sedimentation, and of trophic interactions. Water column numerical models describing turbulent physics and integrated biology / physics will incorporate the understanding gained from the process studies and be rigorously tested against these fundamental measurements, to establish the robustness of parameterisations and the domain of validity of the models. This fundamental research will contribute towards the long term goal of developing robust water column plankton models applicable in the full range of turbulence environments encountered in shelf seas.

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TITLE : IMPACT OF APPENDICULARIANS IN EUROPEAN MARINE ECOSYSTEMS

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APPENDICULARIANS AND THEIR IMPACT ON PELAGIC MARINE ECOSYSTEMS

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INTRODUCTION

Studies on the tunicate *Appendicularia* have long been neglected, mostly because of the sampling difficulties, inadequate conservation and the difficulty to work with living specimens. Appendicularians are present in all oceans, from the surface down to a depth of more than 1000 m (Fenaux 1993). Some 70 species have so far been identified worldwide (Fenaux 1998). The capacity of appendicularians to form blooms is known since the last century (Quoy and Gaimard 1836). In Kingston Harbour, Jamaica, Hopcroft and Roff (1995) observed an average 10.7 fold biomass increase over 24 h, one order of magnitude higher than that of the most representative copepod species. This extraordinary production potential may be explained by a short generation time (2 days to weeks, depending on temperature) and by a high fecundity.

Appendicularians are suspension filter feeders. They have an external filtration system called the house, which is continuously secreted by specialised cells of the animal's trunk. The small pore size of this food concentration filter enables them to efficiently capture and ingest small particles down to sub-micrometer size (Deibel and Powell 1987, Flood *et al*. 1992, *Bedo et al*. 1993, Gorsky *et al*. 1999). The role of small particles in the dynamics of pelagic ecosystems is considered with great interest in contemporal marine research. Particles in a size range of 0.01 to 200 µm make up the so-called "microbial loop", through which a large fraction of oceanic production is recycled. The smaller part of this size range (prochlorophytes, bacterioplankton, sub-micron particles, colloidal matter) are out of reach for the majority of mesozooplanktonic organisms, including copepods.
Appendicularians are capable of short-cutting the size related trophic chain by directly consuming the microbial and colloidal pelagic compartments. By compacting that biomass into body tissue, rapidly sinking faecal pellets and abandoned houses, and thus contributing to marine snow, they are of great relevance to the dynamics of pelagic ecosystems and to the vertical flux of particulate matter.

The general objective of the EURAPP project is to improve the knowledge about the ecological role of distinct appendicularian species in representative marginal seas of Europe. The project focuses particularly on the structure, dynamics and resilience of important components in the marine plankton community and on the contribution of appendicularians to the flux of colloidal and particulate organic matter.

METHODS:

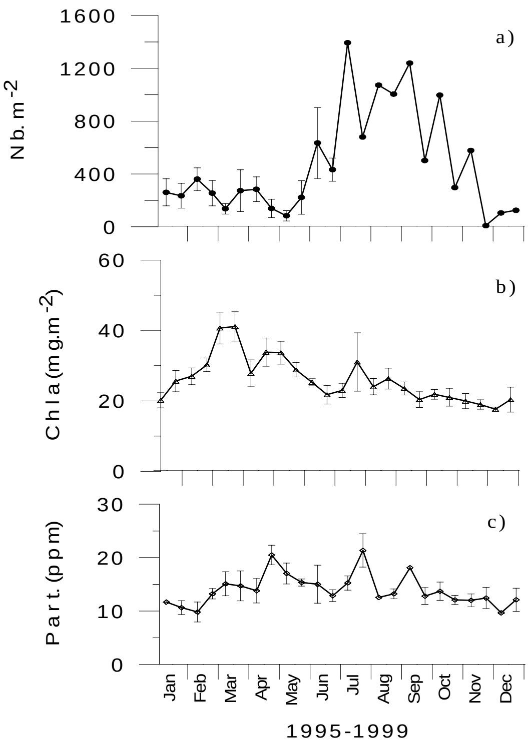
Considerable efforts of the EURAPP project were aimed at the development of new methods and at improving conventional methods and their adaptation to the exceptional fragility of appendicularians. Plankton nets were equipped with new collectors decreasing mechanical damage on collected organisms. Well defined methods of fixation onboard and in the laboratory improved the species determination. New imaging techniques were used for the description and 3-D reconstitution of species (see http://www.obs-vlfr.fr/~eurapp).
Long-term evolution of appendicularian populations was monitored in the marginal seas of Europe (Cantabarian Sea, English Channel, Ligurian Sea and Norwegian fjords). Zooplankton distribution was studied in two distinct fjords using a remote operated vehicle (ROV).
New methods for continuous laboratory culture of appendicularians were developed and used in the different participant countries. Laboratory experiments on the growth, feeding and fecundity are in progress and the results are used for the modelling of the population dynamics. To measure *in situ* filtration rates, the gut pigment technique was adapted by using flash-frozen single animals in a given sample volume instead of frozen animals retained on a filter.
Micro-scaled methods were developed for biochemical analysis on single specimens. Amino and fatty acids and protein content of cultured appendicularians were determined in different feeding conditions. Enzymatic activity of appendicularians was also examined as well as the internal structure of the digestive system. This was realised on toluidine blue stained histological sections and using light and electron microscopy.
Methods were developed for the study of fecundation success under controlled conditions and quantitative fluorescence microscopy was used for the genome size determination.

RESULTS

Field survey

First results from 1999 in the Ligurian sea (northwestern Mediterranean) show that the appendicularians peaked from June to October, during the stratified period, mainly due to the species *Oikopleura longicauda* (fig. 1a). During the winter and spring the appendicularians were less represented in the studied ecosystem. The fritillarians were dominant during the cold mixed period. Since 1996, the population dynamics in the NW Mediterranean is not synchronized with the algal spring bloom (fig. 1b). Nevertheless, the annual variability in the concentration of digestible size particles (2-20 µm) fluctuates only between 10 and 30 ppm (fig. 1c).
Considerable differences were observed between the NW Mediterranean and the English Channel where average mass appearance of *Oikopleura sp*. occurred in April and May and coincided with the flagellate and diatom blooms. These results on appendicularian population dynamics were reported from a ten-year data series (1988-1997) at the site of the EURAPP program (Lopez Urrutia *et al*., 2000).

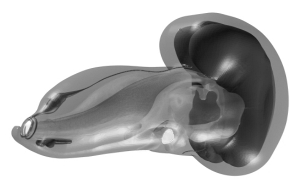
Fig.1: Mean yearly distributions of a) total appendicularian numbers, b) chlorophyl a concentration and c) particle volume. All values are integrated from surface to 75 m depth. Fortnightly means were calculated for years 1995 to 1999 except for particle volume, where only years 1995, 1998 and 1999 were considered. Mean numbers of appendicularians are shown for months January through June the remaining values corresponding to year 1999. Error bars represent standard error with n=3 to n=5.



Biodiversity

Digital processing of a series of histological cross sections allowed to reconstitute a three-dimensional appendicularian trunk showing both the external and internal morphology of organs (fig. 2). Using animation programs software, each organ can be observed individually and from any given angle (see http://www.obs-vlfr.fr/~eurapp).

Fig. 2: Reconstituted trunk of *Oikopleura albicans*. The digitally processed image was obtained from smoothing 340 histologially stained cross sections.



Distinct succession from fritllariid to oikopleurid dominated communities was observed at the Mediterranean site. This confirms historical data from Fenaux, 1963. Several new species were discovered in the Norwegian fjords (Flood submitted). The detailed specific determination is underway. Distinct Norwegian fjords harboured different zooplankton populations. Vertical stratification (Gorsky et al. 2000) and specific distribution of appendicularians was different in each fjord.

Results on genome size revealed an exceptionally small size but also high variability between species (tab. 1). The lowest values came close to that of *Saccharomyces cerevisiae* (16 Mbp).

Tab. 1: Genome size of appendicularians determined by quantitative fluorescence microscopy.

Species	Mean haploid size (Mbp)
Oikopleura dioica	38
Oikopleura longicauda	76
Oikopleura labradoriensis	32
Fritillaria pellucida	152
Appendicularia sicula	20.5

Functioning of appendicularians

Functional feeding response studied for *Oikopleura dioica* showed little differences in maximum ingestion rates (I_{max}=198 ng.C.ind^{-}1.h^{-1} when fed *Isochrisis galbana*) and K_m (38 µgC.L^{-1}) from other filter feeders. Clearance rates, however, remained nearly constant (Acuña and Kiefer, 2000). It was suggested that at low food concentrations, particles are transformed into fast sinking faecal pellets and at high food concentrations, into slow to fast sinking filter houses. Gut passage time (GPT) depended on both temperature (T) and food concentration (FT) and was estimated as: $GPT = 51.67 \bullet e^{-0.0376\ T} \bullet FC^{-0.245}$ (López-Urrutia and Acuña, 1999). Accordingly, at 18ºC, faecal pellets are produced every 2 to 4 minutes depending on food concentration.

Results on the nutritive value of individuals of cultured *Oikopleura dioica*, starved and fed with microalgae, showed unusually high contents of free amino acids (FAA), especially of glycine, which depended on the size of the animal (fig. 3).

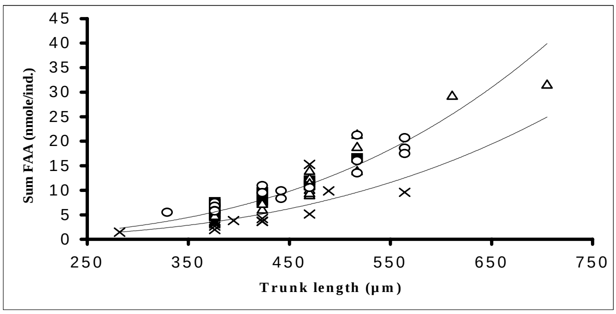


Fig. 3: Correlation between trunk length and total FAA of *O. dioica* when algal fed (open symbols) or starved (crosses).

Electron microscopy study of the digestive tract allowed a better description of the food transport, of the region involved in enzyme secretion, of intracellular digestion and assimilation and of faecal pellet formation.

CONCLUSION

Preliminary results of the field survey and historical data showed differences among study sites in seasonal development of appendicularian populations. *In situ* gut fluorescence measurements showed no correlation with the seasonal chlorophyll a dynamics. The environmental parameters seem to influence the population dynamics and consequently the biodiversity with respect to species succession.

New fecundity success data indicated that a critical sperm dilution seems to be a key factor for the development of an appendicularian population.

On an individual level, high growth rate and short generation time, short gut passage time and high proteolytic activity confirms the exceptional adaptation possibilities of appendicularians to different biotopes. In fact, ingestion and egestion data are coherent with the high

development potential. Appendicularians adapt to unfavourable feeding conditions by successfully feeding on living and nonliving particles. Predator-prey relationships and the environmental parameters (e.g. gametes dispersal) may determine the role of appendicularians on a seasonal basis.
High content of free amino acids and polyunsaturated fatty acids of the appendicularian body make them nutritionally valuable for other zooplankton and above all, for fish larvae.
In the framework of the global climatic change, it is essential to investigate the response of important components of the ecosystem to the environmental changes. The EURAPP program will substantially contribute to this effort by the input of new insights into the peculiar trophodynamics of appendicularians.

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TITLE: MICROPLATE-BASED, MULTIPLE STRAIN BACTERIAL ASSAY FOR MARINE ECOTOXICOLOGY : **MARA**

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MICROPLATE-BASED, MULTIPLE STRAIN BACTERIAL ASSAY FOR MARINE ECOTOXICOLOGY : MARA

INTRODUCTION:

The MARA project addresses the need for improved marine ecotoxicity assays. Such assays are required for better ecological risk assessment in relation to discharge of chemicals into the marine environment and to ensure compliance with existing and forthcoming national and European directives on marine water quality. Ecotoxicity studies are moving from looking at the effects of single chemicals emerging from point sources to studies of complex mixtures and diffuse sources. This requires new and sophisticated tools that can assess and predict the impact of such pollution. In particular there is a need for sensitive bioassays that are more informative, more accurate and faster than existing assay systems. This CRAFT project is aimed at producing a prototype, high-throughput assay based on marine bacteria by early 2001. Patent applications have been made covering the core technologies and proof of concept studies are currently being undertaken.

The prime industrial/economic objectives of MARA are to:

- Prove that assays based on multiple strains of bacteria have a quantifiably improved performance over existing bacterial ecotox assays thus giving the new assay a considerable market advantage
- Produce an assay system that would be at least as cheap as existing systems while offering enhanced performance or could be marketed at a premium on the basis of the improved performance
- Have the assay validated by regulatory authorities leading to its general acceptance and rapid market penetration so that the assay (and its variants) would become the market leader within 5 years
- Generate and protect IPR relating to the use of multiple strains of bacteria
- Deliver a testing system suitable for use by regulators and industry leading to better compliance and self-compliance with water quality directives leading to reduced costs to industry and environmental improvements

PhPlate Stockholm AB is the Prime Proposer SME in this CRAFT project. PhPlate's business is the development and production of microplate based assays. They are the manufacturers of the Phene plate system for bacterial identification

Applied Maths BVBA is a Belgian SME involved in the development and marketing of advanced software for the biosciences

SEAS Ltd is a UK SME providing survey and research services concerning the marine environment.

Don Whitley Scientific is a UK SME who develops, manufactures and sells instrumentation and associated products for microbiological applications.

Scottish Environment Protection Agency (SEPA) is a recently formed UK governmental agency with a wide remit concerned with protecting the Scottish environment.

The Scottish Association for Marine Science is the lead RTD performer and is based at the Dunstaffnage Marine Laboratory on the West Coast of Scotland. The SAMS Marine Biotechnology Group has dedicated molecular and cell biology laboratories and excellent microscopy facilities. Research at Dunstaffnage is being undertaken by Dr Mark Hart. Dr Douglas McKenzie leads the Marine Biotechnology Group and is the co-ordinator of the MARA project.

Karolinska Institutet is the Medical University of Stockholm and a leading European centre for research and development in the biomedical field. The Microbiology and Tumour Centre occupies newly completed laboratories at Karolinska. Research at MTC relating to MARA is being undertaken principally by Dr Inger Kuhn and Ms Jenny Gabrielson. The MTC is led by Prof Roland Moby.

I.1.3. Marine biodiversity

TITLE : **PICODIV**: MONITORING BIODIVERSITY OF PICOPHYTOPLANKTON IN MARINE WATERS

CONTRACT N° : **EVK3-CT-1999-00021**

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PICODIV:
EXPLORING THE DIVERSITY OF PICOPLANKTON

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INTRODUCTION

Picoplankton (defined operationally hereafter as cells that pass through a 3 µm filter) dominate the photosynthetic biomass in many marine ecosystems, not only in the very oligotrophic regions of the world oceans, such as the central Pacific gyre (Campbell et al. 1994) or the Eastern Mediterranean Sea, but also in mesotrophic areas, such as the high chlorophyll - low nutrient equatorial regions. However, picophytoplankton are clearly not exclusively restricted to oceanic environments. In many coastal regions, they are present throughout the year and constitute a 'background' population (Agawin et al. 1998), onto which episodic phenomena, such as the spring bloom develops. In some environments, such as coastal lagoons, picoplankton are a major component of biomass and productivity for most of the year. Picophytoplankton are also very relevant from the human point of view, because some bloom-forming picoplankters, such as *Aureococcus* spp. are toxic (Bricelj and Lonsdale 1997).

Photosynthetic picoplankton encompass both prokaryotic and eukaryotic species:

- Prokaryotes. Only two major genera are important for the picoplanktonic community in marine waters: *Synechococcus* and *Prochlorococcus*. Whereas *Prochlorococcus* dominates over *Synechococcus* in most oligotrophic regions, except at high latitudes, the reverse is true under meso- and eutrophic conditions (Partensky et al. 1999). With such wide ecological distributions, these two genera display a large genetic and phenotypic variability, that is just beginning to be assessed.
- Eukaryotes. In contrast to prokaryotes, the taxonomic diversity of picophytoplanktonic eukaryotes is much broader. In fact, nearly every algal division has picoplanktonic representatives (Figure 1). Still, a vast number of taxa undoubtedly remain unknown and undescribed.

To date fewer than 30 species of picophytoplankton have been described (see Table 1). This number pales in comparison to the more than 4,000 marine phytoplankton species that have been described to date and to the over 100,000 that are believed to exist. A clear proof of our poor knowledge of picophytoplankton diversity is revealed by the discovery of three novel algal classes in the last ten years described from picophytoplanktonic taxa (to put this into perspective, to ignore an algal class corresponds to ignoring the existence of mammals or birds among the vertebrates):

1991 class Pedinophyceae based on *Resultor mikron* 2 µm (Moestrup 1991)
1993 class Pelagophyceae based on *Pelagomonas calceolata* 2 µm (Andersen et al. 1993)
1999 class Bolidophyceae based on *Bolidomonas pacifica* 1.5 µm (Guillou et al. 1999)

Table 1: Chronology of taxonomic picoplankton knowledge.

Year	Name	Class	Size µm
1952	*Chromulina pusilla* Butcher (renamed *Micromonas* Manton & Parke)	Prasinophyceae	1-1.5
	Nannochloris atomus Butcher, *N. maculata* Butcher (renamed *Nannochloropsis* Hibberd)	Eustigmatophyceae	1.5-4
1955	*Nannochloris oculata* Droop (renamed *Nannochloropsis* Hibberd)	Eustigmatophyceae	1.5-4
1957	*Monallantus salina* Bourelly (renamed *Nannochloropsis* Hibberd)	Eustigmatophyceae	1.5-4
1967	*Hillea marina* Butcher	Cryptophyceae	2-2.5
1969	*Pedinomonas mikron* Throndsen (renamed *Resultor* Moestrup)	Prasinophyceae	1.5-2.5
	Scourfieldia marina Throndsen (renamed *Pseudoscourfieldia* Manton)	Prasinophyceae	2-3
1974	*Imantonia rotunda* Reynolds	Prymnesiophyceae	2-4
1977	*Pelagococcus subviridis* Norris	Pelagophyceae	2.5-5.5
1978	*Chlorella nana* Butcher	Chlorophyceae	1.8-2.6
1979	**Discovery of oceanic picoplankton**		
	marine *Synechococcus* Naegeli	Cyanophyta	0.8-1.2
1982	*Nannochloropsis gaditana* Lubian	Eustigmatophyceae	2.5-5
	Nanochlorum eucaryotum Willhelm *et al*. (renamed *Nannochloris* Naumann)	Chlorophyceae	
1987	*Triparma* Booth & Marchant spp, *Tetraparma pelagica* Booth	Chrysophyceae	2.2-4.7
1988	*Prochlorococcus marinus* Chisholm	Cyanophyta	0.5-0.7
	Aureococcus anophagefferens Hargraves & Sieburth	Pelagophyceae	2-4
1990	*Bathycoccus prasinos* Eikrem & Throndsen	Prasinophyceae	1.5-2.5
	Pycnococcus provasolii Guillard	Prasinophyceae	1- 4
1993	*Pelagomonas calceolata* Andersen & Saunders	Pelagophyceae	1.3-3
1995	*Ostreococcus tauri* Courties & Chrétiennot-Dinet	Prasinophyceae	0.8-1.1
1996	*Prasinoderma coloniale* Hasegawa & Chihara	Prasinophyceae	2.5-5.5
	Nannochloropsis granulata Karlson & Potter	Eustigmatophyceae	2- 4
1997	*Aureoumbra lagunensis* Stockwell *et al*.	Pelagophyceae	2.5-5
1999	*Bolidomonas pacifica* Guillou & Chrétiennot-Dinet	Bolidophyceae	1.5
	B. mediterranea Guillou & Chrétiennot-Dinet		
2000	*Picophagus flagellatus* Guillou & Chrétiennot-Dinet	Chrysophyceae	2
	Symbiomonas scintillans Guillou & Chrétiennot-Dinet	Bicosoecid	2

Because so little is known about the taxonomy and systematics of picophytoplankton we have very little data to estimate the levels of its biodiversity under natural conditions and to understand how the picophytoplankton might be affected by environmental variability linked to either anthropogenic influence or to larger scale phenomena, such as those linked to climate change or global warming. However we have some indications that picophytoplankton species (and therefore picophytoplankton biodiversity) may react sharply to changes in marine systems:

- The prokaryote *Prochlorococcus* consists of at least two different genotypes/phenotypes, each one dominates under different environmental conditions: i.e., one is present under the high light/low nutrient conditions of the marine surface layer, and the other under the low light/higher nutrient conditions of the bottom of the euphotic zone. Thus *Prochlorococcus* is able to partition its niche genetically so that it is phenotypically adapted to its environment.
- The abundance of *Synechococcus* in the equatorial Pacific decreases during El Niño Southern Oscillation episodes.

- The potentially toxic brown picoplanktonic alga *Aureococcus* was unknown before 1985, but since then it has bloomed repeatedly in US coastal waters (Bricelj and Lonsdale 1997).

Our ignorance concerning pico-phytoplankton diversity is mostly explained by the fact that, because of their very small size, pico-phytoplankton cells most often lack any distinguishing features and are very difficult to identify by classical methods. In fact many have evolved to small "green or brown ball" morphotypes that mask a broad taxonomic diversity (Potter et al. 1997). Our present state of knowledge regarding picophytoplanktonic biodiversity is in fact analogous to that prevailing ten years ago for eubacteria and archea. Until the early 1990's, the taxonomy and understanding of bacterial diversity was based primarily on species isolated into culture. No-one could have predicted the vast diversity of these organisms in nature (Giovannoni et al. 1990).

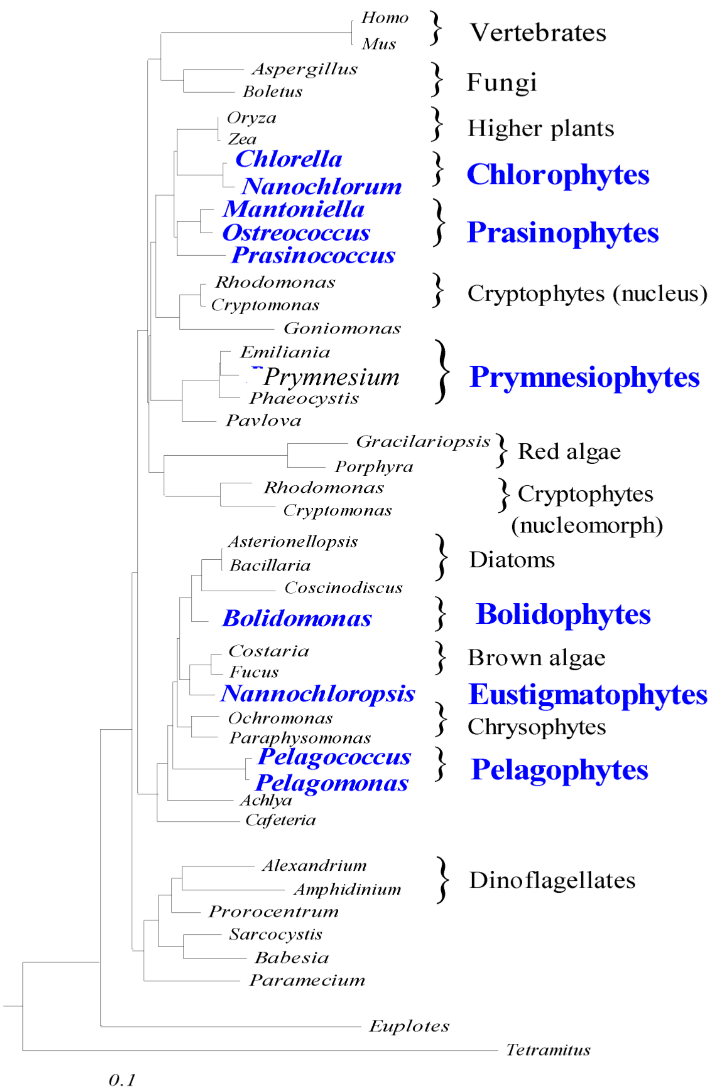

Figure 1: A tree showing the phylogenetic affinities of eukaryotic picophytoplankton species (in bold). Note that only one picoplanktonic Prymnesiophyte has been isolated yet (*I. rotunda)*, but a number of picoplanktonic Prymnesiophyte sequences have been recovered from oceanic samples (Moon-van der Staay et al. 2000). Source: Guillou unpublished

In order to remedy to this very poor state of knowledge concerning a group that, in many ecosystems, accounts for up to 60 to 80% of photosynthetic biomass and production, there is a very urgent need to develop efficient monitoring tools of picophytoplankton diversity. This problem is in fact very analogous to that encountered by microbiologists who cannot tell apart bacteria based on their shape or even on their metabolic requirements. The latter have relied more and more in recent years on molecular biology techniques to identify and detect bacteria in the environment (Giovannoni et al. 1990; Amann and Kuhl 1998). We plan during the course of this project to expand this very successful approach to picophytoplankton.

STRATEGY

Our strategy is encapsulated in the following four steps:

(1) Obtain SSU rDNA sequences for as many as possible picophytoplankton taxa from both cultures and natural samples. Novel taxa will be assessed using a combination of methods including in particular pigment analysis and electron microscopy.
(2) Using this sequence database, develop hierarchical probes recognizing successive taxonomic groupings having picophytoplanktonic representatives
(3) Develop fast and efficient techniques to quantify the fraction of the pico-phytoplankton recognized by the probes in natural samples.
(4) Test and validate these probes on time series of picophytoplankton biodiversity in three coastal ecosystems.

We will focus on the picophytoplankton from **coastal European waters** that has been much less studied in comparison to that of offshore oligotrophic waters. For this purpose we have selected three sites located in the following regions:

- English Channel (Roscoff)
- North Sea (Helgoland)
- Western Mediterranean Sea (Blanes Bay)

These sites have been carefully selected as offering a wide range of environmental conditions representative of EU coastal waters. Moreover, all have been extensively monitored in the past and abundant background information is available on environmental conditions as well as phytoplankton populations. One of them (Helgoland) has been designated as a flagship site for long-term and large-scale marine biodiversity research at a recent European meeting on biodiversity because its long-term sampling program stretches back at least 26 years.

Although these three sites will serve as focal points for our project, we are also taking advantage of oceanographic cruises planned outside this project to examine the diversity of picophytoplankton in other environments. In particular we have begun sample the following ecosystems:

- Mediterranean Sea (PROSOPE 99, MATER99, HIVERN00)
- Red Sea
- North Atlantic Ocean (PROSOPE 99)
- Celtic Sea (PROPHEZE D246)

First, for probe design we need to obtain SSU rDNA sequences covering the full taxonomic spectrum of picophytoplankton. For this purpose, we have adopted a two pronged approach:

a - We are obtaining sequences from **fully characterized laboratory strains**. We are in particular securing all picoplanktonic strains available from international culture collections, such as the CCMP (Center for Cultures of Marine Phytoplankton, Bigelow USA). However, we know that such collections only feature a limited number of picoplanktonic strains, because very little effort has been devoted to this size class to date. Therefore we need to embark on a very strong effort of strain isolation. For this purpose, we are establishing cultures of both prokaryotic and eukaryotic picophytoplankton from the environments listed above using methods that have already proved very successful for this purpose (prefiltration of natural samples, monitoring of cultures by flow cytometry). Once established, the cultures are screened by a variety of techniques (flow cytometry, electron microscopy, pigment analysis, molecular methods) to assess their taxonomic position. Those that obviously contain novel taxa are further purified by dilution or plating and more fully studied (electron microscopy sections), sequenced and described formally.

b - As we know that a large number of planktonic organisms still escape culture due to the lack of optimum culture conditions, we are also using the molecular approach that has been so successful for bacteria i.e., **environmental ribosomal RNA gene cloning** and sequencing. These sequences are being obtained from the same environments from which we obtain cultures. It is highly likely that this will reveal novel groups that we can then target for culturing.

Second, using the sequence database obtained both from cultures and natural samples, we will use or design **hierarchical probes** for each taxonomic level containing picophytoplanktonic representatives (e.g., classes, such as the Pelagophyceae or species, such as *Micromonas pusilla*). These probes will be validated against cultured strains.

Third, we are developing **methods** to assess the fraction of the marine pico-phytoplankton recognized by a given probe. We focus on very recent techniques allowing quantitative and extensive probe measurements (fluorescent in situ hybridization, probe array, quantitative PCR).

Fourth, we will apply these methods in the second phase of the project to assess **picophytoplankton diversity** during a full year at our three coastal sites (English Channel, North Sea, Mediterranean Sea). At the same time, the composition and abundance of the picophytoplankton will be studied with more conventional techniques, such as electron microscopy or pigment analysis, and alternate molecular methods (DGGE). These data will permit a validation of the data obtained by the molecular probe approach. We will interpret then the biodiversity patterns as a function of the other environmental parameters of the site sampled. We will determine in particular whether there is a succession of groups and species (as is the case for the larger nano and micro-phytoplankton) or whether a small group of ubiquitous species are always present and merely change their abundance (but not their diversity) in response to environmental changes.

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TITLE : DEVELOPMENT AND FIELD VALIDATION OF BIOSENSOR METHODS FOR THE ASSESSMENT OF THE EFFECTS OF POLLUTION AND SOLAR UV RADIATION ON COMMERCIALLY AND ECOLOGICALLY IMPORTANT MARINE INVERTEBRATES : **UVTOX**

CONTRACT N° : **EVK3-CT-1999-00005**

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NOVEL CELLULAR AND MOLECULAR TOOLS TO ASSESS THE EFFECTS OF POLLUTION AND SOLAR UV RADIATION ON MARINE INVERTEBRATES

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INTRODUCTION

There is strong evidence that the ozone layer, especially over the Antarctic, is thinning resulting in an increased flux of biologically damaging mid-ultraviolet radiation reaching the earth (UV-B, 280 to 320 nm) (Acevedo and Nolan, 1993). In aquatic ecosystems, within the marine Metazoa, sessile species such as sponges, bivalve embryos and larvae, and sea urchin embryos are the prime candidates for the adverse effects caused by UV-B irradiation. These animals do not possess a protective epidermal layer that is UV-B-adsorbing and characteristic of higher organisms. The major macromolecule affected by UV-B radiation is DNA. Although UV-B radiation is attenuated in seawater harmful effects can still be measured at depths up to 20 m (Smith et al., 1992).

The aim of the UVTOX project is to introduce novel cellular biosensor (marine invertebrate cell culture) and molecular biosensor (optical grating-coupler sensor) techniques in marine environmental research for the estimate of the health state of marine invertebrates in their natural habitats (sponges and sea urchins) or in aquaculture (bivalves). Until very recently marine invertebrate cell cultures were not available. The sponge cell culture has now been established for the first time in one of the participating laboratories (Müller et al., 1999b), and another partner (Le Marrec et al., 1995, 1999) has developed for the first time cultures of bivalve cells (scallop and oyster). In addition, sea urchin coelomocytes have proven as highly sensitive to environmental stressors in the third laboratory participating in this project (Matranga et al., 1999). Systematic studies on the effects of UV-B exposure and their modulation by pollution in the aquatic environment can be performed, using these cell systems.

Several stress-dependent proteins and their respective genes/cDNAs from marine invertebrates have been identified and characterized, and evaluated for biomonitoring in the field (for marine sponges: heat shock protein HSP70, Müller et al., 1998; DnaJ protein homolog, Koziol et al., 1997; glucose-regulated protein GRP78, Schröder et al., 1999b; 14-3-3 protein, Wiens et al., 1998; and thioredoxin, Wiens et al., 1999). However, the methods currently used for the detection of expression of biomarkers (usually Western and Northern blotting techniques, or ELISA methods) are time-consuming and require experienced personnel. Moreover, these methods do not allow the simultaneous measurement of large numbers of samples. One possible strategy to solve these problems is the development and application of the biosensor technology. The aim of the UVTOX programme is to introduce a relatively cheap biosensor method (optical grating-coupler sensor chip/OWLS technology) developed by one of the SME partners (Ramsden et al., 1997) in environmental monitoring. Three molecular biosensors for the detection of the effects of UV-B, alone or in combination with pollutants, on marine invertebrates will be developed: a) an immunosensor for the detection and quantification of *XPB/ERCC-3* gene product, b) a DNA affinity biosensor for the detection of UV-B inducible Y-box binding proteins, and c) DNA damage biosensor. These biosensors will be applied using the invertebrate cell cultures (cellular biosensors), as well as for studies with scallop, oyster, and sea urchin embryos and larvae.

PROJECT METHODOLOGY

The marine sponges studied

Marine sponges are the most abundant multicellular organisms in the marine hard-substrate benthos. As sessile, mainly filter-feeding animals, sponges are exposed to the aqueous milieu in a manner not observed in any other marine metazoan phylum. They are able to accumulate pollutants from the filtered water. Therefore, sponges must possess efficient defence mechanisms which enable them to survive in unfavorable environments. There is growing evidence that the protection systems of sponges against environmental stress are similar to those found in higher metazoa (Müller et al., 1999a). The marine demosponges *Crambe crambe* and *Suberites domuncula* used in this project have also been frequently used for environmental studies in the past (e.g., Bachinski et al., 1997; Schröder et al., 1999a,b).

RESULTS

Sponge cell culture (primmorphs)

The sponge cell culture has been recently established (Müller et al., 1999b). The culture starts from dissociated single cells which subsequently form aggregates; in those aggregates the cells start DNA synthesis and proliferate. The aggregates, termed primmorphs, show a tissue-like appearance and can be cultured for more than five months (*S. domuncula*; Müller et al., 1999b). A specific equipment has been designed to keep primmorphs under pressure.

UV-B inducible proteins: *XPB/ERCC-3* excision repair gene-homolog

Until very recently, no metabolic parameter was available to monitor for the effects of UV-B light on marine invertebrates. In the laboratory of one of the participating groups, markers for UV-B irradiation in marine invertebrates have been identified for the first time (Batel et al., 1998). In the UVTOX project, the following sponge genes/sequences will be used for the planned laboratory and field experiments and have been selected for development of the biosensor: a) the sponge *XPB/ERCC-3* gene product, and b) the Y-box DNA sequence.

The *XPB/ERCC-3*-like cDNA was isolated from the sponge *G. cydonium* (Batel et al., 1998). Fig. 1 shows the alignment of the sponge amino acid sequence with the related amino acid sequences from other metazoan organisms. In humans, the product of the *XPB/ERCC-3* gene, a DNA helicase, is involved in the early step of the DNA excision repair; it corrects the repair defect in xeroderma pigmentosum and in Cockayne's syndrome (Weeda et al., 1990). The *XPB/ERCC-3*-like gene in *G. cydonium* is inducible by UV light. Field studies revealed that the level of expression of this gene was 5-fold higher in *G. cydonium*, collected close to the water surface than the level in animals collected in a cave; intermediate expressions were measured in depths of 20 to 35 m (Batel et al., 1998). The increase of expression of the *XPB* gene paralleled the degree of DNA strand breaks.

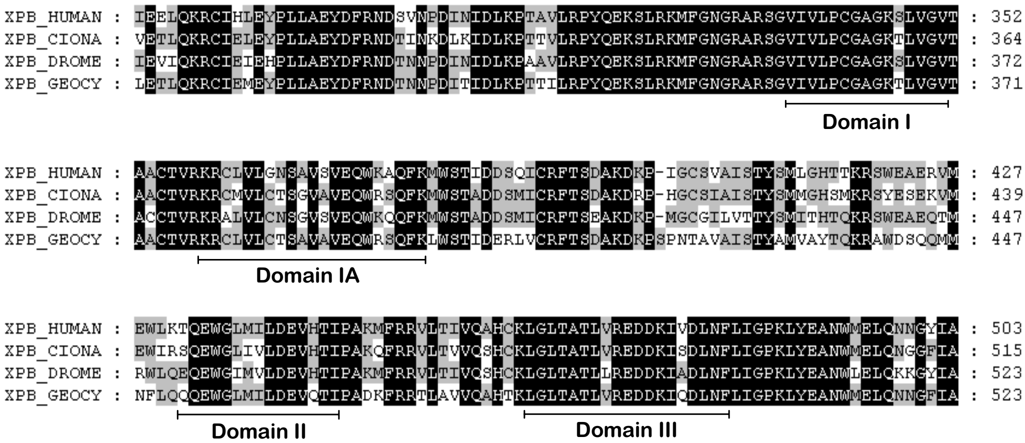

Fig. 1. Alignment of the sponge, *G. cydonium*, amino acid sequence XPB_GEOCY, deduced from the *XPB/ERCC-3*-like cDNA, with related sequences from human [XPB_HUMAN], tunicate [*Ciona intestinalis*, XPB_CIONA] and fruit fly [*Drosophila melanogaster*, XPB_DROME] (sequences truncated). Residues conserved among all sequences are shown in inverted type; residues conserved in at least two of the sequences are shaded. The consensus amino acids comprise the helicase domains (Batel et al., 1998).

The Y-box representing the binding site of specific, stress-inducible proteins has been identified in the marine sponge *S. domuncula*. Y-box binding proteins are found in many vertebrates (Graumann and Marahiel, 1998). Their function is similar to the function of cold shock proteins (CSPs) in bacteria. Interestingly, the expression of these proteins in sponge tissue is strongly increased after UV-B exposure. Therefore, designing of a biosensor that detects binding of Y-box binding proteins inducible by UV-B irradiation to a Y-box oligonucleotide immobilized to the sensor chip surface will provide a powerful tool to detect stress by UV-B exposure in marine invertebrates.

Measurement of DNA damage using Fast Micromethod

The conventional tests for detection of DNA damage are time-consuming and complicated, require greater amounts of sample material and are not suitable for the routine testing of large numbers of samples. We developed novel simple and sensitive methods for the routine assessment of DNA damage (Fast Micromethod I DNA single-strand break assay and Fast Micromethod II DNA double-strand break assay).

THE DNA SINGLE-STRAND BREAK ASSAY (FAST MICROMETHOD I) MEASURES THE RATE OF UNWINDING OF CELLULAR DNA UPON EXPOSURE TO ALKALINE CONDITIONS USING A FLUORESCENT DYE WHICH PREFERENTIALLY BINDS TO DOUBLE-STRANDED DNA (PATENT APPLICATION PENDING: DE 197 24 781 A1 ; MÜLLER ET AL., 1998; SCHRÖDER ET AL., 1999A,B, 2000). THIS METHOD IS PARTICULARLY SUITABLE IF ONLY SMALL AMOUNTS OF MATERIAL ARE AVAILABLE AND A LARGE NUMBER OF SAMPLES HAS TO BE ANALYZED WITHIN A SHORT TIME PERIOD. THE ASSAY CAN BE PERFORMED IN 96-WELL MICROTITER PLATES WITHIN 3 H OR LESS; IT REQUIRES ONLY 30 NG OF DNA PER WELL (~3 X 10^3 CELLS OR ~25 µG OF TISSUE). IT CAN ALSO BE APPLIED FOR FROZEN TISSUE SAMPLES.

In addition to the DNA single-strand break assay, we developed a kit applicable for the routine monitoring of DNA double-strand breaks (Fast Micromethod II; patent application pending). This assay is also suitable for the routine assessment of DNA damage in large numbers of samples. Two special devices for the filtration in micro scale with constant low filtration rates were developed for this test (Fig. 2 A, B).

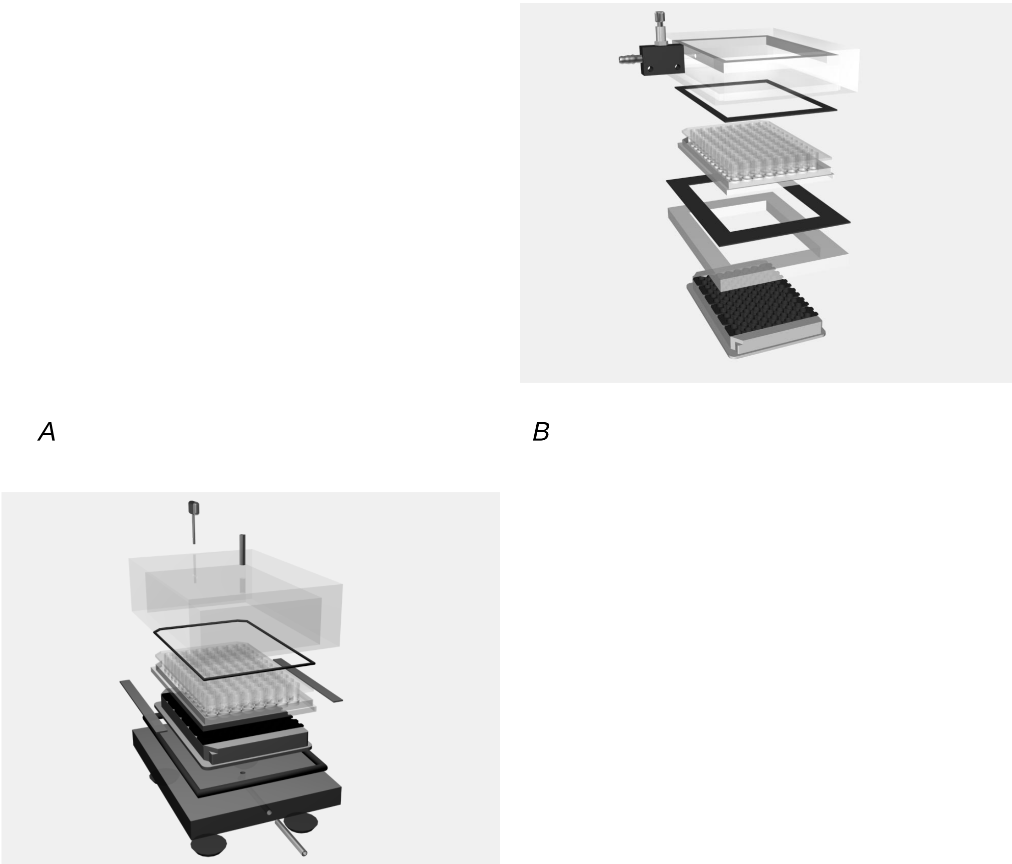

Fig. 2. Special filtration devices for the measurement of DNA double-strand breaks. One of these filtration devices is operating at negative pressure **(A)** and the other one is operating at positive pressure **(B)**. The special construction of both devices allows the filtration in micro scale with constant low filtration rates required for the measurement of DNA double-strand breaks (patent application pending: DE 199 33 078.6).

Our studies using Fast Micromethod I revealed an increased frequency of single-strand breaks in *S. domuncula* after exposure to polychlorinated biphenyls (PCB) (Wiens et al., 1998; Schröder et al., 1999a). Exposure of *S. domuncula* to cadmium chloride also resulted in a strong increase in DNA single strand breaks, as assessed by Fast Micromethod I (Müller et al., 1998; Schröder et al., 1999b). Determinations of cadmium levels in *S. domuncula* collected at five stations in the Northern Adriatic Sea, characterized by a gradient of pollution, revealed significant differences between these stations (Müller et al., 1998). The frequency of DNA strand breaks roughly paralleled the gradient of pollution (cadmium levels) at these sites.

In the UVTOX project, DNA damage upon exposure to UV-B light and upon exposure to defined pollutants and complex mixtures, including xenoestrogens will be determined in cultivated cells and embryos/larvae, using these recently developed, rapid and sensitive microplate assays.

CONCLUSION

There is an urgent need for techniques which allow the rapid assessment of the effects of pollution and increased UV-B radiation, due to ozone depletion, and of their combined effects in the earlier stages of environmental damage. The achievements of the UVTOX project will be the development and introduction of novel cellular and molecular biosensor techniques for the detection of the effects of environmental stress by UV-B exposure without or in combination with stress by pollution in ecologically and/or commercially important marine invertebrates. The development of a methodology for the assessment of UV damage and pollutants in the aquatic environment, and their interactions, will contribute to facilitate the monitoring of environmental risks.

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TITLE : BIOLOGICAL CONTROL OF HARMFUL ALGAL BLOOMS IN EUROPEAN COASTAL WATERS: ROLE OF EUTROPHICATION

CONTRACT N° : EVK3-CT-1999-00015

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BIOLOGICAL CONTROL OF HARMFUL ALGAL BLOOMS IN EUROPEAN COASTAL WATERS: ROLE OF EUTROPHICATION

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INTRODUCTION

Currently there are various initiatives with respect to management of HABs. On a global scale there is GEOHAB (a recent IOC, SCOR and ICES initiative) and ECOHAB, a joint effort of the North-American states. Europe is lagging behind in research on harmful marine algae despite the fact that aquaculture and other use of coastal waters in Europe is intensifying and increasing in economic importance. BIOHAB is the result of a recent initiative (EuroHAB Scientific Initiative Harmful algal Blooms in European Marine and Brackish Waters, 1999) of the EC on the field of HAB's and is part of the IMPACTS cluster. It is imperative that a strong local European scientific basis for coastal water management with respect to harmful algae is built up partly due to BIOHAB. Under the 5th Environmental Action Programme coastal zones are subjected to priority actions by the EU (DG XI). Moreover, coastal zones are also subjected to the Demonstration Programme of DG XI on Integrated Coastal Zone Management (ICZM). Results of BIOHAB are of significance for both EU tasks since BIOHAB addresses one of the key issues of pollutants namely nutrients.

Problems to be solved

Harmful Algal Blooms (HABs) occur in many European marine waters and have increased in frequency concomitantly with a increased nutrient input from land. HABs have a devastating effect on the ecosystem and/or cause direct health problems in humans and animals. Species of interest for BIOHAB belong to different taxonomic groups: dinoflagellates, haptophytes and cyanobacteria. Various algae belonging to these groups produce hepatotoxins, ichtyotoxins, substances responsible for Paralytic Shellfish Poisoning and Diarrhetic Shellfish Poisoning or produce other harmful substances. Some species are not toxin producing, but are harmful in other ways, e g by creating oxygen deficiency.

The success of HABs depends on several biological interactions, which are of a complex nature. The overall objective of BIOHAB is therefore to determine the interplay between (anthropogenic) eutrophication and biological control of the losses and gains of HABs. The overall strategy combines: (1) field and mesocosm studies using natural phytoplankton communities including HABs; (2) laboratory experiments using unialgal cultures.

The ultimate goal is to find ways to manage phytoplankton algal blooms in European coastal waters in such a way that harmful species are avoided or at the least that their negative effects are minimised.

The ultimate goal is to find ways to manage phytoplankton algal blooms in European coastal waters in such a way that harmful species are avoided or at least that their negative effects (e.g. toxin production or oxygen depletion) is minimised. This will require that policies with respect to environmental control and nutrient management for coastal waters are co-ordinated between

EU members, since phytoplankton and nutrients will not stop at national borders. Furthermore, by testing different N/P ratios different scenarios for HAB development will be available thus anticipates to ongoing changes in the nutrient load of the coastal zone.

The co-operation involves several European countries, representing various coastal areas (the Baltic, the North Sea, coastal zone of Norway, the Mediterranean). Results will therefore have more than local interest, considering the great differences between the coastal waters investigated. At the same time, these waters are to some extent uniquely European, with respect to climate, nutrients, salinity conditions etc., which means that practical measures cannot easily be based on research in e. g. Japan, Asia or North America, where harmful algal blooms are also a serious problem.

Scientific objectives and approach

The scientific objectives are :

(1) To determine the susceptibility of HABs to biological control such as grazing (copepods, ciliates, hetero- and mixotrophic dinoflagellates) and infection (virus, bacteria, parasites) when growing under deficient as compared to sufficient nutrient conditions.
(2) Investigate the release of infochemicals by HABs into the seawater with the aim to avoid grazing and infection.
(3) To examine data sets of the general and unique patterns of growth and decay parameters of HAB-species in various coastal regions.
(4) To develop a generic or species-specific model for the development of HABs and their mitigation.
(5) To obtain and grow HAB species-specific pathogens (viruses, bacteria, parasites) which could potentially be used to terminate HABs (bio-control).

The workplan combines laboratory and field experiments with *in situ* studies, to be carried out in 4 different European seas. This includes the low saline Baltic, the eutrophic N-controlled North Sea, the oligotrophic Norwegian Sea, and the P-limited Mediterranean Sea. All areas have repeatedly experienced intensive HABs. Not only the HAB-forming species but also their associated bacterioplankton and viral community (the latter to be isolated) will be studied.

HAB species producing toxins and/or other harmful effects are found in many taxonomic classes, e.g. dinoflagellates (*Alexandrium* spp., *G. aureolum*, Dynophysis), haptophytes (*C. polylepis, P. parvum, Phaeocystis*) and cyanobacteria (*Nodularia* spp. *Aphanizomenon* spp.).

The laboratory experiments will be conducted as semi-continuous cultures under different nutrient conditions. Cell densities will be monitored using state of the art techniques. Algal toxicity and nutrients will be analysed. For specific cell viability tests and genome degradation (indication of apoptosis/-automortality) protocols will be developed. The role of pathogens (viruses, bacteria and parasites) and grazers in terminating HABs will be investigated in separate or combined experiments. All partners will join together for large-scale indoor/outdoor mesocosms experiments and field surveys.

BIOHAB also aims to obtain and grow HAB species-specific pathogens (viruses, bacteria, and parasites) which could potentially be used to terminate HABs. Finally, BIOHAB will develop a generic or a species-specific predictive model for the development of HAB and their mitigation based on the data sets generated.

Expected impacts

Both the Helsinki (HELCOM) and Oslo Paris Commission (OPARCOM) have been established as intergovernmental organisations with their primary task the protection of the marine environments in the Baltic Sea and North Sea.

BIOHAB will result in a sound knowledge of biological factors affecting the gains and losses of key-HAB-species. The information obtained in BIOHAB might provide potential ways of controlling HABs. This will be of potential importance to aquaculture sites, which are of increase (economical) importance in the coastal waters of Europe.

Website for project and related information: www.nioz.nl/projects/biohab

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TITLE: **MI**CROBIAL **D**IVERSITY IN **A**QUATIC **E**COSYSTEMS (**MIDAS**)

CONTRACT N°: **MAS3-CT97-0154**

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MICROBIAL DIVERSITY IN AQUATIC ECOSYSTEMS MIDAS

http://www.icm.csic.es/bio/midas

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INTRODUCTION

The disappearance of species from our planet is perceived as a menace for our future and as a loss of genetic resources for potential useful applications. This loss of diversity is most dramatic and obvious in the case of tropical rain forests. Spectacular epiphytes, colorful birds and bizarre insects are disappearing at alarming rates through human action. Thus, an increasing amount of resources is being put into the study of biodiversity. Several international organizations have sponsored workshops to analyze the problem of shrinking biodiversity and to propose solutions. The European Union has recognized the importance of diversity studies both in the MAST-III work programme and in the Vth Framework Program.

Microorganisms are the most abundant and most genetically diverse living beings and yet, their diversity is essentially unknown. Recently, several molecular techniques have been adapted to yield information on the diversity of microorganisms *in situ*. It is vital that studies are begun with a clear ecological question in mind, so that the appropriate method is chosen and the right community is sampled. In MIDAS we have chosen techniques from different approaches because each one opens a different window into microbial diversity. To our knowledge nobody has tested several of these techniques simultaneously in the same environment to compare the information they provide. We have chosen to use these techniques in environments with special characteristics that make them excellent as test environments. After comparing the techniques, we will use them to study several questions about microbial diversity:

a) Seasonal changes in the abundance of given species in coastal ecosystems.

b) Similarities and differences among contrasting European marine systems.

c) Short-time scale changes and response of bacterial diversity to different nutrient regimes in mesocosms.

d) Effects of viruses and predation by protozoa on bacterial diversity.

We will also isolate bacteria to demonstrate the usefulness of the techniques to retrieve microorganisms of potential commercial application.

OBJECTIVES

The overall aim of MIDAS is to establish a framework (including development and testing of concepts, techniques and strategies) designed to measure diversity and to retrieve microorganisms of potential importance to the biotechnology industry from marine ecosystems. This framework is necessary both to monitor the response of marine ecosystems to human activities and environmental change, and to provide an improved basis, through coordinated European research effort, for exploiting the potential of microorganism diversity in the sea.

The specific objectives of MIDAS are:

1. Develop techniques (specially molecular techniques) to examine diversity of marine microbial ecosystems.
2. Test the developed methods by analyzing patterns of diversity in experimental model ecosystems and in natural environments.
3. Examine the mechanisms responsible for maintaining diversity in natural microbial communities.
4. Develop strategies to isolate microorganisms of potential commercial significance from marine environments. This involves knowing where to look for them and how to isolate them.
5. Analysis of natural microbial assemblages using molecular biology techniques to describe diversity in different marine provinces.

MIDAS will contain the following interacting elements:

- Isolation of microbes of industrial potential and laboratory experiments using cultures of microorganisms.
- Testing of hypotheses of diversity using marine mesocosm experiments containing natural mixed plankton communities.
- Field studies from contrasting locations and modeling results of diversity.

WORKPLAN

The scientific content of MIDAS is subdivided into 6 work packages. The rationale behind the construction of the 6 work packages includes methodological development for bacterioplankton in work package 1, and for phytoplankton in work package 2. Work package 3 will examine some mechanisms which may be responsible for maintaining prokaryotic diversity in marine environments. Work package 4 will develop strategies to isolate microorganisms of potential interest from marine environments. The last two work packages include testing of the methods in natural environments: Work package 5 includes a variety of natural environments and work package 6 comprises work in marine mesocosms. In both work packages we will also try to answer a few questions about diversity. The questions asked are those we thought could be answered with the methods developed during MIDAS.

RESULTS AND DISCUSSION

During the first year of the project we developed protocols to harvest biomass from natural samples, to extract the nucleic acids and to analyze them through a variety of techniques. These included DNA melting and reassociation kinetics, PCR amplification of genes, Denaturign gradient gel electrophoresis (DGGE), RFLP, RISA, cloning and sequencing. Several of these techniques were modified for use with both prokaryotes and eukaryotes. Techniques were also improved for the determination of the abundance of phototrophic microbes through the analysis

of their marker pigments. In the case of heterotrophic bacteria BIOLOG plates were also used to study the metabolic capabilities of natural communities. Finally, a series of additional techniques such as epifluorescence and light microscopy, flow cytometry and incorporation of radioactively labeled compounds were used in combination with the other techniques.
These different techniques were compared during a workshop carried out at the beginning of the second year. The workshop involved 15 days of intensive study of the solar salterns in Santa Pola (Alicante, Spain). Multi-pond solar salterns provide a range of environments with different salinity, from that of seawater up to sodium chloride saturation and sometimes even beyond. As water evaporates and salinity increases, water is pumped or gravity moved to the next pond, such that salinity in each particular pond is kept within narrow limits, essentially constant. Each pond can thus be considered at equilibrium and the biota in any given pond as a well-adapted and established community for that particular salinity. The two extremes of salinity provide one of the most common habitats (seawater) and one of the most extreme habitats in the world (calcium and magnesium chloride saturated brines). As salinity increases the number of different eukaryotic organisms decreases. The total number of bacteria also decreases with increasing salinity, while the number of archaea increases to a maximum in the crystallizer ponds. The salterns, therefore, provided an ideal natural system to test the different techniques developed in MIDAS.

All techniques confirmed the trend for decreasing diversity with increasing salinity. For example, DGGE showed a decreasing number of bands with increasing salinity for bacteria and eukarya. The decrease in archaeal bands was not as pronounced. DGGE for bacteria was carried out with three different sets of primers (amplifying different regions of the 16S rRNA gene). Obviously, the band patterns were different in each case. However, when the three sets of band patterns were used to construct dendrograms, saltern ponds grouped in the same way, indicating that the three sets of primers were giving the same picture of similarities among communities. The same groupings were obtained by another fingerprinting technique (RISA). Therefore, these different fingerprinting techniques can be used with confidence to compare microbial assemblages.

A number of experiments were carried out to follow changes in the microbial community in response to predator manipulations, addition of nutrients, or decreases in salinity. In general, the composition of the bacterial assemblage was fairly robust to all manipulations assayed. It appears that only when differrent carbon compounds are added, does the compositon of the assemblage change.

During the second year, samples were collected from several coastal environments to assess the seasonal changes in the composition of microbial assemblages. In addition, several techniques were used to isolate bacteria of potential interest in pure culture. Molecular techniques were used to monitor the growth of environmentally important bacteria in enrichment cultures. This part of the work is still in progress.

At the beginning of the third year, a second workshop was carried out in the Roskilde fjord in Denmark. Twelve mesocosms were set up and different organic and inorganic nutrients were added to monitor the response of the microbial assemblage with the different molecular techniques. A model linking microbial diversity, nutrient supply, predation and viral lysis had been developed, and the mesocosms provided the experimental setup to test some of the predictions of such model. Preliminary results indicate that a fast response was observed from the picophytoplankton and from the heterotrophic bacterioplankton in the organic matter addition mesocosms. The results from this experiments, however, are still being analyzed.

In conclusion, the project has provided a wealth of information on the mechanisms controling microbial diversity and on the seasonal and spatial scales of variation in the composition of microbial assemblages. The comparison of so many different techniques under different conditions will be extremely useful to interpret what other workers find when a single technique is used. A large collection of new sequences has been obtained from marine environments. These sequences have revealed the degree of microdiversity within bacterian and archaea. New eukaryal sequences have been shown to be widespread and abundant in different areas of the ocean. New archaeal sequences have been retrieved from the salterns. Finally, a wide spectrum of culture techniques has been used to enrich and isolate environmentally relevant bacteria. Thus, the project will not only provide a wealth of new information of microbial diversity, but it will also provide a solid methodological base to for future studies.

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I.1.4 Marine biotechnology

TITLE : LEAD POTENTIAL OF MARINE MICROORGANISMS FROM COASTAL, SHELF AND DEEPSEA SEDIMENTS, A COMPARATIVE ASSESSMENT FOR OPTIMIZED SEARCH STRATEGIES : **MicroMa**r)

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MicroMar, Lead Potential of marine microorganisms from coastal, shelf and deepsea sediments, a comparative assessment for optimized search strategies.

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1. Overview

The objectives of this new project (3/2000) for industrial, life science oriented marine product research are aiming at natural products to be used as leads and/or enzymes, which are:

- biochemically targeted at specific industrial applications with minimal undesirable side effects,
- relying on bio-sedimentary sources which are accessible, in a technical, economic and institutional sense, also for repeated scientific resampling and resupply,
- also produced by cultivation or other methods of bioengineering.

Further objectives are the study of cultivation opportunities in land laboratories but also in the ocean, with

- a characterisation of bacteria and their secondary metabolites, structure elucidation, lead oriented multi-screening and bioassays,
- a general evaluation of responsible uses of marine biodiversity by life science industries and of existing and forthcoming rules and regulations,
- strategies of marine bioprospection on and in the seafloor.

Application and adaptation of well proven methods of seafloor sampling and in-situ monitoring equipment and necessary new developments are scientific and marine technology requirements. But also the logistics, the political and financial implications of natural product research on microbenthic-bacterial communities are important subtasks in finding optimized search strategies: where to explore and analyze, how, and with whom, how to process samples, obtain and optimize cultivation conditions for lead identification in a cost effective and environmentally acceptable way, fast and reliable. Targeted "sampling of opportunity", in regional case studies, is being organized as well as dedicated sampling.

2. Marine Microorganisms

The search in the vast expanses of the ocean and its seafloor for marine organisms, for marine microorganisms in particular, and their production of secondary metabolites and enzymes show so far rather isolated, accidental and geoscience driven actions and results. Biological, ecological and life science oriented, systematical and finally optimized approaches are attempted with MicroMar. The MicroMar workplan, furthermore, is focused on selected European and international subcompartments of the typical marine sedimentary environment on the shelf, slope and deepsea with reduced or no direct terrigeneous influences. The partners are looking primarily at heterotrophic bacteria in the sea floor. Microorganisms living closer and more abundantly nearshore in coastal areas, such as strains of the streptomycetes group, resemble more their terrestrial relatives than e.g. actinoplates, which are more marine and, hence, more abundant and diverse with growing distance from the coast. It is hypothesized and partly verified, that the production of chemically and biologically active metabolites varies accordingly. The recently discovered role of non-extremophile archaea will be looked at as well. Fungi and actinomycetes may be studied when appropriate.

The species diversity of bacteria in the marine environments is being discussed in the light of results from new molecular approaches to detect and identify bacteria. The conventional, culture based methods for identification of bacteria is limited by the fact that in basically all environments examined so far, less than 1%, often even less than 0.01%, of the bacterial population is culturable on standard media. Since the early 1990s the application of molecular techiques (phylogenetic analysis based on PCR amplified 16S rDNA) yielded increasingly more information on the so far unculturable part of the population. The most interesting outcome of these examinations was that the species diversity in all marine systems studied is much higher than it was expected. Direct detection methods, like fluorescence in-situ hybridization (FISH), even revealed dramatic population differences between freshwater based ecosystems and marine systems. In fresh-water, for instance, the ß-subgroup of Proteobacteria is the absolutely dominating subpopulation (up to 80% of all bacteria), while bacteria of the ß-subgroup are hardly ever found in marine systems. This discrepancy between known groups of bacteria in marine systems, often relatives of freshwater or soil microorganisms, clearly indicates the potential of the marine microbiology to identify new bacteria.

3. Expected Impacts

The project contributes to enhance the emerging marine natural product research in European countries and internationally. It promotes the scientific understanding of marine sedimentary microorganisms and their eventual industrial utilisations, and, hence, the competitiveness and employment in European industry and research.The project concentrates on novel marine prospects for advanced life science and marine technology research and development. It integrates multidisciplinary and multisectoral activities in private and public sector partnerships with a strong end user component from business. On the business side there is a large company, a world leader in crop protection and life science, and there are European SMEs to create new products and markets, possibly for bioactive chemicals from the ocean and methods and services to obtain them. Scientific research opportunities of considerable regional European and global significance are addressed: sediments in all waterdepths in the northeast Atlantic and its shelf seas will be investigated and compared as well as in the Eastern Mediterranean and in ocean areas far outside of Europe.

MicroMar will support important objectives of Key Action 3: "Sustainable Marine Ecosystems" by establishing guidelines for management strategies for the exploration and the sustainable future use of ocean sedimentary areas. Application and development of new environmental-safe technologies to survey, monitor and exploit the microbial potential of the sedimentary marine environment are central European tasks. The identification of new lead structures for agrochemical needs and improved health care is an important socio-economic driving force. Knowing and protecting biodiversity in European seas constitute further European policies. Microbiological diversity is at the very basis of life. It merits particular attention as it cannot be seen and may therefore be more easily neglected than visible marine life such as seals or porpoises. The further knowledge and confirmation of the utilization potential of marine soil bacteria will give marine microbiology a much enhanced standing in the public, industrial and academic world.

Deliverables will be:

- optimized marine search strategies in case study regions, based on the occurrence and natural variations of typical marine sedimentary and bio-geochemical ecosystems, on the shelf (including some exemplary coastal sites), on the slope and in the deep sea floor, predictions of such variability, in European seas and beyond, ranking of sedimentary provinces according to lead oriented prospectivity,

- development and utilization of selected and targeted "Sampling of Opportunity" from cooperation with outside researchers, institutions and monitoring groups, who are taking samples and who are doing monitoring work for different marine research and service purposes; thus minimizing expensive ship time, at least in the initial phases of the roject,
- optimized marine search strategies in integrated sequences of adapted and new sampling and monitoring tools, dedicated sampling, including contamination control, bio-sedimentary sample preparation and evaluation, novel prescreening methods, cultivation in the lab and a comparative assessment of in-situ cultivation and metabolic controls,
- identification of easily cultured as well as difficult to grow strains and of specialists; productivity: which organism grows under which condition and produces secondary metabolites and enzymes,
- number, identification and novelty of hits and lead candidates and their derivatives, interesting for agrochemical, animal health, pharmaceutical and further industrial life science applications, concepts for an eventual industrial use of microorganisms in the seafloor, evaluation of technical, legal, environmental and economic production and marketing conditions, emphasizing the safe and environmentally responsible use of the seafloor.

4. Schedule, methods and further work

The project has started on March 1st, 2000 and will last until February 28, 2003. There are three phases, each of approximately of one year duration:

<u>1. The Orientation Phase,</u>

with an initial project start meeting and Milestone One:

- to obtain target "samples of opportunity" and to test sample logistics;
- microbiological and biosedimentary sample preparation and characterisation, onboard, for transport, in the lab,
- establish detailed cultivation and isolation steps, sequencing, phylogenetic taxonomy, etc;
- to conceptually design dedicated sampling and monitoring tools and equipment;
- to establish subsequent laboratory steps by testing with Orientation Phase samples.

Milestone Two; TO + ~ 12M : Evaluation of the Orientation Phase and the decision to go to the Development Phase.

<u>2. The Development Phase,</u>

- to detail design and undertake hardware development of dedicated tools and methods;
- procure cost free shiptime for such efforts, start and perform dedicated autoclave and pollution controlled sampling;
- detailed isolation and characterisation, screening preparation of mixed and isolated cultures;
- continue sampling of opportunity and logistics; initial candidate list for leads and hits.

Milestone Three, TO + ~24M : Evaluation of the two preceeding phases and the decision to proceed to the Strategic Phase.

<u>3. The Strategic Phase,</u>

- Continuation of optimized and specific "sampling of opportunity";

- dedicated sampling and monitoring by well positioned, cable guided autoclave equipment;
- contamination control by autoclave sampling;
- possibly metabolic studies in-situ;
- specific characterisation and prescreening of bulk samples, lab cultivation, finalization of the study of in-situ cultivation, screening including high thoughput screening and lists of hits and leads;
- evaluation alternatives, strategies,responsible uses.

In order to better understand the complex marine ecology for the microbial production of secondary metabolites and enzymes, its spatial and temporal variations must be added to those spot oriented sampling tasks. Furthermore, the chemical concentrations of secondary metabolites produced by microorganisms in their natural environment are usually very low, in the ppb and ppt ranges. Resupply of promising microorganisms by repeated sampling, although technically possible, but ecologically and economically questionable, may thus be limited. Those limitations support the study of in-situ enrichment and cultivation of special bacteria in the seafloor. There are various technical designs available. Dedicated benthic stations could be achieved by adaptation of existing seafloor station concepts to benthic bacterial metabolic laboratories. Additional metabolic parameters will be studied, e.g. controllable changes in supply of oxygen and alternative electron acceptors, availability of different carbon sources and electron donors, as well as biochemical indicators of bio-sedimentary metabolic changes such as NAD/NADH or ADP/ATP.

The first task of microbiological lab evaluation strategies is to identify groups, families and species of microorganisms which are candidates for production of metabolites. The diversity of microorganisms in marine sediments has only been scratched by now, further microorganisms will be detected, also within MicroMar. The expert community expects that those new bacteria, possibly with new biosynthetic potential, will be isolated. The further phylogenetic characterization of these isolates done on the basis of bulk analyses with MALDI will result in a collection of interesting isolates to be included in a more detailed research for compounds.

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TITLE: **MARINE BACTERIAL GENES AND ISOLATES AS SOURCES FOR NOVEL BIOTECHNOLOGICAL PRODUCTS: MARGENES**

CONTRACT N°: **MAS3 - CT97 - 0125**

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MARINE BACTERIAL GENES AND ISOLATES AS SOURCES FOR NOVEL BIOTECHNOLOGICAL PRODUCTS

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SUMMARY

The overall objective of the project is the development and application of a new molecular strategy to turn the hidden biodiversity of marine bacteria into novel biotechnological products (more details are given at: www.gbf.de/margenes). This strategy is based on an integrated molecular approach using bacterial isolates and DNA obtained directly from the marine environment. Environmental DNA is used to access the genetic potential of the non-culturable fraction of the marine microbial community which is usually quite large because only a very small fraction, usually less than 1%, of the marine bacteria can be cultured. The molecular approaches used are cloning and expression of marine bacterial genes, estimation of the structure of bacterial communities by 16S rDNA fingerprinting, and molecular characterization of bacterial isolates. This integrated approach is applied to a set of diverse marine habitats to obtain information about i) the diversity of indigenous bacteria and their genes, and ii) the expression systems necessary to make the genetic information available for biotechnological products such as novel enzymes.

INTRODUCTION

Our current understanding of the evolution of life is that it started in the ocean and was for at least the first two billion years purely microbial. The large size of the marine environment, the variability of the habitats and the enormous amount of microorganisms observed per volume of seawater as units of evolution are additional multiplicators of biological diversity. These basic facts of our biological understanding indicate that phylogenetic and metabolic diversity of marine microorganisms should be greatest among all forms of live. This was demonstrated recently by molecular analysis of DNA obtained directly from marine microbial communities, using comparative 16S rDNA sequence analysis. These communities comprised an enormous diversity of bacteria, including completely new phyla for the domain *Bacteria* and new lines of descent for the *Archaea*. This genetic diversity of very conserved homologous genes represents only the tip of the iceberg in terms of genetic diversity of marine bacteria that should be reflected in many novel biochemical pathways comprised of an almost indefinite number of new enzymes. It is evident from a wide range of studies using molecular methods that the vast majority of the bacterial population in natural environments has yet to be cultured. Therefore, it is a well known dilemma of marine microbiology that marine bacteria are very difficult to grow in the laboratory, e.g. from open ocean seawater samples only about 1‰ of the cells detectable by microscopic methods can be cultured. This means that the classical way to do biotechnology, i. e. the growth of the relevant microorganism to obtain the product, is blocked. There have been significant advancements in the molecular analysis of marine bacterial diversity *in situ*. Therefore, we solve the basic dilemma in marine microbiology by looking at the genetic information of marine bacteria directly, i. e. without any cultivation, in three molecular ways: i) Cloning and expressing the genes, ii) fingerprinting and sequencing 16S rRNA genes from the whole microbial community, and iii) molecular identification of isolated marine bacteria. These molecular analysis are complemented by a functional analysis of the obtained clones and isolates in a high-through-put system (HTS) to select for novel biomolecules such as enzymes and antibiotics.

METHODOLOGY

16S rDNA fingerprinting and sequencing 16S rRNA genes from whole microbial communities

Assessment of high resolution genetic diversity was performed using temperature gradient gel electrophoresis (TGGE) of 16S rDNA amplicons from natural marine communities. TGGE fingerprints of 16S rDNA fragments obtained by the different bacterial communities were generated by using a set of universal PCR primers for amplification (position 968 to 1346, according to *E. coli* numbering). The gel was digitised for image analysis. Band positions were normalised and band intensities quantified for further statistical analysis. In this way we aim to determine the diversity within the sample DNA and in addition detect diversity of gene sequences cloned within several gene banks.

Cloning and expressing of marine microbial genes

Analysis of genetic diversity requires the detection of diversity within genes and this can be achieved by analysis of DNA isolated and cloned directly from seawater without the need to culture specific components of the aquatic biomass. To retrieve as much genetic information as possible we cloned prokaryotic DNA obtained from a variety of carefully selected microbial communities by using a universal non-selective cloning strategy. This approach resulted in the generation of polygenomic libraries that contain functional genes for the investigation of metabolic diversity. Functional expression of genes cloned in the library was determined by screening clones for enzymatic and antibiotic activity using a range of expression vectors.

Molecular identification of isolated marine bacteria

The determination of the taxonomic position of bacterial isolates consisted of a two step genotyping procedure: 1^{st}, Low-molecular-Weight (LMW) RNA profiling of all relevant isolates to genotypically group them at the species level, and, 2^{nd}, 16S rDNA sequencing of the genotypes that could not be identified to the species level by LMW RNA profiling. All these molecular data went into an integrated data base that was used to reduce genotypic redundancy and help identify the most promising clones or isolates for a more detailed analysis of producers of novel antibiotics or enzymes.

RESULTS

Sampling and isolation of strains

Bacterial biomass was obtained from 4 specific habitats (water column of the Baltic proper and Western Mediterranean Sea, coastal and deep sea sediments form the Glénan Island off Brittany and the southern ocean, respectively) for direct molecular analysis and isolation of strains from the respective samples. All in all the total number of samples analyzed for their community structure and the number of isolates obtained or provided in the project is summarized in table 1.

Table 1. Total number of samples and bacterial strains obtained in the project.

Location	Habitat	No. of samples	No. of strains
Western Mediterranean Sea	pelagial	15	325
Baltic Sea	pelagial	25	944
Antarctic Ocean	deep sea sediment	11	105
Atlantic Ocean off Brittany	coastal/tidal sediment	17	1183
		68	**2557**

Community fingerprinting of marine bacterial assemblages

The genetic diversity of the microbial community was determined with 16S rDNA fingerprinting that is able to give a quantitative overview about the abundant species in the community. The analysis of genetic diversity of the single marine microbial communities had to serve as a criterion for the decision on which samples to concentrate with the gene cloning and gene expression experiments. We concentrated on the analysis of the total bacterial fractions from one set of water samples of the central Baltic sea. We analysed two depth profiles (Gotland deep, station Teili) to show a vertical variation of the quantitative taxonomic structure of the bacterioplankton in different depths. We were also interested in the horizontal variability of Baltic surface samples. The cluster analysis of the 16S rDNA fingerprints showed an accurate branching of the planktonic bacterial communities extracted from habitats with similar physico-chemical conditions. All the surface patterns and the top layer profiles (5-30m) clustered together. The deeper Teili samples (90-140m) could be differentiated from the Gotland deep community profiles. The TGGE fingerprints of the Gotland deep showed differences in samples taken above and below the oxic and anoxic interphase. The influences of physico-chemical parameters were also reflected in the total number of bacteria in the different water layers of the Gotland Deep. In general, a decrease of bacterioplankton abundances was observed with depth in the oxic zone and an increase in the anoxic zone. In conclusion, we observed similarities in diversity within samples from similar physico-chemical conditions across the central Baltic and substantial differences along depth.

Furthermore, sediment samples from the deep sea and the Glénan Islands were analysed for their taxonomic structure using 16S rDNA community fingerprints and 16S rDNA clone libraries. 16S rDNA fingerprint analysis indicated that the tidal sediments from Glénan Islands had the highest diversity of all marine habitats studied. Planktonic bacterial communities (Baltic and Mediterrean Sea) appeared to have a low diversity in comparison to all sediment communities studied. The 16S rDNA clone libraries allowed for a very detailed phylogenetic identification of the single members of the microbial communities studied by 16S rDNA fingerprinting. Comparison of the 16S rDNA sequences obtained from the clone libraries with sequences from international databases and from isolates of the same habitat revealed that in both types of habitat, pelagial and sediment, a variety of “uncultured” sequences were found.

This finding was substantially more pronounced in sediments than in the pelagial. Furthermore, in the water column we found quite a lot of sequences from the 16S rDNA clone libraries that corresponded to cultured isolates or to known marine bacteria.

Assessment of the diversity of microbial genes in marine habitats by direct cloning

The specific objective of direct gene analysis is to obtain as many, and as diverse as possible, clone libraries from marine bacterial DNA containing functioning genes, i.e. genes that can be expressed as completely functional proteins. Clone libraries have been constructed from three different samples (one deep sea sediment and two coastal sediment samples). Several shotgun clone libraries were constructed using plasmid vector pZero2. These libraries were not very successful due to the high content of non-recombinant clones. Therefore, the phage vector system Lambda ZapII was used for the construction of new clone libraries. Out of 15 new libraries constructed with the phage vector several were good enough, i.e. they contained more than 99% recombinants with an insert size of > 2kb, to be processed for expression and further screening. 8 clone libraries in Lambda ZapII were constructed from DNA out of a deep sea sediment core. Due to the very low numbers of total clones (below 50 000) only 3 clone libraries were amplified and prepared for expression in *E. coli.* From the Glénan islands another 7 clone libraries in Lambda ZapII were constructed using DNA obtained from the two coastal sediment samples. These libraries resulted in much higher yield of clones (the best libraries had above 1 million) and had a very high percentage of recombinant clones (up to 99%). The best three of these libraries were prepared for expression and screened for activity.

Determination of activities of isolates

Massive enzyme screening was done on marine isolates from sediments from the coast of Brittany, water samples from the central Baltic sea and the western Mediterranean sea. These isolates were screened by HTS for the following enzymatic activities: amylase, arabinase, cellulase, galactanase, pullulanase, xylanase, xyloglucanase, protease and lipase. The number of strains and the number of hits are listed in table 2. A total of 2054 isolates were screened with the HTS resulting in a total number of 1342 hits. The number of hits was on a similar level of about 1 hit per strain for isolates from the coast of Brittany and the Mediterranean sea. Records were far lower for the Baltic sea (165 hits per 944 isolates) and psychrophilic isolates from Antarctic sediments.

Table 2. Enzyme hits of the investigated strains

	no. of strains	**enzyme hits**	**no. hits / strain**
Baltic sea strains	944	165	0.17
Glénan island isolates	749	866	1.16
Mediterranian isolates	325	303	0.93
Psychrophilic isolates	36	8	0.22
Total:	**2054**	**1342**	**0.65**

Molecular and metabolic characterization of single bacterial isolates

Solid identification of the taxonomic position of the marine isolates to the species level was achieved by a combination of Low Molecular Weight RNA fingerprinting and 16S rRNA sequence analysis. From pelagic environments, 204 strains isolated from the western Mediterranean sea and 245 isolates from the central Baltic were characterized. Their phylogeny and ecology was related to their enzymatic activity. The Mediterranean isolates provided a high diversity of species and genera of the *alpha*- and *gamma-Proteobacteria*, the *high*- and *low-G+C Grampositives* and the Cytophaga/*Flavobacteria Bacteroides group.* The correlation of bacterial endo-enzymatic activity showed that the *gamma-Proteobacteria*, the *high*- and *low-G+C Grampositives* represented the enzymatically most active phylogenetic groups, with the genus *Alteromonas* and *Pseudoalteromonas* as the most active and most abundant of the *gamma-Proteobacteria*, and the *Bacilli* as highly active genus of the *low-G+C Grampositives*. For the Baltic sea strains 26 genotypes could be identified that consisted of a large array of species of the *alpha*- and *gamma-Proteobacteria* and one *Bacillus* (*low G+C Grampositive*). 14 novel species were detected, some of them belonging to novel groups. From a coastal environment, 886 isolates from the Glénan islands, were analyzed for a set of phenotypical features. Cluster analysis of these features indicated a high diversity among the isolates. To determine the phylogenetic affiliations of the isolates 16S rRNA analysis is ongoing, with emphasis on isolates of particular interest for enzyme production.

CONCLUSIONS

- Analysis of the overall community structure by DNA fingerprinting revealed that planktonic bacterial communities have a low diversity in comparison to all sediment communities studied.

- From all marine microbial communities assessed by DNA fingerprinting the tidal sediments from Glénan Islands were the most genetically diverse. Successful libraries containing greater than 300,000 clones are currently being screened.

- Several new genera and species of marine bacteria could be discovered using molecular analysis of bacterial isolates, primarily in the phyla *gamma-Proteobacteria* and *Actinobacteria* (*high G+C Grampositives*).

- Some marine habitats, like tidal sediments, were identified using HTS screens as real hot spots for the isolation of bacteria containing endo-enzymes in comparison to the water column of the Baltic and the Mediterranean Sea.

- Using a phage vector system several clone libraries could be constructed from DNA isolated from coastal and deep sea sediment samples.

- Expression of the cloned material was feasible in *E. coli* , but thus far massive screening of expression clones did not result in high rates for endo-enzymes. Detection and screening technology and approaches are being improved.

- These new findings on the ecology and molecular analysis of marine bacteria will enable a better exploitation of marine bacteria and their genes as sources for novel biotechnological products.

TITLE: BIOLOGY OF SPONGE NATURAL PRODUCTS : **SYMBIOSPONGE**

CONTRACT N°: **MAS3-CT97-0144**

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BIOLOGY OF SPONGE NATURAL PRODUCTS

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INTRODUCTION

Sponges are a source for a dazzling variety of secondary metabolites presumably used for protection against predators, overgrowing by space competitors and invading microorganisms. Some of these natural products may be of use for man as drugs, anti-fouling substances or for a variety of other functions. The supply of sponge products with useful properties poses a serious problem for any commercial application, because (1) sponges do not in general occur in large enough populations to allow harvesting, (2) there is no clear knowledge of the distribution of the different types of secondary metabolites among the different species of sponges, (3) most of these chemical substances are difficult or costly to synthesize, and (4) sponges themselves are in general difficult to culture.

It follows that cell culture may be a promising alternative for commercial production of the desired substances. However, for that to be successful, it is necessary to establish the cellular origin of the target compound, since sponges harbour large amounts of microorganisms which may be involved in the production of this compound. The Symbiosponge project hopes to discover chemotaxonomical patterns in the production of secondary metabolites by sponges and to develop efficient research protocols for detection of the cellular origin of these promising marine chemical resources. To this end, the cellular, ecological and chemical aspects of marine sponge secondary metabolite production will be investigated and directed towards: (1) their distribution between and within species from different geographical origin, (2) the understanding of the cellular origin and possible microsymbiont involvement, (3) the understanding of their ecological significance. Ultimately we hope to deliver a standard protocol able to help in taking decisions for the production of promising sponge secondary metabolites.

RESULTS

The project is in its second year and several tasks are in progress or already completed.

Sponge material: Sponge samples (340 samples for 239 species) were collected in five geographic localities (Netherlands, Mediterranean Sea, Oman, Indonesia and Curaçao) in order to identify possible geographic pattern variations. Specimens were photographed, subsampled, fixed for the various types of analyses and subsequently identified. Vouchers of all samples are kept in the collections of the Zoological Museum of Amsterdam.

Extractions and bioassays: Sponge samples preserved in methanol from all collecting locations were stored at -20°C. The samples were extracted according to a standard protocol leading to five extracts of increasing polarity for each sample. All the extracts were submitted to toxicity bioassays against *Artemia salina, Saccharomyces cerevisiae*, a variety of fungi and bacteria. They were also concurrently examined by TLC. Until now 176 samples (corresponding to 166 species) have been extracted and tested.

Isolation and structure elucidation: Selected bioactive extracts were submitted to bioassay guided fractionation using a combination of chromatographic methods. This led to the isolation and the purification of several secondary metabolites, the structures of which were determined on the basis of their spectral properties. For example, new sesquiterpene/quinones and new indole alkaloids were isolated from four different *Hyrtios* species, a novel sphingosine derivative was isolated in high yield (7% dry weight) from *Haliclona vansoesti*, polyunsaturated bromoacids were isolated and identified in various samples of *Xestospongia muta*, the bromopyrrole alkaloids content of several *Agelas* samples was determined, etc. In addition, a systematic search for chemotaxonomical markers in sponges collected during this programme and which belong to the orders Haplosclerida and Halichondrida has been carried out. The extent to which these markers (e.g. isocyanoterpenes, 3-alkylpiperidine derivatives, pyrrole-2-carboxylic acid derivatives) are present in members of these groups could determine the likelihood of being a product of sponges or of symbionts.

Cell fractionation and culture: In order to determine whether the sponge cells or the microsymbionts are responsible for the production of bioactive compounds, sponge tissues of 6 species (*Agelas conifera, Dysidea etheria, Haliclona vansoesti, Ircinia campana, Ircinia felix* and *Xestospongia muta*) were mechanically dissociated into single cells suspensions which were fractionated by differential centrifugation. This allowed the recovery of cell fractions enriched either in sponge cells, bacteria or cyanobacteria. Sponge cells were further fractionated by means of Ficoll and Percoll gradient density centrifugation. Bacteria were mostly located in the low-density fractions. The other fractions were enriched in the different types of sponge cells: choanocytes, archaeocytes and spherulous-like cells. Particularly pure archaeocyte and cyanobacterial fractions were obtained from *Ircinia campana* and *Xestospongia muta* respectively.

Primary cultures of cell suspensions initiated either in filtered seawater or artificial seawater were successful. Sponge cells aggregated, settled and reorganised into a structured network of cells, characteristic of each species. Addition of phytohemagglutinin (1.5%) enhanced the process.

Microsymbiont observation: To locate and visualise the bacteria within the sponges, various preparation and staining techniques were used (e.g. light and fluorescence microscopy, SEM, CSLM, DAPI).

Ecological experiments: To assess the effect of environmental factors on the production of secondary metabolites by selected sponges, experiments were performed to determine the influence of caging (prevention of predation), injuries (simulation of predation), confrontation with territorial coral competitors, depth and light. Moreover, palatability tests with food containing sponge extracts were carried out using fish predators.

CONCLUSIONS

On the basis of the results obtained during the preliminary phase of the project, four Caribbean sponges were selected: *Agelas conifera*, *Haliclona vansoesti*, *Ircinia felix* and *Xestospongia muta*. The major secondary metabolites of each of these species have been identified and analytical methods have been set up for their detection and quantification in small sponge pieces and cell sponge fractions. A complete evaluation of their chemical, biological and ecological properties are on hand.

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TITLE : MARINE CYANOBACTERIA AS A SOURCE FOR BIOACTIVE (APOPTOSIS-MODIFICATION) COMPOUNDS WITH POTENTIAL AS CELL BIOLOGY REAGENTS AND DRUGS

CONTRACT N°: MAS3-CT97-0156

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BIOACTIVE COMPOUNDS FROM CYANOBACTERIA

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SUMMARY

Cyanobacteria have been sampled from the Norwegian and Iberian coast and from the Baltic sea. The samples have been monocultured and made available for bioscreening. Algal extracts have been tested for activity towards primary liver cells, human leukaemia cells, and a number of different cell lines. Thirty extracts were found to have bioactivity, with at least seven of them representing novel substances. The network has until now selected three of these extracts for further characterisation. Different purified fractions of the selected algae have been investigated in order to find their target sites in the pro- and antiapoptotic cell signalling pathways.

Chemical modifications of the known cyanobacterial toxins nodularin and microcystin have been performed in order to make these toxins more cell membrane permeable. Transport peptides have been tested for their ability to transfer the toxins into cells.

INTRODUCTION

Apoptosis is presently one of the most intensely studied biological phenomena, both within basic and applied bio-sciences (Evan and Littlewood, 1998). Hence, drugs that can affect these processes are eagerly searched for both to help research (marine and general) and as potential new drugs in conditions of increased apoptosis (degenerative disorders, HIV) and decreased apoptosis (cancer, certain autoimmune diseases)(Blankenberg *et al*, 2000).

In this respect, the most promising presently available compounds originate from marine micro-organisms, but little is done in Europe (compared to Japan, Australia and North America

including Hawaii) to systematically detect new such compounds and to improve (by hemisynthesis) the pharmacological properties of the existing ones.
Cyanobacteria represent an underexplored resource for drug development and the objectives of this project is to search for novel apoptosis-modifying compounds from marine cyanobacteria and to chemically modify them in order to enhance their actions.

Materials and methods

Sampling and culturing of algae

90 different algae have been sampled and small scale cultured. Algal samples were collected from the Norwegian coast, the coastal areas of the Baltic Sea and the Iberian coast. Algae that scored positive after initial screening were made axenic before large scale culturing for purification and in-depth analysis of bioactivity.
Nodularia spumigena and *Microcystis* have been large scale cultured in order to produce nodularin and microcystin-LA for modification purposes.

Extraction and initial screening of algal samples

A methanol and a dichloromethane/methanol extract were made for each algal sample. Samples were tested for their ability to induce apoptotic cell death in primary liver cells, promyelocytic cancer cell lines and human neutrophils. They were also tested for anti-apoptotic activity. Anti-apoptotic activity was determined by the ability to inhibit Fas-induced apoptosis in Jurkat cells. All samples were also screened for activity towards major members of protein kinase C and protein phosphatases.

Extraction and purification of selected algal samples

In order to extract the active contents of cyanobacteria, freeze dried cells of three selected strains (sample A, B, and C) were extracted subsequently with organic solvents of different polarity (hexane, dichloromethane, methanol) and water. Thin layer chromatography on both silica and reversed phase silica showed numerous different compounds and pigments present in all extracts, especially in the non-polar. For isolation of these components all four solvent extracts of a given strain were combined and applied on a silica column for a first crude separation. The column was eluted with a gradient starting with dichloromethane over methanol to end up with water, using medium pressure liquid chromatography (MPLC).

Retesting and in depth analysis of selected algal samples

The obtained fractions from MPLC were re-tested for apoptotic- and antiapoptotic activity. Fractions were also tested for activation of both purified crude protein kinase C (PKC) and different isoforms of this kinase. Fractions were also tested for their ability to specifically activate either protein phosphatase 1 or 2A.

Purification of nodularin and microcystin for chemical modification

Peptide toxins (microcystin LA and LR, nodularin) were isolated from cyanobacteria strains by means of basic aqueous extraction, followed by reversed phase chromatography (C-8). The fraction containing chromophore visible at 238 nm (absorption of Adda - special amino acid - important fragment of all cyanobacteria toxins including nodularin and microcystin) were collected and purified to homogeneity. Several compounds were obtained and structure of some of them were analysed by means of LC-MS and NMR. This includes nodularin, microcystin LR and LA.

Fluorescent labelling of nodularin and membrane penetration using transportan

Nodularin was dissolved in a buffer and a water soluble carbodiimide was added to preactivate the two COOH groups of nodularin. The fluorescence label ALEXA-Hydrazide 594 was added in 1:1 stochiometry and incubated overnight. The nodularin-ALEXA hydrazide adduct was purified by HPLC and the molecular weight was determined by mass spectrometry. The molecular weight analysis showed that one ALEXA hydrazide had been added to the nodularin, to either of the two COOH groups.

The transport peptide was transportan, with the sequence GWTLNSAGYLLGK((NH-Cys)INLKALAALAKKIL, which is known to penetrate into cells carrying hydrophilic cargoes (Pooga *et al*., 1998). The labeled nodularin was incubated with transportan in equimolar amounts overnight at 4 ^{0}C. The product was not purified but directly applied to Bowes melanoma cells and incubated for 2 hours at 37 ^{0}C. The thiol group of the extra Cys residue of transportan has a high propensity to react with double bonds, so either the Adda double bonds or the (-(carbon-carbon double bond of the MDHB residue of nodularin should have reacted with it. A control sample without the transportan peptide was studied in parallel.

Results and discussion

Bioactivity in algal samples

About one third of all (total 90) screened algal samples had bioactivity as measured by induction of either liver cell apoptosis or lymphoma cells. Four of these extracts appeared to be novel substances, since the morphological appearance of the exposed cells differs from that induced by the known cyanobacterial toxins (Bøe *et al*, 1991). Three of the extracts showed anti-apoptotic activity towards Fas-induced cell death. A majority of the extracts inhibited the action of protein phosphatase 1 and 2A. Phosphatase inhibitory activity is also the target mechanistic action of the known nodularin and microcystins. Finally, the extracts were tested for protein kinase C (PKC) modulatory activity. PKC plays a central role in regulation of a variety of cell functions, including cell proliferation and death. Not surprisingly, a range of diseases have been associated with altered PKC activity (Deacon *et al*, 1997). Five extracts yielded PKC activatory activity and two PKC inhibitory activity. From these initial screenings three extract (named sample A, B, and C) were selected for further purification and in-depth bioactivity analysis.

Testing of purification fractions of sample A, B, and C

An important observation from working with purified fractions of the different algal samples was that the same extract could contain both pro- and anti-apoptotic activity. This might result in direct opposing bioactivity in the same sample thereby cause difficulties when screening for bioactivity.

<u>In sample A</u> two fractions from pressure liquid chromatography (MPLC) was found to induce apoptosis in IPC-81 leukemia cells, this sample did not have any effect on our normal untransformed cell type: primary liver cells. Activation of PKC was found in peak area of fractions, this area did not coincide with IPC-81 inducing fraction.

<u>In sample B</u> both pro- and antiapoptotic activity were detected.The opposing activity was separated by MPLC fractionation. A specific area of fractions was found to contain protein phosphatase inhibitory activity. The same fractions induced apoptosis in leukemia cells. A peak separated from both pro- and antiapoptotic activity fractions was found to stimulate PKC avtivity. This stimulation was fare more potent than TPA, a commonly used and commercially available activator of PKC.

<u>In sample C</u> a number of fractions had apoptosis inducing properties in both leukemia and normal liver cells. The most interesting property of this sample seems to be its ability to strongly induce PKC alfa activity. PKC can be formed by different isoenzymes. The different isoenzymes are thought to have specific activation in different cellular pathways. Specific activators of the different isoforms might therefore prove to be valuable.

Further work will focus on those fractions which have extraordinary activity in the different assays. These will be further separated by MPLC with acetonitrile water gradients on reversed phase silica. Fractions will be collected according to the peaks recorded by UV detection and sent to biological testing again.

Hemi-synthetic modification of cyanobacterial toxins

The cyanobacterial toxins nodularin and microcystin can easily enter liver cells and induce apoptosis (Fladmark *et al*, 1999), but their ability to cross the cell membrane in other cell types is highly limited. These toxins would be highly valuable to use as cell biology tools if they could enter other cell types as well. We have therefore decided to chemically modify these toxins in order to make them more cell membrane permeable. We have chosen nodularin and microsystin LA to prepare some prodrugs which have better bioavalaibity in sense of membrane penetration. Modifications in hydrophilic groups like carboxylates and guanidine group have been performed. We transformed these groups into more hydrophobic moieties. The compounds -derivatives of nodularin and microcystin were chemically modified and later purified to homogenous. Their structures were proved by LC-MS and NMR.

Transport of nodularin labeled with a fluorescent hydrazide into cells using a transport peptide

Fluorescence microscopy of the two parallel samples showed that the Bowes melanoma cells with the transportan adduct were highly fluorescent, whereas the cells treated with the control sample were not. The transportan-nodularin adduct may be mostly distributed in the plasma membrane, but some should also have penetrated into the cytoplasm. Further experiments will be performed with confocal microscopy to determine the distribution of the nodularin adduct in the cells. Activity tests of nodularin with and without the fluorescence label will also be performed.

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TITLE: METHODS TO IMPROVE THE SUPPLY OF MARINE ORGANISMS FOR PHARMACEUTICAL RELATED NATURAL PRODUCTS CHEMISTRY

CONTRACT No: MAS3-CT98-0179

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Introduction:

Marine organisms are a valuable source of new drugs for major diseases such as cancer, but drug development is hindered by the problems of having to collect organisms from the wild. Over collecting of interesting marine organisms is now a major environmental concern and is likely to lead to legislative restrictions on the collection of certain species. Highly variable concentrations of bioactive compounds in the organisms makes it difficult to predict the number of animals needed from the wild. While chemical synthesis of interesting drugs is an obvious solution to this problem, many of the compounds of interest require complex synthesis pathways with many and expensive steps.

Securing supply of interesting marine organisms is a strategic necessity for European companies wishing to research and commercialise marine natural products for pharmaceutical use. Companies solving this problem will have considerable competitive advantage. There is no clear route to this and research is required into a number of possible fruitful approaches. These may offer commercial opportunities to European aquaculture companies wishing to diversify into non-food species and for companies which would support the technologies enabling the supply to be secured.

The prime industrial/economic objectives of FAIRE are to:

- develop methods for the successful culturing of bacterial symbionts of marine invertebrates
- develop, protect and exploit IPR in the area of marine invertebrate cell culture
- develop antifouling methods for line culture of bivalves, increasing profits by up to 100% in the case of scallop farms
- develop, protect and exploit IPR relating to the aquaculture of marine invertebrates for pharmaceutical research and development, diversifying the industry and making it less vulnerable to market fluctuations affecting the core aquaculture activities

Scottish Association for Marine Science is based at the Dunstaffnage Marine Laboratory on the West Coast of Scotland. The SAMS Marine Biotechnology Group has dedicated molecular and cell biology laboratories and excellent microscopy facilities. The group's research strengths are in aquaculture, biofouling and marine microbiology. Research at Dunstaffange is being undertaken by Dr Claire Moss, a specialist in marine invertebrate cell culture and larval biology. Dr Douglas McKenzie leads the Biotechnology Group and is the Co-ordinator of the FAIRE project.

Pharma Mar S.A. is a Spanish-based pharmaceuticals company that is a worldwide leader in the discovery and development of new drugs from marine resources. Pharma Mar has collected and classified more than 20,000 different marine macro-organisms and bacteria from which it has identified a large number of new, pharmacologically active chemical structures. A specific focus of research is the development of marine-derived therapeutics against cancer.

Instituto Biomar S.A. is a Spanish R&D company involved in the discovery and development of novel pharmaceuticals from marine bacteria. Biomar is actively involved in the discovery of lead compounds for anti-cancer, and other drug research. As a group they are particularly interested in developing bacterial culture-methods, especially for microbial symbionts that are involved in the production of natural products in marine invertebrates.

ALVITO Biotechnologie GmbH is a new German company specialising in cell culture, cell biology and biochemical-based services. The company has scientists specialised in cytology, biochemistry, pharmacology/toxicology, genetics and molecular biology. ALVITO is particularly interested in the development of novel cell culture lines - a currently under-exploited niche market.

Loch Fyne Seafarms is a privately owned company located on the West Coast of Scotland, specialising in the production and processing of marine shellfish. LFS is interested in advancing scallop production in Scotland by preventing bio-fouling of the shell surface by barnacles and tube worms. It is also interested in the diversification of the aquaculture industry to include the exploitation of marine organisms which are of value to the chemical and pharmaceutical industries.

Muséum National d'Histoire Naturelle is located in the heart of Paris, France. The Laboratory of Marine Invertebrate Biology and Malacology is involved in fundamental and applied research on the systematics, biology and ecology of the principal groups of marine invertebrate fauna: Sponges, Bryozoans, Cnidarians, Echinoderms, Polychaetes and Molluscs. Cell cultures from marine molluscs are now routinely established in the lab and are being developed as in vitro models for ecotoxicological, pathological and biomineralisation studies.

I.15. Structure and dynamics of shelf ecosystems

TITLE: INFLUENCE OF RISING SEA LEVEL ON ECOSYSTEM DYNAMICS OF SALT MARSHES : **ISLED**

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INFLUENCE OF RISING SEA LEVEL ON ECOSYSTEM DYNAMICS OF SALT MARSHES (ISLED)

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INTRODUCTION

Accelerated sea level rise as a result of the human impact on global climate change, has a dramatic impact on the coastal zone, in particular on ecosystems such as *salt marshes*, which are in one way or another dependent on the tidal movement of the sea. On the long term, salt marshes will only survive if they are able to maintain their elevation relative to mean sea level; otherwise they will be drowned. Without an adequate understanding of the nature, scale and extent of the counteracts to survive, future management and uses of these areas and resources will be extremely difficult. Although we have some understanding of how (i.e. in what direction) the morphology and ecology of the salt marshes will react to increased flooding frequency, we have only scanty information about their rate of response. This is primarily due to our incomplete characterisation of the temporal and spatial scales of the physical, chemical and biological processes involved.

In this project we will focus on identifying, describing and defining the abiotic and biotic processes in salt marsh ecosystems resulting from and counteracting the effect of increased inundation frequencies. These countereffects provide stability and robustness, but when their capacities are exceeded they give rise to non-linear responses. Non-linear behaviour of salt marsh ecosystems has been recognised as a major cause for unpredictable changes. This project involves mechanistic studies and requires a multi-disciplinary approach involving microbiologists, marine biologists, biogeochemists and system ecologists. It is multidisciplinary in its objectives and methods, with a high degree of interaction between the partners. The overall aim is to obtain a comprehensive insight into the dynamics of the system counteracting the effects of sea level rise, and will be integrated into mathematical models fit for future management and conservation of salt marshes.

Firstly, we will determine the historical and current rates of accretion and erosion for predicting the past development and future survival of marshes. We aim to study the ecophysiological processes that lead to changes in competitive abilities of tidal vegetation promoting enhanced trapping of detrital and organic material on the vegetated surface so that long-term changes in the pattern of flooding are counteracted.

Besides these long-term effects of accelerated sea level rise there are also short-term effects on the diversity, biomass, distribution and functional behaviour of salt marsh flora and fauna. The actual zonation pattern of the vegetation in tidal marshes depends on soil characteristics, biotic processes that govern the succession processes in the vegetation, but especially on the flooding frequency of the specific marsh area. The vegetation pattern in estuarine marshes is generally similar regardless of climatic conditions, though the actual constituent species within various zones depend on the specific climatic circumstances. The lower marsh is usually dominated by a vegetation type of low diversity, (in North Western Europe dominated by *Spartina* species). In the higher parts of the marsh (relative to mean sea level) the vegetation is more diverse, with a more intricate relation between species, dependent on competition processes and differences in soil characteristics. In contrast to the vegetation pattern, the faunal diversity decreases with increasing intertidal height and in the uppermost dry zones the benthic fauna is composed of only one or two opportunistic species, but in very high abundance's. Mineral availability in combination with the aeration of the soil, the period during which plants are able to photosynthesise, and changes in the populations of soil dwelling macro- and micro-organisms (e.g. burrow dwelling invertebrates, nitrogen fixing bacteria, denitrification bacteria, mycorhizal fungi) may all result in differentiated changes in the growing potential and hence the competitive ability of the various plant species.

Accelerated sea level rise as a result of global warming is a relatively slow process and difficult to assess in a reasonable length of time. However, civil engineering activities in estuaries may influence the tidal pattern in estuaries and result in changing flooding frequency within a few years' time. In many estuaries in Europe dredging activities are regularly undertaken resulting in an increase in the tidal prism (intended to provide a better access to seaports). The increase in the tidal prism has a direct effect on the flooding frequency of the adjacent tidal marshes. For example the Westerschelde Estuary, the entrance to the Belgian port of Antwerp, will undergo a *unique* major dredging operation within the next few years, starting in 1997. Hydrologists have calculated a resulting increase in the mean level of the high tide in the estuary of 5 - 10 cm at the Dutch - Belgian border. Even in the mouth of the estuary the increase of the mean high tide level will still be 2 cm. As a result, the vegetation of the higher areas of the salt marshes in the area is expected to become less diverse and approach the community structure as currently found in the lower parts of the marsh. Such effect will be noticeable within a couple of years from the start of the dredging operation. Although interesting in itself, the way by which the vegetation changes may be similar to the response to the expected global accelerated sea level rise and therefore enables us with a *natural analogue* to model the changes caused by long-term global change processes

OBJECTIVES

The project Influence of Rising Sea Level on Ecosystem Dynamics of salt marshes (ISLED) has the following goals:

1. To determine the influence of increased flooding frequency on the ecophysiology of tidal marsh plant species and to examine its influence on the activity of sediment micro-organisms in view of the availability of nutrients for the plants.
2. To examine the influence of increased flooding frequency on the infaunal invertebrate community and on bioturbation effects.
3. To study important microbial trophic interactions, including carbon mineralization, related to differences in inundation frequencies.
4. To evaluate the influence of increased flooding frequency on the sedimentation patterns, i.e. its accretion rates and to reconstruct the history of anthropogenic inputs in the studied marshes.
5. To develop a management model to conserve marsh ecosystems as a typical unique landscape in view of accelerated sea level rise.

PROJECT METHODOLOGY

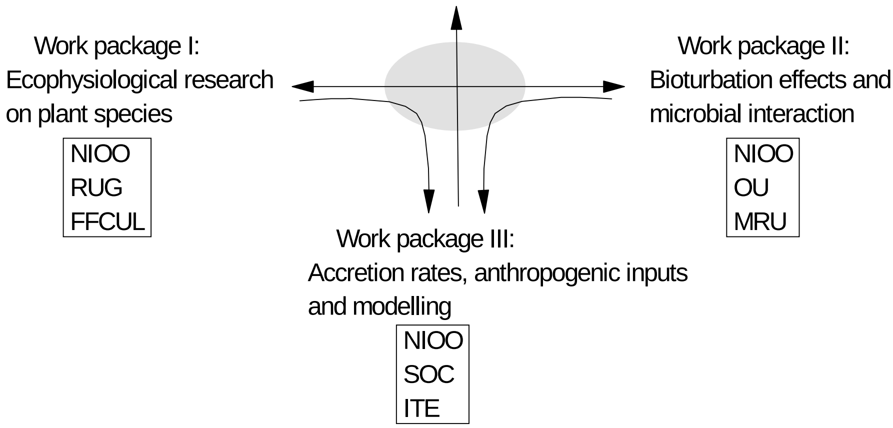
In this project *three different experimental levels* will be considered:

1. Field experiments. In joint field campaigns, twice a year, three marshes different in expected mean level of the high tide along a transect in the Westerschelde Estuary will be examined, and within one marsh a transect from the low- to high-intertidal zones will be sampled.
2. Large-scale mesocosms experiments, in which the expected inundation frequency can be manipulated to examine ecosystem processes under natural circumstances.
3. Pot- and sediment core experiments in the laboratory to examine individual selected processes.

The project will be organised around a number of joint intensive field measurement campaigns, tailored to the time and spatial scales central in the scientific topics to be studied. These campaigns will involve all partners and will be held in three marsh locations at the Westerschelde Estuary, different in effective impact of the intensive dredging operation in increase in flood level and expected inundation frequency. The campaigns will be organised when the growing season of the marsh vegetation will start (May/June) and when biomass is maximal (September/October) in each year of the project. Participation at these joint campaigns is mandatory for all teams; however, the actual duration of the visit will vary between 1 week and 1 month depending on the degree of participation and the type of fieldwork. They will be preceded by a period of method development and intercalibration. Besides these joint field champagnes detailed studies will be set up in order to achieve the tasks of the project. All data from the field sites and the experimental set-ups will be integrated and extrapolated by the exploitation of remote sensing (Compact Airborne Spectrographic Imager) and a Geographic Information System (GIS). It will allow the integration of processes over space and time, and extrapolation of results to cover the effects of sea level rise on the whole system.

The project is divided into three Work Packages, in which the different Tasks for the various partners are given. Data management will be executed by NIOO in close co-operation with all participants and in line with the ELOISE rules. The general flow of information within the project is schematised in which the DATA CENTRE occupies a central role in the flow of information:

Long-term information for management and extrapolation



RESULTS

The project Influence of rising Sea Level on Ecosystem Dynamics of salt marshes (ISLED), which focus on identifying, describing and defining the abiotic and biotic processes in salt marsh ecosystems buffering effects of increased inundation frequencies, made in his first year (1998) the following progress. As in this year no increase of inundation frequencies from the just started major dredging operation in the Westerschelde Estuary could be expected, the data obtained will give the situation in the studied marshes at zero-time.

In two joint field campaigns three marshes different in expected mean level of the high tide along transect in the Estuary were examined. Plant biomass of the three species under study (*Aster, Spartina,Triglochin*) was similar, this in contrast to the root material, being more in the Waarde marsh. Also the highest maximum photosynthesis rates were found in Waarde, where the difference between high and low tide is biggest. A comparison between the mycorrhizal infection of the plants in the Westerschelde and the Tagus estuary was not affected by flooding. Flooding seemed to influence especially biomass partitioning rather than physiological responses. In determining the impact of benthic macrofauna as well as roots on sediment chemistry and biogeochemistry, rates of oxygen uptake and sulphate reduction were considerably high within the marshes but decreased with tidal elevation. The first results on nitrogen cycling suggested that nitrification-denitrification was more important in plots without than with vegetation, due to the lack of N-uptake by the plants. So far there was no clear relationship found between plant, microalgae and bacterial activities, more data will be collected during the next field campaigns and mesocosms studies.

The successful acquisition of airborne remotely-sensed data for all three marshes, in the form of CASI data and colour aerial photographs, was an important aspect of the 1998 experimental programme. Sediment cores have been gathered along land-sea transects on all three marshes and analysis is continuing for both compositional and radiometric parameters. Anomalous levels of zirconium, chromium and titanium have been identified, particularly in the Waarde marsh. Small changes in the surface levels of the marshes are being followed, together with tidal inputs of sediment to each of the three marshes being determined by the use of sediment traps. In the first year no clear picture could be given already on these aspects on accretion rates.

Large-scale mesocosms experiments, in which the expected inundation frequency can be manipulated to examine ecosystem processes under natural circumstances, have been set up at the NIOO-CEMO in Yerseke and at the Laboratory of Biology in Odense. Cultures of the three plant species in monoculture and in mixtures received one inundation frequency as yet, and this frequency will be increased in the second year and again in the third year. For the moment no firm conclusions can be drawn from the first experiments, as most data have still to be analysed.

In joint field campaigns in the second year (1999) two marshes different in expected mean level of the high tide along transect in the Estuary (Ritthem and Waarde) were examined. The environmental differences between the two marshes and between the marsh zones result in differences in biomass of the vegetation, differences in primary production and to a certain extent differences in mycorrhizal infection. These differences may indicate that the inundation regime, but also the salinity may influence the processes in the plant community. In determining the impact of benthic macrofauna as well as roots on sediment chemistry and biogeochemistry, rates of oxygen uptake and sulfate reduction were considerably high within the marshes but decreased with tidal elevation. These measurements did not differ significantly from those obtained in 1998. In the studies on microbial activities and production in the top layer of the two marshes no firm conclusions can be reached on possible effects of sea level rise, but several parameters such as granulometry, organic matter content in sediment and light intensity have an influence on these activities.

In upwelling pot experiments for the measurement of coupled nitrification-denitrification influenced by plant species and different nitrogen (ammonium) loading, supplied in two different pumping rates (10 and 40 rpm), it appeared during flow-through that part of the ammonium was nitrified to nitrate. This process was most pronounced in the *Aster* pots and at higher loading rates. With respect to the mesocosm experiments, there is no apparent influence of changes in the inundation regime on the development of the three plant species (*Aster, Triglochin, and Spartina*). The greenhouse experiments show that plants growing under low light conditions (and flooding causes low light conditions) the plants are bigger, have a lower shoot and leaf production but higher chlorophyll contents. These results indicate that although the plants may be adapted to flooding, an increase in flooding regime may directly affect their primary production.

The compositional and radiometric data at all three marshes reveal changes in the nature of sediment accumulation at the tops of all cores studied. The observations are consistent with an increased sediment accumulation rate in the recent decades in all cores, but it is uncertain when this increase began, and whether it is synchronous at all marshes. Ongoing acquisition of accretion data at all three marshes now reveals that average accretion at Waarde exceeds that at Zuidgors, which exceeds that at Ritthem. This is the sequence expected from the marsh positions on the estuary. Aerial photographs of all three marshes taken between 1950 and 1998 have been processed and the resultant series of images reveal a dramatic retreat in seaward extent on Zuidgors and Waarde (but not Ritthem) marshes during the time series. The Compact Airborne Spectrographic Imager (CASI) data for 1993 and 1998 have been processes consistently and calibrated against plant species ground truth surveys. The comparison of the two images reveals a significant change in vegetation distribution over the Waarde marsh, with vegetation changing over large areas from middle marsh to high marsh type in this 5-year period.

CONCLUSION

To perform intensive fieldwork in three marshes has proven to be too heavy a workload for most of the partners. For 2000 one main field campaign will be scheduled, mainly located in the Ritthem-marsh, where no effect on sea level rise will be expected from the dredging operation, and located in the Waarde-marsh, where a maximal effect had to be expected. The third marsh (Zuidgors) will be only monitored to a minor extent, but with much attention on its accretion rates and its sedimentation patterns. By the reasons given above, a large amount of deep frozen soil cores and plant material is still awaiting sorting and analysis.

Although from the linear interpolation of median water level data in the Westerschelde Estuary an increase of about 20 cm was estimated with a main jump in 1998, so far no firm conclusions can be made on any effects in abiotic or biotic processes buffering an increase in inundation frequencies. It is anticipated that in 2000 effects can be allocated, but it could be that salt marsh ecosystems need a longer time period to react on these influences of rising sea level. In addition, it appears that the pattern of accretion of the studied marshes in space and time is more complex that earlier suggested and may also respond to previous shipping channel realignment and deepening in the mid-1970s.

TITLE: ATMOSPHERIC NITROGEN INPUTS INTO THE COASTAL ECOSYSTEM **: ANICE**

CONTRACT NO: **ENV4-CT97-0526**

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ATMOSPHERIC INPUTS OF NITROGEN COMPOUNDS INTO THE NORTH SEA: INITIAL RESULTS FROM THE ANICE PROJECT

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INTRODUCTION

The productivity of marine planktonic ecosystems is generally considered to be limited by the availability of nitrogen (Kronvang et al., 1993). Terrestrial inputs of nitrogen are often dominated by riverine transport, especially in the winter (Sanders et al., 1997). However, the atmospheric contribution to the total land based nitrogen input has been reported to be in the order of 30% for the total North Sea (North Sea Task Force, 1993). The atmospheric nitrogen load is directly available for algae growth, which makes this contribution more significant because a large fraction of the river run off contributes nitrogen fixed to biological material and hence is not directly available.

Increasing inputs of nutrients to the European coastal zones have resulted in a higher incidence of harmful algal blooms and other eutrophication phenomena and caused deleterious impacts on fisheries and tourism (Lancelot et al., 1989). To understand the impacts of increased nutrient additions to the coastal area, the full magnitude and seasonality of nutrient inputs must be described, including the effect of extreme events such as high atmospheric deposition episodes (Spokes et al., 1993; 2000) which, while small in overall annual budget terms, may be able to trigger algal blooms under nutrient depleted conditions in summer and early autumn. These blooms will be followed by oxygen depletion due to decay of the algae when the growth season is over.

Estimates of nitrogen inputs are often based on measurements over land and sometimes on measurements at open sea. Coastal effects on the total nitrogen depositions are included in a simplistic way, if at all. However, the largest changes in both the physical and chemical properties of an air mass advected from land over sea, and therefore also in the resulting processes, are expected in coastal regions. Since continental sources of atmospheric nitrogen species dominate, sharp gradients in concentrations and associated fluxes are expected across the coastal zone. Reduction of ammonia concentrations in the German Bight by a factor of 4 after 70 km transport out to the sea has been observed (Plate et al., 1995).

The sudden change in the surface properties at the coastline leads to a non-uniform situation. As a consequence, the horizontal homogeneity condition on which current descriptions of the boundary layer physics is based, does not strictly apply. Because the physical processes involved in gas exchange are related to the same physical processes governing momentum exchange, similarity in the range dependent patterns of momentum and gas fluxes can be anticipated. However, while momentum and heat fluxes can generally be regarded as "conservative quantities," gaseous nitrogen compounds are often so reactive that source and sink terms must be taken into account to evaluate their fluxes. Source and sink terms depend on governing chemical reactions, which in turn are dependent on temperature, humidity, and the time scales compared to the horizontal and vertical mixing rates.

The ANICE project

Atmospheric Nitrogen Inputs into the Coastal Ecosystem are addressed in the ANICE project (De Leeuw, 1998). ANICE focuses on quantifying the deposition of atmospheric inputs of nitrogen compounds (HNO_3, NO_3^-, NH_3 and NH_4^+) into the sea, both near the coast and in open water, and the governing processes. The Southern North Sea is studied as a prototype. Because the physical and chemical processes are described, as opposed to empirical relations, the results can also be applied to other regional seas like the Mediterranean, the North Atlantic continental shelf area and the Baltic.

The *aim* of the ANICE project is to improve transport-chemistry models that estimate nitrogen deposition to the sea. To achieve this, experimental and modelling work is being conducted which aims to improve understanding of the processes involved in the chemical transformation, transport and deposition of atmospheric nitrogen compounds, with emphasis on the influence of coastal zone processes. Most current models use grids that are too coarse to describe the governing processes with sufficient accuracy, particularly in coastal regions.

Experimental work within ANICE consists of a long-term observational program using scientific equipment mounted on commercial ferries, complemented by two intensive field experiments . The latter focus on process studies and provide information on the concentrations in air and water and their spatial and temporal variability. The field experiments were undertaken in June 1998 and in August 1999. The ferry measurements started in May 1998 and lasted about 1.5 years.

An interesting feature of the ANICE project is the use of two atmospheric chemistry transport models, ACDEP (Hertel et al., 1995) and METRAS (Schlünzen, 1990; Schlünzen et al., 1996). An aerosol module is developed for ACDEP, based on a 1-D model for the coastal zone (Vignati, 1999), to account for emission, diffusion and deposition, as well as heterogeneous processes. ACDEP is used to estimate atmospheric inputs of nitrogen to the whole North Sea, integrated over periods varying from 6 hours to a year. ACDEP also provides the ‘large’ scale lateral boundary conditions for the calculations in the coastal model domain using METRAS. METRAS in turn, will be used for studying scenarios for specific days, to calculate the atmospheric nitrogen input to coastal waters with a high resolution in space (some 100 meters) and time (minutes). METRAS is coupled with the Chemical Transport Model (MECTM) which includes the complex RACM gas phase chemistry (Stockwell et al., 1997) and the aerosol model SEMA (Von Salzen and Schlünzen, 1999a, 1999b, 2000).

The combined modelling effort is expected to lead to a major improvement in the estimate of atmospheric inputs into the North Sea, which can subsequently be used in effect studies. The models are complementary because of the different scales, the different mixing schemes and the different initialisations.

RESULTS AND DISCUSSION

The initial results of the ANICE project (De Leeuw et al., 2000) support the primary hypothesis on the importance of coastal effects on nitrogen inputs to the regional seas. Gaseous nitrogen compounds are primarily produced over land and very high concentrations were observed close to the coast in off-shore winds. The ANICE experimental results indicate that these concentrations decrease rapidly with increasing fetch resulting in a reduction to 'background' levels when the air mass is transported across the North Sea, over a distance of only about 200 km. The gases are highly soluble and are therefore either directly deposited to the surface or taken up by aerosols where they are accommodated through chemical reactions. The reactions with aerosols obviously affect the gaseous air-sea fluxes as evidenced from the shape of the gas concentration profiles (Geernaert et al., 1998). The aerosol dry deposition flux is in part due to different physical processes and depends on particle size, and thus the dry deposition velocities for gases and particulate nitrogen compounds are different. Moreover, the direct gas fluxes are determined by the partial pressure difference of the gaseous species in the water and in the air directly above the water, both of which have been observed during the ANICE experiments to vary strongly in both space and time. In model calculations such variations, especially those in the sea, are usually not taken into account. Examples presented in De Leeuw et al. (2000) show that neglecting spatial variations may lead to a significant overestimation of dry deposition of NH_3.

Both NH_3 and HNO_3 play a role in heterogeneous chemistry, as discussed in the introduction. In particular, HNO_3 reacts directly with sea spray aerosol. Current atmospheric chemistry transport models do not include such reactions. Work is underway in several groups to implement heterogeneous chemistry involving HNO_3. In ANICE this occurs through the implementation of heterogeneous reaction schemes in both METRAS, using the SEMA model, and in ACDEP using a 1-D model. The latter is in part based on the model of Vignati (1999), which was applied to show the large influence of the reaction between nitric acid and sea spray on both the concentrations and the gradients of HNO_3, over a fetch of only 25 km. The strong variations imply that a coarse grid model will not be able to correctly predict the effect of coastal processes at very short fetches on the nitrogen input, unless these processes are included through a coastal sub-grid or a proper parameterisation.

The experimental efforts included simultaneous measurements of bubbles generated in the water by breaking waves and aerosols just above the water surface. The comparison of the respective size distributions leads to conclusions on the bubble-mediated aerosol source function and the amount of fresh sea spray available for heterogeneous reactions. These small scale measurements are supported by lidar measurements on atmospheric boundary layer properties that influence the atmospheric mixing of both gases and aerosols. The lidar measurements also reveal aerosol plumes generated by waves breaking at the sea surface or in the surf zone. In off-shore winds, plumes of surf generated sea spray aerosol were observed to be transported out over the sea and were visible over distances of at least 5 km, in good agreement with model calculations using CAT (Vignati et al., 2000). Hence, also in off-shore wind large amounts of sea spray (sea spray aerosol concentrations may be enhanced by 1-2 orders of magnitude; De Leeuw et al, 2000) are available for heterogeneous reactions, at least in the study area along the Dutch coast. Other experimental efforts included micro-meteorological studies on fluxes of NH_3, HNO_3, CO_2, as well as momentum, heat and water vapour, which are used in studies on air-sea exchange processes. The modelling effort includes the construction of a model that accounts for effects of non-homogeneity across the coast line (Geernaert and Astrup, 1999).

Effects on regional scales were addressed through co-ordinated measurements at the WAO site on the UK coast and at MPN near the Dutch coast, as well as through ferry measurements. The large concentration differences measured on the ferry during different periods shows the necessity of long term measurements to establish a representative data set on nitrogen inputs into the southern North Sea. The analysis of cases with connecting air flow between the WAO site and MPN shows the change in aerosol particle size distributions and chemical properties, see Figure 1. The 16 June case was modelled using the SEMA box model in a Lagrangian sense. The encouraging results give confidence on the use of SEMA in METRAS for fine gritted estimates of the nitrogen inputs into the southern part of the North Sea. METRAS prognostically calculates the three-dimensional fields of wind, temperature, humidity, cloud and rain water content and derives exchange coefficients from first order closure theory. This scheme has been thoroughly tested with favourable results. Finally, the ACDEP model extended with a detailed aerosol module will be used to calculate the nitrogen inputs for the whole North Sea. Computed annual mean ammonia concentrations, reflecting mainly the emission areas over the Netherlands and Great Britain, are qualitatively in reasonable agreement with the 1999 ferry measurements. Generally the concentrations range between 0.1 and 1 $\mu g\ m^{-3}$, with highest concentrations in the coastal regions of England and the Netherlands/ Germany. The annual nitrogen deposition calculated from the loads ranges between 0.3 and 1.3 tonnes N km^{-2}.

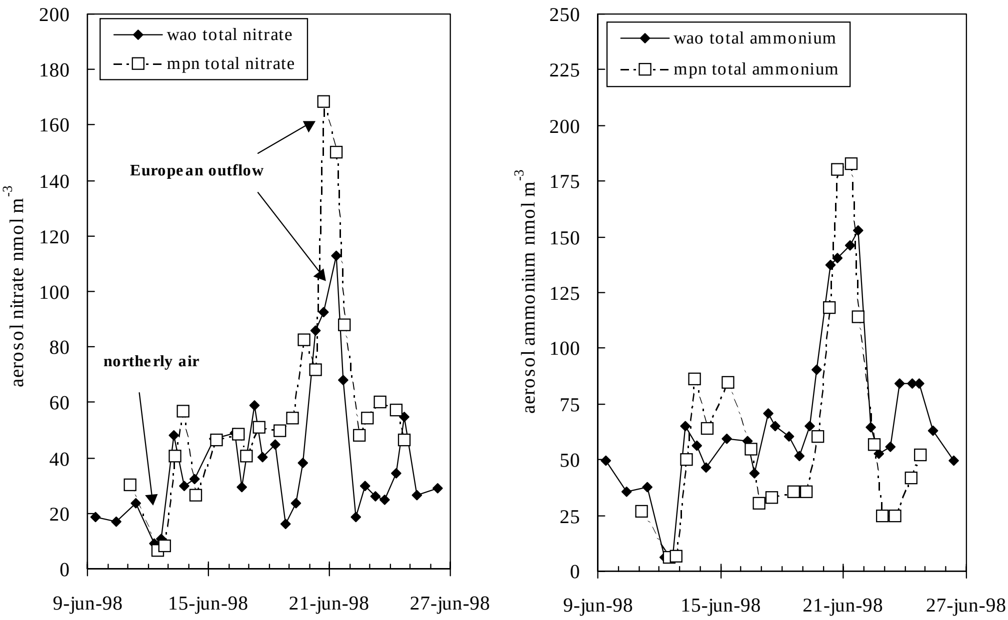

Figure 1. Comparison of aerosol nitrate and ammonium concentrations at WAO and MPN during the 1998 ANICE field campaign. Connecting air flow was observed on 16 June (WAO to MPN) and on 20-21 June (MPN to WAO). Effects on aerosol composition are evident, e.g. from the significant reduction of both nitrate and ammonium between MPN and WAO.

The updated atmospheric chemistry transport models will be used for the assessment of the impact of atmospheric nitrogen on coastal ecosystems. Both episodic and chronic nitrogen inputs will be considered and the assessment will be based on comparisons of phytoplankton nitrogen requirements, other external nitrogen inputs to the area of interest to ANICE and the direct nitrogen fluxes provided by ANICE.

In the study presented above, only inorganic nitrogen has been considered. A limited effort is made in the ANICE project to characterise DON in rain and in aerosols. Dissolved organic nitrogen species are ubiquitous in rain and aerosols and comprise between ~20 and 80% of total dissolvable nitrogen. Further analysis is currently underway to quantify these species. This data will both increase our knowledge of the size distribution of this, largely chemically uncharacterised, material and improve our estimates of the total flux of combined nitrogen species to the surface waters of the North Sea.

ACKNOWLEDGEMENTS

ANICE is part of ELOISE and is supported by EC DG XII, contract ENV4-CT97-0526, as well as by internal funding of participating institutes. Meetpost Noordwijk is owned and maintained by the Dutch Ministry of Public Works. The Weybourne Atmospheric Observatory is run by the University of East Anglia. Scandinavian Seaways kindly gave the permission to mount scientific equipment on board the ferry "*Prince of Scandinavia*" for the long term measurements. The Noordwijkse Reddingsbrigade provided their life boat as well as able seamen for water sampling in the area between the Dutch coast and MPN. Dr. Uwe Schwarz, Stefan Reis and Dr. Burkhard Wickert at Stuttgart University provided the high resolution emission data that are used in the ACDEP and the METRAS models. The meteorological data used in ACDEP were obtained from Egil Støren from the EMEP MSC-W at the Norwegian Meteorological Institute and the data for METRAS from the German Weather Service, Offenbach.

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TITLE : EFFECTS OF CLIMATE INDUCED TEMPERATURE CHANGE ON MARINE COASTAL FISHES: CLICOFI

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GROWTH PERFORMANCE, FECUNDITY AND RECRUITMENT IN MARINE FISH: UNDERSTANDING CLIMATE EFFECTS BASED ON PHYSIOLOGICAL AND ECOLOGICAL STUDIES IN ATLANTIC COD (*GADUS MORHUA*) AND COMMON EELPOUT (*ZOARCES VIVIPARUS*)

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INTRODUCTION

The impact of decadal-scale climate variations on marine communities and populations has been well documented (Cushing, 1982; Beamish, 1995; Bakun, 1996). Therefore, warming of the atmosphere as currently reported is likely to affect, directly or indirectly, marine populations during all life stages. However, a mechanistic cause and effect understanding has not yet been established. The present project reports on the development of a concept that may support such an understanding. This concept is based on data collected on ecological and physiological processes in a latitudinal gradient and their respective modelling. Within the context of a wider comparison between and within species the study focusses on two model species characterized by northern and southern distribution limits in the North Atlantic: eelpout (*Zoarces viviparus*), as a typical non-migrating inhabitant of the coastal zone, with a k-selektive reproduction strategy (viviparous) and cod (*Gadus morhua*), as a typical inhabitant of continental shelf with a high importance for fisheries. Both species have a boreal distribution, the southernmost distribution limits are in the Wadden Sea of the southern North Sea (eelpout) and in the northern part of the Biscay (cod).

A comparison between summer water temperature and abundance of *Zoarces viviparus* in the German Wadden Sea over the last 40 years, shows a distinct relationship between hot summer events and a high mortality (re-calculated from data of J. Ulleweit and R. Knust, unpubl.) and indicates the sensitivity of this species to temperature. For cod, the effect of temperature changes is not necessarily the same in all areas of distribution (Daan, 1994). Those dwelling in colder waters (Labrador, Greenland, Iceland and Barents Sea) seem to augment stock size, recruitment and growth rates when temperature is increasing. Those dwelling in warmer regions (North Sea, Georges Bank, Newfoundland) do better at cooler periods. Temperature is held responsible for most of the observed differences in growth (Brander, 1994). In the Arcto-Norwegian cod stock off N. Norway and in the Barents Sea, year classes of higher abundance were associated with high temperatures and a 20-fold change of year-class abundance at age 3 was recorded (Nakken, 1994). According to temperature extremes it seems that cod reaches a temperature dependent limit in the Southern North Sea during summer and that cold temperatures in the Barents Sea also limit performance and recruitment of this species. In consequence, global warming might have opposite effects on fish populations on the northern and southern edges of their distribution. The effect is large enough to be relevant for short-term catch forecasts.

For a mechanistic understanding of cause and effect relationships the EU project CLICOFI brings together current efforts to improve our understanding of the past and present impact of climate induced temperature changes on fish populations through the combination of retrospective studies, field data analysis, ecological and physiological experiments, genetical investigations and modelling. As far as modelling is concerned the action exerted by climate variability on fish populations, due to its intrinsic complexity, seems to baffle any effort aiming to be general and handy at the same time (Claireaux et al., 1995; Brander, 1996; Lindahl et al., 1998). As a consequence, the most practical solution to any specific problem still remains a semi-empirical and essentially data-driven approach. Ecological and physiological studies are put into the perspective of such a modelling approach. They focus on the analysis of growth and fecundity of cod (*Gadus morhua*) and eelpout (*Zoarces viviparus*) from different climatic regions in field campaigns and laboratory experiments.

PROJECT METHODOLOGY :

Modelling of historical data

The pattern of temporal correlations between cod recruitment and sea surface temperatures, in conjunction with the atmospheric pressure variability provided by the North Atlantic Oscillation (NAO) index, was investigated by means of a combined use of PCA (Principal Component Analysis) (Ghil and Vautard, 1991) and CCA (Canonical Correlation Analysis) (Rencher, 1992), using temperature time series collected in the area surrounding the Kola peninsula (Barents sea), in the North Sea and in the Baltic Sea. For each of the three areas the time series of sea surface temperatures (Figure 1, left panel), cod recruitment (Figure 1, right panel) and NAO were submitted to PCA and checked for the inclusion in the first three principal components of the largest fraction of the total information. The correlation between pairs of single components from the time series were computed within each area, to trace the presence of hidden correlations between the dissected, independent features of the series. Eventually, in order to obtain some synthetic (and comparable) descriptors of the correlation pattern within each area, a canonical correlation study was carried out over the principal components' triplets. This allowed a straightforward comparison of global correlation patterns between temperature and cod recruitment in the different areas.

Ecological studies

Growth performance and fecundity of cod and eelpout were calculated from literature data and from field data collected during expeditions in 1998 and 1999 in various regions (Norwegian coast, Arctic, White Sea and Southern North Sea). Growth rates were evaluated from reading growth rings on otoliths. The number of eggs in the gonads of cod (Bleil and Oeberst,1993) and the number of larvae of eelpout were estimated as fecundity parameters. Growth experiments at different temperatures were carried out with Norwegian coastal cod (CC), Arctic cod (NEAC) and with cod from the Southern German Bight. Offspring of broodstocks from CC and NEAC (spring 1997) as well as wild caught cod from the German bight were raised under laboratory conditions. The fish were individually tagged and kept at different water temperatures and fed with commercial dry food (Nor/Aqua) in surplus. Fish were measured once every 3 to 6 weeks.

Physiological studies
HSP70 were measured in the livers of cod (from the Arctic, Norwegian coast or German Bight) grown at various temperatures. HSP70 levels in the liver were measured with an indirect non-competitive ELISA. Upper lethal temperatures were determined in Baltic and North Sea eelpout as the temperature values at which loss of equilibrium or the onset of spasms were observed during an acute temperature increase. For an analysis of the temperature dependence of pO_2 in body fluids and the establishment of adequate methodology pO_2 was monitored online in gill blood of cod using optodes (PreSens, Neuburg/Donau, Germany). Oxygen consumption in Antarctic eelpout (*Pachycara brachycephalum*) and North Sea common eelpout (*Zoarces viviparus*) were measured by intermittent flow or flow through respirometry. Critical temperature thresholds were determined by succinate analyses in liver tissue of fish exposed to various temperatures. Cod used for haemoglobin studies were collected in the Southern North Sea (February 1999), and in the Baltic Sea (Kiel bight, Bornholm, and South of Gotland and North of Gdansk. Blood was sampled from anaesthetised fish.

RESULTS

Temperature effects on the ecology of cod and eelpout: mathematical modelling of historical data, field and laboratory studies

Highly significant correlations between cod recruitment and temperature fluctuations over time emerge for all three regions. In the Kola region this correlation takes place along two distinctly independent correlation channels which correspond to the two significant canonical correlations between cod recruitment and temperature. In North Sea and Baltic regions the data highlight the presence of only one statistically significant mechanism of correlation between cod recruitment and temperature. The NAO and temperature spaces are fully interconnected (canonical correlations near unity between NAO and temperature spaces) in all three areas, thus confirming, from an empirical data-driven point of view, the importance of this global-scale climate mode for local temperature dynamics. Despite the strong link existing between NAO and temperature spaces, cod recruitment and NAO spaces did not show any significant canonical correlation. This result shows that temperature is the climate “variable” most directly acting on cod populations.

Fecundity and growth performance in both eelpout and cod depend on the regional climate and decrease with increasing latitude. Eelpout collected in the White Sea had a significantly lower fecundity than those of the same age from the German Wadden Sea. Cod populations living in colder waters displayed a significantly lower growth performance than cod from the southern North Sea. The growth experiments confirmed this climate dependent growth performance in cod for Norwegian coastal cod, Arctic cod and cod from the German Bight. In all populations highest growth rates were found between 10 ˚C and 11 ˚C. The growth curves estimated in laboratory studies for German Bight and Norwegian coastal cod fit those calculated from field data. Such an analysis proved impossible for Arctic cod, due to the incomplete data basis on mean water temperatures for that area.

Thermal tolerance and energy budgets of cod and eelpout in a latitudinal cline

A recent hypothesis links the phenomenon of cold induced mitochondrial proliferation in marine ectotherms with the level of thermal tolerance. Once temperature drops below a low or exceeds a high critical threshold (Tc) aerobic energy production becomes limiting for tissue function (e.g. ventilation or circulation) indicated by decreased oxygen levels in the body fluids and the transition to an anaerobic mode of mitochondrial metabolism as well as progressively insufficient cellular energy levels (see Pörtner et al., 1998, 2000 for review). This Tc was found at 22 ˚C in North Sea eelpout and at about 9 ˚C in Antarctic eelpout (van Dijk et al., 1999). Oxygen consumption analyses in North Sea and Antarctic eelpout revealed an exponential rise with increasing temperature. When North sea eelpout were acclimated to cold temperatures their temperature specific rate of oxygen consumption rose to similar levels as in Antarctic eelpout. Nonetheless, owing to lower levels of Arrhenius activation energies in the metabolic rate of the North Sea species a drastic factorial rise in metabolic rate only occurred beyond 22 ˚C whereas the Antarctic eelpout displayed such a drastic increase already when temperature approached 9 ˚C (van Dijk et al., 1999). Obviously, this metabolic increment is correlated with critical temperatures. In contrast, thermal sensitivity during short term heat exposure was not defined by changes in liver HSP70 in cod. However, 10 months of acclimation to 8, 12 and 15 ˚C showed that sex and temperature both had a significant influence on HSP70 level. Females had higher levels than males and animals grown at 12°C had 25 % higher levels than those at 8 or 15 ˚C. A significant correlation was found between HSP70 levels and hepato-somatic Index. HSP70 levels were similar in all cod populations (Arctic, Norwegian coast or German bight).

The HbI(1) allele frequency distribution showed a marked gradient from west to east from 66% at the White Bank to 1% at Gotland and Gdansk and confirmed previous gene frequency analysis in that it shows an increased fraction of HbI_{22} in the Baltic Sea from east to west with falling temperature. Animals were significantly larger in the North Sea than in the Baltic Sea. Furthermore, heterozygotic were larger than the homozygotic animals, and HbI_{22} animals had the lowest weights and smallest sizes. In the Baltic Sea, however, HbI_{22} fish were larger and heavier than HbI_{11} fish, and there was an increase in weight and length from west to east in the Baltic Sea. Oxygen affinities between the hemoglobin genotypes revealed no differences according to temperature or habitat.

CONCLUSIONS

The following tradeoffs appear to be involved in the adjustment of thermal tolerance limits to the ambient temperature regime (Pörtner et al., 2000). Cold acclimation and adaptation on the population level leads to higher mitochondrial densities and thereby, elevated mitochondrial maintenance metabolism at constant level of eurythermy. Even within a species these differences may be genetically fixed between populations. They may explain a species specific or even population specific sensitivity to heat. They may also explain the reduction in growth performance and fecundity observed. The agreement between field and experimental studies suggests that the differences in growth between populations are likely not related to food limitations in the field. Some of these features still need to be confirmed for Atlantic cod and common eelpout

As a preliminary picture for Atlantic cod and common eelpout, warming will lead to a northward shift of populations. These species will experience increased growth performance and fecundity at more northern latitudes as water temperatures rise. Owing to a change in energy budgets productivity of cod may increase to a larger degree than expected from a typical Q_{10} effect alone. If polar water temperatures remain the same as of today the limitation of growth performance and fecundity by cold temperatures in the North will lead to a more restricted range of geographical distribution and reduced abundance. Further research will need to follow this line of thought. The specific tradeoffs involved in cod and eelpout performance in a latitudinal cline require further study at the mitochondrial and whole animal level. Changes in the energy budget and the mechanisms behind need to be quantified for a more complete understanding of the tradeoffs involved in temperature adaptation and, finally, the limits of adaptational processes involved.

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TITLE : SUBMARINE GROUNDWATER-FLUXES AND TRANSPORT-PROCESSES FROM METHANE RICH COASTAL SEDIMENTARY ENVIRONMENTS: **SUB-GATE**

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SUBMARINE GROUNDWATER-FLUXES AND TRANSPORT-PROCESSES FROM METHANE RICH COASTAL SEDIMENTARY ENVIRONMENTS: SUB-GATE

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INTRODUCTION

Although known for more than several decades, the major importance of submarine groundwater discharge (SGD) for hydrological budgets and the release of nutrients, natural radio nuclides, anthropogenic constituents, and methane to the coastal zone was revealed just only within the last few years. Especially in regions of low surface run-off, submarine groundwater discharge account for a significant fraction of the fresh water inflow (e.g., Valiela and D'Elia, 1990; Buddemeier, 1996; Moore, 1996). In contrast to several descriptive reports about the magnitude of submarine groundwater discharge still very little is known about discharge rates, the spatial distribution of SGD and the chemical composition of the effluents.
Submarine groundwater discharge is a phenomenon occurring in many European coastal environments (Buddemeier, 1996; Burnett, 1998). Nevertheless, it is only investigated in a few areas of the European coastal waters. Major target areas of the Sub-GATE project are the western Baltic Sea (Eckernförde Bay, DE and off Als, DK), and the western Irish Sea. For the muddy sediments in these areas SGD has not only an implication for the water budget but also for the release of methane and nutrients from the sediment to the water column.
The input of methane, nutrients and pollutants in coastal environments has both ecological and economical implications for the respect regions. Enhanced growth of macroalgae due to inflow of high loads of nutrients via SGD causes increasing water turbidity (Laroche et al., 1997). A decrease in water quality tremendously affects the touristic value of a coastal region and it has negative implications on fish stock habitats.

PROJECT METHODOLOGY : LANDSIDE AND MARINE FIELD STUDIES AND NUMERICAL MODELLING

Submarine Groundwater Discharge is caused by a complex pattern of land-ocean interactions. In the project methodology this is reflected by field investigations of the landside and the marine environment. Besides the combination of these data sets, numerical modelling is an essential tool for an integrative investigation of SGD and its environmental impact.
Landside investigations include geological and hydrogeological studies of the catchment area and the aquifer system. For 54 wells reports about the sedimentology and hydrogeology of groundwater wells were considered and chemical composition of groundwaters were analysed. The spatial distribution of the landside aquifer system was mapped. Based on the field work a hydrological model was established to calculate groundwater renewal rates and estimates of submarine groundwater discharge rates.
Studies of the marine environment are focussed on geological characterisations of the subsurface by shallow seismic, sediment acoustic, and sediment coring, investigations of natural radio nuclides as Radon and Radium in the water column and the sediment, direct flow

measurements by in situ systems (Landers), and geochemical analysis of sediment and pore water composition at groundwater discharge locations (so called pockmarks) and sites unaffected by SGD. To address biological implications of SGD the benthic biota is investigated at these sites. A submarine well system was applied for sampling submarine freshwater.

During several cruises with RV “Alexander von Humboldt” (IOW, Warnemünde, DE), RV Littorina and RV Alkor (IfM, Kiel, DE), and RV “Prince Madog” (Univ. Wales, UK) a large data set was established. For example, for the major target area of Eckernförde Bay more than 250 sediment sites were sampled and an extensive grid of seismic lines was established.

In addition to hydrological modelling by MODFLOW, numerical multi-phase modelling of fluids and gas (dissolved and free methane) by MUFTE-UG was applied to characterise the transport regime at submarine groundwater discharge sites and the release of methane from the sediment by fluid advection.

RESULTS

For Eckernförde Bay, the main target area of the project, some results of landside investigations, marine research and modelling efforts are briefly reported. For the marine environment data acquisition by shallow seismic and sediment sampling by vibro-coring allowed the development of a geological model which describes the post glacial development of the bay with specific emphasis to the distribution of late holocene sands, the potential aquifer for submarine groundwater discharge, and clay layers indicating the transgression sequence. Based on this results a sediment-physical description of known and potential discharge sites was derived.

For quantification of freshwater discharge rates the geochemical composition of porewaters was analysed. Steep gradients in the Chloride concentrations were observed which allowed to compute freshwater advection rates. By this mean discharge rates of a few hundred litre per square meter and day were deduced. In situ measurements of outflow at the seafloor of discharge sites support these advection rates.

Geochemical analysis of methane, sulphate and other major and minor constituents of the pore water composition support the importance of the freshwater efflux for the methane cycle in surface sediments. In the bottom water of pockmark sites enhanced concentrations of methane were observed, suggesting the flushing of methane by freshwater advection and discharge. The distribution of methane, dissolved in the pore water and as free gas within the sediment was computed by numerical multiphase transport modelling. This allowed to deduce the complex pattern of sediment flushing by freshwater and the production and redistribution of methane within the sediment

As an additional tracer for groundwater discharge the Radium and Radon distribution in the water column was derived. In contrast to other study areas (e.g., the East Atlantic Bight off the US), this revealed that Radium is not a suitable tracer for groundwater discharge from muddy sediments. In contrast the Radon signal traces discharge sites and enhanced Rn inventories in the bottom water were observed. On the scale of the entire area of the bay this suggests that the overall efflux of freshwater in the by can be estimated by the Radon inventory.

The spatial distribution of discharge sites within Eckernförde Bay was deduced by investigations of more than 250 sites within the bay. In addition to the formerly known few discharge sites this revealed the widespread occurrence of SGD within this coastal environment.

The combination of methane measurements and different techniques like hydrological budgets of the landside, direct in situ measurements, pore water modelling, and Radon inventories of the lower water column the amount of freshwater discharge in the bay and its effect on the methane and nutrient cycle will be estimated.

CONCLUSION

For the Eckernförde Bay the multidisciplinary efforts of the Sub-GATE project showed that sites affected by submarine groundwater discharge are much more widespread that previously expected. High discharge rates are suggested by combination of geochemical and hydrological techniques and numerical modelling. Associated to the efflux of water are high concentrations of methane. Due to the combination of landside and marine research a conceptual model for the characterisation of SGD in coastal zones was developed. The applicability of this model is currently tested in other target of the Sub-GATE project.

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TITLE : INTEGRATED NITROGEN MODEL FOR EUROPEAN CATCHMENTS : **INCA**.

CONTRACT N° : **EVK1-CT-1999-00011**

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INTEGRATED NITROGEN MODEL FOR EUROPEAN CATCHMENTS (INCA): ASSESSMENT AND MODELLING OF NITROGEN LOADS IN KEY EUROPEAN ECOSYSTEMS.

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OVERVIEW

The INCA project is based on the INCA (*I*ntegrated *N*itrogen in *Ca*tchments) model, a process based representation of plant/soil system and instream nitrogen dynamics. The INCA project aims to use the model to assess the nitrogen dynamics in key European ecosystems. The work will involve the investigation of the likely impacts of environmental change on streamwater N concentrations and loads, both in the individual ecosystems and at the pan European scale. Furthermore, the results from the modelling work will be linked to economic models to assess the monetary costs of environmental degradation caused by N in rivers and the feasibility of harmonising N regulation policies across Europe. The project, which began in April 2000, will last for 3 years and involves 8 partners from the UK, Finland, Norway, Denmark, Germany, The Netherlands, France and Spain.

PROBLEMS TO BE SOLVED

Across the European Union there are concerns about nitrogen (N) in lowland and upland fresh water systems, estuaries and marine areas (Johnes, 1999). In such systems, additional N inputs can cause europhication, leading to rapid aquatic plant growth. Such increases in growth are often viewed as a nuisance as certain plant species may grow at the expense of others and, within freshwaters, the microbial breakdown of the dead plant matter can lower oxygen levels which is detrimental to invertebrate and fish populations. The problems of freshwater eutrophication are usually associated with lowland, intensively farmed areas where fertilisers provide a significant source of N and/or urban areas where domestic and industrial effluent is discharged to the receiving watercourse and groundwaters. However increasing N deposition from the atmosphere has lead to increased problems in upland regions. For example, it is known that increased atmospheric N deposition leads to soil and streamwater acidification (Skeffington and Wilson, 1988). In addition, there is evidence that many of Europe’s forests are N saturated, leaching N in excess of plant and microbial demand (Emmett et al., 1995). Whilst management strategies have been implemented to control N in river systems, these have tended to address single issues: either diffuse or point sources, or upland or lowland areas. However, the N concentrations and loads in rivers reflect the integration of the catchment N sources: fertiliser inputs, atmospheric deposition and sewage discharges. Superimposed on these anthropogenic inputs are contributions from the vegetation and mineralisation (and subsequent nitrification) of organic N in soils. Furthermore, the combination of the multiple catchment N sources has a downstream effect, influencing the options for further water utilization and impacting the water quality of estuarine and marine areas. Thus, given the holistic nature of the N problem, an integrated management approach is required. To support such an approach, modelling tools are needed (Neal et al 1997, 1998). Models are useful as they formalize ideas regarding the factors and processes controlling water chemistry. Moreover, they provide initial estimates of the likely impacts of environmental change on water chemistry.

SCIENTIFIC OBJECTIVES AND APPROACH

Based on mass balance and reaction kinetics, the INCA model accounts for the multiple sources of N and simulates the principle N mechanisms operating, including mineralisation, immobilisation, nitrification and denitrification (Whitehead et al., 1998a, b). The model is dynamic and N concentrations and fluxes are produced as a daily time series. Also, the model is semi-distributed. As such, it does not model the catchment land surface in a detailed manner; rather, different land use classes within sub-catchments are modelled simultaneously and the information fed sequentially into a multi-reach river model.

Replication of a model application across key ecosystem types throughout Europe will provide (i) an assessment of the river catchment N sources and sinks and (ii) estimates of the likely impacts of N deposition, land use and climate change scenarios on N fluxes, both in the plant/soil system and instream. The results will be achieved by the creation of extensive databases describing the hydrology and N dynamics of the study areas and the creation of a generic version of INCA. Application of INCA to different ecosystems will provide some internal testing of the model. Furthermore, the model will be used to evaluate scale-up and connectivity using Monte Carlo analysis and measured N flux estimates at the plot and catchment scales. The model will also be used in conjunction with simple economic models to assess the costs and benefits of N controls in Europe.

EXPECTED IMPACTS

The project will directly contribute to EC policies including the Nitrate Directive (91/676/EEC) aimed at controlling diffuse nitrate pollution of public water supplies throughout Europe; the EC Habitats Directive (92/43/EEC) which aims to provide for the preservation of rare and valuable remnants of natural habitat in member states, including both terrestrial and aquatic habitat types; the Integrated Pollution Prevention and Control Directive (96/61/EEC) and the proposed Water Framework Directive (COM (97) 49 Final). The model will also provide some estimate of the N loads entering estuarine and marine areas from some of the study areas.

The INCA project addresses the problem of providing high quality water in Europe through an improved understanding of river system functioning and the development of a pragmatic scientific and management tool. Thus the INCA model will assist end users such as the European Environment Agency and its national counterparts in the improved management of N in river catchments, which may lead to reduced eutrophication problems in streams, rivers, lakes and marine areas. The results of the project will also provide information for policy makers in the continued development of EC policy regarding the environment. As such the project will contribute to an improvement in water supplies for industrial use, public consumption and benefit environmental conservation thereby enhancing the quality of life for Europeans.

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PROJECT TITLE: BRIDGING EFFECT ASSESSMENT OF MIXTURES TO ECOSYSTEM SITUATIONS AND REGULATION **: BEAM**

CONTRACT N^{O}: **EVK1-CT1999-00012**

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RISK ASSESSMENT OF CHEMICAL MIXTURES IN THE AQUATIC ENVIRONMENT: AN OUTLINE OF THE *BEAM* PROJECT

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INTRODUCTION

Contamination of fresh and marine waters by anthropogenic chemicals is rarely limited to only one single toxicant but typically involves a multitude of pollutants. Decades of scientific research have revealed that combination effects may occur as a consequence of multiple exposure. Toxic effects of mixtures usually exceed those of the most active component alone. Moreover, even in situations where all the components of a multiple mixture are present in concentrations below individual NOEC-values (No Observed Effect Concentrations), a severe overall effect of the mixture may result [1, 2]. These findings demonstrate the need to consider joint effects of toxicants in risk assessment procedures and regulatory measures for the protection of aquatic life.

For the predictive assessment of the toxicity of mixtures from existing knowledge about the toxicity of single compounds two different concepts are available: *Concentration Addition* and *Independent Action*. In laboratory tests with single species both have been proven to provide reliable tools for prediction: *Concentration Addition* for mixtures of substances with an identical mechanism of action, and *Independent Action* in the opposite case of mixture components with strictly dissimilar specific mechanisms of action [2-5]. Experiments with heterogeneous mixtures of aquatic priority pollutants indicate that the application of both concepts may at least allow to define a reliable "prediction window" [6]. Additionally, multi-species tests with algal communities have revealed that the applicability of these concepts is not generally restricted to single species tests [2].

Despite extensive research into the field of mixture toxicology, combined effects of pollutants are still a highly controversial issue, and current procedures for the prospective or retrospective assessment of chemical risks still focus on single pure toxicants. The incorporation of existing scientific evidences on the predictability of combination effects into regulatory strategies is hampered by two crucial gaps:

- There is too little knowledge available at the stage of risk assessment on how to use existing toxicity information for single substances in order to account for expectable combination effects.
- There is lack of environmental realism in the existing scientific approaches to the assessment of mixture toxicity regarding both the types of mixtures actually occurring and the suitability of methods for the purpose of a routine assessment.

To bridge both these gaps is the intention of the European research project BEAM *(Bridging Effect Assessment of Mixtures to Ecosystem Situations and Regulation*) which started in spring 2000 and is scheduled for a three years period.

RESEARCH OBJECTIVES

The specific objectives of BEAM are:

- To achieve more environmental realism in the scientific hazard assessment of complex exposure situations by addressing the following questions:
  (*i*) what are realistic exposure scenarios with respect to mixtures, (*ii*) how relevant are combination effects due to mixtures that are typically found in the environment, and (*iii*) may the toxicity of such mixtures be predictable from single substances toxicity data?
- To provide three new tools for the assessment of mixture toxicity:
  (*i*) a swift biotest suitable for analysing effects of mixtures of pollutants on natural biological communities at ecological time scales, (*ii*) a chemometric/QSAR methodology for grouping toxicants with unknown modes of action according to the predictive concepts of *Concentration Addition* and *Independent Action*, and (*iii*) a framework of biometrical demands for the design of mixture toxicity experiments and the predictive assessment of mixture toxicity in terms of data quality and quantity.
- To explore the options for implementation of predictive mixture toxicity assessment into regulation by:
  (*i*) compiling and reviewing existing approaches, (*ii*) developing decision rules for the applicability of the concepts of *Concentration Addition* and *Independent Action,* (*iii*) defining a framework for the risk assessment of mixtures of aquatic pollutants, (*iv*) developing protocols for the classification of ecological water quality in relation to mixture fate and effects.

PROJECT METHODOLOGY AND WORKPLAN

The BEAM consortium is performing joint research on the problem of mixture toxicity since 1997. In an interdisciplinary effort BEAM uses a combination of experimental and theoretical approaches, drawing expertise and methods from biometry, chemometrics and QSAR, analytical chemistry, experimental ecotoxicology, mixture pharmacology and regulatory toxicology.

The BEAM workplan (Fig. 1) is structured into ten workpackages (WP). Two of them will work out exposure scenarios for pollutant mixtures by using two different approaches. From an experimental TIE-analysis (*Toxicity Identification and Evaluation*) of sediments (WP1), site-specific contamination patterns will be determined at selected locations in both freshwaters (river Elbe, Germany) and marine waters (Swedish west coast). More general exposure patterns for European surface waters will be gained from a theoretical approach by compiling monitoring data and applying fate and distribution models to selected lists of aquatic priority pollutants (WP2). Biological effects of selected pollutant mixtures as well as the~~re~~ir individual

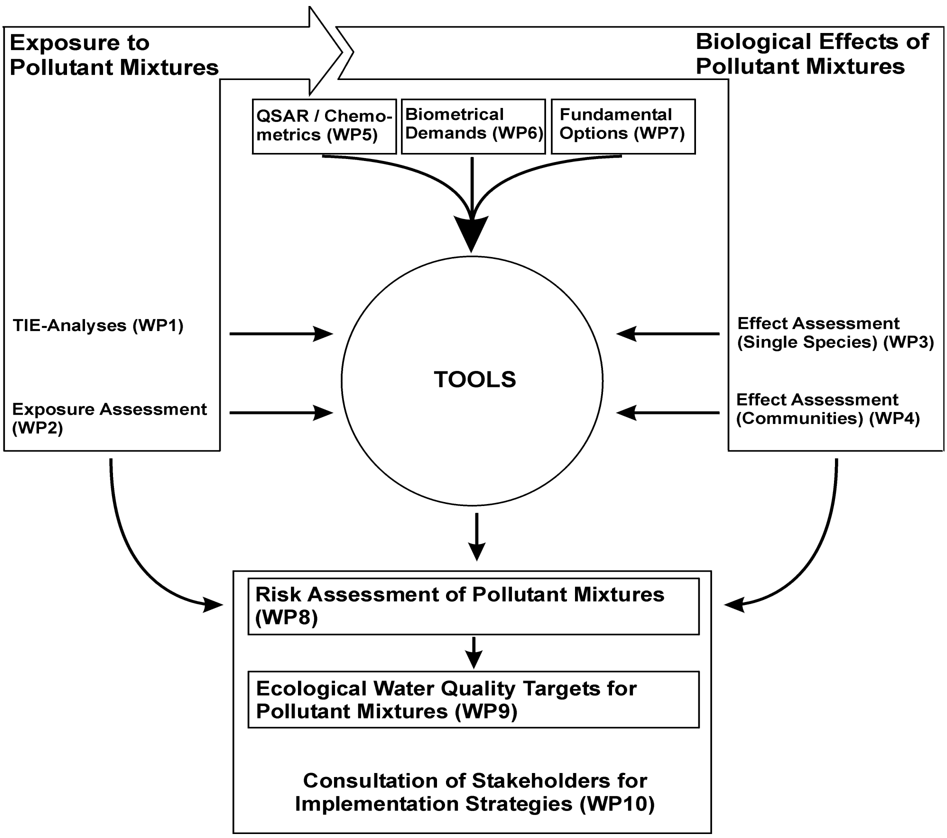

Fig. 1: Structure of Workpackages (WP) in the BEAM Project

constituents will be experimentally studied in single species tests with algae and bacteria (WP3) and in multi-species tests with natural periphyton communities (WP4). Work on multi-species mixture toxicity will include methodological developments towards a swift assay with increased testing capacity. Experimental data will be used for a comparative evaluation of the predictive power of both concepts, *Concentration Addition* and *Independent Action*, for the toxicity of heterogeneous mixtures in realistic exposure situations in fresh and marine waters. ~~.~~

Missing knowledge on the modes of action of many environmental pollutants may be a severe obstacle to the choice of the most appropriate concept for a predictive mixture toxicity assessment. However, the combined use of various chemometric methods and QSAR ~~modeling~~modelling (*Quantitative Structure Activity Relationships*) has been proven as promising tool to overcome this difficulty [2, 7]. Experimental BEAM data will be used for a further refinement and evaluation of this new approach (WP5). Statistical accuracy and precision of mixture toxicity predictions depend on the quality and quantity of available toxicity data for single substances. Experimental BEAM data as well as external data bases will be used as an input to simulation studies in order to work out a framework of minimum biometrical demands (WP6). Existing experimental evidences and theoretical considerations offer two fundamental options for the predictive risk assessment of pollutant mixtures in a regulatory context. Either that concept which is expected to provide the most accurate prediction is selected on a case by case basis. Alternatively the general assumption of *Concentration Addition* might be justified as a “realistic worst case estimation” [8]. The acceptability of this simple pragmatic approach depends on the quantitative differences between the predictions derived from both concepts. Simulation studies on these differences will be performed for a range of scenarios (WP7).

BEAM results on exposure and effects will be combined in order to perform a risk assessment for pollutant mixtures in the aquatic environment (WP8). Methodologies which are used for characterising and comparing risks of single pollutants (e.g. risk quotient approaches) will be analysed for their applicability to a risk assessment of mixtures. Finally, experimental, methodological and theoretical findings of BEAM will be condensed towards the establishment of protocols for the derivation of ecological water quality targets for toxicant mixtures on the basis of toxicity information for the single components (WP9). It will be evaluated, how the chemical characteristics of a surface water body, in terms of mixtures of pollutants present, may affect the characteristics of aquatic ecosystems in relation to the definitions of “High”, “Good” and “Fair” status according to the EU Framework Directive for Community Action in the Field of Water Policy. Work on risk assessment procedures (WP8) and water quality targets for mixtures (WP9) will be performed under participation of a stakeholders consulting group (WP10). Experts from European policy and regulatory advisors, chemical industries as well as environmental NGO´s will be invited to join this group. Thereby a successful achievement of the aim to *bridge the gap between science and regulation* shall be insured.

EXPECTED IMPACTS

The exploitation of BEAM results will allow to implement mixture toxicity assessment into EU regulations, ensuring better pollution management of water resources and the sustainable use of water bodies. This will indirectly improve quality of life, health and safety. BEAM will increase EU-competitiveness, knowledge and skills in the field of environmental risk assessment of chemical mixtures. The participation of a stakeholders consulting group will ensure the effectiveness of the exploitation process.

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TITLE : BIOGEOCHEMICAL INTERACTIONS BETWEEN THE DANUBE RIVER AND THE NORTHWESTERN BLACK SEA : **EROS-21**

CONTRACT N° : **ENV4 CT96-0286**
IC20-CT96-0065

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THE NORTHWESTERN BLACK SEA : A PILOT SITE TO UNDERSTAND THE COMPLEX INTERACTION BETWEEN HUMAN ACTIVITIES AND THE COASTAL ENVIRONMENT

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INTRODUCTION

Coastal ecosystems are important spawning and nursery grounds for marine organisms and are of great value for recreational activities. Coastal areas are also the recipient of increasing amounts of nutrients and contaminants from human activities, including industrial effluents, agricultural runoff, and municipal sewage. As a consequence, remarkable changes have been observed in coastal waters all around Europe during the last decades. The EROS (European River Ocean System) project is part of a long-term research to understand the biogeochemical processes and their alteration by human activity in the European coastal zone. The ultimate objective is to develop a mathematical model of the land-ocean system to be used as a scientific tool for reducing the degree of uncertainty within current coastal zone management decisions are taken in order to achieve sustainable development of coastal and land-locked ecosystems.

Since 1994, the EROS team is focusing its research to the lower reaches of the Danube and its delta along with the north-western Black Sea. The Black Sea, with its unique features, such as being the largest enclosed catchment basin receiving freshwater and sediment inputs from rivers draining half of Europe and parts of Asia, represents the ultimate example of deterioration of the coastal and marine environment in Europe. It was chosen as a pilot site to understand the complex interactions between human activities and the marine environment. The Black Sea ecosystem has indeed experienced several changes during the last decades, driven by human perturbations in the coastal ecosystem itself and in the drainage basins of the rivers. Among these, the Danube river receiving the effluents from eight European countries is affecting the north-western Black Sea ecosystems and represents the most significant source of river-borne pollution flowing into the Black Sea.

Assessing and predicting the response of the Black Sea ecosystem to human perturbations is the ultimate objective of EROS 21, bringing together biologists, geochemists and physicists of Western, Eastern and Central Europe. More specifically, the project addresses the following questions : What would be the consequences of a reduction of the river input of nutrients for the eutrophication and the ecosystem structure of the north-western Black Sea ? What are the riverine sources, levels and fates of key organic/inorganic pollutants in the Black Sea environment ? What are likely to be the short- and long-term consequences of the regulation of river discharge for sediment erosion/transport/deposition and water stratification ?

PROJECT METHODOLOGY : HISTORICAL DATA, FIELD RESEARCH AND MATHEMATICAL MODELLING

The complex interactions between the continental and marine coastal systems require definition and integration of the most natural and human-induced driving forces controlling the ecosystem variability. The EROS approach was to review existing data in the Danube drainage basin and the north-western Black Sea, conduct process-level studies under field and laboratory-controlled conditions and combine them with remotely sensed satellite data to develop and validate numerical models describing ecosystem and biogeochemical transformations along the aquatic continuum of the Danube - north-western Black Sea.. A number of field investigations were organised in the lower reaches of the Danube and its delta, at strategic coastal areas south of the Danube mouth to monitor the seasonal evolution of nutrients and algal blooms and in the north-western Black Sea. Two large expeditions were conducted in the Black Sea in summer 1995 and spring 1997 aboard the Ukrainian research vessel « Professor Vodyanitsky ». A total of 150 stations were sampled in the area of Danube influence along eutrophic-oligotrophic gradients as well as in the central basin. A sediment trap was deployed from August 1995 to October 1996 to measure sedimentation rates and the nature and composition of sinking particles. Process-level studies were conducted on the rate of microbial degradation of organic matter both in the water column and in the sediment. These experiments were combined with isotope measurements in an attempt to discriminate biodegradable organic matter of continental or marine origin. For the first time, investigations on the structure and functioning of the planktonic food-web were conducted in an integrated comprehensive way : studies of the relationship between phytoplankton communities and nutrient availability (N:P:Si) were combined with experimental investigations of the feeding ecology of key planktonic organisms (protozoa, copepods, *Noctiluca*) and of the gelatinous carnivores *Mnemiopsis* and *Aurelia*. The rate of sedimentation and the processes occurring within the sediments and at the water-sediment interface were studied as well. For the first time, a benthic lander was successfully used in the Black Sea to measure nutrient exchanges at the sediment-water interface. The knowledge gained is progressively integrated in a hierarchy of 'offline' coupled physical-biogeochemical models of different spatio-temporal and trophic resolution which describe biogeochemical transformations along the aquatic continuum from the Danube river over the north-western shelf to the central Black Sea basin as a function of meteorological forcing and human activities.

RESULTS

Sensitivity of the Black Sea ecosystem to human perturbations : phenomenological analysis and mathematical modelling of historical data

Since the early sixties noticeable and well-documented alterations have been observed at various trophic levels of the Black Sea ecosystem. In less than 30 years the Black Sea ecosystem has been evolving from a highly biodiverse ecosystem, characterised by rich biological resources to a low biodiversity ecosystem dominated by a dead-end gelatinous food-chain (e.g., Mee, 1992). During the late 1980's there was an almost total collapse of fisheries, which coincided with an unprecedented increase of the jellyfish *Aurelia* but also of the exotic combjelly *Mnemiopsis* unintentionally introduced in the Black Sea in the mid-1980's. At the time, it was thought that these carnivores - lacking predators and feeding on zooplankton, fish eggs and larvae - were dramatically reducing recruitment of fish to the adult stocks and were claimed responsible of the ecosystem destabilisation.

EROS re-analysis of existing data in combination with numerical experimentation making use of the conceptual mechanistic model BIOGEN developed in the scope of EROS (Van Eeckhout and Lancelot, 1997), however, suggests that the successful development of the undesirable gelatinous carnivores results from a combination of human perturbations having occurred almost synchronously in the drainage basin of the Danube river and in the marine system: manipulation of hydrologic regimes of out-flowing rivers (Bondar, 1977), urban and industrial expansion and the intensive use of agricultural fertilisers (Bologa et al., 1984; Gomoiu, 1990), introduction of exotic species such as the gelatinous Ctenophore *Mnemiopsis* sp. (Vinogradov et al., 1989; Mutlu et al., 1994), selective and excessive fishing (Ivanov and Beverton, 1985; Stepnowski et al., 1993; Bingel et al., 1993; Gücü, 1997). Among these however, human-induced changes in the Danube river basin were the dominant driving force.

Human-induced changes in the Danube catchment conducted since the 1970's were dramatically modifying the quantitative and qualitative nutrient environment of the coastal phytoplankton, increasing nitrogen and phosphorus (due to cultural eutrophication) and lowering silicate (due to hydraulic management). This nutrient change was stimulating, after 1970, the development of numerous phytoplankton blooms during the summer period in this phosphorus-limited marine environment and generally, the phytoplankton community was dominated by non-diatom species some of them of little palatability for mesozooplankton. As an immediate response to increased primary production, large developments of herbivorous copepods and carnivorous predators (fishes) were first observed (Porumb, 1970). The higher fish availability led to an increase of fish catches and as a matter of consequence of the fishing pressure (Zaitsev, 1993, Gücü, 1997). By decreasing fish stocks, fishing pressure indirectly stimulated the development of the predatory gelatinous carnivores *Aurelia* and the alien *Mnemiopsis*, competing with the fish for copepods. This situation was accelerated in an explosive way due to the lack of known predators of these gelatinous carnivores and to their voracious feeding of fish eggs and larvae. Most of organic matter produced by primary producers was thus deviated to a trophic dead-end. The organic matter, derived from dead organisms further enhanced the development of bacterial populations with subsequent oxygen deficiency problems and increasing the bulk of organic matter reaching the sediment.

Present-day functioning of the Black Sea ecosystem : signs of improvement

Since 1995, however, some positive signs of coastal Black Sea ecosystem recovery were observed by the EROS project at different levels of the Black Sea ecosystem:

First of all, phosphorus and nitrogen loads have considerably decreased, but concentrations in the Danube river are still comparable to other polluted European rivers, such as the Rhine, Rhone and Po (Cociasu et al., 1997). However, budget calculations, based on the *in situ* measurements of phosphate benthic fluxes, indicate that sediments are contributing as much phosphate to coastal phytoplankton growth than Danube waters (Friedl et al., 1997).
As expected, no clear trend could be observed in the evolution of silicate concentrations in the Danube after the dramatic decrease that followed the building of the iron gates in 1972. A value of 100 µM is typical for dissolved silica in the Danube (Cociasu et al., 1997). Such a concentration is significantly lower than the average value of 175 µM characterising the world's rivers (van Bennekom and Salomon, 1980).
Inorganic contaminants as well were found to now reach concentrations similar to those recorded in the Mediterranean and Baltic Sea although historically extremely high levels of contaminants were reported for the north-western Black Sea. In contrast, the Danube river has

been found to continue representing an important source of organic pollutants to the north-western Black Sea, especially for its northern mouth (Chilia Branch). The concentrations of petroleum hydrocarbons, organochlorine compounds and of the sewage marker trialkylamines were two orders of magnitude higher in the Danube mouth than in the open sea. The chemical composition of the organochlorine compounds revealed a recent increase in use of pesticides in the riparian countries. Their concentration level recorded was one order of magnitude higher than those reported for the Mediterranean Sea.

At the ecosystem level, some planktonic and benthic species considered to be extinct or very rare nowadays became again very common in the Black Sea. In the same direction, a slight recovery of the mussel (*Mytilus galloprovincialis*) populations was observed from 1995 to 1997. On the other hand, the abundance of the undesirable jellyfish has levelled out and the number of anchovy eggs and larvae has increased (Shiganova, 1997)

Incidentally these events are corresponding to the economical collapse which recently occurred in the Eastern and Central European countries. However, to which extent the present-day catastrophic socio-economic situation induced such a rapid response of the coastal ecosystem cannot be assessed by a simple correlation between events. The complex interactions between the continental and marine systems require a more in-depth analysis of the natural science, social science and economic science interfaces.

CONCLUSION

Observational achievements already made by the EROS project indicate that the north-western Black Sea represents well a unique region where the effect of curative measures upon the quality of a given coastal ecosystem and its biological resources can be tested because of :

- the high sensitivity of the north-western Black Sea ecosystem to human disturbances, as demonstrated by historical records tracing the environmental and ecological changes over the past 30 years, and
- its rapid response to human-induced changes as suggested by the improvements recorded after the economical collapse faced by the Eastern and Central-Europe countries.

However, the complex interactions revealed between the continental and marine systems and between different human-induced activities superimposing on natural variability cannot be understood by simple correlation between events. The ongoing hierarchical development of coupled physical-mechanistic biogeochemical models developed in the scope of EROS for describing biogeochemical transformation and ecosystem change along the Danube river-Black Sea aquatic continuum in response to natural and human-induced perturbation will encompass this complexity. When fully validated it will constitute a valuable tool for answering the following environmental questions:

- What is the time needed for a given ecosystem to recover after a voluntary reduction of nutrient loads to the coastal area ?
- What is the buffering capacity of the ecosystem to nutrient reduction ?
- What is the synergy between human-induced changes such as eutrophication, overfishing and introduction of exotic species leading to ecosystem destabilisation ?

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TITLE : OCEANOGRAPHIC APPLICATIONS TO EUTROPHICATION IN REGIONS OF RESTRICTED EXCHANGE : **OAERRE**

CONTRACT N° : **EVK3-CT1999-00002**

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OAERRE: OCEANOGRAPHIC APPLICATIONS TO EUTROPHICATION IN REGIONS OF RESTRICTED EXCHANGE

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SUMMARY

OAERRE aims to understand the physical, biogeochemical and biological processes, and their interactions, involved in eutrophication in coastal marine Regions of Restricted Exchange (RREs), especially lagoons and fjords. The scientific issues addressed include the controls on horizontal and vertical exchange in RREs and the response of coastal ecosystems to nutrient enrichment.

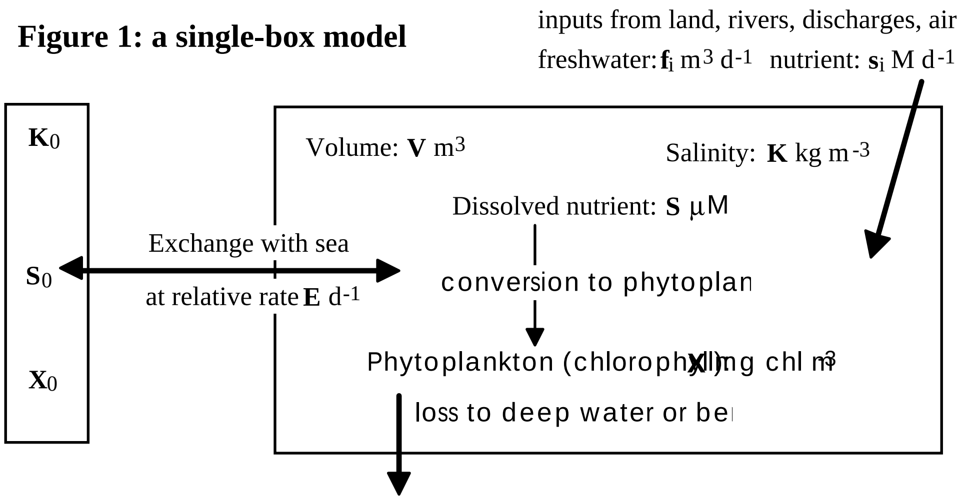
INTRODUCTION

Eutrophic conditions have been defined by the CEC as:

'enrichment of water by nutrients especially compounds of nitrogen and phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms and the quality of the water concerned.'

Figure 1 summarises some important processes involved in eutrophication in Regions of Restricted Exchange (RREs). In this model, all interaction with the adjacent sea take place on one side of the RRE and are summarised by the lateral exchange rate **E**, the fraction of the box contents that is replaced during a day. A number of processes are involved in this exchange, including tidal flows, estuarine circulation and the intermittent flushing of deep water. In all cases the main driving forces are the horizontal pressure gradients in the restricted entrance. These are set up by, for example, freshwater inflow to the RRE, and variations in the sea-level or the density field outside the RRE. The exchange is limited by friction, expansion losses, baroclinic/topographic interactions, and/or hydraulic controls. The relative magnitude and importance of the different limitations depends on the geometry of the restriction relative to the basin. Local wind stress must also be taken in account.

Figure 1: a single-box model



In stratified RREs, vertical exchange $\mathbf{E_v}$ is important. It results from turbulent mixing due to boundary stirring or internal motions, upwelling or entrainment resulting from lateral flows, and displacements caused by flushing of deep water. Vertical exchange resulting from internal waves is poorly understood but may be a crucial mixing process. Some RREs are sufficiently broad (> 20 km at 60°N) for the weak Coriolis acceleration (due to the rotating Earth) to influence currents. In such cases water flows must be observed or modelled in three dimensions, and exchange may depend critically on interaction between wind- and density-driven circulations and dynamically-controlled fronts linked to the restricted opening.

A simple box model, based on Figure 1, is recognised by some EU states for use in screening for eutrophication potential. The key feature is the assumption of total conversion of nutrient into phytoplankton chlorophyll at (system) yield **q**, giving:

maximum chlorophyll: $\mathbf{X_{max}} = \mathbf{X_0} + \mathbf{q}.(\mathbf{S_0} + (\mathbf{s_i}/(\mathbf{E}.\mathbf{V})))$ mg chl m^{-3}

Concentrations of nutrient and phytoplankton in the sea are $\mathbf{S_0}$ and $\mathbf{X_0}$. Total daily input of nutrient into the box, from natural and anthropogenic sources, is $\mathbf{s_i}$. **V** gives the volume of water in the RRE. Light is assumed not to be limiting.

Organic matter produced by phytoplankton can sink into deep water, where, in stratified systems, its decomposition depletes oxygen and replenishes nutrients. Alternatively, production can be transferred to the benthos by filter-feeders , or by sinking and deposition into a sea-bed 'fluff' layer available for transfer to organisms or the consolidated sediment. Micro-organisms in the fluff and consolidated layers mineralise the organic matter, releasing nutrients and consuming oxygen. Sediment oxygen demand can be simply parameterised by the term $\mathbf{E_b}.\Delta\mathbf{O}/\Delta\mathbf{z}$, where the oxygen gradient $\Delta\mathbf{O}/\Delta\mathbf{z}$ depends on consumption within the sediment and on concentration in near-bed waters. The value of the exchange parameter $\mathbf{E_b}$ is critical; some argue that it is largely the results of physical processes, especially shear turbulence in the Benthic Boundary Layer (BBL), others that it depends mainly on macrobenthic pumping. In the latter case, anaerobic conditions, which kill most benthic animals, will diminish oxygen flux; however, they also encourage bacterial denitrification, leading to permanent loss of nutrient-nitrogen. In shallow waters, benthic micro- and macro- algae can contribute significantly to the production of organic material. Finally, stress exerted by currents flowing over the sea-bed can resuspend particles, increasing turbidity and re-mineralisation of organic matter . In regions of tidal movement, resuspension and deposition usually balance over a tidal cycle. In RREs of low tidal energy, deposition may dominate, with intermittent resuspension events resulting from wind-waves or currents caused by winds or sea-level changes.

Changes in 'the balance of organisms', due to nutrient enrichment, may be largely due to the effect of changes in the ratios of the elements N, P and Si on phytoplankton in a given physical environment. Aquatic ecologists have recently come to appreciate the importance of the 'microbial loop', in which pelagic production passes by way of bacteria to protozoa before returning to the classical food chain at the level of mesozooplankton. 'Microplankton' includes all planktonic bacteria, micro-algae and protozoa. The ratio η of the biomass of pelagic heterotrophs to microplankton influences **q** and, also, how much organic material is available to sink and thus potentially cause sediment or deep water de-oxygenation. Like the balance between diatoms and flagellates, the value of η shows natural changes with the seasons, but its sensitivity to nutrient enrichment is unknown. The idea of characterising any microplankton as a mixture of two components offers a means of parameterising key microbial properties of the pelagic ecosystem in terms of the two system parameters η and ψ. The latter gives the ratio of the biomass of flagellates and related organisms to total microplankton biomass.

The scientific literature contains increasing evidence of ways in which the highlighted factors influence ecosystem functioning at higher trophic levels as well as determining their assimilative capacity for nutrients. One - optimistic - hypothesis suggests that RREs will cope with increased nutrient enrichment by diverting a greater proportion away from phytoplankton, and by increased denitrification. A pessimistic view stresses the possibility of irreversible switches in food web structure, as production is diverted through flagellates and as macrobenthic animals are killed by increased organic sedimentation. Modelling allows such hypotheses to be investigated.

OBJECTIVES & METHODOLOGY

OAERRE's objectives are (1) Observations of the physics of vertical and open boundary exchange in RREs, leading to improved parameterisation of these processes in research and simplified models. (2) Study of the phytoplankton and pelagic micro-heterotrophs responsible for production and decomposition of organic material, and of sedimentation, benthic processes and benthic-pelagic coupling, in RREs, with the results expressed as basin-scale parameters. (3) Construction of closed budgets and coupled physical-biological research models for nutrient (especially nitrogen) and organic carbon cycling in RREs, allowing tests of hypotheses about biogeochemistry, water quality and the balance of organisms. (4) Construction of simplified 'screening' models for the definition, assessment and prediction of eutrophication, involving collaboration with 'end-users', and the use of these models to analyse the costs and benefits of amelioration scenarios.

OAERRE is a collaboration amongst physical, chemical and biological oceanographers, and coastal resource managers, with intensive studies relating to eutrophication at sites that exemplify a range of hydrographic and enrichment conditions. Four of these sites are fjords: Kongsfjord (Svalbard); Gullmaren (Skagerak); Himmerfjärden (Baltic); and the Firth of Clyde (Scotland). Two are bays: Golfe de Fos (French Mediterranean); and Ria Formosa (Algarve). Research cruises to fjords will using standard shipborn instrumentation (ADCP and CTD with optical sensors and water sampling bottles), plus turbulence probes, towed undulating CTD, and benthic sampling devices. Bays will be sampled using small boats and divers. Moorings will be equipped with a range of physical, acoustic, chemical and (bio-) optical sensors. The Gullmaren mooring will contain a profiling sensor package. Process studies will provide estimates of key ecosystem parameters, especially concerning physical exchange, nutrient cycling, the relative importance of autotrophic and heterotrophic processes, and the 'balance of organisms' amongst the benthos and the smaller plankton. Remote sensing will be used to study larger RREs and to provide boundary conditions.

Table 1: OAERRE sites

Site	where	key features	used by OAERRE
Kongsfjord	Svalbard (Spitzbergen)	Arctic conditions; little pollution; exchanges with ocean across narrow shelf	as low temperature, 'control' site; 3 cruises
Gullmaren	Sweden (Skagerak)	well-described fjord with shallow sill, and tendency to flagellates	intensive time-series (18 month mooring deployment) and near-real-time data telemetry; 7 cruises
Himmerfjärden	Sweden (Baltic)	fjord with low salinity; cyanobacterial blooms; N-stripped discharge	site with extreme P:N ratio; 4 cruises
Firth of Clyde	Scotland (west coast)	broad fjord, eutrophic in inner part; classical food web	exchange processes in broad fjord; 2 main cruises, regular visits by smaller vessel; moorings
Golfe de Fos	France (Mediterranean)	shallow bay, weak astronomical tide, intermittent wind-driven exchange	study of low energy bay; detailed study of benthic processes including production; mooring
Ria Formosa	Portugal (Algarve)	shallow lagoon behind barrier islands, tidally energetic, high drying proportion, but low flushing in parts	study of energetic, eutrophic, lagoon; difficult estimation of flushing

Research models, involving 1D, 2D and 3D representations of physical, biogeochemical and biological processes in RRE, will draw on and advance the state of the art, and will be tested against historic and new data. One model will be used for trial "real-time" simulations. The research models, and the process studies, will underpin the development of 'screening' models for eutrophication and related aspects of water quality. These 'screening' models are simplified representations of RREs in terms of a few boxes, in which the major processes are described in terms of a few 'system parameters'. They will be designed to be safe through use of the precautionary principle, and to be transparent to a range of end-users, especially those responsible for maintenance of water quality. Output will be subjected to socio-economic scenario analysis to demonstrate the use of these models in evaluating options for ameliorating environmental impact.

DISCUSSION

At the time of writing this short account, OAERRE has yet to commence. However, it is understood that its start date will be soon. Its expected achievements of are: better understanding of lateral and vertical exchange in RREs; better understanding of effects of physical environment and enrichment status on pelagic and benthic system parameters in RREs; better understanding of the holistic functioning of RREs; and improved European water-quality objectives and standards for marine coastal eutrophication.

The studies in OAERRE that will help to define safe loadings of an RRE with nutrients are relevant to the EC Urban Waste Water Treatment Directive and similar directives, soon to be consolidated in a Water Framework Directive. They are also relevant to aquacultural aspects of the Common Fisheries Policy. OAERRE relates to key action 3 (Sustainable Marine Ecosystems) of Framework V, especially to the themes concerned with: better assessment of naturally occurring mechanisms of ecosystem functioning; transport pathways and impacts of pollutants and key elements in the marine environment; reducing the effects of anthropogenic activities on the marine environment and recovering degraded marine systems; and integrated studies of land-ocean interaction. The project will contribute to the aims of ELOISE, including the objective-foci concerned with interactions between pollution, eutrophication, and physical disturbance and development of mathematical models aimed at prediction and upscaling.

TITLE : MARINE EFFECTS OF ATMOSPHERIC DEPOSITION : **MEAD**.

CONTRACT N° : EVK3-CT1999-00014

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MARINE EFFECTS OF ATMOSPHERIC DEPOSITION (MEAD)

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MEAD has recently been funded through the Fifth Framework Programme of the European Union. Here we present the rationale and overall aims for the project.

PROJECT SUMMARY

The coastal seas represent one of the most valuable resources on the planet (Constanza *et al*., 1997). However, this ecosystem is threatened as a result of human pressure. We rely on the coastal zone for mineral resources, waste disposal, fish and recreation. In Europe high population densities and high levels of industrial activity mean that the pressures arising from these activities are particularly acute. Globally we have probably doubled nitrogen fixation as a result of human activities (Galloway *et al.*, 1995) and increased nitrogen emissions five fold (Prospero *et al*., 1996). On a regional scale in Europe the increases have been greater than this (Galloway *et al*., 1995), leading to real concern over eutrophication threats to coastal waters (Nixon, 1995). There is a large reservoir of essentially unreactive N_2 gas in the atmosphere which we do not consider now. We subsequently only consider fixed nitrogen, that which is readily bioavailable - nitrate, ammonium and organic nitrogen (Cornell *et al.,* 1995; Seitzinger and Sanders, 1999).

Atmospheric inputs deliver 20->50% of the total input of nitrogen from land to coastal waters (Paerl, 1995; Richardson, 1997) and differ in many ways from riverine inputs (Jickells, 1998):

- They are chemically different, rivers are dominated by nitrate and P and Si while atmospheric inputs include reduced and oxidised nitrogen in equal amounts, but no significant P or Si.
- Riverine inputs are delivered to the coastal seas at their margins whereas atmospheric inputs can be delivered directly to the surface of the central areas of coastal seas and hence exert an impact in regions less directly affected by riverine inputs.
- Atmospheric delivery goes on all year whereas riverine inputs are dominated by the high flow winter and spring periods when coastal water phytoplankton activity is low.
- Atmospheric inputs are also highly episodic with high deposition events associated with particular meteorological conditions (Owens *et al*., 1992; Michaels,1993; Spokes *et al*.,1993).
- Interaction between seasalt and nitrogen species such as HNO_3 are complex and non-linear (Quinn *et al.,* 1988; Liss and Galloway, 1993; Gard *et al*.,1998; Geernaert e*t al*., 1998; Keene. and Savoie, 1998; Pryor *et al*., 1999) but act to focus atmospheric deposition to the coastal seas by changing aerosol particle size and increasing deposition velocities.

Thus we believe atmospheric inputs to be quantitatively important and potentially different in their effect on coastal ecosystems to riverine inputs, hence requiring different management strategies. However, we have almost no information on the direct effects of atmospheric deposition on marine ecosystems, though clearly such a large external nitrogen input should lead to enhanced rates of phytoplankton growth. In the case of ammonia, even the direction of air-sea exchange is uncertain (Quinn et al., 1988). **We hypothesise that high atmospheric deposition events in summer to nutrient depleted, well lit, surface waters in coastal seas may play a role in stimulating algal blooms which may be harmful and/or contribute to bottom water oxygen depletion.** There is essentially no information with which to test this hypothesis (Paerl, 1985; Richardson, 1997). Policy makers are concerned to regulate nitrogen inputs to coastal waters to avoid unacceptable damage to ecosystems. However, current scientific understanding is inadequate to allow rational decisions to be made over whether increased regulation is necessary, and if so how to do this most cost-effectively.

MEAD is focussed on the Kattegat, a well studied area where we know atmospheric deposition to be a very important source of nitrogen and where various eutrophication problems appear to be evident. However, the products of MEAD should be applicable to other coastal seas.

The Contribution of MEAD to solving this Problem.

To improve our understanding of the effects of atmospheric deposition events in coastal waters, MEAD uniquely brings together atmospheric and marine scientists with appropriate expertise in physics, chemistry, biology, meteorology, modelling and the management of coastal ecosystems. The innovative approach of focussing this diverse group onto this specific problem will, we believe, yield exciting scientific insights and important new understanding of how to manage this issue.

Observational programmes will be initiated, building on existing activities to yield the most cost effective return. These programmes will provide the observational data to develop parameterisations needed in the water column and atmospheric models. They may also allow the direct observation of the effects of a small number of high deposition events, but such observations are not in themselves the primary goal of this component of the work.

An atmospheric transport model for the coastal region will be extended and improved to include better descriptions of the complex meteorological regime created by the abrupt changes in roughness and static stability between the terrestrial and marine environments. This novel model will include a detailed description of the atmospheric chemistry involved in the interaction of marine and terrestrial air-masses. This model, which will be specifically formulated to allow high spatial resolution in the coastal region, will be nested within a larger long-range transport model.

The marine modelling component will involve adapting an existing hydrodynamic model of the Kattegat to include nutrient cycling, lateral and vertical nutrient fluxes and the biological response, including appropriate representation of grazing, remineralisation and sedimentation. It will be one of the first models designed specifically to investigate the effects of atmospheric deposition events and will be parameterised using data from the water column biogeochemical programme.

We will also utilise existing and archived monitoring data on phytoplankton abundance in the coastal Kattegat in a retrospective analysis to identify incidences of blooms and test for any relation between these and atmospheric deposition based on existing atmospheric deposition network sampling. We will then utilise the data from the new generation of ocean observing satellites to expand this monitoring work to follow the blooms in space and time. We will also build up a climatology of the high atmospheric deposition events. This retrospective approach is, we believe, entirely novel.

The final component of MEAD involves merging the modelling and retrospective analysis to produce a model to forecast eutrophication resulting from atmospheric deposition and conduct scenario modelling to consider management strategies for emissions to reduce these effects. This will involve at least one local agency responsible for coastal management to ensure that the tools developed are appropriate to their needs. The great advances in computing power mean that the provision of such models to the user community is now a realistic possibility opening the way for a completely new approach to managing coastal seas. MEAD will yield important new understanding of the coastal region from observations and models. The most innovative part of this unique collaboration is the development of the conceptual understanding (and quantitative description) of the link between atmospheric deposition and water column biogeochemistry.

MEAD has recently been funded as part of the Energy, Environment and Sustainable Development thematic programme of the Vth Framework Programme of the European Union and is within the IMPACTS cluster. Research within the IMPACTS cluster aims to quantify the effects of human activities on contrasting marine ecosystems around Europe. The resulting knowledge will underpin Europe's emergent management models on the cost-benefits of pollution reduction, rehabilitation of degraded ecosystems and the sustainable development of the marine environment. Sustainable development requires the regulation of human activities and/or anthropogenic emissions to protect ecosystems. Coastal seas are particularly vulnerable because of their heavy exploitation for fisheries and mineral resources, their use for recreation and as the repositories of waste discharges from rivers, the atmosphere and from direct inputs. Coastal zones have been identified for priority action under the EU 5th Environmental Action Programme.

MEAD is specifically concerned with potential eutrophication problems in coastal seas and the role of atmospheric deposition in these problems. The significance of atmospheric deposition has only relatively recently become apparent and new components of this deposition such as organic nitrogen are areas of intense research interest. It is now clear that atmospheric deposition represents a large input to coastal seas. Furthermore atmospheric deposition can be effectively transported to the centre of coastal seas and delivered at times when other nutrient inputs are reduced. However, the meteorological and chemical processes controlling transport and deposition over coastal seas are extremely complex and not well understood.

While we now know that atmospheric deposition can be a large source of nitrogen to the coastal seas, we do not know its effects on coastal biogeochemistry. The composition and nature of atmospheric deposition mean that it cannot be assumed that the effects will necessarily be similar to those of riverine nitrogen inputs. In particular high atmospheric deposition events under nutrient depleted warm summer conditions may have unique and damaging effects. Sustainable management of a system that is perturbed by extreme events requires different strategies to those for systems perturbed by chronic contamination. Our current ignorance about the role of atmospheric deposition in general, and high deposition events in particular, prevents rational and well informed policy development in this area.

Thus one goal of MEAD is to document the relationship between atmospheric deposition and its effects. MEAD will also provide a pre-operational modelling scenario tool designed to allow policy makers to evaluate strategies to minimise the damage to the coastal seas from high atmospheric deposition events. Such strategies might involve further controls on NO_x emissions across Europe or regulation of farm manure handling practices to minimise ammonia emissions. The optimisation of such strategies should allow planners to strike the balance between regulation of human activity and environmental protection most effectively. Although MEAD is inevitably focussed on a particular coastal region, the Kattegat, the overall aim is to produce a broad increase in scientific understanding of coastal zone processes. The modelling tools developed will be, in part, generic and transferable to other regions. One of the partners in MEAD is also the statutory regulatory authority for coastal management in part of the Kattegat, and through this partner, we will ensure that the products of this work are directly made available to the user community and relevant to their needs.

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TITLE : FEED-BACKS OF ESTUARINE CIRCULATION AND TRANSPORT OF SEDIMENTS ON PHYTOBENTHOS: **F-ECTS**

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INFLUENCE OF PHYTOBENTHIC COVERAGES ON THE ERODIBILITY OF AN ESTUARINE SEABED

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INTRODUCTION

The scope of work of F-ECTS is the study of the active role of estuarine phytobenthic communities (rooted macrophytes, non-attached macroalgae and microflora) in modifying the characteristics of the ecosystem they live in and in mediating the human impact on the Coastal Zone habitats (Bergamasco, 1998).
Complicated processes link in fact these communities with hydrodynamics, nutrient cycling and sediment transport. The project aims at studying the different interactions with a new inter-disciplinary approach in order to produce a reliable conceptualization and parameterization of the main processes to be included in an existing SPM transport model.
In particular for the Lagoon of Venice, selected as pilot case study, the modeling activities within F-ECTS are currently enabling the set up of linked modules for the simulation of the feed-back loops between the physical processes and the phytobenthic habitat.
These tools are expected to be considered as a support in the management of the lagoon where the degradation of the morphological structures, also triggered in the last decades by man-induced erosion processes, reveals itself in a deepening of the lagoon with a general trend towards the “marinization” of the environment.

THE FIELDWORK APPROACH

During the first year of the project, two seasonal field campaigns were carried out in the pilot case study (the Lagoon of Venice - Italy). Specific biological, sedimentological and physical data were simultaneously collected in-situ to provide inputs for the modelling of the sediment transport and the ecological processes in the Lagoon extending an existing SPM lagrangian model.
Eleven subtidal and intertidal sites were identified and selected for field investigation during the planning of the campaigns, their location and physical and “environmental” characteristics being regarded as representative of the whole Lagoon of Venice.
Attention was focused on three transects going from the mainland to the three lagoon inlets. This enabled to take into account for all kind of different physical and environmental conditions such as sediment type (from sand to pelites), hydraulic regime, bathymetry, nutrient loading, light climate and macrophytobenthic associations. Moreover, for what the phytobenthos concerns, areas with different ecological role were investigated: in particular both seagrass-dominated and macroalgae-dominated areas were considered, as well as areas where the natural saltmarsh environment ("barene") still survives, together with the ones where the human impact is most relevant.

The main purpose of the fieldwork was to determine the stability and benthic flux of bottom sediments at the above described sites during the periods of expected maximum and minimum benthic biological activity (summer and winter respectively) and examine the differences resulting from changing physical and biological settings. The Sea Carousel was used for this purpose.

Station	Name	Transect	Depth	Covering	Notes
10	Bacàn	North	Subtidal	Rooted macrophytes (massive) - Sand	Northern lagoon inlet
20	S. Giacomo	North	Subtidal	Bare – Soft bottom	Intensive small boat and ferry traffic in the near canal
30	Cona	North	Intertidal	Macroalgae/rooted macrophytes (spotted)	Near to freshwater outlet – Surviving natural saltmarsh
40	Centrega	North	Intertidal	Rooted macrophytes / filamentous macroalgae	Surviving natural saltmarsh
50	Saline	North	Intertidal	Rooted macrophytes / filamentous macroalgae	Surviving natural saltmarsh
60	S.M. del Mare	Center	Subtidal	Rooted macrophytes (massive) and macroalgae	Central Lagoon inlet
70	S. Leonardo	Center	Subtidal	Bare – Soft bottom with shells	Fishing impact Large oil tankers passage
80	S. Spirito	Center	Subtidal	Macroalgae (massive) – Fine sediments	Northern watershed – Very low water currents
90	Bocca Chioggia	South	Subtidal	Rooted macrophytes/ bare sandy bottom	Southern Lagoon inlet
100	Petta di Bò	South	Intertidal	Rooted macrophytes	Typical southern lagoon environment
110	Punta Vecia	South	Intertidal	Rooted macrophytes	Eroding / artificial saltmarsh rebuilding

Table 1 - The main characteristics of the selected fieldwork sites.

Sea Carousel is a benthic annular flume designed and developed for field use in subaqueous settings by the Geological Survey of Canada (Amos, 1992). The Carousel is 1.0 m in radius with an annulus 0.15 m wide and 0.30 m high. Flow in the annulus is induced, through eight small paddles, by rotating a movable lid which is driven by a digital stepping motor powered from the surface. For the F-ECTS deployments the Carousel was equipped with:

- an ADV current meter to log continuously the three components (azimuthal, radial and vertical) of the flow at 25 Hz;
- three optical backscatter sensors, two of these located on the inner wall of the annulus at 0.03 and 0.18 m above the bed, the third one to detect the ambient turbidity near the bed;
- a fluorometer installed at a height of 0.20 m from the bed inside the flume;
- a sampling port, to collect water from inside the flume at a height of about 0.20 m above the bed.

In particular, the data collected with the Sea Carousel were processed to evaluate the erosion threshold, interpreted as the point at which the surface of the bed begins to erode and the erosion rate, as a function of the applied bed shear stress.

By using the Sea Carousel within a biological perspective, many mechanisms of interaction between the sediment erodibility and the biologically-driven processes were investigated and evaluated such as the dependence of the critical shear stress for erosion on the kind and amount of phytobenthos covering and on the previous depositional history of the surface sediment itself.

Furthermore, during the field campaigns the sedimentological and the ecological teams performed a joint programme of experiments. One of these was to simultaneously measure physical, chemical and biological parameters at most stations. The objective was to study in situ the effect of the hydrodynamics on the efflux of dissolved nutrients from the sediments and its link with the benthic bacterial activity and benthic diatom production.

The production of benthic diatoms was measured by traditional ^{14}C-method (adding $^{14}HCO_3$ to the newly harvested sediment samples), where the algae take up the marked inorganic carbon for photosynthetic purpose and incorporate it into the cells. The surplus of isotope is washed away by acidification of the sample, where the isotope is transformed to $^{14}CO_2$ and leave the sample.

The measurements of bacterial activity were carried out as a ETS-method. Here, the bacteria use the INT-formazan as the terminal electron acceptor for respiratory purposes. During the oxidative phosphorylation the INT changes from hydrophilic to hydrophobic, and is stored inside the bacteria cells. This amount of INT can then be extracted by methanol and quantified spectrometrically.

OVERVIEW OF PRELIMINARY RESULTS

Patterns of stability during the summer and winter surveys

There is a clear trend in the stability of the mudflats of the Lagoon of Venice. The greatest stability is found in the north, the least in the central and southern regions with a trend of decreasing stability from the inner parts of the Lagoon to the mouth (Amos, 1999). The most significant aspect of the fieldwork was the changes in erosion threshold between the summer and the winter surveys (Fig. 1). During the summer survey the mean (Lagoon-averaged) value was 1.10 (± 0.20) Pa. This value is within the range of values found in other coastal environments around the world, altough it is quite high in the response to the high water temperatures found there (25-30 °C). During the winter, this value dropped down to 0.69 ((± 0.25) Pa, about 42% drop, wich could depend on the lowering in the biostabilization resulting from the cooler water temperatures (between 6 and 10 °C). This was most evident in the northern and shallower part of the Lagoon; the central and southern parts showed values of erosion threshold similar to those of the summer.

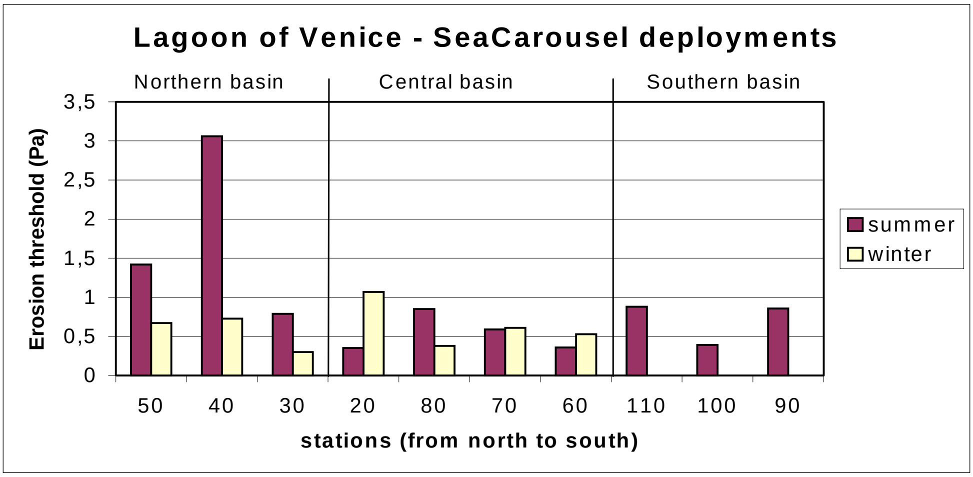

Fig. 1 – Project F-ECTS: Comparison between the erosion threshold values measured during the Sea Carousel deployments (Note: In station 10 no sediment resuspension was induced due to the massive coverage of *Cymodocea nodosa*).

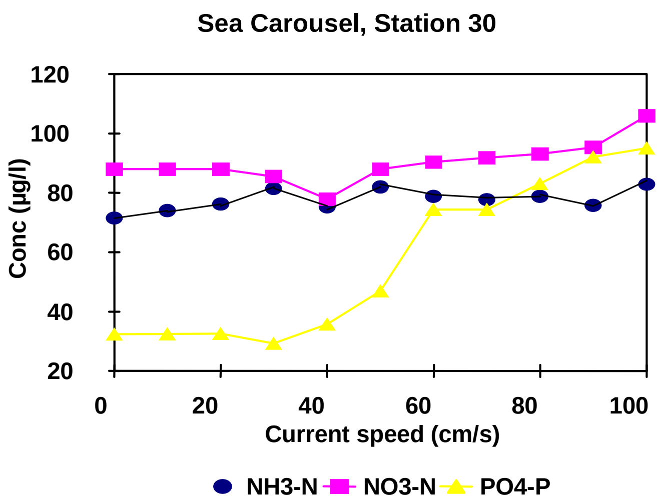

Fig. 2a. Measurements of ammonia, nitrate and phosphate effluxes from the sediment porewater to the water column as a results of increasing current speed created by the Sea Carousel.

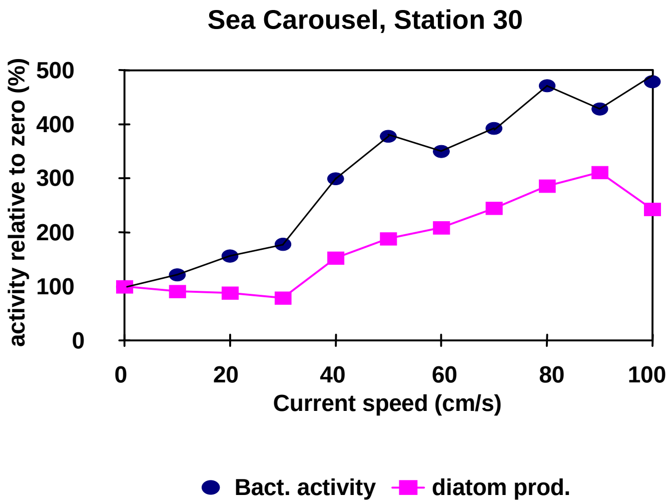

Fig. 2b. Measurements of bacterial ETS-activity and benthic diatom production as a results of increasing current speed.

Values of bulk density were greater in the winter, but their trends do not follow the trends in sediment stability. Consequently this physical property does not seem to have a primary control on bed stability. Rather, it is the colonization of plants and biostabilization by bacteria and algae which appear to have the most significant effect on bed stability.

Feedback studies between hydrodynamic, sedimentology and biological activity

Under controlled physical conditions with respect to the water column, current velocity, temperature and light, nutrient effluxes from the sediment to the water column were followed during the resuspension experiments in the Sea Carousel. As an example the results presented in Fig. 2a for station 30 show that depending on the increasing of the current velocity phosphate starts to escape from the sediment porewater (Flindt, 1999). At this station this happens when the current velocity exceeds 40 cm s^{-1}, in the same range of the critical shear stress. The concentration in the water column consequently increases from growth-limiting conditions to 3 times higher. Both the heterotrophic bacterial activity and the benthic diatom production is stimulated by increasing current velocity (Fig.2b). The benthic diatom production increases of about 300% relative to initial conditions without any current velocity and the bacterial respiratory activity increases of about 500%. In a shallow system like the Lagoon of Venice where the tidal excursion creates high current velocities in extended areas these processes may be very essential for the turnover of nutrients.

CONCLUSIONS

Observational achievements carried out within the F-ECTS project indicate that the biomediation can assume a very high relevance on the medium-to-long term morphological changes especially in human-impacted estuarine areas.

The results of the surveys showed that the winter mean stability of the Lagoon bed was significantly lower (55%) than during the summer period: this can be attributed to the diminished influence of biostabilisation.

Furthermore, clear trends emerge between stability and benthic biological communities that vary with species: areas dominated by filamentous algae (*Cladophora sp.*) in the northern lagoon exhibited strength values that are amongst the highest ever detected by SeaCarousel; in some stations (e.g. station 10), the massive coverage of seagrasses (*Cymodocea nodosa*) did not allow any sediment resuspension during the field experiments.

The experimental findings suggest that there are fundamental differences in bed properties of the northern lagoon with respect to the central basin. In the northern one a surface layer of rapidly increasing strength due to the phytobenthic coverage is well established and the region is not in immediate danger of disappearing; this layer was largely absent in the central basin, where the sediments present the characteristics of continued bed erosion, according to the deepening and the consequent loss of habitat experienced within this region of the Lagoon in the last three decade.

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TITLE : KEY COASTAL PROCESSES IN THE MESOTROPHIC SKAGERRAK AND THE OLIGOTROPHIC NORTHERN AEGEAN : A COMPARATIVE STUDY : **KEYCOP**

CONTRACT N° : **MAS3-CT97-0148**

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KEY COASTAL PROCESSES IN THE MESOTROPHIC SKAGERRAK AND THE OLIGOTROPHIC NORTHERN AEGEAN : A COMPARATIVE STUDY (KEYCOP)

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INTRODUCTION

The overall objective of the KEYCOP project is to understand and model the processes that determine the flux of carbon, nutrients and trace substances in the water column and sediment in different hydrographic regimes and the vertical and horizontal fluxes between the pelagic and benthic systems. The project's objective should be seen in the light of numerous publications in recent years identifying hydrographic control of the shape of the pelagic food web and biological production processes at various marine sites (e.g. Kiørboe *et al.*, 1988; Cushing, 1989; Kiørboe, 1993; Neilsen and Richardson, 1993; Richardson *et al.*, 1998; Richardson *et al.*, 2000). The shape of the pelagic food web and magnitude of production, ultimately, control the amount of material sedimenting from the water column to the benthos. Thus, understanding the factors controlling production and food web structure will improve our ability to predict the fate of substances entering or being produced in a given region.

Predicting the fate of substances in a given environment has obvious implications in terms of being able to understand the interaction of anthropogenically introduced nutrients and contaminants with the marine environment. This project does not, however, deal explicitly with identifying the fate of introduced contaminants or nutrients. Instead, the project aims to systematise knowledge concerning the processes affecting fluxes in the marine coastal environment. On the basis of earlier site-specific studies, a series of general hypotheses concerning these processes and their influence on fluxes were developed at the outset of KEYCOP. In general, however, these hypotheses deal with:

- the importance of turbulence in controlling small-scale processes in the plankton (hypothesis 1-4),
- how stratification and nutrient pulsing control vertical particle flux and the microbial loop (hypotheses 5-7),
- how the plankton community structure and material fluxes vary with the degree of mixing (hypotheses 8-10),

- how organic production controls metal speciation and complexation (hypothesis 11) and
- the importance of lateral processes in controlling sedimentation rates and water-sediment interactions (hypotheses 12-13).

In the project, the identified hypotheses are tested for their validity and robustness using data collected in two very different marine systems and during different seasons. The hypotheses identified as being robust under different conditions can then be used to improve (post-KEYCOP) modelling efforts to describe fluxes in marine coastal environments.

STUDY AREAS

The two study areas, the mesotrophic Skagerrak and the oligrotrophic Aegean, have been chosen for the KEYCOP project because of their very different trophic states. The specific sampling periods and geographic positions of the sampling stations in each study area have been chosen with the aim of data collection under differing hydrographic regimes.

SKAGERRAK: The dome shaped pycnocline in the Skagerrak has been well described in earlier studies and pelagic production processes related to the depth of the pycnocline here (Kiørboe *et al.*, 1988). Thus, it was possible to identify specific sampling sites in the Skagerrak which would allow sampling under different hydrographic regimes on the basis of these earlier studies. A transect running from just off the Danish coast at Hirtshals to a point of the Swedish coast at Koster was chosen.

On the first KEYCOP Skagerrak cruises, intensive measurement programmes were carried out at the two ends of the transect. At intermediate stations, hydrography as well as distribution of state variables and plankton biomasses were recorded. In addition, some rate measurements (e.g. primary production) were determined. For the second series of Skagerrak cruises scheduled to take place in August-September, 2000, it is planned to carry out intensive measurements of all parameters and fluxes at an intermediate station on the transect as well as it is predicted that this station will be characterised by a very shallow pycnocline as compared to the other two stations.

Timing of the two Skagerrak sampling campaigns was designed to allow a relatively mixed (late winter-early spring) situations and period of maximum stratification (late summer).

THE AEGEAN: This study area was less well investigated than the Skagerrak and it was necessary to conduct a pilot cruise in September 1998 in order to select the sampling positions. This was done on the basis of hydrographic measurments as well as on a preliminary analysis of the side scan sonar imaging and acoustic profiling of the sea bed.

The northern Aegean displays very large horizontal discontinuities, such as the frontal region where Black Sea waters (BSW) enter the Aegean Sea, exiting from the Dardanelles as a surface current. The selection of the two main stations, was mainly based on two very different water columns: KA1 is characterised by the absence of the thin, low-salinity surface layer of modified BSW that forms the Dardanelles plume in the North Aegean. The full height of the water column is occupied by water of Levantine origin, and the stratification is almost entirely determined by temperature. Thus, the water column at KA1 is essentially one water mass, and the water processes can only be related to the level of stratification and mixing, and not to the interaction of two different water masses.

KA6, on the other hand, is within the core of the BSW plume, and is characterised by very high stratification at the interface between low and high-salinity layers. The two different water masses have distinct biogeochemical characteristics, as the Aegean is oligotrophic and the Black Sea mesotrophic. At KA6 the observed distributions of biogeochemical properties represent not only effects of stratification and mixing, but of the interaction of two distinct water-masses as well.

KA6 falls closer to frontal conditions than KA1 (normally out of the front), however, the true frontal conditions are met along the transect KA1 to KA6. Maintaining a 48-hour station within the frontal zone itself would be almost impossible, due to the high variability of its position.

The benthic stations were selected taking into account not only their location inside and outside the frontal system (as it was determined in the pelagic leg) but also the depth and type of substratum and form groups of similar depths and similar sediment types. Thus benthic stations are located following the KA1 – KA6 transect as well as a depth gradient in an East-West and North-West direction away from the frontal system.

RESULTS

KEYCOP is just past the half-way stage and as a consequence most of the data are still being worked up. We would like to illustrate the basic approach by using the modelling part of the project.

Marine ecosystem modelling can be viewed has having two complimentary roles. The first is a heuristic role, whereby it is used to corroborate a hypothesis, illuminate areas, which require further, study and identify where further empirical data is required. The second role is as a predictive tool, whereby the model is used to aid marine resource management and assess the impact of man on the marine ecosystem. The KEYCOP modelling strategy will focus on the heuristic role and as such, it's overall objective is,

- *To investigate, via numerical modelling, the seasonal evolution and controls exerted by vertical mixing processes on the flow and cycling of carbon and nutrients in the water column and sediments in the different hydrodynamic and nutrient regimes of the Skagerrak and northern Aegean Sea.*

The basic modelling tool to be used is a coupled physical biogeochemical water column model similar to that described in Allen *et al*. (1998). It is a synthesis of a one-dimensional vertically resolved water column model with a Mellor Yamada 2.5 turbulence closure and the European Regional Seas Ecosystem Model ERSEM (Baretta *et al*., 1995). ERSEM is a generic model, which describes both the pelagic and benthic ecosystems and the coupling between them in terms of the significant biogeochemical processes affecting the flow of carbon, nitrogen, phosphorus and silicon. The ecology described is not site specific and responds to the physiochemical environment that it is placed within. Having all significant pathways for energy in the modelled system means that it responds to the physical and chemical forcing in a way that is quantitatively correct under a wide range of conditions. A schematic representation of this model system is shown in Figure 1, illustrating the interactions between the physical, pelagic and benthic components of the system.

More specifically we will use ERSEM to investigate via simulation,

- *The short term response of the ecosystem to changes in the turbulence regime, including effects on the size and composition of the foodweb, the effects on grazing rates and detrital fluxes during the periods of the KEYCOP cruises*
- *The seasonal and annual response of the ecosystem and flows of carbon and nutrients including the benthic pelagic coupling to changes in the physio-chemical environment.*
- *The potential importance of the appendicularians, as a potential mechanism for the rapid export of fine detrital material from the euphotic zone.*

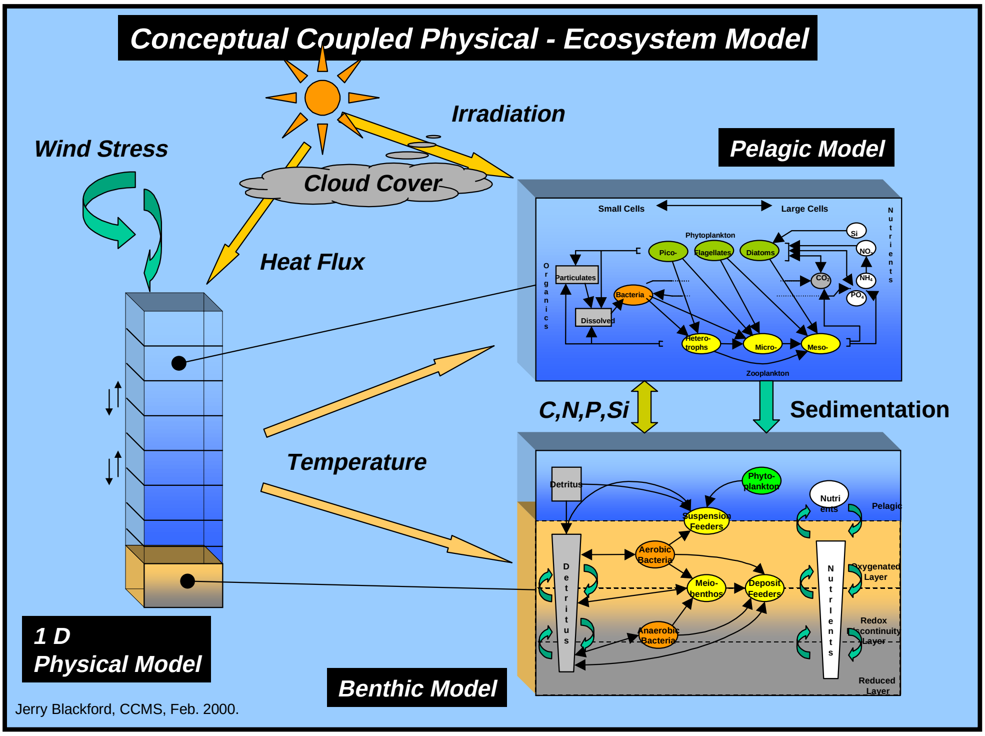

Figure 1

Simulation Workplan

Four simulation sites will be chosen two in the Skagerrak and two in the Aegean, reflecting regional differences and data availability. The following simulations will be made,

a) Short time scale simulations (up to two weeks in length) to look at the processes taking place during the data acquisition periods, focusing on the short term effects of changes in the turbulence regime on ecosystem functionality.

b) Seasonal and annual time simulations, to quantify the biogeochemical cycling of carbon, nitrogen and phosphate in the Skagerrak and N Aegean, including the benthic pelagic coupling.

c) An appendicularian model will be developed and used to simulate the data collected during KEYCOP. This model will then be embedded in ERSEM and run for the Skagerrak sites to investigate the potential significance of appendicularians as a mechanism for modifying the carbon and nutrient fluxes to the seabed.

The aforementioned simulations will be validated using data from the KEYCOP cruises, other data sets held by the KEYCOP partners and data sets in the public domain (e.g. ICES, NOWESP).

1.1 *DESCRIPTION OF THE MODEL SYSTEM*

Physical model

The General Ocean Turbulence Model, GOTM, (Burchard and Kristensen, 1999) is a one dimensional-water column model will allows for different combinations of momentum and tracer equations and gives a choice of standard turbulence parameterisations. GOTM will be coupled with ERSEM and used to calculate the vertical temperature, salinity, diffusion and momentum structure of the water column.

Ecological Model

ERSEM considers the ecosystem to be a series of interacting complex, physical, chemical and biological processes that exhibit coherent system behaviour (Baretta *et al*., 1995). A 'functional' group approach to describe the ecosystem whereby biotas are grouped together according to their trophic level (subdivided according to feeding method or size). Biological functional group dynamics are described by both physiological (ingestion, respiration, excretion and egestion) and population processes (growth, migration and mortality). State variables were selected to keep the model relatively simple without omitting any component that exerts a significant influence upon the energy balance of the system. A detailed description of ERSEM and its sub-models is beyond the scope of this report and further details can be found in (Baretta *et al*., 1995; Ebenhoh *et al*., 1997; Baretta-Bekker *et al*., 1995, 1997; Broekhuizen *et al*., 1995).

Pelagic Foodweb

A schematic diagram of the pelagic foodweb to be used in KEYCOP is shown in Figure 2. This has been modified form the original foodweb (Blackford, 1995) in the light of data collection undertaken in KEYCOP. The phytoplankton pool is described by five functional groups based on size classes collected in KEYCOP and trophic position as follows; diatoms (P1), size class 20 - 200 µm, eaten by micro and mesozooplankton; ultraplankton (P2), size class 0 – 1.2 µm, eaten heterotrophic nanoflagellates; picoplankton (P3), size class 1.2 to 3 µm, eaten by heterotrophic nanoflagellates and microzooplankton; nanoplankton (P4), size class 3.0 – 8 µm, grazed by microzooplankton and mesozooplankton; dinoflagellates (P5), size class > 8 µm, grazed by microzooplankton, mesozooplankton and krill.

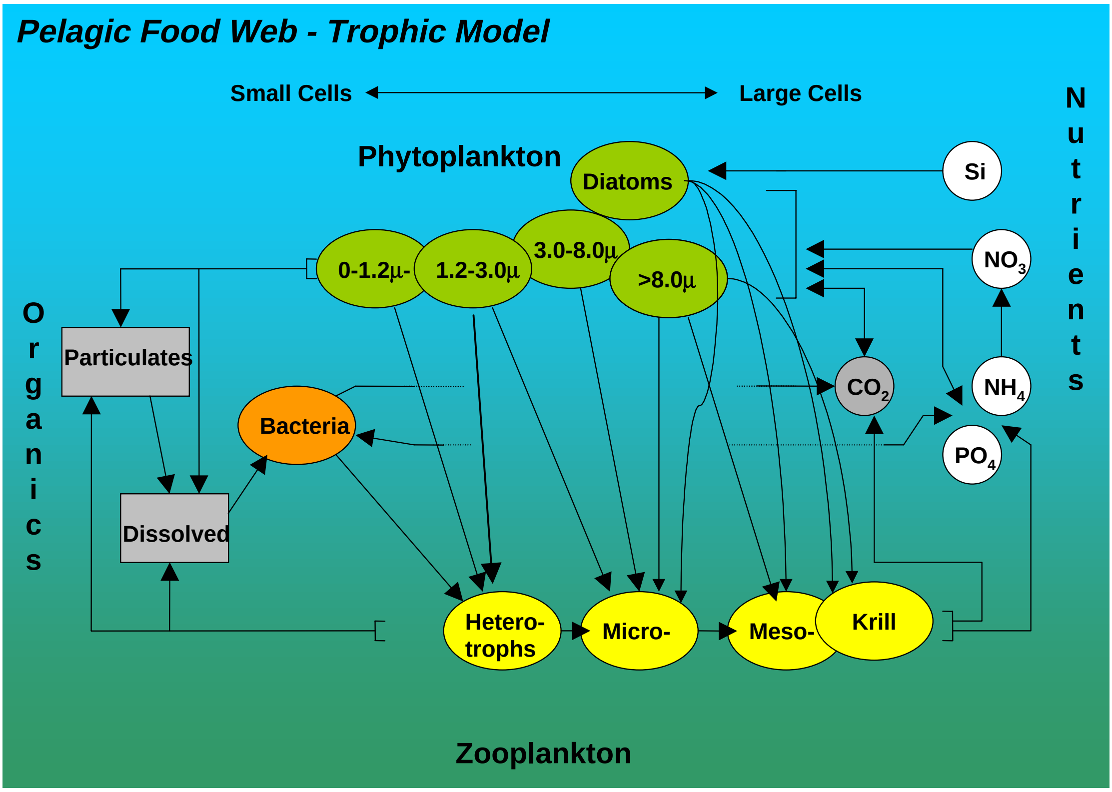

Figure 2

The heterotrophic components of the model are defined as: Bacteria act to consume DOC, decompose detritus and can compete for inorganic nutrients with phytoplankton. Heterotrophic flagellates (Z6) feed on bacteria and picoplankton, are grazed by microzooplankton and are cannibalistic. Microzooplankton (Z5) consume nanoplankton, diatoms, dinoflagellates and heterotrophic flagellates, are grazed by mesozooplankton and are cannibalistic. Mesozooplankton (Z4) consume diatoms, nanoplankton, dinoflagellates and microzooplankton and are cannibalistic. Krill (Z3) are assumed to be herbivorous crustacea (~1 cm in size) feeding on diatoms and dinoflagellates (Falk-Petersen *et al.*, 1990). The particulate organic carbon variables will be subdivided into fast and slowly sinking detritus.

In addition a submodel of appendicularians will be constructed which will be parameterised and validated using the experimental work undertaken in KEYCOP. Appendicularians will be represented as non-selective filter feeders, consuming everything between 0.2µm and 30µm, so they eat everything in this size range. An additional functional group will be added to represent the detrital pool created by the houses shed by appendicularians.

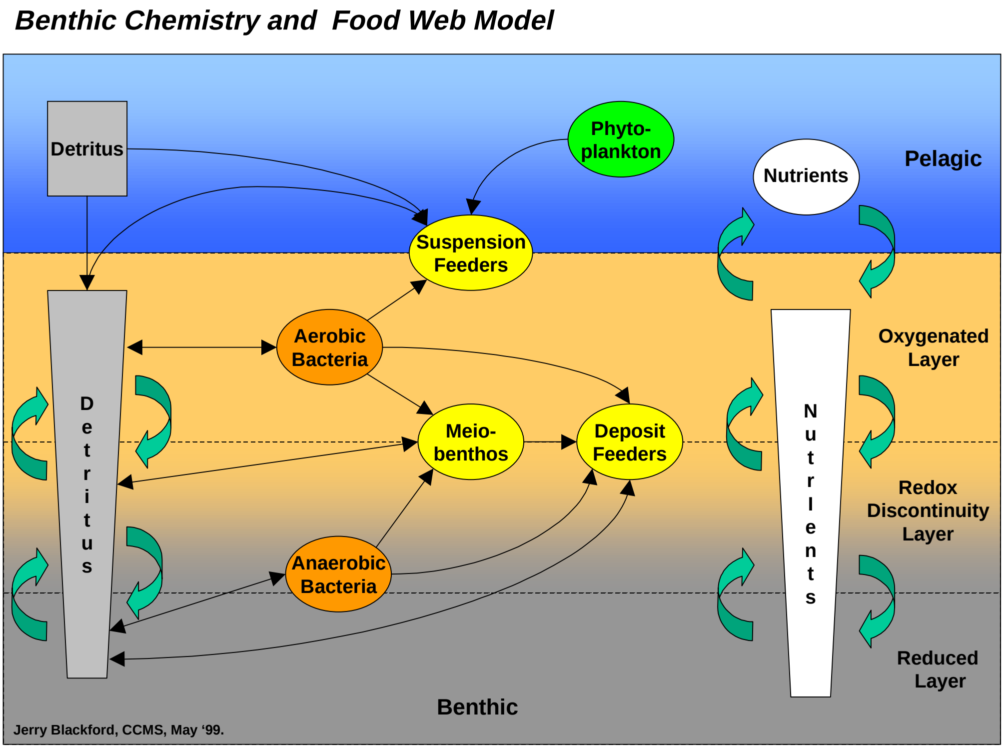

Figure 3.

Benthic Ecosystem Model

The benthic submodel (Figure 3) describes nutrient and carbon cycling via both aerobic and anaerobic bacterial pathways, a zoobenthic foodweb, bioturbation/bioirrigation and the vertical transport in the sediment of particulate matter due to the activity of benthic biota (Ebenhöh *et al*., 1995; Blackford, 1997). The benthic pelagic coupling is described by the inputs of settling organic detritus into the benthos and diffusional nutrient fluxes into and out of the sediment, which are influenced by zoobenthic activity.

SUMMARY

KEYCOP is having its midterm review in June 2000. At the review the hypotheses being tested will be evaluated and the progresss assessed. We expect the data obtained from the four cruises to be of fundamental importance in understanding nutrient fluxes and their effects on biota in two very distinct types of European areas. The modelling component will enable us to make predictions concerning nutrient cycling. Such predictions will be relevant to mangement issues related to nutrient inputs to European coastal waters.

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TITLE IMPORTANCE OF DISSOLVED ORGANIC MATTER FROM TERRESTRIAL SOURCES FOR THE PRODUCTION, COMMUNITY STRUCTURE AND TOXICITY OF PHYTOPLANKTON OF THE EUROPEAN ATLANTIC AND BALTIC COASTAL WATERS; ROLE OF MICRO-PREDATORS FOR TRANSMISSIONS OF TOXINS TO COMMERCIAL SHELLFISH AND FISH LARVAE : **DOMTOX**

CONTRACT NUMBER: **MAS3-CT97-0149 and IC20-CT98-0109**

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DOMTOX

BACKGROUND

Two major hypotheses have been invoked to explain increase in importance of toxic dinoflagellates and cyanobacteria blooms in coastal waters: (1) Continuous relative depletion of silica in river discharge would led to diatoms being replaced by non-siliceous forms. (2) Organic compounds from land drainage have favoured auxotrohic species, such as dinoflagellates. DOMTOX focuses on hypothesis (2).

Some toxic dinoflagellates are mixotrophic and have been hypothesised to feed on cyanobacteria and heterotrophic bacteria, as well as ciliates. On this basis, it was hypothesized that micropredators' phagotrophy is a route for the transfer and accumulation of toxins in commercial molluscs and fish larvae.

OBJECTIVES

(i) To investigate whether uptake of dissolved organic nutrients from terrestrial sources promote the growth of toxin-producing microalgae and/or cyanobacteria, and thus influence the community structure.
(ii) To investigate whether dissolved organic nutrients from terrestrial sources can promote toxin production in toxin-forming microalgae and cyanobacteria.
(iii) To determine whether the predation of cyanobacteria may be a source of toxins in toxic dinoflagellate blooms, and to investigate whether the predation of cyanobacteria by ciliates and toxic mixotrophic dinoflagellates is a pathway for the transfer of toxin to commercial filter-feeding shellfish and fish larvae.

MAIN ACTIONS AND RESULTS TO DATE

Effects of DOM from land sources on the biomass, productivity, community structure and toxicity of potentially toxic phytoplankton from near-shore to open sea

In May, June and July 1999, seawater was collected in the Gulf of Riga, along the gradient from the Daugava River to the open Baltic Sea. Five stations were visited with the Estonian R.V. "Arno". Four samples per station were taken. After the second and the third cruises, aliquots of water collected in the upper layer of the station situated in the centre of the gulf and the station situated in the open Baltic were differentially enriched. Incubation was carried at the Pärnu Biological Station, Estonia. The dissolved organic matter (DOM) from land origin used for these experiments was extracted a few days before inoculation from water of the Pärnu River. Data are presented in a report entitled "Gulf of Riga Report" .

Effect of DOM from land sources for the growth and toxin content in the cyanobacteria *Microcystis aeruginosa*, strain MAGR-2, isolated from the Gulf of Riga

DOM >1000 daltons was extracted in early May 1999 from the river Pärnu, Estonia. The extract contained 601 µM DON (dissolved organic nitrogen). Two experiments were carried out.

Experiment 1. The culture medium was prepared with water sampled in the Gulf of Riga. Addition of nutrients (NO_3, PO_4, and DOM as DON and DOP) was done on the beginning of experiment. Seven different enriching treatments (triplicates) were implemented. During the first week (Days 5-6), the best growth was related to the addition of NO_3+PO_4 and PO_4+DOM, while the weakest growth occurred in the treatment +DOM (Figure 1). At the end of the experiment, the best growth was found in the PO_4+DOM treatment. Although results are not really clear, it was assumed that in nitrogen-limiting conditions, *Microcystis aeruginosa* strain MAGR-2 can use DON as nitrogen source, and that DOM >1000 daltons can contribute to the nutrition of that species.

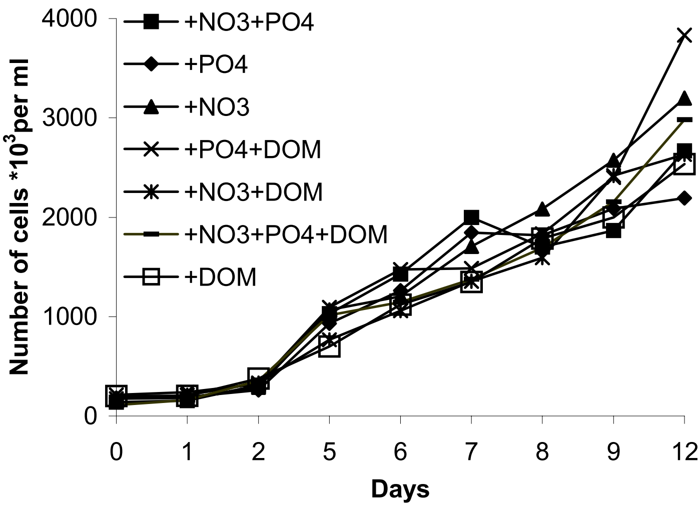

Figure.1. Cell concentration versus time, in *Microcystis aeruginosa* cultured in varied nutrient treatments

Experiment 2. Batch cultures (4 litres) were carried out in 5-liter P.C. bottles. Nutrients were added daily during 7 days. Growth was monitored daily by measuring *in vivo* fluorescence and cell counting. Biomass, toxin content, bacterial production and concentrations of NH_4 and urea were analysed on critical days. Results show that the presence of DOM sustained growth (Figure 2).

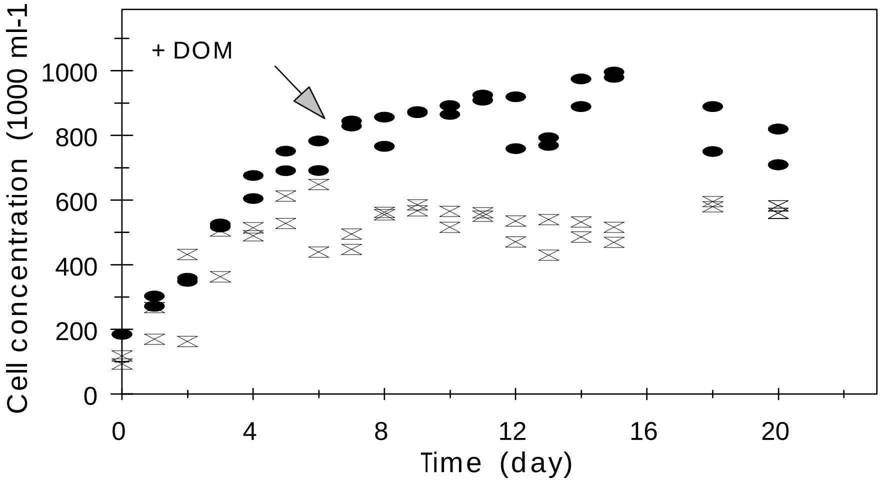

Figure 2. Cell concentration versus time, in *Microcystis aeruginosa* strain GR-2 grown in the presence and the absence of DOM from land origin

Effect of DOM for the growth and toxin content in several Baltic strains of the cyanobacteria *Nodularia spumigena*

The toxin profile and the genetic identity of *N. spumigena* strains isolated from Askö (Stockholm archipelago) and the Sound of Kalmar have been first investigated. The toxins produced by these strains are nodularins. However, the nodularin produced appeared to be different in strains from Askö compared to those from the Sound of Kalmar. The genetic identity of these strains is not correlated with their origin, *Nodularia spumigena* KAC 13 is isolated in the Sound of Kalmar but is nevertheless closer related to the strains from Askö (Table 1). That these strains are identical to those that occur in the open Baltic Sea has also been confirmed.

Table 1. Type of nodularin produced by and genetic type of *Nodularia spumigena* strains from different areas of the Baltic Sea. *KAC; Kalmar Algae Collection

Strain	Isolated from	Nodularin type	Genetic type
KAC 10*	Kalmar	A	α
KAC 12	Kalmar	A	α
KAC 13	Kalmar	A	β
KAC 67	Askö	B	β
KAC 68	Askö	B	β

In previous DOMTOX studies the toxin content of *N. spumigena* was found to be higher in phosphate depleted stationary phase of cultures than in exponentially growing cells. This was in contrast with the only earlier report that the toxicity of *N. spumigena* does not depend on nutrient status.

Effect of DOM for the growth and toxin content in *Prymnesium patelliferum*

The hypothesis that *P. patelliferum* could use nitrogen from terrestrial DOM (DON), either directly or indirectly via phagocytosis of bacteria was tested in semi-continuous culture. Five different ratios of nitrate and DON-nitrogen (1, 0.75, 0.5, 0.25 and 0) were supplied at a total nitrogen concentration of 20 µM.

P. patelliferum used the added DON with an efficiency slightly lower than 0, *i.e.* the DON additions had a slightly negative effect on the cell yield on nitrate. Possibly, this was caused by growth of bacteria on the humic acids, and subsequent nitrogen uptake by those bacteria. Alternatively, there could have been a direct negative effect of bacteria growing on the humic material. This hypothesis is supported by the fact that axenic cultures showed a higher maximum specific growth rate than non-axenic cultures.

Toxicity was determined by measuring the haemolytic activity of a methanol cell extract, as compared to a standard, saponin. *P. patelliferum* appeared to be toxic, but the toxicity was not correlated to the fraction of humics in the available nitrogen (Figure 3). It was inferred that terrestrial DON will not promote the growth of *P. Patelliferum* directly, and neither will it promote or induce its toxicity.

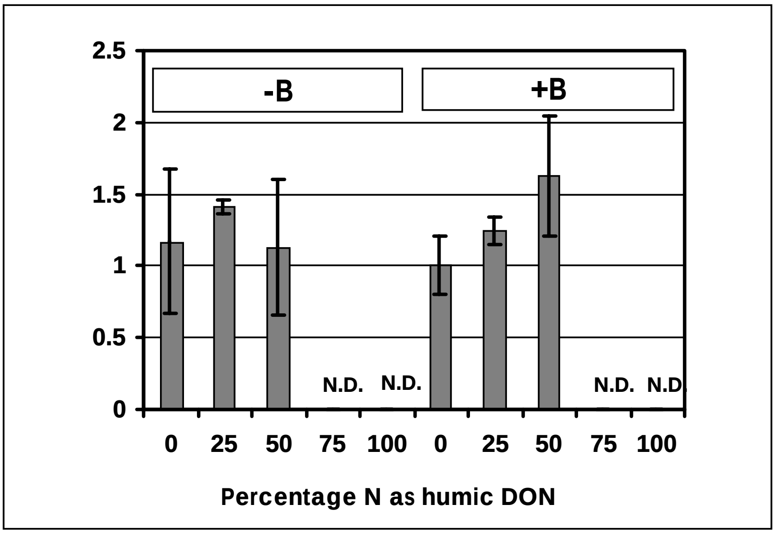

Figure 3. Toxicity of *Prymnesium patelliferum*, measured as haemolytic activity, at 5 different DON : NO_3 ratios in cultures where no bacteria could be detected at the start of the experiment (-B) and in cultures with bacteria (+B). N.D. = not detectable

Influence of DOM from land origin for growth and toxin content in *Alexandrium minutum* AM89BM

Since vitamins and trace metals are well known to be growth regulators in algae and are often present in DOM, the effect of addition of DOM with those of addition of vitamins + trace metals was investigated. These additions were combined with three different NO_3:PO_4 ratios. Inorganic nutrients present in the DOM extract were taken in account to calculate the addition of NO_3^- and PO_4^3. Cultures were sampled during exponential growth phase, at the end of growth phase and at stationary phase.

As previously reported, addition of DOM enhanced the final biomass. However, addition of vitamins + trace metals more markedly enhanced the biomass than did the addition of DOM (Figure 4). Toxin samples are still under analysis.

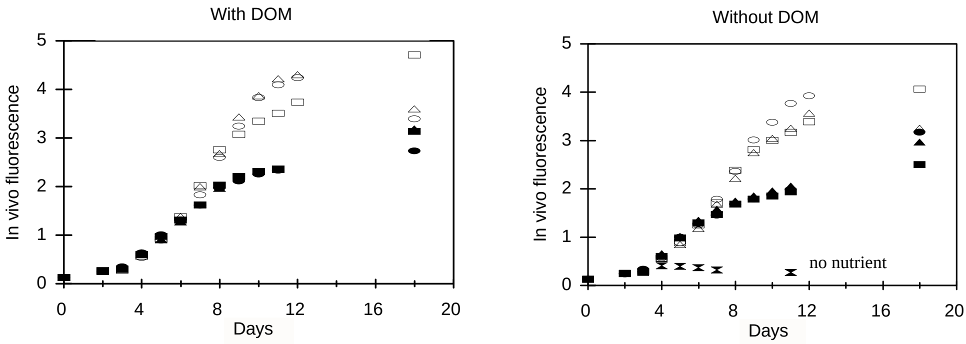

Figure 4. *In vivo* fluorescence versus time, in *Alexandrium minutum* grown under different inorganic N and P enrichments, with and without addition of DOM from land origin or vitamins+trace metals. Open symbols: with addition of vitamins+trace metals; black symbols: no addition of vitamins+trace metals. Squarres: N:P = 80; ovals: N:P = 16; triangles: N:P = 5.5. The control condition without any enrichment is quoted "no nutrient" on the figure.

Influence of DOM from land origin on the growth and the toxin content in *Alexandrium tamarense* KAC-2

Preliminary experiment led to conclude that *A. tamarense* requires the presence of bacteria in order to grow. Hence, continuous cultures (0.1 day^{-1}) were carried out in media prepared with filtered (0.45 µm) and autoclaved water taken from the Baltic Sea. The treatments corresponded to a gradient of dissolved organic nitrogen (in the DOM extract) and nitrate concentrations in the media from 0 to 100 %.

A. tamarense used DOM as a nitrogen source, either directly or indirectly (via bacteria). Cell yield was highest when NO_3:N-DON ratios were between 0.25 and 0.75 (Figure 5). However, even in the medium with no nitrate, the cultures were able to reach significant cell concentrations with N-DOM as sole source of nitrogen. *A. tamarense* reached higher cell concentration in the treatment NO_3:N-DON = 50. Thus, DOM possibly provides an unknown growth factor that stimulates the growth.

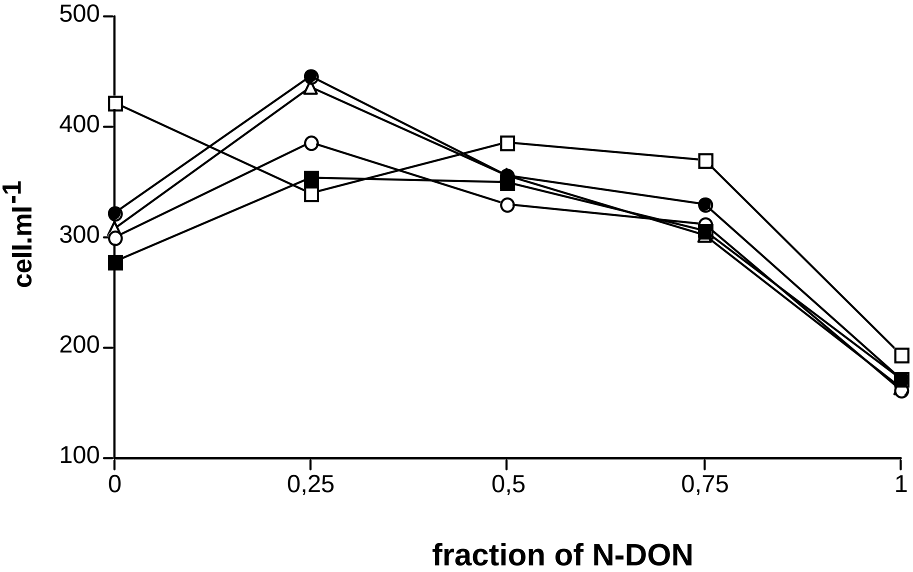

Fig. 5: Cell densities of *A. tamarense* grown as semi-continuous cultures in the different treatments (ratios of nitrate/N-DON with a constant total N concentration of 20 µM). Lines correspond to sampling days: —□— day 50, —■— day 53, —○— day 57, —●— day 61, —△— day 65.

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TITLE : EFFECTS OF EUTROPHICATED SEAWATER ON ROCKY SHORE ECOSYSTEMS STUDIED IN LARGE LITTORAL MESOCOSMS : **EULIT**

CONTRACT N° : MAS3-CT97-0153

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ARE ROCKY SHORE ECOSYSTEMS AFFECTED BY NUTRIENT ENRICHED SEAWATER SUCCEEDING TWO YEARS EXPOSURE

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INTRODUCTION

The overall objective of this study is to predict the response of rocky shore ecosystems to increased nutrient availability. Specific objectives are:

- to investigate structural and functional changes of rocky shore ecosystems as a result of nutrient enrichment.
- to assess the response of community metabolism to increased nutrient supply through measurements of changes in oxygen, nitrogen and phosphorus in enclosed sub-systems.
- to test if increased nutrient load stimulates the production of individual algal populations and communities (e.g. periphyton, ephemeral and perennial algae) and total system productivity.
- to examine whether the composition of the faunal community changes in response to structural changes of the algal assemblage.
- to examine possible correlation's between feeding preferences by key herbivores and algal food quality.

Increased nutrient inputs from land have led to eutrophication of many European coastal areas, which have caused widespread environmental deterioration (e.g. Sand-Jensen and Borum 1991, Duarte 1995). Well-known effects of eutrophication include loss of seagrass cover, increased dominance of ephemeral macroalgae and noxious blooms of Phaeocystis (Lancelot 1990, Wennberg 1992, Duarte 1995).

Eutrophication effects are rather well described from phytoplankton and seagrass based ecosystems. In contrast, the present knowledge of the effects of increased nutrient inputs to rocky shore ecosystems is largely qualitative. Rocky shores are important components of the coastal ecosystems along the Atlantic coasts of Europe and in the Baltic. These ecosystems are dominated by macroalgal communities, which, together with their epiphytes, provide substantial amounts of organic matter. The high primary production of rocky shore ecosystems supports a complex and highly productive animal community, consisting of both sessile and motile invertebrates that live on the produced algal matter. The majority of the fauna is made up by herbivores such as mussels, periwinkles, amphipods and isopods, and their community structure is expected to depend on the composition of the algal community.

Increased anthropogenic nutrient input to coastal ecosystems is expected to stimulate the growth rate of photosynthetic organisms (Moncreiff *et al.* 1992) on rocky shores and, therefore, also the total productivity of the ecosystem. Increased production do, however, not occur solely by the increased growth rate of the existing vegetation, but also due to structural changes in the algal communities where slow-growing types are replaced by faster-growing types (Borum *et al.* 1990, Bokn *et al.* 1992, Pedersen & Borum 1996).

The critical nutrient loading needed to exceed the buffering capacity of an ecosystem and, thus, lead to undesirable effects is presently unknown. Quantitative knowledge is, therefore, needed in order to be able to predict the responses of rocky shore ecosystems to increased nutrient inputs. Experimental ecosystems (i.e. mesocosms) are now regarded as being an essential tool when studying marine pollution because they bridge the gap between single-species tests (laboratory) and the natural environment (field experiments). Mesocosm experiments have some clear logistical advantages; experimental installations such as settling panels, cages, electrodes, etc. are better protected against accidental or deliberate damage, and sampling is very well controlled.

METHODS

Eight land-based mesocosms designed for hard-bottom littoral communities were prepared for the EULIT project in order to investigate the response of rocky shore ecosystems to enhanced nutrient loading. Littoral communities were initiated by transferring small boulders with associated macroalgae and fauna from the Oslofjord into the mesocosms two years ahead of the start of the project. Natural development during the pre-project period have resulted in a luxuriant flora and fauna resembling that in the out-site fjord. The mesocosms are fed with water from 1 m of depth in the Oslofjord and the water turnover rate is 2½ hours. The inflowing water also acts as a source for spores, zygotes and larvae. A tidal regime resembling that in the fjord (±0.36 m amplitude) is maintained and waves are regularly generated.

Pilot projects were conducted in 1997 in order to develop relevant techniques and statistical design for the EULIT experiments ahead of the nutrient manipulation. Nutrient addition started in May 1998, thus providing an appropriate length of the experimental period with 2 ½ years of nutrient enrichment. The nutrient enrichment of the mesocosms corresponds to: +0, +1, +2, +4, +8, +16 and +32 μmol DIN L^{-1} above the natural concentration, keeping the molar NP-ratio at 16. The concentrations of dissolved and total nutrients and carbon are measured on an average weekly basis in the in- and out-flowing water. Measures of community structure and processes, such as species composition, biomass, growth and grazing, are studied during intensive field campaigns (spring, summer and autumn) each year. Automatic registration equipment ensures continuous measurements of oxygen concentration, insolation, temperature and salinity in all mesocosms.

Recruitment and succession is followed by mounting artificial substrates (granite chips and ceramic tiles) acting as substrate for settling macroalgae and periphyton in the mesocosms over varying length of time. Recruitment, growth and succession among macroalgae are measured by means of 10x10 cm granite chips. Long-term (LT) response is measured by mounting 36 chips in each of four basins (+0, +2, +8 and +32 μmol N L^{-1}). Three chips are harvested every second month during summer months. The short-term (ST) responses are measured by harvesting three randomly selected chips every month, replacing them with new, sterile chips. The LT and ST chips are analyzed for biomass (DW and AFDW), taxa composition, cover degree of sessile organisms and counts of mobile fauna.

Macroalgal cover and biomass are assessed using different methods depending on the sampling area (i.e. steps, walls, bottom and wave bar of the mesocosms). Cover of macroalgae on the steps is measured within quadrates of a grid system (frame) established in each basin. The cover is determined with help of a sub-sampling grid divided in to 25 sub-squares and algal cover is quantified in 16 quadrates, 4 on each step, in each basin. Each quadrate is further photographed during each sampling time for documentation. The biomass of macroalgae growing on the steps is finally computed by combining the cover estimates with estimates of species specific biomass per unit of cover ratios obtained through destructive sub-sampling. The biomass of macroalgae growing on the walls, bottom and wave bar is obtained from destructive sub-sampling alone. These sub-samples also provide material for the determination of carbon, nitrogen and phosphorus content in macroalgal species from each basin.

The biomass and species composition of macroscopic epiphytes on fucoids, are investigated by scraping of all epiphytes on 8-10 randomly selected specimens of *Fucus* spp. collected in each mesocosm.

Growth and production of individual key species of macroalgae and the periphyton community are measured using species specific methods (increase in length or biomass for macroalgae, increase in chlorophyll content for periphyton communities).

The effect of epiphytes on host algae are investigated by positioning 8 pre-weighed young individuals of *Fucus* spp. free of visible epiphytes in all basins, next to 8 specimens with natural epiphytic cover and comparing the growth rate of the hosts over time.

Fauna abundance and biomass are estimated in each basin during each campaign. The amount of large motile and all sessile animals are determined by frame counting on the steps in all basins. Smaller mobile animals (macrofauna and meiofauna) are estimated by collection from traps and/or artificial substrates. The total abundance of each fauna group is estimated from knowledge of the total proportion of different substrates in each mesocosm. Biomass is estimated from standardized weight analyses of the different species.

The abundance and biomass of blue mussels on the wave bar are estimated by sub-sampling from 8 x 8 cm frames during all campaigns. The fauna associated to the blue mussel colony is analyzed within the same process. Habitat *versus* food preference is studied in a mesocosm experiment carried out at +0, +16 and +32 μmol N L^{-1}. Aquarium experiments are used to test more specific hypotheses regarding food quality and survival and growth of *Gammarus* sp.

System metabolism is measured from continuous measurement of oxygen concentrations in each mesocosm. Water-flow, oxygen concentration and temperature are continuously measured in each basin, while salinity is measured in one mesocosm; water depth is measured in three mesocosms. Light intensity in the air, air temperature and barometric pressure are measured are measured continuously. The daily variation in oxygen concentration is used to calculate ecosystem gross primary production and ecosystem oxygen consumption for each day. Measurements of metabolism in sub-systems (communities) are performed in transparent enclosures with a volume of 20 L that are incubated in the mesocosms.

RESULTS

The effects of nutrient enrichment were few and very small during the first year of nutrient addition, while the effects were more obvious during the second year. The algal communities responded to nutrient enrichment by increasing their tissue nutrient concentrations – the higher the nutrient enrichment, the higher the tissue nutrient concentrations.

The growth rate of the periphyton communities were stimulated by nutrient enrichment and they increased their biomass with increasing nutrient richness, particularly during May and July 1999. The response was fast since their C:N and C:P almost remained constant across nutrient treatments. Macroalgae were also stimulated by nutrient enrichment, but the response differed depending on algal type. Fast-growing macroalgae such as *Ulva lactuca* had their growth rate stimulated much by nutrient enrichment, while the response was much less evident among the fucoids and non-existing among the laminarians.

Recruitment and succession on artificial substrates measured over short time scales (ST) have so far only revealed small variations with different nutrient enrichment indicating that the recruitment potential are equal among treatments. Recruitment and succession on artificial substrates measured over longer time scales (LT) have, in contrast, shown that the developing algal community differs significantly with nutrient treatment. Thus, fucoids tend to dominate the communities at low nutrient enrichment, also resulting in quite high biomass, while fast-growing algae become almost entirely dominating at high nutrient enrichment.

The macroalgae communities have been strongly dominated by brown perennial species since the start of the project as these long living species accounted for, on average, 82% of the total macroalgae biomass. The structure of the macroalgal communities have not change significantly as a result of nutrient enrichment during the first16 months of nutrient addition. Although nutrient addition has promoted a marginal increase in macroalgal diversity due to a slight increase in the abundance of fast-growing algal types (i.e. periphyton and ephemeral macroalgae), the abundance of slow-growing macroalgae (fucoids) remain unaffected by the enrichment. The total biomass of the macroalgal communities was lower in 1999 than in 1998, but the biomass did not vary systematically as a function of nutrient enrichment. Differences in nutrient additions did not affect the size spectra of the autotrophic biomass either.

Epiphytic macroalgal communities were dominated by red macroalgae at low nutrient enrichment and by green species at high nutrient addition. Despite these differences, the biomass of macroscopic epiphytes on *Fucus* spp. did not increase with increased nutrient enrichment.

The fauna communities have only changed little in response to nutrient treatment. Apart from an increase in the abundance of the common periwinkle, *Littorina littorea* and a weak increase in the abundance of the small isopod *Jaera* spp at high nutrient loading, no significant changes in the Solbergstrand mesocosm fauna have appeared after two years of nutrient addition. Apparent grazing rates by herbivores are very low and not detectable in all basins. Thus, grazing seems not to be important as a loss process for the algal communities and, therefore, not as a structuring force either.

Total system metabolism (i.e. system productivity measured from oxygen balances) tended to increase slightly with increased nutrient loading, although not significantly so. When the metabolism of sub-communities of green, red and brown algae were measured along the nutrient gradient, no difference in weight specific production could be detected for any of the groups. As there was no effect of nutrient treatment on the weight specific production or on the biomass of the three alga groups, it seems logical that there is no effect on the basin gross primary production. Whole basin respiration rates were much lower than the production rates and did not differ in response to nutrient treatment either.

Monitoring of the export of matter from the mesocosms through the pipeline outlets showed that the total export of macroalgae was small and relatively insignificant compared to the total biomass and productivity of algae. However, the export of certain algal types (e.g. sheet-like and filamentous algae) was relatively large compared to their biomass and production. A considerable fraction of the fauna produced in the mesocosms (especially amphipods and isopods of the family Idoteidae) was exported.

CONCLUSIONS

Increased nutrient loading enhanced nutrient uptake and subsequently the tissue nutrient concentration in all algal types investigated in this study. Accumulation of periphyton biomass on empty substrates and community growth rates were greater at high nutrient loading, indicating that the periphyton communities were nutrient limited during mid-summer under ambient conditions. Fast-growing macroalgae, such as *Ulva lactuca*, showed the same response to increased nutrient availability (except the highest level), whereas slow-growing, perennial macroalgae, i.e. species of *Fucus* and *Laminaria*, only responded marginally to nutrient enrichment. Thus, increased nutrient loading will stimulate the growth and productivity of fast-growing algal types (i.e. periphyton and ephemeral macroalgae) while the productivity of more slow-growing macroalgae may remain unaffected by increased nutrient availability.

Tissue nutrient concentrations, CN, CP and NP-ratios indicated that phosphorus was the main limiting nutrient in ambient seawater from the Oslofjord during summer for both the periphyton community and the macroalgae. Hence, increased P-loading is expected to stimulate growth and productivity of these algal types.

However, no significant changes in macroalgal community structure have been observed over the 16 months of nutrient enrichment, although the growth of fast-growing algae were stimulated by increased nutrient loading. The recruitment and succession experiments have shown that the recruitment potential are similar along the nutrient gradient and, that post-recruitment processes (i.e. stimulated growth of certain species, differential grazing etc) must be responsible for the observed dominance of ephemeral algae at high nutrient loading in these primary communities. Then why haven’t we seen any such changes in community structure within the established communities?

First of all, the established communities are heavily dominated by large perennial species. These species are only expected to disappear with increased nutrient loading if shading from epiphytes become severe. Our results showed that epiphytic load (biomass) was unaffected by increased nutrient loading. Secondly, the growth rate of ephemeral species was indeed stimulated by increased nutrient availability, but why then, do they not become more dominating with time? One obvious reason could be that most herbivores prefer ephemeral algae due to higher nutrient content and lower content of phenolic compounds etc. However, our results showed that apparent grazing was very low and certainly insignificant relative to the production of matter. Herbivores are thus not responsible for the lack of dominance by ephemeral algae.

Instead, physical disturbance created by the wave bar in the mesocosms may be much more important for the structuring of the algal communities than initially expected. Thin and delicate macroalgae (i.e. the ephemerals) are detached from their substrate and exported from the basins much more easily than the perennial species (i.e. *Fucus, Ascophyllum, Laminaria*). Accordingly, the export of ephemeral algae almost equaled the productivity, which does not of course, allow significant changes in biomass in the basins to occur.

As the structure of the algal communities did not change significantly over time, it is not expectable that the fauna communities would change since availability of food and its quality are more or less similar along the nutrient gradient.

In summary, we can conclude that increased nutrient loading to hard-bottom littoral communities dominated by perennial algae will stimulate the growth of ephemeral algae as expected. The stimulated growth will, however, not result in significant changes of the community structure in areas with wave exposure, because the ephemeral algae will be removed by physical disturbance before they will cause the large perennial algae to disappear due to increased shading. If the substrate are completely cleared for perennial algae (by storms, ice-scouring, outbreak of grazers etc) ephemeral algae may be able to colonize and become dominant if the nutrient loading is high enough. Well-established communities of perennial algae seem, therefore, to be important as buffers to increased nutrient loading. Increased nutrient loading are only expected to be followed by blooms of ephemeral algae in sheltered areas such as small semi-enclosed bays, harbours etc.

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TITLE : LONG-TERM CHANGES IN BALTIC ALGAL SPECIES AND ECOSYSTEMS : **BIOBASE**

CONTRACT N° : **MAS3-CT98-0160**

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THE BALTIC SEA – YOUNG AND POOR

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INTRODUCTION

The Baltic Sea is the World's largest brackish water sea and it provides a unique salinity gradient ranging from brackish to marine from the Baltic Sea, through the Danish belts to the Kattegat-Skagerrak area. Furthermore, the area is relatively young as a brackish water area as the Littorina Sea came into being approximately 7.500 BP. At that time the salinity in the Bothnian Bay and the Gulf of Finland was higher (ca. 8 psu) than present day salinities as can be seen from *Littorina littorea* in deposits. Also marine, benthic algae from adjacent floras could penetrate into the Littorina Sea presumably with an advantage to those species already adapted to changing or low salinities, for example the littoral species. The Littorina period came gradually to an end about 3.000 BP as the Baltic Sea became colder and less saline. This change in the environmental conditions was a serious adaptive challenge to the organisms during the transition period: they could either adapt to the adverse conditions or become extinct (Russell 1985). The present day macroalgal flora in the Baltic Sea is a filtrate of the Skagerrak-Kattegat flora with a characteristic attenuation in species number and well-documented physiological adaptations sometimes associated with morphological changes. In some cases genetic differentiation has been found, but no apparent hybridization has occurred in the contact zones.

BIOBASE has the last two years explored an overall three-pronged objective: 1) to establish a comprehensive culture collection of marine, benthic algae from the Baltic proper and from the transition area, 2) to conduct experimental studies of ecotypic responses to salinity and temperature and to analyse growth, dispersal, and distributional data based on literature, which has accumulated during the last 100 years, and 3) to analyse genetic population structure including phylogeographic structure for selected key species/complexes, *Ceramium tenuicorne, Enteromorpha intestinalis and E. compressa, Chorda filum, Fucus serratus* and *Fucus evanescens.* The latter species was introduced in southern Scandinavia in the last century and recently recorded in the western part of the Baltic Sea.

RESULTS

Culture collection

The comprehensive culture collection consists at the moment of more than 40 species in unialgal cultures. Some of the most common species, for example *Pylaiella littoralis, Enteromorpha intestinalis/compressa, Scytosiphon lomentaria, Chorda filum,* and *Ceramium tenuicorne* comprise of more than 20 strains collected along the entire distribution area from Skagerrak-Kattegat into the Baltic proper.

Salinity and temperature ecotypes

The experiments on salinity and temperature tolerances as well as optimal requirements for growth and reproduction in the green alga *Enteromorpha intestinalis* are in progress and focus at the moment on material from Varberg (local salinity 25 psu) and Gräsö (local salinity 5 psu). The results indicate that the Varberg strain is euryhaline as the specific growth rate does not change with salinity. The Gräsö strain, however, shows some adaptation to low salinity by having significantly reduced specific growth rate at 30 psu compared to lower salinities.

Experiments were undertaken to test growth and reproduction in the red alga *Aglaothamnion tenuissimum* from Kristineberg (eastern part of Kattegat) under various salinity regimes. The results show that in addition to a detailed knowledge on the nuclear phase of the plants studied, it is also necessary to know the gender of the gametophytes before meaningful comparisons can be made.

Dispersal analyses

The relationship between species richness and structural stability, *e.g.* variation in species number, turnover and sum of changes in relative abundance in natural macroalgal communities in three districts in Danish waters was examined annually in 120 macroalgal communities over a nine year period. Variation in species number and turnover of species from year to year declined significantly with increasing species richness, and the sum of changes in relative abundance was not related to species richness. We were able to separate the effect of species richness from possible effect of salinity, eutrophication and availability of light and substratum. Communities within the three districts followed the same stability pattern although they differed in terms of environmental stress and variability. The overall conclusion is that species richness increases the stability of macroalgal communities.

The relationship between local abundance and geographical-range size of benthic macroalgae in four Scandinavian brackish regions (Hardanger Fjord, Kattegat, Baltic proper, and the entire Baltic Sea area) of different size and gradients in salinity was studied. We found significant positive relationships between abundance and range for the entire assemblage of macroalgae as well as for red, brown, and green algae alone in all four regions. The positive relationships were strongest in the most homogenous regions. The positive relationships also existed for algae of characteristic morphology and size. In the entire Baltic Sea area the positive relationships were significantly stronger for large, canopy-forming algae than for small species, while no significant differences among morphological type and size groups were found in the other smaller regions (Sand-Jensen et. al., submitted).

In Danish esuaries species richness of marine macroalgae increased significantly with reduced nutrient richness and greater vertical extension of macroalgal growth was apparent in more transparent waters from the inner estuaries to open coastal waters. Rank-abundance curves did not change between individual sites across the environmental gradients. However, summed rank-abundance patterns for several sites within larger areas showed significantly steeper initial slopes and low evenness in the inner estuaries than in open waters. This pattern was due to high rank consistency of dominant species and variable presence of rare species among sites in inner estuaries of low habitat complexity. In open waters of high habitat complexity, rank consistency was low, and the summed species abundance across sites had a high evenness. The results imply that the scale of the study, habitat complexity and community variability observed at a particular scale, is the main determinant of abundance patterns.

Population differentiation (species level)

Enteromorpha intestinalis and *E. compressa*

Enteromorpha intestinalis and *E. compressa* are morphologically very plastic with many overlapping characters and therefore difficult to distinguish from each other. Branching of the thalli is often regarded as the most important morphological difference between the two species and *E. intestinalis* is unbranched while *E. compressa* can be highly branched. Both species have been collected in a large number of sites in the Baltic Sea area and in some extra-Baltic localities. ITS1 and ITS2 sequences were obtained for indiviuals from all sites for a safe species identification. The results show that the solid morphological feature for species identification (branched/unbranched) no longer can be maintained.

Ceramium phylogeny and biogeography

Species of *Ceramium* that are represented in Scandinavian waters have been analysed using molecular tools and crossing experiments in unialgal culture. Our molecular data are based on sequences of the rubisco spacer (the intergenic spacer between the large and small subunit genes coding for the Rubisco enzyme) in the chloroplast genome and the ITS2 (internal transcribed spacer) and parts of the LSU (large subunit) of nuclear ribosomal DNA. We have analysed 45 samples from our culture collection and are in the process of including herbarium specimens of taxa that were originally described from Scandinavian waters. Our results suggest that a revision of the Scandinavian species of *Ceramium* is needed.

Fucus serratus x *F. evanescens* hybrids

Hybrids between the two species were produced in the laboratory and putative hybrids were collected in the field. Controls showed no parthenogenesis in the dioecious *F. serratus,* but selfing occurred in the monoecious *F. evanescens.* All crosses, as well as intraspecific controls, resulted in viable offspring although eggs in all putative field hybrids were irregular both in size and number per oogonium. DNA was extracted from all field and culture individuals for microsatellite analysis. Preliminary data suggest that the microsatellite loci used in our analysis were inherited in a mendelian fashion. Furthermore, an analysis of naturally-occurring hybrids using locus C187 suggested polyploidy, as the hybrids possessed alleles 217 and 221 from *F. serratus* and allele 235 from *F. evanescens.*

Spatial genetic structure (population level)

Enteromorpha intestinalis and *E. compressa*

The development of microsatellite primers has been extremely difficult, but the problems have now been solved and we have found six polymorphic and two monomorphic loci in the Baltic Sea area. All the samples will now routinely be screened for the the 6 loci.

Ceramium tenuicorne

A total of 126 cultures have been established from samples of *C. tenuicorne* along the gradient from Skagerrak to the Baltic proper. We sequence the spacer region between cytochrome oxidase subunit 2 and 3 (cox spacer) in the mitochondrial genome (Zuccarello et al. 1999) to investigate the phylogeography within *C. tenuicorne* along the gradient. In addition, we use RAPDs (random amplified polymorphic DNAs; Williams et al. 1990) to investigate population-level differentiation within *C. tenuicorne*. Our preliminary results suggest a lower level of variation in the Kattegat-Baltic area as compared to Oslofjorden.

Fucus serratus and *F. evanescens*

Extensive samplings have been made in the Skagerrak-Kattegat-Baltic Sea and in some extra-Baltic localities. In *F. serratus* a grand total of 134 alleles were detected at nine microsatellite loci. Analysis of a subset of *F. evanescens* individuals from localities throughout the North Atlantic and Kattegat using two loci specific for *F. evanescens* and five loci specific for *F. serratus* revealed no polymorphism. The surprising lack of variability over such a large geographical scale (Maine, USA – Iceland – Blushøj, Denmark – Kiel, Germany) may be the result of the monoecious nature of *F. evanescens* and/or indicative of a recently evolved species. Because of this lack of variability, we will focus exclusively on *F. serratus* for population genetic analysis.

Moderate microsatellite polymorphism was revealed when considering *F. serratus* in all (15) localities, with a mean of 5.80 alleles per locus and a mean genetic diversity (H_e, Nei's expected heterozygosity) of 0.485. Both values, however, depend on sample size.

Estimates of Wright's F_{is} or inbreeding coefficient (Weir & Cockerham 1984) suggest occurrence of random mating in *F. serratus.* All mean F_{is} values of localities with samples >50 were not significantly different from 0, *i.e.* no departure from Hardy-Weinberg expectations, but five localities displayed significant heterozygous excess when considering loci A198 and B128. Wahlund's effect was demonstrated when examining F_{is} in those localities with >50 individuals as the magnitude of heterozygous deficiency increased when the sample size increased. This effect may indicate a hidden spatial or temporal genetic structure and this will be investigated further when more data are available.

Pairwise estimates of Wright's F_{st} also can be used to estimate genetic differentiation. When investigating localities with >50 individuals, no genetic differentiation was revealed among populations separated by c. 1 km, but highly significant differentiation was present among population separated by >70 km. The Oslo populations were not differentiated from the Tjärnö population (70 km). All other populations were highly differentiated including the two Spanish population separated by 140 km.

Autocorrelation analysis was conducted using three 1x1 m plots and one 5x100 m plot at Blushøj (Denmark) and one 5x100 m plot at Ribadeo (Spain) and Oslo (Norway). In all cases there was no correlation suggesting that the genetically homogenous patch is larger than 100 m. The autocorrelation analysis and pairwise F_{st} values show that the spatial panmictic unit appears to be somewhere between 1 and 70 km. Distinct clustering of the localities also was observed. Using a neighbor-joining method three distinct clusters were revealed: Ribadeo, La Coruña (Spain) and the Skagerrak-Kattegat localities. The three clusters were also apparent when using a multiple correspondence analysis to assess overall relationships among samples. This analysis also showed much greater differentiation within Ribadeo and La Coruña populations than within each Skagerrak-Kattegat population suggesting that the Spanish populations were much older (maybe glacial refugia) than the Skagerrak-Kattegat populations.

CONCLUSIONS

- The benthic macroalgae in the semi-enclosed Baltic Sea show low genetic variability reflecting its recent geological and biological history.
- Species rich macroalgal communities show enhanced stability.
- Species range and local abundance are significantly related.
- The molecular analyses in the genus *Ceramium* suggest that the number of species in the Baltic Sea and adjacent waters are lower than earlier considered.
- The number of haplotypes in *Enteromorpha intestinalis* in the Baltic Sea is low compared to the British Isles.
- *Fucus evanescens* show no polymorphism with regard to microsatellite loci.
- Skagerrak-Kattegat populations of *Fucus serratus* display much less differentiation both within and between populations than found in Spanish populations, again suggesting a very recent evolution.

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TITLE: STRATIGRAPHICAL DEVELOPMENT OF THE GLACIATED EUROPEAN MARGIN: **STRATAGEM**

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STRATIGRAPHICAL DEVELOPMENT OF THE GLACIATED EUROPEAN MARGIN: STRATAGEM

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SUMMARY

STRATAGEM will address the problem of a lack of regional approach to the stratigraphical development of the European glaciated margin. The project will study the relatively near sea-bed geology of the European Atlantic margin from Ireland to Norway, principally employing seismic techniques but also carrying out studies on cores. These studies will focus on defining the sedimentary packages on the margin and produce a unified stratigraphic framework and common terminology for the mid-Cenozoic to Recent sediments of the region. A geological model for the evolution of the margin will also be produced, taking a regional view of both the processes that have produced the present-day margin, and of the forcing factors that have controlled those processes. Oil-industry-standard reports will be produced for both the stratigraphy and the model. These reports will form the basis for future studies on the margin by both academia and the industry.

PROJECT OBJECTIVES

The timing of geological events on the north-west European margin is a problem of international significance that impacts directly on its exploration, exploitation and sustainable development. The successful management of this region and its resources is highly dependent upon a thorough understanding and ability to model the nature and frequency of the processes that have shaped, and continue to shape, this margin. In particular, hydrocarbon exploration along the glaciated margin is a strategic activity for several European countries, and solving problems associated with margin development has important socio-economic and environmental implications for these nations and the whole of Europe. The safety of structures and people working in this difficult offshore setting requires knowledge of the nature of the risk inherent along the margin.

One way of providing a measure of risk assessment is to develop a timescale, or stratigraphy, for geological events that have affected the margin, especially those involving slope instability which provides a real potential threat to the exploitation and sustainable management of the continental margin. This problem of developing a mid- to late Cenozoic stratigraphy has been only partly addressed by the oil industry, and to date it has largely been a case of problem solving and risk assessment only in restricted areas. The margin is here taken to include the mid- to outer shelf, slope and deep-water basins as indicated in Figure 1.

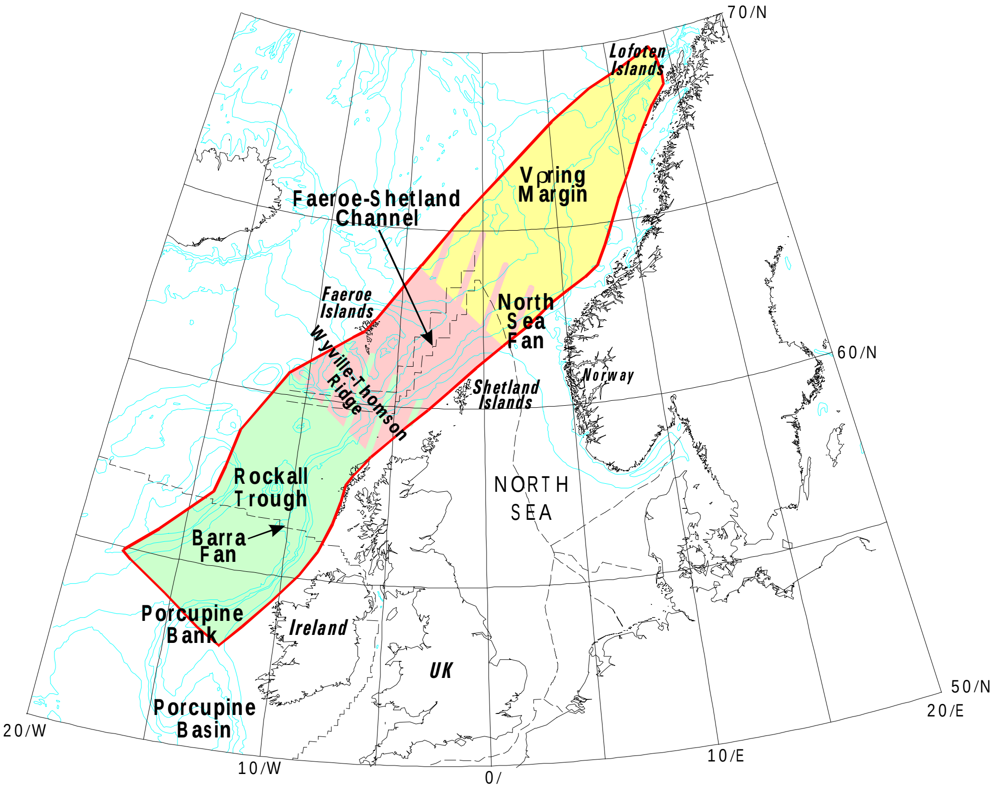

FIGURE 1 THE STRATAGEM STUDY AREA

STRATAGEM will address the entire European glaciated margin south of Lofoten, providing a context for all present and future studies in the region, and therefore having a real impact on sustainable management of the margin well beyond the life of the project. From the Consortium’s experience, this proposal is very much demand-led and will help answer real problems faced by oil companies working in this North-East Atlantic frontier region, and who have particular concerns regarding health and safety issues. On this basis, STRATAGEM will provide:

- ***A unified mid- to late Cenozoic stratigraphic framework for the glaciated European margin on a range of timescales***, with increased resolution in the younger part of the succession. This will provide a framework and context for all margin studies (including other projects in the European Margin cluster), as well as a common terminology. This whole time-range will be addressed throughout the margin, but because of the nature of the geology, the Plio-Pleistocene part will be emphasized in the north.

- ***Definition of the architecture and spatial variability of the strata*** which comprise the margin, and account for its present morphology. This will be presented as maps for the whole region, with additional illustrative material.

- ***An improved understanding of the sedimentary processes*** responsible for the development of the margin, and an assessment of the relationship of these processes to the major forces that have controlled them: climate and glaciation, ocean circulation, sea level, and both regional and local tectonics.
- ***A Margin Evolution Model.*** This will involve addressing a wide range of scientific questions related to Cenozoic glacial and pre-glacial geology, and can be anticipated to lead to the development of new concepts in margin development and advancement of the state-of-the-art. Objectives include consideration of, *inter alia*, the following:

    - What were the processes and causes of the build-out of vast prograding wedges and trough mouth fans of presumed Plio-Pleistocene age?
    - What was the timing and extent of influence of oceanic currents on the development of the margin?
    - What was the effect and timing of glacial erosion on the outer shelf?
    - Can the extent of Neogene uplift along the hinterland of the glaciated European margin, and its influence on the margin, be defined?
    - Can the southernmost point at which glaciers reached the shelfbreak on the European margin be mapped?
    - Were there times of increased frequency of instability on the margin, and if so what were the likely causes of this phenomenon?

METHODS

The workplan devised comprises 7 workpackages (WPs): three of these divide the study area into 3 regions, with 2 WPs integrating the results of these regional studies, and 2 further WPs related to data management, quality assurance and quality control (see Figure 2).

Execution of the project is based primarily on expert skills in seismic-sequence interpretation and sedimentological and biostratigraphical analysis. Some of the data is to be collected as part of STRATAGEM; these new data will be collected on 4 cruises in critical areas where available data coverage is missing. Detailed appraisal and planning will be an important component of the earliest stages of the project. Existing data will be provided by the partners from their databases, from previous co-operation with the industry, or from new agreements with industry specifically relating to STRATAGEM.

Chronology for the seismic-sequence interpretation will be based on existing biostratigraphical interpretations of cores, wells and boreholes, as well as on new data including released commercial well data and site-investigation boreholes. These sample data will also provide sedimentological information for input to the Margin Evolution Model. Using all of these data, we will develop a unified stratigraphic framework for the glaciated European margin based on the concept of seismic-sequence analysis, whereby key regional unconformities are used both to sub-divide successions and to provide a first-order correlation between sedimentary sequences.

In addition, post-rift backstripping modeling will be carried out on transects along the length of the European glaciated margin. These calculated models will constrain the more-qualitative Margin Evolution Model, and provide insights into factors such as uplift and subsidence that have been among the crucial controls on margin development. Comparison with other glaciated margins, such as Greenland and Antarctica, will place our study in a global perspective.

The data management will be carried out by Svitzer, an SME who work in the offshore oil and gas industry. They will not only set up a database for the project, and establish and maintain the STRATAGEM web page, but will most importantly provide expert map-compilation services to ensure that the end-products of WPs 4 and 5 are of a standard that industry would expect.

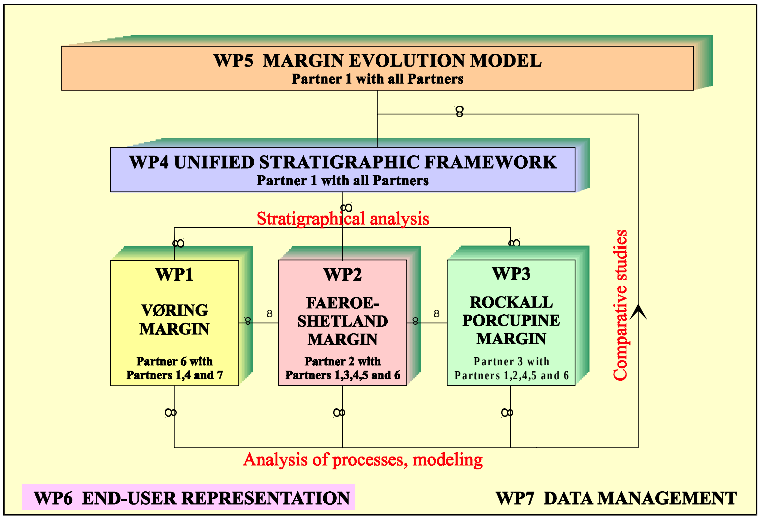

Figure 2 STRATAGEM project organisation

The STRATAGEM project is punctuated by 5 workshops. These major workshops will be critical milestones for product delivery and decision-making regarding the agreed framework. Furthermore they present the primary opportunity for End-User Representatives to ensure that the approach of maximum value to the industry is being achieved. It is also intended that representatives from other projects in the European Margin cluster will attend, as well as other interested workers. It is anticipated that the principal themes will be:

- Workshop 1 – Assessment of existing and available data and planning of cruise target areas.
- Workshop 2 – Assessment of early interpretations to ensure comparable approach from all partners.
- Workshop 3 – Mid-project review; assess implications of new data.
- Workshop 4 - Agree final stratigraphic framework, and consider model.
- Workshop 5 – Agree model and details of final report.

CO-OPERATION

During the conception of STRATAGEM, this Consortium has had detailed discussions with the oil industry, principally in the form of the joint association of four Joint Industry Projects (JIPs) active on the north-west European margin:

- The Seabed Project in Norway.
- The GEM Network in the Faeroes.
- WFA (Western Frontiers Association) in the UK.
- PIP (Petroleum Infrastructure Project) in Ireland.

Altogether, these JIPs represent 27 European oil companies, and the joint association has provided strong backing for STRATAGEM, and data exchange agreements have been made with them. Furthermore it is intended that the JIPs will provide personnel from within their midst to participate in the project and act as End-User Representatives.

STRATAGEM also forms part of the EC 5th Framework cluster OMARC (Ocean Margin Deep-Water Research Consortium), ensuring good co-operation between a range of geological and biological ocean-margin projects.

RESULTS

As this 3-year project began on 1st March 2000, the project remains in its initial exploratory stages and there are no results to report. However, the full extent of the database available is being assessed and plans are in hand for cruises during 2000. Presently planned cruises will take place on the Voring margin (*Jan Mayen* – University of Tromso), the North Sea Fan (*Hakon Mosby* – University of Bergen) and the Rockall-Faeroes margin (*Pelagia* – NIOZ). The first workshop will take place in late May in Copenhagen, and the second will be held during the late autumn of 2000.

I.1.6. Sedimentary processes

TITLE : ENVIRONMENTAL CONTROLS ON CARBONATE MOUND FORMATION ALONG THE EUROPEAN MARGIN : **ECOMOUND**

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CARBONATE MOUNDS ALONG THE EUROPEAN CONTINENTAL MARGIN: IMPLICATIONS FOR ENVIRONMENTAL CONTROL ON THEIR FORMATION

Scientific Party

INTRODUCTION

Prominant carbonate mound reefs have been features of Earth's history ever since Cambrian times and are believed to extinct since Mesozoic times (Reitner and Neuweiler, 1995). These mounds frequently form giant host rocks for hydrocarbon accumulation. However, their formation and environmental controls are the subject of much discussion and disagreement. The discovery of spectacular **modern carbonate mounds** along the European continental margin (Hovland et al. 1994, Henriett et al. 1998) provides an outstanding opportunity to study the processes that create carbonate mounds. Our present day knowledge of reef growth and reef formation is limited to the shallow water reef environments intropical regions and to a few observations of "reefs" from the cool water coral margin off Europe. Data and observations on modern carbonate mounds are entirely missing.

Modern mounds are up to 300m high and many of them are made up of carbonate. However mud mounds exist as well in almost the same settings. In particular seabed and sub-seabed mounds of strongly different dimensions, and therefore of possibly different origin and controlled by different formation processes, have been recognised in the Porcupine Seabight, the SE and SW Rockall Trough (on the flanks of the Porcupine and Rockall Banks), the N Rockall Trough the Faroe-Shetland Channel and along the Norwegian Margin.

These biogenic accumulations are for the most part located within the depth range of the habitable zone of corals and apparently support a rich deep-water reef ecosystem (Freiwald et al. 1999). The acceptance of a simple model of mound formation is unsatisfactory owing to the observed variation in size, morphology (linear ridges, ring shapes etc.) and the prolific number of these mounds with their very localised clustering. An explanation is not as yet available for their yet poorly-documented and regionally-studied distribution, shape, composition and formation in relation to the seismic and sedimentary characteristics and local oceanographic conditions.

Two major areas for investigations have been selected where clusters of carbonate mounds have been reported: The Porcupine and Rockall Basins off western Ireland and the UK. Furthermore we will include the Faroe-Shetland Margin and two sites along the Norwegian Margin located offshore the Troms district and on the Mid Norwegian Margin, where huge *Lophelia* mounds have been described (Hovland et al. 1994, Freiwald and Wilson, 1998).

INITIAL RESULTS

Carbonate mounds, with cross sections between 100-1800m at their basis and rising up to 350 m above the seabed and surrounded by a 60-90 m deep circular moat, have been reported in the Porcupine Seabight in water depths of 650-1000m (Henriett et al. 1998). More recently, carbonate mounds up to 350 m high were discovered on the continental slope north of the

Porcupine Bank in water depths of 500-1100 m. In addition, carbonate mounds have been described in a zone up to 9 miles wide zone in water-depths of 500-1000 m in the south-western Rockall Trough (Hovland et al. 1994). These mounds also rise up to 350 m above the seabed and appear associated with slumped or faulted margin sediments.

Based on data generated by high resolution seismic profiling and a few sidescan sonar records, the mounds of both the Porcupine Seabight and Rockall Trough appear to extend parallel to the slope, at the present boundary level between Eastern North Atlantic Water (ENAW) and Mediterranean Outflow Water (MOW) (New at al. in press, Freiwald et al. in press).

It has been argued that corals stands may have been destroyed by mobile sands and subsequently have been transported and reworked by vigorous currents parallel to the slope (Freiwald and Wilson 1998). The debris from these processes has then formed a relatively sheltered habitat for patchy development of new corals. Indeed, currentinduced structures around the mounds are evident on sidescan and underwater TV records. In addition TV records show abundant living corals and organisms covering present-day mounds.

Coring on and around the mounds has been extremely limited so far. In the Porcupine Seabight and Margin the mounds seem to be composed of (carbonate) debris, from the breakdown of abundant ahermatypic *Lophelia* and *Madrepora* cold-water corals and associated fauna. This has also been found at the Darwin Mounds in northern Rockall Trough and along the Faroe-Shetland Margin. Piston-core sampling of mounds in Porcupine Seabight and the SE and SW Rockall Trough margins showed the surface sediments to be built up at intermittent intervals with carbonate debris, including the cold-water corals *Lophelia pertusa* and *Madrepora oculata*, and shell fragments, separated by clayey layers. This indicates intermittent mound growth, related to increased carbonate sedimentation, separated by periods of quieter sedimentation, involving mound formation by a combination of pelagic and detrital sedimentation.

Initial studies of the $\delta^{18}O$ and $\delta^{13}C$ isotopic composition of skeletal material of mound biota showed a clear marine signature controlled by ocean circulation. Differences of the $\delta^{18}O$ signal within individual species have been related to variations in growth rate and are thought to reflect variability in food supply during skeleton growth and possibly of ambient seawater temperature. So far, the limited data set on isotopic signals from the Norwegian, Faroe and Porcupine Seabight mounds have failed to show any controls on formation other than those related to water masses.

Preliminary studies on organic matter show low total organic matter in both the Porcupine Seabight and SE Rockall Trough mound sediments, with an isotopic signature reflecting a mixture of terrestrial and marine sources. Biomarker studies of a SE Rockall Trough mound showed a distribution of fatty acids and n-alkanes suggesting a considerable land-derived contribution of terrigenous lipids, in addition to plankton-derived material.

In the NW Rockall Trough and around the Faeroe-Shetland Margin the development and growth of these mounds and reefs have been related to enhanced food supply through re-suspension of particles on the slope by either the breaking of internal waves or the presence of strong near-bed currents. Similarly, strings of cold-water reefs and mounds of several kilometers long and tens of meters high, bathed in ENAW water, occur up to 70°N along the entire Norwegian Margin (Freiwald et al. 1999). Availability of suitable food, a homothermal water mass and sufficiently strong currents to prevent sedimentation were considered to be the most important forcing mechanisms, in contrast other hypotheses suggesting an origin through the upward seepage of hydrocarbon-charged porewaters from the sediment below.

GOALS:

The ECOMOUND project will investigate the external oceanographic controls and forcing mechanisms on carbonate mound formation and build up. It will offer crucial conceptual innovation for the understanding of this newly discovered ecosystem and its importance for the evolution of the continental margin.

- Aside from new area-related knowledge concerning the distribution, size, shape of mounds and their relation to sedimentary settings and water mass conditions, an overall synthesis will be made of the environmental controls governing mound formation and biological distributions on the European Margin.

- The direct coupling of hydrographic, geological and geochemical data sets on the scale of this study will yield new insights in the dynamic effects and processes involved in particle transport, settling and re-suspension governing mound growth along the margins of NW Europe.

- The role of mound biota in the sequestration and biotransformation of organic matter and the sources and composition of organic matter involved in mound development will be evaluated in contrasting mound areas. Microbially-mediated processes contributing to mound formation will be identified.

- A largely unknown habitat will be explored which comprises an essential part of the European Margin.

- Temporal and spatial variability of carbonate production and lithogenesis in and around mounds will be defined and its relationship to environmental forcing established.

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TITLE : **THE MOUND FACTORY - INTERNAL CONTROLS : GEOMOUND**

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CARBONATE MOUNDS OF PORCUPINE AND ROCKALL BASINS, OFF SOUTHWEST IRELAND : GIANT BIOGENIC BUILD-UPS AT THE CROSSROADS BETWEEN GEOLOGICAL AND OCEANIC FLUXES ?

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INTRODUCTION

Carbonate mounds are throughout the Palaeozoic a prominent reef type driven by microbial automicrite formation. They generally are believed to be extinct since the end of the Mesozoic (Reitner and Neuweiler, 1995). Fossil mounds are important reservoirs of oil and gas in a number of hydrocarbon provinces. The discovery of maybe one of the world's most prolific recent carbonate mound provinces along Europe's margins, southwest of Ireland and right within the present deep water areas of interest for hydrocarbon exploration - urges for investigating the potential but still eluding links between giant mound occurrences and hydrocarbon resources.

Porcupine and Rockall Basins indeed display - within the North Atlantic realm and perhaps in a global perspective - a unique association and diversity of carbonate mound provinces, which may yield the key to address the question of mound genesis and its significance in a global oceanic plot, from a process-oriented point of view. The giant mounds on the present seabed surface, 200 to 250 m high, the extensive cluster of hundreds of buried reefs embedded in drift sediments, the whole range of mounds towering from a deeply ravinating unconformity on the eastern slope of Porcupine Basin and in the southern Rockall Basin are not mere curios, but significant build-ups. They may put Man on the track of hitherto unknown Biosphere processes, thriving at the confluence of fluxes from both internal (geological) and external (oceanic) origin. In many aspects and *mutatis mutandis*, carbonate mounds might be for the Margins what sulphide mounds are on the Ridges: the product of biologically controlled geological processes, of global significance.

Considering the close association between mounds and various surface expressions of fluid expulsion, "mound events" may hold clues towards fluid expulsion phases and hence towards prospectivity of basins. The apparent association between various mound clusters and past events of slope failures suggests mounds may also hold a key towards slope stability.

The geological and ecological controls on the mound formation in the Porcupine/Rockall area form the research objective of the EU FP5 twin projects GEOMOUND (Geological Controls on Mound Formation along the European Margin) and ECOMOUND (Environmental Controls on Mound Formation along the European Margin). They are closely associated with the projects ACES (Atlantic Coral Ecosystem Study) and "Deep-Bug" and are partners of the projects COSTA and STRATAGEM, within the FP5 project cluster "OMARC".

MOUND MORPHOLOGY AND SETTING

Different types of mound morphology and palaeoenvironmental setting have been unveiled and intensively surveyed in recent years off southwest Ireland, in water depths ranging from 650 to 1000m. The mounds cluster in 4 major geographical locations: on the SE and SW rim of Rockall Basin, in the northern part of Porcupine Basin and on the eastern continental slope of Porcupine Basin.

The first mound occurrences reported from industrial data by Hovland *et al.* (1994) on the northern slope of Porcupine Basin have led to the discovery of a fascinating and complex setting: a large, multi-phased buried slope failure with imbricated walls and high-energy sediment fill, forming the basement of that large cluster of surface mounds. "Hovland" Mounds are outcropping mounds with a conical shape that occur as single mounds or in elongated clusters. One single mound may have a cross-section of 1km and a height of 100m above the seafloor.

The buried depression below the "Hovland" mound group is fringed in upslope direction by an extensive, crescent-shaped province: the "Magellan Mound Province" (Henriet *et al.*, 1998). Magellan Mounds occur in large numbers in a very well delineated area and are characterized by a wide variety of mound shapes and sizes. Seventeen mounds in the Magellan Province reach to and pierce the present-day sea floor, over an area of 250 km^2 . The vast majority however are buried by up to 90m of sediment.

On the eastern margin of Porcupine Basin, a range of large mounds are towering from a strongly erosive unconformity: the "Belgica" Mounds. They partly root from an enigmatic, locally very thick acoustically transparent horizon, of unknown age and composition. "Belgica" Mounds are outcropping or buried conical mounds (single or in elongated clusters) and occur on the eastern slope within a bathymetric interval of 200 m over a distance of 20 km. A recent survey has led to the discovery of a totally different "mound" within the "Belgica" mound range, spilling like a mud volcano over the seabed. This mound is about 100m high and 750m across. A video track revealed this "mound" might host the richest deep coral environment in the North Atlantic (D. Billett, SOC, pers. comm.).

The ranges of mounds on the southwest and southeast slopes of Rockall Basin bear some similitude with the "Belgica" mounds range, though in general the southern Rockall mounds are larger, with a height up to 200 or 300 m. They consist of irregular structures with multiple peaks. Moving upslope towards the northeast, mound size decreases until the mounds eventually disappear at about 55°40N.

OF MOUNDS AND CORALS

The mounds are colonized by deep-water coral associations, based on species such as *Lophelia pertusa L.* and *Madrepora oculata L.* which are widespread globally at appropriate depths in the oceans. The presence of these often vibrant biological communities, associated with large biogenic structures at water depths where biological activity was previously thought to be limited, has gained significant attention not just from the scientific community but also from the media and a broad public. The mounds also represent an important fish nursery zone for deep-water fish stocks, with fishing activity concentrated over the mounds.

The scale of the giant mounds in the Porcupine Seabight and certain areas of the Rockall Trough represents a startling phenomenon. These mounds represent a significant sink for carbon and could play an unquantified role in global climate regulation. Furthermore, the enhanced sedimentation rate necessary to produce giant mounds could provide the potential for high-resolution records of climate change.

INTERNAL GEOLOGICAL CONTROL...

A key question in such setting is *what triggers* mound genesis, and *who are the actors* controlling their growth. The apparent coincidence between mound clusters and subjacent structural controls (Hovland *et al.*, 1994), the proximity of at least one “mud volcano”-type mound, the systematic spatial association of mound clusters and actively prospected deeper hydrocarbon reservoirs provides a hint towards the possible value of such mounds for explaining patterns of fluid flow and geochemical evolution of fluids in a sedimentary basin, in space and time. Methane - in solution or in gas phase, or temporarily buffered in a hydrate reservoir - may effectively have played a role in the genesis of such mounds. This is a tantalizing hypothesis which can only be verified through well-prepared drilling.

Sedimentary build-up processes might have been controlled by microbial communities, which also may have played an active role in the possible lithification of mound sediments through automicrite formation. The Porcupine mounds are no doubt active laboratories within the present-day oceanic realm, where the role of bacteria in carbonate mound formation and slope consolidation can be elucidated - a role since long suspected from fossil evidence. Microbial build-ups and the role of bacteria in fossil reef and mound development in fossil mud mound settings is discussed in several papers and has been reviewed by Monty *et al.* (1995). For the first time, this tempting hypothesis might be tested on the modern counterparts. Bacteria play an important role in the early diagenesis of sediments and detritus (Turley and Lochte, 1990) and may play a major role in the genesis of these mounds (indirectly as nutrient suppliers for frame builders or directly in sediment fixation). The local development of bacterial blooms may induce changes in the chemistry and the mineralogy of sediment components through redox reactions.

The apparent spatial association of the “Hovland” and “Magellan” Mound provinces with a major buried slope failure asks for the elucidation of the potential signals which such mounds may provide regarding past or incipient slope failures. The possible interaction between fluid expulsions and gas hydrates is widely discussed in relation to slope instabilities. Although there are no signs of gas hydrates in the area of interest, we cannot exclude related processes in the past. Fluid flow may stabilise sediment slopes by triggering cementation, but early cemented sediments may also block fluid flow to the surface, thus decreasing the shear strength of the underlying sediment through fluid trapping.

...AND EXTERNAL CONTROL

On the other hand, these mounds are located on a margin which throughout Neogene-Quaternary times has repeatedly flipped between glacial and temperate environments. Moreover, this basin has been influenced by fluctuating intensities of the MOW intermediate water mass. Such observations consequently also argue for a complex but equally eluding external control. Facies changes in the mounds may be bound to environmental factors such as (i) sealevel changes, (ii) changes in temperature profile, (iii) local currents bringing suspended food to particular sides of the mound, (iv) fortuitous development of local hard substrates with corals. These external controls will be thoroughly investigated by ECOMOUND.

STRATEGY AND INITIAL ACTIONS

Within the fist few months of its project life, GEOMOUND has already started a wide range of actions :

- collection and prime analysis of industrial geophysical (2D, 3D) data and well data
- preparation of various cruises for the 2000 field season
- prime analysis of long cores, acquired shortly before the start of the project
- a multibeam survey is underway
- a proposal for ODP drilling has been submitted in association with the partner projects ECOMOUND, Deep-Bug and ACES
- a proposal for a joint TOBI-BRIDGET survey has been submitted in association with the ECOMOUND project
- a proposal for submersible dives has been submitted in association with ECOMOUND, ACES and French teams
- the collection of long cores with R/V Marion-Dufresne in 2001 is in an advanced planning stage.

The GEOMOUND strategy will involve the construction of a high-resolution Spatial Information Grid, straddling Porcupine Basin and southeastern Rockall Basin and including key mound occurrences and proven fluid migration sites (e.g. the Connemara oil field). This integrated approach will result in the establishment of the most robust data framework possible for possibly assessing the genesis of the carbonate mounds and establishing the potential link between phases of mound growth and hydrocarbon seepage.

The ultimate product will be a fluid migration model for this basinal area. Theoretical work dedicated to the way methane flows to the surface (classical Darcy flow, diffusion, solitary waves, shock waves), which depends mainly from the rheological properties of the sediments, will be combined with molecular transport studies (diffusion) of gaseous hydrocarbons in water-saturated rock samples to assess the potential relevance of this transport mechanism in the feeding of surface systems. The data integration and model construction will be performed by means of commercial 2D and 3D basin modelling software capable of simulating sediment deposition, subsidence and uplift and, in particular, single- and multi-phase fluid transport processes.

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TITLE : SILICON CYCLING IN THE WORLD OCEAN : THE CONTROLS FOR OPAL PRESERVATION IN THE SEDIMENT AS DERIVED FROM OBSERVATIONS AND MODELLING : **SINOPS**

CONTRACT N° : **MAS3-CT97-0141**

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COMBINING A DATA BASE OF OBSERVATIONS WITH A GLOBAL BIOGEOCHEMICAL OCEAN MODEL FOR AN IMPROVED QUANTIFICATION OF THE MARINE SILICON CYCLE

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INTRODUCTION

A change in greenhouse gas induced climate forcing is expected due to enhanced emissions of carbon dioxide and other radiatively active trace gases into the atmosphere. State of the art climate models are able to overall reproduce the present day climate. For realistic future scenarios, however, it is essential that also the sensitivity of the models is correct, i.e. that they can simulate rates of climate change satisfactorily. For the marine part of the climate system, the only means to test this ability lies in attempts to reconstruct past climates with these models and to validate their results with data from the marine sediment record. A substantial role within this context play the foraminiferal record (carbon and oxygen isotope data as measured in calcareous shells of benthic and planktonic microorganisms) and the calcium carbonate record. Both records depend on the preservation of calcium carbonate in the deep sea. There are wide spread regions in the world ocean which are of climatic relevance, such as the Southern Ocean, which at least over longer periods of time contain only few calcium carbonate sediments and thus only reduced information about the past ocean status. Therefore the opal sedimentary record is urgently needed as an additional tracer in paleoceanography. Opal or biogenic silica is produced in the surface ocean for siliceous frustules of diatoms and radiolarians through uptake of silicic acid. Biogenic silica enters the sediment via the export production and the vertical particle flux.

A rigorous interpretation of the record of opal in the sediment as a paleoclimate and paleoproductivity tracer requires a profound knowledge of the modern processes that control the production, i.e. the silicification in surface waters during the photosynthesis of siliceous phytoplankton, the sinking, and the accumulation of biogenic silica in the sediments. Before the last decade, little emphasis has been placed by oceanographers on the marine silicon cycle which is by far less explored than that of nitrogen and phosphorus as biogenic elements directly linked to the carbon cycle. Because diatoms contribute to a large extent to the export of biogenic matter to the deep sea, and given the potential of opal as a tool for paleo-productivity reconstructions, studies of the silicon cycle were more and more incorporated into oceanographic programs during the last decade. Substantial progress has been made in our understanding of the processes that control the modern marine biogeochemical cycle of silicon (Nelson et al., 1995; Tréguer et al., 1995). Besides the observational part, also progress in biogeochemical ocean modelling has been made and models of the marine silicon cycle became available (Maier-Reimer, 1993; Heinze et al., 1999). A logical next step was to combine data and model. More specific the objectives of the current project are:

1. To provide a data base of observations and a general circulation model which allow comprehensive studies of the silicon cycling in the oceans.
2. To reproduce the first principles of silicon cycling in today's world ocean by use of an ocean general circulation model.
3. To offer an explanation for the present opaline sedimentary distribution.
4. To test our understanding of opal by a paleoceanographic application of the model and to compare the simulations with the observed sediment record.

PROJECT METHODOLOGY :
THE DATA BASE, THE BIOGEOCHEMICAL MODEL, OPTIMISATION OF THE MODEL

In order to achieve these goals the following methodology was persued. By one partner a comprehensive data base on marine silicon cycling was collated including as variables silicic acid in the water column as well as in the sediment pore waters, opal weight percentages in the sediment (relative to total sediment and relative to a calcite free basis), and opal fluxes through the water column as derived from sediment trap data (Dittert et al., in preparation). This data base was compiled through use of the PANGAEA information system as developed and maintained by the Alfred Wegener Institute of Polar and Marine Research at Bremerhaven (http://www.pangae.de). Numerous data sets were newly included in the data base after collecting data directly from the originators.

In parallel, the Hamburg Oceanic Carbon Cycle Circulation Model (HAMOCC) was employed to integrate a model control run providing the basis for further optimisation. The control run and the dedicated postprocessing (plot) software were documented in a user's manual and made available to the public (Heinze and Maier-Reimer, 1999).

Both the publically available silicon data base on the PANGAEA-system (data also available on a special CD-ROM), as well as the public model version are central parts of the dissemination of results to the scientific community.

The control run was compared in detail to the silicon data base of observations and model deficiencies were diagnosed. Not any pure silicon model data were checked against observations but also carbon cycle tracers, phosphate, and oxygen values in order to make sure, that in our multitracer approach all relevant oceanic tracer species which are modelled are represented well.

In a further step, a suite of sensitivity experiments was carried out concerning biogenic parameter variations which affect the marine silicon cycling and the respective silicon tracers in the water column and the bioturbated sediment layer (in the model: the top 10 cm of sediment).

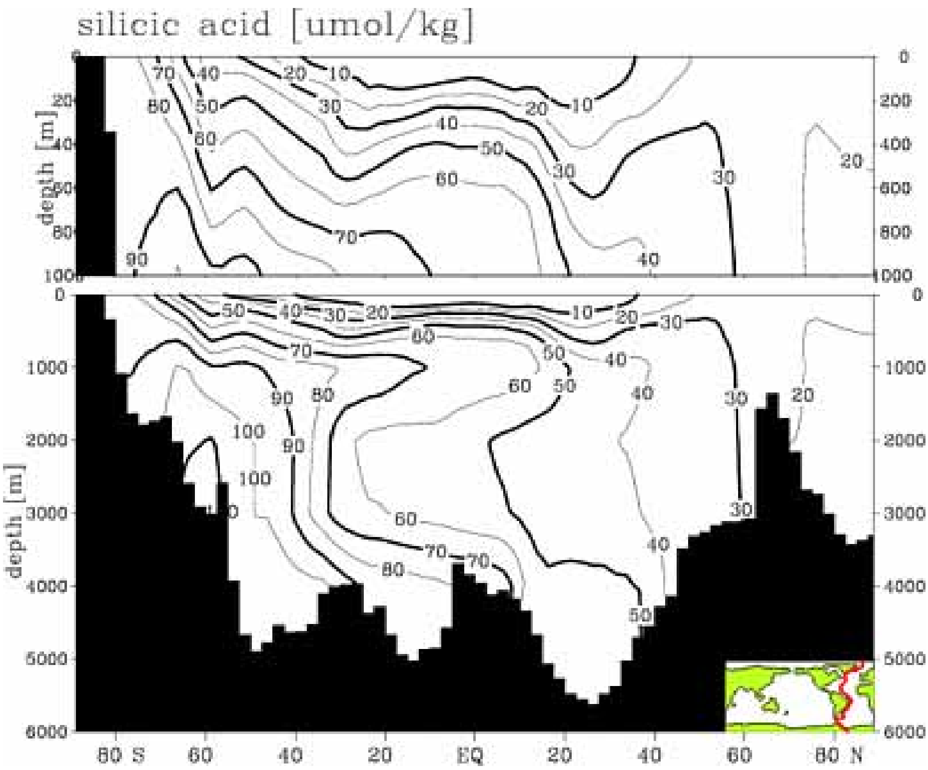

Figure 1: Section of silicic acid along the western Atlantic Ocean for the model reference run.

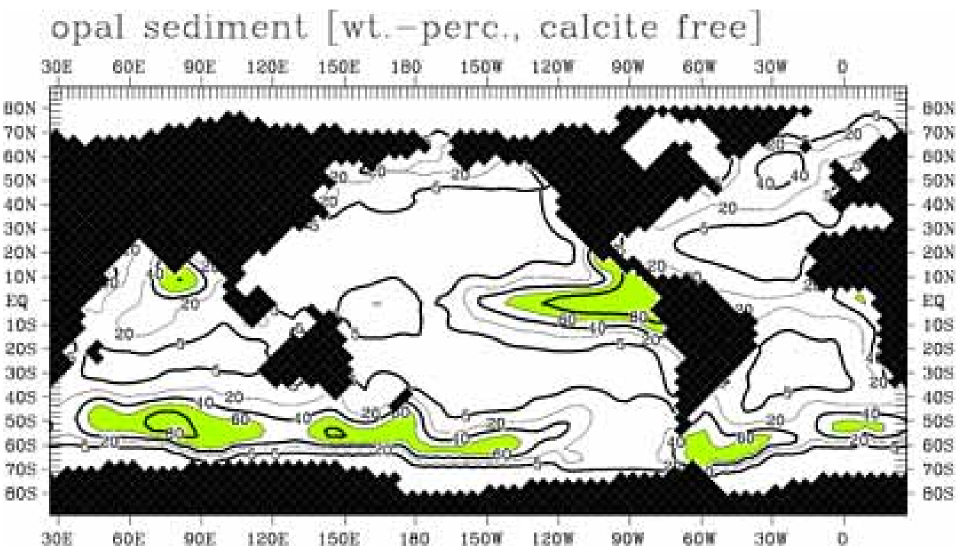

Figure 2: Model result for the opal sediment (on a calcite free basis). Average over the bioturbated zone (10 cm thick).

RESULTS

Improved control run:

On the basis of the standard model run as presented in Heinze et al. (1999), an improved reference run was integrated with a revised model source code. This source code is much faster than the previous one due to an implicit simultaneous treatment of the porewater reactions and porewater diffusion. The particle flux scheme was modified allowing now the separate specification of particle settling velocities and opal redissolution kinetics within the water column at each grid point independently (e.g., as a function of the local temperature). Compared to the earlier standard run, the new model has much more realistic silicon throughput rates (i.e. input rates through weathering of continental crust and output through opal sediment accumulation) of 5.5 teramoles Si per year corresponding to Tréguer et al. (1995). Examples for the new model standard run are given in Figures 1 and 2. A comprehensive comparison between modelled and observed silicon data distributions was carried out. The most prominent disagreement was found for the opal fluxes through the water column which are overestimated by the model as compared to the sediment trap data by about one order of magintude. The further analysis will clarify whether besides other minor model deficiencies in particular this mismatch can be removed through another choice of parameters or revised parameterisations of processes.

Sensitivity experiments:

A suite of sensitivity experiments was carried out (Heinze et al., in preparation) concerning variations in the following biogeochemical parameters: the half saturation constant in the formulation of silicic acid uptake kinetics in the surface ocean (change of polar preformed silicic acid concentrations, Fe limitation), the maximum uptake velocity of silicic acid, the settling velocity of opal particles (reduction with depth, dependent on opal export production), the opal redissolution kinetics in the water column and in the sediment porewaters (T dependency), the dilution of sediment with inert clay material, and the input rate of silicic acid from the continents (equal to the global opal accumulation rate when the model approaches equilibrium). These experiments provide the basis for a systematic model optimisation. A linear model was set up for determination of the optimal model parameters so that (within the limits of the linear model) the different model parameters can be adjusted simultaneously for an optimal fit to the silicon tracer data (as provided by the data base collected in the PANGAEA-system) in the various compartments. A singular value decomposition technique (based on the work of Lanczos, 1961) is applied in order to find a meaningful best fit solution for the model parameters. The optimal set of parameters will be then used in the full threedimensional nonlinear model.

The optimal model code will be used for new runs with velocity fields for two paleo time slices: the last glacial maximum and the mid-Miocene (open Panama isthmus). Preliminary studies have already been carried out with the source code of the updated improved model reference run.

CONCLUSION

A comprehensive data base on silicon data in the water column and the bioturbated sediment layer (solid sediment, sediment pore waters) was compiled. The biogeochemical ocean model HAMOCC was integrated with new parameterisations for the vertical particle flux and the treatment of the porewater reactions and diffusion. A revised reference run was set up and a serious of sensitivity experiments was carried out which are evaluated systematically in an optimisation procedure. While the major part of the observed silicon data can be reprodruced satisfactorily, a mismatch between modelled and observed opal particle fluxes through the water column exists. The final work in the project will focus on the question whether this disagreement can be removed or at least clarified. The optimal model configuration will be applied to two velocity fields for past time slices (last glacial maximum, mid-Miocene) to check the progress in our understanding of opal as a paleo climate tracer.

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TITLE : AN INTERNET DATABASE OF SEAFLOOR SAMPLES FROM THE EUROPEAN SEAS : **EUMARSIN**

CONTRACT N° : **MAST-CT98-0182**

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EUROPEAN MARINE SEDIMENT INFORMATION NETWORK (EUMARSIN) - AN INTERNET DATABASE OF SEAFLOOR SAMPLES FROM THE EUROPEAN SEAS

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INTRODUCTION

With the acceptance of the International Law of the Sea and the 200-mile economic zone, the area of the seas under EU jurisdiction is almost three times the extent of the land area. Although the European Seas represent the most thoroughly explored sector of the world's oceans, there are still large areas about which very little is known. Initially, marine research programmes in Europe were focussed on the national requirements of individual countries. For example, marine geological surveys were principally aimed towards providing regional information, such as the nature of seabed sediments, that could be used to produce geological maps of the seafloor. In recent years, however, the projects carried out by marine institutes have attained a transnational character as they address problems such as quality of coastal waters, impact of human activities on the marine environment, transport pathways of sediments and contaminants and circulation of water masses, all of which require multinational cooperation.

In order to meet these modern challenges, in 1996 the geological surveys of the EU member states and Norway formed the Association of European Geological Surveys (EuroGeoSurveys, Annells, 1996). The 14 marine geology departments of EuroGeoSurveys formed a network to develop a strategy for improving our knowledge of the European marine environment. The team recognised the need for an inventory of all available sea-bed samples as a first stage in assessing future priorities in marine geoscience and proposed the European Marine Sediment Information Network (EUMARSIN) project for funding to EC in the framework of the MAST III program. The proposal was accepted and the EUMARSIN project started in November 1998 with a duration of two years.

The EUMARSIN project aims to stimulate use of the data resource of the EuroGeoSurveys partners and provide a tool to benefit the integrated management of the European coastal and marine environment. The project team is developing a public-domain Internet metadatabase available on the World Wide Web, which will give access to information obtained by the EuroGeoSurveys partners during their work in the European seas. In addition, each partner is responsible for contacting other marine organisations in their respective countries to invite them to contribute metadata for seafloor samples that they have collected during their own investigations of the European seas.

EUMARSIN has complementary links with the MAST III project, EUROCORE, which is compiling metadata for samples and cores from the world's ocean basins, providing they have been collected and stored by organisations in the EU member states and Norway. These links have led to the development of strictly defined areas of responsibility during the data compilation phase, but the results have been integrated in a single metadatabase using common metadata formats, which will be accessed through the World Wide Web by a single entry point. The name of the integrated EUMARSIN/EUROCORE metadatabase is EU-SEASED and its Website address is http://www.eu-seased.net.

PROJECT OBJECTIVES AND METHODOLOGY

The EUMARSIN project objectives include the formulation and implementation of an acceptable set of terms of reference for the marine sediments held by organisations in the EU countries and Norway. These terms of reference were developed by the EUMARSIN partners at a joint meeting with the EUROCORE project in June 1999, when a set of parameters for recording mandatory and optional metadata were agreed. The term 'metadata' is defined as that used widely in the context of digital spatial data, that is 'background information about the content, quality, condition and other characteristics of data' so that the user can determine the data's fitness for purpose. The EU-SEASED metadatabase follows the guidelines set by international standards committees such as the emerging International Organisation for Standardisation (ISO) TC211 Metadata Standard. These standards provide a common set of terminology and definitions for the documentation of spatial data. The standard does not specify how this information is organised in a computer system or in data transfer, or the means by which the information is transmitted or communicated to the user.

For each sample in the EUMARSIN metadatabase there are therefore a record number, measuring ID, the measuring area type, such as whether the record is of a sample point, track or a map area, the sample co-ordinates, sampling device and the data source holder. The optional metadata fields give access to further information about the quality of the sample such as the sample state, it's storage condition and the types of analyses that can be obtained. The analytical measurements may include lithological description, particle size, and mineralogical and geochemical analysis. The metadatabase will also provide information on availability of sample/core photographs or X-rays that may have been taken. Finally, there is information on the water depth at which the sample was collected, the total depth of penetration and a basic description of the predominant sediment type and, if available, the age of the basal sediments that were collected. Each sample is linked to information about the project and cruise during which it was collected and any relevant reports or publications that describe further work that has been carried out on the data.

Another important feature of the EUMARSIN metadatabase is that it provides information about all maps related to the European seafloor that have been published by the European geological surveys. These maps include classifications of the seabed sediments, Quaternary sediments and bathymetric isolines that the user can relate to the available sample metadata.

To further concert the EUMARSIN metadatabase with other data management procedures that have been applied to European and other international projects, the EUMARSIN metadata is tuned with the EuroGeoSurveys' Geological Information Exchange System (GEIXS) and the National Oceanic and Atmospheric Administration (NOAA) of the United Sates Department of Commerce through the use of references to codes for common metadata fields.

The EUMARSIN project also aims to develop an information infrastructure that will establish electronic information exchange between the project partners, in order to manage and maintain the EUMARSIN services. This information exchange includes a newsletter that presents articles related to the European seabed which are of popular and scientific interest. There will also be information on research cruises in which the partners have participated and notices and reports on Conferences, Workshops and Meetings. A Bulletin Board will allow both project partners and other users to discuss, for example, the availability of equipment for hire and the suppliers of marine scientific and operational services. Links will be established to other Websites that share a common interest in the marine environment. The information exchange will be included on the EU-SEASED Website and will be updated on a regular basis to maintain contact between the project partners and to inform users of the current work of the EuroGeoSurveys marine departments. These products and the metadatabase will be maintained after the end of the EC-funded phase of the project.

Finally, the EUMARSIN products will be distributed on the Internet and on CD-ROM to bring them to the attention of decision-makers, scientists and technologists in the public and private sectors as well as the general public. By doing so the project will achieve it's most important objective, that of re-use of the valuable data resource that has been accumulated by European organisations with interests in the marine environment.

RESULTS

Before the projects commenced, the EUMARSIN and EUROCORE partners reached agreement on data compilation responsibilities to avoid duplication of effort. It was agreed that the EUMARSIN project would deliver metadata for all seabed samples and cores held by the European geological surveys and in addition they would gather information from commercial organisations and hydrographic surveys. The EUMARSIN project concentrated on providing metadata from the European seas, mainly from the continental shelf (defined as <200 metres water depth) but including survey data in deeper waters. The EUROCORE partners took responsibility for compiling metadata from European universities, research institutes and marine stations from anywhere in the world providing the data was collected by, and held at, a European institution. The geographical area for EUROCORE data gathering was therefore concentrated in the deeper waters seaward of the continental shelf. In countries where the EUROCORE project did not have a partner (Finland, Sweden, Denmark, Ireland, Belgium and Greece) it was agreed that the EUMARSIN partners would include data from both the continental shelf and deeper waters during the data-gathering phase of the project.

The initial phase of the EUMARSIN project was to identify all seabed sample metadata held by the project partners. Once the metadata format had been agreed, the project partners then concentrated on external data gathering. By the end of May 2000, the EUMARSIN partners had identified data for approximately 103,000 samples held by the geological surveys and a further 130,000 external samples from over 300 organisations who had been invited to contribute their metadata. These data have been gradually added to the EU-SEASED metadatabase throughout the past 8 months and are expected to be completed on schedule.

The metadata can be accessed on the EU-SEASED Website through both an alpha-numerical search and retrieval interface and by a geographical interface which allows the user to define an area of interest on a scrollable map showing the distribution of the seafloor sample/core locations. Once the area of interest has been defined the user is able to access metadata for individual samples or groups of samples. An on-line data entry system has been developed which will allow the geological surveys, and other organisations who wish to add their metadata to the metadatabase, to submit new information as it is acquired in the future.

CONCLUSION

The EUMARSIN project is on schedule to complete its objectives by the end of October 2000. It is expected that the metadatabase will contain metadata for approximately 230,000 seabed samples collected by geological surveys and other research organisations, universities, hydrographic offices and commercial companies in Europe. In future years the metadatabase will be regularly updated by the project partners and an on-line data entry system will allow other organisations to contribute their metadata either retrospectively or as new data is acquired. Information about the activities of the project partners in the marine environment will provide a basis for regular contributions to the EU-SEASED Website. By making this valuable data resource publicly available through the Internet, it is anticipated that secondary use of the information will be increased, and that the EU-SEASED metadata will continue to inform a wide range of users in the marine environment.

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I.2. Extreme ecosystems

TITLE: DEEP-SEA HYDROTHERMAL VENTS: A NATURAL POLLUTION LABORATORY **(VENTOX).**

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DEEP-SEA HYDROTHERMAL VENTS: A NATURAL POLLUTION LABORATORY (VENTOX)

ABSTRACT

The aim of the VENTOX project is to carry out innovative research into the specialised adaptations and processes found in representatives of the mid-Atlantic deep-sea hydrothermal vent fauna and its associated microbial populations. Studies will focus on the ability of deep-sea vent organisms to survive under extreme physical and chemical conditions. From a fundamental perspective, considerable effort will go into the fine scale characterisation of the physical and chemical environment around deep-sea vents in the mid Atlantic, defining the corresponding micro-scale distribution of vent communities, and quantifying the conditions which they are exposed to and the toxicant burdens found in their tissues. These measurements will then provide the basis for a wide range of molecular, biochemical and physiological studies which will be carried out on vent crustaceans, mussels and gastropods, to provide valuable new insights into their heavy metal, pH, temperature, CO2 and sulphide tolerances, bacterial and megafaunal production rates, rates of DNA damage and repair (note, vent emissions contain many substances that are known to inflict damage on the genetic material of other groups of organisms, including humans), and the identification of endosymbiont-mediated detoxification processes which must underpin survival in this highly toxic, extreme marine environment.

From an applied perspective, the hydrothermal environment provides a useful analogue for polluted environments elsewhere, with the notable and significant difference that evidence of the complex biological communities which live around deep-sea vents can be traced back in the fossil record to at least the Mesozoic era, thus allowing sufficient time for the evolution of adaptations to combat environmental toxicity; this contrasts with the situation for non-vent marine species which are exposed to anthropogenic pollution which, by comparison, is a relatively recent phenomenon (<100 years). While the fundamental aim of VENTOX is to extend our knowledge of the conditions experienced by vent organisms and the special adaptations they have evolved to resist toxicant stress, an important secondary objective is the generation of findings and material which will be of relevance to SME-based exploitation in the fields of biochemistry (repair enzymes), deep-sea pressure technology (flow-through pressure chamber, IPOCAMP) and microbial biotechnology (e.g. detoxification and bioremediation).

An important part of VENTOX will be the establishment of a shore-based laboratory in the Azores (LABHORTA), to which cages of deep-sea vent organisms will be transferred at intervals throughout the year to provide research material for the scientists working on the project. The problem of gaining access to vent animals without a research ship has been overcome by the use of acoustically operated cages which can be recovered by a small surface vessel as weather conditions allow. Another innovative component of the VENTOX project will be the use of the IPOCAMP hyperbaric chamber in which vent organisms are going to be studied under their natural pressure conditions, this has important implications in terms of their biochemistry, physiology and cell biology. VENTOX thus represents both a major technical and philosophical step forward for deep-sea vent biology, which has moved from being a largely descriptive science into an experimental one.

TITLE : ATLANTIC CORAL ECOSYSTEM STUDY**: ACES**

CONTRACT N° : **EVK3-CT-1999-00008**

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THE ATLANTIC CORAL ECOSYSTEM STUDY (ACES): A MARGIN-WIDE ASSESSMENT OF CORALS AND THEIR ENVIRONMENTAL SENSITIVITIES IN EUROPE'S DEEP WATERS

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INTRODUCTION

Coral reefs are something we usually associate with warm, tropical waters and exotic fish, but not with the cold, deep and dark waters of the North Atlantic where corals were regarded as oddities on the seafloor. It is now known that cold water coral species also produce reefs which may rival their tropical cousins in terms of the species richness of associated marine life (JENSEN and FREDERIKSEN 1992). Increasing commercial operations in deep waters, and the use of advanced offshore technology have slowly revealed the true extent of Europe's hidden coral ecosystems. The discovery of extraordinary 10km-long chains of the reef-building corals *Lophelia pertusa* and *Madrepora oculata* in 300m water depths on the Norwegian Shelf (MORTENSEN et al. 1995, FREIWALD et al. 1999) have deeply challenged conventional views. The same coral assemblage is also found associated with large seabed structures in the Porcupine Seabight, where they are so abundant that their skeletal remains have, over the millrnnia, contributed to carbonate mound structures up to 300m high in 700 - 1200m water depths (HENRIET et al. 1998). The potential of cold-water corals to contribute to the formation of these large seafloor features and their high biological diversity have attracted considerable public attention through reports in numerous national TV and newspaper features.

In the Northeast Atlantic, the geographic distribution of deeper water coral (DWC) ecosystems can be traced from the slopes and banks off the Iberian Peninsula as far north as the Scandinavian Shelf. To cover the variation in environmental factors and interactions at ocean boundaries which enable the development of DWC ecosystems, the ACES scientific community will focus on selected key flagship areas along this latitudinal gradient - Galicia Bank, Porcupine Slope, Rockall Trough, Skagerrak, Norwegian Shelf. Our aim is a margin-wide environmental baseline assessment of the status of Europe's deep-water coral margin to provide recommendations for essential monitoring and methodology requirements for future sustainable development. The evolution of new management concepts for the sustainable use of deeper-water marine ecosystems on a margin-wide scale is a grand challenge that can only be achieved on a joint European scale.

To meet that challenge, ACES will focus on three main scientific objectives which will provide the scientific data necessary to carry out our final objective which is to provide impartial practical recommendations for enlightened management of this spectacular deep-water ecosystem.

SCIENTIFIC OBJECTIVES AND EXPECTED ACHIEVEMENTS

Objective 1: ***To map the structural and genetic variability, the framework-constructing potential, and the longevity of DWC ecosystems***

High resolution maps of DWC buildups are essential to determine the spatial distribution and the status of the ecosystem in the various working areas. The framework-constructing potential in DWC largely depends on the annual extensional growth rate, the intensity of secondary thickening of the coral skeleton and the intensity of post-mortem destruction by endolithic borers. We aim to utilise molecular genetic techniques to assess the spatial genetic structure and population dynamics of *Lophelia* at several scales (between regions, within regions and within individual coral reefs). **Achievements:** The outcome will be the first digitized atlas based on acoustic and video imaging of different DWC buildup types along Europe's margin. Detailed growth rate, dating, and ultrastructural studies performed on *Lophelia* and *Madrepora* skeletons will provide results on the longevity of coral frameworks for each of the DWC working areas. The genetic techniques will enable us to identify levels of genetically effective migration between different geographic areas. It will also allow us to identify which *Lophelia* reefs contain the greatest genetic diversity. Both the larger scale imaging data and the detailed studies of the corals themselves will be integrated into a status check of the structural integrity of the DWC ecosystems.

Objective 2: ***To assess hydrographic and other local physical forcing factors affecting BBL sediment particle dynamics and POC supply in the vicinity of DWC ecosystems***

DWC ecosystems are often found at or near oceanographic boundaries - even in fjords - but the detailed effect of hydrographic conditions on DWC buildups remains a matter of speculation. The poleward flowing warm and saline NE Atlantic slope current is a well documented feature at the shelf break which extends from the Iberian to the Norwegian Sea margin. Predominantly poleward (northward) slope currents at an eastern boundary tend to drive downward near seabed currents in the frictional layer. This has implications for the transport of suspended material in the benthic boundary layer (BBL) and hence for the nutrition and distribution of corals. In addition, the hypothetical contribution of hydrocarbon enrichment to DWC ecosystem nutrition will be assessed. **Achievements:** The outcome will be to provide a hydrographic description for the NE Atlantic margin where corals occur, including information on local hydrodynamics (currents) and water mass properties (temperature, salinity, density) at or near DWC ecosystems. The detailed description of suspended particle dynamics in relation to BBL flow and its impact on coral nutrition and feeding (either derived from advected particulates or hydrocarbon sources) is key to understanding the function of the whole DWC ecosystem. This will be achieved by molecular analysis of biologically labile POM fluxes within the coral systems and surrounding surface sediments.

Objective 3: ***To describe the DWC ecosystem, its dynamics and functioning; investigate coral biology and behaviour and assess coral sensitivity to natural and anthropogenic stressors***

This objective takes a whole ecosystem approach addressing not only important aspects of coral biology such as reproduction, recruitment and feeding behaviour, but also interspecific biotic interactions such as the importance of coral stands as refugia (particularly for juvenile commercially important fish species) in promoting the high associated biodiversity of the coral ecosystem fauna. Detailed food web analysis will help elucidate individual species response to local variations in physical forcing and BBL organic carbon characteristics related to the presence of the coral framework. Coral sensitivity to natural and anthropogenic stressors will be determined both *in situ* and in controlled laboratory experiments. **Achievements:** A comprehensive understanding of important aspects of coral biology and the elucidation of the role of the coral framework in promoting increased biodiversity when compared with the adjacent seabed. The description of coral ecosystem food web relationships and resource partitioning related to variations in local physical forcing and BBL organic carbon inputs. The identification of coral health indicators in the field and the development of experimental systems and protocols for toxicological and physical stress testing in the laboratory.

Objective 4: ***To assign a sensitivity code, identify the major conservation issues (and increase public awareness), and make recommendations for the rationale use of deep-water resources on the European Margin***

The principal aim is to translate the scientific discoveries and conclusions from the objectives above into a form which is accessible to endusers. A major goal is to ascertain the sensitivity and vulnerability of DWC ecosystems in the key flagship areas. Consultation with environmental managers, industry and NGO's will be an important step in the identification of the principal conservation issues and enduser requirements. Recommendations based on sensitivity coding will inform stakeholders on the necessary measures which will permit future rationale resource development in the vicinity of DWC ecosystem. **Achievements:** This will be the first sensitivity coding of a deepwater ecosystem based on impartial scientific evidence. It will represent an extension of existing EU policy regarding Coastal Zone Management to the deep-sea province and will act as an important primer for the future development of management tools for use in the deep sea. It will develop a partnership between end users including offshore industries, environmental managers, legislators and scientists. It will produce guidelines and practical recommendations in the form of a deep-water ‘coral reef manual’ for future environmental monitoring and the rationale mangement of the deep-water coral ecosystems. An educational version of the ‘reef manual’ will supplement other methods of public dessimination of information about the coral ecosystem.

ACES KEY FLAGSHIP SITES

The discovery of giant coral reef structures with their associated biological diversity is another milestone in the scientific and industrial exploration of the deep continental slopes and shelves. Deep-water coral systems are found along Europe‘s Atlantic Margin, from the Iberian Peninsula to the Norwegian Shelf, and also occur in shallow-water coastal regions along the Swedish Bohuslän coast and in Norwegian fjords.

The ACES community has selected five key flagship DWC areas along Europe‘s NE-Atlantic frontier (see Tab. 1). These study sites will be investigated with standardised methods to achieve a high degree of comparability - a major prerequisition for providing broad-based recommendations for the protection of these unique ecosystems.

Table 1. Overview and site characteristics of ACES targets along Europe's NE-Atlantic Frontier.

Site	Galicia Bank	Porcupine Slope	Rockall Trough	Kosterfjord (Sweden)	Sula Ridge (Norway)
Latitude	43° N	51° N	59° N	59° N	64° N
Water Depth	700 - 900 m	600 - 1200 m	1000 m	85 - 150 m	248 - 315 m
Seabed Structure	Coral thickets on pelagic ooze sand waves	Large thickets on top of giant carbonate mounds (up to 300m high)	Coral bushes on numerous, small carbonate mounds (ca. 5m high)	Shallowest DWC occurrence reported.	Largest known DWC reef in the NE Atlantic with 13 km lateral extension.

Galicia Bank (43° N).- This seamount lies 120 miles off the northwest coast of NW Spain and has its summit at 500 m water depth. It is approximately 1500m long with a very steep eastern slope of bare rock. The west slope levels out at about 800m to an extensive sandy plateau. Current speeds are high, producing a seafloor of coarse foraminiferal sand that is formed into mega-ripples with a wavelength of about 25m and an amplitude of 50 cm. Surprisingly, the corals *(Lophelia* and *Madrepora)* occur in this dynamic sandy area rather than on the bare rock slopes. The corals form longitudinal patches of about 1 m wide and 1 m high, and can run for over 10 m, oriented parallel to the mega-ripples. Old coral trunks form the attachment point for the living animals. The patches, which are as frequent as the mega-ripples, provide a habitat for many other animals like fish, crabs and sea urchins. Near the foot of the seamount's summit there are extensive beds of crinoids (sea feathers). The presence of the crinoids and the extensive development of coral growths suggest that the oceanic water here is richer in food than is generally thought. This site lies close to the "40° N" boundary, an oceanographic division that may represent the transition from the comparatively oligotrophic southern part of the NE Atlantic to the more eutrophic northern part.

Porcupine Slope (51° N).- In water depths of 600-900 m, this area contains numerous clusters of hundreds of carbonate mounds up to 2 km in diameter and 250 m in height (HENRIET et al. 1998). The processes of mound formation are beyond the scope of ACES, but these mounds are sites of extensive *Madrepora* and *Lophelia* buildups. Recent video observations have shown that the coral growths also support an extensive epifauna of sponges and gorgonians and are home to numerous fish. The work on the ecological dynamics of the DWC belt in the Porcupine Seabight can also be assessed via a comparative study of an adjacent suspesion feeding community. Centred at approximately 1250 m depth there is a dense belt of hexactinellid sponges (*Pheronema carpenteri)* around much of the Seabight (RICE et al. 1990). Both the sponge belt and coral growths elsewhere are suggested to benefit from topographic intensification of internal wave energy (FREDERIKSEN et al. 1992). The sponges, as with coral accumulations, also appear to enhance the biological diversity of their associated faunal community (BETT and RICE 1992).

Rockall Trough (59° N).- There is a mound field located at a depth of approximately 1000 m in the northeast corner of the Rockall Trough, immediately to the south of the Wyville-Thomson Ridge (BETT, 1999). The "Darwin Mounds" lie on the surface of a large sediment drift complex (HOWE 1995, STOKER et al. 1998), which in the area of the mounds comprises a 15 cm layer of rippled foraminiferous sand, indicating appreciable current activity in the area. The numerous mounds (100+) in the field are generally 100 m across and about 5 m high and are characterised by the presence of *Lophelia* growths (the occurrence *Madrepora* has not yet been confirmed at this site). An apparently unique feature of the Darwin Mounds are their "tails". These features are detectable by sidescan sonar but have no obvious physical expression at the seabed. The tails are, however, readily detected visually by the presence of particularly abundant populations of the xenophyophore (giant protist, 20 cm in diameter) *Syringammina fragilissima.* The tails all have a common orientation, from northeast to southwest, and appear to represent a "downstream" feature. Their orientation is consistent with a branch of the slope current being deflected from its northeast course over the Wyville-Thomson Ridge and circulating round the local topography of the basin, exiting to the southwest into the general deepwater circulation of the Rockall Trough. The tails and their xenophyophore populations provide an opportunity to study the near-field effects of coral based communities.

Kosterfjord (59° N).- While the major occurrences of cold-water coral bioherms in the northeast Atlantic are located in relatively deep, open sea locations along the Atlantic Margin, there are also a few occurrences in relatively shallow fjords in Norway and Sweden. The confirmed locations in Sweden are restricted to a deep trough in the Kosterfjord area outside the northern part of the Swedish west coast (WAHRBERG and ELIASSON 1926, JÄGERSKIÖLD 1971). There are some indications that degradation of coral growths have taken place in this region, however, at one of the Kosterfjord sites the occurrence of live *Lophelia* bioherms has recently been confirmed using an ROV. This bioherms are located in water depths of 80-90 m and contain hundreds of live *Lophelia* colonies, ranging in size from a few centimetres to several metres. This site will be the primary area of investigation for the present work. The Swedish *Lophelia* sites differ from the other ACES locations in many respects (depth, absence of strong tidal forces, highly stratified water column, seabed morphology, isolation etc.), the identification of factors common to the different locations should provide strong evidence of which environmental parameters are key to the successful development of DWC ecosystems.

Sula Ridge (64° N).- As far as is known, the *Lophelia* reef complex on Sula Ridge represents the largest DWC bioherm in European waters, having a lateral extension of 13 km and a coral framework thickness of 15 - 35 m (MORTENSEN et al. 1995). Reef evolution on Sula Ridge commenced at 8600 YBP, shortly after the Younger Dryas cold spell (HOVLAND et al. 1998). To achieve that degree of framework accretion during the Holocene, the *Lophelia* growth rate must fall within the magnitude of tropical shallow-water coral growth rates, at about 2 cm per year. This value has been confirmed by stable isotope analysis studies by MIKKELSEN et al. (1982) and FREIWALD et al. (1997). Although located on a mid-shelf position, the Sula reef complex lies in the path of a topographically-guided stable filament of the N-Atlantic Current (LJØEN and NAKKEN 1969). This filament forms a dense and saline underflow beneath the less saline surface water of the Norwegian Coastal Current. The discovery of this large DWC ecosystem in the economic zone off Norway caused immediate public concern and has led to environmental protection through Government legislation.

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TITLE: SAPROPELS AND PALAEOCEANOGRAPHY: Palaeoceanographic, Palaeo-climatic, Palaeo-environmental and Diagenetic aspects of sapropel formation in the eastern Mediterranean: **(SAP)**

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SAPROPELS And PALAEOCEANOGRAPHY:
Palaeoceanographic, Palaeo-climatic, Palaeo-environmental and Diagenetic aspects of sapropel formation in the eastern Mediterranean
(SAP)

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INTRODUCTION

During the last twenty years the scientific community has considered the Mediterranean Sea to be an important natural laboratory for research in physical, chemical and biological oceanography and marine geology. This relies on the particular features of the Mediterranean Sea, being an semi-enclosed marginal sea, having a negative hydrological budget, and thus being an unstable biogeochemical palaeo-environment. Furthermore, its geological history is long and complex; and during the late Holocene has fashioned a unique cradle of civilisation.

The Mediterranean Sea obtained its present morphological character in the lower Pliocene. A peculiarity of the Mediterranean basin is the presence of several sills with depths between a few dozens to several hundreds meters. These sills, from west to east, divide the Mediterranean Sea into a series of smaller basins: the Alboran Sea, the Balearic Basin, the Tyrrhenian Sea, the Eastern Mediterranean and the Aegean Sea.

The geographical position of the Mediterranean Sea is unique: at present it is surrounded by arid regions to the south and east and by more humid regions in the north. All these features influence the individual basins in different ways, and salinity and temperature gradients that increase eastward are reported. The individual basins will have a different evolution during a major climatic change : i.e. the transition from a glacial stage to a interglacial stage. The end member of the model to the east (the Black Sea), the most isolated, has changed its condition from a fresh water environment to a brackish water one, with permanent anoxic conditions in the water column. The end member of the transect to the west (the Atlantic Ocean) only underwent minor changes in temperature and salinity. During this time the eastern Mediterranean remained a marine environment where regional and global variations in climatic signals were recorded in the sediments. Due to its unique regional basin environment, the recordings of regional and global change have been preserved much better in the sediments of the eastern Mediterranean than in those of the open ocean.

Mediterranean sediments are ideal for high resolution studies, because of the excellent control on stratigraphic time (due to ash layers and sapropels, most of which have been extensively dated). This, in combination with palaeomagnetic, isotopic (both stable and radiogenic), micropalaeontological, and geochemical markers, tuned with astronomical parameters, gives an unprecedented precision for chronostratigraphy over the last few million years. Such high precision allows the accurate comparison, in high detail, of identical time intervals in the sedimentary record of different cores.

Pelagic sedimentation in the Mediterranean

The deep sea pelagic record in the Eastern Mediterranean is notable for the existence of numerous layers of dark-coloured pelagic sediments rich in organic carbon, known as sapropels. Most sapropels occur during the warming trend or just at warm peaks of the isotopic curve. A few sapropels however fall in cold episodes. Different models have been proposed for the origin of Eastern Mediterranean sapropels, related to climate changes which occurred in the Late and Middle Pleistocene. Sapropel deposition is a short duration regional phenomenon and no sapropels occur in the same period of time in the Atlantic Ocean or in the Holocene western Mediterranean.

The origin of the formation of sapropels has been debated from the time of their discovery until the present-day. Bottomwater anoxia due to stagnation, and/or enhanced productivity have been put forward as possible explanations. In view of the rare presence of benthic organisms in sapropel intervals, bottomwater anoxia seems likely to have occurred. There is, however, considerable evidence for enhanced productivity as well. All models are related to global climatic variations that have occurred, such as those referred to as 'astronomical forcing' models. These climatic variations may have resulted in distinct changes in the physical water mass circulation, and/or chemical fluxes to the water column. The former could have caused water column stagnation or a change from anti-estuarine to estuarine circulation.The latter may have been caused by an increased amount of nutrient input, for example through enhanced river input. All of these potential changes result in nutrient accumulation, and consequently enhanced biological productivity, and/or enhanced preservation. The Eastern Mediterranean remained a marine environment during sapropel formation, and recorded and amplified the climatic signal in its sedimentary record as shown by oxygen isotope stratigraphy of planktonic foraminifera.

With the tools developed and the cores recovered during previous MAST programmes and the SAP-project, it is now possible to compare in a high resolution multi-disciplinary study the spatial and temporal variability in sediments, deposited under varying environments (high/low sedimentation rate, varying water depths, oxic/anoxic conditions, and terrestrial versus marine input, etc.). This is being done for sediments from the last 10 000 years, followed by studies from older sediment units at decreasing spatial resolution. In addition, we are comparing the composition of sediments deposited under similar pelagic fluxes, but preserved under contrasting bottomwater redox conditions. The strength of our approach is not only the comparison between deposition in two contrasting environments (oxic versus anoxic), but also the highly interdisciplinary research done on sediments from boxcores, multicores, gravitycores and a large diameter long piston core, using a high resolution sampling interval. Despite the large amount of disciplinary sub-samples needed per sample, these cores allow almost all studies to be done on identical samples.

The understanding of the conditions leading to the onset and ending of sapropel formation are of vital importance for the long term management and sustainable use of the (eastern) Mediterranean.

OBJECTIVES

The overall aim of SAP is to better understand the (paleo) functioning of the eastern Mediterranean and to determine the role in the global environment by studying the characteristic biogeochemical processes. SAP also contributes to a better understanding of how the oceans function and how marine geoscientists can better interpret the marine sedimentary record. The improved understanding of the functioning of the palaeoceanographic and sedimentary system of the eastern Mediterranean will be a valuable contribution to the European (palaeo)oceanographic and marine (geo)science communities, and to the future management for the sustainable use of the Mediterranean.

The specific objectives of SAP are to:
1a. Determine composition, preservation, and environmental signals recorded in sediments deposited under different (oxic and anoxic) bottomwater conditions in the eastern Mediterranrean
1b. Determine composition, preservation, and environmental signals in some intervals of sediments from a core in the S Aegean Sea and a core in the western Mediterranean
2. Determine the relative contributions of aeolian and fluvial material and the associated biogeochemical fluxes to sediments of the eastern Mediterranean
3. Quantify salinity and nutrient fluxes from the sediment to the deep water of the eastern Mediterranean

RESULTS

Cruises
During two subsequent cruises supplemental samples and data were collected. The first cruise was held in December 1998 with *RV Urania*. Box cores and gravity cores in three areas of the Ionian Sea were collected: the Apulian Plateau, the Anoxic Lakes Region and the Calabrian Rise. In the summer of 1999 an additional 8-week cruise with the Russian vessel *Professor Logachev* took place. Sampling sites included the Bannock, Urania and Atalante Basins, high and low sedimentation sites, mud dome areas and the Aegean Sea.

Database
Work during the two first years of SAP has focussed on the establishment of the data base necessary for the construction of east-west, north-south and water depth transects for the sediments of the eastern Mediterranean basin, and for comparisons with adjacent areas. A multidisciplinary dataset, containing sedimentological, micropaleontological and organic and inorganic geochemical data for 7 SAP-sites is now available. The WWW site with information on the SAP project can be found at: http://hp715.gp.terra.unimi.it/ ~conisma/SAP.htm. Pages with SAP-data are only accessible to SAP-partners. At this site all users, so not only SAP Partners, can access other data for four hundred cores from all over the Mediterranean Sea.

Oxic-anoxic comparison
A comparison has been made of sediments deposited in a ‘normal’ Mediterranean environment (ABC26) including environmental transitions changing from anoxic to oxic going from the sapropel to the post-sapropel era, with sediments deposited under continuously anoxic environmental conditions (BC15; Rutten et al., 1999). Results indicate that:
(a) In the anoxic environment there has been no oxidation front that would otherwise have removed part of the paleo-environmental signals
(b) In the anoxic environment biogenic silica is well-preserved whereas it is not preserved at all in the oxic environment
(c) In the anoxic environment aragonite may be better preserved than in the oxic environment
(d) Barite seems to be better preserved in the high-sulphate, high water-depth, hypersaline anoxic environment
The results are as yet inconclusive for a potential difference in preservation of organic matter between the anoxic environment and the residual (i.e. un-oxidized) part of the S1 sapropel found below presently oxic conditions.

Original S1 thickness

SAP will employ the Ba/Al ratio rather than organic carbon content or visual appearance to define the levels of initiation and cessation of sapropel S1 deposition, using the approach outlined by Thomson et al. (1999). This is shown for the SAP-core UM42 (Figure 1). The location of the original top of sapropel S1 is indicated by the position of the upper Mn/Al peak and the upper boundary of the sediment layer with high Ba/Al ratios. The residual visual S1 unit is located below the lower Mn peak and is characterized by elevated concentrations of organic C.

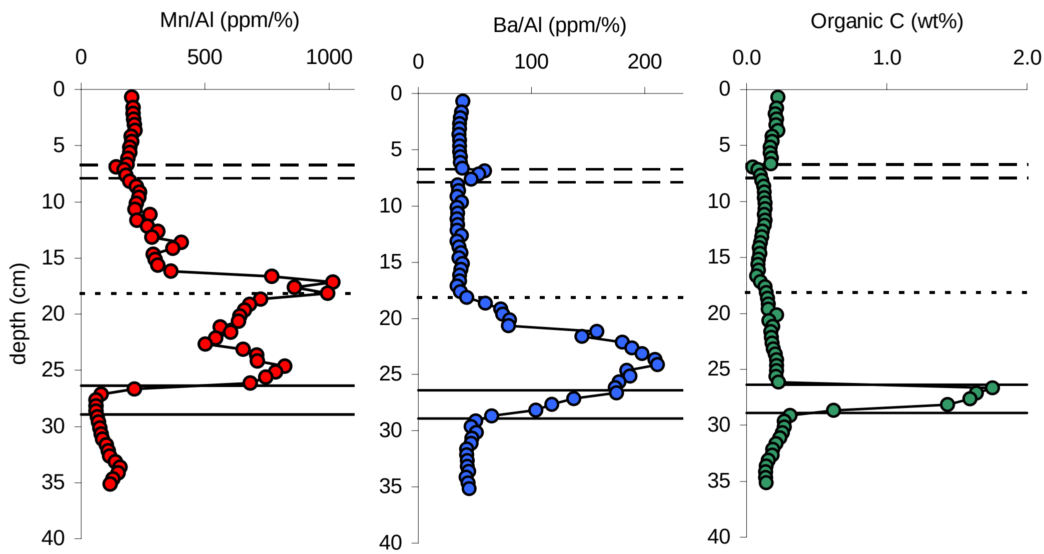

Figure 1. Mn/Al (ppm/%) and Ba/Al (ppm/%) weight ratio profile and organic C (wt%) profile for core UM42. Tephra layer: long dashes. Top of oxidized S1: short dashes. Present S1: drawn lines.

Timing of S1

Advantage is being taken of cores for which comprehensive radiocarbon data were already available from other workers to supplement the data that could be gathered by SAP alone. A SAP-paper (Mercone et al. 2000) presents a synthesis of the new Ba/Al ratio data gathered for these cores with available radiocarbon data. Several important conclusions on S1 deposition emerge in this paper:

(a) Slightly different S1 durations, as defined by the Ba/Al ratio, are indicated for slowly-accumulated S1 units (5300-9500 years) and for rapidly-accumulated S1 units (6000-9500 years). This is believed to be due to post-depositional bioturbation effects which mix more of the S1 signal upwards in slowly-deposited sediments after bottom waters return to a condition of full oxygenation. These estimates for S1 duration are longer than most literature values, but coincident with times of wet conditions around the Mediterranean basin according to a recent revised interpretation of Mediterranean terrestrial pollen records (Rossignol-Strick, 1995; 1999).

(b) The unexpected observation (Murat et al. 1990) that S1 C_{org} contents increase with core water depth is matched by a similar increase in Ba/Al ratio with water depth. This observation is explained by shallower sites being nearer land and having more rapid overall sediment accumulation rates than deep-water sites that tend to be from the centre of the basin. In shallow water situations the biogenic Ba flux is therefore diluted by the detrital Ba flux. The available data can be modelled as a constant biogenic Ba flux superimposed on a variable detrital Ba flux that is governed by overall accumulation rate.

(c) A first attempt has been made to derive a timescale for S1 formation as a function of water depth, independent of position. This shows that S1 formation was underway before 9000 years and was complete by 6000 years. This finding is at odds with the recent suggestion that sapropel formation might start earlier at shallow water depths than in deeper waters (Strohle and Krom, 1997).

Aegean Sea and Western Mediterranean

Initial work on the comparison of Aegean Sea sediments with those of the open eastern Mediterranean basin was undertaken on a core collected during the PALAEOFLUX project (core LC 21, recovered in 1995 by *Marion Dufresne* Cruise 81 from the south-east Aegean Sea and archived at SOC). Because insufficient material was available for the full suite of SAP analyses, an important aspect of the work on core LC 21 was to prove the suitability of its site in anticipation of new coring on the 1999 SAP *Logachev* cruise. The site of core LC 21 was revisited and core SL60PC was recovered for intensive SAP work in the coming year. Core LC 21 differs from those from the deeper eastern Mediterranean basin investigated elsewhere in SAP in that the visual S1 sapropel unit it contains is clearly in two parts, of roughly equal thickness (De Rijk et al.1999).

Initial work on the comparison of western Mediterranean Sea sediments with those of the eastern Mediterranean basin was undertaken using core MT15 archived at Utrecht. Preliminary estimates of the sedimentation rate at this site, indicate that the interval deposited in sapropel S1 times is most likely located between 60 and 100 cm depth.

Aeolian and fluvial input

Distinct ash layers are known to occur in eastern Mediterranean sediments. Most of these have been dated and can be referred to eruptions from known volcanic fields.A clear volcanic signal is visible in several of the cores studied by SAP (e.g. core BC19, which contains evidence of the Santorini ‘Minoan’ eruption of 3356 yrs BP). Ti/Al profiles versus depth are thought to reflect the dust input into the eastern Mediterranean. Low and high sedimentary Ti/Al ratios, i.e. low and high dust fluxes respectively reflect humid and arid climatic conditions in the larger Mediterranean region. Clearly, the period of deposition of organic-rich sediments (sapropel S1) coincides with a humid climate, whereas the period before and after it is characterized by more arid climatic conditions.

Other diagnostic and more sophisticated constituents that can be used to detect dust versus riverine terrestrial input are Sr and Nd/Sm isotopes (Freydier et al., 2000). The initial sapropel interval is characterized by relatively high εNd0 values and relatively low $^{87}Sr/^{86}Sr$ ratios. Generally, in sapropel and non-sapropel sediments, values for εNd0 and $^{87}Sr/^{86}Sr$ tend to be higher respectively lower going from the more western to the more eastern sites. In addition, in each core, sapropel sediments tend to show this same trend relative to non-sapropel sediments within the same core. This all points to the relatively enhanced influence of Nile material to the sediment in an eastward direction and during sapropel times.

Salinity and nutrient fluxes

Quantification of salinity and nutrient fluxes to the deep-water of the eastern Mediterranean requires accurate pore water data. Pore waters for key-areas have been collected during several cruises in 1998 and 1999 (SINSAP98 with RV *Urania*, Medinaut with RV *Nadir*, and Medineth/Smilable with RV *Logachev*). Data analysis is currently underway.

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TITLE: BALTIC AIR-SEA-ICE STUDY : **BALTEX-BASIS**

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Summary

The Baltic Sea Air-Sea-Ice Study (BASIS, 1998-2000) is a process study of the BALTEX/ GEWEX programme. An extensive, marine meteorological, sea ice and oceanographic winter experiment was carried out in February-March 1998, in order to study physical processes and parametrizations for optimization of coupled atmosphere- ocean models. The report outlines the study and gives sample results.

INTRODUCTION

The Baltic Sea is located in the seasonal sea ice region with an ice cover forming and melting each year. By acting as a thermal insulator and a mechanical cover, the sea ice influences the sea-atmosphere interaction in a dramatic way. Sea ice also has a large climatic importance as a sensitive indicator of a climatic change and can act as a feedback mechanism for the change. As to the winter navigation, sea ice is of first-rate importance to various Baltic Sea countries. Mathematical modelling is vital for understanding air-ice interaction and weather and predicting the processes affecting the climate and winter navigation, but the models also need verification and optimization by observations. Accordingly, the overall objective of BASIS is *to create and analyse an experimental data set for optimization and verification of coupled atmosphere-ice-ocean models*. The specific objectives cover investigations of:

- atmospheric boundary layer (ABL), especially close to the sea ice margin
- momentum and thermal interaction at the air-ice-sea interfaces
- sea ice and its dynamics
- ocean boundary layer (OBL)
- ocean water and heat budget.

Field observations and process studies result in validation and development of coupled atmosphere-ice-ocean models.

FIELD CAMPAIGN

The field experiment was carried out in the Gulf of Bothnia, Baltic Sea, in February-March, 1998. For studies of the ABL, airborne measurements were made by both a research aircraft and a helicopter. The observations covered atmospheric turbulence and radiation, and clouds. Ground-based ABL measurements included radiosonde soundings at five stations at the coasts of the Gulf of Bothnia. An ice going research vessel (*R/V Aranda*, Finland) in the sea ice formed the central platform for the surface meteorological, ice and oceanographic studies. Meteorological buoys, meteorological masts, sonic anemometer, radiation sensors, and ship weather station were used to observe the ABL and surface properties at the Ice Station. A station with meteorological mast, turbulence instruments and radiation sensors measured the atmospheric surface boundary layer in the land-fast ice at the Swedish coast, and one more special meteorological turbulence and surface station was operating in the Finnish coast. The sea ice was investigated by remote sensing, drifting buoys (9 drifters) and surface-based measurements. The ocean boundary layer was studied by CTD, ADCP and current meters, thermistor chains, and the OBL turbulence was investigated by ultrasonic and electromagnetic eddy flux measurements below the ice. In detail, the experiment and the data are introduced in the BASIS Data Report (Launiainen, 1999).

SPECIFIC STUDIES

Various specific studies as a co-operation applying the BASIS joint data set are under way. The studies cover the following topics:

1. Temporal and spatial variability of fluxes over ice and water (by UHAM, FIMR, and UUPP)
2. Turbulence structure over sea and ice (UUPP, UHAM, FIMR)
3. Characteristics of ice as seen by Radarsat satellite and aircraft (SMHI, UHAM)
4. ABL modification on the basis of aircraft observations and modelling (FIMR, UHAM, UUPP)
5. Observations and modelling mesoscale circulations at the ice edge zone (UUPP)
6. Parameterization of subgrid-scale fluxes over broken sea ice cover (FIMR, UHAM, UUPP)
7. Heat fluxes in the ice and snow (FIMR, SMHI)
8. Variability of sea ice kinematics (SMHI, FIMR, CUT)
9. Wind forcing on the sea ice drift (FIMR)
10. Process studies of ice-water fluxes and parameterization of turbulence below the ice (UHOK, FIMR, SMHI)
11. Ocean heat budget during BASIS (SMHI, UHAM)
12. Structural properties of sea ice (UHOK, FIMR)
13. Study on HIRLAM model reanalyses and radiosonde data (UHANN)

In addition to the studies listed, Contractors are proceeding with studies based on their own data.

Results

Here we present samples of results from BASIS studies. The samples are selected to represent the main progress fields: atmospheric boundary layer, ice and surface processes and properties, and modelling.

Atmospheric boundary layer

The local ABL over sea ice has been studied on the basis of the data collected at the Ice Station at R/V Aranda (FIMR). The results address the turbulent surface fluxes and air-ice coupling. The ice surface temperature, which is especially difficult to estimate by the ordinary means strictly, was estimated by various independent methods (i.e. based on the air temperature profile, on longwave radiation, on temperature profile drawn from the sonic anemometer, or as estimated by a thermodynamic ice model). In surface turbulence studies, various methods were compared for determination of turbulent fluxes. Particular attention was paid on determination of the roughness lengths of momentum and temperature. For over the sea ice, the results yield a new formula for the momentum to temperature roughness ratio (Launiainen et al., 2000).

The temporal and spatial variability of surface fluxes have been studied on the basis of the observations at the three coastal/ice-based and by the research aircraft Falcon. The aircraft and surface-based observations showed a reasonably good agreement. The surface-based stations alone were not enough to explain the spatial variability over the Gulf of Bothnia, but the aircraft observations demonstrated the sensitivity of the fluxes to the ice conditions and ice concentration (Figure 1). Considering the outer ABL, the formulae to calculate the depth of the ABL have been validated. The values of the constants in the PBL height theory were re-estimated, and including the free-flow stability the surface-layer theory shows reasonable results. The BASIS rawinsonde soundings have been applied to verify the HIRLAM (High Resolution Limited Area Model) analyses. In many cases the model profiles of wind and air temperature were good, but the low-level jets were not well modelled by HIRLAM.

Ice and surface processes and properties

The atmospheric forcing on sea ice drift was studied by applying the data from the buoys deployed on sea ice (Uotila, 2000). Trajectories of the sea ice drifters are presented in Figure 2. The statistical dependence between the wind and drift was strongest in the centre of the basin, where the sea level variations correlated with the buoy motion. The drift was simulated by a dynamic sea ice model with 18 km and 5 km resolutions, and various methods were applied to calculate the wind forcing. The best wind forcing was achieved by applying a method dependent on atmospheric stability and ice conditions.

Derivation of surface wind from Radarsat SAR data was studied and reported (Mugnier-Pollet, 1998). Other studies address snow and ice properties, concentration, ice kinematics, and ice roughness. The Radarsat data yielded interesting results addressing the relation of the backscatter coefficient and the incident radar beam angle. The SAR ice concentration maps were constructed (SMHI) and a CD ROM including 12 images was delivered to the BASIS data bank.

Ocean boundary layer

Stratification, currents, and heat, salt and momentum transfer in the OBL were studied. The bulk transfer coefficients of heat and momentum indicated dependence on the stratification and current direction, but the mean water-ice fluxes were small, indicating a strong stable stratification below the ice. Compared with previous studies in polar regions, the heat flux and transfer coefficients show a large variety.

Modelling

Modelling studies address the heat budget of the Baltic Sea, the sea ice dynamics and thermodynamics, and the ABL. The modification of the ABL over the Gulf of Bothnia has been modelled using the aircraft observations as a reference. Two ABL models have been applied in the work: a 2-D model (in FIMR) and a 3-D model (in UUPP), and the simulations have addressed on-ice and off-ice flows. The development of a low-level jet during the on-ice flow was sensitive to the parameterization of turbulent mixing, and the subgrid-scale leads had an important contribution to the heating of the ABL during the off-ice flow. In addition to the mesoscale simulations, a non-hydrostatic LES model has been used (UHAM) as a one-dimensional column version to simulate the stable boundary layer over the sea ice. The studies will continue addressing the unstable flow over broken ice and open water.

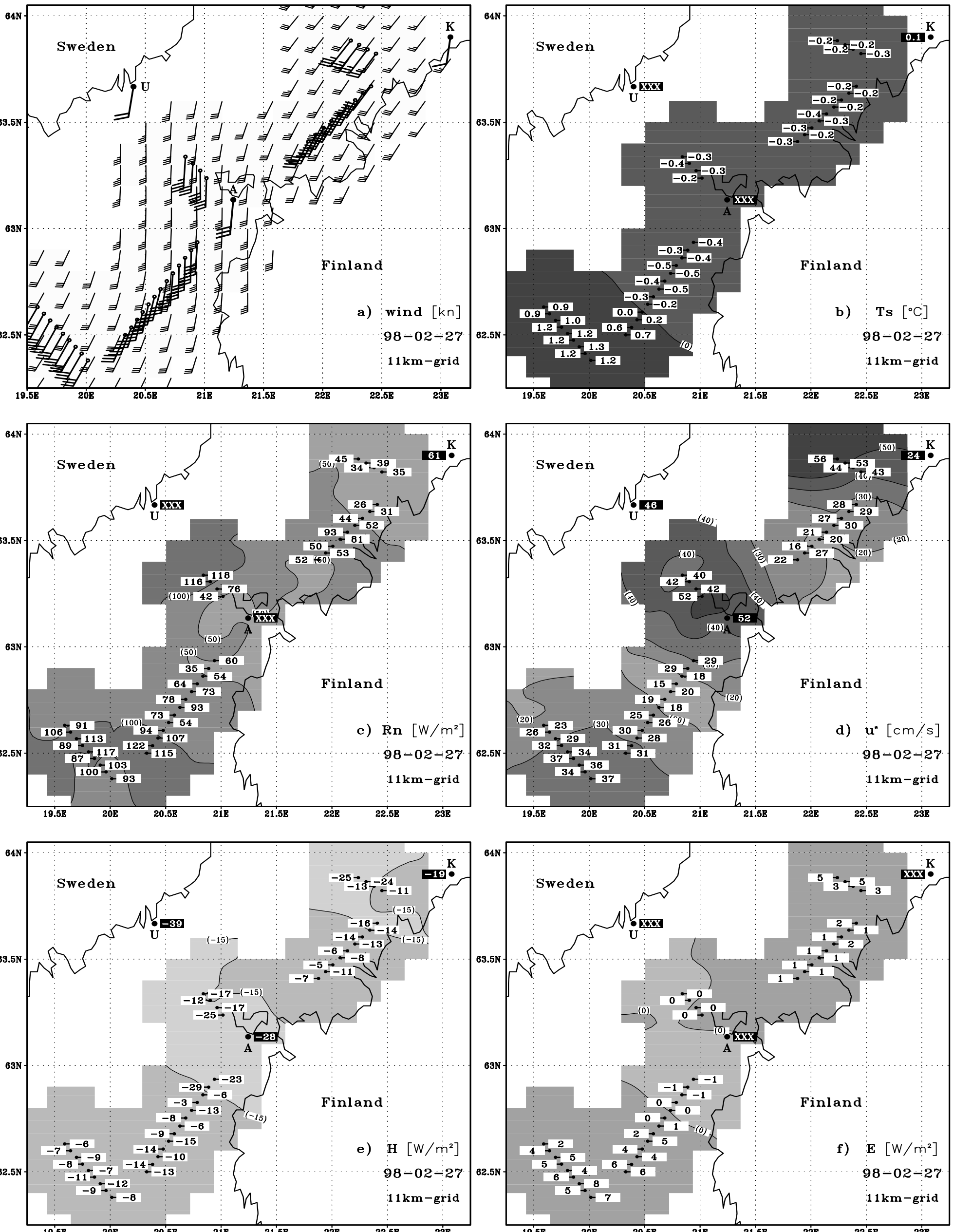

Figure 1. Spatial distribution of (a) wind speed, (b) surface temperature, (c) net radiation, (d) friction velocity, (e) sensible heat flux, and (f) latent heat flux, as measured by the research aircraft Falcon and the coastal stations during on-ice flow over the Gulf of Bothnia, on 27 February, 1998 (from Brümmer et al., 2000).

CONCLUSION

In the light of the results, a lesser part of which are published still so far, we foresee the project main goals to be satisfied successfully. For example, some new air-ice process parametrizations are already in test use in Finnish coupled air-ice research models.

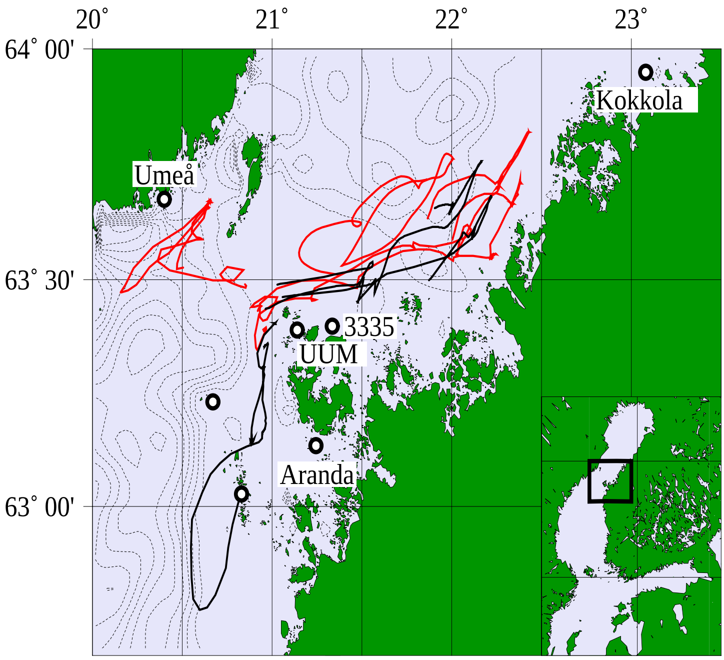

Figure 2. Trajectories of nine drifters between 19 February and 5 March 1998, and the locations of the coastal (Umeå and Kokkola) and ice-based (Aranda, UUM, buoy 3335) meteorological stations.

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I.3 Supporting initiatives

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SEA-SEARCH: YOUR ON-LINE GATEWAY TO MARINE AND OCEANOGRAPHIC DATA, INFORMATION AND KNOWLEDGE IN EUROPE: WWW.SEA-SEARCH.NET

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ABSTRACT:

The Sea-Search project, officially known as EURONODIM, comprises the organisation of a European co-operative network for oceanographic data & information management, that will operate in a coherent and operational mode to strengthen the quality, service and overall performance of ocean and marine data & information management in Europe, both on a national and international level. Sea-Search is to develop, maintain and electronically publish a portal / navigation site on the internet and to provide 3 major meta-data products/directories; to keep track of ocean and marine data & information; and to improve the overall awareness, overview and access to ocean and marine data & information in Europe; through 1. European Directory of Marine Environmental Data (EDMED) 2. Cruise Summary Reports (ROSCOP) 3. Research Projects Database. The Sea-Search network is also to exchange experience and to cooperate in development, promotion and implementation of data & information management practices and methods; to develop and organise an overall capability for handling, processing, quality-controlling and archiving a variety of oceanographic and marine data types, anticipating differences in capabilities of individual partners and the evolvement of new data types.

INTRODUCTION:

National Oceanographic Data Centres play a key role in data management of oceanographic data & information. Nowadays these Data Centres are developing their services very much pro-active towards users. The Data Centres are making their data holdings accessable by Internet and as data management partner are supporting many national and European research projects in setting up good data management systems and dissemination activities.

A very important initiative in this respect is the EURONODIM project, better known as SEA-SEARCH, a Concerted Action within the framework of the EC-MAST III programme.

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SEA-SEARCH is a European cooperative network of 16 national oceanographic data centres and marine information services from 14 European countries, including the EC-Joint Research Centre. Each SEA-SEARCH partner is specialised in managing and giving access to extensive resources of oceanographic and marine data & information and is providing data management services & support to a wide range of institutes and research projects, both nationally as internationally.

The primary goal of SEA-SEARCH is to provide users with a central overview and access to ocean and marine data & information in Europe. This is accomplished by operating a network of partner websites and a joint European website (www.sea-search.net)., that is being developed into the key resource or 'portal site' for oceanographic data & information in Europe.

The SEA-SEARCH website hosts an array of catalogues, overviews and links and acts as central gateway to ocean and marine information & data resources of SEA-SEARCH partners and other related organizations in Europe.

Sea-Search provides:

- an Internet gateway for users, searching for ocean and marine data & information in Europe
- a support infrastructure for organizations and projects, dealing with ocean and marine data & information, for indexing, disseminating and promoting their data & information resources to a wide user community
- a valuable resource for data management expertise and support

Key Resources of Sea-Search:

Together the SEA-SEARCH partners maintain a number of major catalogues and overviews, that can be searched through the central SEA-SEARCH website:

* EDMED - Marine Environmental Datasets

The European Directory of Marine Environmental Datasets (EDMED) was initiated in 1991, recently upgraded, and has established itself as a de-facto European standard for indexing and searching datasets relating to the marine environment, covering a wide range of disciplines. It is a high level inventory, covering Datasets and Data holding centres. At present EDMED covers already more than 2300 Datasets from more than 500 Data holding centres. SEA-SEARCH partners are responsible for keeping their national contributions up-to-date.

* ROSCOP - Research Cruises

The Directory of Research Cruises is based upon ROSCOP, a global standard for indexing oceanographic cruises by research vessels. It gives insight in data collection activities and research institutes, involved in these cruises. The reports are prepared by chief scientists and the national collation is done by SEA-SEARCH partners. The SEA-SEARCH website gives access to the European Directory of Research Cruises, that are also transferred to ICES for global compilation. Next to Search options the SEA-SEARCH website will also provide entry facilities for chief scientists to submit ROSCOP forms online.

* EDMERP - Marine Environmental Research Projects

The European Directory of Marine Environmental Research Projects (EDMERP) gives information on ongoing research projects, data collection activities, involved organisations and scientists, and resulting products. Compared to EDMED and ROSCOP this directory is a relatively new item, that will expand in coverage and importance rapidly, undertaken by the SEA-SEARCH partners.

* EC Marine Research Projects

This is a special issue of the Marine Environmental Research Projects directory, covering already more than 300 marine research projects, that are funded through the EC MAST and ENVIRONMENT programmes. This directory will also keep track of new marine research projects, that are initiated within the EC 5th framework.

* Marine Data Networks in Europe

Influenced and supported by international initiatives like EC programmes, EuroGOOS and others a number of marine data networks have been and are being implemented, e.g. in the fields of operational oceanography, marine geology, ocean geology. The SEA-SEARCH website maintains an overview and relevant web links to these marine data networks in Europe.

* Marine Data & Information Centres in Europe

An up-to-date overview and web links to centres in Europe specialised and involved in management, processing, archival, dissemination and related support services for ocean and marine data & information.

Strategy of Sea-Search:

The Sea-Search network and its central website are seen as an umbrella and bundled marketing effort for creating awareness and giving overview and access to all ocean and marine data & information activities by organizations in Europe. So Sea-Search is not only covering traditional oceanographic research, but also aiming at giving shelter and indexes / links to data initiatives in 'operational oceanography' and 'remote sensing' communities. For a major part this is accomplished by having Sea-Search partners joining and supporting various data and network initiatives with their data management and dissemination activities.

Visit the website at : www.sea-search.net

TITLE: MEDITERRANEAN DATA ARCHAEOLOGY AND RESCUE OF TEMPERATURE, SALINITY AND BIO-CHEMICAL PARAMETERS : **MEDAR/MEDATLAS II**

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MEDITERRANEAN DATA ARCHAEOLOGY AND RESCUE OF TEMPERATURE, SALINITY AND BIO-CHEMICAL PARAMETERS (MEDAR/MEDATLAS II)

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SUMMARY

The objective of the MEDAR/MEDATLAS II project (1998-2001) is to rescue, safeguard and make available a comprehensive data set of temperature, salinity and bio-chemical parameters collected in the Mediterranean and Black Sea, through a wide co-operation of the Mediterranean countries. The data are collected at national levels and checked for quality according to the common protocol, at four regional qualification centre. The data sets will be integrated in a common database and transferred to a modellisation centre for calculating the climatological statistics. At the present stage of the project, a preliminary benchmark of data sets from all participants has been processed, to test the structure and the protocol.
In addition to the technical tasks, sharing the knowledge and know-how among the participants is an important issue of the project. It aims to enhance the overall capacity of the Mediterranean and Black Sea data centres network, in oceanographic data management, in the perspective of the forthcoming operational oceanography.

INTRODUCTION

Managing the marine resources and following up the environmental changes in the sea waters requires the availability of long times series of observations. Key parameters are dissolved oxygen, nutrients, temperature and salinity. Oxygen deficiencies in the upper layers can result in diminution of higher life forms, and pathology in living organisms. Nutrient fluxes that control primary production and the bio-diversity, and can affect aquaculture and fishing activity. Temperature and Salinity, which are the primary indicators of climate changes and allow the computation of other derived quantities such as density, sound velocity, current.

Appropriate data management is necessary to insure the availability of such data. It is currently recognised that, about 30% of data remaining dispersed in the scientific laboratories are in danger to be lost after 10 years. Unfortunately, an observation in variable environment can never be remade.

Therefore, the objective of the MEDAR/MEDATLAS II project is to rescue, safeguard and make available a comprehensive data set of such parameters collected in the Mediterranean and Black Sea, through a wide co-operation of the Mediterranean countries: 15 National Oceanographic Data Centres or National Designated Agencies (NODC/DNA, 3 modelling centres and 2 international organisations. This project follows to previous successful pilot projects MAST/MEDATLAS (MAS2-CT93-0074) in which the presently most exhaustive database of temperature and salinity was produced, and MODB (MAS2-CT93-0075-BE) where the Variational Inverse Model for preparing objectively analysed data was developed.

The present project is organised in four regional tasks for data compilation and qualification, and four thematic tasks for preparing a global cruise inventory, an integrated data product, an updated climatology and a reference protocol manual.

MEDAR Cruise Inventory

The first task has been to inventory and compile information on oceanographic cruises where Temperature, Salinity, Oxygen, Nitrate, Nitrite, Ammonia, Total Nitrogen, Phosphate, Total Phosphorus, Silicate, Chlorophyll-a, Alkalinity, pH, H2S observations have been made. In addition to national sources, a review of the project and bibliographical sources has been identified to complete this information. The resulting global cruise inventory, developed by the Russian NODC/World Data Centre-B, in collaboration with ICES and the other participants, has been published on internet (www.meteo.ru/nodc/project/ftp/load.htm), with tools to select the cruise reports by countries (fig 1).

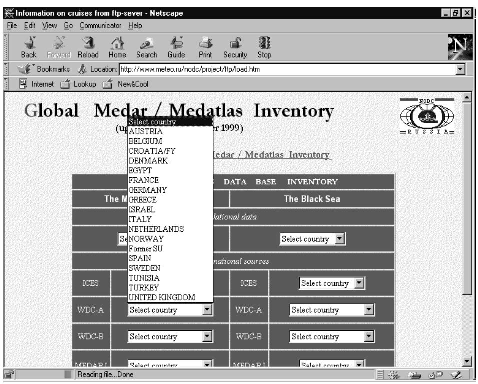

Fig. 1:On line tool for retrieving cruise information

METHODOLOGY FOR DATA PROCESSING

The compilation of data from each source laboratory is done in each country, by the National Oceanographic Data Centre (NODC) or Designated National Agency (DNA). In order to prepare an integrated database from data sets issuing from these various sources, it was necessary to insure comparability and compatibility by using a common protocol for formatting and quality checking (1). Formatting at the MEDATLAS format is made by each participant, and the quality checks on the observations, are performed at four regional qualification centres, by using available expert software tools, designed according to the protocol. As a result, the data are eliminated or a quality flag is added to each numerical value.

All the profiles are submitted to a series of automatic and visual checks. A first series of checks (QC1) is dedicated to the location, date, and data below the bottom depth (fig 2).

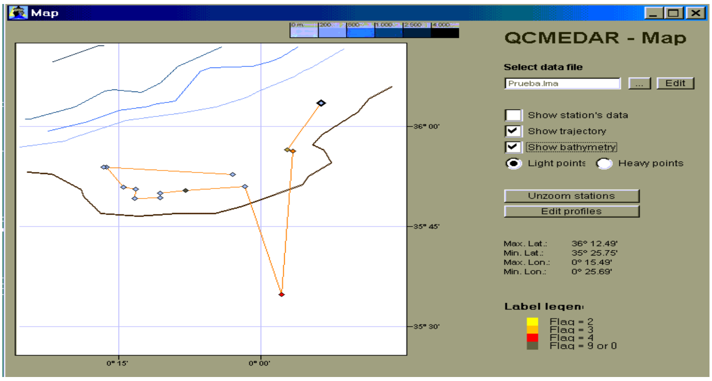

Fig 2: User interface for controlling the Location and Date

A second series (QC2) checks that the data points are not: constant profiles, out of the regional broad range values, do not create a vertical instability, a spike, incoherent with the existing climatological statistics. After the automatic checks, the profiles are visualised in waterfall individual profiles and superposed profiles to check the internal coherence (fig. 2).

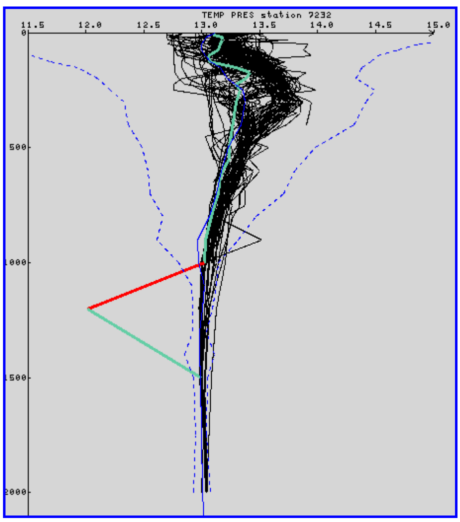

Fig 3: Example of Quality Checks of Temperature Profiles

FIRST BENCHMARK OF DATA TO TEST THE STRUCTURE

A first benchmark of newly rescued data from all sources has been circulated to test the whole structure and the protocol, format, codes, software etc. Several systematic format or codes errors have been detected and new clarifications will be inserted in the revised protocol manual The data location of some bio-chemical parameters of this benchmark are displayed on fig 4.

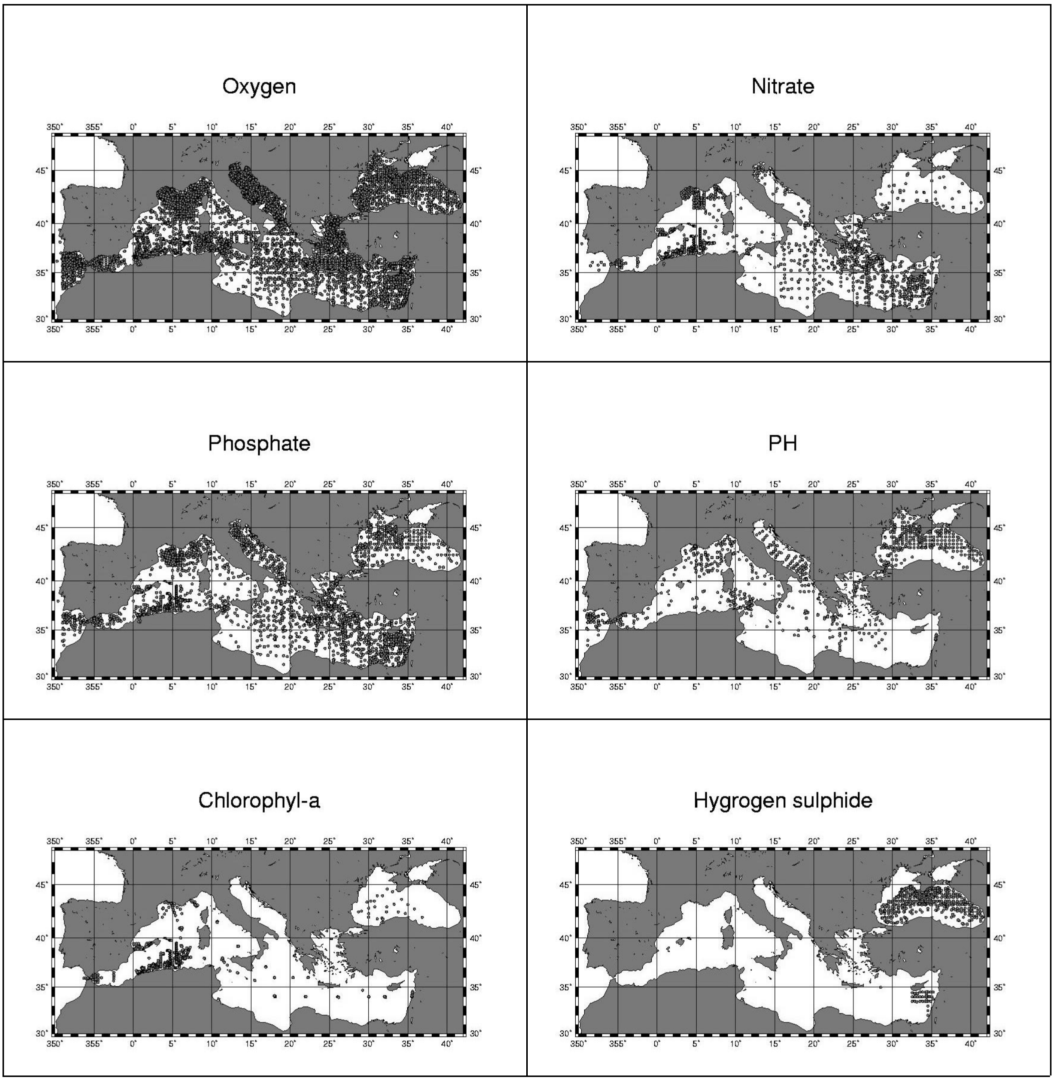

Fig 4 : Bio-chemical parameters of the first benchmark - test of the structure

Climatologies and Maps

In parallel to the observational data management, several improvements have been made to the variational inverse model analysis tool, which includes now an automatic error computation, an automatic coordinate transformation, and automatic statistical parameters. Moreover, the gridding technologies and available software have been reviewed, the data decimation pre-processing technique has been developed and is ready to use, the horizontal and vertical grid, the relevant analysis regions and time resolution have been defined, an optimiser and a seminormed background field facility have been added. Some preliminary tests on the database have been performed and the new release of the analysis tool has been made available on Internet http://modb.oce.ulg.ac.be/Medar (Fig. 5):

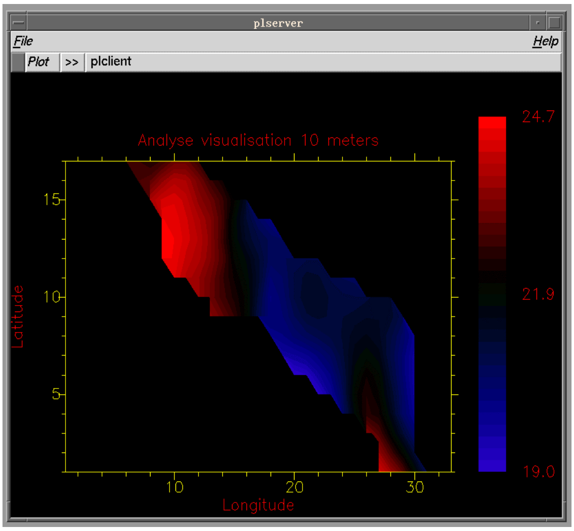

Fig. 5: Output of the DIVA software - Horizontal distribution of analysed data

DISCUSSION AND NEW DEVELOPMENTS

It is noticeable that two main objectives of the project are progressing: making available geo-chemical data and completion of the void areas at the East and South of the region, thanks to the joint effort of all the participants. Collaborations have also been developed with NODC-Croatia, INSTM Tunisia, DNA Georgia IODE and MAST NODC network, and with GODAR Project (World Data Centre-A) (2), EUROGOOS, MEDGOOS (3), SHOM. However the data rescue is not an easy task and difficulties arise not only to convince the scientists to publish their data, but also from the number of duplicates found in the historical data sets. Archiving information on cruises, maintaining national responsibilities and making use of expert software can allow working out this problem. In any case, preservation and documentation of the data sets is improved by short time lag between data collection and release, and should be encouraged by all the funding agencies.

One key point of the project is the sharing of know-how and expertise among Mediterranean and Black Sea data managers. A double QC workshop (in Brest and Athens) organised by IFREMER, IOC, NCMR/HNODC, RIHMI-WDC and IEO was dedicated to training in data formatting and QC. From many discussions of the workshop, the QC methodology will be improved. The software developed by IEO on PC has been disseminated after the workshop to all the participants and the IFREMER software on Unix is used for final checking and inter-comparisons. Another workshop will be dedicated to objective analysis.

Another important point has been the development of networking. A distributed network of thematic and regional websites has been opened to public (4), with interfaces for data request. National websites for hydrological data information and exchange are under development, and a few of them like for the Israeli site, already open.

By all these actions, is expected to contribute to the preservation of the existing data, to the further exploitation of the experimental fieldwork, and the improvement of the overall level of data quality.

ACKNOWLEDGEMENTS

The MEDAR Group acknowledges with gratitude the many contributors who have made this work possible. The scientific laboratories contributed to this database by sending copies of the collected observational data and documentation to their NODC/DNA. The financial support was provided by the Commission of the European Communities under the contracts MAS3-CT98-0174 & IC20-CT98-0103, and by the participating organisations. We thank Dr. Elisabeth Lipiatou of the DGXII/MAST Office for her efficient assistance.

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TITLE : A SEARCHABLE INTERNET DATABASE OF SEABED SAMPLES FROM THE OCEAN BASINS HELD AT EUROPEAN INSTITUTIONS: **EUROCORE**

CONTRACT N° : **MAS3-CT98-0175**

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USING THE INTERNET TO ACCESS THE EUROPEAN SEAFLOOR SAMPLE ARCHIVE: THE EUROCORE AND EU-SEASED PROJECTS

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INTRODUCTION

Marine sediment cores and other seabed samples are a raw data resource of immense scientific value and many thousands of bottom samples have been collected by European research institutes, Geological Surveys, universities and exploration and survey companies. Such data have a wide range of applications and are the fundamental data source for information on seabed character and recent sedimentation. Such information is vital to a large number of end-users in governments, industry and academia. Research into global climate change, palaeoceanography, slope stability, oil exploration, pollution assessment and control, surveying for laying telecommunication cables and offshore pipelines and national resource assessment and many other science areas (see Table 1) all rely on data obtained from marine sediment samples.

Table 1. End-users of sediment cores and seabed data

• research into global climate change	• palaeoceanographic research
• studies of slope stability	• geochemical studies
• geochronological studies	• benthic surveys
• studies of sedimentary processes and dynamics	• productivity studies
• seafloor mapping and surveys (ground truth)	• oil exploration
• environmental protection and monitoring	• pollution assessment and control
• surveying for laying telecommunication cables and offshore pipelines	• undergraduate and postgraduate training
• studies of acoustic response and defence applications	• national resource assessment
• siting of seafloor structures	

Sediment cores and other bottom samples are normally collected for a primary data requirement and after they have been analysed for the purpose for which they were collected, they are normally stored under controlled conditions for further use. Consequently, samples are stored at a large number of locations dispersed throughout the countries of the European Union and provide a legacy of continuing usefulness and importance. However, secondary use of this important data resource is currently seriously impeded by lack of knowledge of what cores are available and where they are stored.

EUROCORE is a project developed by a consortium of European core repositories to provide greater accessibility to the European sediment core archive and hence promote secondary usage of this important, but currently underexploited, raw data resource. The project began in November 1998 and is funded for three years.

PROJECT METHODOLOGY

EUROCORE has combined with another concerted action called EUMARSIN (European MARine Sediment Information Network) to form a combined project called EU-SEASED. This is a new European Union MAST III Supporting Initiative that aims to set up a central-access searchable internet database of seabed samples from the ocean basins held at European institutions. The project comprises a consortium of European national Geological Surveys and national marine core repositories which will populate the database; and a Dutch data management company, responsible for database construction and server maintenance. The EU-SEASED database will be accessible through the World Wide Web and will only list metadata (data on the data, i.e. location, sampler type, water depth, geographic and physiographic area, contact details etc.). Access to the actual samples and any related accessory datasets will be for negotiation between the requestor and the repository where the sample is held. Although EU-SEASED is an integrated project, a clear division of labour has been agreed between the EUROCORE and EUMARSIN partners with respect to data gathering (Figure 1).

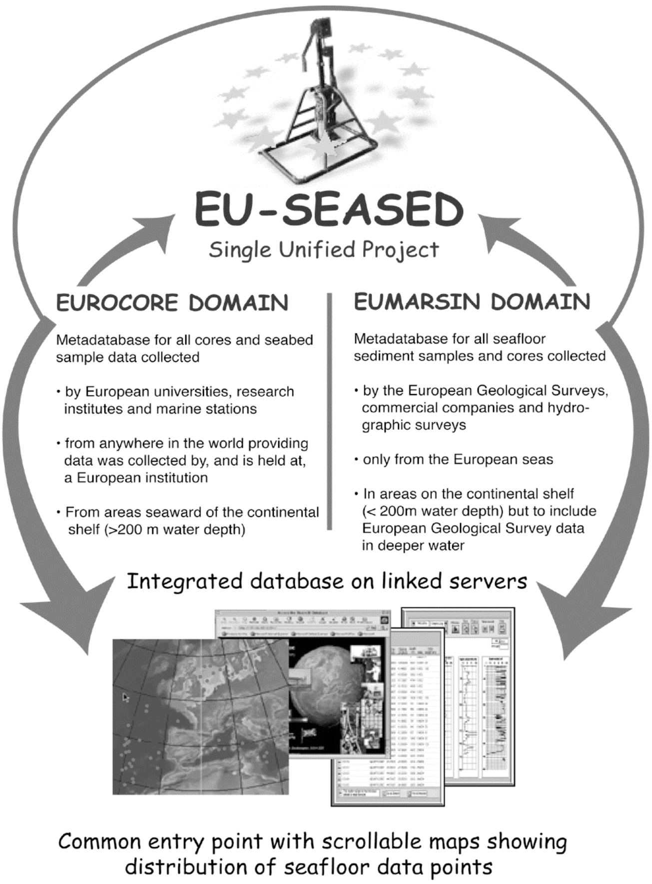

Figure 1: Schematic diagram showing the separate domains (division of labour) of the EUROCORE and EUMARSIN projects in populating the EU-SEASED database

The EUROCORE project, comprising seven of Europe's main core repositories, utilises already existing national networks and takes a strongly proactive approach in data gathering, using questionnaires and data scouts to actively seek out metadata for the database (Figure 2). A proactive approach is important as many smaller core-holding institutions may not have the resources to collate their data and EUROCORE provides the means by which this can be done.

Thoughout the project, there is close collaboration and consultation between the EUROCORE and EUMARSIN partners and with other national and international bodies involved in marine sample data management, such as World Data Centre A in Boulder, Colorado, USA, which has hosted the Index for Marine Geological Samples database since 1977. This database is a co-operative effort between mainly American institutions that maintain marine sample repositories to provide information on the contents of their collections to help researchers locate marine sediment and rock material for further analysis. Only three European repositories (one British and two German) contribute metadata to the Index of Marine Geological Samples. Consequently until the advent of EU-SEASED, it has always been far easier to determine what cores have been taken in European waters by American ships and are stored at American institutions, than to find out what cores have been taken by European ships and stored at European institutions.

Within EU-SEASED it has been recognised that the functional specification and the front-end options and capabilities of the database must be end-user driven. Hence there has been extensive consultation amongst partners, all themselves users of seabed data, concerning the metadata format for data entry. This culminated in a workshop held at the offices of the Marine Information Service, Rijswijk, in the Netherlands in April 1999, and resulted in the production of an agreed standard form for metadata collection. Two types of metadata were identified: mandatory metadata fields and optional, but recommended if available, metadata fields (Table 2).

Table 2. EU-SEASED metadata fields for data collection

MANDATORY METADATA FIELDS

Record number, measuring ID, measuring area type, co-ordinates, sampling device, data source holder

Mandatory only for EUROCORE samples : Sample state, sample storage condition

OPTIONAL BUT RECOMMENDED METADATA FIELDS

Internal reference number, objective of measurement, treatment/analysis, measured parameters, surface/subsurface sample, geographical area, monitoring site, physiographic province, navigation system, core/sample length, water depth (corrected/uncorrected/not known/reference area), core/sample penetration, core/sample diameter, date of collection, project/cruise name, research/survey vessel, project/cruise report, basal age or period, predominant sediment type, sample recovered (volume or weight of dredges or grabs), list of maps, references, comments

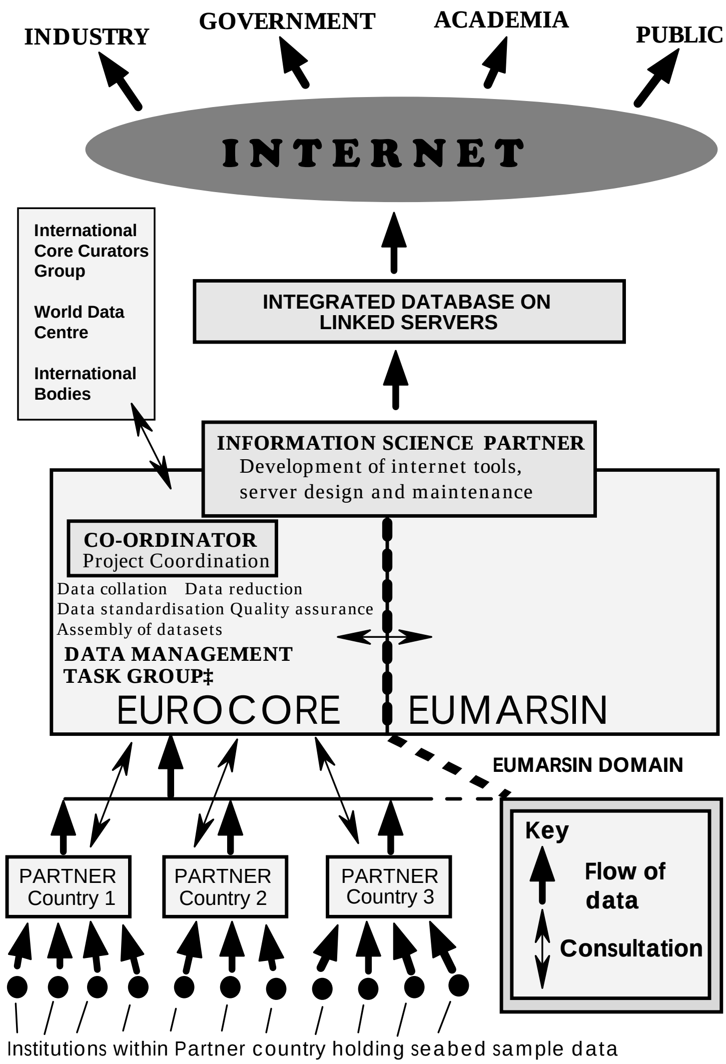

Figure 2: Diagram showing EU-SEASED project organisation and the flow of data and consultation in the project and with end-users. Membership of the data management task group comprises the information science partner (MARIS), the Coordinator and data management specialists from project partners.

Agreement on the functional specification of the database and the boundary conditions on metadata format has allowed the metadata collection phase to begin. The initial phase (months 0-18 of the project) involves the compilation of metadata on bottom samples held by EUROCORE partner institutions and submission of this data to the data management partner for compilation of the database file. The second phase of data collection (months 12-36) involves trawling for metadata on cores and bottom samples held by other sample-holding institutions in partner countries. All institutions within the European Union that hold bottom samples are invited to participate in the project. Access to the actual samples and any associated analytical datasets will always be controlled by the sample-holding institutions and EU-SEASED acts as a virtual 'shop window' for Europe's repositories and bottom sample collections; facilitating greater secondary usage of resources and providing the potential for greater scientific return and value for money on initial data collection. The database website has been established on the internet (http://www.eu-seased.net) and database operation will begin from Month 18 of the project. Metadata on a total 58,267 cores and bottom samples had been mounted on the database by Month 19 (May 2000) and metadata submission is continuing.

The basis of the central database is a Windows NT server. It will be available to users worldwide through unrestricted Internet access using an intuitive graphical user-interface, probably involving scrollable electronic maps on which the data points will be located. Over time, it is hoped to add GIS functionality, allowing enhanced search capabilities, such as identifying bottom samples within user-specified corridors, certain distances from land and undersea features etc. In addition, it is planned to add a number of community-based pages to enhance the website and increase its value to users. These include an online newsletter on marine sedimentary themes, a regularly updated conference/meetings listing, a bulletin board and a comprehensive sedimentary resources (links) page. Future development of the database could include adding other types of point (e.g. seafloor photographs, heat flow stations), line (e.g. seismic profile) and area (e.g. sidescan sonar coverage, high-resolution swath bathymetry) type seafloor data. During the project, an exploitation strategy will be developed to provide the means of updating the database in future years as more cores are collected.

A vast amount of analytical data on cores has been published or is otherwise in the public domain. Another objective of EUROCORE is to demonstrate how this data can be integrated into the database to enhance it's usefulness to users. Therefore EUROCORE will set up a demonstration model to access datasets from the meta-directory level. The demonstration model will be developed around a selected subset of cores that already have published related datasets available (e.g. graphic logs, photographs of the split cores, geochemical data, geotechnical or stratigraphic data, bibliography etc.). These files will be downloadable over the internet (Figure 3).

RESULTS

The first 18 months of the EUROCORE project has been devoted to compiling bottom metadata according to an agreed format at partner institutions, and those within partner countries that also hold core data and have agreed to participate in the project. A number of underexploited, even dormant, collections, have been identified and the number of bottom sample stations available probably exceeds well over 100,000. The functional specification of the metadatabase system has also been finalised and the website established. Software development and testing relating to data-input facilities and query options has shown that the database model will be effective and can be developed to meet end-user needs. Already searches can be made by inputting corner co-ordinates for search 'boxes'. A mapping interface will be available from the Autumn of 2000.

CONCLUSION

Marine sediment cores and seafloor samples form our primary raw data resource on the nature of the seafloor and the earth history it records. Previously, fuller exploitation of European bottom sample collections has been seriously impeded by lack of knowledge of what material is available and where it is stored. EUROCORE, in association with EUMARSIN, will populate the EU-SEASED database with metadata for bottom samples held at repositories throughout the European Union and make this data available, in searchable format, to end-users worldwide, via the World Wide Web. Scientists and others interested in the seafloor will have a time-efficient and cost-effective way of accessing the European seafloor sediment archive. The potential savings to scientific research, education, industry and governments (and ultimately the European taxpayer) will be considerable.

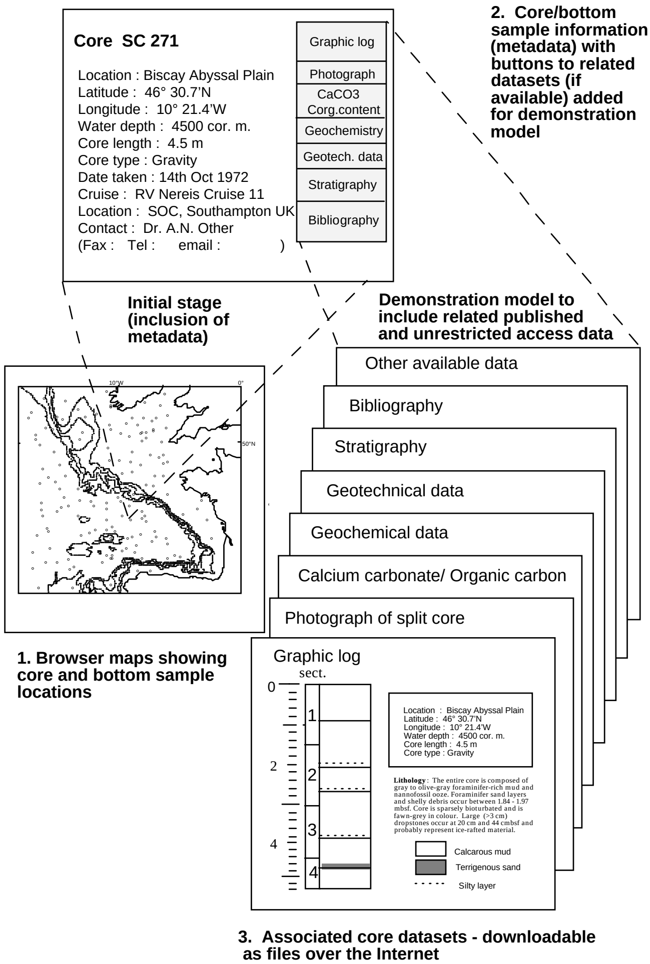

Figure 3: Diagram showing how the EUROCORE demonstration model for direct access to datasets from the meta-directory level WWW pages may look and relate.

TITLE : EUROPEAN SHELF SEAS OCEAN DATA ASSIMILATION AND FORECAST EXPERIMENT – PHASE 1: **ESODAE PHASE 1**

CONTRACT N^{o} : MAS3-CT98-0187

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EUROPEAN SHELF SEAS OCEAN DATA ASSIMILATION AND FORECAST EXPERIMENT (ESODAE) – PHASE 1
http://www.met-office.gov.uk/sec5/ESODAE/ESOHOME.html

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INTRODUCTION

This two-year EC-funded Concerted Action, which commenced on 1 February 1999, has, as its objective, the development of a plan for a European Shelf Seas Ocean Data Assimilation and Forecast Experiment (ESODAE). The European Shelf Seas are taken to include all the shallow seas from Norway, round the Shetland Islands, Scotland, Ireland, south west of the UK to southern Brittany and including the North Sea. The Concerted Action forms ESODAE Phase 1, whilst the actual experiment will constitute a later phase.

The primary ESODAE experiment will aim to be carried out in conjunction with the Global Ocean Data Assimilation Experiment (GODAE), one of the aims of which is to provide lateral boundary conditions for runs of shelf models such as those which will be part of ESODAE. GODAE will take place in the 2003-2005 timeframe. This will form the final Phase of ESODAE, and will comprise the end target ESODAE activity.

OBJECTIVES

The specific objective of the current effort is to design a framework to carry out an experiment so as to:

- develop models for the North West European Shelf that analyse and forecast the ocean in depth (temperature, salinity and current structure), including data assimilation

- run a selection of available models for the North West European Shelf in a consistent framework (with regards to area, boundary, tidal and meteorological forcing and, where possible, observational input) at the various participating institutes for a period of at least 3 months;

- exchange model products between the various participants involved in such an experiment and to jointly assess performance;

- carry out model sensitivity studies to boundary and meteorological forcing and impacts of data assimilation.

The Concerted Action aims to contribute towards networking and exchange of data, models and assimilation schemes between institutes involved in development of ocean forecasting in Europe. It is also intended to increase awareness of current developments in pre-operational modelling of the area of interest. The overall goal will be to design an experiment to provide a practical demonstration of the overall capabilities of ocean analysis/assimilation and forecasting models for the North West European Shelf. The Concerted Action is thus aimed at fostering a spirit of cooperation in developing improved ocean forecasting services for the North West European Shelf region.

Particular issues that are being addressed include:

- the performance of different model systems in analysing and forecasting conditions on the North West European Shelf, including the shelf break;

- the needs of the various modelling systems for boundary and forcing data;

- the application of techniques of data assimilation in shelf models so as to maximise the use of available data;

- planning for exchange of model products and, in collaboration with EC-funded SeaNet initiative for the fixed station network, for data exchange.

THE ESODAE TASK TEAM

The plan for ESODAE is being developed through an ESODAE Task Team which consists of the proposers of the Concerted Action, and other members of the ocean observing and modeling community who wish to take part. . To date (May 2000) 5 Task Team meetings have been held. The Team keeps under review existing efforts and developments in operational and pre-operational ocean shelf modelling, and has responsibility for the overall planning of ESODAE. It will also looks to interaction with other EC-funded projects and, for example with the EuroGOOS North West Shelf Task Team.

Particular Tasks being tackled by the task Team include:

I. ESODAE Science and Coordination Planning

I.1 Identification of overall scope and concept design.

I.2 Detailed review of existing candidate models and systems amongst participants, including model performance.

I.3 Selection of experimental area and identification of work necessary to standardise models to this.

I.4 Review of appropriate data assimilation techniques and plans for incorporation and development of these into candidate models.

I.5 Identification of appropriate lateral and surface boundary forcing and sources of boundary data, in particular surface meteorological forcing.

I.6 Identification available observations, including fixed and other in situ observations and remotely-sensed data.

I.7 Identification of analysis and forecast periods as well as the duration of the experiment

I.8 Identification of methods of data exchange and boundary forcing for both models and observed data.

I.9 Identification of key products and methods of assessment of models and products.

I.10 Identification of appropriate sensitivity experiments.

II ESODAE Administration, Task team Meetings and Workshops

II.1 Planning and holding of ESODAE Task Team Meetings and Workshops, including writing and publication of Meeting and Workshop Reports.

II.2 Development and maintenance of an ESODAE Web page. (This can be found at: http://www.met-office.gov.uk/sec5/ESODAE/ESOHOME.html)

II.3 Administration of the ESODAE Concerted Action

III. Development, writing of the ESODAE experimental plan for ESODAE

ESODAE WORKSHOPS

ESODAE planning also being guided via input from its Workshops, which form part of the Concerted Action activity. The first of these Workshops took place at RIKZ in The Hague from 19-21 January 2000 and covered issues related to 'Observations, Data Assimilation and Lateral and Surface Boundary Forcing. The report of the Workshop will be available at EurOcean 2000. The second ESODAE Workshop, which is scheduled to take place in Aberdeen, Scotland, from 20-22 September 2000, will focus on user issues and needs and look to encourage user involvement in the ESODAE experiment proper. The topic of the final ESODAE Phase 1 Workshop has yet to be decided, but is aimed to coincide with presentation of the ESODAE Plan to the wider community. The Workshops are open to all interested scientists and other representatives of agencies and institutes.

ESODAE - THE WAY AHEAD

As noted in the introduction, the primary ESODAE experiment will aim to be carried out in conjunction with the Global Ocean Data Assimilation Experiment (GODAE). The Task team is currently developing the ESODAE Plan, around the following guidelines:

- The area to be modelled includes both the entire NW European continental shelf (out to and including the shelf break) and also in higher resolution, other parts of the NW shelf, including southern North Sea. It is anticipated that only one or two shelf -wide models will be run, and that these will provide boundary data to the higher -resolution limited areas models

- Models to be run include both 3D baroclinic (shelf-wide and North Sea) and 2D storm surge models for the North Sea.

- There will be a range of models available of both types. ESODAE Phase 1 is identifying potential candidate models.

- Existing data assimilation methodologies will be drawn upon where feasible and appropriate

- Not every participating Institute need run every configuration of model or assimilation approach. Within the overall framework of the experiment, institutes could choose to specialise in one, or a number of, specific task(s).

- The experiments will focus on the value of assimilation of relevant data (including boundary forcing and observations)

- At the same time it is recognised that development and tuning of model systems will be required in order to optimally establish pre-operational forecast model systems for the NW European shelf seas. This will need to be carried up in an initial follow-up to ESODAE Phase 1 by potential participants, with funding initially sought through national and other sources.

- The ESODAE participants will exchange data as necessary - including the surface and lateral boundary forcing required by any of the models.

- TASKS: at least one participating centre needs to provide effort in each of the following areas

  - Run models to provide deep ocean boundary forcing for use by the shelf-wide models
  - Run a shelf wide baroclinic model, including deep ocean boundary conditions, freshwater inflows, data assimilation (SST, tide gauge, ...), to provide central North Sea boundary conditions for high resolution nested models of southern N Sea
  - Provide surface forcing (NWP) at the best available time and space resolutions for each of shelf-wide and southern North Sea, for both baroclinic and storm surge models.
  - Run southern North Sea nested models, including data assimilation.
  - Run available data assimilation techniques - for any of the model scales

- It is not envisaged that new techniques or models will be explicitly developed as part of this project, but rather that existing models and existing techniques will be brought together and applied on the available near-real time datasets of observations

- Assessment will focus on intercomparison and verification of model outputs and of products, the latter via explicit interaction with users. Indeed, interaction with users and gaining feedback from them will form a key part of the experiment. ESODAE Phase 1 aims to identify a portfolio of existing and potential users and products.

ESODAE – AN INVITATION

Involvement in present and future ESODAE activities by members of the ocean modelling, observational and user community is welcomed. If you are interested , please contact the coordinator at the address on the title page.

TITLE : MEDITERRANEAN MODELLING NETWORK AND ARCHIVING PROGRAMME: **MEDNET**

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THE MEDITERRANEAN MODELLING NETWORK

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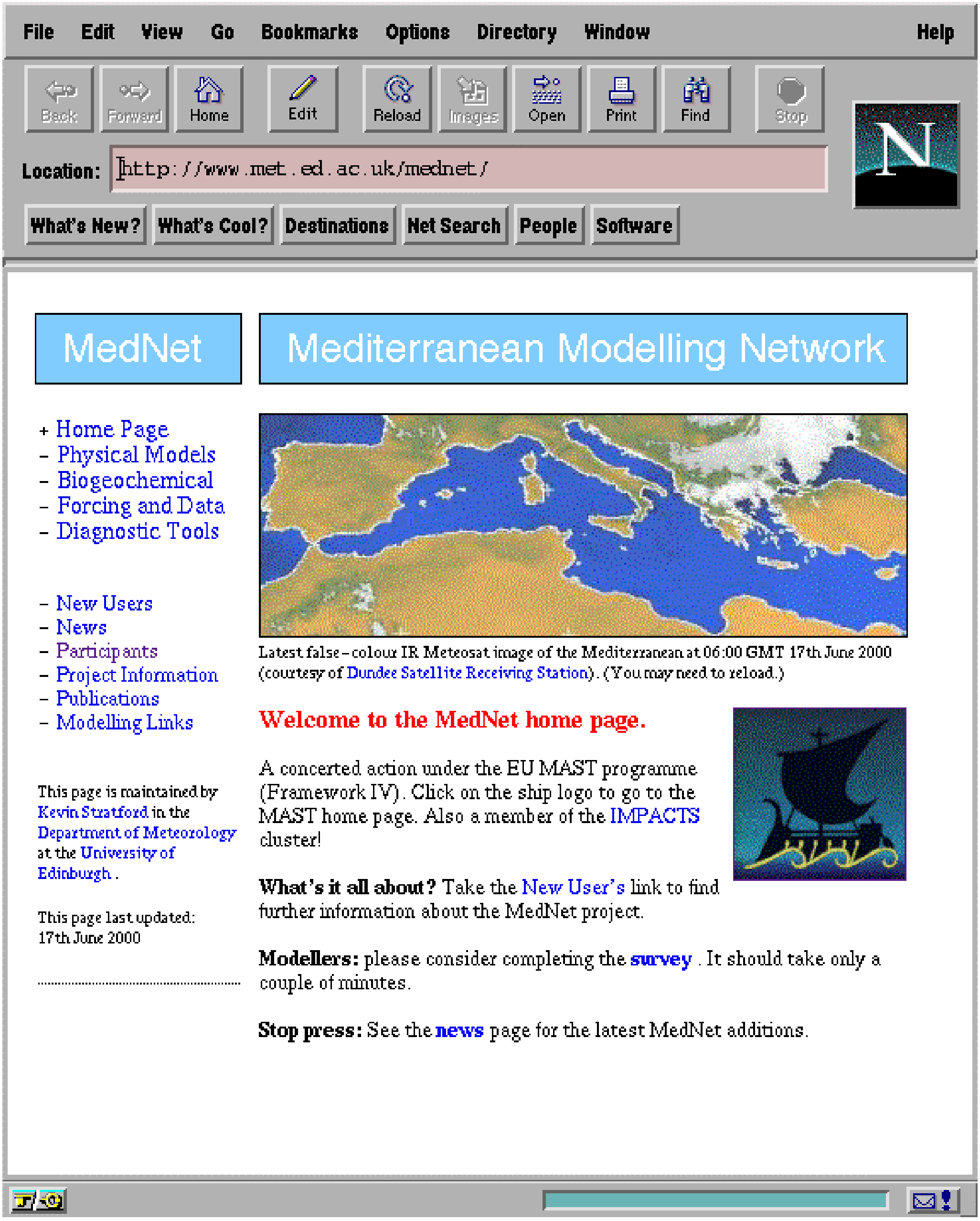
INTRODUCTION

Over the past decade, EU-funded projects have taken the lead in the development, for the first time, of detailed computer models of the Mediterranean Sea and the marginal seas therein (particularly the Adriatic and the Aegean). These projects have led to considerable progress in understanding and simulating both the physical circulation and the ecosystem dynamics of the region on timescales from a few days to hundreds of years. A variety of new models have been developed, each specifically tailored to suit particular aspects of the Mediterranean.

The aim of the MedNet concerted action is to document the models that have been built and to create an archive containing these model codes and some of their results which can be used by scientists and others to support their ongoing work and for future reference. We have also included links to much of the additional data such as bathymetric data, historical hydrographic data and meteorological data which can be used for initialising and running the models. During the lifetime of the project we will provide support for new users of Mediterranean models as far as we are able, for example by collaborating with PIs in new Mediterranean projects funded under framework V. All the products of MedNet are to be available via the World Wide Web (WWW). The central MedNet web site can be visited at http://www.met.ed.ac.uk/mednet/.



(1) MODELLING THE MEDITERRANEAN SEA

PHYSICAL MODELS

Ocean models (often referred to as general circulation models, or GCMs) solve the equations of motion for the constituent fluid, which is partitioned in some way on a discrete grid. The model flows are driven by wind and exchanges of heat and freshwater at the surface (tides are often ignored in this type of model). In the Mediterranean, the exchange of freshwater (a balance between precipitation, evaporation and river run-off) is particularly important; a large excess of evaporation means that the surface waters become very saline, an important factor in determining the density and hence the dynamics.

A number of different models will be made available by MedNet, each with strengths in different areas. The most common model resolutions are 1/4 and 1/8 degree for models of the whole Mediterranean with some models having higher resolution in regional seas.

The MedNet programme is also involved with the further development of the existing models, particularly in the area of parallel coding, to take advantage of ever-increasing computer power. This will allow the use of more sophisticated models to address new issues such as biogeochemistry.

BIOGEOCHEMICAL MODELS

The cycling of dissolved nutrients (such as nitrates and phosphates) by both the biological and physical processes in the ocean and its subsequent impact on the climate and environment represents one of the major challenges facing oceanographers in the near future. At present, rather simple models are available which are able to capture some of the main features of this cycling, such as the spring bloom. However, such models are still in their infancy, particularly in regard to the representation of the coupling between the physical circulation, which supplies nutrients from the deep water, and biological activity which this generates (e.g., Oschlies and Garcon, 1998). Both simplified nutrient cycling models and sophisticated biological models have been run in the Mediterranean sea. This will be an area where much further work is required matching the best physical models with adequate ecosystem data to simulate the annual cycling withing the Mediterranean.

(2) RESULTS

The main deliverable of MedNet is in the form of a working web site which others can use to aid thier own research, and a forum for new Mediterranean researchers. Much of this is now in place and can be visited at the above web address. The final MedNet archive will help to maintain and enhance the base of expertise gained over the last decade in the area of modelling across the EU.

Many of the scientists involved in MedNet are also actively involved in researching the Mediterranean sea circulation. Topics of particular interest include, the Eastern Mediterranena Transient. The path of deep water circulation in the Mediterranean using tracer data such as CFCs
and Tritium. The assimilation of satellite altimeter and hydrographic data into models of the Mediterranean circulation. The use of offline and online models to reproiduce the cycling of nutrients in the Mediterranean sea. The stability of the thermohaline circulation of the

Mediterranean and the likely impact of changes to the net Evaporation caused by damming of rivers and climate changes over the past few decades.

PROJECT WEB PAGE

http://www.met.ed.ac.uk/mednet/

TITLE : NATIONAL FLEETS OF RESEARCH VESSELS IN EUROPE : **NATFLEET**

CONTRACT N° : **MAS3-CT98-0186**

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NATIONAL FLEETS OF RESEARCH VESSELS IN EUROPE : NATFLEET

SUMMARY

“Research, technological development and demonstration (RTD) in the fields of energy, environment and sustainable development is essential for the social well-being of Europe’s citizens and the implementation of Union policies.” Oceans are one of the most important earth systems. Moreover, they play a key role in our climate, hold living and non-living resources and have an enormous impact on our living environment. The need for marine research is obvious. To conduct the necessary research, research vessels are indispensable.

Ships are highly sophisticated systems and therefore cost intensive. They should be used as efficiently as possible. One of the goals of the EU is to encourage the transnational use of public or private facilities which address critical needs in order to further improve their exploitation while avoiding unnecessary duplication, and to cover emerging priority needs.

To support this aim and to prepare the basis for future European steps the study will

- survey the existing fleet of multi-role research ships in Europe
- assess the likely future scientific requirements for research vessels
- present the likely future capacity of multi-role research ships into the 21st century in terms of demand and supply as well as quality and quantity

The results of the study could be utilised

- to locate and to fund the required ship time especially for smaller institutes
- to be integrated into national replacements or refitting plans
- to support the European view when planning to renew or to replace national ship capacities

To achieve this goals the study will be divided into three phases.

- Within the first phase the data base and the relevant information and the extent of the interrogation will be defined.
- Secondly, the data will be gathered mainly by questionnaires sent to marine scientists and research vessel operators. The data will be checked regarding their soundness and completeness. Additional interviews will be held, if necessary.

- During the last phase an analysis will take place and conclusions for the development of ships' capacity (in terms of quality and quantity) will be derived.

The draft plan presenting the future capacity development of research ships into the 21st century taking into account the national fleets of research vessels in Europe could then be the basis for further plans and measures to encourage the transnational use of ships’ facilities and to address critical needs in order to further improve their exploitation while avoiding unnecessary duplication, and to cover emerging priority needs.

ACTUAL STATUS

The three partners are gathering data from different marine scientists and ship operators at the moment. As they are about to start the analysis it is too early to give any results. The termination of the study is planned for late autumn this year.

TITLE : **BERGEN MARINE FOOD CHAIN RESEARCH INFRASTRUCTURE**

CONTRACT N° : **ERBFMGECT 950013**
HPRI-1999-CT-00056

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MOBILITY OF RESEARCHERS AND ACCESS TO MARINE INFRASTRUCTURES: AN EXAMPLE FROM NORWAY

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SUMMARY

The article summarises the activities conducted under the project “Bergen Marine Food Chain Research Infrastructure” during the period 1996-1999. The project has been funded by the European Commission through the Training and Mobility of Researchers (1996-2000) and Improving Human Potential (2000-2003) Programmes. This is an umbrella project through which a total of 72 short-term research projects have been conducted, involving 120 users from 51 institutions in 14 countries.

INTRODUCTION

Activities oriented to facilitate the mobility of researchers and to optimise the use of existent research infrastructures across Europe were introduced in the 2nd Framework Programme (FP2) with the Large Installations Plan (1989-1992; FP2), a pilot scheme that funded 17 facilities. Since then, the concept has been present and evolving through all the subsequent Framework Programmes. The Human Capital and Mobility Programme (1990-1994; FP3) funded 42 facilities, and under its successor, the Training and Mobility of Researchers Programme (1995-1999; TMR, FP4), the number of research facilities funded increased to 116. A similar scheme continues under the “Improving the Human Research Potential and the Socio-Economic Knowledge Base” (IHP) programme from the current Framework Programme (FP5; 2000-2002) which has funded 111 facilities after the first call for proposals. In FP5 the concept of improving the transnational access to research infrastructures has been expanded and implemented specifically in each of the four Thematic Programmes.

The Bergen Marine Food Chain Research Infrastructure is one of seven marine infrastructures currently funded by the IHP programme. Started in 1996 under the TMR programme as the Large Scale Facility for Marine Pelagic Food Chain Research, the Infrastructure has hosted more than 100 scientists from 14 EU countries and Associated States.

RESEARCH AREAS AND INFRASTRUCTURE

The Bergen Marine Food Chain Research Infrastructure addresses the study of marine ecosystems and the production of marine organisms both in natural environments and in culture systems. These main research fields are organised in 12 areas of scientific focus represented by recognised research groups at the University of Bergen and the Institute of Marine Research :

Microheterotrophs and Viruses
Phytoplankton and Primary Production
Zooplankton and Secondary Production
Reproductive and Developmental Biology
Larval Fish Physiology
Larval Fish Ecology
Marine Juvenile Production
Diseases of Marine Organisms
Environmental Health
Molecular Marine Biology
Oceanographic and Ecological Processes
Marine Biodiversity and Habitats

The research is carried out using different combinations of installations from a set of highly integrated complementary installations comprising a wide range of experimental facilities and natural environments :

Experimental facilities:
- Seawater laboratories
  - Fish tanks and silos
  - Walk-in cool rooms for studies with fish larvae and other small organisms
  - Quarantine units for work on fish pathogens, xenobiotics and alien organisms
- Outdoor fish tanks and pens
- Experimental ecosystems
  - Floating mesocosms
  - Seawater basins
  - Semi-enclosed lagoon

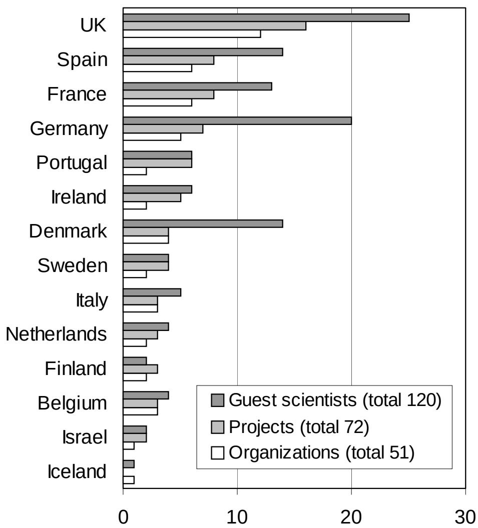
Contrasting natural environments accessible from:
- Marine Biological Station
- Research vessels for coastal (fjord) and oceanic research
- Remotely Operated Vehicle (submersible to 2000m depth)

Supporting infrastructure as:
- Laboratory for live feed production
- Zooplankton concentrator for large-scale supply of natural live feed
- Analytical instruments
- Sampling devices

ACTIVITIES (1996-1999)

A total of 72 research projects have been conducted at the Infrastructure through the first contract (1996-1999), involving 120 users from 51 institutions in 14 countries (fig 1, tab 1). These projects differed in several aspects: research area, installations utilised, degree of scientific cooperation with local teams, size of the research group (1-11 persons). Projects conducted by one individual person were typically represented by postdocs or by PhD students. These users stayed typically for the maximum period allowed by the programme (3 months) and had a high degree of integration to the local teams. Senior scientists stayed usually for shorter periods, often at the start of experiments that were then continued by younger participants. The research groups utilising the experimental ecosystems were typically large (up to 11 participants) and diverse, including users from up to 4 institutions in 3 different countries, besides their cooperation with local teams. More than 1/3 of the users were women. Most of the projects conducted during the first 4 years of the LSF/RI activity focused on basic research related to the planktonic food chain and on research directly relevant to the aquaculture of marine species and salmonids.

Fig 1. Transnational access during 1996-1999 grouped by country of origin of the users' home organisations. Projects involving researchers from different countries are allocated to the working place of the team leader.



Fourteen articles have been published in peer-reviewed scientific journals in 1997-2000 (tab.2) and over 23 oral and poster presentations have been given at international symposia on work carried out at Bergen Marine. However, most of the results are still under elaboration and the publication rate is expected to increase during the next years.

Besides the measurable output in form of scientific publications, the project has also increased the international cooperation network of guest and host scientists. Results from some of the projects conducted at Bergen Marine have been included in PhD dissertations and otherwise increased the carrier opportunities of their participants. Other projects have worked as pilot experiences resulting in larger international cooperative projects.

The number of proposals received has been increasing every year and the activity is attracting new users. Projects to be conducted during 2000 at Bergen Marine include users from 11 research institutions not represented before, including scientists from the new Associated States from Eastern Europe.

Table 1. Home institutions of guest researchers (1996-1999)

Belgium	Catholic University of Louvain University of Ghent University of Liege
Denmark	Roskilde University University of Copenhagen Kaldbak Marine Biological Laboratory National Environmental Research Institute
Finland	Helsinki University Finish Game and Fisheries research Institute
France	Université de Paris VI, Observatoire Océanologique, Villefranche Université de Bretagne Occidentale Université d'Angers Université de la Mediterranée CNRS, Laboratoire Arago, Banyuls Institut National de la Recherche Agronomique (INRA), Toulouse
Germany	Alfred Wegener Institute Universität Kiel Universität Hamburg Universität Mainz Universität Osnabrück
Iceland	Marine Research Institute
Ireland	University College, Cork Fisheries Research Centre
Israel	Israel Oceanographic and Limnological Research Ltd.
Italy	International Marine Centre Istituto de Ricerche sulle Risorse Marine e l'Ambiente University of Calabria
Netherlands	Netherlands Institute for Sea Research (NIOZ) University of Nijmegen
Portugal	Universidade do Algarve Universidade de Aveiro
Spain	Universidad de Valencia Universidad de Las Palmas de Gran Canaria Universidad de Vigo Universidad Complutense de Madrid Instituto Español de Oceanografía, Málaga Instituto de Investigaciones Marinas, Vigo
Sweden	Lund University Göteborg University
UK	University of Stirling University of Glasgow University of Leicester University of Wales, Bangor University of Aberdeen University of Liverpool, Port Erin Marine Laboratory University of St. Andrews, Gatty Marine Laboratory FRS Marine Laboratory, Aberdeen Marine Biological Association Plymouth Marine Laboratory Centre for Environment, Fisheries and Aquaculture Science, Lowestoft Marine Harvest McConnell

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European Commission

EUR 19359 — EurOCEAN 2000
The European Conference on Marine Science and Ocean Technology
Project synopses - Vol. I: Marine processes, ecosystems and interactions

Luxembourg: Office for Official Publications of the European Communities

2000 — 416 pp. — 17.6 x 25 cm

ISBN 92-828-9713-3