SSD

SCIENCE FOR A SUSTAINABLE DEVELOPMENT

COMBINED EFFECT OF CHANGING HYDROCLIMATE AND HUMAN ACTIVITY ON COASTAL ECOSYSTEM HEALTH

"AMORE III"

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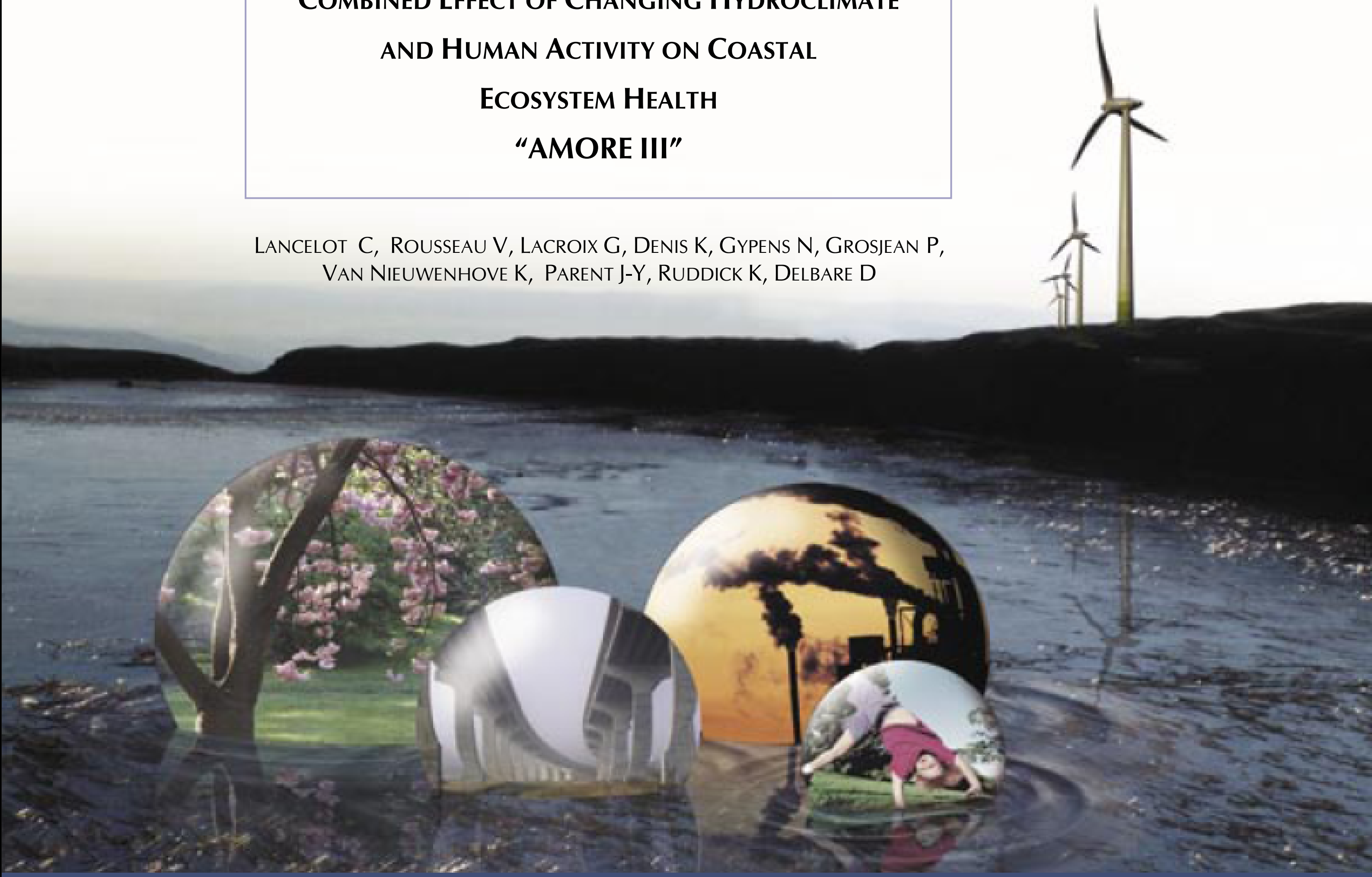

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SCIENCE FOR A SUSTAINABLE DEVELOPMENT (SSD)

North Sea

FINAL REPORT PHASE 1

COMBINED EFFECT OF CHANGING HYDROCLIMATE AND HUMAN ACTIVITY ON COASTAL ECOSYSTEM HEALTH

“AMORE III”

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ABSTRACT

AMORE (Advanced Modeling and Research on Eutrophication) is an interdisciplinary consortium composed of biologists, bioengineers, biostatisticians and physical and ecological modelers aiming to the development of Sustainability Science for the management of coastal zones in the Channel and the Southern Bight of the North Sea with a focus on the Belgian coastal zone (BCZ).

Previous achievements since 1997 by the AMORE consortium demonstrated that the BCZ is a key pilot area for addressing causes of natural variability and effects of human activity on coastal eutrophication and the sustainable use of economic activity. The BCZ is indeed submitted to local (Scheldt, Ijzer) and transboundary (Seine, Somme, Rhine/Meuse) river inputs of anthropogenic nutrients that modify the N:P:Si nutrient balance and the ecosystem structure of the coastal area, favoring the blooming of undesirable *Phaeocystis* colonies over diatoms. However the actual contribution of local river inputs to the BCZ eutrophication and the related geographical spreading of *Phaeocystis* are largely determined by large-scale climatic phenomena such as the NAO (North Atlantic Oscillation) that determines the weather conditions over Northwestern Europe.

The research project AMORE III addresses the dual control of changing human activity and climate on eutrophication processes in the BCZ and the feedback effect of eutrophication on goods and services provided by the BCZ. As service, AMORE III focus on the atmospheric CO_2 absorption capacity of the BCZ while the newly-deployed offshore mussel farming is considered as a good. Mussel farming is a recent economic activity in the BCZ planned in three offshore areas (Nieuwpoort, Oostdijck and Westhinder) with varying proliferation of *Phaeocystis* colonies. While some negative effect of *Phaeocystis* colonies has been reported for mussel beds, their impact on mussel farming in floating cages is unknown but probably depends on *Phaeocystis* magnitude (colony size and number).

As a scientific contribution towards the sustainable use of the BCZ, AMORE III provides new ecological knowledge, technological developments and ecological modeling to:

- Assess the dual role of changing hydro-climate (wind strength and direction, temperature) and nutrient river loads in determining the geographical spreading and the magnitude of *Phaeocystis* blooms in the BCZ as well as the role of the coastal area as buffer of increased atmospheric CO_2; predict how these might change in the near future (2015) based on realistic scenarios of changing climate and river nutrient loads;

- Assess the impact of *Phaeocystis* colony spreading on offshore mussel farming and make recommendations for an optimized management;

- Define ecological quality criteria for measuring ecosystem changes and the effectiveness of management and policy applications.

The research methodology involves and combines (i) laboratory-controlled process-level experiments, (ii) the collection of historical and new field data, (iii) the set up of (near) real-time monitoring of phytoplankton distributions and (iv) numerical tools (statistical and dynamic models). In this methodology, the existing MIRO&CO-3D ecological model plays a central role as integrator of new knowledge gained from experimental studies and as tool for eutrophication assessment and prediction as well as decision support. Complementary the (near) real-time phytoplankton monitoring is expected to provide a quasi synoptic view of phytoplankton biodiversity in the BCZ and adjacent waters and a powerful tool for model validation in areas where monitoring stations are absent as well as for identification of regions

affected by undesirable *Phaeocystis* colony blooms. Application of multivariate statistical methods to this new data set is expected to support the model-sensitivity-based formulation of ecological quality criteria and provide early warning of ecosystem shifts. Progress achieved on all these items is detailed in the present report.

Phytoplankton ecology

Phytoplankton eco-physiological studies were focused on the mechanisms controlling *Phaeocystis* colony formation, a weakly constrained parameterization in the existing MIRO&CO-3D although crucial for determining their occurrence. Results suggest that *P. globosa* colony formation is related to the growth dynamics of the haploid population present in the water column. In particular, a light threshold of about 50 µmol m^{-2} s^{-1} and nutrient enrichment are necessary for the occurrence of syngamy and colony formation. Besides light and nutrients, a possible role of *Chaetoceros* spp. in triggering colony formation cannot be excluded.

Possible factors controlling the recurrent diatom/*Phaeocysti*s/diatom succession in the BCZ were approached based on a statistically analysis of the 13-yr existing phytoplankton record at St 330 in the central BCZ. Disappointing the statistical analysis of phytoplankton records did not provide strong support to the regulation of the observed recurrent seasonal succession of diatom communities and *Phaeocystis* by temperature, nutrients and ambient light conditions. The small neretic diatoms and *Chaetoceros* seem however better adapted than *Guinardia* to lower temperature in late winter-early spring. Different resistance of the diatom communities and *Phaeocystis* colonies to grazing has been hypothesized as a factor shaping the phytoplankton succession.

Phytoplankton monitoring

AMORE III acquired a new instrument combining the technology of flow-cytometry (FlowCAM) and image analysis (PhytoImage). Laboratory experiments with pure cultures of phytoplankton species allowed defining specific conditions under which the combination of the FlowCAM and PhytoImage is a useful tool for monitoring phytoplankton in the North Sea. First we showed that a 800μm cell with a 2x magnification, or a 300μm cell with a 4x magnification, used both with a fluorescence triggering mode, are the most appropriate settings for the FlowCAM. Second, specific training sets and recognitions algorithms were designed in PhytoImage, not only for a given geographical area, but also for each season. Third our system can be presently used only for detecting relative abundances within the digitized sample. Absolute abundances, biomasses, etc. still need a careful calibration of the number of colonies or particles counted by the FlowCAM/PhytoImage in function of the density of these particles in the samples and the flow rate in the measurement cell.

Finally, the actual combination of the FlowCAM and PhytoImage has been successfully tested on sea (up to 4-5 Beaufort), and can provide (near-)real time data with a quick, but simplified process. If needed, the series can still be reanalyzed with the full process back to the laboratory.

Detrimental (or beneficial) effect of *Phaeocystis* colonies on mussel feeding

The potential effect of *Phaeocystis* colonies on the production of offshore mussel (*Mytilus edulis*) farming was assessed based on laboratory bio-assays involving mussels fed with different concentrations and sizes of *Phaeocystis* colonies and on the field measurement of indicators of physiological state before, during and after *Phaeocystis* blooms. The physiology status of offshore mussels defined by its biochemical composition (protein, glycogen and lipid) showed seasonal variation in spring-summer that could be possibly attributed either to a negative effect of *Phaeocystis* colonies or mussel spawning or their combination. The combined use of laboratory feeding experiments and additional field observations in late summer – early fall when a secondary *Phaeocystis* blooms is sometimes observed will allow concluding on the effect of *Phaeocystis*.

First feeding experiments with *Isochrysis* cells showed that clearance rate normalized with respect to mussel size is a good parameter to compare mussel feeding capacities. They also showed that *Isochrysis* is a good prey for mussels and can be considered as a reference for investigating mussel feeding on *Phaeocystis*. Based on these results, a mussel size between 30 and 40 mm was chosen for running feeding experiments on *Phaeocystis* colonies. From the available experiments a positive relationship was observed between the average mussel clearance rate on *Phaeocystis* colonies and the average size of the corresponding offered *Phaeocystis* colonies up to an average size of 300 µm. Clearly, as field *Phaeocystis* blooms in BCZ are dominated by large colonies (500 - >1000 µm), more bio-assays involving a dominance of >500 µm colonies are needed to confirm this trend and eventually determine a threshold size above which colonies are detrimental for mussel growth.

Ecological reference for *Phaeocystis* disturbance

A cell reference of 4 10^6 cells L^{-1} for a well-balanced (healthy) *Phaeocystis* ecosystem as well as nutrient thresholds for *Phaeocystis* disturbance in the BCZ have been developed based on microscopic observations of grazable *Phaeocystis* colony number and corresponding colonial cells and historical MIRO model simulations making use of RIVERSTRAHLER simulations of nutrient loads for pristine conditions, assuming a watershed covered by primary forest. The obtained nutrient load of 60 kT N y^{-1} to BCZ might be used as a target for the implementation of nutrient reduction policies. In addition, the *Phaeocystis* reference can now be used to flag in the BCZ ecosystem *Phaeocystis*-problem and non- problem areas based on either real-time monitoring by the FlowCAM/PhytoImage tool or MIRO&CO-3D simulations, the latter for present-day and nutrient reduction scenarios.

Ecological model development

New numerical development were operating on either the ecological code MIRO or the development, implementation and forcing parameterization of the MIRO&CO-3D.

Sensitivity tests on the complex ecological model MIRO have secured the reduction of phytoplankton cell variables from 3 to 2 without changing the phytoplankton dynamics. The phytoplankton module could be replaced by this aggregated version when running MIRO in highly-resolved physical models. The reduction of the microbial loop complexity (from 5 to 3 state variables) is ongoing but results obtained are not satisfying yet.

The CO_2 module was successfully implemented in the existing MIRO&CO-3D model and the resulting sea surface pCO_2 simulations were validated by comparison with existing data. Result analysis point the role of river loads and hence eutrophication in determining the capacity of coastal zones in absorbing atmospheric CO_2.

The implementation of the MIRO&CO-3D model on the BCZ grid allowed simulations with a better resolution (750m x 750m) and so a better assessment of the role of Belgian rivers on the BCZ eutrophication. Preliminary results have been obtained and are currently analysed and validated. This is promising for the future applications (nutrient reduction scenarios on BCZ).

High frequency total suspended matter (TSM) has been obtained from remote sensing images (MODIS) thanks to the STEREO2-RECOLOUR project and has been used as updated input to the kPARv1 module for water column light (PAR) attenuation calculation. The importance of TSM variability on PAR attenuation has been demonstrated by comparison of MIRO&CO-

3D results with *in situ* data. Model simulations obtained with this new TSM forcing show that the spatial variability of phytoplankton bloom timing is closely related to the suspended matter load. Furthermore the interannual variability of the bloom timing depends on the combination of factors such as TSM, incident surface PAR, and nutrients.

Response of the BCZ to nutrient reduction

Scenarios exploring the effect of selected riverine nutrient reductions (phosphorus and/or nitrogen) on the nutrient/*Phaeocystis* distribution and on the eutrophication status of Belgian waters have been performed with the MIRO&CO-3D model using the new criteria for scaling *Phaeocystis* disturbance. Results confirm the need to prioritise N reduction to reduce undesirable *Phaeocystis* blooms.

Furthermore a module for calculating transboundary fluxes in the MIRO&CO-3D domain has been implemented at the C&SNS grid scale and nutrient transports have been computed for the years 1993-2004. Results of these scenarios are under analysis and preliminary results on the transboundary nutrient transport have been reported to OSPAR meetings.

1. INTRODUCTION

Coastal zones are threatened by several socio-economic drivers operating in both the watershed (urbanization, agriculture, industrial activity) and the coastal zone itself (e.g. fisheries and aquaculture). The effects of these human activities superimpose to natural variability driven by the climate. Coastal zones are also providing goods (Costanza et al., 1997) like fisheries but also some ‘carbon sink’ service against increased atmospheric CO_2 (Gypens et al., 2004; 2009). Crucial questions such as how will changes in human activity and climate alter the coastal ecosystem functions and services are still unanswered (Crossland et al., 2005). Decreasing this uncertainty is needed for implementing the right mitigation strategies that will guarantee the sustainability of coastal resources and maintain the socio-economic activity in the coastal zones.

Results gained during the Belgian Federal AMORE-I and II projects (Lancelot et al., 2004; 2007; Rousseau et al., 2006) indicate that the Belgian Economic Exclusive Zone (EEZ) is a key pilot area for addressing the combined effect of natural variability and human activities on the goods and services of the coastal ecosystem. The Belgian EEZ, here called BCZ is submitted to local (Scheldt, Ijzer) and transboundary inputs of land based nutrients (Fig.1) that modify the N:P:Si nutrient balance and the ecosystem structure of the coastal area, favoring the blooming in spring of undesirable *Phaeocystis* colonies over desirable diatoms (review in Rousseau et al., 2006). Accordingly, *Phaeocystis* has been identified as an indicator species of water disturbance (Tett et al., 2007) and recommendations for decreasing its abundance to numbers representative of non-problem areas and good ecological status have been made in the scope of the implementation of the OSPAR Strategy to combat eutrophication (OSPAR, 2005) and the Water Framework Directive of the European Union (EU WFD; 2000/60/EC). A recent analysis of last decade nutrient and phytoplankton time-series suggests that the actual contribution of local river inputs of anthropogenic nutrients to the eutrophication of the BCZ and the related geographical spreading of *Phaeocystis* colonies are determined by large-scale climatic phenomena such as the North Atlantic Oscillation (NAO) which determines the weather conditions over Northwestern Europe (Breton et al., 2006). Specifying this is crucial for guiding Belgium authorities to take measures to protect the BCZ against the adverse effects of eutrophication in the scope of its obligation as EU member and Contracting Party of the OSPAR Convention for the Protection of the Marine Environment of the northeast Atlantic.

Offshore mussel farming is a recent economical activity in the BCZ, set up for facing actual problems in the Belgian fisheries sector. This activity is planned in three areas (Nieuwpoort, Oostdijck, Westhinder) however characterized by varying proliferation of *Phaeocystis* colonies. Some negative effect of *Phaeocystis* colonies has been recorded for mussel beds (Tracey, 1988; Petri et al., 1999; Pieters et al., 1980) but their impact on mussel farming in floating cages is unknown but probably depends on *Phaeocystis* magnitude (colony size and number).

As a scientific contribution towards the sustainable use of the BCZ, the AMORE III project aimed to provide new ecological knowledge, technological developments and numerical experimentation to:

- Assess the dual role of changing hydro-climate (wind strength and direction, temperature) and nutrient river loads in determining the present-day geographical spreading and the magnitude of *Phaeocysti*s colony blooms in the BCZ as well as its capacity to absorb atmospheric CO_2; predict how these might change in the near future (2015) based on realistic scenarios of changing climate and river nutrient loads;

- Assess the impact of *Phaeocystis* colony spreading on offshore mussel farming and make recommendations for an optimized management;
- Define ecological quality criteria for measuring ecosystem changes and the effectiveness of management and policy applications.

Results obtained will allow assessing the contribution of the Belgian continental waters and coastal effluents to the actual eutrophication status of the BCZ and their influence on the nutrient environment and ecological status of adjacent EEZ's (Dutch and UK). This will therefore provide a substantial scientific support to the Belgian authorities for international negotiations related to the implementation of the EU WFD and the OSPAR convention quality objectives. Moreover, crossed nutrient reduction and climate change scenarios combined with the formulation of ecological quality criteria will point the nutrient(s) to be targeted by international and national (federal and regional) nutrient reduction policies as well as the environmental quality to be reached and maintained.

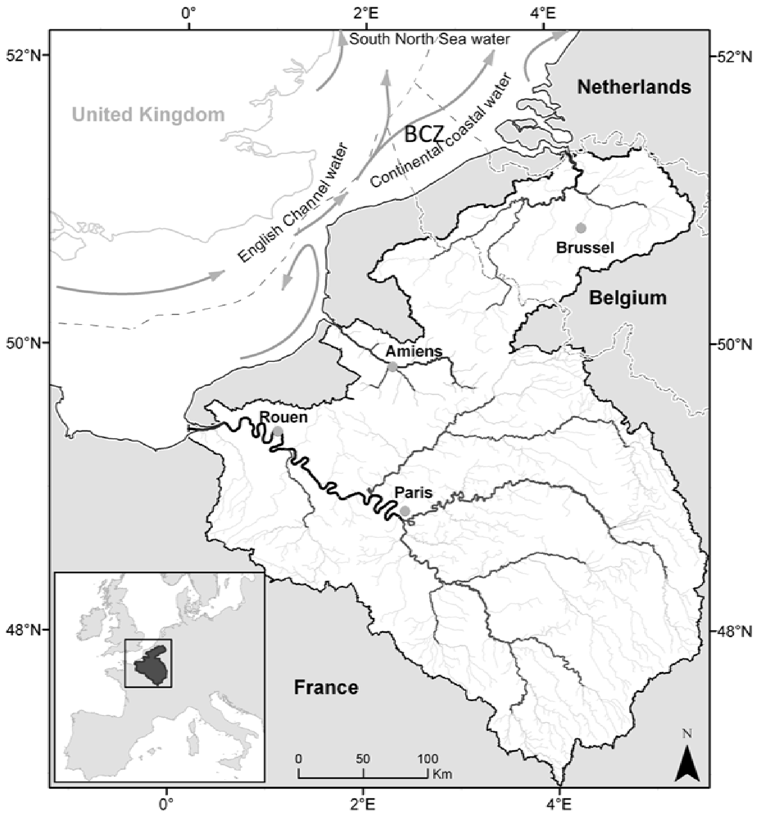

Figure 1: Map showing the eastern Channel and Southern Bight of the North Sea and their 'three river' watershed (Seine, Somme and Scheldt). BCZ represents the Belgian EEZ.

2. METHODOLOGY

Recognizing the multiplicity of processes, changes and forces behind the dynamics of coastal ecosystems, the AMORE methodology involves and combines field observations, process-level studies and mathematical modeling in an iterative way. In this frame, the ecological model plays a central role as integrator of new knowledge and prediction tool. Basically new knowledge is first integrated in the ecological module MIRO and tested in a multi-box frame (Lancelot et al., 2005) prior to being integrated in the fully coupled physical-ecological 3D-MIRO&CO model (Lacroix et al., 2007). The improved prediction capacity of the latter is evaluated based on a comparison of simulations with existing or newly collected field data.

As part of our long term strategy, experimental and numerical work performed during AMORE III is based on failure and weakness revealed with the multi-box MIRO and the 3D-MIRO&CO models applied in the Eastern Channel and Southern Bight of the North Sea in the scope of the AMORE I and II projects. These are:

- The imperfect knowledge of *Phaeocystis* origin and of the mechanisms triggering colony formation;
- The imperfect description of phytoplankton succession patterns in the BCZ and their environmental controls;
- The insufficient geographical coverage and time resolution of available phytoplankton records preventing the full validation of MIRO&CO simulations and the assessment of eutrophied vs non-eutrophied regions;
- The insufficient resolution of the physical component of MIRO&CO for properly addressing the dual role of anthropogenic nutrients and climate change impacts on the only BCZ domain;
- The lack of ecological quality criteria for scaling ecosystem changes and defining thresholds of environmental quality;

These needs added to the introduction of a new economical activity at sea (offshore mussel farming; Fig.2) have dictated the definition and organization of AMORE III experimental and numerical activities.

2.1 Experimental work

2.1.1 Process studies

Research need for resolving the *Phaeocystis* life cycle is based on experimental results obtained during AMORE II (Lancelot et al., 2004) and pointing the existence of an haploid-diploid life cycle where blooms of diploid *Phaeocystis* colonial cells alternate with haploid flagellates (Rousseau et al., 2007). New bio-assays were set up with both *P. globosa* pure strains and field populations to identify the biotic and abiotic factors inducing colony formation.

The potential effect of *Phaeocystis* colonies on the production of offshore mussel (*Mytilus edulis*) farming was assessed based on laboratory bio-assays involving mussels fed with different concentrations and sizes of *Phaeocystis* colonies and on the field measurement of indicators of physiological state before, during and after *Phaeocystis* blooms. Actually two farmers are operating in three production areas (Fig.2). Oostdijk (OD), and Westhinder (WH) are two experimental sites while D is the main culture area.

2.1.2 Field observations: phytoplankton distribution fields

Sophisticated statistical tools (VEGAN package of the R software http://www.r-project.org) were used for deciphering phytoplankton seasonal patterns in the BCZ making use of the existing phytoplankton time series obtained at station 330 (N 51 26.05; E 2 48.50) from 1988 to 2000 (Rousseau, 2000; Breton et al., 2006).

The setting up of an appropriate strategy for monitoring the timing and geographical extent of key phytoplankton blooms in the BCZ is strongly needed for (i) MIRO&CO-3D validation, (ii) the mid and long term assessment of the ecosystem health in response to nutrient reduction policies and (iii) the management of offshore mussel farming. The weekly sampling at St 330 between 1988 and 2000 was found relevant to identify the recurrence of diatom/*Phaeocystis* successions in the BCZ but irrelevant for deciphering changing patterns over seasons and years. Monitoring in such hydrodynamically variable areas requires powerful tools to improve the spatial and temporal resolution of the sampling as the traditional microscopy analysis of discrete samples prevents the treatment of large number of samples. The new imaging methods developed to automatically enumerate particles and automatic recognition based on image analysis have shown high potential for automatic recognition of digitized particles (Bell and Hopcroft, 2008; Benfield et al., 2007; Grosjean et al., 2004; Irigoien et al., 2009). Accordingly, real-time monitoring of phytoplankton species in the BCZ was developed based on a digitization device, the FlowCAM, a digital camera microscope combined with flow cytometry, and the software PhytoImage for image analysis and recognition of particles.

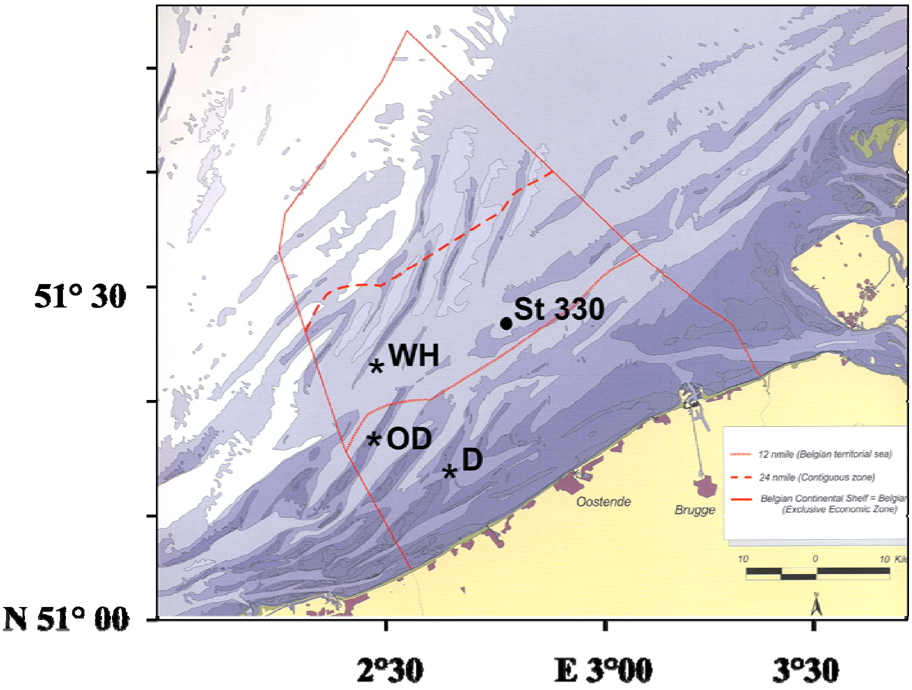

Figure 2: Map of the Belgian Economical Exclusive Zone with location of the eutrophication monitoring station 330 and of the three offshore cultivation areas D (main culture in front of Nieuwpoort), Oostdijk (OD) and Westhinder (WH)

2.2 Numerical work

Constructing a mathematical tool for understanding and advising on mitigation strategies for combating eutrophication problems in the *Phaeocystis*-dominated BCZ and assessing its impact on the BCZ capability for absorbing atmospheric CO_2 is the long-term purpose of the AMORE consortium. The main mathematical tool is the three-dimensional MIRO&CO-3D model (Lacroix et al., 2007) that couples the 3D hydrodynamical model COHSNS (Lacroix et al., 2004) determining the physical transport of water and its components and the ecological model MIRO describing interactions between the ecosystem components as previously tested in a multibox frame (OD-MIRO; Lancelot et al., 2005).

In the present report, numerical experimentation are using the ecological MIRO implemented in the multi-box configuration (Lancelot et al., 2005) or in the 3D frame (MIRO&CO-3D; Lacroix et al., 2007), depending on the purpose (Fig.3).

Present-day assessment of bloom dynamics and delimitation of the eutrophied maritime zones involved the use of the 3D model. However, increasing the grid resolution of MIRO&CO-3D from ~5km to <1km was found necessary in order to increase the geographical resolution of the BCZ for testing applications related to the evaluation of EU WFD implementation and define the geographical limits of BCZ areas affected or not by eutrophication (OSPAR strategy to combat eutrophication).

On the contrary historical reconstruction and projections were first appraised in the 0D configuration as this involved the online coupling with river nutrient loads calculated by the RIVERSTRAHLER model (Billen et al., 2001; 2005). The advantage of using modeled fluxes of nutrient inputs as forcing is considerable in terms of coastal eutrophication management as RIVERSTRAHLER calculates nutrient transfers along the river system as a function of meteorological conditions, geomorphology and nutrient emissions on the watershed.

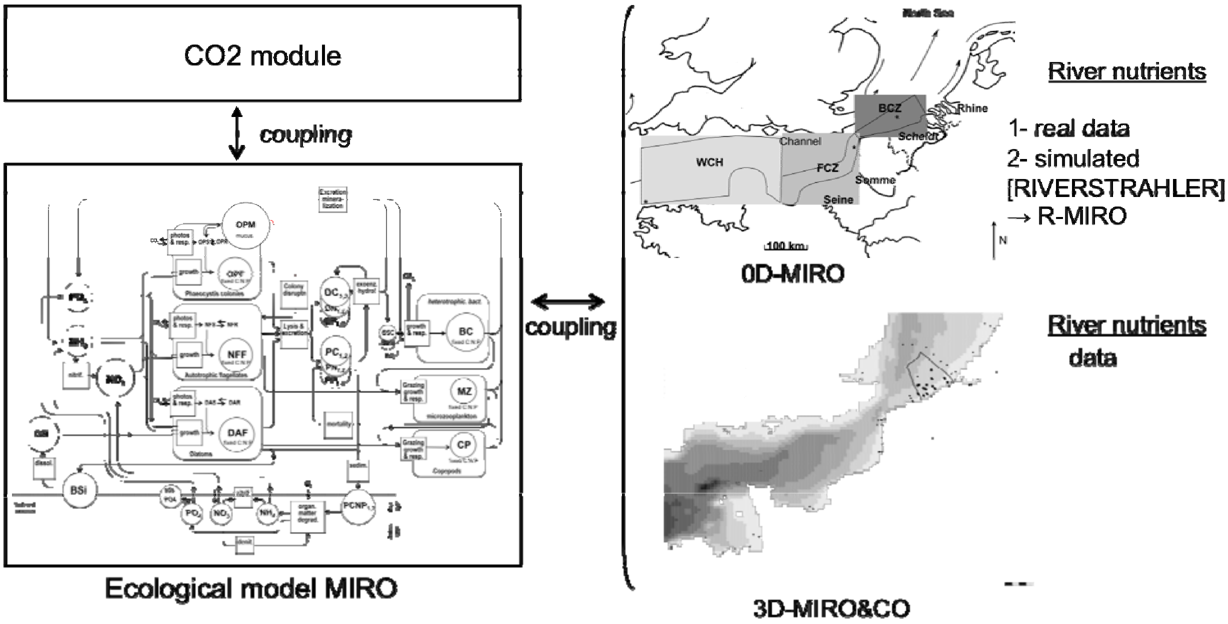

Figure 3: Schematic description of the mathematical tools.

3. RESULTS

3.1 Phytoplankton blooms eco-physiology

3.1.1 Phytoplankton community succession in the BCZ

Two major taxa, diatoms and *Phaeocystis globosa* (hereafter *Phaeocystis*), are forming phytoplankton blooms in the BCZ with a very little contribution of the other groups, i.e. cyanobacteria, euglenophytes, cryptophytes, chlorophytes or dinoflagellates (Breton et al., 2006; Muylaert et al., 2006). Diatoms are present the year round with various species succeeding during the vegetative season (Rousseau et al., 2002) while *Phaeocystis* colonies occur mainly as a unique spring event (Breton et al., 2006) with few fall outbursts during some years.

The phytoplankton time series data obtained at St 330 (N 51 26.05; E 2 48.50) between 1988 and 2000 (Breton et al., 2006) were analyzed and classified into communities based on hierarchical classification using complete linkages and Bray-Curtis distance matrix on double square root transformed abundances. This classification was applied on the more abundant diatom species recorded, *i.e.* those representing 80 % of the total cumulated cell density. Four phytoplankton communities were identified: three diatom groups and *Phaeocystis* colonies which appear as a separate group (Fig. 4). The three diatom groups distinguish (i) the small bentho-pelagic diatom species *Paralia sulcata*, *Thalassionema nitzschioides*, *Asterionellopsis glacialis*, *Plagiogramma brockmannii*, *Thalassiosira levenderi* and *Skeletonema costatum*, (ii) *Chaetoceros socialis*, *C. debilis* and *C. curvisetus* and (iii) the large-sized diatom *Guinardia delicatula*, *G. striata* and *Rhizosolenia shrubsolei*.

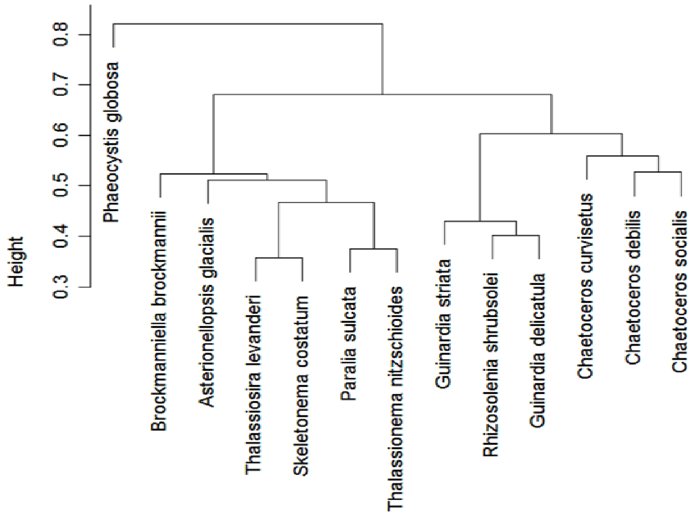

Figure 4: Dendrogramm cluster obtained after hierarchical analysis using Bray-Curtis distance of the 1988-2000 phytoplankton cell density data at St 330.

The four assemblages identified with the statistical analysis show a recurrent seasonal succession pattern with however varying onset time and duration (Fig. 5). The phytoplankton spring succession is invariably initiated in late winter-early spring by the small bentho-pelagic diatom species that are progressively replaced by *Chaetoceros* spp. and then by *Guinardia-Rhizosolenia* spp. and *Phaeocystis* colonies. In the late phase of the spring bloom, *Phaeocystis*, *Guinardia-Rhizosolenia* are the more often co-occurrent before completely disappearing in late spring-early summer. Later in summer, the large *Guinardia-Rhizosolenia* again constitutes the bulk of phytoplankton. Interestingly enough the summer-fall bloom succession is composed of same species dominances and exactly mirrors the spring event (Fig. 5).

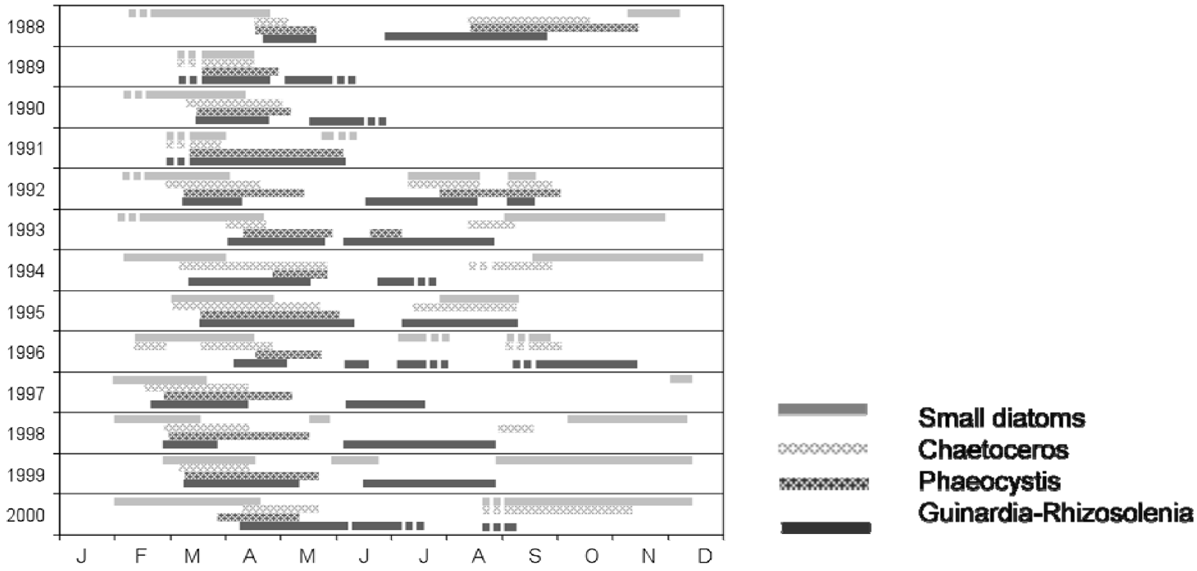

Figure 5: Timing and duration of the three diatom communities and *Phaeocystis* population identified after hierarchical analysis using Bray-Curtis distance of 1988-2000 phytoplankton data at St 330.

3.1.2 Ecological niches of the diatom and *Phaeocystis* communities

Ecological preference of *Phaeocystis* and the three diatom and communities, identified on basis of the environmental conditions prevailing during their growth period, are surprisingly similar (Table 1). Globally, the different communities encompass a wide and overlapping range of salinity, temperature, nutrients and ambient light conditions.

Temperature in the early phase of the spring bloom suggests however, that bentho-pelagic small diatoms are better adapted to blooming at low temperature (3.2°C) than the other communities.

More than bottom-up controls, a different ‘resistance’ of the diatom communities and *Phaeocystis* to grazing could well shape the phytoplankton species succession in the BCZ. A high copepod grazing pressure on small bentho-pelagic diatoms has indeed been suggested to explain their disappearance at the end of the spring bloom (Breton, 2000; Daro et al., 2006). On the contrary, large *Phaeocystis* colonies (Gasparini et al., 2000; Weisse et al., 1994) and *Guinardia-Rhizosolenia* (Breton, 2000) are not or little grazed by copepods. The transient disappearance of *Guinardia-Rhizosolenia* could however been explained by *Noctiluca* grazing during summer (Daro et al., 2006).

Table 1: Range of salinity, temperature, nutrients and water colum light intensity (PAR_{WC}) during the exponential growth of small bentho-pelagic diatoms, *Chaetoceros*, *Guinardia-Rhizosolenia* and *Phaeocystis* communities. From time series data at St 330 in the BCZ.

Community	Salinity	Temp. °C	NH_4 µM	NO_3 µM	$Si(OH)_4$ µM	PO_4 µM	PAR_{WC} µmol m^{-2} s^{-1}
Small bentho-pelagic diatoms	31.9-35.0	3.2-19.3	0.1-6.1	1.6-39.2	0.4-13.4	0.4-1.8	10-48
Chaetoceros	31.3-35.0	3.9-19.3	0.9-5.0	1.3-42.6	0.9-7.4	0.3-1.7	21-62
Phaeocystis	31.4-34.7	7.7-19.3	0.7-4.6	1.7-41.3	0.4-6.7	0.1-1.4	15-56
Guinardia-Rhizosolenia	31.4-35.0	6.6-17.9	0.1-6.6	1.6-40.5	0.4-9.4	0.2-1.4	13-110

3.1.3 Mechanisms triggering *Phaeocystis globosa* colony formation

Rousseau et al. (2007) demonstrated the existence of a haploid-diploid life cycle in *P. globosa* where blooms of diploid colonial cells are formed after syngamy of haploid flagellates. Experimental work has been conducted on both *P. globosa* pure strains and field populations to identify the factors inducing this process.

As a first step, crossing experiments were conducted by mixing five pure strains of haploids identified as *P. globosa* and all originating from the Southern Bight of the North Sea, under various conditions of light (20-100 µmol quanta m^{-2} s^{-1}), nutrients and vitamins enrichment. During these experiments, no colony production was observed possibly due to inadequate conditions for syngamy, absence of compatible mating types or the occurrence of different *Phaeocystis* species in the investigated strains.

As an alternative, laboratory experiments were conducted with natural populations of *Phaeocystis* haploids collected in the central BCZ during the pre-bloom period, i.e. in absence of the colonial form. Seawater samples were incubated at different light and nutrient conditions and the appearance of *Phaeocystis* colony was monitored under light microscopy. After a 3-weeks incubation, massive colony formation was observed above a light intensity of 49 µmol quanta m^{-2} s^{-1} when nutrients and vitamins (F20 medium) were added (Fig. 6a). This suggests that light intensity and nutrients would play a key role in colony generation from haploid flagellates.

Besides light intensity and nutrients, a possible role of *Chaetoceros* spp. in triggering *Phaeocystis* colony formation cannot be excluded. Small *Phaeocystis* spherical colonies (10-80 µm in diameter) are indeed regularly found within *Chaetoceros curvisetus* and *C. debilis setae* in the early phase of the *Phaeocystis* bloom. This coupling is supported by the positive relationship existing between the *Chaetoceros* cell density and the number of young colonies < 80 µm in diameter (Fig. 6b). Protection against grazing of young colonies within Chaetoceros setae and/or requirement of a solid substrate for anchorage and/or allelopathy are three different hypotheses to be tested to explain the presence of small colonies within *Chaetoceros* setae.

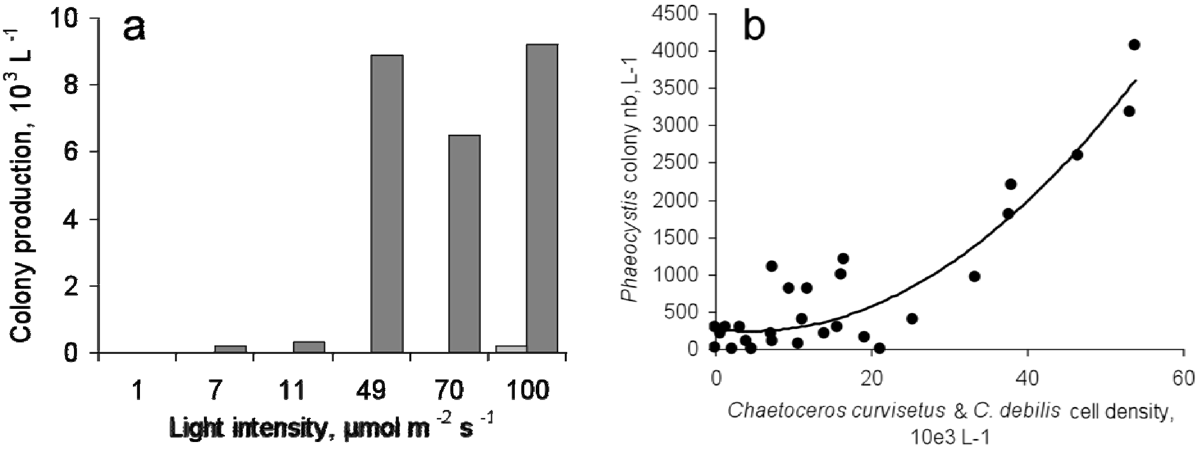

Figure 6: a) *Phaeocystis* colony production from natural populations of haploid flagellates sampled during the pre-bloom period and incubated at different light intensities and enrichment conditions (light grey: no enrichment; dark grey: enrichment with nutrients and vitamins);
b) Relationship between the number of *Phaeocystis* spherical colonies <80 µm and the cell density of *C. curvisetus* and *C. debilis* present at the stage of colony formation.

3.2 Real-time and automatic identification of phytoplankton

3.2.1 System for automatic analysis of phytoplankton samples

Real-time monitoring of phytoplankton species is based on a digitization device, the FlowCAM - a digital camera microscope combined with flow cytometry (http://www.fluidimaging.com/default.aspx)- and a software for image analysis and automatic recognition of the particles, PhytoImage (http://www.sciviews.org/zooimage/index.html) that we had to couple. The FlowCAM detects phytoplankton particles flowing through a glass cell using one of three triggering modes: fluorescence detection, scattering detection and time-lapse video (“auto-image” mode). In addition, the optical tool measures 21 parameters on each detected particle (e.g. area, size, fluorescence …) and extracts their images collected together in so-called ‘collages’. In the system developed in AMORE III, FlowCAM data are transferred in real-time through a TCP/IP (Ethernet) connexion on a remote computer that runs PhytoImage. On the remote computer, collages are reprocessed for a more accurate and more complete analysis of the particles: 30 additional features are measured (e.g. moments, shape…) and ‘vignettes’ are extracted. These are small images of each detected particle that our taxonomist can use for identification. A representative subset of all vignettes is identified manually by our taxonomist and constitutes the training set. Correspondence between measurements performed by the FlowCAM and by PhytoImage with the taxonomic group is established using machine learning techniques (learning phase). Finally, summary statistics and ecologically meaningful derived variables (abundance, size spectra and biomass per taxa) are calculated.

3.2.2 Analysis of cultivated phytoplankton species

Data acquisition for the automatic recognition of North Sea phytoplankton taxa has been preliminary made on several phytoplankton strains. Pure cultures of five phytoplankton species (the diatoms *Chaetoceros compressus*, *Ditylum brightwellii*, *Skeletonema costatum*, *Thalassiosira rotula* and *Phaeocystis*) were digitized for a large range of parameter combinations (flow cell depth, optical magnification and image triggering mode). Training sets associated to each combination were analyzed using six different machine learning algorithms (linear discriminant analysis, k-nearest neighbour, learning vector quantization, neural network, partitioning trees and random forest).

In all cases, random forest provides the highest performance (lowest global error rates). Moreover, the fluorescence mode provides the best results for each association. We then defined the best cell depth – magnification combinations as 800µm – 2x, 300µm – 4x and 100µm – 10x (Fig. 7), the latter being however of limited use on board due to the very fragile flow cell.

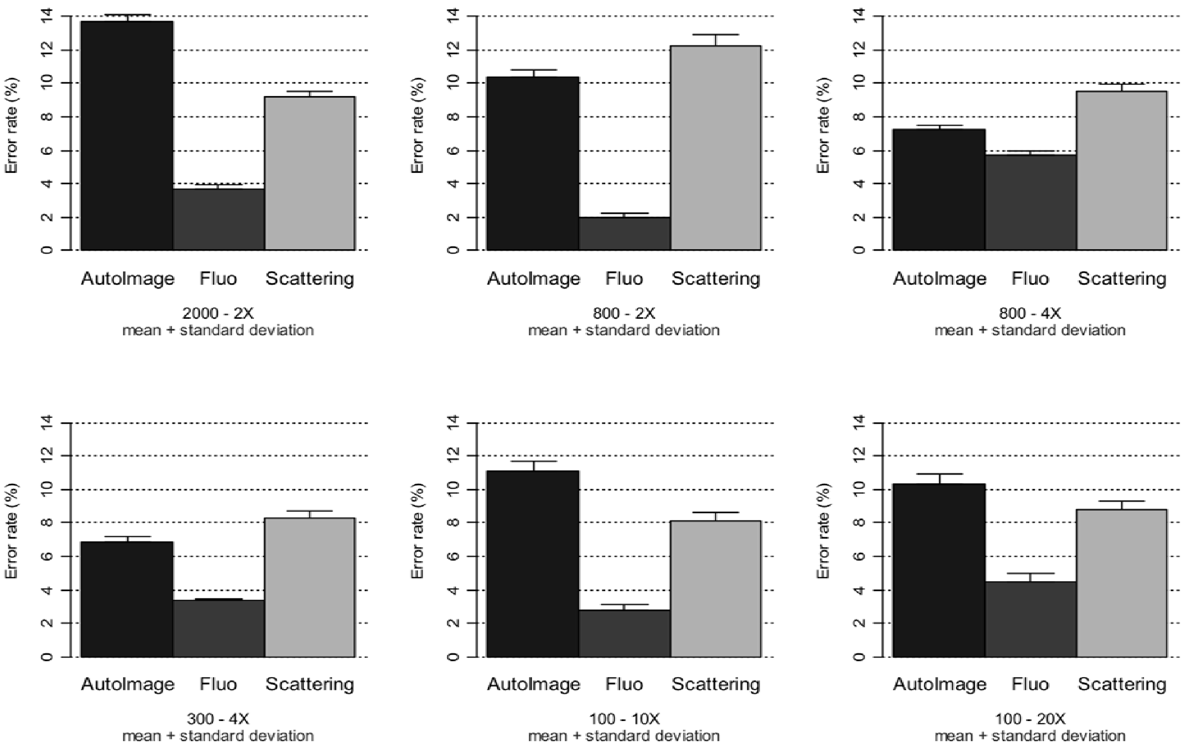

Figure 7: Variation of the global error rates with phytoplankton pure cultures, different cell depth/magnification and with the best machine learning algorithm random forest.

A comparison of particles enumeration in pure culture obtained by the FlowCAM and by traditional manual method shows a slight systematic underestimation by the FlowCAM for all modes. This underestimation is taken into consideration by calculating the correction coefficients 1.17 (800µm – 2x) and 1.42 (300µm – 4x), obtained from regression analysis between manual and FlowCAM enumeration of particles per mL (Fig. 8). Furthermore, the ability of PhytoImage to discriminate and give correct proportions of artificial mixtures of the five phytoplankton species used in culture was conclusive, being less than 5% relative difference in all cases.

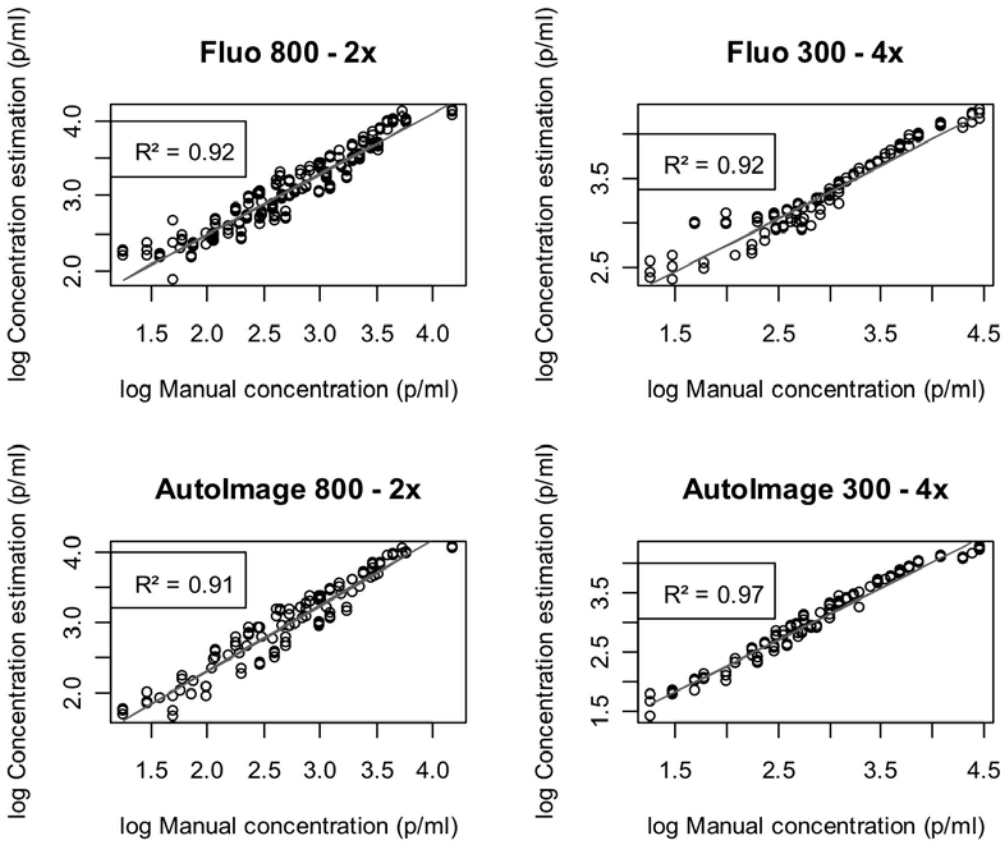

Figure 8: Comparison between manual and FlowCAM counts of particles. The underestimation by the FlowCAM is corrected by a linear relationship (line).

3.2.3 Field (near) real-time monitoring of phytoplankton

Field measurements with the FlowCAM have been made aboard the RV Belgica during four cruises in the BCZ (July 2007, March, April and July 2008). Providing some usage precautions (securing the equipment, not using the fragile 100µm cell), the FlowCAM was usable at sea up to 4-5 Beaufort. Contrasted stations were sampled and analyzed in fluorescence mode to provide images of natural samples. Specific training sets for BCZ and different seasons are in construction.

First tests for real-time process were made. Image acquisition by the FlowCAM varies from 0.5 to 16 particles per second, depending on the concentration and flow cell width. In the real time process, PhytoImage works on the 21 features extracted by the FlowCAM exclusively. A complete reanalysis cannot process more than 6 particles per second using a recent powerful computer, in comparison of 750 to 1300 particles per second processed by PhytoImage when it only uses the 21 parameters provided by the FlowCAM (estimation based on simulations using an artificial continuous feeding of data).

Images from cruises were analyzed in PhytoImage and were manually classified in different training sets to recognize as much groups as possible for the real-time application. First results obtained with two training sets (summer and spring) show that error rates obtained with the real-time analysis (Fig. 9) are significantly higher than with a complete analysis. Thus, real-time monitoring on board (quick analysis) should probably always be followed by a complete analysis (lowest error rates) back to the laboratory.

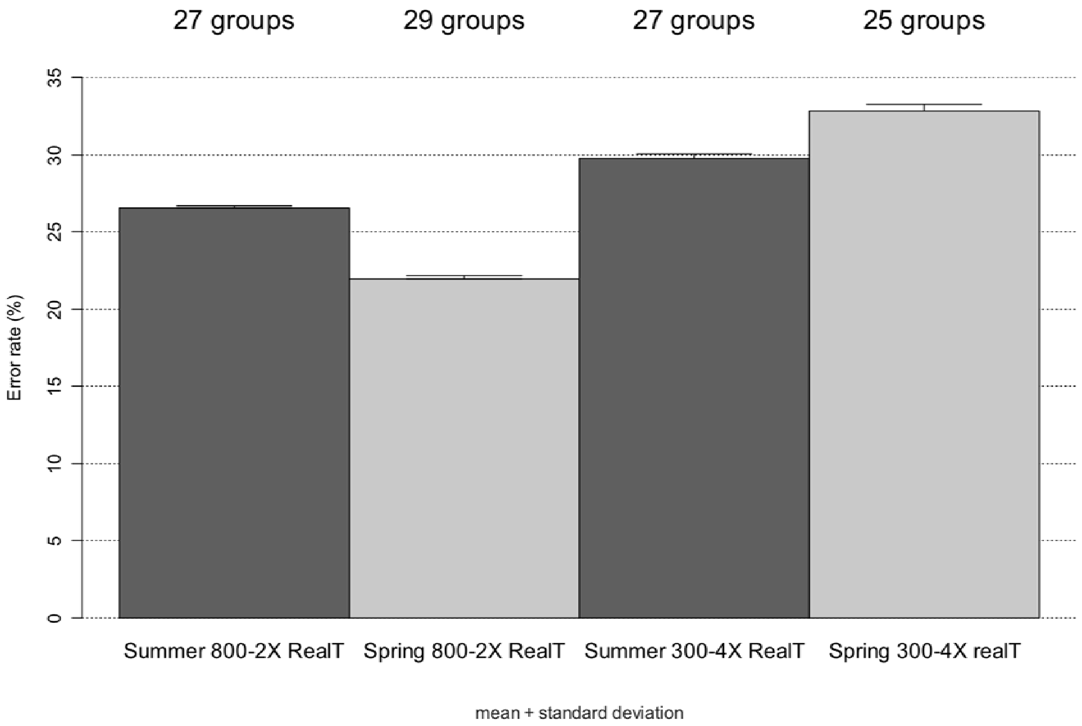

Figure 9: Variation of global error rates of summer and spring training sets configured for the real-time monitoring of plankton calculated by random forest.

3.3 Impact of *Phaeocystis* colonies on offshore mussel farming

3.3.1. Physiological status of offshore mussels along *Phaeocystis* blooming

Seawater and mussel samples were regularly collected in 2008 in the mussel area D (Fig.2) for the measurement of *Phaeocystis* colony presence and of the mussel physiological status respectively. The latter was determined based on the biochemical assessment of the organism's energy reserves available for metabolism, assessed by measuring the total lipid, protein and glycogen content in the mussel (De Coen et al., 1995).

Fig. 10 compares the seasonal evolution of phytoplankton (total Chl *a* and *Phaeocystis* colonies; Fig.10a) and of the biochemical composition of mussels (Fig.10b,c). Clearly the spring phytoplankton bloom and *Phaeocystis* colony occurrence are overlapping with the mussel spawning period (April-July; Fig.10) that makes complex the interpretation of data. The mean lipid and glycogen composition of mussels is however close to the reference levels given by the National Institute of Nutrition and Seafood Research in Norway (Table 2). This added to the observed more than twice higher mussel protein content (Table 2) suggests little effect of *Phaeocystis* colony presence on the mussel metabolism as a whole. This contrasts with results obtained in intertidal waters (mussel beds) suggesting a negative influence of *Phaeocystis* colonies on glycogen storage (Smaal and Twisk, 1997)

The protein and lipid content shows a similar trend yet more pronounced for protein than for lipids: a massive increase in early spring at the time of the phytoplankton bloom onset and the maintenance of elevated values during the spring bloom and spawning period (Fig.10a,c). Still a significant decrease of protein and lipid is observed in mid-June that could be explained by a massive spawning. This is supported by the concurrent decrease observed in the glycogen content (Fig.10b,c) and agrees with the general observation that blue mussel spawning events are sequential and highly synchronized (Pieters et al., 1980).

Glycogen levels (Fig.10b) are slowly build up during all the phytoplankton period (i.e. from April to October; Fig.10a,b) with elevated values after the spawning period. This pattern might be explained by the fact that stored glycogen is immediately catabolized for the synthesis of proteins and lipids that compose the gametes.

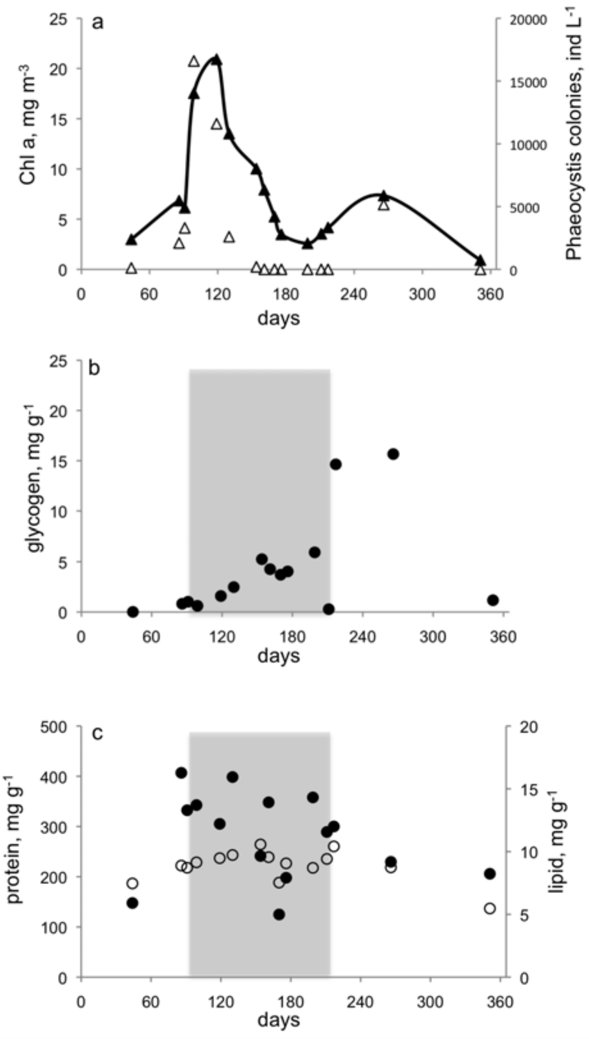

Figure 10: Seasonal evolution of the phytoplankton bloom (a), of glycogen (b) and lipids (open dots) and proteins (filled dots) (c) content of mussels. Grey rectangles represent the mussel spawning period.

Table 2: Biochemical composition of BCZ mussels compared to refernce levels given by the National Institute of Nutrition and Seafood Research in Norway (NIFES)

Mussel origin	Lipid, mg g^{-1}	Glycogen, mg g^{-1}	Protein, mg g^{-1}
NIFES	10	<6	100
BCZ	8.85 +/-1.27	4.10+/-6.86	211.85+/-87.19

3.3.2 Mussel feeding bio-assays under laboratory-controlled conditions

Reference clearance rates

Feeding experiments were first conducted with *Isochrysis galbana* cells chosen as reference food particles and making use of the Okumus et al. (2002) method. Clearance rates obtained for 7 mussel size classes between 1 and 70 mm and 3 prey concentrations between 0.5 and 3.5 10^6 cells L^{-1} are shown on Fig.11. Results confirm that *Isochrysis* is a good food for mussels and can be considered as a reference for investigating mussel feeding on *Phaeocystis.* From Fig.11, it can be concluded that clearance rates depend on the initial concentration of algae as obtained by Okumus et al. (2002) for *Mytilus galloprovincialis*. However, clearance rates of mussels >30mm show no significant difference for cell concentrations >1.5 10^6 cells L^{-1} (Fig.11). This allows to chose the mussel size between 30 and 40 mm for running feeding experiments on *Phaeocystis* colonies.

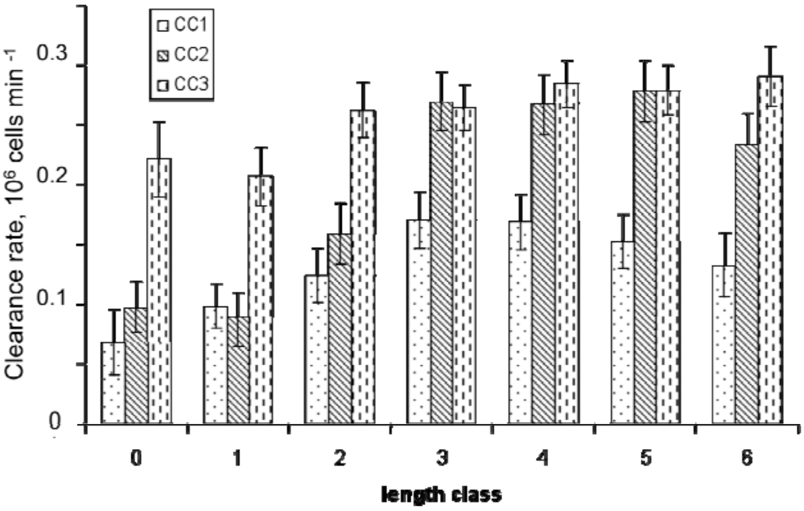

Figure 11: Clearance rates measured for mussels of 7 different size classes (0=0-9mm; 1=10-19mm; 2=20-29mm; 3=30-39mm; 4=40-49mm; 5=50-59mm and 6=60-69mm) and for 3 prey concentrations (CC1: 0.5-1.5, CC2: 1.5-2.5, CC3: 2.5-3.5 10^6 cells L^{-1}).

Mussel clearance rates on *Phaeocystis* colonies

Six experiments were run, each consisting of a set of 7-10 bio-assays containing each a mussel of the length class 30-39 mm. Clearance rates were measured for different concentrations and sizes (25-1000 mm in diameter) of *Phaeocystis* colonies. Results obtained with the largest colony size distribution show mussel ingestion on all colonies of size ≤ 1000 µm (Fig.12). Comparison between clearance rates and the size frequency of colonies suggests in addition that the mussel clearance rate increases with the number of colonies indifferently from the size (Fig.12).

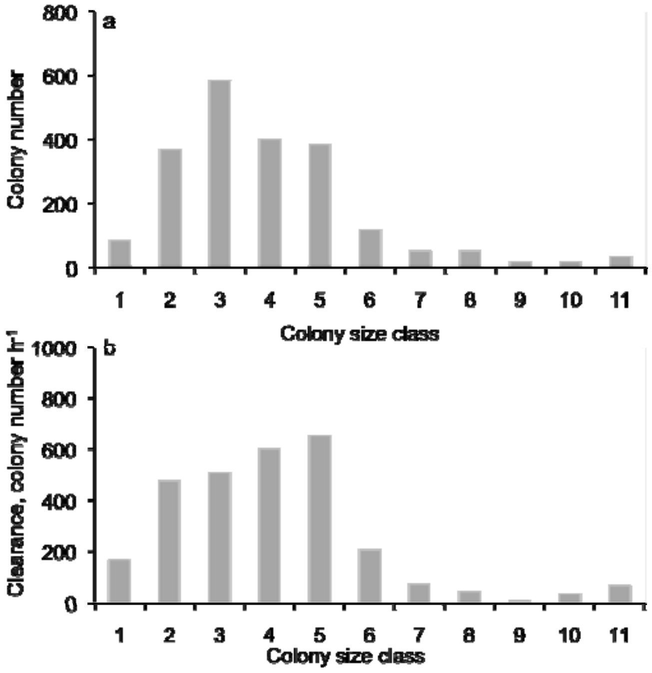

Figure 12: Size frequency of *Phaeocystis* colonies (a) and their mussel clearance rate (b). 1:0-99; 2:100-199; 3:200-299; 4:300-399; 5:400-499; 6:500-599; 7:600-699; 8:700-799; 9:800-899; 10:900-999, 11>1000 µm.

When combining all experiments a positive relationship is observed between the average mussel clearance rate on *Phaeocystis* colonies and the average size of the corresponding offered *Phaeocystis* colonies up to an average size of 300 µm (Fig.13). Clearly, as field *Phaeocystis* blooms in BCZ are dominated by large colonies (500 - >1000 µm), more bio-assays involving a dominance of >500 µm colonies are needed to confirm this trend and eventually determine a threshold size above which colonies are detrimental for mussel growth.

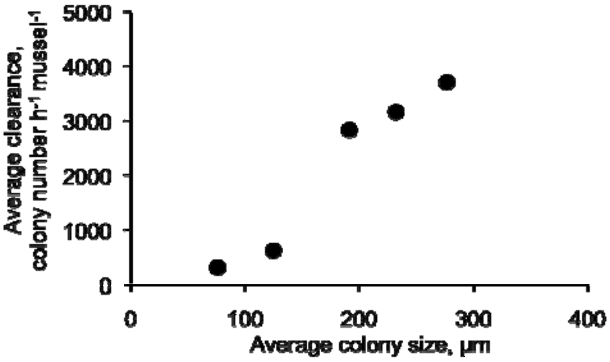

Figure 13: Relationship between the average mussel clearance rate on *Phaeocystis* colonies and the average colony size.

3.4 Ecological modeling and analysis

The role of model simulation in understanding system complexity and in decision-making is nowadays recognized. Models offer the ability to test conceptual understanding of how components of a given system are linked, and to simulate complex biogeochemical interactions in a quantifiable repeatable manner. Modeling, however, is continuously evolving, being useful only if new insights subsequently lead to validation or rejection of hypotheses upon more detailed examination. Thus information extracted from model simulations is strongly linked to the chosen structure (trophic resolution) of the model and to the parameterization of the interactions between the components. When properly validated, models can be useful in both hindcast and forecast modes and used for testing environmental policy alternatives and their impact. Here we report on new modeling developments and applications.

3.4.1 New model development

3.4.1.1 Simplified MIRO

The complexity of highly resolved biogeochemical model (elevated number of state variables) such as MIRO (38 state variables) prevents their use for long-term simulations or application to highly resolved grid domain due to elevated computation time. Hence model sensitivity analysis aiming at decreasing the number of state variables without loosing model realism is relevant.

The possible aggregation of R (reserves) and S (small metabolites) phytoplankton state variables (see Lancelot et al., 2005 for model description) in one unique state variable (R+S) without changing initial assumption behind the phytoplankton model was investigated by adapting parameters related to the dynamics of R and S and by comparing simulations obtained by nominal and simplified MIRO. Several set of parameters were tested and the best fit parameter was then chosen by using the Taylor diagram (Fig.14). Similarly, visual comparison shows a similar evolution for the main MIRO state variables (nutrients, phytoplankton, zooplankton and bacteria) of the BCZ ecosystem and allows the use of this simplified version of MIRO when required by a higher complexity of the hydrodynamic model.

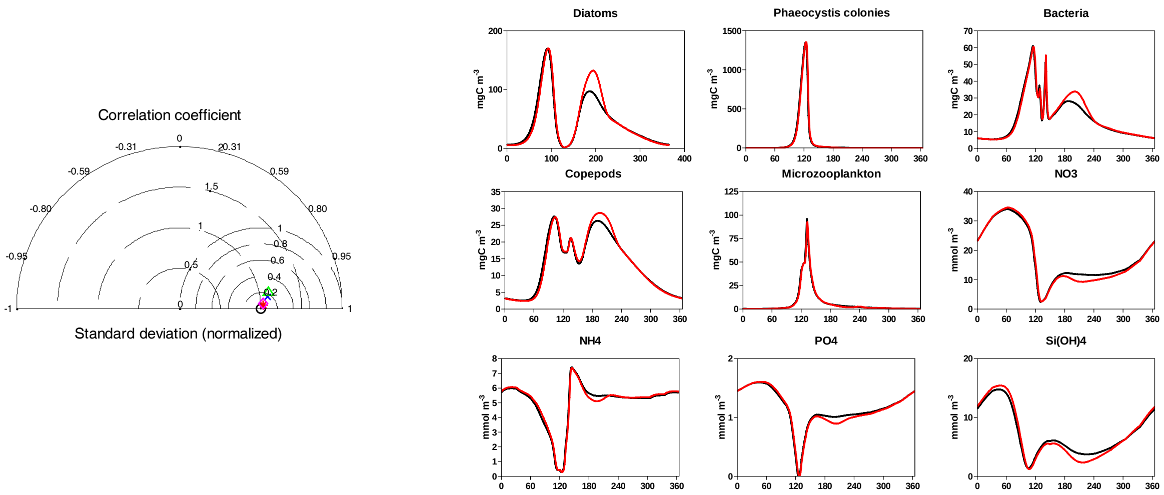

Figure 14: Taylor diagram comparing statistical results obtained for different set of parameters (Left); Seasonal evolution of the main components of the BCZ ecosystem simulated with the simplified (red curve) and nominal (black curve) MIRO model (right)

A similar approach was used to simplify the description of the microbial loop originally composed of bacteria and 5 classes of detrital organic matter (monomers SBC, SBN, dissolved polymers HC1, HC2, HN1, HN2, HP1, HP2, particulate organic matters PC1, PC2, PN1, PN2, PP1, PP2 with 1 and 2 used for fast and slow biodegradability). Considering the biodegradability criterion more important than the particle size, we investigated the possibility of aggregating the dissolved and particulate fraction of organic matter in one unique state variable i.e. TOC1=HC1+PC1, TOC2=HC2+PC2 etc… Results obtained are however not yet satisfying and require additional sensibility tests for defining some aggregated parameters.

3.4.1.2 Increased grid resolution of MIRO&CO in the BCZ domain

In order to better appraise the role of direct nutrient loads in the BCZ and distinguish them from transnational fluxes, the MIRO&CO-3D model has been implemented on the high resolution (750m x 750m) BCZ grid covering the region between 51°N, 2.1°E and 51.92°N, 4.2°E. This grid has been nested within the C&SNS (Channel & Southern North Sea) grid by imposing hydrodynamical variables (elevation & 2D current [5'], 3D temperature & salinity [1h]) and nutrients [daily] as boundary conditions to the smaller grid (Fig.15). ‘Belgian’ rivers have been implemented based on the ‘Coastal Catchments Areas’ used for BE reporting to OSPAR.

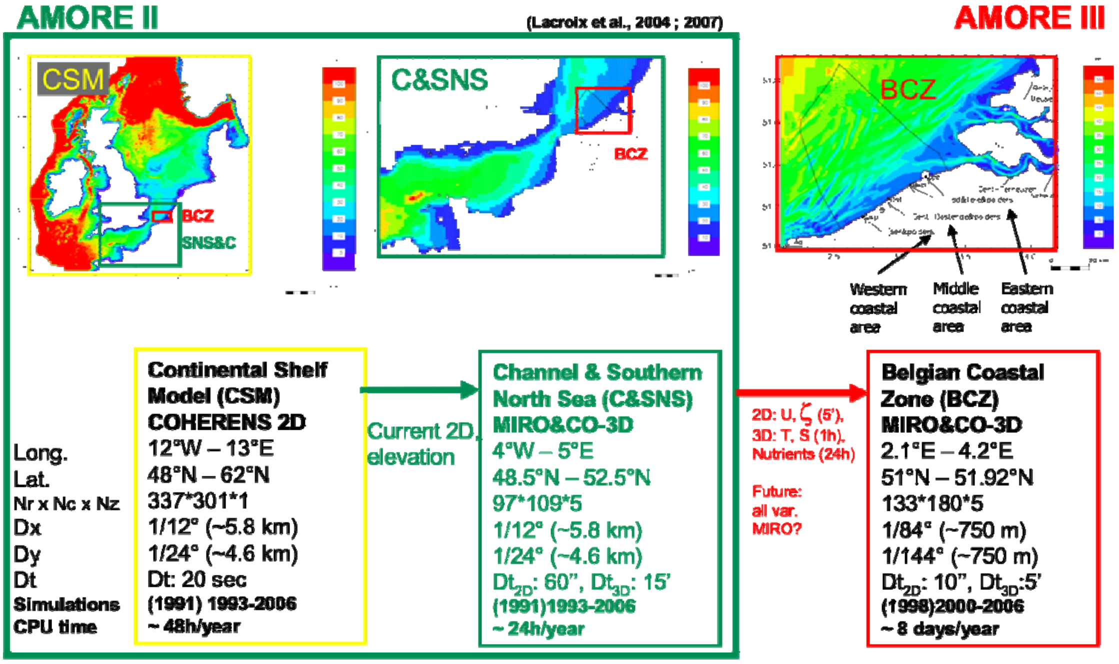

Figure 15: Diagram showing the BCZ grid implementation within the existing CSM (Continental Shelf Model) and C&SNS (Channel & Southern North Sea) grids developed in the scope of the AMORE II Project (Lancelot et al., 2007).

Simulations with the fully nested MIRO&CO-3D (CSM-C&SNS-BCZ) have been performed for 2000-2006. Comparison between results (Fig. 16) shows that simulated salinity is lower when using the higher resolution grid, supporting the need of a high-resolution grid for resolving the impact of Belgian rivers on the BCZ eutrophication.

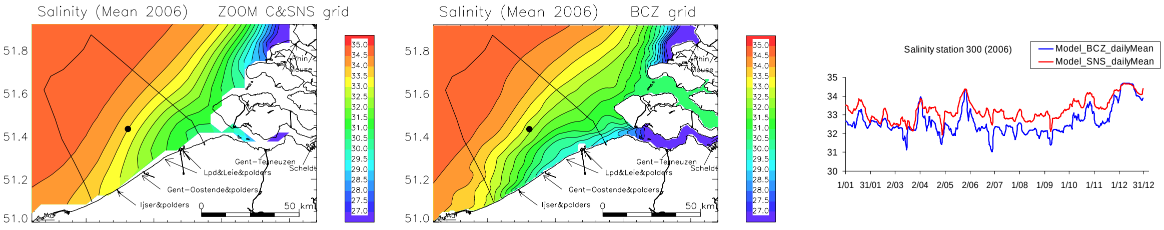

Figure 16: 2006 mean surface salinity obtained with MIRO&CO-3D. Left: zoom of results on C&SNS grid, middle: results on BCZ grid. Right: time series of simulated salinity at St 330 for C&SNS BCZ grid.

3.4.1.3 Improved parameterisation of light availability in the MIRO&CO-3D domain and testing on phytoplankton bloom occurence

Water column light attenuation in MIRO&CO-3D is described as a function of TSM (Total Suspended Matter), CDOM (Coloured Dissolved Organic Matter), Chl *a* and depth and is computed with the kPARv1 module. This model has been validated with PAR attenuation measurements available for the BCZ (2002-2005). The correlation is significant (r^2 = 0.8191, n=44; Fig. 17 left) giving high confidence in the kPARv1 module. While CDOM (through its relationship with salinity) and Chl *a* are state variables of MIRO&CO-3D and are thus calculated at each time step, TSM was derived from 1997-2002 SeaWiFS images and corresponds to a seasonal climatology, spatially averaged to match the model grid cells. Such a limitation prevents resolving interannual variability. The necessary TSM high frequency was obtained from cloud-filled remote sensing images (MODIS) provided by the ongoing STEREO2-RECOLOUR project. A preliminary validation with CEFAS data (Fig. 17 right) shows that the annual cycle and the range of values for PAR attenuation are well represented.

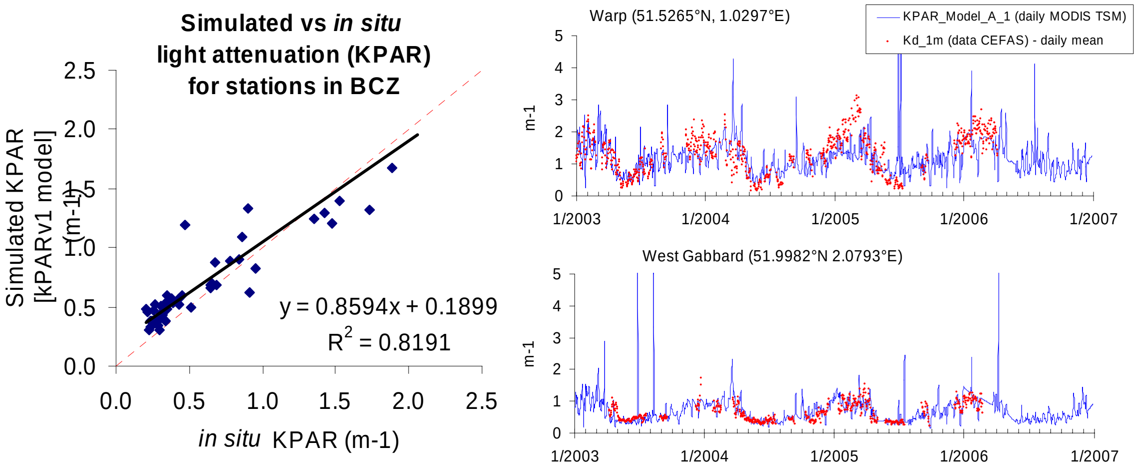

Figure 17: Left: Comparison between KPARv1 modeled and field measurements of KPAR in BCZ during 2002-2005; Right: Temporal evolution (2003-2006) of KPAR estimated with the KPARv1 module forced with daily MODIderived TSM and in situ measurements for 2 stations of the UK monitoring programme.(CEFAS ; Mills et al., 2003)

Simulations of MIRO&CO-3D (C&SNS grid) have been performed with daily/weekly and with daily/weekly/seasonal climatology (average on 2003-2006) MODIS-derived TSM to study the impact of (i) TSM forcing frequency and (ii) interannual variability on phytoplankton bloom development (Fig.18).

Time series of surface Chl *a* at St 330 constrained with daily (AC1) or weekly (AC7) climatology TSM are very similar although slightly different from that forced with the seasonal climatology (ACS) TSM forcing (Fig. 18 upper). Chl *a* concentration computed with daily TSM forcing shows little difference compared to that obtained with daily climatology of TSM forcing (A_1 and AC1, Fig. 18 below). A comparison with in situ data from daily MERIS Chl *a* shows relatively good agreement of the bloom timing obtained with new daily TSM forcing.

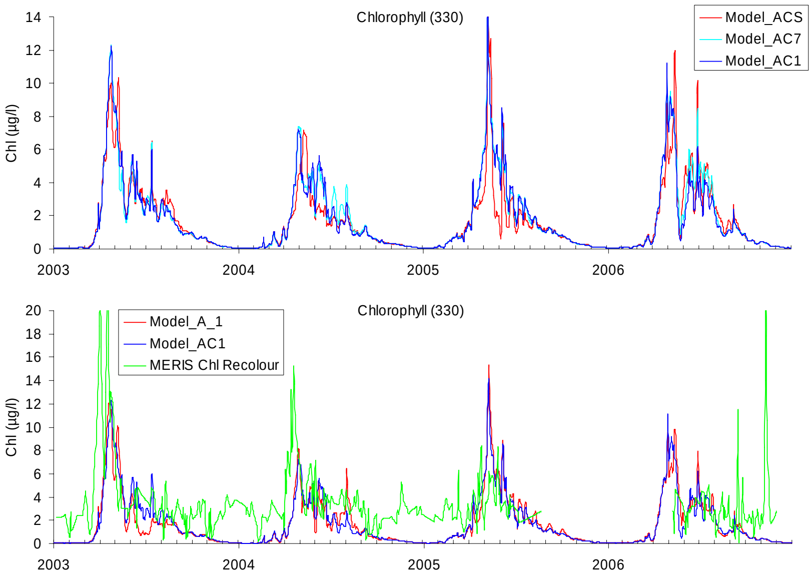

Figure 18: Time series of surface Chl a (St 330) obtained with daily (AC1)/weekly (AC7)/seasonal (ACS) climatology of MODIS-derived TSM (upper) and daily (A_1)/daily climatology (AC1) of MODIS TSM and in situ daily MERIS Chl a (lower).

3.4.1.4 Implementation and testing of a CO2 module [MIRO-CO2&CO-3D]

In order to assess the present-day capability of the eutrophied BCZ in absorbing atmospheric CO2 (if, where and when) the CO2 module developed in AMORE II (Gypens et al., 2004) was successfully integrated in MIRO&CO-3D model (Lacroix et al., 2007) for simulating the surface CO_2 partial pressure (pCO_2) and the air-sea CO_2 fluxes in the C&SNS model grid. The upgraded MIRO-CO2&CO-3D was run for the period 1994-2004 and validated by comparison with pCO_2 data collected in 2003-2004 in the model domain (Schiettecatte et al., 2007). A general agreement was found between model results and data. Particularly well captured by the model is the significant spatial and seasonal variability of pCO_2 in the Western Channel and the Southern Bight of the North Sea with pCO_2 varying between 100 ppm and 600 ppm (Fig. 19). Important under- and over-saturation are shown in spring and summer respectively due to the dominance of either auto- or heterotrophic activities and the highest values are found close to the river mouths.

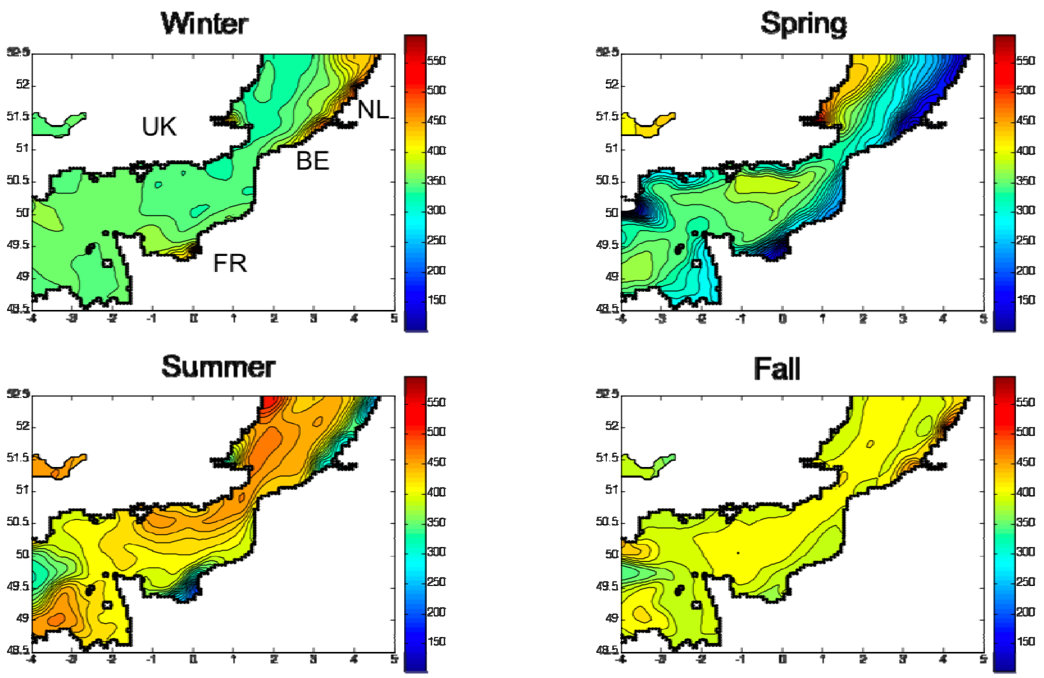

Figure 19: Weekly averaged simulated pCO2 for 4 periods representative of the winter, spring, summer and fall.

Similarly, the simulated distribution of annual air-sea CO_2 fluxes shows significant geographical variability with sources for atmospheric CO_2 simulated near estuaries but moderate (or even zero) sinks in offshore waters (Fig. 20). A focus on the seasonal evolution of air-sea CO2 fluxes simulated at 3 stations sampled along a coastal-offshore transect in the BCZ suggests that this spatial variability is controlled by carbon and nutrient river loads.

The seasonal amplitude is lower offshore than near shore characterized by an important spring phytoplankton bloom but, on an annual base, the offshore region acts as a sink for CO2 while near shore, a significant source is simulated (Fig.20).

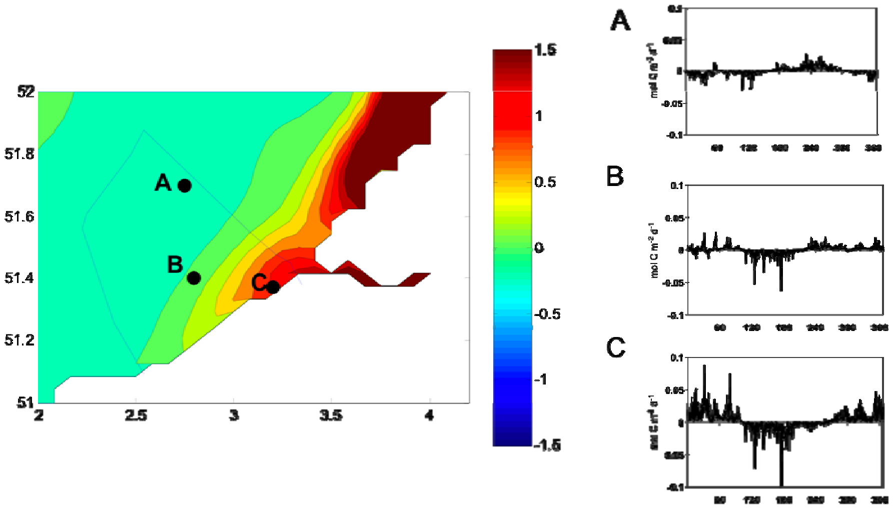

Figure 20: Spatial distribution of annual air-sea CO_2 fluxes (in molC m^{-2} y^{-1}) simulated for 2004 in the Southern North Sea with a focus (right panel) on daily air-sea CO_2 fluxes computed at three stations along a offshore-coastal gradient in the BCZ area.

3.4.2 0D-MIRO and MIRO&CO-3D applications

3.4.2.1 Ecological quality criteria: definition and application

Identification of ecological quality criteria connected to the anthropogenic pressures (i.e. nutrient river loads) was addressed by combining field observation analysis and model simulations. The proposed methodology included first the definition of a reference *Phaeocystis* cells from which the disturbance could be scaled. This reference was derived from the *Phaeocystis* feature that creates ecosystem disturbance i.e. the capacity of *Phaeocystis* colonies which once formed can reach sizes unmanageable for indigenous copepods. Based on analysis of *Phaeocystis* observations available at St 330, the reference was set at 4 10^6 cells L^{-1} (Lancelot et al., 2009) and corresponds to the maximum *Phaeocystis* cells contained in the grazable colonies (<400µm) recorded in the BCZ between 1988 and 2000.

As an alternative approach towards the definition of 'natural' *Phaeocystis* marine ecosystems, reconstruction of nutrient loads and phytoplankton bloom was made for pristine conditions using a coupled river-coastal model. For this application, the RIVERSTRAHLER model, describing carbon and nutrient transformations along the river system as a function of meteorological conditions and land use and human activity on the watershed, was coupled to the biogeochemical 0D-MIRO model describing diatom and *Phaeocystis* blooms in the marine domain (the R-MIRO model; Lancelot et al., 2007). Model simulations obtained for pristine conditions (when all human activity has been erased and the watershed is covered by primary forest) show *Phaeocystis* colony cells similar to the established field reference of 4 10^6 cells L^{-1} suggesting that natural *Phaeocystis* ecosystems are well balanced and transfer efficiently their production to higher trophic levels.

The ecological reference was then used to identify the contemporary eutrophication status of the BCZ and its future evolution after nutrient mitigation measures. The R-MIRO was then run for the past period 1950-2000 and a future 2015 scenario mimicking the implementation of the Water Framework Directive (WFD). *Phaeocystis* and diatom trends simulated in BCZ were analysed with respect to changing nutrient loads and compared to the ecologically-based *Phaeocystis* colony criterion (4 10^6 cells L^{-1}) as well as to the existing field observations.

Both the field observations obtained during the 1988-2000 period and the 1950-2000 historical reconstruction of eutrophication in the BCZ simulated by the R-MIRO model were pointing *Phaeocystis* colony cell maxima well above the reference value.

Considering that the return to the pristine reference value was not achievable, another approach was then proposed based on historical R-MIRO simulations of annual primary and copepod productions which ratios (CP:PP) are an indicator of trophic efficiency. The analysis of the changing CP:PP ratios in relationship with simulated winter concentrations and annual inputs of nutrients showed that the decrease in trophic efficiency was related to the imbalanced DIN and DIP inputs (NP>20) explained here by the intended decrease of DIP loads after 1985 while DIN were maintained elevated . On this basis a critical DIN load estimated at 60 kT y-1 was proposed to re-equilibrate the N:P balance and favor diatoms over *Phaeocystis* colonies. This target should be kept in mind for assessing the effectiveness of future nutrient mitigation measures implemented by Belgium in the scope of the WFD compliance.

3.4.2.2 Nutrient reduction scenarios at the river outlet

Scenarios exploring the effect of selected riverine nutrient reductions imposed at their outlet have been performed with the MIRO&CO-3D model (C&SNS grid) for the period 1993-2004. The impact of nutrient reduction scenarios (N and/or P, 50%/70%/90%) on (i) surface winter nutrient distribution, (ii) Chl *a* maximum and (iii) *Phaeocystis* maximum has been analysed. As expected, the reduction of N and/or P has a direct effect on winter N and P distribution. For *Phaeocystis*, the percentage of total Belgian waters (BCZ; Fig.21) in which *Phaeocystis* can cause eutrophication problems ('high biomass') has been estimated using the new criteria for *Phaeocystis* disturbance in the scope of this project (Lancelot et al., 2009).

Scenario results show that a reduction of N (combined with a P reduction or not) by at least 70% is needed to allow significant decrease of surface area with undesirable *Phaeocystis* biomass (Fig. 21). Interestingly enough a decrease of P alone has no effect on the surface area affected by undesirable *Phaeocystis* colonies.

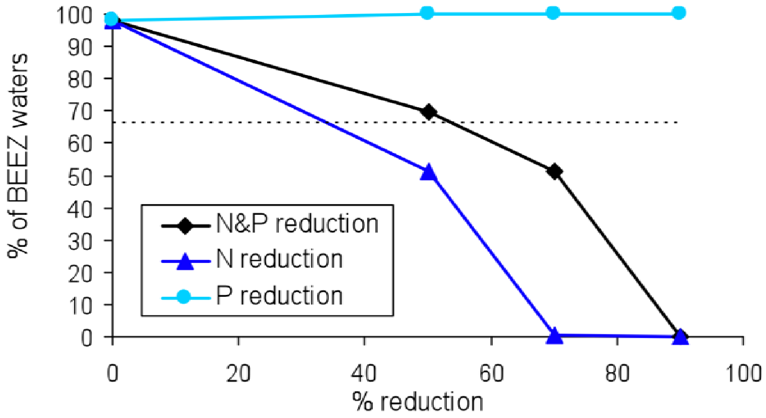

Figure 21: Percentage of the BCZ waters with high biomass of *Phaeocystis* colonies computed with MIRO&CO-3D for different scenarios of river nutrient input reduction.

The MIRO&CO-3D model has also been used to estimate the eutrophication status of Belgian waters according to OSPAR criteria (OSPAR, 2005) and by using the new criteria for *Phaeocystis* disturbance (Lancelot et al., 2009). Fig 22 shows the eutrophication status of BEEZ in the nominal (STD) situation and for each nutrient reduction scenario.

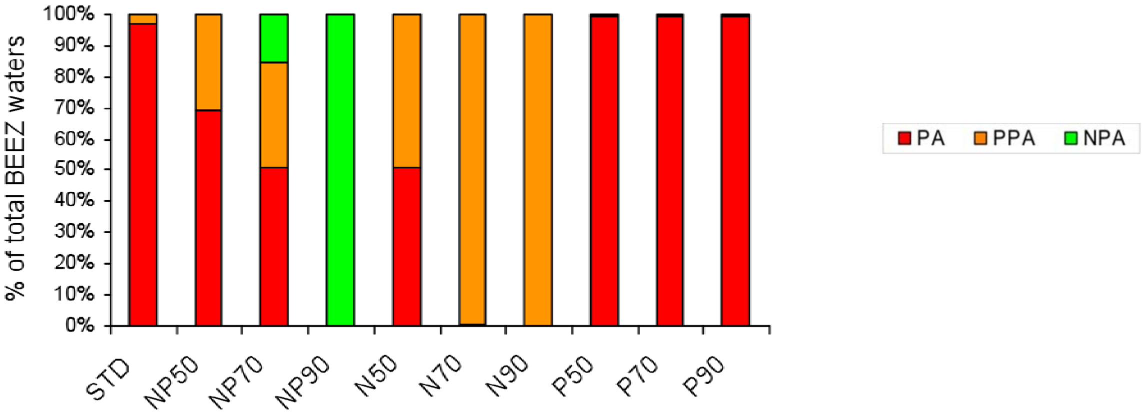

Figure 22: Relative coverage (%) of BEEZ whose eutrophication status is Problem Area (PA), Potential Problem Area (PPA) or Non Problem Area (NPA). STD: standard run

Finally, a module allowing the computation of transboundary fluxes (daily, weekly, monthly & annually) has been implemented in the C&SNS grid, allowing the estimation of nutrient transport across several transects in the Channel and the Southern North Sea and across BE boundaries with FR, UK & NL for the period 1991-2004 (Fig.23). Results are under analysis but first estimations of transboundary nutrient fluxes for the period 1991-2004 show important interannual variability.

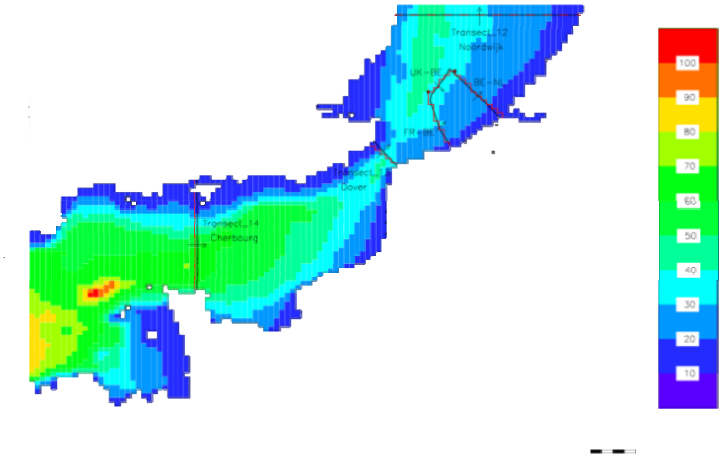

Figure 23: Position of transects for the nutrient transport computation

4. SUPPORT TO THE DECISION

The AMORE III project provided the essential supporting research needed as scientific basis for the report of Belgium on the "Second application of the Comprehensive Procedure for the Belgian marine waters" in the framework of the OSPAR (Oslo and Paris Commissions for the prevention of marine pollution) Convention. This report, a national obligation under the terms of the OSPAR convention, was edited by the Federal Public Service Health, Food Chain Safety and Environment (Directorate General Environment DG-ENV, Marine Environment Service) and included major contributions from AMORE scientists, including the majority of text and figures. AMORE scientists participated in a number of formal and informal meetings and email exchanges with the DG-ENV in the drafting and revisions of this report, including the meeting of the OSPAR Eutrophication Committee held in London on 23-25 October 2007. Understanding of the ecosystem achieved within the AMORE III project has stimulated improvements in the OSPAR procedures, including the adoption of a 90 percentile parameter for Chl *a* instead of the "maximum Chl *a*" previously used as well as a clearer understanding of the relevance and relationship between winter DIN and DIP thresholds and the Chl *a* thresholds used within OSPAR.

The MIRO&RIVERSTRAHLER modeling work on conditions over the last 50 years and hence preceding the large increase in nutrient loads occurring in the 1970s provides some indications for OSPAR of concentrations of nutrients and Chl *a* that could correspond to "pristine" conditions. The notion of pristine conditions (+50%) is the basis of thresholds that are set by OSPAR for eutrophication problem area definition.

The AMORE research into criteria for ecological disturbance by colonial *Phaeocystis* formation is considered by OSPAR as a possible way of objectively defining a threshold for definition of eutrophication problem areas.

The MIRO&CO-3D model was used to assess the probable impact of targeted nutrient reduction on the eutrophication status of Belgian waters in the framework of the OSPAR Eutrophication Committee's Intersessional Correspondence Group on Ecosystem Modelling (ICG-EMO). AMORE represented Belgium in this group and participated actively in the model intercomparison workshop held by OSPAR ICG-EMO in Lowestoft on 10-12 September 2007. The MIRO&CO-3D model is also being used to assess, within OSPAR, transboundary nutrient transport, of relevance for determining the causes of and responsibilities for eutrophication problem areas.

Although OSPAR is at present the primary intergovernmental framework for assessment of eutrophication in the North Sea, the European Union Water Framework Directive (WFD) is beginning to adopt the OSPAR procedures. Convergence between the EU-WFD and the OSPAR Eutrophication Strategy is now high (except for the geographical extent of waters covered) and the AMORE research will thus be supporting more and more Belgium's WFD assessment and will play a role in the implementation of the recently adopted Marine Strategy Framework Directive.

AMORE scientists participated in the WG-MASC (MArine Shellfish Culture) and WKEUT (Workshop on time series data relevant to Eutrophication Ecological Quality Objectives) working group of the International Council for Exploration of the Seas (ICES), including attendance at meetings held in 2008 and 2006 respectively. AMORE III provides the link between mussel culture and *Phaeocystis* algae relevant to WG-MASC.

5. PERSPECTIVES

5.1 Phytoplankton eco-physiology

Research effort for elucidating mechanisms triggering *P. globosa* colony formation will be pursued based on laboratory-controlled and field experiments. In particular, factors controlling the syngamy of *P. globosa* haploid flagellates from strain BCZ05 isolated from BCZ will be continued by conducting bio-assays under different light levels and using the culture medium recommended by Noel et al. (2004) to induce ploidy change in another Haptophyte. Field conditions in which colony formation occurs such as water column light climate, nutrient concentrations and ratios, will be measured at high temporal resolution during the prebloom period.

5.2 Real-time monitoring of phytoplankton species

The real-time digital imaging flow cytometry will be installed aboard research vessels for direct measurements during oceanographic cruises. Results of image analyser will be compared with those obtained from microscopic analysis of discrete samples.
Digital images of phytoplankton monitored at sea using the flow cytometer will be stored in a database. The latter will be reanalyzed with the first version of the automatic recognition and measurement algorithm (http://www.sciviews.org/zooimage web site). This will provide data for further improving the algorithm by comparison of automatic identification with traditional microscopy analysis. Real-time, or near real-time analysis of the samples is expected from this very moment. Yet, all previous images stored in the database are still accessible for reanalyzes, should the classification algorithm be improved during the coming years.

Opportunities provided by (near)-real-time monitoring of phytoplankton thanks to the system developed in AMORE III will be leveraged by the concurrent study of multivariate statistical methods to provide early warnings (i.e., near-real-time) of ecosystem changes. Among those techniques, “centerD2” (Ibanez 1981, 1991) and “structural change” (Zeileis, 2001) allow early detection of changes in multivariate space-time series. Comparisons between results obtained with these techniques and the model predictions will be used to help identification nutrient-driven thresholds and accompanying variables responsible of the development of undesirable algal blooms.

5.3 Impact of *Phaeocystis* colonies on offshore mussel farming

Considering the observed overlapping between *Phaeocystis* bloom and mussel spawning in spring, the planned survey of the physiological status of offshore mussels will be pursued focussing on end-summer period (end August- mid September) when secondary Phaeocystis colony blooms might occur. Based on mussel feeding data on *Phaeocystis* colonies reported on Fig. 13, laboratory bio-assays will explore mussel feeding on larger colonies. Afterwards this experimental design will be repeated with smaller mussels (length classes 0 and 1). Long-term experiments of mussel feeding on Phaeocystis colonies will be performed
The effect of *Phaeocystis* colonies on the physiological state of mussels will be investigated by feeding mussels with various colony concentrations and sizes over a long term period (at least one month). At the end of these long term experiments, measurements of mussel biomasses, filtration rates, gill histology, biochemical composition will be performed. Altogether results obtained will give insight on the influence of *Phaeocystis* colonies on adult and juvenile mussels. This will help to understand the observed seasonal variation of the physiological state of offshore mussels.

5.4 Ecological modelling and analysis

Based on results obtained on phytoplankton eco-physiology (section 3.1.2), and recent literature survey, a conceptual model of phytoplankton successions will be constructed and tested. The model will include 5 phytoplankton groups representing the small bentho-pelagic diatom species, *Chaetoceros, Guinardia, Phaeocystis* haploids and colonies, each described as in MIRO. Sensitivity tests with derived-parameters will allow identifying important mechanisms behind the observed seasonal phytoplankton succession including colony formation.

5.5 Upgrading and validation of MIRO&CO-3D

When available the new MIRO module will replace the current one in MIRO&CO-3D (Lacroix et al., 2007) and this new version will be tested for its coherence with respect to simulations obtained with the original MIRO.

The new version of MIRO&CO-3D will be run in its upgraded physical description over the 1991-nowcast period with the real meteorological forcing (6 hourly reanalyzed forecasts data from UK meteorological office) and real river loads [daily flow and (bi)-monthly nutrient discharges].

Simulations will be compared with various existing or retrieved data sets for the period. Time series of biogeochemical data (inorganic nutrients and Chl *a*) at different stations of the Belgian (IDOD/BMDC), the Dutch (RIKZ) and French (SOMLIT, REPHY, SRN) will be used to assess the model ability to reproduce the seasonal cycle at different locations. Data obtained during the AMORE III monitoring surveys will be used for assessment of the model capability for resolving the seasonal and geographical distribution of diatom and *Phaeocystis* blooms. Chl *a* maps obtained from remote sensing (MERIS and MODIS, where applicable) will be used to assess the reliability of the model in reproducing the surface phytoplankton distribution over the whole domain (Channel and Southern North Sea).

The availability of additional data sets for validation will be further explored during AMORE III. In particular, high temporal (20') time series of nutrients and chlorophyll *in situ* measurements from CEFAS (UK) would allow assessing the model reliability for resolving seasonal cycle close to the Thames mouth and to the northern boundary conditions of simulated domain. Spatially average (0.5°x0.5°) nutrients and chlorophyll concentration obtained from ICES and CPR (Continuous Plankton Record) will offer the opportunity to assess the model reliability in the English Channel.

5.6 Model assessment of future realistic nutrient reduction policies

The possible ecological improvement obtained after implementation of realistic nutrient mitigation measures such as waste water treatment plant upgrading or implementation of good agricultural practices or their combination will be assessed by replacing measured nutrient loads forcing in MIRO&CO-3D by those simulated by the SENEQUE/RIVERSTRAHLER model (Thieu et al. 2009). The average climatological year 2000 will be used for the meteorological forcing and results will be appraised in terms of changing winter nutrient stocks and diatom/*Phaeocystis* colony blooms with respect to a contemporary reference (2000) and to a pristine historical reference. The latter will be obtained by running the SENEQUE-RIVERSTRAHLER model under conditions where all human activity has been erased and the watershed is covered by primary forest. The ecological quality threshold of 5 10^6 cell L^{-1} will be used as reference for a well-balanced *Phaeocystis* ecosystem. Similar runs conducted with the validated MIRO-CO2&CO-3D will assess the potential impact of nutrient reduction policies on the buffering role of the BCZ for increased atmospheric CO_2.

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