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

AMORE III

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FINAL REPORT

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

AMORE III

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TABLE OF CONTENT

SUMMARY

A Context and objectives

AMORE (Advanced MOdeling and Research on Eutrophication) is an interdisciplinary consortium composed of biologists, bioengineers, biostatisticians and physical and ecological modelers aiming to develo 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).

Achievements performed since 1997 by the AMORE consortium demonstrated that the BCZ is a key pilot area for addressing effects of natural variability and human activity on coastal eutrophication and the sustainable use of economic activity. The BCZ is submitted to local (Scheldt, Ijzer) and transboundary (Seine, Somme, Rhine/Meuse) river inputs of anthropogenic nutrients (N and P) 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 eutrophication in the BCZ 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. Regarding services, AMORE III focuses 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 in other regions, 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 results and tools to:

- Assess the role of changing nutrient river loads in determining the present-day geographical extent and the magnitude of *Phaeocystis* 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 river nutrient loads reduction i.e. simulating the implementation of the European Union (EU) Water Framework Directive (WFD);
- Assess the impact of *Phaeocystis* colony blooming 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;
- Set-up a new observation instrument (FlowCAM and Zoo/PhytoImage) for the efficient real- time monitoring of phytoplankton taxa in the North Sea.

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. The complementary 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. The 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 long term ecosystem shifts.

B Progress achieved

Phytoplankton ecology

Phytoplankton eco-physiological studies were focused on the mechanisms controlling *Phaeocystis* colony formation. Parameterisation of colony formation in the existing MIRO&CO-3D is crucial for correctly simulating colony occurrence, but the underlying science basis for such parameterisation is relatively weak. Results suggest that *Phaeocystis* colony formation is related to the growth dynamics of the haploid population present in the water column before the bloom. 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. setae in providing a solid substrate for anchorage to facilitate mating and for protecting newly-formed small *Phaeocystis* colonies from grazing cannot be excluded.

Possible factors controlling the recurrent diatom/*Phaeocysti*s/diatom succession in the BCZ were approached based on eco-physiological laboratory studies in combination with the analysis of the 13-yr existing phytoplankton record at St 330 in the central BCZ and additional seasonal geographical surveys. Three phytoplankton communities bloom regularly in the BCZ: the ‘small bentho-pelagic diatoms’ including *Chaetoceros* spp., the ‘*Guinardia-Rhizosolenia’* and the colonial haptophyte *Phaeocystis*. The ‘small bentho-pelagic diatoms’ occurs in spring and fall while the ’Guinardia-Rhizosolenia’ appears mainly during summer except offshore where they are present all through the growing season. While a slightly different time laps is to be observed between the seasonal patterns of these two diatom groups along the nearshore-offshore gradient, *Phaeocystis* colonies appear as a unique synoptic spring event. Disappointingly, neither the statistical analysis nor the eco-physiological studies could provide clear conclusions regarding the regulation of the observed recurrent seasonal succession of diatom communities and *Phaeocystis* by temperature, nutrients and ambient light conditions. However, one finding was that the small neritic diatoms and *Chaetoceros* seem better adapted than *Guinardia* to the most extreme temperature (<8°C and >17°C). Different resistance of the diatom communities and *Phaeocystis* colonies to grazing is now seen as an additional factor shaping the phytoplankton succession.

Phytoplankton real-time monitoring

The automatic recognition of plankton is a new research area, which is in rapid evolution. For the first time, real-time monitoring of phytoplankton has been deployed at sea using a FlowCAM and the Zoo/PhytoImage software (http://www.sciviews.org/zooimage). This system collects surface seawater even when the ship is cruising and digitizes image of particles for automatic classification and counting in real-time. A classifier for North Sea phytoplankton in the BCZ has been designed to separate 25 groups. This has been tested

along transects and can be used to plot maps of phytoplankton distribution in real-time after combination with position information. These data could be exploit to optimise the choice of stations for collection of discrete samples, e.g. along transects, where interesting changes in phytoplankton communities are observed. Such data could also provide critical information on phytoplankton species composition, which could be combined with satellite-derived Chl a maps to give information on whether the detected blooms correspond to harmful algal species (HAB).
The ‘in situ’ sampling system has been designed to avoid damage to fragile phytoplankton colonies, except for partial disruption of *Phaeocystis* colonies around or larger than 2000 µm which are still detected but whose size is slightly altered. This FlowCAM configuration is not adapted for zooplankton sampling although copepods are sometimes digitized indicating the ability of the system to digitize motile plankton. Thus, the sampling procedure could probably be expanded for the detection of both phytoplankton and zooplankton in size range from 50-3000µm.

The global accuracy of the classifiers varies from 79.5% for real-time processing to 83.5% for the post-processing in the laboratory. The 25 groups included in the classifiers are the most abundant ones. Minor groups, which resulted in less than 30 items in the training set, have been excluded from the classifiers. These could be added if new vignettes for these groups are digitized in future cruises. The training set is thus a dynamic collection of phytoplankton ‘examples’ which can be improved with new groups according to particles digitized with the FlowCAM. The determination of the number of cells per phytoplankton colonies is also possible, after calibration of linear conversion models. Such models have been studied for two taxa: *Phaeocystis* and *Pseudo-nitszchia*; they would still require a more systematic analysis to be used for all taxa.

The errors made by the classifiers are not random but systematic; it is thus possible to model such error, in order to perform statistical corrections. We have already started to implement new algorithms for error correction and for identification of suspect particles (items whose probability of misclassification is very high) and we will continue to develop the software in this direction. In fact, it will never be possible to reach 100% accuracy, but it appears easier to spot which particles are wrongly classified. In other words, a fraction of the particles are hard to classify but they could, at least, be tagged as suspect for further manual validation. Tools to ease manual validation of those suspect particles are also in development. First trials show a possible improvement of global accuracy by 15% (thus, up to 90-95%) with manual validation of roughly 20% of the most suspect particles. Overall, the FlowCAM coupled with Zoo/PhytoImage is able to accurately determine the abundance of major phytoplankton taxa present in the BCZ and could be used to validate ecological models more rapidly than manual counting of discrete preserved samples.

The FlowCAM generates a lot of data. About seven gigabytes of images are generated per hour along transects. These data are currently stored in distinct files on external hard disks. In the future, it would be better to improve the phytoplankton monitoring system by storing and managing this large amount of data in a dedicated database.

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 offshore mussels fed with different concentrations and sizes of *Phaeocystis* colonies and on field measurement of indicators of mussel physiological state (glycogen, lipid, protein) before, during and between *Phaeocystis* blooms.

Altogether, our laboratory feeding experiments and field measurements reveal that <1000 µm *Phaeocystis* colonies which are prevailing in spring in the BCZ, are cleared from the water column by offshore mussels, as much as there are abundant and with no size distinction. Field measurement of mussel energy levels during two contrasted years (high *vs* low *Phaeocystis* abundance) suggests that the presence of *Phaeocystis* colonies has little effect. Therefore, it might be concluded that offshore mussels can assimilate small-sized *Phaeocystis* colonies, while large-sized colonies are cleared from the water column, but released as pseudo-faeces. The latter was also observed during the feeding experiments.

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 MIRO model simulations. The later make use of historical RIVERSTRAHLER simulations of nutrient loads for pristine conditions, assuming a watershed covered by primary forest. This suggests that a threshold nutrient load of 60 kT N y^{-1} to the 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 'undesirable *Phaeocystis* areas' based on either real-time monitoring by the FlowCAM/PhytoImage tool or by MIRO&CO-3D simulations, the latter for present-day and nutrient reduction scenarios.

Ecological model development

Numerical model improvements were made in the development, implementation and forcing parameterization of the MIRO&CO-3D model.
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. Results show the role of river loads and hence eutrophication in determining the capacity of coastal zones in absorbing atmospheric CO_2.

The successful implementation of the MIRO&CO-3D model on the BCZ grid allowed simulations with a better resolution (750m x 750m) and has potential for a better assessment of the role of Belgian rivers on the BCZ eutrophication.

High frequency data for total suspended matter (TSM) has been obtained from remote sensing images (MODIS) thanks to the STEREO2-BELCOLOUR-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.

Transboundary fluxes and effect of nutrient reduction scenarios on the eutrophication status of the BCZ

A module for calculating transboundary fluxes in the MIRO&CO-3D domain has been implemented and nutrient transports have been computed for the years 1991-2004. The net annual fluxes of water and nutrients through the BCZ are following the SW-NE residual pattern of circulation in this area. However at the tidal scale, fluxes are more intense and variable in direction making the BCZ a zone of transit for nutrients transported mainly from the English Channel, but also from the French, Belgian and Dutch rivers. Despite the implementation of nutrient reduction measures, the Southern Bight of the North Sea remains an area of significant nitrogen enrichment due to river inputs with some input also from Atlantic waters (e.g. in 2002). The same is observed for phosphorus, though in lesser proportions, and the resulting increase in the N:P nutrient status of the Southern Bight of the North Sea may be seen as a signature of the human activities in the North Sea watershed.

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. However RIVERSTRAHLER scenarios testing the effect of different realistic nutrient reduction options such as those recommended by the EU WFD for reaching a good ecological status of transitional waters in 2015 (improvement of waste water treatment WWT, good agricultural practices or their combination) suggest little reduction of nitrogen loads to the coastal sea when compared to phosphorus and hence an exacerbation of the nitrogen and phosphorus imbalance. In confirmation of this the coupling of RIVERSTRAHLER simulations with the MIRO&CO model shows that the implementation of realistic mitigation measures, despite improvements, will leave the BCZ as a “Problem Area”, especially regarding nitrogen.

C Support to Sustained Development

AMORE research is delivering solid scientific support for sustainable development of the Belgian coastal zone as regards excess nutrient (N and P) loads (eutrophication) and recent aquaculture development (offshore mussel farming). AMORE III has made an important contribution to the formulation of quality descriptors based on improved knowledge of the ecosystem functioning. This knowledge is used for the assessment of problem areas in the BCZ and for the formulation and testing of nutrient reduction options.

Altogether both the AMORE observation and modeling activities conclude that the eutrophication problem of the BCZ (and adjacent waters) visible as *Phaeocystis* blooms are due to an excess of nitrogen loads when compared to phosphorus. Our model scenarios clearly show that the ongoing implementation of WFD measures for WWT improvement and good agricultural practices will exacerbate the problem by reducing phosphorus with a little effect on nitrogen. Therefore options for reducing nitrogen from diffuse sources need to be taken for re-establishing the coastal ecosystem equilibrium. As these involve societal changes, these measures need to be explored with all stakeholders including citizen and scientists.

Ecosystem goods and services provided by coastal zones (carbon sink, stocks of fish, recreation…) are receiving increased attention and measures for maintaining them are already taken. However due to the high diversity of marine ecosystem uses, some measures chosen for maintaining one service might be disadvantageous for another one. A good example suggested by AMORE research concerns measures taken for combating eutrophication in the BCZ where the reduction of nutrient river loads might well be beneficial to the coastal ecosystem by decreasing the magnitude of harmful *Phaeocystis* colony blooms but at the same time be negative for climate change mitigation by reducing the atmospheric carbon uptake capacity of the BCZ. Indeed MIROCO$_2$&CO simulations suggest for the BCZ a complex link between nutrient loads and both harmful algal (*Phaeocystis* colonies) blooms and the carbon sequestration capacity. This highlights a hitherto neglected coupling between nutrient reduction policies and mitigation of greenhouse gases emissions. Altogether this

strongly suggests that future measures taken for the reduction of *Phaeocystis* colony blooms in the BCZ might not be beneficial to the carbon sink service and *vice versa* if no measure is taken.

Keywords

Southern North Sea, Belgian coastal zone, eutrophication, *Phaeocystis*, real-time phytoplankton monitoring, ecological modeling, offshore mussel farming, air-sea CO_2 exchange, nutrient mitigation

1. INTRODUCTION

Coastal zones are threatened by several socio-economic drivers operating in both the watershed (urbanization, agriculture, industrial activity; Fig.1) 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, 2011). 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 best mitigation strategies that will guarantee the sustainability of coastal resources and maintain the socio-economic activity in the coastal zones.

Results gained during the BELSPO 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 (Seine and Somme) inputs of land based nutrients from the rivers (Fig.1). These nutrient loads modify the quantity and N:P:Si balance of nutrients and subsequently the ecosystem structure of the coastal area, favoring the blooming in spring of undesirable *Phaeocystis globosa* (hereafter reported as *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 (WFD) of the European Union (2000/60/EC). Historical model reconstruction of eutrophication problems in the BCZ since 1950 (Lancelot et al., 2007) shows a clear increase of *Phaeocysti*s blooms in the eighties when nutrient loads (N and P) were maxima. Unexpectdly these blooms are maintained nowadays in spite of a 50% reduction of P loads. A recent statistical analysis of last decade nutrient and phytoplankton time-series suggests that the actual contribution of local river inputs of anthropogenic nutrient to the eutrophication of the BCZ and the related geographical spreading of *Phaeocystis* colonies are determined by large-scale climatic phenomena such as the 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 (Fig.2) is a recent economical activity in the BCZ, set up in 1998 for facing actual problems in the Belgian fisheries sector and possibly reducing eutrophication problems. At the moment two farmers are operating in three production areas (Nieuwpoort (D1), Oostdijck and 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).

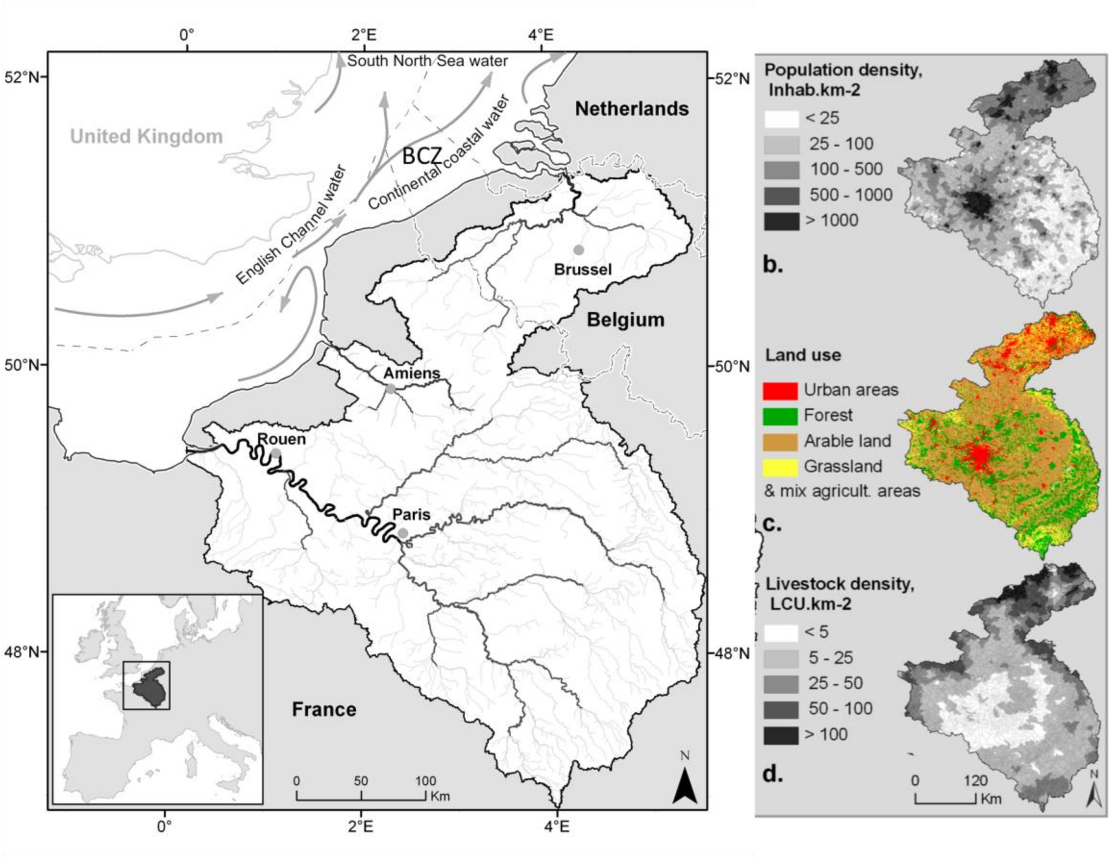

Figure 1: Map showing the eastern Channel and Southern Bight of the North Sea and their 'three river' watershed (Seine, Somme and Scheldt) including socio-economic indicators. The limits of the Belgian EEZ is shown as BCZ. Adapted from Thieu et al., 2010.

Figure 2: Mussel boy and pontoon with cages carrying mussel ropes

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 role of changing nutrient river loads in determining the present-day geographical spreading and the magnitude of *Phaeocystis* 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 river nutrient loads mimicking the implementation of the EU WFD;
- 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;
- Set-up a new observation instrument (FlowCAM and Zoo/PhytoImage) for the real-time monitoring of algal blooms in such a dynamic area.

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.

When operating the new observation device will improve knowledge on phytoplankton diversity change in the area and support monitoring effort and strategy.

2. METHODOLOGY AND RESULTS

2.1 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 effect of changing anthropogenic nutrient loads 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) has dictated the definition and organization of AMORE III experimental and numerical activities.

2.1.1 Experimental work

2.1.1.1 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 pure strains and field populations of *Phaeocystis* haploids flagellates to identify the biotic (infochemicals potentially release by vernal diatoms *Coscinodiscus concinnus, Skeletonema costatum, Ditylum brightwellii, Thalassiosira rotula, Chaetoceros compressus*) and abiotic (light, nutrients including trace elements and vitamins) factors inducing colony formation. The added hypothesis that a haploid cell threshold must be reached to allow syngamy was also tested.

The potential effect of *Phaeocystis* colonies on the production of offshore mussel (*Mytulis 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 all mussels were collected from the D1 site (Fig.3) by farmers during their routine work at sea.

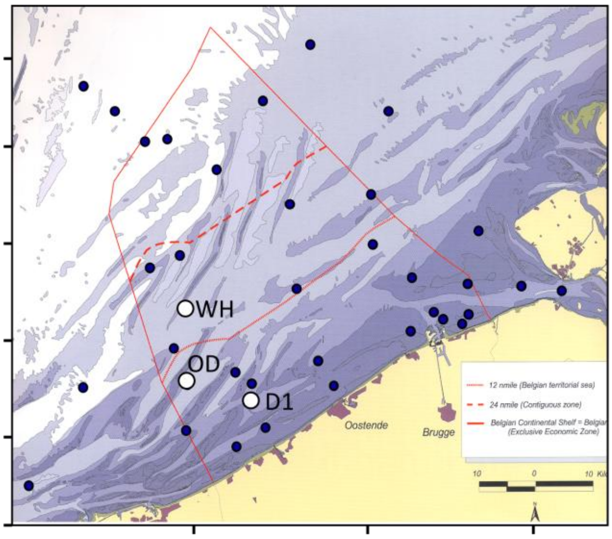

Figure 3: Map of the BCZ with location of the phytoplankton monitoring stations (•) and of the three mussel cultivation areas **O** *: D1 (main culture in front of Nieuwpoort), Oostdijk (OD) and Westhinder (WH)*

2.1.1.2 <u>Field observations: phytoplankton distribution fields</u>

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 (Breton et al., 2006) as well as new collected data during spatio-temporal surveys. Some 35 stations (Fig. 3) were visited resulting into 922 records.

2.1.1.3 <u>Technical development: the FlowCAM and Zoo/PhytoImage</u>

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) 3D-MIRO&CO validation, (ii) the mid and long term assessment of the ecosystem health in response to nutrient reduction policies and (iii) the future 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 increase 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 identification of digitized particles (Bell & 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 Zoo/PhytoImage for image analysis and classification of particles. The FlowCAM includes a video camera coupled with a microscope which detects particles circulating through a glass cell (so-called, the flow cell) using one of three triggering modes: fluorescence detection, light scattering detection and time-lapse video (‘autoimage’ mode). In addition, it measures 17 parameters on each detected particle (area, perimeter, fluorescence, etc.) and extracts a rectangular region of interest (ROI) around the particle in the whole image that is pasted into a new composite image called a ‘collage’. Examples are shown in Fig. 4.

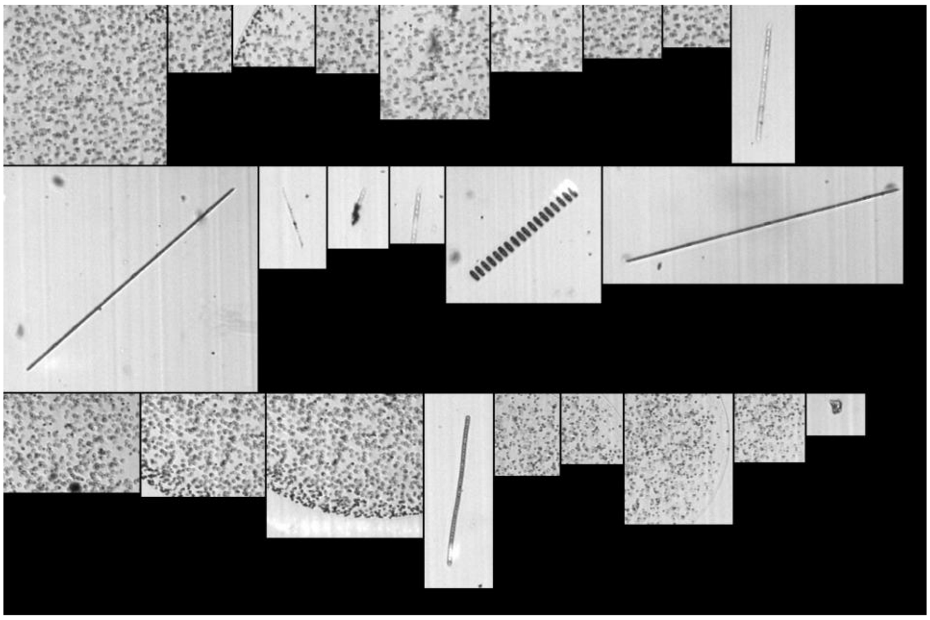

Figure 4: FlowCAM collages. Several portions of very large Phaeocystis colonies are visible, as well as various elongated diatom colonies.

ZOO/PhytoImage is an open source software developed at UMONS for the automatic recognition of plankton digital image (http://www.sciviews.org/zooimage). FlowCAM data images are processed either in real-time or can be processed later in the home laboratory.

The best combination of physical and optical parameters for using the FlowCAM for monitoring North Sea phytoplankton were experimentally determined. The 600 µm flow cell coupled with a 2x magnification in ‘autoimage’ mode appears to be the most appropriate setting for the FlowCAM in that context. The autoimage mode for North Sea applications was best set at five images digitized per second.

Automatic determination of particle concentration has been validated by comparison with manual enumeration, and reproducibility was checked during an intercalibration experiment where results obtained with three different FlowCAM were also compared. No significative difference was observed between manual and automatic enumerations nor between predictions by the different FlowCAMs

2.1.2 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 basic mathematical tool is the three-dimensional MIRO&CO-3D model (Lacroix et al., 2007a) 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 and the related air-sea CO_2 exchanges as previously tested in a multi-box frame (0D-MIRO; Gypens et al., 2004, Lancelot et al., 2005). Several improvements were brought to the physical model and ecological module on basis of new ecological knowledge or application need.

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.5).

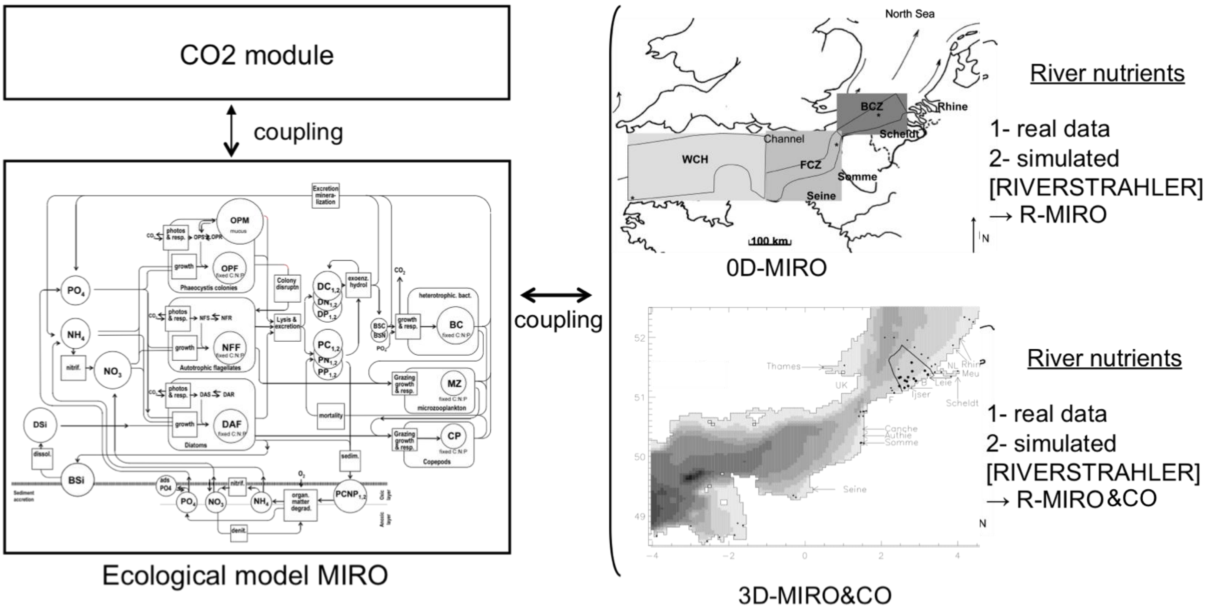

Figure 5: Schematic description of the mathematical tools.

Present-day assessment of bloom dynamics and delimitation of the eutrophied maritime zones in the BCZ involved the use of the 3D model. However, increasing the grid resolution of 3D-MIRO&CO 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 as requested by the OSPAR strategy to combat eutrophication (OSPAR, 2005).

Historical reconstruction and projections of the BCZ eutrophication status and the related atmospheric CO_2 absorption/release were first appraised in the 0D configuration prior to 3D as this involved the online coupling with river nutrient loads calculated by the RIVERSTRAHLER model (Billen et al. 2001; 2005; Thieu et al., 2010). 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.

2.2 Results

2.2.1 Real-time monitoring of phytoplankton with image analyzer

2.2.1.1 Set up of the FlowCAM-Zoo/PhytoImage tool

Training set- The training set elaboration is one of the most essential and critical steps for automatic recognition of plankton. For monitoring experiments, the training set should contain as many groups as possible in order to be representative of natural communities. The final training set is usually a compromise between taxonomical precision and global accuracy (Culverhouse et al. 1996, Tang et al. 1998, Davis et al. 2004, Grosjean et al. 2004, Bell & Hopcroft 2008, Gislason & Silva 2009, Irigoien et al. 2009). A training set dedicated to North Sea phytoplankton communities in the BCZ was developed using vignettes obtained from natural samples and manually classified by our taxonomist. This training set was used to train the computer using the 'random forest' algorithm (Breiman, 2001) in order to create a classifier that will be used to automatically discriminate particles.

From the 31 original groups, 4 classes containing less than 30 particles were eliminated. Yet *Chaetoceros danicus* and "bubble" are kept despite the low number of vignette because they are very easy to discriminate. The two groups of *Thalassiosira spp* on one hand and the *Guinardia striata* and *Eucampia zodiacus* groups on the other hand were pooled together because they were hardly discriminated with the automatic classifier. The final training set contains 25 groups: 19 phytoplankton taxa, 3 zooplankton and 3 other groups (Fig. 6).

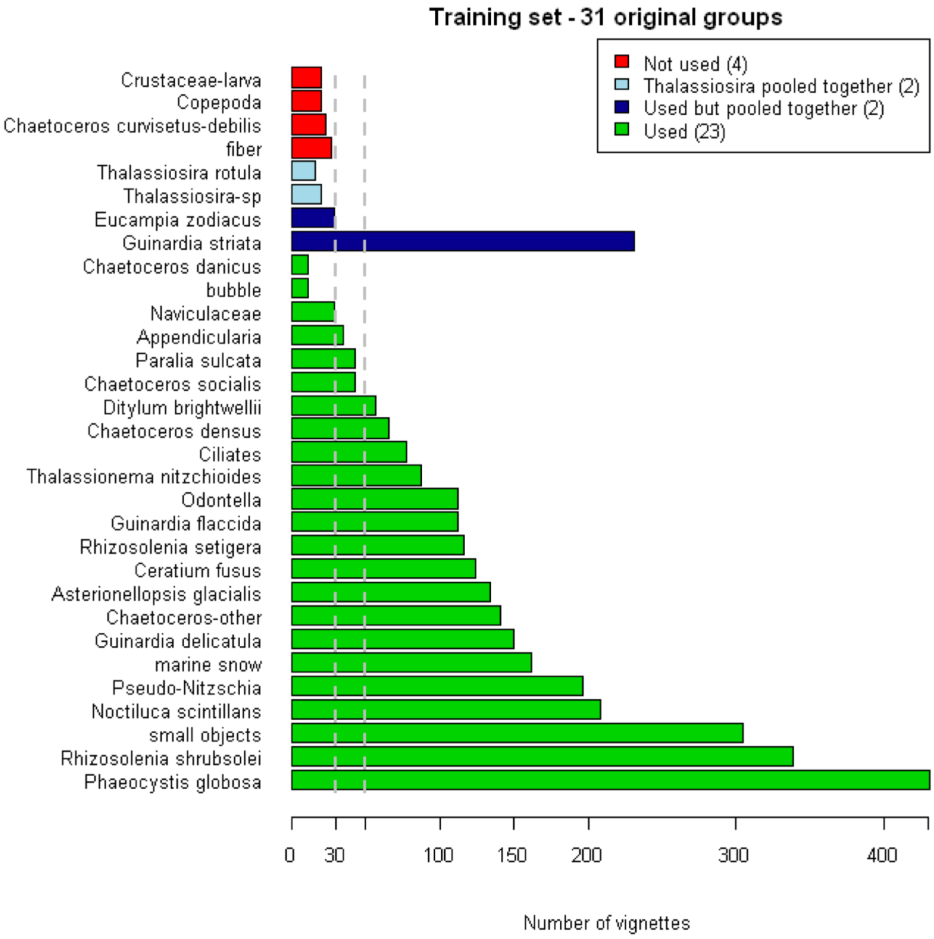

Figure 6: Groups in the training set and in the classifier used for real-time monitoring

Cell number per colony- For colonial phytoplankton (diatom chains, *Phaeocystis* colonies), the FlowCAM digitizes colony as an independent particle instead as an assemblage of single cells. The missing ecologically important information on the number of cells per colony is then counted manually on the vignettes by our taxonomist. This is relatively easy for diatoms like e.g. *Pseudo-nitzschia* spp while it is more tedious for large colonies of *Phaeocystis* (Rousseau et al. 1990).

From the different models tested to relate the manually count colony cells to the particle/colony equivalent spherical diameter (ESD) measured by the FlowCAM, best results were obtained with a simple least-square linear regression:

Pseudo-nitschia cells colony^{-1}= 0.023 * colony ESD + 0.91 ($P < 0.001$, $r^2 = 0.88$)

Phaeocystis cells colony^{-1}= 2.11 * colony ESD – 400.9 ($P < 0.001$, $r^2 = 0.87$)

These relationships estimate the number of cells per colony with an accuracy varying between 39% and 49%. The accuracy increases up to 95% if we accept an estimation of +/- one size class in logarithm. It means that an error of one cell is accepted for colonies containing between 2 and 5 cells; 2 cells for colonies containing between 6 and 11 cells; 3 cells for colonies containing between 12 and 22 cells; etc., and +/- 800 cells on colonies containing between 4800 and 5800 cells (*Phaeocystis*). Such an error is rather small in comparison with the size reached by *Phaeocytis* colonies. Incidentally the relation between the diameter of *Phaeocystis* colonies and the number of cells estimated by Rousseau et al. (1990), based on microscopic observations could not be applied here because the relationship was based on measurements of intact colonies and are not usable with FlowCAM images which often digitize parts the largest *Phaeocystis* colonies.

2.2.1.2 Field real-time monitoring of phytoplankton

Setting up- During eight cruises aboard Belgica from 2009 to 2010, the real-time system has been progressively set up and tested in different conditions from calm to rough sea. The final system can now be operated at full speed of the ship (12 knots) and supports rough seas (up to 9 Beaufort with waves of 4 m high). The FlowCAM presents also some limitations for its use in real-time during long transects.

The original pumping system and hydraulics ahead of the FlowCAM was adapted to be as less destructive as possible for the fragile phytoplankton colonies, while allowing a constant flow rate of seawater in the digitizing device, even when operated in rough seas. Long and thin phytoplankton colonies (e.g. *Asterionellospis glacialis, Chaetoceros* spp*, Guinardia* spp*, Pseudo-Nitzschia* spp*, Rhizosolenia* spp) are kept intact, as determined by comparison with the gentlest sampling technique using a bucket, but the system partly damages *Phaeocystis* colonies ≥ 2000 µm. Yet these fragile colonies are digitized with the FlowCAM and correctly classified by Zoo/PhytoImage, only the size of the colonies is altered. A quantitative estimation of this taxon in the field is thus possible, given some correcting factor for size.

Based on the established training set, two classifiers were implemented, one for the real-time analysis using the basic 17 features measured by the FlowCAM (with 79.5% of accuracy) and the second one for a more complete post-processing using all 48 features, as measured both by the FlowCAM and by Zoo/PhytoImage. The latter provides a slightly higher accuracy (83.5%), but more importantly, it raises the opportunity to better discriminate similar objects thanks to the additional measurements, and it also offers a validation mode where our taxonomist can further correct classification (semi-) manually.

Field surveys- The reliability of the new tool is shown by Fig.7 that plots the geographical distribution of phytoplankton particles in September 2010 and compares the estimated phytoplankton cell abundance estimated with the latest development of the FlowCAM and Zoo/PhytoImage tool with the fluorescence signal measured concomitantly.

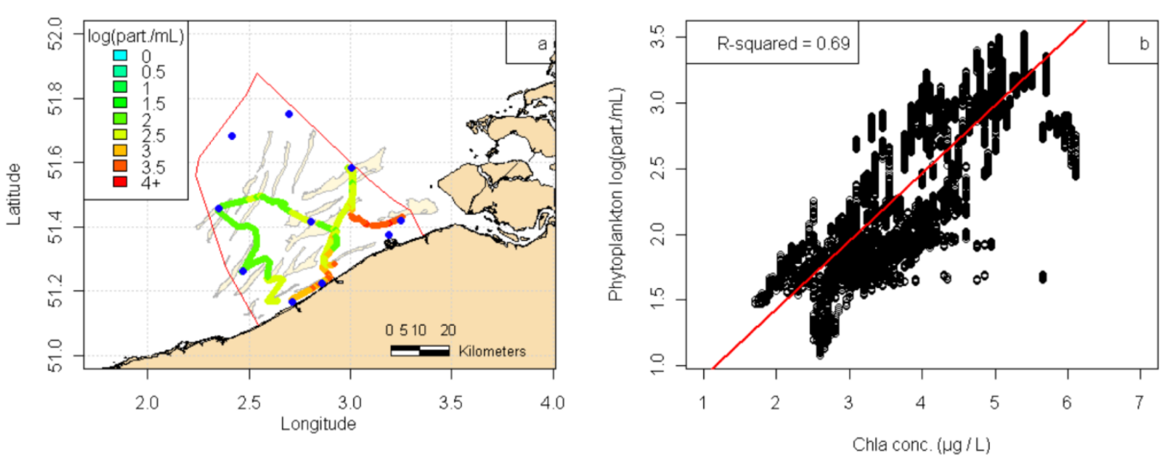

Figure 7: Distribution of total abundance of phytoplankton particles (in log scale) estimated by the FlowCAM and Zoo/PhytoImage during the Belgica cruise of September 2010 (a) and relationship between these measurements and Chl a concentration measured aboard the RV Belgica by natural fluorescence (b). The least-square regression line is in red. Blue dots show the position of fixed stations.

The highest phytoplankton abundances were, as expected, retrieved close to the coast, in an area influenced by the nutrient-enriched freshwater of the Scheldt (Fig.8a). Phytoplankton abundance is rather homogeneous in the rest of the BCZ (Fig.8a).

No *Phaeocystis* colonies were detected in September 2010 in agreement with previous records (Breton et al. 2006). Diatom colonies were identified along the transect but, quite interestingly, with a taxa-specific geographical distribution (Fig.8). *Chaetoceros* spp (Fig.8b) is the most abundant and its distribution matches rather well the distribution of total phytoplankton (Fig.8a). On the contrary *Rhizosolenia* spp (Fig.8c) and *Pseudo-Nitzschia* spp (Fig.8d) show highest abundance in areas outside the direct influence of the Scheldt plume although high abundance were retrieved in nearshore waters.

Altogether, these first results demonstrate the feasibility of the developed new instrument in describing phytoplankton diversity patchiness and pointing species dominance in relationship with river influence in the BCZ. This could help scientists to develop better sampling strategy when working at sea. More generally it provides, in complement with remote sensing, quasi-synoptic data for ecological model validation in areas where discrete sampling is seldom and insufficient to capture the highly-variable dynamics of populations.

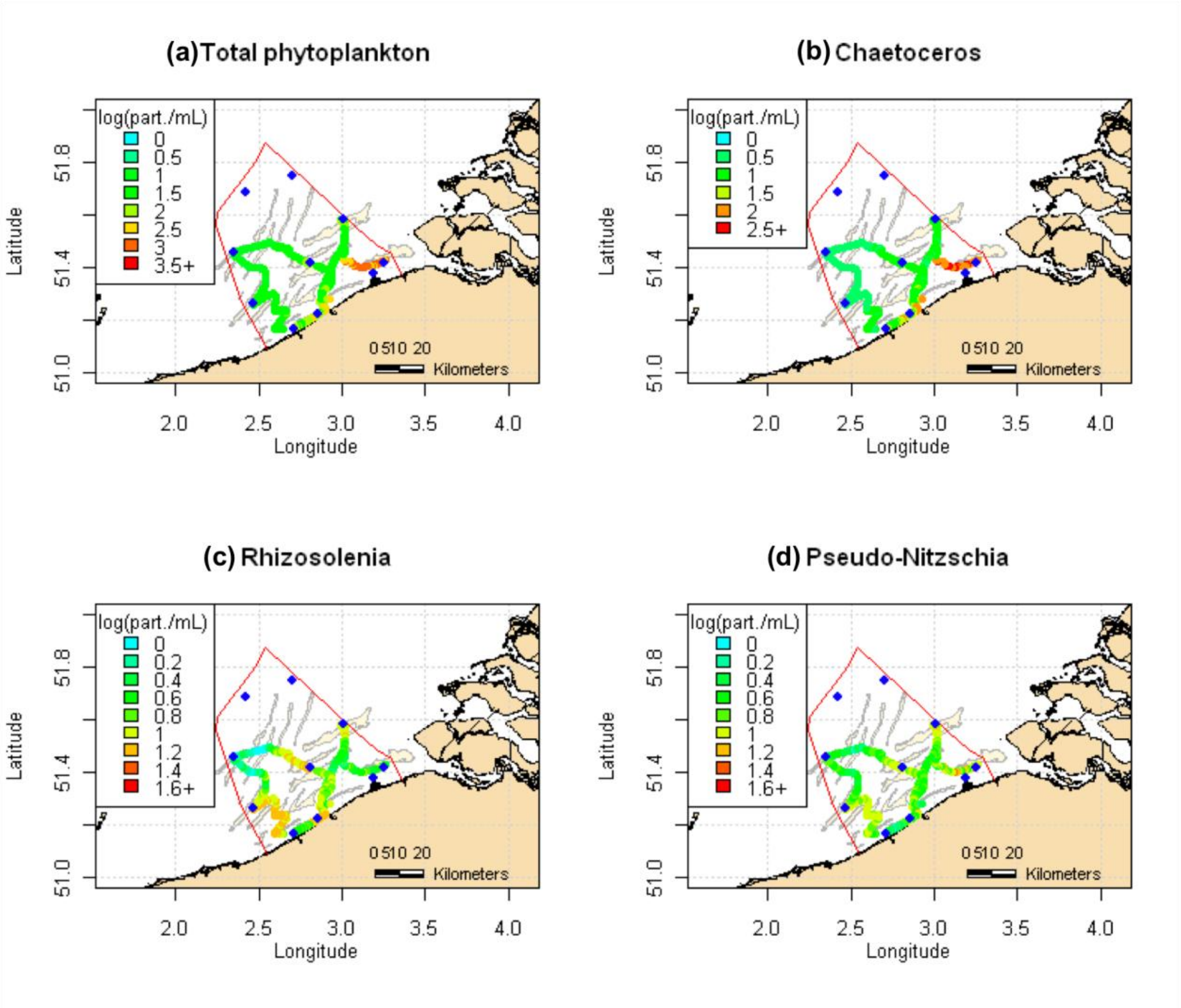

Figure 8: Abundances (in log scale) of total phytoplankton (a), Chaetoceros spp. (b), Rhizosolenia spp. (c) and Pseudo-nitzschia spp (d) recorded during the Belgica cruise in September 2010.

2.2.2 Phytoplankton eco-physiology

2.2.2.1 Phytoplankton community succession in the BCZ

Three communities were identified based on our statistical analysis of phytoplankton data: (i) the small bentho-pelagic diatom species *Paralia sulcata, Thalassionema nitzschioides, Asterionellopsis glacialis, Plagiogramma brockmannii, Thalassiosira levenderi, Skeletonema costatum,* and *Chaetoceros socialis, C. debilis* and *C. curvisetus* (called hereafter 'small bentho-pelagic diatoms'); (ii) the large-sized diatom *Guinardia delicatula, G. striata* and *Rhizosolenia shrubsolei* (called hereafter '*Guinardia-Rhizosolenia')* and (iii) *Phaeocystis* colonies which appears as a separate group.

As shown on Fig.9, these three phytoplankton communities are all occurring in the studied area with however a slight different timing in their seasonal pattern along the nearshore-offshore gradient. The general succession pattern previously evidenced at St 330, i.e. the diatom-*Phaeocystis* spring succession but also the 'small bentho-pelagic diatoms' – '*Guinardia-Rhizosolenia'* – ''small bentho-pelagic diatoms' (Rousseau et al., 2002, 2006) are clearly retrieved at salinity 32-34. Few but elevated densities of *Guinardia-Rhizosolenia* can however occur in early March in these intermediate waters.

In the other salinity classes, this sequence of diatom communities is much less apparent. For salinity 27-32, all three communities are blooming simultaneously except during summer when '*Guinardia-Rhizosolenia'* is the main blooming assemblage. For salinity >34, the diatom succession is still observed but the large '*Guinardia-Rhizosolenia*' community is blooming during nearly all along the vegetative season. Interestingly, *Phaeocystis* colonies occur as synchronous unique blooms in the whole area with the highest cell densities recorded in the intermediate salinity waters 32-34. Later in summer, the large *Guinardia-Rhizosolenia* cells constitute the bulk of phytoplankton.

As previously observed at St 330 (Rousseau et al., 2000) the bloom inception happens when the water column light climate PARwc reaches 8-12 µmol quanta m^{-2} s^{-1}. Incidently this PARwc threshold is reached offshore in early March but in early April nearshore, explaining the one-month delay between the near and off-shore bloom onset (Fig. 9).

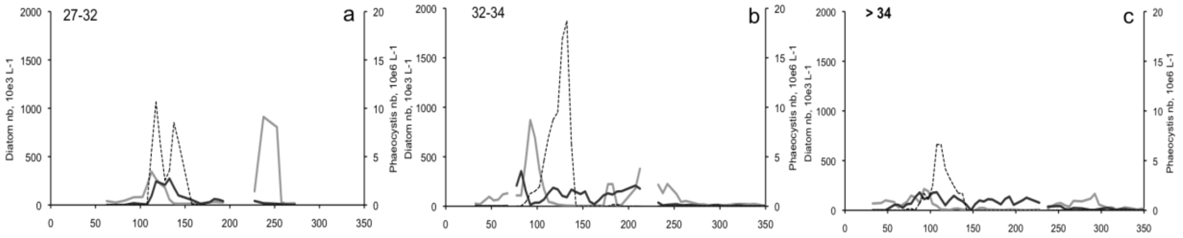

Figure 9: Spatio-temporal distribution of diatom and Phaeocystis communities in the BCZ and adjacent waters for the salinity classes 27-32 (a), 32-34 (b) and >34 (c). 'Small bentho-pelagic diatoms': light gray line; 'Guinardia- Rhizosolenia': dark gray line and Phaeocystis colonies: dotted black line. Data were averaged for 5-day period.

2.2.2.2 Mechanisms triggering *Phaeocystis* colony formation

Bioassays performed with natural populations of haploid flagellates suggest that *Phaeocystis* colony formation in our area relies on the haploid cell growth dynamics. Invariably, for all experimental sets (except those run in Summer), massive colony formation was observed after 3-weeks incubation of haploid cells provided sufficient light (>49 µmol quanta m^{-2} s^{-1}) and nutrients and vitamins (Fig. 10a) in agreement with the light threshold of 45 µmol m^{-2} s^{-1} determined by Peperzak (1993) in his nutrient-repleted cultures.

In addition, our experimental results suggest that a cell threshold must be reached to allow mating and syngamy (Fig. 10b). Indeed for this individual experiment, a threshold of 2000 haploid cells L^{-1} (forming 1000 colonies L^{-1}) is required to allow mating and syngamy. We conclude that increasing probability of meeting and mating are possible mechanism explaining the existence of such a cell threshold for syngamy.

Interestingly enough, the potential to form colonies exists in the BCZ and the marine Scheldt estuary (salinity > 18) but also all along the year (January-March, June, September-December) except during summer.

In addition to light intensity and nutrients, a possible role of *Chaetoceros* spp. in promoting *Phaeocystis* colony growth in the field cannot be excluded. In the early phase of the bloom, small *Phaeocystis* spherical colonies (10-80 µm in diameter) are indeed regularly found within *Chaetoceros curvisetus* and *C. debilis* setae. This coupling is supported by the positive relationship existing between the *Chaetoceros* cell density and the number of small colonies (< 80 µm in diameter; Fig. 11). Protection against grazing of early-formed colonies within *Chaetoceros* setae, requirement of a solid substrate for anchorage allowing meeting and mating are different hypotheses that could explain the presence of small colonies within *Chaetoceros* setae.

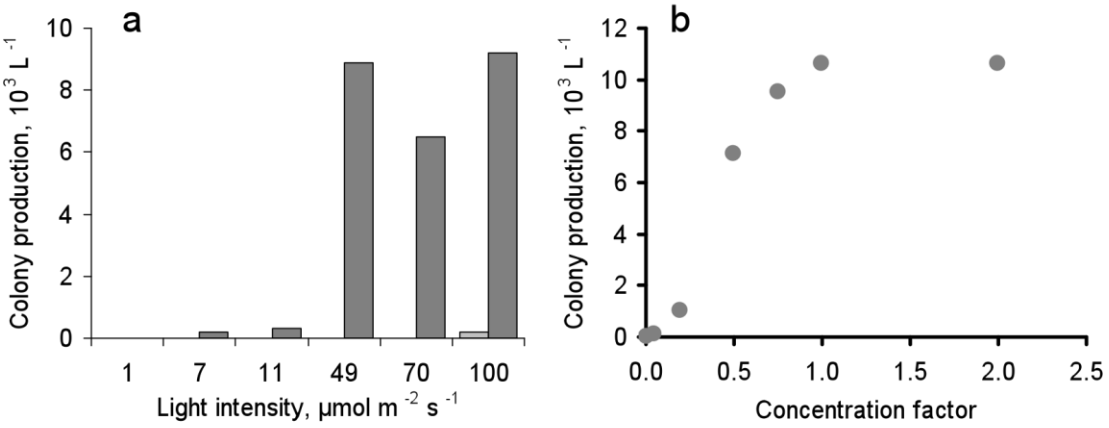

Figure 10: a) Phaeocystis colony abundance obtained from natural populations of haploid flagellates sampled during the pre-bloom period and incubated at different light intensities and enrichment conditions (light grey: no enrichment; dark grey: enrichment with nutrients and vitamins); b) Relationship between colony formation rate and the concentration of haploid cells during the dilution experiment.

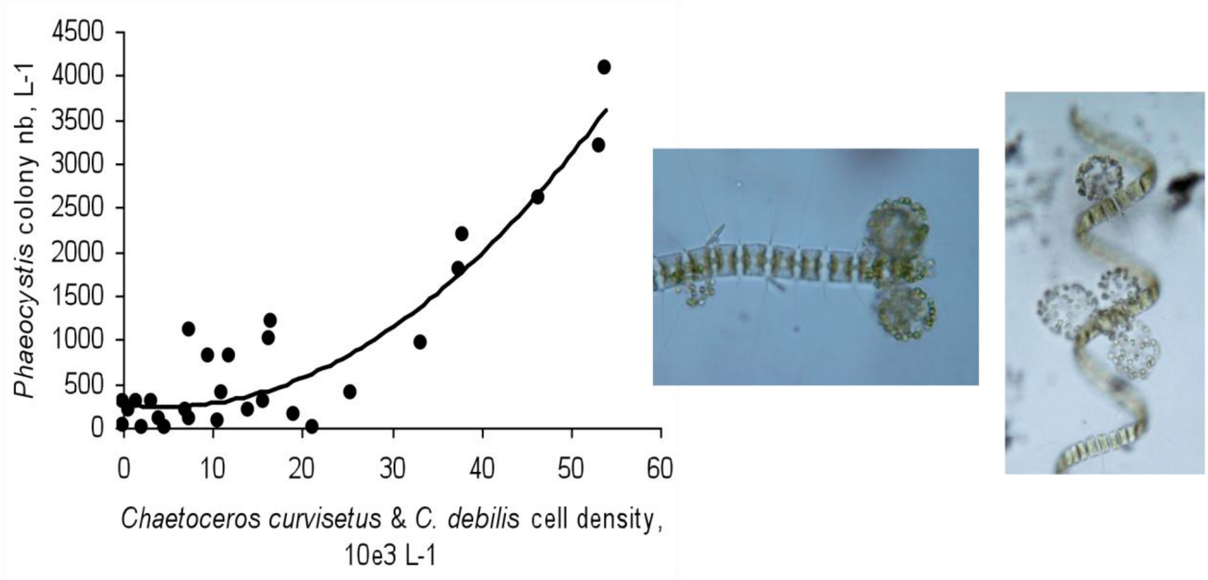

Figure 11 Relationship between the number of Phaeocystis spherical colonies <80 µm and the cell density of C. curvisetus and C. debilis present at the stage of colony formation at St 330 (1988-2000) in the BCZ. Microscopy photographs by Jean-Yves Parent.

2.2.2.3 Auto-ecology of diatoms and *Phaeocystis* communities

Temperature and light were the two investigated bottom up factors possibly acting on phytoplankton successions in the BCZ and that might be changed in response to expected climate change.

Fig.12 shows the temperature-growth regulation curves experimentally obtained for *Phaeocystis* colonies and for different species representative of the two main diatom communities blooming in the BCZ (Fig.9). Unexpectedly, despite a better competitive advantage of the ‘small bentho-pelagic diatoms’ below 8°C and above 17°C both the diatom groups are characterized by a marked optimum temperature for growth in the range 14-15°C (Fig. 12). Interestingly, *Phaeocystis* colonies are better adapted to lower temperature and have an optimum temperature for growth of 12°C, which corresponds to the seawater temperature during spring. Altogether, this suggests that temperature variability has little impact the diatom-*Phaeocystis* succession in these coastal waters.

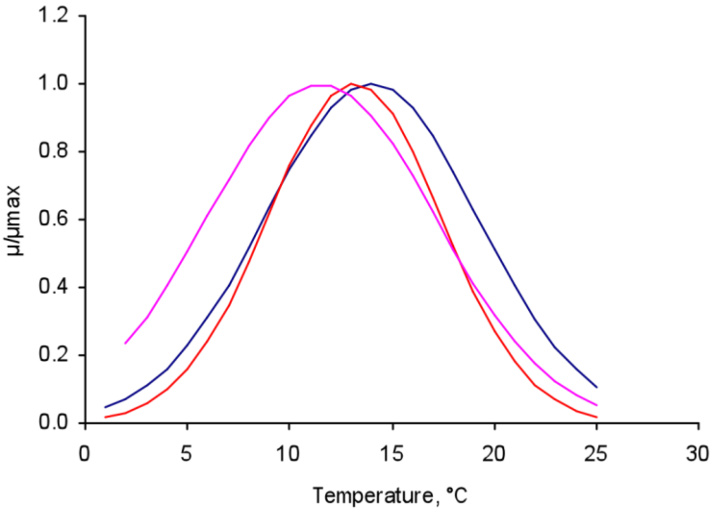

Figure 12: Growth (day^{-1}) – temperature dependance for ‘small bentho-pelagic diatoms’ (blue); ‘Guinardia-Rhizosolenia’ (red) and Phaeocystis colonies (pink). Experimental data are fitted with a Gaussian function.

Besides temperature, light adaptation of the diatom communities and *Phaeocystis* colonies has been investigated as a possible explanation of their spatio-temporal distribution. A better adaptation to low light could indeed explain the occurrence of the ‘small bentho-pelagic diatoms’ in early spring and fall when the available light in the water column PARwc is in average 12-18 µmol quanta m^{-2} s^{-1}. The predominance of the ‘*Guinardia-Rhizosolenia’* in late spring-summer and in clear offshore waters where PARwc is in average 15-40 µmol quanta m^{-2} s^{-1}, might be related to a better photosynthetic adaptation to high light.

Photosynthetic parameters measured during photosynthesis-irradiance experiments show a high variability of both photosynthetic efficiency (α^B) and capacity ($Pmax^B$) for the three communities (Fig. 13). Globally high α^B are obtained under low PARwc meaning that the species are adapted to their light environment (Fig. 13a). The lack of species-specific trend suggests that these coastal phytoplankton communities are well adapted to the highly fluctuating light environment that characterized these tidally-mixed shallow waters. Like specific growth, the photosynthetic capacity depends on temperature (Fig. 13b).

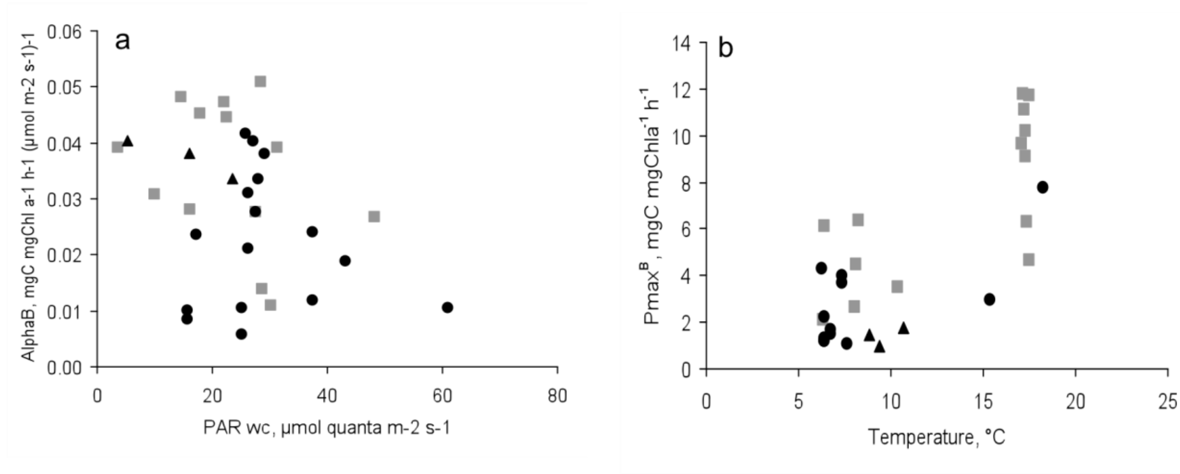

Figure 13: Photosynthetic parameters of the different phytoplankton communities: photosynthetic efficiency (a) and capacity (b). 'Small bentho-pelagic diatoms': Grey squares; 'Guinardia-Rhizosolenia'.: black dots and Phaeocystis colonies: black triangles.

2.2.3 Effect of *Phaeocystis* colonies on offshore mussel (*Mytilus edulis*) growth

Two questions were experimentally addressed: do mussels ingest *Phaeocystis* colonies and if yes how would this prey impact on offshore mussel growth. The first question was investigated by running laboratory-feeding experiments with various *Phaeocystis* colonies as diet while the second one was addressed by a field survey of mussel quality during and outside the *Phaeocystis* blooming period.

2.2.3.1 Mussel clearance rates on *Phaeocystis* colonies

Six experiments were run, each consisting of a set of 7-10 short-term bio-assays containing each a mussel of the length class 30-39 µm. Clearance rates were measured for different concentrations and sizes (from 25 to >1000 µm in diameter) of *Phaeocystis* colonies.
Results obtained with the largest colony size distribution show mussel ingestion on all colonies of size ≤ 1000 µm. When combining all experiments a positive relationship is obtained between mussel clearance rates and the total number of *Phaeocystis* colonies with no size distinction (Fig.14).

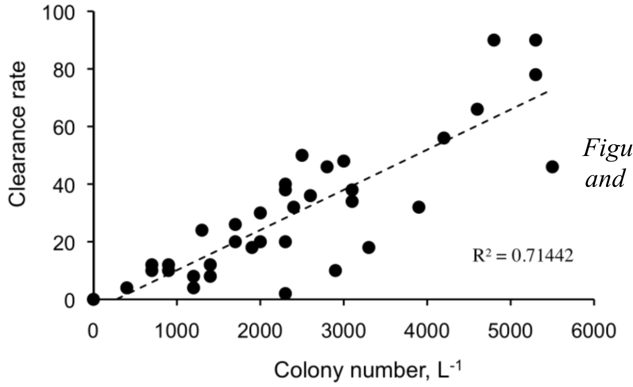

Figure 14: Relationship between mussel clearance rate and the total number of colonies

In agreement specific clearance rates calculated the different size classes of colonies tested (Fig.15) show comparable values for each colony size class i.e. 1.5 h^{-1} with some slightly higher values for colonies between 600 and 900 µm.

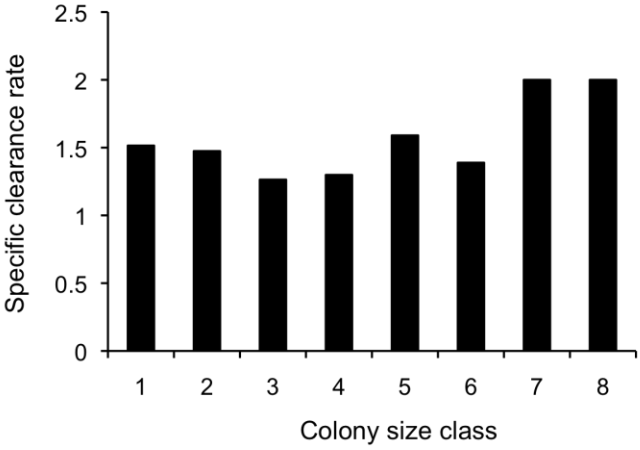

Figure 15: Specific mussel clearance rate (h^{-1}) for the different size classes. 1:<100; 2:100-199; 3:200-299; 4:300-399; 5:400-499; 6:500-599; 7:600-699; 8:700-799; 9:800-899.

2.2.3.2 Effect of *Phaeocystis* bloom on offshore mussel quality

Seawater and mussel samples were regularly collected in 2008 and 2009 in the mussel area D1 (Fig.3) for the measurement of *Phaeocystis* colony number and size 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. 16 compares the seasonal evolution of *Phaeocystis* colonies and of the biochemical composition of mussels recorded in 2008 and 2009. Clearly the spring *Phaeocystis* colony occurrence overlaps with the mussel-spawning period (April-July; grey area in Fig.16) that makes complex the interpretation of data as the effect of reproduction causing differences in energy allocation in mussels can mask a positive or negative impact of the *Phaeocystis* colony presence.

Phaeocystis colonies were quite more abundant in 2008 than in 2009 (Fig.16d) while their size distribution was quite similar, being composed in majority of <1000 µm colonies. Despite this variation in food availability, little difference is to be observed in mussel energy levels between 2008 and 2009, with however protein and to a less extent glycogen showing higher concentrations during the *Phaeocystis* bloom of 2008 (Fig. 16a-c).

Glycogen levels slowly build up during the *Phaeocystis* bloom while accumulating in late summer, i.e. after the spawning. This pattern can be explained by the fast use of stored glycogen for gametogenesis (Pieters et al., 1979). After the spawning period the compensation for protein and lipid storage is no more needed and glycogen can accumulate. The large decrease in glycogen level in autumn can be explained by the glycogen conversion into lipids and proteins during gametogenesis (Fearman et al., 2009; Pieters et al., 1979; Gabbot, 1976). In agreement the seasonal evolution of lipids mirrors that of glycogen (Fig16a,c).

The high variability observed in the mussel protein content (Fig.16b) might be attributed to the fact that blue mussels are sequential spawners (Pieters et al. 1980).

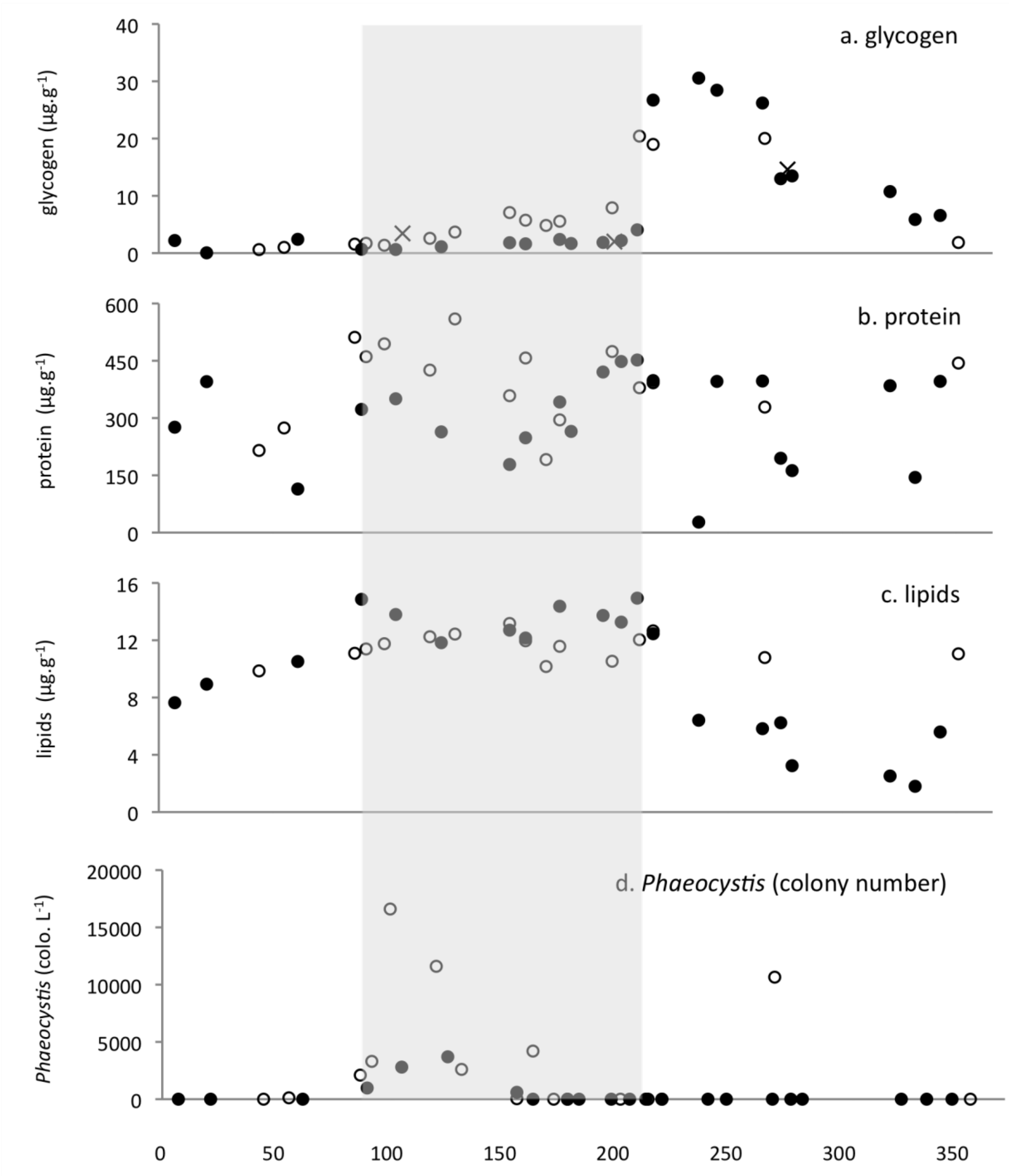

Figure 16: Seasonal evolution of mussel specific glycogen (a), protein (b) and lipid (c) content and of Phaeocystis colonies (d) in 2008 (○) and 2009 (•).

Altogether, our laboratory feeding experiments and field measurements suggest that <1000 µm *Phaeocystis* colonies prevailing in spring in the BCZ can be cleared by offshore mussels, with no size distinction. Field measurement of mussel energy levels during two contrasted years suggests little effect of the presence of *Phaeocystis* colonies. It might thus be concluded that mussel do not assimilate large *Phaeocystis* colonies but releases them in the ambient as pseudo-faeces. The latter were indeed observed during the feeding experiments.

2.2.4 Ecological modeling

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.

2.2.4.1 New model development

Increased grid resolution of MIRO&CO in the BCZ domain - In order to better appraise the role of direct nutrient loads in stimulating phytoplankton blooming 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 all MIRO state variables [daily] as boundary conditions to the smaller grid (Fig.17). The 'Belgian' rivers have been implemented based on the "Coastal Catchments Areas" used for BE reporting to OSPAR (Scheldt, Gent-Terneuzen Canal, eastern coastal area, middle coastal area and western coastal area).

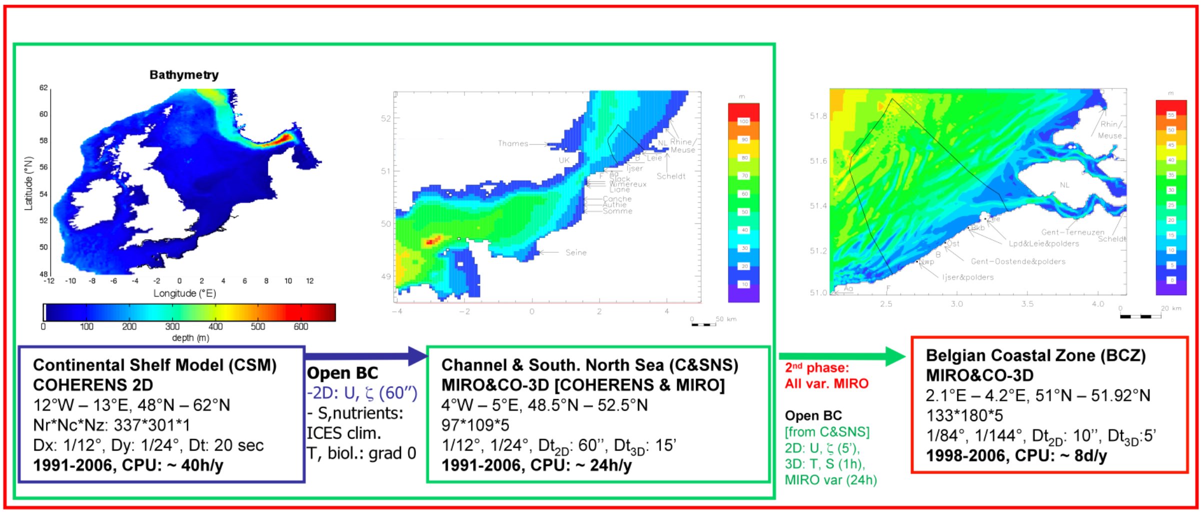

Figure 17: Belgian Coastal Zone (BCZ) implementation within the existing Continental Shelf Model (CSM) and Channel and Southern North Sea (C&SNS) grids.

The global performance of the model remains the same regarding nutrients and phytoplankton dynamics when using the high-resolution grid of the BCZ. Yet the latter shows more refined spatial distributions of phytoplankton than the normal resolution grid (Fig.18), which is then more appropriate for addressing nearshore problems.

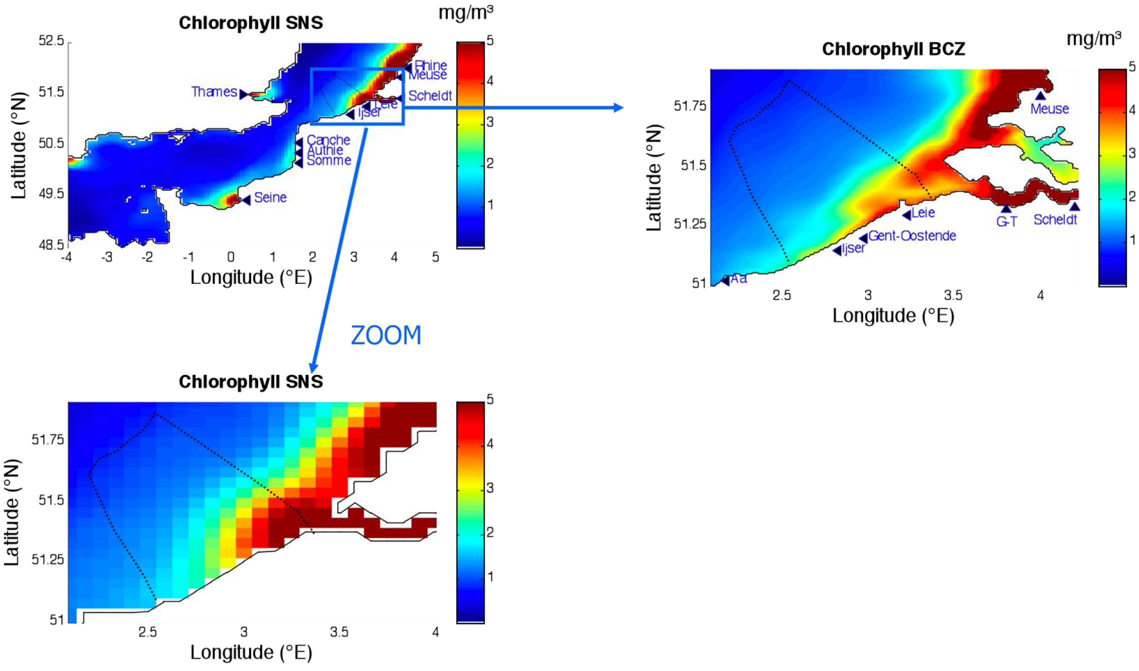

Figure 18: Comparison of MIRO&CO simulations of Chl a obtained with the high resolution grid (top right) and the usual grid (bottom left).

Improved parameterisation of light attenuation in the MIRO&CO-3D domain – Light availability in the water column is a necessary factor controlling phytoplankton development. Water column light attenuation in MIRO&CO-3D is computed with the kPARv1 module based on TSM (Total Suspended Matter), CDOM (Colored Dissolved Organic Matter), Chl a and depth. This model has been validated with PAR attenuation measurements available for the BCZ (2002-2005; $r^2 = 0.8191$, n=44). 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 is now obtained from cloud-filled remote sensing images (MODIS) provided by the STEREO BELCOLOUR2-RECOLOUR project. As shown by Fig. 19 that compares the computed light attenuation coefficient KPAR with observations recorded by the CEFAS buoys in UK waters, the use of daily satellite-derived TSM allows the capture of the observed high variability. In some cases, however, the highest field values are not well reproduced by the calculated KPAR. This may require more refinement in the treatment of MODIS satellite images that provide the TSM concentrations.

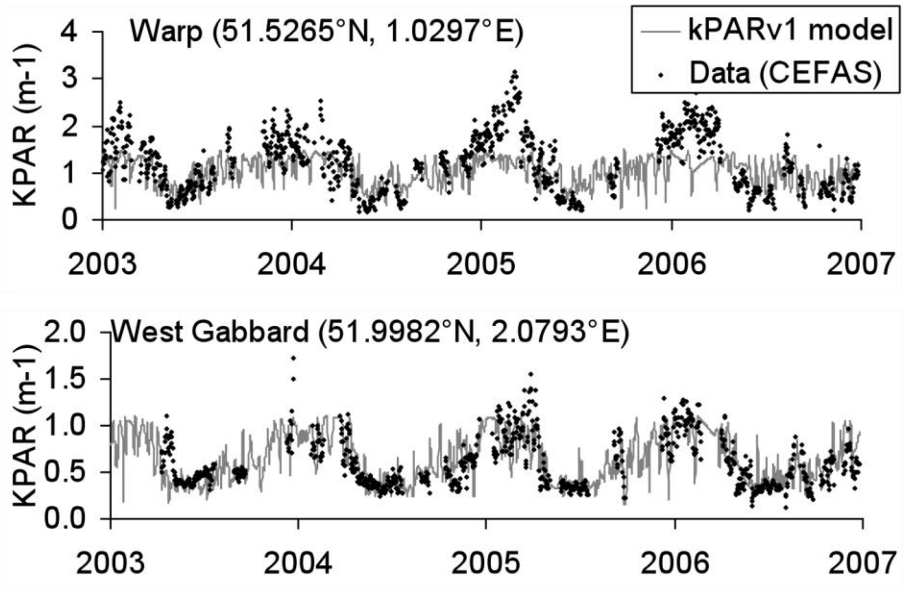

Figure 19: Seasonal evolution of observed and calculated KPAR (kPARv1 model). The latter is based on daily TSM, as provided by MODIS data after 2003 [from the BELCOLOUR-2/RECOLOUR projects]. In situ data provided by CEFAS (Mills et al., 2003).

Implementation and testing of a CO_2 module: MIRO-CO2&CO-3D - In order to assess the present-day capability of the eutrophied BCZ in absorbing atmospheric CO_2 (if, where and when) the previously developed CO_2 module (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), the air-sea CO_2 fluxes and other components of the carbonate system (pH, saturation state of calcite (Ωa) and of aragonite (Ωr)) 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), available from the Belgian marine data centre (http://www.mumm.ac.be/datacentre/).

Fig. 20 compares weekly averaged modelled and measured surface pCO_2 during different periods in 2003-2004, covering the seasonal cycle. Both observations and model results point large spatial and seasonal variations of surface pCO_2 in the whole domain, with higher under- and over-saturation of CO_2 with respect to atmospheric equilibrium (~377 ppm in 2004) in the vicinity of the river mouths. As a general pattern, the simulated spatio-temporal variability of surface pCO_2 is lower in the offshore waters of the Eastern Channel (ECH) than in the Southern Bight of the North Sea (SBNS) with values ranging between ~100 and ~700 ppm (Fig. 20).

In winter, measured and simulated pCO_2 (from ~350 to ~370 ppm) are close to atmospheric equilibrium in the whole domain except in the vicinity of the river mouths characterized by CO_2 over-saturation (~500 to ~670 ppm) (Fig. 20c). In spring (Fig. 20d,e), the spatial gradients and the temporal variability of surface pCO_2 are more intense than during the other seasons (Fig. 20a,b,c,f). High horizontal gradients are simulated in early spring when offshore waters are still close to the atmospheric CO_2 equilibrium, near-shore waters are significantly under-saturated (pCO_2 down to 100 ppm; Fig. 20d) and over-saturation prevails in the vicinity of rivers discharging in the SBNS (Scheldt, Rhine and Thames), in agreement with observations (Fig. 20d). Four weeks later (Fig. 20e), a significant under-saturation is simulated in the whole domain, due to spring primary production (not shown), except in the turbid Thames plume where CO_2 over-saturation prevails in the model and is higher than in the field measurements. During summer (Fig. 20a), measured pCO_2 values are above atmospheric equilibrium in the whole domain, with the highest values near-shore. Simulated pCO_2 fields show similar patterns but the model fails to reproduce the marked over-saturation measured in the near-shore waters of the Scheldt plume (Fig. 20a). In autumn, CO_2 over-saturation is simulated in the whole domain, with the highest over-saturation near the river mouths (mainly in the Rhine and Scheldt plumes) in agreement with observations (Fig. 20b,f).

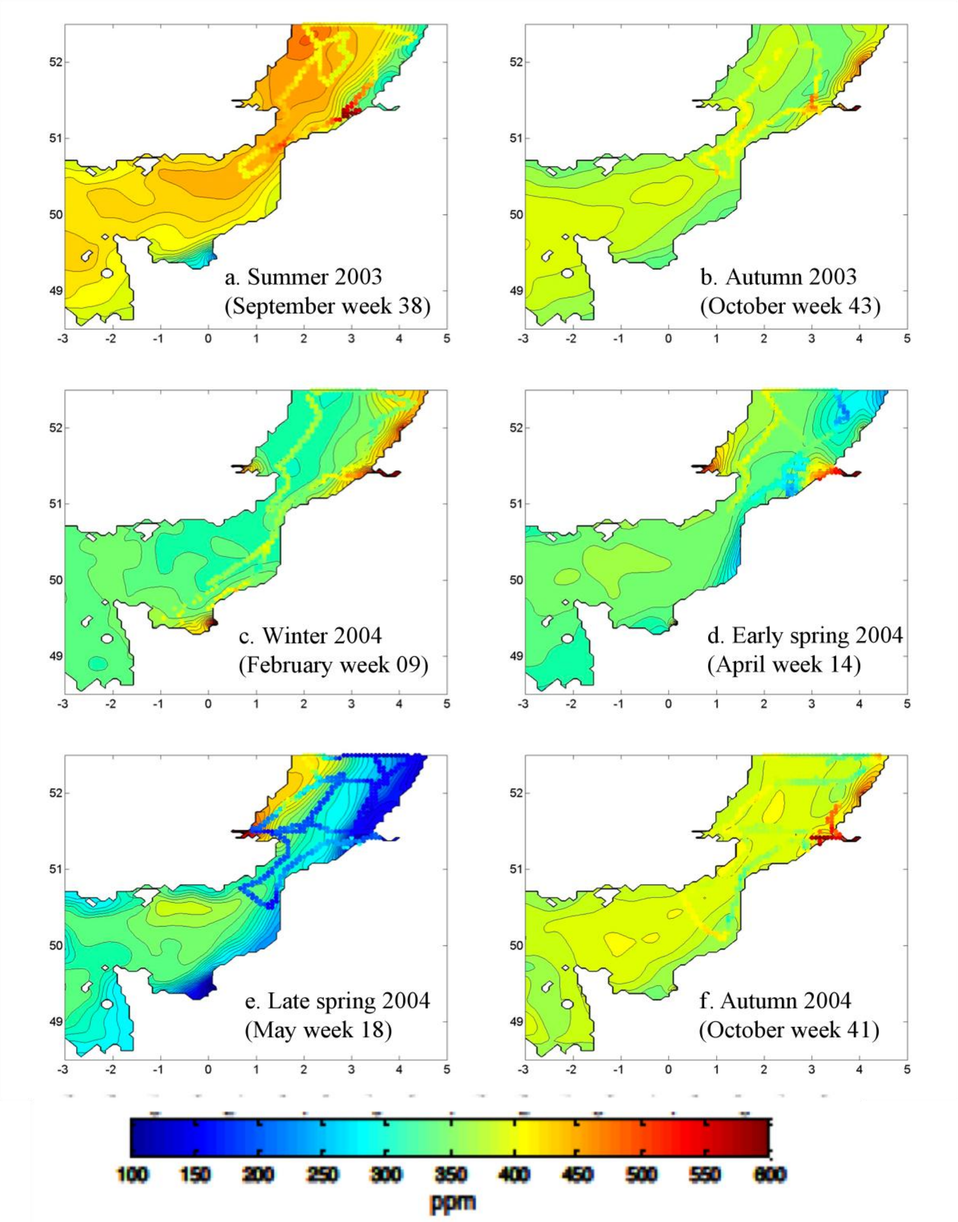

Figure 20: Spatial distribution of observed (dotted) and modeled surface pCO_2 (ppm) for different weeks of years 2003 and 2004 (Gypens et al., 2011).

Altogether the model describes satisfactorily the main patterns (timing, magnitude, amplitude, horizontal gradients) of the spatial and temporal variability of surface pCO_2 in the SBNS. The largest differences between observations and simulations are found in the near-shore waters of the Seine and the Scheldt estuaries in summer and in the vicinity of the Thames in late spring. Discrepancies between field measurements and model results could partly result from SST and/or the suspended particulate matter (SPM) forcing used in the model. These two quantities show important spatial and temporal variability and that might not be captured by the model that actually considers a weekly and a seasonal resolution for SST and SPM, respectively.

On an annual scale, simulations in 2004 show that estuarine plumes act as a source of CO_2 to the atmosphere, and coastal waters act as a sink for atmospheric CO_2 and offshore waters as neutral or moderate sinks of atmospheric CO_2 (Fig. 21a). The corresponding annual mean of pH is about 8.08 over the whole domain (ranging spatially between 7.89 and 8.18; Fig. 21b). The annual mean of Ωa in 2004 is 3.83 and 3.72 in the ECH and the SBNS, respectively (ranging spatially between 2.63 and 4.67; Fig. 21c), and the annual mean of Ωr is 2.46 and 2.41 in the ECH and the SBNS, respectively (ranging spatially between 1.63 and 2.96; Fig. 21d). The general spatial pattern of pH, Ωa and Ωr is characterized by higher values nearshore than offshore in particular in the plume of the Seine and the combined plume of the Scheldt and Rhine.

Over the 1994-2004 period, air-sea CO_2 fluxes show significant inter-annual variability, with oscillations between net annual CO_2 sinks and sources. The inter-annual variability of air-sea CO_2 fluxes simulated in the SBNS is controlled primarily by river loads and changes of biological activities (net autotrophy in spring and early summer, and net heterotrophy in winter and autumn), while in areas less influenced by river inputs such as the ECH, the inter-annual variations of air-sea CO_2 fluxes are mainly due to changes in sea surface temperature and in near-surface wind strength and direction. In the ECH, the decrease of pH, of Ωa and of Ωr follows the one expected from the increase of atmospheric CO_2 (ocean acidification), but the decrease of these quantities in the SBNS during the considered time period is faster than the one expected from ocean acidification alone. This seems to be related to a general pattern of decreasing nutrient river loads and net ecosystem production (NEP) in the SBNS.

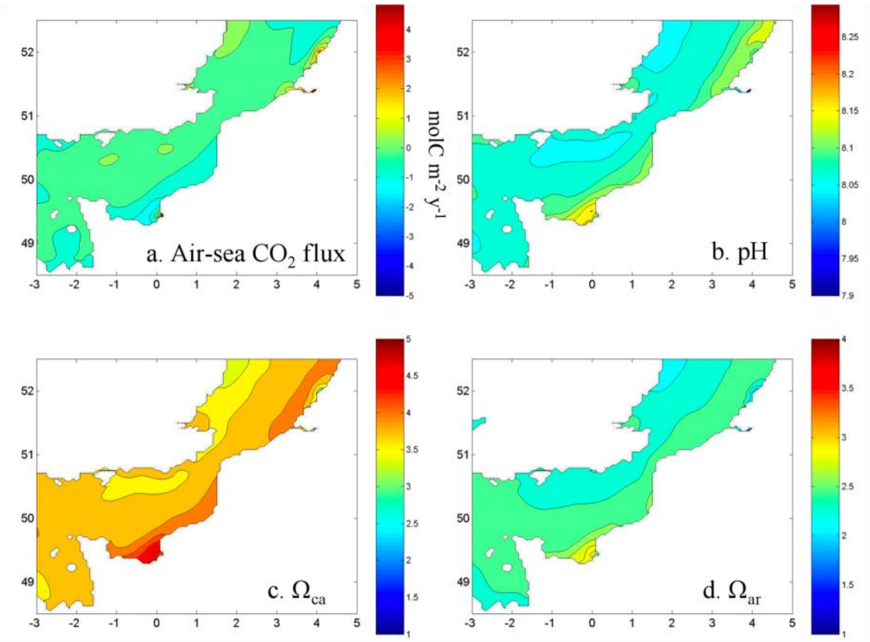

Figure 21: Spatial distribution of annual means of (a) air-sea CO_2 flux (mol C m^{-2} yr^{-1}) (b) pH, (c) saturation state of calcite (Ωa) and (d) saturation state of aragonite (Ωr) simulated for 2004, in the English Channel and Southern Bight of the North Sea.

Annually, the combined effect of carbon and nutrient loads leads to an increase of the sink of CO_2 in the ECH and the SBNS, but the impact of the river loads varies spatially and is stronger in river plumes and nearshore waters than in offshore waters. The impact of organic and inorganic carbon (C) inputs is mainly confined to the coast and generates a source of CO_2 to the atmosphere and low pH, of Ωa and of Ωr values in estuarine plumes, while the impact of nutrient loads, highest than the effect of C inputs in coastal nearshore waters, also propagates offshore and, by stimulating primary production, drives a sink of atmospheric CO_2 and higher values of pH, of Ωa and of Ωr.

2.2.4.2 Model applications

An ecological quality criterion for scaling *Phaeocystis* - 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 blooms 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; details in 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 abundance of *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 full implementation of the WFD on the river watersheds. *Phaeocystis* and diatom trends simulated in BCZ were analyzed 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 (N:P>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.

Nutrient reduction scenarios at the river outlet - Scenarios exploring the effect of selected riverine nutrient reductions imposed at their outlet have been first 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 analyzed. 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 (Fig.22) in which *Phaeocystis* can cause eutrophication problems (‘high biomass’) has been estimated making use of the new criterion for *Phaeocystis* disturbance established 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. 22). Interestingly enough a decrease of P alone increases the surface area affected by undesirable *Phaeocystis* colonies.

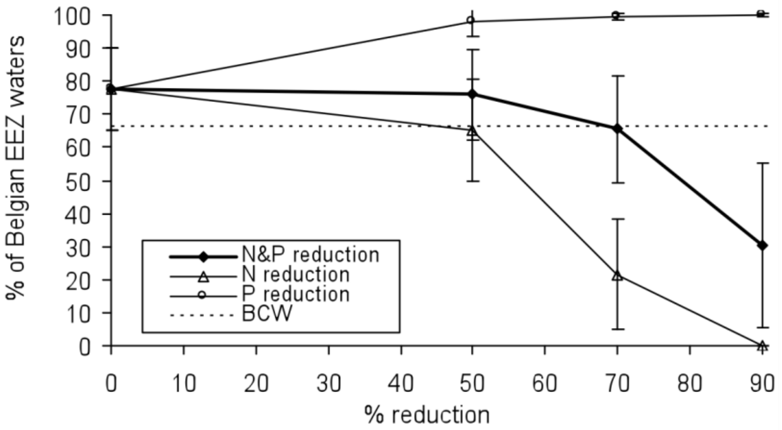

Figure 22: Percentage of the Belgian EEZ (i.e. BCZ) waters with high biomass of Phaeocystis colonies computed with MIRO&CO-3D for different scenarios of river nutrient input reduction.

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 criterion for *Phaeocystis* disturbance (Lancelot et al., 2009). Fig 23 shows the eutrophication status of BCZ in the nominal (STD) situation and for each nutrient reduction scenario.

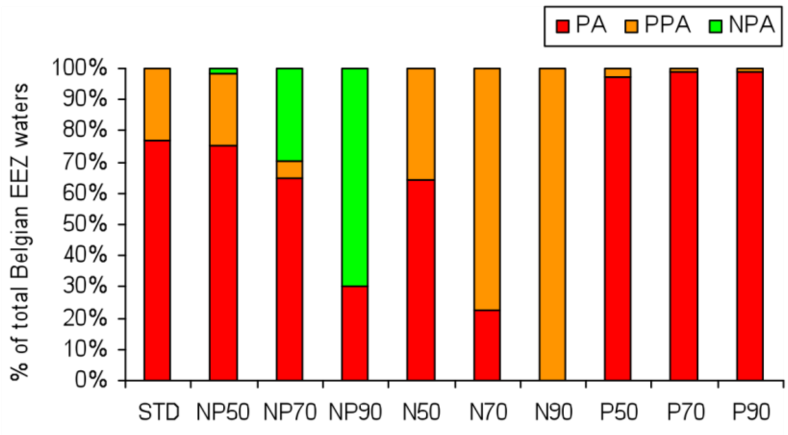

Figure 23: Relative coverage (%) of Belgian EEZ (i.e. BCZ) whose eutrophication status is Problem Area (PA), Potential Problem Area (PPA) or Non Problem Area (NPA). STD: standard run.

Transboundary nutrient fluxes - Nutrient transport across several transects in the Channel and the Southern North Sea and across BE boundaries with FR, UK & NL have been estimated for the period 1991-2004 making use of the MIRO&CO-3D model in which a module has been implemented for the computation of transboundary fluxes in the C&SNS grid (Fig. 24). Transboundary nutrient fluxes estimated for the years 1991-2004 point to an important variability between years.

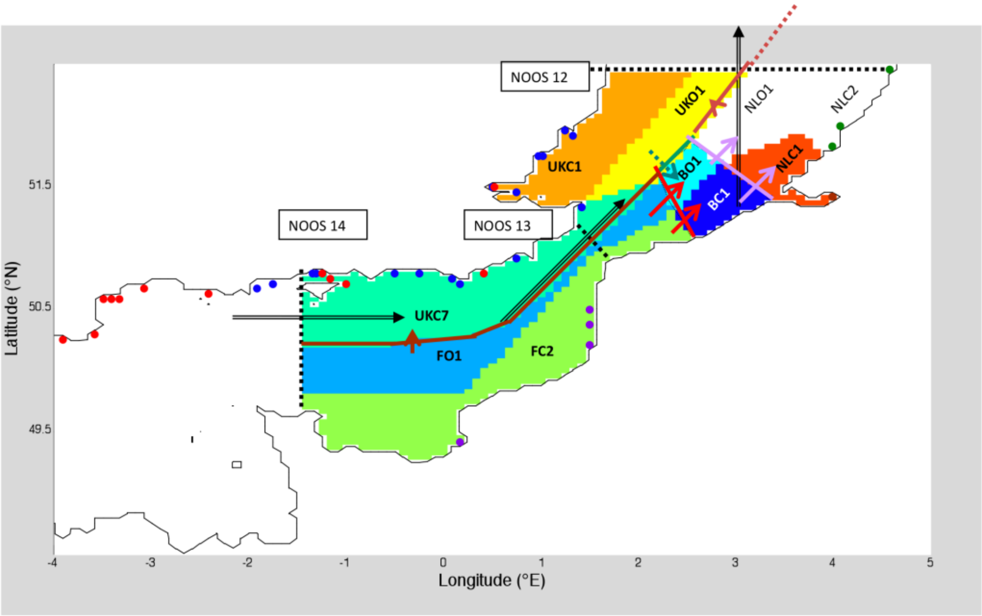

Figure 24: Position of NOOS (North West European Shelf Operational Oceanographic System) transects and Target Areas for the computation of transport nutrient fluxes. Arrows show the direction and intensity of residual annual mean water transport between target areas in the Channel and the Southern Bight of the North Sea.

Globally, transboundary nutrient fluxes estimated for 2002 (Table I) show that the water leaving the SNS to the north contains much more nitrogen (Ntot) than the water imported from the Channel indicating that the SNS is enriched in N from the rivers. The same is observed for phosphorus (Ptot), though in less proportions (Table 1). The resulting N:P ratio

of water masses leaving to the northern North Sea increases slightly (+14%) as a signature of the human activities in the surrounding countries.
The net fluxes of nutrients follow the net water circulation patterns (Fig.24). However, it should be pointed out that the tidal movement imposes at shorter time scales much more intense gross fluxes of water and nutrients through the BCZ.

Table I: Annual mean fluxes of water and nutrients through the NOOS transects in 2002. Direction of fluxes is from SW to NE. The 'relative increase' is the proportional enrichment at NOOS12 with respect to NOOS14 (attributed to river loads).

Fluxes of water and nutrients through three transects				
Transect name	**NOOS14**	**NOOS13**	**NOOS12**	**Rel. incr. (%)**
Volume (m3 s-1)	93219	94751	97919	5
Ntot (kTN y-1)	692	855	1218	76
Ptot (kTP y-1)	59	66	91	54
N:P ratio (molN molP-1)	26.0	28.7	29.6	14

The methodology used to determine the original sources of marine nutrients is the tracking experiment (Fig.25). It is a sensitivity analysis where a 1% decrease in N or P loads is imposed on a specific source (a river or a group of rivers). It is assumed that such a small change does not affect significantly any process while it is observable. The nutrients are thus affected by transport and by all the biogeochemical processes (primary production, sedimentation,...). The results are compared to a reference simulation, and the relative changes in marine concentrations are attributed to their respective sources. Fluxes of nutrients from specific sources through chosen transects can also be estimated. A spin up run of several years is sometimes necessary to trace back remote effects, as the nutrients from one source may take time to be eventually found on the other side of the model domain.

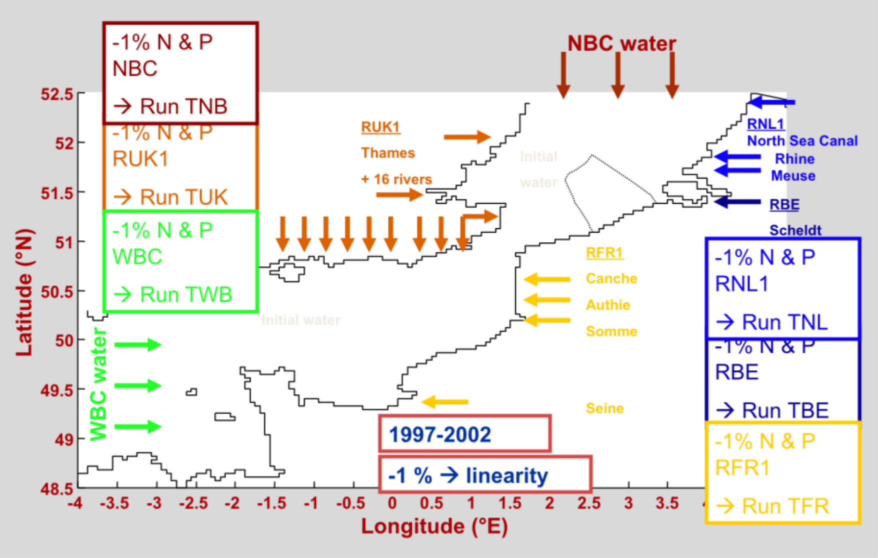

Figure 25: Representation of the different scenarios for the tracking experiment: each scenario corresponds to the reduction by 1% of the loads from a chosen group of rivers (indicated by the different colors: UK1, NL1, BE and FR1 rivers), or to the reduction by 1% of the concentrations brought at the boundaries (NBC and WBC).

The relative influence of the national rivers and boundaries in specific Target Areas delimited in Fig.24 show, for instance, that in the BC1 area (nearshore Belgian Coastal zone; Fig.24) the Channel waters are the most important source for both DIN and PO_4, on an annual average in 2002 (Fig.26). French rivers are the most important sources of DIN, followed by an equal contribution of the Belgian (Scheldt) and NL1 (Rhine/Meuse) rivers. Globally the rivers are in 2002 a much lesser PO_4 source than the English Channel waters, except for UK1 rivers in the UK coastal zone. Yet, the Dutch and French rivers are discharging in the BC1 a small but significant amount of PO_4. In the BO1 area (Belgian offshore zone; Fig.24), the Channel remains the most important contributor for both DIN and PO_4, with an important contribution from the French rivers regarding DIN (Fig.26). Such model results may orient discussion on how to reduce eutrophication in the BCZ, provided the same analysis is conducted at a seasonal scale instead of at the annual scale.

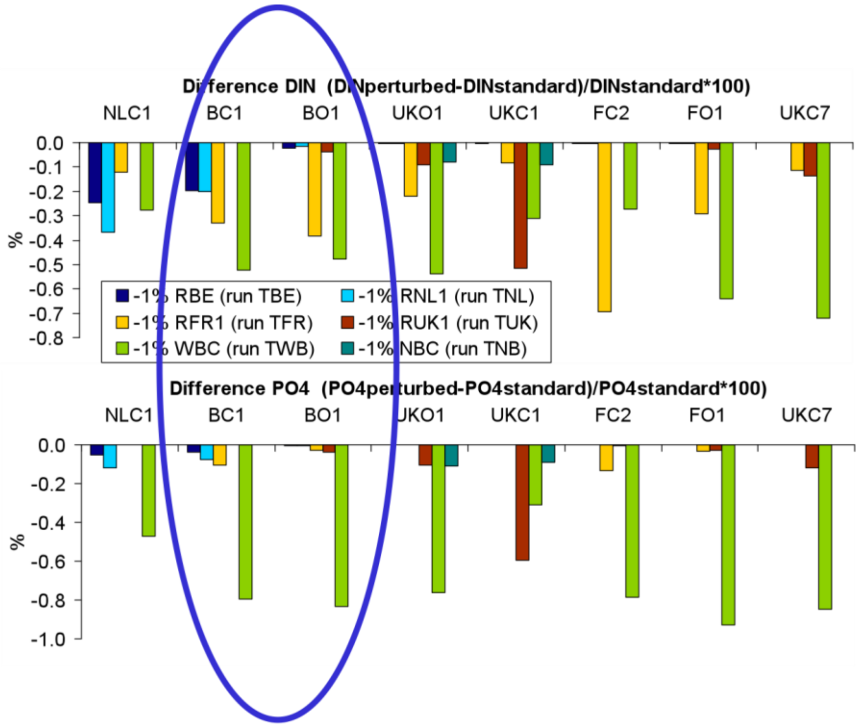

Figure 26: River and boundary influence regarding the DIN and PO_4 origin in specific Target Areas (the blue oval indicates the two Belgian Target Areas: the nearshore BC1 and offshore BO). Each coloured bar represents the influence of a group of rivers or the influence of one boundary (see Fig.24 for geographical limits of Target Areas)

Effect of realistic nutrient reduction measures on the eutrophication status of BCZ and adjacent waters at the 2015 horizon - Recommendation for reduction of nutrient river loads to the North Sea such as e.g. the 50% reduction of N and P loads with respect to the 1985 situation targeted by OSPAR (1987), are often taken without considering if the requested nutrient reductions necessarily to be obtained by upgrading wastewater treatment plants and/or changing agricultural practices is technically feasible and economically sustainable. As a first step to address the feasibility, the coupled river-ocean model SENEQUE/RIVERSTRAHLER-MIRO&CO (hereafter referred as SR-MIRO&CO) was implemented in the C&SNS marine domain (Fig.1). The SR-MIRO&CO model calculates on the one hand nutrient export at the river outlet as a function of nutrient inputs to the drainage network (Billen and Garnier, 1999; Garnier et al., 2002; Thieu et al., 2009) and on the other hand the subsequent nutrient enrichment and ecological response of the *Phaeocystis*-dominated coastal sea to the simulated river loads. This tool has been successfully applied in the BCZ for the reconstruction of diatom-*Phaeocystis* blooms over the 1950-1998 period, making use of 0D-MIRO (Lancelot et al., 2007; 2009). Here, as a further development, our goal is to test and estimate the effect of few realistic nutrient mitigation options taken in compliance with the WFD (upgrading of wastewater treatment plants WWT, use of different agricultural practices AGR and their combination MIX; see Thieu et al., 2010 for details) on the nutrient and eutrophication status of the BCZ and adjacent areas. All scenarios are run making use of year 2000 characterized by average meteorological conditions, as a reference. The upgrading of wastewater treatment plants WWT scenario corresponds to a reduction of 70 and 90% for N and P respectively for a treatment capacity >20,000 IE (inhabitant equivalent). The agricultural scenario AGR focuses on N reduction and responds somewhat to the Council Directive 91/676/EEC (nitrate directive). It combines integrated fertilization with the introduction of nutrient winter catch crops (WCC) assuming a homogeneous typology of the agricultural landscape, making use of agronomical census and results (model simulation and local experiments) available for N trapping efficiency of WCC (Thieu et al., 2010).

The efficiency of nutrient reduction scenarios implemented in the 3S watershed on the BCZ eutrophication status is appraised by analyzing the obtained changes in winter nutrients (dissolved inorganic N and P, respectively DIN and DIP; Fig.27) and *Phaeocystis* excess (Fig.28). The latter is defined by integrating *Phaeocystis* abundances > 6 10^6 cells L^{-1} i.e. the ecological quality criterion of Lancelot et al. (2009) + 50%.

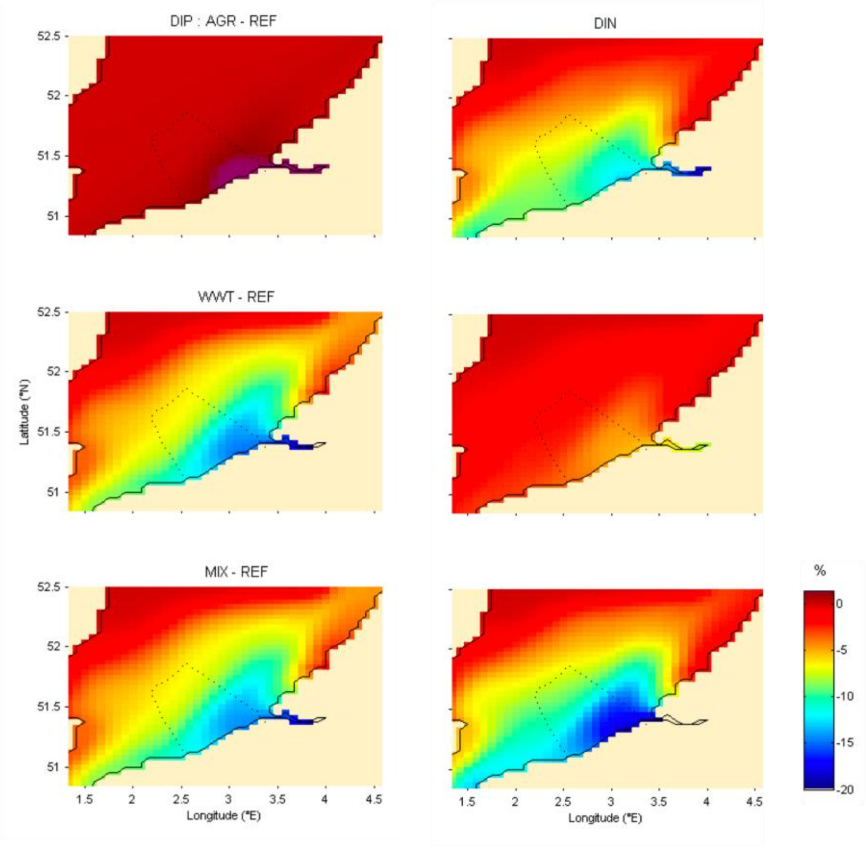

Figure 27: Relative differences in SR-MIRO&CO simulations of winter nutrient concentrations obtained for each reduction scenario (AGR (top), WWT(middle) and MIX(bottom)) and the reference scenario REF in 2000 (left: DIP and right: DIN)

Clearly a change in agricultural practices or in WWT efficiency at the level of the watersheds will have an influence on the winter enrichment of the BCZ (Fig. 27). With the exception of the AGR scenario where DIP concentrations slightly increase along the Belgian coast, all scenarios suggest a significant decrease in DIP and/or DIN concentrations, especially close to the coast. Interestingly enough the upgrading of the only WWT has a clear impact on the DIP enrichment in the BCZ while little reduction is to be observed for DIN (Fig.27). The reverse is true for the AGR scenario that predicts the only reduction of DIN. Therefore the best reduction of BCZ nutrient enrichment is obtained with the MIX scenario that suggests a maximum reduction of respectively 15% and 20% in DIP and DIN concentrations.

Fig. 28 suggests that the three tested nutrient reduction measures would have different measurable effects on *Phaeocystis* blooming in the area. There are two observable effects: the magnitude and extent of undesirable *Phaeocystis* area, and the importance of the '*Phaeocystis* natural' (undisturbed) area where *Phaeocystis* abundance is always below the criteron of $6\ 10^{6}$ cells L^{-1} (uncolored area in Fig.28). Globally the AGR scenario increases the surface of the *Phaeocystis* undisturbed area by comparison with the REF scenario but has no significant effect on the extent of undesirable *Phaeocystis* blooms in the BCZ (Fig.28). It also increases the surface of the undisturbed area by comparison to the REF scenario, which is an added positive consequence. Though the WWT scenario results in a very small decrease of *Phaeocystis* spring peak on average in the BCZ (not shown), it shows the highest excess of undesirable *Phaeocystis* in the vicinity of the Scheldt mouth due to the increased N:P ratio delivered by river loads. The MIX scenario shows a dual response: an increase in the undisturbed area similarly to that obtained by AGR, but a more intense undesirable *Phaeocystis* blooming in the nearsore zone, like obtained with the WWT scenario (Fig.28). The latter effect is due to a higher N:P ratio of loads simulated by the MIX compared to the AGR scenario, which favours *Phaeocystis* over diatom not only in the spring but also in the summer. This supports the conclusions from previous model applications pointing out the priority of decreasing N-loads before P-loads (Gypens et al. 2007, Lacroix et al. 2007a).

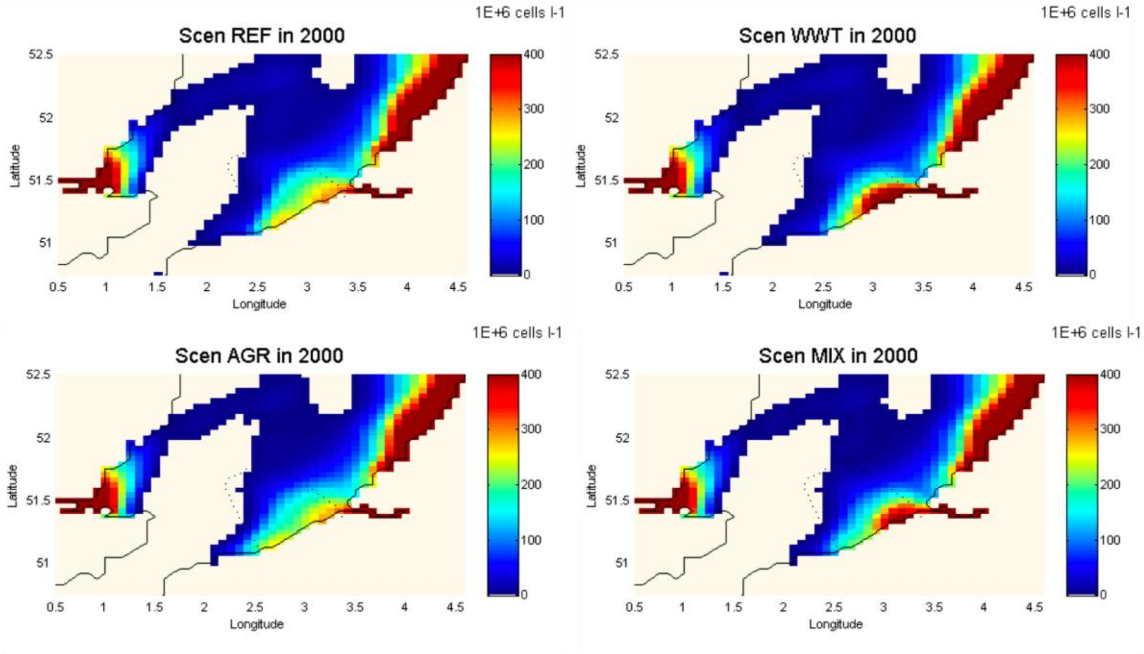

Figure 28: Annual maps of undesirable (colored) and desirable (uncolored area) Phaeocystis bloom for the reference scenario and the three nutrient reduction scenarios in 2000.

3. POLICY SUPPORT

AMORE research is delivering solid science support for sustainable development of the Belgian coastal zone facing excess nutrient (N and P) loads (eutrophication) and recent aquaculture development (mussel offshore farming). Of importance is the contribution to the formulation of quality descriptors based on improved knowledge of the ecosystem functioning and used for the assessment of problem areas in the BCZ and to the formulation and testing of nutrient reduction options.

3.1 Policy support activities

During the period covered by the report 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 meetings of the OSPAR Eutrophication Committee held in London in 2007 and 2009. 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 critical *Phaeocystis* abundance for healthy ecosystem has been estimated to $4\ 10^6$ cells L^{-1} +50%, a figure that can now be used to appraise the effect of nutrient reduction measures taken for decreasing *Phaeocystis* blooms.

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 as well as in the 3rd ICG-EMO Workshop "Transboundary nutrient transport”, organized by MUMM in Bruxelles, 7-9 September 2009. In this scope, the MIRO&CO-3D model was 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. For instance AMORE achievements contributed to the recent Eutrophication Quality Descriptor report throughout the participation of C Lancelot in the Task 5 expert group set up in 2009 by the European Commission to provide the guidance on eleven Good Environmental Status (GES) Descriptors that form the basis for evaluating ecosystem function.

AMORE scientists participated in the WG-MASC (MArine Shellfish Culture) and WKEUT (Workshop on time series data relevant to Eutrophication Ecological Quality Objectives) and HAB working groups of the International Council for Exploration of the Seas (ICES), including attendance at meetings held in 2008, 2006 and 2009 respectively. AMORE III provides the link between mussel culture and *Phaeocystis* algae relevant to WG-MASC.
Last but not least, the RIVERSTRAHLER/MIRO(&CO) tool developed in collaboration with the Université Pierre et Marie Curie (UPMC) allows to test and appraise the effects of short- and long-term management options at the basin scale. Such a science-based mathematical tool is being used in the scope of the EU FP7 AWARE project (ULB participant) to test a new decision support system that involves the participation of citizen, stakeholders, decision/policy makers and scientists. Ongoing scenarios already show that measures recommended by the WFD (upgrading of WWT and agricultures practices) will not be sufficient for restoring ecological quality of the BCZ because of the little effect on N loads. Alternate options to reduce N emission involving societal change (i.e. organic farming) and/or life style are being discussed with the citizen and the stakeholders and tested by the scientists.

3.2 Recommendations

Altogether both the AMORE observation and modeling activities are concluding that the eutrophication problem of the BCZ (and adjacent waters) visible as *Phaeocystis* blooms are due to an excess of nitrogen loads when compared to phosphorus. Our model scenarios clearly show that the ongoing implementation of WFD measures on WWT improvement and good agricultural practices will exacerbate the problem by reducing phosphorus with a little effect on nitrogen. Options for reducing nitrogen from diffuse source need to be taken for re-establishing the coastal ecosystem equilibrium. As these involve societal changes, these measures need to be explored with all stakeholders including citizen and scientists.

4. DISSEMINATION AND VALORISATION

4.1 Data base

Data obtained during the AMORE III project have been delivered to the IDOD data base of BMDC. These data are detailed in Table II and include measurements performed aboard the RV Belgica and related to the seasonal mussel and phytoplankton monitoring performed at Station D1 in the BCZ. The data are or will be (after embargo) available on the website http://www.mumm.ac.be/datacentre/.

Table II: AMORE III data set delivered to BMDC

Institution	Sample number	Data
ULB	204	- Core: *Phaeocystis* and diatoms cell densities; Taxonomic composition (62 diatom species) - Ancillary: Temperature, salinity, Chl *a* and major nutrients (NO_3, NH_4, PO_4, $Si(OH)_4$) concentrations, suspended matter content
UMons	153	Flow CAM collages of plankton images Automatic recognition of detected particles
ILVO	43	Temporal monitoring at station D1: *Phaeocystis* colony number and size

4.2. Zoo/PhytoImage software

The Zoo/PhytoImage software (1.0-1) has been upgraded based on AMORE III progress. The new version (1.2-2) allows now real-time monitoring of phytoplankton species, and integrates new statistical approaches including: the real-time recognition of particles detected by the FlowCAM ; the real-time plotting of abundance of the automatically recognized groups ; a method to automatically determine phytoplankton colony cell numbers and a method to classify vignettes (images containing only one particle of interest) according to the automatic identification.

This version has been uploaded to the official distribution web site (http://www.sciviews.org/zooimage/) after having been beta tested, i.e. a dynamic process usually done in Open Source software development. The latest version in development is available through SVN access at the zooimage forge site on R-Forge (https://r-forge.r-project.org/projects/zooimage/).

4.3 Participation to international conferences and workshops

Between 2007 and 2010, AMORE scientists actively participated to 18 international conferences and 12 workshops, disseminating AMORE results through oral (invited and key-note) presentations (OP) and posters (P). They took part in the following Open Sciences conferences: European Geosciences Union (EGU) in Vienna in 2007 (1 invited OP), 2008 (2 P) and 2009 (1 OP); American Society of Limnology and Oceanography ASLO in 2008 (Orlando, USA; 1 OP), 2009 (Nice, France; 3 OP); 2010 (Santa Fe, USA; 1 OP) and upcoming 2011 (San Juan, USA; 1 OP); American Geophysical Union AGU in 2010 Foz do Iguaçu, Brazil (1 invited OP).

The AMORE team contributes to international research programme (IGBP, GEOHAB) conferences among which the Joint IMBER/LOICZ Continental Margins conference in Shanghai in 2007 (1 OP, 2 P); the Third GLOBEC Open Science Meeting in Victoria, Canada in 2009 (1P), the Second Open Science GEOHAB Meeting on HABs and eutrophication in Beijing, China in 2009 (1OP) and the 14th International conference on Harmful Algae in Hersonissos-Crete, Greece in 2010 (1P).

AMORE results were also presented during topic conferences focusing on ecological modelling, management of coastal eutrophied ecosystems and statistical methods. Among them, the 6th European Conference on Ecological Modelling, ECEM07 in Trieste, Italy in 2007 (2 OP); Advances in Marine Ecosystem Modelling Research AMEMR in Plymouth, UK in 2008 (3OP, 1P), the 41st and 42nd International Liege Colloquium on Ocean Dynamics, Liège in 2009 (3 OP) and 2010 (1P) respectively; the EUTRO2010 International Symposium on Research and Management of Eutrophication in Coastal Ecosystems in Nyborg, Denmark in 2010 (1 key-note; 1 invited OP, 1 OP) and the R-User Conference in Rennes, France in 2009 (1OP).

Besides these international conferences, the AMORE consortium took part to specific workshops among which those of OSPAR ICG-EMO (2007 and 2009) and TBNT (2007), of Cost 735 in Vienna (2008), Liège (2010) and Istanbul (2010); on ‘Climate change and impact on eutrophication’ in London (2007); on ‘*Phaeocystis* blooms: causes and consequences: Recommended practices and future directions’ in Boulogne-sur-mer (2007); the 5th EuroGOOS Conference, Exeter, UK (2008); BeNCoRe conference State of the Art and future of Belgian Coastal Research, Leuven (2007); the 10th VLIZ Young Scientists’ Day, Ostende (2009).

As member of working group, AMORE scientists contributed to the Working Group Physical Biological Interactions (WGPBI – ICES) in Huelva (2009) and to the SCOR 130 meeting in Baton Rouge, USA (2009).

4.4 Demonstration

Some 11 scientists, technicians and students were trained during workshops and courses to properly use the FlowCAM and the software Zoo/PhytoImage. These demonstrations were organized by UMons at national and international (EU, USA) levels.

4.5 Added Value

Committees

Several AMORE scientists are involved in international committees and working groups such as IGBP (C. Lancelot), the OSPAR Eutrophication Committee EUC (K. Ruddick); the OSPAR Intersessional Correspondence Group on Eutrophication Modelling ICG-EMO (G. Lacroix) ; the ICES Working Group on modelling of Physical-Biological Interactions WGPBI (G.Lacroix) ; the ICES EuroGOOS Planning Group on the North Sea Pilot Project (G. Lacroix, K. Ruddick).

K. Ruddick is the coordinator of the HAB monitoring of the MARCOAST project (GMES). C. Lancelot is member of the Eutrophication expert group (MSFD Descriptor 5) for the implementation of the EU Marine Strategy. P. Grosjean is member of the SCOR working group 130 “Automatic Visual Plankton Identification”.

Participation in other federal and EU Projects

At the Belgian federal level, the scientific work developed in AMORE is used in the IAP IMOTHY (ULB) where the ecological MIRO model is the basis for developing an explicit

river-coastal sea model of the Scheldt estuary. The Ocean colour maps produced in the frame of the STEREO BELCOLOUR 2 (MUMM, ULB) project are used for validation of 3D MIRO&CO simulations.
At the european level, AMORE participates to the EU FP6 ECOOP (MUMM) where the MIRO&CO-3D model has been implemented within a nesting chain to produce 4-day forecast. ULB is principal investigator of the FP7 AWARE where the BCZ is a typical case study for improving the participatory role of citizen, scientists and stakeholders in decision with respect to nutrient emission reductions. MUMM participates to the FP7 MyOcean where MIRO&CO-3D simulations are used to test metrics for validation of biogeochemical products and CoBiOS where MIRO&CO-3D and satellite products will be integrated to produce operational information on high biomass blooms. UMons developed close collaborations with IFREMER for automatic identification of phytoplankton with the FlowCAM/Zoo/PhytoImage in the framework of the French phytoplankton monitoring program REPHY.

4.6 Press Communication/Education

Communication

AMORE scientists gave 6 interviews for radio broadcast amongst which "Semences de curieux" on the PREMIERE and 4 TV. Amore results were used in 2 articles in the newspaper Le Soir in the “Actu Sciences” and “Sciences & Santé”. A press lunch on coastal eutrophication has been organized in April 2010 at ULB by the communication interface.

Education

The AMORE team collaborated to educational projects such as the VLIZ e-learning education project “Planet Ocean” and to the ULB podcast on marine eutrophication (http://www.ulb.ac.be/actulb/podcast.php).

Public demonstrations of the FlowCAM and Zoo/PhytoImage system were made aboard the RV Belgica during the Navy Days in Zeebrugge (2009) and in UMH during the Matinée des Chercheurs (2009).

5. PUBLICATIONS

5.1 Peer-reviewed publications

5.1.1 Published

2007

Gypens N., Lacroix G. and Lancelot C. 2007. Causes of variability in diatom and *Phaeocystis* blooms in Belgian coastal waters between 1989 and 2003: a model study. Journal of Sea Research, 57(1): 19-35. Doi: 10.1016/j.seares.2006.07.004.

Lacroix G., Ruddick K., Park Y., Gypens N., Lancelot C. 2007. Validation of the 3D biogeochemical model MIRO&CO with field nutrient and phytoplankton data and MERIS-derived surface chlorophyll a images. Journal of Marine Systems, 64(1-4): 66-88. Doi: 10.1016/j.jmarsys.2006.01.010.

Lacroix G., Ruddick K., Gypens N., Lancelot C., 2007. Modelling the relative impact of rivers (Scheldt/Rhine/Seine) and Channel water on the nutrient and diatoms/*Phaeocystis* distributions in Belgian waters (Southern North Sea). Continental Shelf Research, 27(10-11): 1422-1446. Doi: 10.1016/j.csr.2007.01.013.

Lancelot C., Gypens N., Billen G., Garnier J. and Roubeix V. 2007. Testing an integrated river-ocean mathematical tool for linking marine eutrophication to land use: The *Phaeocysti*s-dominated Belgian coastal zone (Southern North Sea) over the past 50 years. J. Mar. Syst. 64(14): 216-228.

Rousseau V., Chrétiennot-Dinet M.-J., Jacobsen A., Verity P. and S. Whipple. 2007. The life cycle of Phaeocystis: state of knowledge and presumptive role in ecology. Biogeochemistry. 83:29-47.

Vasas V, Lancelot C, Rousseau V, Jordán F. 2007. Eutrophication and overfishing in temperate nearshore pelagic food webs: a network perspective. Mar Ecol Prog Ser. 336:1-14

M.A van Leeuwe, J. Stefels, S. Belviso, C. Lancelot, P.G. Verity and W.W.C. Gieskes (eds). *Phaeocystis,* major link in the biogeochemical cycling of climate-relevant elements. Eds. Springer (reprinted from Biogeochemistry, volume 83). 2007.

2008

Gypens, N., Lancelot, C., Soetaert, K., 2008. Simple parameterisations for describing N and P diagenetic processes: Application in the North Sea. Progress in Oceanography, 76:89-110.

Roubeix, V., Becquevort, S. and Lancelot C. 2008. Influence of bacteria and salinity on diatom biogenic silica dissolution in estuarine systems. Biogeochemistry: 47-62

Roubeix V., Rousseau V. and C. Lancelot. 2008. Diatom succession and silicon removal from freshwater in estuarine mixing zones: From experiment to modelling. Estuarine Coastal Shelf Science. 78:14-26

Roubeix V, Lancelot C. 2008. Effect of salinity on growth, cell size and silicification of an euryhaline freshwater diatom Cyclotella Meneghiniana Kutz. Transitional waters Bulletin 1, 31-38.

Ruddick, K., G. Lacroix, C. Lancelot, B. Nechad, Y. Park, S. Peters and B. Van Mol, 2008a. “Optical remote sensing of the North Sea” in “Remote Sensing of the European Seas”, edited by V. Barale and M. Gade, Springer Science, pp 79-90.

Ruddick, K., G. Lacroix, Y. Park, V. Rousseau, V. De Cauwer, and S. Sterckx. 2008b. Overview of Ocean Colour: theoretical background, sensors and applicability for the detection and monitoring of harmful algae blooms (capabilities and limitations), p. 331-383. Real-time coastal observing systems for marine ecosystem dynamics and harmful algal blooms. Oceanographic Methodology Series. UNESCO publishing.

2009

Astoreca, R., Rousseau, V., Ruddick, K., Knechciak, C., Van Mol, Barbara, Parent, J.-Y., Lancelot, C. 2009. Development and application of an algorithm for detecting *Phaeocyst*is globosa blooms in the Case 2 Southern North Sea waters. Journal of Plankton research, 31(3):287-300.

Conley, D.J, Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, Sybil P., Havens, K. E., Lancelot, C., and Gene E. Likens. 2009. Controlling eutrophication by reducing both nitrogen and phosphorus. Policy Forum, Science 323: 1014-1015.

Gypens, N., Borges, A.V., Lancelot, C. 2009. Effect of eutrophication on air-sea CO_2 fluxes in the coastal Southern North Sea: a model study of the past 50 year. Global Change Biology, 15:1040-1056.

Irigoien X., Fernandes J.A., Grosjean Ph. Denis K. Albaina A. & Santos M., 2009. Spring zooplankton distribution in the Bay of Biscay from 1998 to 2006 in relation with anchovy recruitment. J. Plankton Res., 31(1):1:17.

Lancelot C., Rousseau, V. and N. Gypens. 2009. Ecologically-based reference for *Phaeocystis* colonies in eutrophied Belgian coastal waters (Southern North Sea) based on field observations and ecological modeling. J Sea Res., 61 :43-49.

2010

Borges A.V. and N. Gypens (2010) Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification, Limnology and Oceanography, 55, 346-353

Lenhart H-J., Mills D.K., Baretta-Bekker H., van Leeuwen S.M., van der Molen J., Baretta J.W., Blaas M., Desmit X., Kühn W., Lacroix G., Los H.J., Ménesguen A., Neves R., Proctor R., Ruardij P., Skogen M.D., Vanhoutte-Brunier A., Villars M.T., Wakelin S.L., 2010. Predicting the consequences of nutrient reduction on the eutrophication status of the North Sea. Journal of Marine Systems, 81: 148-170

Struyf E., A. Smis, S. Van Damme, J. Garnier, G. Govers, B. Van Wesemael, D.J. Conley, O. Batelaan, E. Frot, W. Clymans, F. Vandevenne, C. Lancelot, P. Goos and P. Meire, Historical land use change has lowered terrestrial silica mobilization, Nature Communications, DOI:10.103/ncomms1128.

2011

Arndt S., Lacroix G., Gypens N., Regnier P., Lancelot C., 2011 Nutrient dynamics and phytoplankton development along an estuary-coastal zone continuum: A model study. Journal of Marine Systems, 84: 49-66.

Gypens N., Lacroix G., Lancelot C., Borges A. 2011. Seasonal and inter-annual variability of air-sea CO_2 fluxes and seawater carbonate chemistry in the Southern North Sea, Progress in Oceanography (2010),doi: 10.1016/j.pocean.2010.11.004

5.1.2 In revision

Lancelot, C.,V. Thieu, A. Polard, J. Garnier, G. Billen, W. Hecq, N. Gypens. Cost assessment and ecological effectiveness of nutrient reduction options for mitigating Phaeocystis colony blooms in the Southern North Sea: an integrated modeling approach. STOTEN: Accepted with minor revision.

Lacroix G, Sirjacobs D., Ruddick K., Park Y., Beckers J-M., Lancelot C. Spatial variability of the spring bloom timing in the Southern North Sea investigated by MIRO&CO-3D and remote sensing. (in revision).

5.2 Assesment report and book chapters

Ferreira J G., Andersen J H., Borja A, Bricker S B., Camp J, Cardoso da Silva M, Garcés E, Heiskanen A-S, Humborg C, Ignatiades L, Lancelot C, Menesguen A, Tett P, Hoepffner N, Claussen U. 2010. Marine Strategy Framework Directive. Task Group 5 Report. Eutrophication. JRC Scientific and Technical Reports EUR 24338 EN-2010.

Voß, M, Baker A, Bange H A, Conley D, Cornell S, Deutsch B, Engel A, Ganeshram R, Garnier J, Heiskanen A-S, Jickells T, Lancelot C, McQuatters-Gollop A, Middelburg J, Schiedek D, Slomp C. 2011. Nitrogen turnover processes and effects in coastal and marine ecosystems. In: The European Nitrogen Assessment Report. In press

Billen G, Silvestre M, Grizzetti B, Leip A, Garnier J, Voss M, Howarth R, Bouraoui F, Darracq A, Behrendt H, Lepisto A, Kortelainen P, Johnes P, Curtis C, Humborg C, Smedberg E, Kaste O, Ganeshram R, Beusen A, Lancelot C. Nitrogen flows from European regional watersheds to coastal marine waters. In: The European Nitrogen Assessment Report. In press

Lancelot C, Muylaert K. 2011. Trends in estuarine phytoplankton ecology. In Estuaries Treatise Chapter 7.3. In press.

5.3 Conference Proceedings

Lacroix G., Park Y., Ruddick K. and Lancelot C., 2007. Spatial and interannual variability of the spring phytoplankton bloom in the North Sea investigated by modelling and remote sensing. Proceedings of the 6th European Conference on Ecological Modelling (ECEM'07), Trieste, 27-30 November 2007.

Sirjacobs D., Alvera-Azcarate A., Barth A., Lacroix G., Nechad B., Park Y., Ruddick K., Beckers J-M., 2008. Reconstruction of missing satellite total suspended matter data over the Southern North Sea and English Channel using Empirical Orthogonal Function decomposition of satellite imagery and hydrodynamical modelling. Proceedings of the Ocean Optics 2008 Conference, Barga, Italy, 6-10 October 2008.

Borges A., Ruddick K., Lacroix G., Nechad B., Asteroca R., Rousseau V., Harlay J., 2010. Estimating pCO2 from remote sensing in the Belgian coastal zone. Proceedings of the 2010 ESA Living Planet Symposium, Bergen, 29/06-01/07/2010.

Terseleer, N, Gypens, N and Lancelot, C. Evaluating the river de-eutrophication gain on the magnitude of *Phaeocystis* blooms in the Southern North Sea between 1985 and 2005: a model study. 14th International conference on Harmful Algae in Hersonissos-Crete, Greece in 2010. Submitted

5.4 Master Thesis

Xavier Tezzo. Master Bioingénieur en Agronomie 2008. Identification automatisée du phytoplancton de la Baie Sud de la mer du Nord par imagerie numerique en cytometrie de flux (FlowCAM®) associée a des techniques de classification supervisee. Université Libre de Bruxelles.

Ines Cabrita Andrade dos Santos. Master Bioingénieur en Sciences et Technologies de l'environnement 2010. Elaboration et validation d'un algorithme spécifique de la production

primaire dans les eaux côtières eutrophisées de la Baie sud de la mer du Nord. Université Libre de Bruxelles.

Sébastien Milleville. Master Bioingénieur en Sciences et Technologies de l'environnement 2010. Modélisation des emissions de sulfure de diméthyle (DMS) associées aux efflorescences de *Phaeocystis globosa* en Baie Sud de la mer du Nord. Université Libre de Bruxelles.

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7. REFERENCES

Bell J.L. and R.R. Hopcroft. 2008. Assessment of ZooImage as a tool for the classification of zooplankton. J. Plankt. Res. 30 (12): 1351-1367

Benfield M.C., Grosjean P., Culverhouse P.F., Irigoien X., Sieracki M.E., Lopez-Urrutia A., Dam H.G., Hu Q., Davis C.S., Hansen A., Pilskaln C.H., Riseman E.M., Schultz H., Utgoff P.E. and G. Gorsky. 2007. RAPID Research on Automated Plankton Identification. Oceanography 20(2): 172-187

Billen, G. and J. Garnier. 1999. Nitrogen transfer through the Seine drainage network: a budget based on the application of the RIVERSTRAHLER Model. Hydrobiologia 410: 139-150.

Billen G., Garnier J., Ficht A. and C. Cun. 2001. Modeling the response of water quality in the Seine river estuary to human activity in its watershed over the last 50 years. Estuaries 24(6B): 977-993.

Billen G., Garnier J. and V. Rousseau. 2005. Nutrient fluxes and water quality in the drainage network of the Scheldt basin over the last 50 years. Hydrobiologia 540 (1-3): 47-67.

Breiman L. 2001. Random forests. Machine Learning, 45:5-32.

Breton E., Rousseau V., Parent J.Y., Ozer J. and C. Lancelot. 2006. Hydroclimatic modulation of diatom/phaeocystis blooms in nutrient-enriched Belgian coastal waters (North Sea). Limnol. Oceanogr. 51(3): 1-14.

Costanza R. et al., 1997. The value of the world's ecosystem services and natural capital. Nature 387:253-260.

Crossland C. J., Kremer H.H., Le Tissier M.D.A., Lindeboom H.J., Marshall-Crossland J.I. and N. N. Rabalais. 2005. Coastal Fluxes in the Anthropocene, Springer-Verlag.

Culverhouse P.F., Simpson R.G., Ellis R., Lindley J.A., Williams R., Parisini T., Reguera B., Bravo I., Zoppoli R., Earnshaw G., McCall H. and G. Smith. 1996. Automatic classification of field-collected dinoflagellates by artificial neural network. Mar. Ecol. Prog. Ser., 139:281-287.

Davis C., Hu Q., Gallager S., Tang X. and C. Ashjian. 2004. Real-time observation of taxa-specific plankton distributions: an optical sampling method. Mar. Ecol. Prog. Ser., 284:77-96.

De Coen W.M., Janssen C.R. and G. Persoone. 1995. Biochemical assessment of Cellular Energy Allocation in Daphnia magna exposed to toxic stress as an alternative for the conventional “Scope for Growth” methodology. ANPP-International symposium, biological markers of pollution, September 21-22, 1995, Chinon, France

Fearman, J.-A., Bolch, C.J.S., Moltschaniwskyj, N.A. 2009. Energy storage in mussels, Mytilus galloprovincialis: the influence of diet quality. Journal of Shellfisheries Research. April, 2009

Gabbot, P.A. 1976. Energy Metabolism. In: Bayne, B.L. (Ed.) Marine mussels: their ecology and physiology. Pp. 293-356

Garnier J., G. Billen, E. Hannon, S. Fonbonne, Y. Videnina and M. Soulie. 2002. Modeling transfer and retention of nutrients in the drainage network of the Danube River. Estuarine, Coastal and. Shelf Science, 54: 285-308.

Gasparini S., Daro M.-H, Antajan E, Tackx M., Rousseau V., Parent J.-Y. and C. Lancelot. 2000. Mesozooplankton grazing during the Phaeocystis globosa bloom in the Southern Bight of the North Sea. J. Sea Res. 43: 345-356

Gislason A. and T. Silva. 2009. Comparison between automated analysis of zooplankton using ZooImage and traditional methodology. J. Plank. Res., 31(12):1505-1516.

Grosjean P., Picheral M., Warembourg C. and G. Gorsky. 2004. Enumeration, measurement, and identification of net zooplankton samples using the ZOOSCAN digital imaging system. ICES J. Mar. Sci. 61: 518-525.

Gypens N., Lancelot C. and A. Borges. 2004. Carbon dynamics and CO_2 air-sea exchanges in the eutrophied coastal waters of the Southern Bight of the North Sea: a modelling study. Biogeosciences 1: 147–157.

Gypens N, Lacroix G and Lancelot C. 2007. Causes of variability in diatom and Phaeocystis blooms in Belgian coastal waters between 1989 and 2003: a model study. Journal of Sea Research 57, 19-35.

Gypens N., Borges A. V. and C. Lancelot. 2009. Effect of eutrophication on air-sea CO2 fluxes in the coastal Southern North Sea: a model study of the past 50 years. Global Change Biol. 15: 1040-1056.

Gypens N., Lacroix G., Lancelot C., Borges A. 2011. Seasonal and inter-annual variability of air-sea CO2 fluxes and seawater carbonate chemistry in the Southern North Sea, Progress in Oceanography (2010),doi: 10.1016/j.pocean.2010.11.004

Irigoien X., Fernandes J.A., Grosjean P., Denis K., Alba A. and M. Santos, 2009. Spring zooplankton distribution in the Bay of biscay from 1998 to 2006 in relation with anchovy recruitment. J. Plankt. Res. 31(1) 1-17.

Lacroix G., Ruddick K., Ozer J. and C. Lancelot. 2004. Modelling the impact of the Scheldt and Rhine/Meuse plumes on the salinity distribution in Belgian waters (Southern North Sea). J. Sea Res. 52: 149-153.

Lacroix G., Ruddick K., Park Y., Gypens N. and C. Lancelot. 2007. Validation of the 3D biogeochemical model MIRO&CO with field nutrient and phytoplankton data and MERIS-derived surface chlorophyll a images. J. Mar. Syst. 64(1-4): 66-88

Lancelot C., Rousseau V., Becquevort S., Parent J.-Y., Déliat G., Leblanc C., Daro M.-H., Gasparini S., Antajan E., Meyer A., Ruddick K., Ozer J. and Y. Spitz. 2004. Study and modelling of eutrophication-related changes in coastal planktonic food-webs: A contribution of the AMORE (Advanced MOdeling and Research on Eutrophication) consortium. Final Report, Brussels: Belgian Science Policy.

Lancelot C., Spitz Y., Gypens N., Ruddick K., Becquevort S., Rousseau V., Lacroix G. and G. Billen. 2005. Modelling diatom and Phaeocystis blooms and nutrient cycles in the Southern Bight of the North Sea: the MIRO model. Mar. Ecol. Prog. Ser. 289: 63-78

Lancelot C., Gypens N., Billen G., Garnier J. and Roubeix V. 2007. Testing an integrated river-ocean mathematical tool for linking marine eutrophication to land use: The Phaeocystis-dominated Belgian coastal zone (Southern North Sea) over the past 50 years. J. Mar. Syst. 64(14): 216-228.

Lancelot C., Rousseau V., Gypens N., Parent J.-Y., Bissar A., Lemaire J., Breton E., Daro M.-H., Lacroix G., Ruddick K., Ozer J. , Spitz Y., Soetaert K., Chrétiennot-Dinet M.-J., Lantoine F. and F. Rodriguez. 2007. Advanced modeling and research on eutrophication AMORE II. Final Report. , Brussels: Belgian Science Policy, D/2007/1191/5.

Lancelot C., Rousseau V. and N. Gypens. 2009. Ecologically-based reference for Phaeocystis colonies in eutrophied Belgian coastal waters (Southern North Sea) based on field observations and ecological modeling. J. Sea Res. 61: 44–49

Mills D.K., Laane R.W.P.M., Rees J.M., Rutgers van der Loeff M.M., Suylen J.M., Pearce D.J., Sivyer D.B., Heins C., Platt K., Rawlinson M. 2003 Smartbuoy: a marine environmental monitoring buoy with a difference, in: Dahlin, H. et al. (Ed.) (2003). Building the European capacity in operational oceanography: proceedings of the 3rd

International Conference on EuroGOOS 3-6 December, 2002, Athens, Greece. Elsevier Oceanography Series, 69: pp. 311-316

OSPAR. 2005. Common Procedure for the Identification of the Eutrophication Status of the OSPAR maritime area, OSPAR agreement 2005-3.

Peperzak L. 1993. Daily irradiance governs growth rate and colony formation of Phaeocystis (Prymnesiophyceae). J. Plankton Res. 15:809-821

Petri G., Donat F.H and M. Vareschi. 1999. Investigations on uptake and utilization of Phaeocystis globosa colonies by the blue mussel Mytilus edulis. Senkenbergiana marit. 29: 117-118.

Pieters, H., Kluytmans, J.H., Zurburg, W. and Zandee, D.I. 1979. The influence of seasonal changes on energy metabolism in Mytilus edulis (L.): 1. Growth rate and biochemical composition in relation to environmental parameters and spawning, in: Naylor E., Hartnoll R.G. (Ed.) (1979). Cyclic Phenomena in Marine Plants and Animals: Proceedings of the 13th European Marine Biology Symposium, Isle of Man, 27 September-4 October 1978. European Marine Biology Symposia, 13:pp. 285-292

Pieters H., Kluytmans J.H., Zandee D.I. and G.C. Cadee. 1980. Tissue composition and reproduction of Mytilus edulis in relation to food availability. Neth J. Sea Res. 14: 349-361.

Rousseau, V., Mathot, S. & Lancelot, C., 1990. Calculating carbon biomass of Phaeocystis sp. from microscopic observations. Mar. Biol., 107 : 305-314.

Rousseau V., Leynaert A., Daoud N. and C. Lancelot. 2002. Diatom succession, silicification and silicic acid availability in Belgian coastal waters (Southern North Sea). Mar. Ecol. Prog. Ser., 236: 61-73.

Rousseau V., Lancelot C and D. Cox. 2006. Current Status of Eutrophication in the Belgian Coastal Zone. Rousseau V., Lancelot C. and D. Cox (Eds). Presses Universitaires de Bruxelles, Bruxelles. Pp 121. D/2006/1191/45

Rousseau V., Chrétiénnot-Dinet M-J, Jacobsen A., Verity P. and S. Whipple. 2007. The life cycle of Phaeocystis: state of knowledge and presumptive role in ecology. Biogeochemistry, 83:29-47.

Schiettecatte, LS Thomas, H Bozec, Y Bozec, Y. 2007. High temporal coverage of carbon dioxide measurements in the Southern Bight of the North Sea. Marine Chemistry. 106: 161-173

Smaal, A.C. and F. Twisk. 1997. Filtration and absorption of Phaeocystis cf globosa by the mussel Mytilus edulis L. J. Exp. Mar. Biol. Ecol. 209:33-46

Tang X., Stewart W.K., Vincent L., Huang H., Marra M., Gallager S.M. and S.C. Davis. 1998. Automatic Plankton Image Recognition. Artif. Intell. Rev., 12:177-199.

Tett P., Gowen R., Mills D., Fernandez T, Gilpin L., Huxham M., Kennington K., Read P., Service M., Wilkinson M. and S. Malcolm. 2007. Defining and detecting indesirable disturbance in the context of marine eutrophication. Marine Pollution Bulletin 55: 282-297.

Thieu V., Billen G., Garnier J. 2009. Nutrient transfer in three contrasting NW European watersheds: the Seine, Somme, and Scheldt Rivers. A comparative application of the Seneque/Riverstrahler model, Water Research, 43(6):1740- 1754.

Thieu V., Garnier J., G. Billen. 2010. Assessing impact of nutrients mitigation measure along rivers continuum to southern bight of the North Sea. Science of the Total Environment, 408:1245–1255.

Tracey G.A. 1988. Feeding reduction, reproductive failure, and mortality in Mytilus edulis during the 1985 « brown tide » in Narragansett Bay, Rhode Isalnd. Mar. Ecol. Progr. Ser. 55 : 73-81