Executive Summary:

Primary production (PP), i.e. the process by plants and algae fix atmospheric CO2 using the sunlight as energy, forms the basis of all ecosystem processes. Understanding and management of marine ecosystems is thus impossible without accurate knowledge of how primary production responds to environmental change. In spite of this, primary productivity measurements are not part of the routine monitoring programs by most EU Member States, are not included in the Water or Marine Strategy Framework Directives (WFD & MSFD), and are seldom included in any European operational oceanography programs. The most likely reason is that the current methodology, based on the measurement of uptake of radioactive labeled CO2 is not suitable for routine applications. The PROTOOL project will now make this possible because it developed hardware, software and parameter estimation algorithms to measure primary production autonomously, with the aim of installing the equipment on ships of opportunity. Developing the tools to do this was the main aim of PROTOOL, and these goals have been realized so that implementation is the next phase.

The hardware consisted of:
1) an automated 'active' fluorometer to measure photosynthetic activity and phytoplankton biomass using the main pigment chlorophyll-a as a proxy,
2) a spectral light reflectance setup allowing remote sensing at shipboard level (in order to obtain water quality parameters and
3) an in-line absorption meter (PSICAM) to measure the light absorption by the algae and to obtain inherent optical parameters which can be used to derive information on the water constituents.

Although delay occurred in the development of the PSICAM (sold as OSCAR), the instrument is now ready for sale and several customers have already ordered the instrument. Prototypes of the fluorometer and reflectance units were extensively field tested and a final commercial version of the fluorometer can now be ordered and has been tested in the Baltic Sea with good success. The reflectance unit is a combination of existing radiometers using a special setup and our Finish partner Syke developed a positioning instrument and software to control it. Manufacturing instructions can be downloaded from their website.

The instruments and their prototypes developed were tested in extensive field campaigns. The testing was necessary for several purposes: First it has to be investigated if the instruments performed well during the field trials (e.g. how reliable were they). Second, the fluorometer does measure the efficiency of the photosynthetic process and measures the rate of photosynthetic electron transport (ETR), and calibration against CO2 fixation experiments were necessary in order to obtain calibration constants. In addition data were needed to develop algorithms in order to obtain the relevant water quality parameters from the OSCAR and reflectance modules. Several successful algorithms were developed to estimate phytoplankton biomass (chlorophyll) from both instruments but a light attenuation coefficient algorithm needs further development.

The field test at several locations revealed that photosynthetic activity followed a clear diurnal pattern, which would be missed using standard measuring protocols. Data obtained for the Dutch estuaries demonstrated that the seasonal patterns in daily primary production were accurately captured and that estimates of annual production can be obtained with a

high degree of accuracy, but occasional calibration is advisable. A meta-analysis of these calibration factors, supplemented with data obtained during PROTOOL cruises resulted in regional algorithms to predict the calibration factors (the quantum requirement of C-fixation).

PROTOOL also developed software to treat the data stream. At this moment a 14C primary production database (in MS access) and a combined FRRF/spectral reflectance database (in mySQL) are developed in which several fitroutines and algorithms are implemented. These will be made available on the web but are currently available on request only.

Project Context and Objectives:

Coastal seas, shelf seas and oceans are under severe anthropogenic pressure as a direct result of intensive fishing, pollution and eutrophication and indirectly via global climate change. Already about 50%, approximately 3 billion people, live near the coastline, a percentage which is expected to double by 2025, hence the anthropogenic pressure on coastal seas will rise. Such pressures have altered the ecosystem foodweb structure, and resulting 'regime shifts' in many marine ecosystems in the Pacific the Northern Atlantic Ocean, the North Sea, the Wadden Sea and the Baltic Sea have been documented. These regime shifts can have opposite effects on primary production and stimulate harmful/toxic algal blooms, causing deterioration in food quality of higher trophic levels and a decline in key ecosystem services, such as the fisheries. Of all factors, eutrophication appears to be the most common cause of regime shifts observed to date, especially in coastal seas, and has been a subject of international concern. Measures have been established to half the loads of phosphorous (P) and nitrogen (N), and these measures have been partially successful, at least in the southern North Sea: P but not N loadings have been substantially lowered leading to large changes in the N:P-ratio, an important structuring factor in phytoplankton communities, but also in increasing occurrences of coastal P-limitation. It is important to stress that lowered nutrient loading ('oligotrophication') is hardly studied. It cannot be assumed that it is simply the reverse process of eutrophication, as demonstrated in freshwater ecosystems. Instead, oligotrophication may represent an alternative stable state, which can prove to be a major problem in efforts to improve the ecological status of aquatic ecosystems.

Primary production (PP) by plants and algae forms the basis of all ecosystem processes and determines the upper bound of the carrying capacity. Understanding and management of marine ecosystems is thus impossible without accurate knowledge of how primary production responds to environmental change. Unfortunately, the biomass of phytoplankton (measured as chlorophyll a (chla) is not a good indicator to estimate aquatic primary production because of rapid and variable turnover times of the phytoplankton cells. In this respect, there is a large difference between the functioning of terrestrial and marine aquatic ecosystems. Both ecosystems fix approximately 50% of globally fixed carbon (C), however, the total amount of C locked into living biomass in marine ecosystems is only approximately 1% of total global C. Consequently, the turnover of C in marine ecosystems must be much faster than for terrestrial systems. In spite of this, primary productivity measurements are not part of the routine monitoring programs by most EU Member States, are not included in the Water and Marine Strategy Framework Directives (WFD resp. MSFD), and are not included in any European operational oceanography programs. We find this finding startling because understanding the ecological status of an aquatic ecosystem is not possible without first understanding the magnitude of primary production. Although the MSFD stresses the importance of monitoring schemes to uphold marine biodiversity, healthy fish stocks, and to guide measures to counteract pollution, overexploitation, and eutrophication, measuring primary productivity is no longer mentioned explicitly in the directive, probably because there is currently no infrastructure to support these measurements on an international level. Better estimates of PP are also necessary to better understand the role of phytoplankton in the global C-cycle: current estimates suggest that net annual marine phytoplankton PP is approximately 50 Gigaton, but the uncertainty is large and ranges from

35-75 Gton/a. As the anthropogenic CO2 production is approximately 6 Gton/a, a more accurate estimate of marine PP is essential to understand both the human and phytoplankton role in the global C-cycle.

In marine environments, primary productivity is normally measured via the incorporation of radioactive carbon dioxide in the form of bicarbonate, an approach that has hardly changed since the development of this technique by Steemann Nielsen in the 1950s. This technique is cumbersome, expensive and not suited either for routine monitoring purposes. Such fundamental limitations clearly justify why most monitoring programs lack measurements of primary production. However, recent conceptual and technological advances have enabled photosynthetic activity to be measured using alternative, 'variable fluorescence' techniques. Several studies have demonstrated a linear relationship between photosynthesis rates estimated from variable fluorescence (which measures photosynthetic electron transport, ETR) and from C-fixation measurements demonstrating the potential of the technique to measure primary production. Although individual studies indeed shows a tight coupling between C-fixation and ETR, there exists considerable variability between studies in the nature of the linear relationships: i.e. the regression coefficients differ between studies. Further knowledge into the nature of this variability (making predictions of the regression coefficients possible) is therefore necessary and will be part of this project. The great advantage of fluorescence-based measurements is that they can be automated and incorporated into remote monitoring devices. This project contributes to the development of a pan-European automated primary production system suitable for use on research vessels, ships-of-opportunity and moorings. This would greatly enhance the in situ capabilities of the EU flagship initiative entitled Global Monitoring for Environment and Security (GMES).

Our project will specifically build on these recent advances in using variable fluorescence techniques to yield primary productivity measurements. Our primary aim is to use the variable fluorescence technique as a basis to develop new automated technology to measure primary productivity in estuaries, coastal areas and seas. Importantly, we propose to develop a modular platform of maximum application for establishing a fully autonomous system but also for upgrading instrument platforms already in existence (e.g. FerryBox network). In addition we will validate active fluorescence-based productivity measurements in a number of coastal areas and seas with high temporal and spatial resolution by predicting primary production on new datasets using earlier derived parameters within the project.

In order to accomplish our goals, we have divided the project in 5 subprojects (SPs):
SP1: instrument development
SP2: field testing and primary productivity measurements
SP3: conversion factors
SP4: Intercalibration
SP5: data center

SP1: instrument development
The objectives for SP1 are the following:
- Develop a PROTOOL Fluorometer (WP2): this fluorometer will be developed in 3 stages: the first version (baseline model) will be put together from existing equipment, and with this module most of the field work will be done. This included the development of a PC controlled LED light panel by

partner 8 (PSI). The performance of the baseline model should be input for the development of the prototype and the final version. The final version should be ready near the end of the project and will undergo only limited field testing.
- Development of a PROTOOL absorption meter, the Point Source Integrating Cavity (PSICAM, WP3). The PSICAM should measure and quantify the spectral characteristics of the different water constituents (CDOM, suspended matter, algal absorption and algal biomass (as chla). From these measurements the rate of light absorption should be distinguished, a parameter necessary to quantify the rate of photosynthetic electron transport when using the bio-optical model.
- Development of a PROTOOL reflectance module (WP4). This module performs hyperspectral remote sensing at shipboard level. The measurements should be used to obtain the important water quality (WQ) parameters chla and the light attenuation coefficient (Kd).

Hardware is not the only part of SP2. Some of the parameters are not measured directly but should be retrieved from the raw data using specially designed algorithms. This requires validation and for this reason field work has to be done.

SP2, field testing has a dual role.
The objectives of the subproject are:
- To test the developed equipment under realistic field condition to check on its performance. Because there is a large diversity of water types in the EU the PROTOOL approach has to be able to work in all water types present in the EU. Therefore work was carried out in Estuaries (WP5), the brackish Baltic Sea with a large contribution of cyanobacteria, which are normally a challenge to active fluorometers, WP6), the North Sea (WP7, including work on the Celtic Sea and some Channel data) and the oligotrophic waters of the north east Atlantic (WP8). Originally we planned to measure in the Channel and Gulf of Biscay, but because the ferry used for this work was taken out of service, a number of cruises on the north east Atlantic were performed as an alternative.
- To obtain data necessary for calibration and for validation of the algorithms developed for the different instruments in SP1. Due to the different types of water bodies we envisage an approach that might result in regional algorithm.

SP3, conversion factors
PROTOOL adopted the Fast Repetition Rate fluorescence (FRRf) approach to measure marine primary productivity from seas and oceans. However, FRRf measurements suffer from two fundamental limitations: PP is measured (i) relative to the number of 'photochemical reaction centers' and not relative to the volume of water sampled; and (ii) in a 'currency' of electrons evolved (the so-called electron transfer rate, ETR) and not CO2 fixed. Therefore conversion factors must be applied to all FRRf-ETR measurements to yield measurements of primary production in units of C per unit time per unit area. Thus the fundamental aim of WP9 was to establish novel algorithms to enable implementation of these conversion factors.

In order to convert the ETR to CO2 uptake knowledge of the 'quantum requirement for carbon fixation (QR) is required (assuming that one absorbed photon leads to the production on 1 electron finally extracted from water). Three parallel approaches were taken;

- A meta-analysis was performed to compile existing QR data sets with environmental and taxonomic parameters to develop predictive algorithms
- Collect new high resolution data to support past data sets during dedicated PROTOOL cruises, in particular for special marine regions (Baltic Sea and the Eastern Scheldt). In addition,
- Perform additional laboratory culturing of phytoplankton to experimentally identity the role of key factors, e.g. nutrient availability upon the QR of different algal taxa.

SP4, intercalibration
In order to facilitate the development of algorithms necessary for the different modules it is good to know if the measurements carried out during the fieldwork is comparable. For this reason a number of intercalibration exercises were carried out:
- A pigment intercalibration exercise. This involved sending out pigment samples (both standards and field samples) collected by one partner to the other participating partners in the intercalibration exercise.
- A primary production intercalibration. This intercalibration was aimed at testing the mathematical procedures followed to come to an estimate of estimates of daily primary production from a raw dataset. NIOZ mailed a dataset including irradiance data, data from a scintillation counter, added amount of radioactive bicarbonate, in situ and incubation temperatures, light attenuation coefficients and total dissolved inorganic carbon. The participants were asked to compute the photosynthetic characteristics, daily and integrated water column primary production.

SP5, datacenter and dissemination
Proper data management is essential for the success of the project. Because of the large amount of data generated by the different PROTOOL modules and versions, automated fitting routines will be developed and special emphasis will be given to treatment of outliers, which will be manually checked. After quality control and quality assurance (QaQc), data will be added to the project database. We plan to host the database on the dataservers of the NIOZ and make the data available via a web interface.

The NIOZ will develop two databases:
- A C14 database, developed in MS Access. This database will hold the raw data obtained from the 14C-uptake experiments. It contains fitting routines, written in the R (see http://cran.r-project.org online) to fit the photosynthesis irradiance (P/I) data and obtain the parameters which describe the shape of the P/I-data (maximum rate of photosynthesis, photosynthetic affinity and light saturation parameter. It contains three different models to fit the P/I data. When the light attenuation coefficient is known and the hourly irradiance, it should be able to compute daily primary production in the water column and integrate the daily primary production estimates over a defined interval.
- A MySQL database which will hold the data obtained from the different TriOS-Ramses sensors (data from the reflectance module, including GPS coordinates). There should be a software routine which flags unreliable data and after passing the filter criteria it should computes spectral reflectance and from this it the chla concentration and light attenuation coefficient according to 4 different algorithms (using an R-script). The database will also hold data from the PROTOOL fluorometer. From the FRRF data the RLC curve parameters will be calculated and from this it will compute daily water column primary productivity and integrate it over a set time.

In addition the datacenter will develop the PROTOOL website which will give general information about the project and which will publish results. It will also contain a protected part for the project partners. Dissemination of results in variable form will be put on the website (e.g. newsletters, papers etc).

Project Results:

WP2: the PROTOOL fluorometer

The goal of the project was to develop and test the device for measuring and calculation of Primary production using variable fluorescence measurement and to implement the device with all other supportive devices into ferry box and other ships of opportunity to measure primary production in a simple and cheaper way with results comparable to present instruments (e.g. Fastracka device produced by Chelsea Instruments, Great Britain). During the project several generations of the instrument were constructed and tested: the first FRR prototype, the laboratory experimental version used for routine testing and finally the industrial flow-through version that was successfully incorporated into the ferry-box. Equipment proved to be functional and its parameters are in accordance with the expectations. The flow-through device is fully functional and is now under the implementation process. The design of the initial and the experimental versions of the FRRF fluorometers was derived from the standard fast fluorescence kinetics measuring devices produced by PSI company. Major changes were in the light generating head (LED selection and arrangement) and in the software, where the device could provide single turnover flashlets in high frequency and with complex setup for the protocol with increasing actinic light (AL) levels. The first version of the instrument was built with two excitation wavelengths (blue and red), where blue was exciting Chla fluorescence and photosystem II reaction centers PSII, while the red actinic light was used for excitations into the phycocyanin/phycoerythrin phycobilisome (PBS) antenna. Three instrument prototypes were delivered by PSI to LPIM, NIOZ, UESSEX and the fourth with three wavelengths was built for SYKE. In 2010 new versions of the modified prototypes with red excitation substituted by amber were delivered. The final ('industrial') flow-through version of the FRRF device was developed and introduced at spring 2012 and was tested in the laboratory as well as at the ferry box of R/W Aranda at the SUPREMO II-12 cruise SYKE Finland.

Hardware. The device runs under LINUX operation system and it can be remotely operated via internet connection. The Fast Repetition Rate fluorometer (FRRf) consists of 2 main parts: FFL-040 Fluorometer and Operator PC with web server. The FRRf instrument is built into the IP67 alluminium box case. It contains 2 holes for measuring tubes, power switch, light source intensity indicator and waterproof connectors. Left (measuring) port contains the measuring and actinic lights together. Right (adaptation) port contains actinic light only. This port is also used for circulation of the sample during the measurement. Actinic light intensity and light spectrum are the same for both ports. Temperature stabilization of the sample by the double sided tube is possible in the adaptation port. Circulating water in the outer tube maintains stable temperature of the sample. Measuring light sources (590nm and 460nm) are operated during the FRR induction sequence of the experiment. High power LED diodes controlled by high speed electronic driver circuit trigger measuring flash train of µs-scale light pulses (flashlets). Timing of these triggers may be controlled with 100ns accuracy. AD converter acquisition trigger is synchronized with light triggers also with 100ns accuracy. Both measuring light sources use 6 LEDs from each side of the tube. These LEDs are placed in perpendicular direction to the detector port. LED chips are placed close to each other for getting the best homogeneity of the light in the measuring tube. Double side excitation as well as optical parameters of the glass tube also improve the homogeneity of the light inside the measuring tube. LEDs illuminate 20mm of the

sample in the middle of the detector port. Use of the high power LEDs must follow special design precautions for proper cooling of individual chips. Heating of the LEDs by current flow lowers the LED light intensity, especially in the amber measuring light source (see the LED documentation below). Actinic light sources (amber 590nm and blue 460nm) are operated during the rapid light curve (RLC) measurement. High power LED diodes create the simulation of the ambient light for measuring the ETR curves from the sample inside the tube. Actinic light sources are placed on the opposite site of the detector channel. It contains the secondary LED optics for collimating the light into the tube. This improves the actinic light homogeneity inside the measuring tube. Whole measured sample must be illuminated during the RLC protocol. Actinic light is switched ON for a long time. LED strip with alternated amber and blue LED chips is used for the illumination of the whole measuring tube. On 20 cm of the tube length is used in total 16 amber and 16 blue LED chips of the same type as the measuring light (LZ1-00B200 and LZ1-00A100, respectively).

The signal coming from the measuring tube reaches the detector port window. This window is located opposite to the actinic light source. Optical glass protects the emission filters against scratching. O-ring sealing makes a waterproof protection. Signal originating from the sample travels approximately 1.5cm and then continues through the emission filter set FB690-10. PIN diode with effective area 66mm2 is used as a light sensor. The 16 bit A/D converter with the maximal sampling rate 1MHz includes also the electrical gain circuit. There is a support for 4 gain settings giving the approximately 40x multiplication of the measured signal. Output from the detector is already read on the absolute scale. It means gain settings influence only the dynamic range and the noise of the measured signal. These 4 inter-calibration constants are measured during the calibration process. Measuring tube with a fluorescence dye inside must be inserted in the measuring port.

Graphical User Interface. The FFL – 040 fluorometer communicates with Operator PC via RS232 serial port. USB to serial converter is included for converting the data flow to USB bus standards. Data readout, data storage, pump and solenoid switching logic is controlled from the Operator PC. Specific communication protocol is implemented for this purpose, enabling to operate more FFL-040 fluorometers connected to one Operator PC. Operator PC reads out the calibration constants from all the connected FFL-040s.

Measurement with flow-through system runs under the control of the FRRF Operator PC. It is currently the standard HP laptop computer with Linux system installed. This software includes also the web server application (Java6 compatible) running for online network access. All data measured with the flow-through fluorometer FFL-040 are stored on Operator PC. Client from the remote PC can run the fluorometer GUI on its terminal easily by starting the Java runtime application on client's computer. User can profit from remote control of the measuring process on his/her client PC. Fluorometer GUI (graphical user interface) controls the state of the measuring process. Any change in FFL-2012 state is automatically updated in all connected clients.

The fluorometer GUI starts automatically after switching ON the FRR (Fast Repetition Rate) Linux controller Operator PC. Prior to the start of the automated measurements, few protocol parameters can be set to allow more user-defined variability. Automated setting of these parameters has not

been implemented yet, but will be available in the commercial types of this device. The software plots actual data flow. FRR induction sequence fit is executed with the ST1 data. Results of this fitting procedure are presented. Different parameters are obtained for Blue and Amber colors. Standard deviation is calculated for all computed values. Statistical parameters and p-value inform about the quality of the fit. Fitting process is executed automatically by running the R-script file in the R language. This script can be reprogrammed in the future for improving the fitting procedure. All measured data are stored on the hard disk of the Operator PC. Left side list shows these data sorted in the time order. Data from each sampling are presented here as one line. All plots, similarly like in the online window are accessible for these data. Data on the Operator PC can be accessed by the remote PC via the ftp communication protocol. FRR flow-through fluorometer system consists of FFL-040 fluorometer, FRRF Linux controller with data storage and optional pumps and valves for water flow control.

WP3 Point Source Integrating Cavity

Important for the determination of primary production in the sea and for general water quality assessment is the exact measurement of the light absorption by various water constituents, as e. g. the absorption by phytoplankton itself. Goal of this work package was to build a compact, commercial instrument based on the point-source integrating-cavity absorption meter approach that can be used as an underwater in situ sensor as well as a flow-through bench instrument for ferry box implementation. The instrument consists of an integrating sphere, a special LED-light source and a miniature spectrometer. The design of the light source and the optical connection of the light source and the detector with the sphere were challenging. The first version of the instrument was tested in the field and the validity and reliability of the measuring concept and the new construction were proofed.

The aims of this work package was to develop, first, a modular baseline version of the PSICAM absorption meter that is used to test the PSICAM principal in an automatic flow-through mode connected to a ferry box system and to develop and evaluate necessary calibration, cleaning, and maintenance procedure. Second, an autonomous version of the PSICAM shall be build as a stand-alone commercial instrument which can be part of the final PROTOOL-MD system.

During the project an instrument was developed for in situ measurements of the light absorption of water constituents like phytoplankton with the additional option to use it as a flow-through instrumentation. The instrument's concept and design was based on a lab-version (PSICAM) already in use and extensively tested. The special PSICAM design has the advantage of being very sensitive due to a long optical path length and is not affected by scattering problems due to its integrating sphere approach. Some details of the optical design that shall lead to a better performance than the former lab version were difficult to implement and delays in the construction were encountered due to delays in production of some important optical parts. During the project a simple version of the new instrument (OSCAR) was tested in the field and this instrument performed well compared to the available lab-version. The functionality of the instrument regarding calibration, and easy maintenance, and the used in different environments (using different cavity sizes) is superior and will make in situ and flow-through measurements of light absorption possible that up to now where difficult to perform and labour-intensive or subject to substantial errors.

WP 4 Reflectance module

Aims

The aim of this work package was to develop an independent module to obtain the spectral water-leaving reflectance (water colour) from ships-of-opportunity. The module supports integration with the other PROTOOL modules (WP2 and 3) through database connectivity. Three aspects define how reflectance complements the PROTOOL package:

- Light absorption and scattering processes in the surface layer of the sea can be derived from the measured reflectance
- Remote optical sensors (e.g. on satellites) are used to measure reflectance of large sea areas, allowing primary productivity measurements to be placed in a spatial context
- Because the sensors are placed outside, the module records the actual intensity of sunlight available for photosynthesis

The development of the Reflectance module is described in detail in PROTOOL public report D4.15. The module provides support for the use of commercially available spectroradiometers (TriOS RAMSES), which are commonly used for in situ reflectance measurements.

The development of a supporting platform for moving ships-of-opportunity included:

- research into the optimization of the spatiotemporal coverage, notably avoiding capture of sun glint and minimizing the recording of reflected sky radiance.
- development of an automated processing scheme to produce reflectance spectra from the spectroradiometric measurements
- research into the performance of algorithms that interpret reflectance in terms of useful water quality parameters, such as the biomass of phytoplankton in terms of the pigment chlorophyll-a

The algorithm development is described in detail in PROTOOL public report D4.16. Highlights of the developed hardware/software solutions as well as the algorithm results are provided below for each of the approaches listed above.

Optimizing spatiotemporal coverage

Reflectance is measured as the ratio of upwelling over down-welling light. In above-water measurements, a radiance sensor with a narrow field of vision is pointed at the water at an oblique angle. The signal measured with this sensor consists of light that has been reflected in the water column, but also a contribution of light that was reflected at the water surface. The latter does not carry information about light absorption and scattering processes in the water itself. To correct for this contribution, a separate sensor is pointed at the sky to record the spectral quality of the sky radiance. A third sensor captures all downwelling irradiance. Previous studies (Austin 1974, Mobley 1999) have shown that the best results are obtained when the water leaving radiance is captured at an angle away from the sun, to avoid sun glitter which is usually brighter than the radiance reflected in the water column.

Because the solar angle as well as course of the ship that they are mounted on is not constant, we need to periodically adjust the viewing angle of the sensors. Alternatively, we could mount several sets of sensors on different positions of the ship, looking at the sea surface from different angles. This is a costly option compared to the solution that was developed in this project.

GPS modules provide all information that is needed to calculate the position of the sun, as well as the direction in which the ship is moving. The platform developed in this WP uses this information to correct the viewing angle of the spectroradiometers at regular intervals. The sensors are mounted on the axis of a stepper motor which is placed in a weather-proof enclosure. The software that controls the viewing angle of the sensors is also used to periodically calibrate the centre angle of the platform to guarantee optimal operation. Further, the software triggers measurements and receives and stores the raw signals measured by the sensors. The conditions under which measurements are triggered can be manually set, so that the user can choose to collect only measurements taken under optimal conditions. The software as well as a hardware description are available as open-source documents at http://sourceforge.net/projects/rflex/.

Deriving reflectance

For every measurement, it is necessary to estimate the contribution of sky radiance reflected at the water surface to the water leaving radiance. After the viewing angle has been optimized to avoid direct sun glint, this contribution is still variable and depends on illumination conditions (clouds, sun angle) and the roughness (waves) of the sea surface (Doxaran et al. 2004, Toole et al 2000). The sky radiance contribution has previously been modelled after wind speed, as a proxy for wave slope. Unfortunately, this method can easily lead to inaccuracies, particularly in relatively clear waters where the water leaving radiance is weak compared to the reflected sky radiance. The existing methods also do not provide any means to judge, without expert involvement, whether the obtained reflectance is valid or not. Invalid measurements occur when reflectance is measured under broken cloud cover, in rain or fog, or when exposed ship heaving during storms.

The newly developed method interprets the sky radiance reflectance by comparing the hyperspectral patterns in the downwelling radiance and the radiance measured from the water surface. The method (Simis and Olsson, submitted) optimizes the sky radiance reflectance factor and evaluates whether spectral patterns present in the downwelling light are removed from the resulting reflectance. The result of the method is the optimized reflectance, as well as several product quality flags. Tests on the Baltic Sea suggest that up to 50% of the measurements taken from a large ferry are successfully retrieved and flagged as useful for further interpretation.

Deriving water quality products

Two quantities that can be derived from reflectance are of central importance to estimate primary productivity: the absorption of light by photosynthetic pigments, and the number of photons available for light harvesting, in the mixed layer. Relatively little attention was given to the retrieval of the vertical diffuse attenuation coefficient that predicts light intensity at depth given the irradiance measured at the surface (which the reflectance module provides). This coefficient can be modelled after light absorption and backscattering and solar elevation (Lee et al. 2005), and relies primarily on the accurate inversion of reflectance to these bulk inherent optical properties. More complicated is the retrieval of partitioned absorption, such as the absorption of light by phytoplankton (often scaled to the concentration of the pigment chlorophyll-a). Quantifying this absorption from reflectance is one of the central themes in spaceborn remote sensing of the global ocean. In coastal seas, the optical composition of the water is complex, and

reflectance algorithms must be designed to overcome this complexity. We have obtained satisfactory results in retrieving phytoplankton pigment absorption in both the turbid estuaries and the open North Sea and Baltic Sea, during field testing in this project, using an adaptive band ratio algorithm (Ruddick et al. 2001) for turbid waters and a neural network inversion scheme in the open sea areas. These results are described in detail in report D 4.16.

References

Austin, R. (1974). The remote sensing of spectral radiance from below the ocean surface. Optical Aspects of Oceanography, 317-344

Doxaran, D., Cherukuru, R.C.N., & Lavender, S. (2004). Estimation of surface reflection effects on upwelling radiance field measurements in turbid waters. Journal of Optics A: Pure and Applied Optics, 6, 690-697

Lee, Z.P., Du, K.P., & Arnone, R. (2005). A model for the diffuse attenuation coefficient of downwelling irradiance. Journal of Geophysical Research-Oceans, 110

Mobley, C.D. (1999). Estimation of the remote-sensing reflectance from above-surface measurements. Applied Optics, 38, 7442-7455

Ruddick, K.G., Gons, H.J., Rijkeboer, M., & Tilstone, G. (2001). Optical remote sensing of chlorophyll a in case 2 waters by use of an adaptive two-band algorithm with optimal error properties. Applied Optics, 40, 3575-3585

Simis, S.G.H., Olsson, J. Unattended processing of shipborne hyperspectral reflectance measurements. Submitted.

Toole, D.A., Siegel, D.A., Menzies, D.W., Neumann, M.J., & Smith, R.C. (2000). Remote-sensing reflectance determinations in the coastal ocean environment: impact of instrumental characteristics and environmental variability. Applied Optics, 39, 456-469

Sub-Project 2 (SP2): Field testing and Primary Production mapping

For PROTOOL to succeed, fieldwork is necessary to 1) test the different developed PROTOOL equipment (baseline, prototype and final versions) under relevant field conditions, and 2) obtain the field data necessary for validation of the algorithms developed as part of sub-project 1 (instrument development). Most of the testing took place on research vessels, although reflectance modules have been used on the ferry Finnmaid on the Baltic Sea. In addition during this SP2 we obtained data from which the conversion factors (electron requirements for C-fixation) could be obtained. This data was part of the input for SP3, in which WP9 was devoted to evaluating, understanding and predicting the conversion factors needed to obtain rates of C-fixation from ETR.

WP5 Field testing in the Dutch estuaries.

Aim and site description

Estuaries are an important testing ground for the PROTOOL products. Estuaries carry out important ecosystem services (e.g. nutrient removal, nursery grounds for fish, sustain fisheries by virtue of primary production) but estuaries also face immense anthropogenic pressure because they concentrate the material released from the watershed in the rivers into a relatively small area. Hence, careful management of estuaries is a necessity, and this requires knowledge of primary production.

The Western Scheldt and Eastern Scheldt estuaries are two coastal areas investigated by the water quality monitoring program of the NIOZ. Apart

from water quality parameters like dissolved and particulate nutrients, suspended matter (SPM) and phytoplankton pigments it also measures phytoplankton primary production at intervals (Kromkamp and Peene 2005; Kromkamp and Peene 1995; Wetsteyn, et al. 2003). In addition to this monitoring program also the Ministry of the Infrastructure and Environment (RWS) carries out an extensive measuring program with more parameters including heavy metals and toxic substances, but they do not carry out primary production but rely on data provided by the NIOZ. In both estuaries diatoms are the dominant phytoplankton group, although in summer flagellates and cryptophytes are important groups in the Eastern Scheldt. Picoplankton varies between 15-30% of the biomass in the Eastern Scheldt (no data available for the Western Scheldt).

The Western Scheldt estuary is the largest estuary in NW Europe. It has a full salinity gradient running from Antwerp to the mouth near Vlissingen/Breskens. Because of the large tides and the bend in the estuary the water is very turbid with Secchi disk visibility ranging from less than 0.5 m in brackish/freshwater zone to 1.5 m near the mouth of the estuary. In general nutrient concentrations are too high to be limiting phytoplankton growth so the system can be regarded as eutrophic. The Eastern Scheldt is a mesotrophic coastal embayment of the Netherlands with relatively clear water (Secchi depth visibility normally ranges between 2.5-4 m) and nutrient concentrations are likely to be limiting during the spring bloom. Because of the high density of shellfish (mussel farming as a bigh industry) the regeneration of nutrients during summer is very intense, and nutrient limitation during this period is not likely.

The aim of the work here was to test the PROTOOL modules and compare estimates of primary productions using the different techniques. Due to delay in the development of the PSICAM, this instrument was not field tested in the Dutch estuaries.

Reflectance results

The reflectance module was implemented by putting a system of 3 sensors on the bow of the ship and manually adjusting the viewing angle relative to the sun. The biweekly/monthly cruises delivered a large quantity of data and a series of filters were developed which were implemented in a MySQL database (see D11.24 for more information). Approximately 25% of the data do not pass the criteria for several reasons (instrument failure, sunglitter, measurement of different parts of the sky). The data obtained from the reflectance module correlate well with measured chla concentrations (r2=0.71) and show a considerable mesocale variability which is not detected if only samples from the standard station are analysed. A good algorithm for the light attenuation coefficient is under development but not finished yet at the time of writing of this report.

Primary production

The PROTOOL FRR baseline fluorometer was used to map the parameter (Fv/Fm) which is the maximum photosynthetic quantum efficiency of PSII. This parameter is an indication of the algal condition, and a decrease in the maximum value might be related to nutrient limitation, photoinhibition or other stress parameters. Fv/Fm was low in winter in both estuaries, a possible effect of the low temperatures in winter. In the Western Scheldt the decrease in winter was more pronounced, especially in the brackish water region of the estuary, suggesting that salinity stress also added to the lowered Fv/Fm values. Lowered values

during the growth season were mainly observed in the Eastern Scheldt, an indication that they might suffer from nutrient limitation.

Primary production form the FRRf measurements was calculated using 3 algorithms, including two new ones developed during the project and which remove the need for some assumptions (rate of light absorption in the Absorption algorithm and the concentration of PSII in the Sigma algorithm, see D9.22 and Oxborough et al 2012). All algorithms effectively captured the seasonal dynamics in daily primary production as measured with the 14C technique. Seasonal dynamics in the quantum requirements for C-fixation showed very similar behaviour in the two new algorithms with highest values outside the main growth season, although here there was a different pattern between both estuaries, where values were lower again in December and January in the Eastern Scheldt. FRRf daily production estimates were higher than the measured ones when using the default value for the quantum requirement (QR) (4 absorbed photons/C-fixed). Annual average QRs for the Eastern Scheldt were 4.9 ± 0.9, 6.4 ± 0.9 and 6.1 ± 0.9 for the Kolber & Falskowski (1983), Absorption and Sigma algorithm respectively. Annual estimates of primary production using these QR produced very accurate estimates of the measured annual primary production (deviation generally less than 15% for the two new algorithms). Hence for the Eastern Scheldt the PROTOOL FRRfluorometer approach seems a valid say to estimate primary production.

For the Western Scheldt the situation was more complex. Again all algorithms accurately captured the seasonality in daily primary production, but due to the fact that was variation in the average annual QR (which seem to show a (cyclic pattern?), the accuracy of the was less (generally between 50 and 150% of the measured primary production). However, over a 5 year period the PROTOOL FRRF derived estimates were close to the measured ones with an overestimate of 12% by the Kolber & Falkowski to an underestimate of 11% by the Sigma Algorithm.

Conclusion: QR requirements should be determined during the growth season. If the QR are obtained the PROTOOL fluorometer is a valuable tool to measure (changes in) primary production and seems a monitoring suitable technique. In combination with high spatial resolution of water quality parameters obtained with the Reflectance module detailed maps of primary production can be obtained.

References

Kolber, Z., and P.G. Falkowski (1993). Use of active fluorescence to estimate phytoplankton photosynthesis in situ. Limnology and Oceanography. 38:1646-1665.
Kromkamp, J. C., and J. Peene (2005). Changes in phytoplankton biomass and primary production between 1991 and 2001 in The Westerschelde Estuary (Belgium/The Netherlands). Hydrobiologia 540:117-126.
Kromkamp, J., and J. Peene (1995). Possibility of net phytoplankton primary production in the turbid Schelde Estuary (SW Netherlands). Marine Ecology-Progress Series 121(1-3):249-259.
Oxborough, K, et al. (2012). Direct estimation of functional PSII reaction centre concentration and PSII electron flux on a volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data. Limnology and Oceanography-Methods 10:142-154.

WP 6 Field testing on the Baltic Sea
Aims

The Baltic Sea is a brackish, shallow sea suffering from the effects of eutrophication and pollution combined with limited water exchange with the open ocean. The sea is considered one of the most polluted seas in the world and vulnerable to intensive shipping and fishing industries. Diatoms and dinoflagellates dominate the spring bloom while cyanobacteria often dominate in summer. Besides the occurrence of cyanobacteria blooms the sea has a unique optical composition due to high dissolved organic matter loading while the particle population of the open sea is dominated by weak light scatterers (phytoplankton rather than suspended sediments). These properties require dedicated techniques in the PROTOOL modules:
- reflectance processing schemes optimized for clear coastal waters (WP4, D4.16, WP6)
- unmixing of the dissolved organic matter absorption from the measured total absorption (WP3, WP6)
- fluorescence excitation light sources targeting algal and cyanobacteria pigments (WP2, WP6)
Various stages of PROTOOL development were tested in the Baltic Sea during a series of dedicated bio-optical research cruises with R/V Aranda. A detailed overview of the testing activities is given in PROTOOL public report D6.

Tested modules
The prototype absorption meter (PSICAM by TriOS, marketed as OSCAR, see WP3) was tested with discrete samples during two summer cruises and found to have sufficient sensitivity to characterize the phytoplankton component, albeit masked by strong dissolved organic matter absorption. A separate absorption unit for dissolved organic matter using cross-flow filtration of sea water, capillary waveguide techniques, and off-the-shelf spectrometers and light source was built, with dedicated software, and tested in the ship flow-through system. This system helps address the issue of unmixing the total absorption in the Baltic Sea. The final model PSICAM that was demonstrated in the public PROTOOL workshop in Helsinki was not field-tested in the Baltic Sea.

The fast-repetition rate fluorometer by PSI was tested on all summer cruises during the various stages of development, including the final version optimized for flow-through measurements, with operation of valves, flexible measurement protocols, and centralized data storage and analysis. Based on the various field tests, the instrument is considered suitable for implementation in the Baltic Sea.

The reflectance module was developed at SYKE and therefore most extensively tested on the Baltic Sea, during both spring and summer cruises. The final version includes full software support and network connectivity to allow centralized data storage. The software developed for the reflectance module should be able to also control the TriOS PSICAM (which is also supported by the manufacturer's software) as the data transfer protocols are identical. This has not yet been tested.

Supporting studies
The optical requirements (excitation and emission configuration) for fluorescence induction instruments sensitive to both algae and cyanobacteria, was assessed using laboratory phytoplankton cultures and extensive simulation and statistical modelling. The resulting publication (Simis et al. 2012) describes the optimal configuration and pitfalls in instrument design, as well as some fluorescence characteristics in cyanobacteria that should be kept in mind when conducting fluorescence

induction measurements. The PSI fluorometer conforms to the described specifications.

The automated processing of clear-water reflectance measurements was subject of research into new processing algorithms, as described in WP4 and PROTOOL report D4.16 in particular. The new algorithm (Simis and Olsson, submitted) supports the automatic removal of measurements carried out under suboptimal conditions, eliminating the need for expert involvement in data treatment.

The interpretation of the electron transport rate obtained from the fast-repetition-rate fluorometer in terms of carbon fixation required studies into the sources of variability in the conversion from one currency into the other. These studies were carried out both on a broad scale, comparing results from different seas (Lawrenz et al. submitted) and specific to the Baltic Sea with data collected during bio-optical research cruises as well as ferry-based sampling over a longer period. Several fluorometer types and techniques were used to compile the dataset. These results are described in detail in PROTOOL public report D6. Major identified sources of variation in the conversion factor were sampling depth and the share of (pico)cyanobacteria in phytoplankton biomass. Further investigation of the relation with phytoplankton composition is still ongoing (pending additional lab-results).

Implementation on ships of opportunity

The reflectance module was subjected to two tests on a ship-of-opportunity crossing the Baltic Sea. Improvements were implemented after the first test in summer 2011 and the module performed optimally during the second 2-month trial period in May-June 2012. The implementation can be achieved with only electricity supplied from the ship. An improved version of the deck box (with fewer electronic parts and embedded computer) is still being developed at SYKE.

Although the PSICAM and FRRF were not tested in the ship-of-opportunity itself, the flow-through system on R/V Aranda is identical to those in the Algaline ferrybox fleet. Thus, the FRRF was demonstrated to perform autonomously for the 7-day duration of the last bio-optical research cruise. The PSICAM has not yet been tested in autonomous flow-through mode.

Currently, an application is being considered by the Finnish Environment Ministry to acquire and install the functionality of the PROTOOL modules on a ship-of-opportunity, to support renewal of monitoring methods. The acquisition takes place by public procurement.

References

Lawrenz, E., Silsbe, G., Capuzzo, E., Ylöstalo, P., Forster, R.M., Simis, S.G.H., Prášil, O., Kromkamp, J., Hickman, A.E., Moore, C.M., Forget, MH., Geider, R.J., Suggett, D.J. Predicting the electron requirement for carbon fixation in seas and oceans. Submitted.

Simis, S., Huot, Y., Babin, M., Seppälä, J., & Metsamaa, L. (2012). Optimization of variable fluorescence measurements of phytoplankton communities with cyanobacteria. Photosynthesis Research, 112, 13-30

Simis, S.G.H., Olsson, J. Unattended processing of shipborne hyperspectral reflectance measurements. Submitted.

WP 7 Field testing on the North Sea

Introduction and aims

The production of organic carbon compounds from inorganic carbon dissolved in seawater is essential for supporting all food webs on Earth. In the sea, the majority of this conversion is driven by light and takes place in the surface sunlit layer known as the euphotic zone. The rate of carbon fixation or primary production varies according to several factors, but is primarily under the control of light and temperature as well as the availability of nutrients. Wind and tide-driven turbulence is also important in controlling the exposure of photosynthetic organisms to irradiance and nutrients. Most primary production is due to phytoplankton, but when sufficient light is available at the seabed then production by seaweeds, seagrasses and microphytobenthos may also be important. The aim of this WP of the PROTOOL project was to examine the photosynthetic physiology of North Sea phytoplankton, using automated optical techniques. Our knowledge of primary production in the North Sea is limited to a small number of in situ studies, with few reports of production measurements in the past decade, despite the importance of this region in supporting fisheries.

The work by HZG (formerly GKSS) and Cefas in WP7 concerns the measurement of GPP by variable fluorescence but many rate and state variables must be measured in order to understand carbon (and nutrient) flows in the North Sea. This variable often cannot be measured automatically, so, realistic alternatives must be sought, and this is where the PROTOOL approach has made significant advances in the past three years. The PROTOOL lab measurements and cruises in this work package were designed to provide a comprehensive coverage of the physical, chemical, optical and biological status of the North Sea in the period 2010-2012.

The aim of this work package was to first test and implement the PROTOOL-MD versions on research vessels in the North Sea. These included tests of the PSICAM prototype and Reflectance-module baseline version. Later it was planned to test and implement the PROTOOL-modules (prototypes) in a FerryBox system on a SOOP cruising the North Sea to obtain temporal and spatial resolution series of primary production and to obtain the for the calculation necessary optical and non-optical parameter, and conversion factors to convert rates of ETR into rates of C-fixation. The main activity of this WP was to test and demonstrate the viability of the PROTOOL modules in the North Sea and obtain optical parameter and conversion factors and estimates of primary production.

PROTOOL Equipment tested

Several research cruise to the North Sea were used to test prototypes of the different modules (PSICAM, R-module, various types of fluorometers), to test the implementation in existing ferry-box system on the research vessel, and to test improvements for their operational use (like prevention of fouling, cleaning etc.).

On the same cruises, the parameters necessary for the calculation of primary production were collected and techniques improved to retrieve these regional parameters from continuous measurements by the three modules, this included biological (e.g. chlorophyll and suspended matter concentration) and optical parameter (e.g. phytoplankton and detritus absorption). Two prototype systems (PSICAM and LWCC/CDOM) were fully implemented in the available HZG-Ferry Box system. This included full software control of both system and connection to the Ferry Box fluid system. The test on SOOP were replaced by tests on research vessel equipped with ferry-box system, as the final modules were available only

late in the project and then the ferry-box lines operated by HZG were not available due to changes in the routes and ships in the earlier for this purpose proposed container ship lines. A general problem was that the container ships (in opposite to other lines with ferries) do often not take researchers on the ship. For tests on the available ferry-box lines for the North Sea fully implemented and autonomously running instrumentation with no risk of seawater leakage were a prerequisite, therefore the instrumentation was tested only on research vessels.

Results

A large set of optical and physiological data were collected on the ship cruise. One goal was to test whether the important state variables can be estimated from automated measuring system, like ferry-boxes that include online measurements of chlorophyll fluorescence, optical measurements, like absorption of water constituents (PSICAM), or remotely determined surface reflectance. All system can be used, e.g. to determine the concentration of chlorophyll a, some can be used to determine the factors that determines the light attenuation in water, like suspended and dissolved matter concentration, or their results can be used to estimate the light attenuation coefficient directly. Another problem examined was the estimation of the fluorescence blank values that in automatic system are difficult to measure and have to be estimated by easier to measures parameter. In case of the chlorophyll a the results showed that its concentration can be estimated not only by fluorescence measurement but as well by inversion of the reflectance measurement as well as from absorption measurements done with the PSICAM. Similarly the concentration of suspended matter and the absorption of dissolved matter, as well as the attenuation coefficient can be estimated by these automatic methods. A combination of the methods would make these estimations more robust and would allow good estimates of the necessary state variables for primary production calculations from automated instrumentation.

The fluorescence blank varied strongly in different parts of the North Sea and was not much related to abiotic factor like temperature and salinity, but more related to e.g. CDOM concentration. A typical issue that had been handled in all optical measurements was the susceptibility of the measurements to contaminations and bio-fouling, which was observed with the fluorescence and the flow-through PSICAM system.

Conclusion

The results of this study showed that the proposed approach of PROTOOL to estimate primary production bear good prospects. They showed…

- that the mass-specific optical properties relevant for PP calculations are rather conservative in the North Sea, therefore,
- that these properties and other relevant parameter like chl a and TSM concentration can be determined by automated continuous absorption measurement directly from the absorption values,
- that, similarly, these properties can be remotely sensed by continuous reflectance measurements, these included also the attenuation coefficient in the water column,
- that fluorescence blank value are a source for significant error in the determination of photosynthetic efficiencies when phytoplankton biomass is low and are not easy to estimate from other properties like temperature, salinity, etc. contamination (of optical surfaces) and bio-fouling had significant influence on the measurements and more work is need to avoid or correct for these effects in automated optical systems

WP 8 Field testing on the Atlantic Ocean

A fundamental aspect of the PROTOOL project was field testing of the different PROTOOL modules on research ships and ships of opportunity. In addition, it aimed to compile comprehensive data sets in order to produce maps indicative of biological activity over wide spatial and temporal resolution in coastal and open ocean waters. The collected data was then to be used to evaluate the conversion factors developed in SP3. Due to the limited availability of the PROTOOL modules during the early part of the project, mapping became the focus of WP8. The aim was to make measurement in clear waters where absorption by phytoplankton dominates the light attenuation with depth (ie Case 1), thus providing 'open ocean' field data for calibration and derivation of conversion factors. This was to augment the measurements made in other field regions (ie WPs 5, 6 and 7) where primary productivity is higher. Plant pigment, taxonomy and primary productivity measurements, together with simultaneously collected FFRF data, were the main focus of the work.

Initially WP8 was planned in the English Channel and Bay of Biscay by augmenting the ferrybox system on the Pride of Bibao ferry (PoB), but unfortunately in September of 2010 the ferry route ceased operations. Nevertheless, some important physical and biological data were collected throughout 2010 prior to its discontinuation and this has been compared with data collected along the route in 2003 and 2004. The results show that there are very distinctive patterns in phytoplankton distributions and successions along the Pride of Bilbao route in the Bay of Biscay and English Channel that have been seen repeatedly over 3 different years. In the Bay, the spring bloom of diatoms appears to be temperature controlled (greater than 11 C) and timing varies little from year to year, the bloom occurring in mid-April. After this, silicate concentrations fall close to zero suggesting that silicate may be limiting. Coccolithophores immediately follow the diatom bloom and again their year on year occurrence shows the same general pattern with some variation in abundance. Following the coccolithophore bloom in May, the nutrients are extremely low and phytoplankton growth during the summer is nutrient limited. From the spread of the pigment zeaxanthin it would appear that prochlorophytes, which thrive in low nutrient environments, dominate during the summer and autumn.

Moving northward off Ushant, coast populations are mixed and more variable due to the energetic tidal mixing. Here, diatoms and coccolithophores bloom in normal succession and dinoflagellates are seen in most seasons. Within the English Channel nutrients do not seem to be limiting as here sporadic populations occur throughout the summer. However in 2010, nitrate was depleted by the unusual occurrence of a bloom of the toxic dinoflagellate Karenia mikimotoi along the western Channel front. Such a bloom was also evident in 2003 but not in 2004 and its occurrence may be wind related as throughout much of the spring and early summer in 2010 and in 2003 the winds in the Channel remained southwesterly whereas in 2004 they were northwesterly. While various suggestions have been put forward as to the reasons for the infrequent occurrence of Karenia mikimotoi in the central English Channel none so far have been conclusive.

Interestingly the year-to-year variations in the central Bay of Biscay seem to be relatively minor, suggesting that this region is not influenced too much by changes in the NAO index. This is not true further north off Ushant and in the English Channel where stronger winds and hence more energetic mixing influence the occurrence and duration of the

blooms. The results from the PoB work have been written up and submitted to Deep Sea Research*.

Following the work on the Pride of Bilbao the NERC partners fulfilled their commitment to PROTOOL by making measurements on three oceanographic cruises to the North Atlantic; all in Case 1 waters. RRS Discovery cruise D365, Extended Ellett Line was primarily a hydrographic survey between 11 May and 2 June 2011 from Iceland to the UK, running south along 20µW and then across to Scotland. RRS James Cook JC62, Porcupine Abyssal Plain cruise took place 24 July – 2 August, 2011 from Falmouth UK to Cork, Ireland. Finally, RRS James Cook JC71, Porcupine Abyssal Plain between 29th April – 12 May, 2012 from Avonmouth, UK to Glasgow, Scotland. The three cruises were particularly targeted to represent northern and central North Atlantic regions and the final cruise was a repeat of the second to look at annual variation.

Using a NERC FastTracka FFRF and a PROTOOL PSI light source and flow through cuvette developed for the FastTracka by NIOZ, a PROTOOL type module was connected to the research vessels seawater supply. Continuous measurements were made using this arrangement in UK and international waters, with limited sampling in Irish waters during James Cook cruise 62 when permission was available. These measurements were augmented with pigment and taxonomy sampling from the sea water supply and also with the collection of samples at depth from a CTD rosette. Personnel from NIOZ joined NERC on the final cruise making measurements of primary productivity and additional FFRF measurements. In this way milestones 8.1 and 8.2 were achieved. The photosynthetic pattern observed (upregulation of the maximum rate of photosynthesis with maxima near midday) and occasional photoinhibition of the photosynthetic efficiency alpha during clear days resembled the pattern observed in the Baltic Sea, and were confirmed based on static measurements (measurements on single samples, thus not in flow through mode). A reflectance unit and the OSCAR were also used in flow through mode on the JC071 cruise to the PAP site, but data still have to be analysed. Fouling of the inner wall of the cavity did not seem to be a problem.

Conclusion. The scientific interpretation of the biological results is still at an early stage, but it is clear that throughout the open ocean North Atlantic blooms are patchy. During D365 phytoplankton activity was relatively high in the central regions of the Iceland Basin with chlorophyll –a concentrations approaching 4 mg m-3. Other dominant pigments in the Iceland Basin eg fucoxanthin, 19-hexanoyloxyfucoxanthin and 19- butanoyloxyfucoxanthin with concentrations up to 0.8 mg m-3, 0.5 mg m-3 and 0.8 mg m-3 respectively suggest the presence of diatoms and coccolithophores, but peridinin is low suggesting an absence of dinoflagellates. In contrast, results from the PAP site during both years suggest that phytoplankton activity in this area is low during the late spring and early summer, with chlorophyll-a concentrations less than 2 mg m-3. There was however some slight activity south of Iceland during JC71 and here dinoflagellates were the dominant species with cell counts approaching 0.05 x106 cells L-1. There was also a prevalence of diatoms at a single station off the coast of Scotland in the Northern Irish Sea with cell counts of 0.1 x106 cells L-1

- D. Smythe-Wright*, A. Daniel, S. M. Boswell, D. Purcell, M. C. Hartman, S. E. Hartman and D. J. Hydes. (2012). Biological regimes in the Bay of Biscay and English Channel. Submitted to Deep Sea Research.

WP9: conversion factors S&T results and foreground (WP9)

A core goal of PROTOOL is to implement autonomous high-resolution measurements of marine primary productivity (MPP) from seas and oceans. Fast Repetition Rate fluorescence (FRRf) is a powerful means by which this goal can be accomplished; FRRf measurements and implementation into oceanographic programs has already revolutionized the way in which MPP is quantified but suffers from two fundamental limitations: MPP is measured (i) relative to the number of 'photochemical reaction centers' and not relative to the volume of water sampled; and (ii) in a 'currency' of electrons evolved (the so-called electron transfer rate, ETR) and not CO2 fixed. Therefore conversion factors must be applied to all FRRf ETR measurements to yield measurements that are meaningful to stakeholders, e.g. fisheries and climate scientists, who need to know the mount of CO2 fixed by phytoplankton into organic matter per unit volume of water. Therefore, the fundamental aim of WP9 was to establish novel algorithms to enable implementation of these conversion factors.

To convert the ETR to CO2 uptake requires knowledge of the 'electron requirement for carbon fixation', which is the reciprocal of the Quantum Requirement for C-fixation (assuming 1 absorbed photon results in the production of 1 electron (ER(e,C), mol e-: mol CO2 uptake). Many studies in the past have attempted to reconcile independent measurements of ETR and CO2 uptake from the same water sample (and hence estimate ER(e,C)); from these exercises, values of ER(e,C) appear highly variable both within and between studies most likely as a result of taxonomic and environmental variability. Therefore, we implemented a parallel approach to (i) compile existing ER(e,C) data sets to develop predictive algorithms, (ii) collect new targeted and high resolution data to support past data sets, in particular for special marine regions (Baltic Sea and the Eastern Scheldt). In addition, support this approach with (iii) additional laboratory culturing of phytoplankton to experimentally identity the role of key factors, e.g. nutrient availability upon ER(e,C) to support interpretation of (i) and (ii).

A meta-data approach was used to evaluate the dependency of ER(e,C) upon 'easy to measure environmental variables', such as light, temperature, nutrients etc. (to establish algorithms that could then predict ER(e,C) from these variables. Data from 14 studies comprised of ten research cruises and four time series studies covered a broad range of different geographical areas of the world's seas and oceans, including the temperate, tropical and subtropical Atlantic Ocean, Massachusetts Bay (USA), Bedford Basin (Canada), Ariake Bay (Japan), the Celtic and Irish Sea, the North Sea, Gulf of Finland, the Baltic Sea, UK and European shelf waters and the Pacific Ocean. In fact much of this data was from the northeast European shelf and therefore directly relevant to PROTOOL's immediate goals. Once the data sets were integrated and treated for uniformity, ca. 350 data points were examined by a stepwise multivariate approach. Firstly, a simple correlative exercise for each separate study, which demonstrated that the relationships between ER(e,C) and environment were dependent upon how data within and between data sets were grouped; secondly, a principal components analysis, in combination with cluster analysis, to form clusters of sites with similar environmental conditions; and thirdly, multiple stepwise regression to evaluate the relationship between each cluster and the environment (and hence generate the required algorithms). This approach detonated that a global algorithm (all data together) could not be produced BUT that at the regional cluster level (11 clusters in all) could yield algorithms that could explain as much as 60% of the variance of ER(e,C) from measurements of specific environmental factors. As such, we have produced the first ever

algorithms to obtain CO2 uptake from ETRs at high resolution and, for several regions, with a high level of confidence.

In producing these novel algorithms, we were able to subsequently develop a simple program (.xls macro) whereby researchers could input environmental data for their region of interest and extract appropriate values for FE:C. An important part of this 'tool' is that it is can be continually (iteratively) refined as new data becomes available; as such, we also produced (i) the database on line with which the original algorithms for FE:C were developed and (ii) an optimized protocol for collecting FE:C data so that new (but standarised) information can be uploaded for to the database data. All of htrse various components represent a major step change in the use of FRRf that is unprecedented in its 20 year history. Importantly, we acknowledge that presently our algorithms are restricted to examining surface waters. Even though the FE:C database is constructed from data collected across different depths, data does not unfortunately exist to determine whether FE:C will be very different (and predictable) with depth within any given body of water that has structure, e.g. a subsurface nutricline. A conceptual exercise was therefore performed to evaluate the influence of water structure on application of values of FE:C. Overall, we found that use of a constant value for FE:C within a water column with a surface nutrient limited layer and a deeper nutrient replete layer may result in error of upto 10-15% in the depth integrated value of CO2 uptake. Clearly, this value is small but significant and therefore warrants further targeted attention in future studies.

Ability to predict ER(e,C) from environmental data is in part dependent upon the size f the available data set and thus a component of PROTOOL was to collect additional data for specific 'regions of interest'. Two regions were focused upon and both yielded novel insight. Several campaigns in the Baltic Sea/Gulf of Finland (2010-2011) resulted in ca. 70 new data points for FE:C across large environmental and taxonomic gradients. Our previous analysis demonstrated that ca. 25-40% of all variance for ER(e,C) could be explained by environmental condition for this region; therefore, we examined whether taxonomy may comprise a significant source of variance. Indeed, a comparison of ER(e,C) against a bio-optical signature of cyanobacterial abundance demonstrated that a further ca. 45% of ER(e,C) variance could be explained. This study was extremely important in demonstrating how current algorithms MAY need to build in taxonomic information to become more accurate. Monthly data was also collected throughout the project for the Eastern Schedlt since no data for this important region was previously available. Strong seasonal patterns for ER(e,C) were observed (with higher values for ER(e,C) being observed during most productive periods); however, surprisingly, a large proportion of variability of ETRs could be explained by 60-80% of the variance for CO2 uptake and hence a single value of ER(e,C) may be appropriate for this region. Such a magnitude of predictability is well within the levels of confidence returned by the more complex multivariate relationships between ER(e,C) and environment (above) when spanning wider marine regions but demonstrates that more simpler predictive algorithms may be feasible where regions are targeted and the data sets substantial. In addition to these campaigns, several phytoplankton species were grown in the laboratory to further examine the role of taxonomy (and the interaction of nutrient availability) upon regulation of FEC. These various data sets are still being processed and will be integrated into the ER(e,C) databases once completed.

In order to address whether the concentration of photochemical reaction centres per unit volume of seawater (=[RCII]) could be estimated, we took a two-step approach. Unfortunately, [RCII] is exceptionally difficult to measure since standard techniques are slow and require material in concentrations that vastly exceed what is found in nature (by several orders of magnitude); therefore past FRRf studies have assumed constant values for [RCII] for specific groups of phytoplankton. Measurements of [RCII] have shown these assumed constants to be highly erroneous. Initially, past measures of [RCII], largely derived from phytoplankton cultures were compiled into a database to determine whether [RCII] could be predicted from 'easy to measure' environmental variables (e.g. light, temperature), i.e. an analogous approach as for FE:C. However, [RCII] could not be confidently correlated with any variables (either in isolation or combination), except that higher values of [RCII] could be negatively correlated with cell size. In part this inability to 'predict' [RCII] likely reflects that few samples of direct [RCII] measurements exist. Therefore we secondly adopted an entirely new approach to attempt to derive [RCII] from FRRf induction parameters via first principles. This approach proved extremely successful:

A novel algorithm was generated that could predict [RCII] from measurements of the maximum and minimum fluorescnece yields and the effective absorption cross-section standardized to an instrument specific constant. Two past data sets of [RCII} measurements (one laboratory-based with high taxonomic diversity but constant biomass and one field based with high biomass diversity but constant taxonomic composition) and both demonstrated that this algorithm could explain 90% of variation of [RCII] measured conventionally. Importantly, the instrument-specific constant must be determined by calibrating the fluorescnece signals against conventional measures of [RCII] (as was performed for some of the PROTOOL instruments); once one instrument is calibrated, other instruments can be cross-calibrated based on factory settings of the excitation optics. Given that [RCII] can now be derived from FRRf, it was possible to incorporate the approach directly into existing algorithms to determine ETR (to result in a modified ETR that is per unit volume of seawater and not per [RCII]). This approach heralds a step change in the way in which FRR fluorometry can be used to measure primary productivity and inevitably will broaden the extent in which this technology can be applied to address research questions and management problems.

WP10. Intercalibration

This WP had the main task to carry out pigment calibration and a 'calibration' of the primary production measurements (it was not possible to send out a set of radioactive filters to also include a comparison of the radioactive counting efficiency as disintegrations per minute, but we expect this to an insigficant source of error. Because of the development of the new algorithms to obtain absolute rates of electron transport from the PROTOOL fluorometer a workshop was organised in June 2012, followed by a repeat of the exercise in August 2012. These data are not analysed yet.

Pigment calibration: rationale

Over the last half-century methodology for plant pigment analysis has moved from simple spectroscopic determinations to High Performance Liquid Chromatography (HPLC) coupled to diode array or mass spectrophotometric detectors. In spite of continuous developments in instrumentation, the analysis of phytoplankton pigments is still a challenge for HPCL techniques. The use of different chromatography columns eg C8 and C18

reversed-phase stationary phases, the method of pigment extraction from the plant material and the way in which the extract is clarified poses difficulties. In general, C18 columns exhibit shape selectivity whereas monomeric C8 columns show special selectivity towards compounds with subtle differences in polarity. Evidence now suggests that methanol or ethanol are better solvents than acetone and that extraction time should be at least 16 hours with ultracentrifugation used for clarification. Even when such parameters are the same there appears to be subtle differences in methodologies that result in variability in reported concentrations. In view of this it was felt important that PROTOOL had a good understanding of the deficiencies in pigment data from multiple sources. Many of the parameters and coefficients used in the PROTOOL calculations are normalized to Chlorophyll-a, consequently, any data used for their derivations and validation must be comparable. To this end WP10.1: pigment inter-calibration was included in the project. The aim was twofold.

Firstly, to document the pigment methods used by the partners using a simple questionnaire. Of particular interest was information regarding extraction solvent, filter disruption method and timing, ways of clarification and also the type of column and gradients used for HPLC analysis, all of which have been the subject of some debate. Secondly, to analyse known standard concentrations and field samples prepared and distributed by the NERC partner. Partners were not constrained to a specific methodology, as the idea was to see what level of variance occurred and subsequently agree the adjustments required to each partner's data to achieve a common baseline. The field samples were purposefully collected at temporally and spatially different sites so that a range of values for different phytoplankton groups was covered.

Results

Results from the questionnaire showed that although all partners were using similar HPLC methodology for the collection and harvesting of pigments, there were some slight variations with storage and solvent extraction and a number of differences in instrumentation. Generally, all partners were processing pigment samples by collecting water samples that were immediately filtered through GF/F filters and subsequently flash frozen with liquid nitrogen followed by storage at -80 C or alternatively storing directly at -80 C. The reason for this difference depended on the availability of liquid nitrogen at the field stations. Solvent extraction was either by 100% methanol or 100% acetone with the occasional use of 96% ethanol by 1 partner. Extraction times showed some variance. Four of the five partners left samples extracting for between 18 - 24 hours, while one partner extracted for just one minute with cell disruption. Three of the four partners who used longer extraction times also used sonification to disrupt the cells. Probably the most important difference was the use of C18 or C8 columns as this causes differences in separation efficiency and the possible mis-identification of individual pigments and their allomers.

Initially, just one cross calibration of known standards and field samples for a range of plant pigments was envisaged and this was done in late 2010/early 2011. The standard mixtures were prepared from DHI standards by pipetting known volumes of each of the individual standards into a vessel and making this up to 12 ml. An aliquot of this mixture was then diluted to make an intermediate standard and then further diluted to make one at a low level. From these three homogenous mixtures 10, 1 ml aliquots were prepared and each of the 5 partners received duplicate

samples of the three known concentrations. In this way it was possible to ensure that all partners received identical samples. The pigments chosen represented those that are commonly measured and/or those that are difficult to separate or have interferences. The field samples represented 3 replicates from 5 stations within the Baltic Sea and 13 samples from 7 depths (4.5, 9.5, 27, 40, 76, 101 and 125 m) from the PAP (49µN, 16.5µW) site (duplicates except for 27m). At first sight, results from this first exercise showed considerable scatter in the data, but it was possible to explain some of the variability and when this was taken into account the results improved considerably. For example, some reported chlorophyll-a results included divinyl chlorophyll-a, whilst others gave separate values for the two compounds. In addition some of the carotenoids were underestimated and it is possible that this is due to the way allomers of the different pigments are treated, in particular fucoxanthin which has two distinct allomers.

Unfortunately one partner was unable to participate in the first exercise due to instrument problems and while results for a range of different pigments were reported they were not necessarily the same pigments from all partners, which made comparison difficult. In addition, the concentrations of some of the pigments in the PAP samples were below detection limits for some of the partners. Consequently a second exercise, concentrating on a smaller number of pigments and using a fresh set of field samples, with somewhat higher concentrations than those distributed in first exercise, was undertaken in January 2012. Each partner received 8 samples for analysis, i.e. 3 prepared standards of high, intermediate and low concentration made from pure concentrations of DHI standards of Chlorophyll-a, Divinyl Chlorophyll-a, Chlorophyll-b and Fucoxanthin; 4 field samples; and a vial of DHI pigment mixture. The field samples (A-D) were taken from the PAP site in the North Atlantic at 49.751N, 11.701W on 25th July 2011. Samples A and B were from 20 m depth, sample C from 10 m and sample D from 35 m. These samples were 25 mm GFF filters through which 1.6 – 2.0 L of water had been filtered and the filters flash frozen with liquid nitrogen before final storage in a -80 freezer. All 8 samples were sent to the partners in dry ice at the end of January 2012.

The second exercise proved to be more conclusive. Results from the highest standard concentration showed fairly good agreement (except for divinyl chlorophyll-a) with much closer results for Chlorophyll-a than during the first exercise. For the intermediate standard it was clear that all laboratories were underestimating the concentrations by an average of 30 %; again divinyl chlorophyll-a was a problem. The lowest level standard was only reported by the NERC partner and here at only 25 -30% of the actual pigment value. For the other laboratories the values were below detection. This result is significant showing that none of the partners were able to measure low concentrations satisfactorily.

Conclusions pigment calibration

The conclusion from this work package is that the partners are able to measure chlorophylls a and b at the 50 ug L-1 level to a reasonable accuracy in known standard solutions. This is not true however for concentrations 1 and 2 orders of magnitude lower and this maybe due to extrapolation errors. All the PROTOOL partners produce standard calibration curves against which samples are compared and since inaccuracies occur at lower concentrations it is possible that non-linear calibration might be the cause. What is interesting is that in the second round errors do not appear to come form the use of C8 and C18 columns.

Three of the five PROTOOL partners use C8 (although only two report data here) in order to separating chlorophyll-a and divinyl chlorophyll-a and, where this is not achieved, it is to be expected that the reported chlorophyll-a data would be higher, but this does not seem to be the case. However, the finding could also reflect the generally lower values reported by the partner using the C18 column. The spread of the data from the analysis of the field samples was better in the second inter-calibration exercise. Generally speaking values for the individual pigments were within the same range but no conclusions can be drawn that one laboratory had consistently higher levels for all pigments than another. During both exercises the data for fucoxanthin was less than ideal and this needs some investigation.

Calibration of primary production measurements
This exercise was reported as deliverable D10.1a (and can be retrieved from the PROTOOL website). From this primary production exercise it could be concluded that the differences in calculation of daily primary production by the different participants were very small. Two major reason explain these (small) differences: the mathematical procedure used to fit the data (the algorithm used by the statistical packages used to fit the PI-data) and the fact that most partners did not correct for surface reflection of incident light. It is therefore suggested that all partners will adopt the same calculation procedure, and it has been included in the C14 database/fitting package.

Calibration of fluorometers
The development of the new Sigma and Absorption algorithms prompted us to organize a calibration workshop in June 2012 at the NIOZ. The main aim of this workshop was to obtain the instrument specific calibration factor KR (KR=[RCII]·sigma(PSII)/Fo·E(LED) where [RCII] is the concentration of the photosynthetic reaction centre units of PSII, Fo the minimal fluorescence, sigma(PSII) the functional absorption cross section and E(LED) the intensity of the measuring beam of the LEDs. When KR is known the absolute rate of ETR can be calculated without assuming a PSII concentration per mg of chla. More info is given in D9.22 and D5.17. However, because the workshop was so recent, not all information is processed yet, but we expect this to result in 1-2 scientific publications. Because there were some problems with the chla analyses (to be solved yet) we repeated in a small committee (NIOZ and K. Oxborough from Chelsea Instruments) some of the measurements. The obtained KR value for the Fasttracka-I, used in the NIOZ PROTOOL fluorometer baseline version was used in the calculations of the daily primary production estimates (see D5.17).

WP11: Data Centre and dissemination
The Data Centre had the main task to develop de website and the databases to store the results of the primary production measurements (14C and FRR fluorometer) and the Reflectance data. In addition dissemination was coordinated by this WP.

Website
Design and implementation of the website is completed, the website is online since February, 2010 and can be found at http://www.protool-project.eu. The restricted part is used by the partners for sharing documents concerning the PROTOOL project. We added a link to the PROTOOL blog on the home page, which contains the newsletters mailed to a selected party of possible interested persons.

Databases

The NIOZ developed two databases from which several fitting routines (R – scripts) can be accessed.

The 14C-database, developed in Microsoft Access is a database to deal with the raw data obtained from the 14C-measurements. It can use the scintillation counter data together with the necessary fieldparameters (temperature, chla concentration, total DIC, radioactive DIC added) and use these data to obtain the parameters which describe the shape of the photosynthesis irradiance (PI) curve (e.g the maximum rate of photosynthesis, the slope of the PI-curve, the light saturation parameter Ek). 4 different fitting routines are implemented (Eilers and Peeters 1988; Platt and Gallegos 1980; Platt and Jassby 1976; Webb et al. 2007). The database also holds the surface irradiance values (hourly averages) and light attenuation coefficients from these data, the PI parameters, the chla-concentration the hourly daily primary production is estimated at the different depth intervals (50 depth intervals between the surface and the depth to which 0.5% of the light penetrates are calculated). Summation over depth and time of day gives the daily primary water column production ('column production'), and by linear interpolation between measuring dates an integrated primary production over a set period can be obtained. If the morphology of the waterbody is taken into consideration (i.e. the surface are per depth interval) the primary production in the water column can be calculated taking this morphology into consideration as wel ('Komberg' method).

The FRRF/Reflectance database is developed in MySQL, but has a MS Access user interface. It contains both the TriOS sensor data to calculate the reflectance as the FRRF-data. The current input routine is targeted to the PROTOOL baseline fluorometer format, but we will change this to the other formats used as well.

The TriOS sensor data are first going through a set of filter criteria and those measurements which do not pass the filter are flagged. For the Dutch estuaries this is approx. 25% of the data. After passing through the data the reflectance is calculated and from the reflectance spectra the chla concentrations is calculated according to 4 different algorithm (the open ocean SeaWIFS algorithm (O'reilly et al. 1998), the Modis algorithm (Carder et al. 2003), the Gons algorithm (Gons et al. 2002) and the adaptive two-band algorithm from Ruddick (Ruddick et al. 2001). Only the Ruddick algorithm proved satisfactorily in the Dutch estuarine waters. We tried a Kd algorithm (Gons et al. 1998), but we were not satisfied with the result, so new ones are in development but not ready at the time of writing.

The FRRf-data from the FasTTracka-I fluorometer are imported in the MySQL database, and several queries are incorporated to retrieve the FvFm, sigmaPSII and other parameters from the measurements. The RLC data are fitted according to the new approach developed by PROTOOL (Silsbe and Kromkamp 2012) from which the parameters Pmax and alfpha are obtained. These can then be used together with the irradiance data and the Kd data to calculate daily primary production, using the Kolber & Falkowki, Sigma and Absorption algorithm (Oxborough et al. 2012). This package was used to create the data presented in D5.17 (field work Dutch estuaries).

At the moment the databases are still locally installed. An empty version with a test set of data will be made available via the website (on

request). Within half a year we plan to make the data publically available via a web interface.

Dissemination. The website gives information about the project, and on the results tab the reports and other outreach can be downloaded. PROTOOL also has its own twitter account (@protoolproject), although this operated at low key. In addition PROTOOL made a weblog for the newsletters, and these can be accessed via a link on the homepage http://www.protool-project.eu/blog/.

Cited references

Carder, K. L., F. R. Chen, Z. P. Lee, S. K. Hawes, and J. P. Cannizzaro. 2003. MODIS Ocean Science Team Algorithm Theoretical Basis Document. In M. Greenbelt [ed.], ATBD 19, Case 2, Chlorophyll a. Goddard Space Flight Center.

Eilers, P. H. C., and J. C. H. Peeters. 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecological.Modelling. 42: 199-215.

Gons, H. J., G. E. Edwards, and J. Kromkamp. 1998. Optical teledetection of the vertical attenuation coefficient for downward quantum irradiance of photosynthetically available radiation in turbid inland waters. Aquatic Ecology 31: 299-311.

Gons, H. J., M. Rijkeboer, and K. G. Ruddick. 2002. A chlorophyll-retrieval algorithm for satellite imagery (Medium Resolution Imaging Spectrometer) of inland and coastal waters. Journal of Plankton Research 24: 947-951.

O'reilly, J. E. and others 1998. Ocean color chlorophyll algorithms for SeaWiFS. J. Geophys. Res.-Oceans 103: 24937-24953.

Oxborough, K., C. M. Moore, D. J. Suggett, T. Lawson, H. G. Chan, and R. J. Geider. 2012. Direct estimation of functional PSII reaction centre concentration and PSII electron flux on a volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data. Limnology and Oceanography-Methods 10: 142-154.

Platt, T., and C. L. Gallegos. 1980. Modelling primary production., p. 339-351. In P. G. Falkowski [ed.], Primary productivity in the sea. Plenum Press.

Platt, T., and A. D. Jassby. 1976. The relationship between photosynthesis and light for natural assemblages of coastal maine phytoplankton. Journal.of.Phycology. 12: 421-430.

Ruddick, K. G., H. J. Gons, M. Rijkeboer, and G. Tilstone. 2001. Optical remote sensing of chlorophyll a in case 2 waters by use of an adaptive two-band algorithm with optimal error properties. Appl. Optics 40: 3575-3585.

Silsbe, G. M., and J. C. Kromkamp. 2012. Modeling the irradiance dependency of the quantum efficiency of photosynthesis. Limnology and Oceanography-Methods 10: 645-652.

Webb, E. A., R. W. Jakuba, J. W. Moffett, and S. T. Dyhrman. 2007. Molecular assessment of phosphorus and iron physiology in Trichodesmium populations from the western Central and western South Atlantic. Limnol. Oceanogr. 52: 2221-2232.

Potential Impact:

This chapter of the report discusses the potential benefits from the PROTOOL project, and has hence a science fiction character. Despite the fact that we cannot see in the future a few things are clear, which will be described below.

From previous contacts it was clear that the PROTOOL approach is welcomed by the HELCOM countries, and this interest was strengthened during the final end-user workshop organized by SYKE and NIOZ in Helsinki. Nevertheless it is uncertain at what rate our product will be implemented in future monitoring within the framework of the Marine Strategy Framework Directive, but one thing is clear: PROTOOL fulfilled its promise and developed a new method to measure primary production in an automated and autonomous was.

The PROTOOL project generated quite some interest in parties relevant responsible for water management of marine waters. Among these are the Centre for Environment, Fisheries & Aquaculture Science (CEFAS, UK), the Directorate North Sea (DNZ) of the Dutch Ministry for Infrastructure and Environment (RWS) and the 'Waterdienst' of RWS. DNZ has invited the coordinator as a partner in a preparative study on 'new and innovative monitoring strategies' which should lead to a new EU project proposal.

Implementation of the PROTOOL ideas has taken place in at least two projects:
- Within the frame work of the Sea and Coastal Research (ZKO) program of the Netherland Organization for Scientific Research (NWO) the PROTOOL approaches are adopted (slightly modified) by the IN-PLACE project which develops a coastal monitoring station/strategy.
- The Waterdienst from RWS asked the NIOZ to evaluate the PROTOOL approach as an alternative method to the standard way of measuring primary production in the monitoring program MONEOS, which is studying the changes in the Westerschelde as a result of dredging activities.

Economic aspects

PROTOOL developed new equipment. Really new are the OSCAR developed by TriOS optical sensors and the FFL040 fluorometer developed by Photon System Instruments (PSI).

The CEO of TriOS writes:
The sales perspectives for the new developed PSICAM (sold as OSCAR) have to be seen as very positive. The current design and functionality has an outstanding position in the world market, as there is no other commercial instrument available yet, which follows the PSICAM principle in a hyperspectral design.

Based on the fact that already more than 10 units are ordered from customers, the annual sales is expect to increase to 40-50 units a year within 2-3 years from now.

The key-market at the moment is scientific institutes and organizations, as well as marine monitoring applications.

TriOS is a leading supplier of instruments for this applications, the market entry of a new product is well known. The basic way is via conferences, workshops and mouth-to-mouth propaganda of satisfied customers. In addition the instrument will be presented in various exhibitions (like Oceanology International). Finally a wide sales network of international distributors will contribute to the sales as well.

In a further step, the new instrument will be made available also for industrial applications, like in chemical or pharmaceutical research and production. If these applications can be made successfully, the number of sold units can properly be doubled.

On a long-term run the successful marketing of the new PSICAM has positive impacts on the economic situation of TriOS and will lead into securing and generation of new qualified jobs inside the company but also at various suppliers.

The CEO of PSI writes the following about the newly developed PROTOOL fluorometer:
The PSI flow through fluorometer system FFL-2012 starts a new production line of monitoring fluorometers intended for the Gross Primary Production measurements. During the project a new design with additional functionality was developed and tested, which hadn't been yet on the market. There is no device on the market now, which offers such an FRRF measurement capability (multicolor) and online data availability (web server included as the one developed during the PROTOOL project). We aim to acquire 25% of the European market share of ferry box flow-through instrumentation within the next 5 years (200,000 EUROS/year). We expect an increase of 2 employees during next year in our production unit and 1 research staff for the further development. The device will be also presented on conferences worldwide to interest new customers all around the world.

There is a potentially large market for a miniaturized version of the FRRF fluorometer for installation on buoys, gliders and monitoring stations. We want to apply a registered design intellectual property right on our design and software.

These two statements clearly show that PROTOOL created economic success, and a future PROTOOL-2 is expected to do the same as the consortium has already plans for a follow-up and like to work on miniaturization of the PROTOOL equipment and develop similar equipment for the platforms mentioned by PSI (buoys, gliders and fixed monitoring stations).

The third piece of equipment developed, the Reflectance module was less innovative and dealt with a reconfiguration of existing equipment, although SUKY developed a positioning sensor platform, but at the moment no plans exist to market this. The hardware description (excluding sensors) and software for shipborne reflectance measurements are open source resources hosted at http://sourceforge.net/projects/rflex/. However, the R-module is a very valuable addition to our monitoring equipment, and when widely adopted it can be a the reason for a surge in sales of the TriOS RAMSES sensors which form the hard of the R-module.

We expect that the science produced by PROTOOL will have socio-economic impact: The generation of a new algorithm to quantify the concentration of photochemical reaction centres, [RCII] (and so in turn an '[RCII]-free ETR algorithm') as well as region-specific algorithms to convert ETRs into CO2 uptake rates will radically transform the nature and scale of FRR flourometry in aquatic sciences. These developments represent THE long awaited breakthroughs for the field and enable ETRs to be measured free from the practical constraints that have for so long limited widespread implementation of FRR fluorometers as 'productivity sensors'. Benefits include generation of higher resolution (and hence more accurate) marine primary productivity (MPP) estimates that can be used to improve the accuracy of forecasts of environmental change upon MPP though ecosystem models. More accurate MPP estimates also promise to improve remote sensing algorithms that provide real-time broad scale estimates of MPP.

Such improvements in capacity to measure and predict MPP have broad societal implications but importantly, we see such improvements absolutely fundamental in driving the future market of FRRf technology for MPP-based studies. The limitation is no longer on the inherent assumptions to the algorithm but on the technology. Variants of FRR fluorometers have already been developed (e.g. the PSI fluorometer as part of PROTOOL) to enable quantification of the contribution of different functional groups to be determined. Discussions are also now underway to develop this technology to examine MPP from benthic systems. Ultimately, such developments and the widening of the FRRf market(s) will require that instruments become smaller, more power efficient and able to transmit data wirelessly.

Steps towards more widespread implementation have already begun by cross calibrating FRR fluorometers in use at sea (e.g. Arctic, Australia, and Antarctic waters) with those calibrated as part of PROTOOL. Similarly, we have begun to explore the possibility of applying an analogous approach in generating conversion factors for ETRs to CO2 uptake (and hence E:C) for freshwater ecosystems. Adoption of FRR fluorometers in freshwaters for evaluating ecosystem health and primary productivity has not been as rapid as for marine systems despite the huge dependence by society upon freshwater health and availability. It is likely that a different dedicated set of algorithms for E:C will be required given that environmental control of primary productivity in freshwaters is very different as for marine systems; thus further development of application of PROTOOL for lake systems presents a significant future opportunity.

Wider implications with regard to the development realized by the PROTOOL project

WP10 contributes to the international debate on pigment analysis. There has been some concern about the inter-comparibilty of pigment data over recent years, in particular the need for a robust data set for in situ calibration of ocean colour data. The compilation of information from further inter-calibration exercise will help analysts unravel the inconsistencies in reported pigment data and help develop single methodologies for individual pigments and water bodies.

WP8 provides comprehensive pigment and taxonomy data for the Bay of Biscay and the North Atlantic. Data for these regions is relatively sparse and this high resolution data over three years provides information on seasonal and annual variability. The data will be available to the international community and will be useful for scientific studies of changes in phytoplankton diversity and phenology and also for the in situ calibration of ocean colour satellite observations.

FRRF methodology in fresh water research: A Czech perspective from a Czech member of the PROTOOL consortium

For the technological development of the monitoring systems in The Czech Republic the PROTOOL approach was the main missing part in the information about hydrobiology of the water resources and standing water bodies in Czech Republic. The C14 primary production data are only scarce and done on specific places for scientific purpose only. The PROTOOL approach would make it easy to perform primary production measurement for routine monitoring, and because it can operate autonomously it does not require great man power to obtain and store the data.

The application of the methodology in our freshwater resources would change the monitoring abilities not only to scientists following the C14 production information, but implementation in the national network of the lakes ponds and standing waters primary would yield the necessary primary production information hardly needed. Although the network of the data for our water resources exists at the Hydrometeorological Institute, unfortunately the primary production measurements are missing there.

For the hydrobiology scientists in the Czech Republic it would mean to start manage the databases and interpret the data instead of harvesting them by not very efficient C14 method producing radioactive waste. Nevertheless we are not there yet and large amount of the work on conversion factors for freshwater algae has to be done for direct application of the FRR fluorescence methods to the freshwater habitats.

This perspective voiced by a Czech member of the PROTOOL consortium is valid for most EU memberstates.

PROTOOL in a global context

Not only monitoring agencies can use the PROTOOL approach to get the necessary information needed for evaluating the ecological status of their seas. Phytoplankton plays a crucial role in the global C-cycle. Current estimates state that phytoplankton is responsible for about 50 Gton of CO_2 net primary production: about 50% of the total global fixed CO_2 is fixed by photosynthetic organisms, despite the fact that this is realized by less than 1% of the living C on Earth. This implies that phytoplankton is not a good indicator of primary production because of the rapid turnover times, a fact established by several studies. This

fact also causes a large uncertainty in the estimates of net phytoplankton primary production (NPP). The estimates of NPP by phytoplankton vary twofold from ~35-75 GtonC/year. Part of this uncertainty lies in the fact that the phytoplankton biomass estimates from optical remote sensing (which use chlorophyll-a as proxy) is still difficult, especially in the more turbid case-2 waters. But more important is that no primary production measurements are available. Only two permanent monitoring stations exist: the HOT (Hawaiian Ocean Time Series) in the Pacific ocean in the vicinity of Hawai and BATS (the Bermuda Atlantic Time Series).

Both sites reside in the ultra-oligotrophic region of the ocean, whereas most NPP takes place in the temperate oceans and coastal seas. Hence, NPP estimates are based on satellite based estimates of chla and a primary production algorithm which normally fits the rate of photosynthesis as a high order temperature polynomial. No corrections are made for nutrient or high light stress, changing taxonomic composition etc. Until more frequent measurements are made the global estimates of C-fixation by phytoplankton will contain a large margin of error. Because part of the CO2 fixed by the phytoplankton sinks to the ocean floor, algae remove atmospheric CO2 for thousands of year (this process is called the biological pump). The biological pump causes slows down the rise of global warming, a reason why large scale fertilizations geoengineering plans exist, which might be disastrous if carried out at large scale because the effects of the foodweb are unknown and might cause a collapse of the foodweb: imagine the disaster if krill stocks would collapse! Hence, more monitoring of phytoplankton primary production is necessary, and this can be done by placing our equipment on ships of opportunity.

The potential to use ships of opportunity is also recognized by the Scientific Committee on Oceanic Research (SCOR), a global NGO of scientists working on the oceans (see http://www.scor-int.org/ online). They developed the program OceanScope in which they set out a roadmap to use ships of opportunity to sampling platforms. The complete report can be found at:
http://www.scor-int.org/Publications/OceanScope_Final_report.pdf

In the Preface they state:
Over the last several decades oceanographers of all disciplines have noted significant changes taking place in the marine environment: steady increases in upper ocean temperature, major shifts in plankton distributions, increases in dissolved CO2 and acidity and possible circulation changes. These and other ocean issues were the subject of major reviews at the OceanObs'09 conference in Venice, Italy on 21-25 September, 2009 (see http://www.oceanobs09.net/). There was general agreement that the ocean, especially the water column, continues to be severely under-sampled. Calls for new and better sensors and measurement strategies, along with closer coordination among the many different ongoing ocean-observing programs, were strong recurring themes.

OceanScope sets out a roadmap to setup a large network of commercial ships equipped with ferryboxes, and as commercial ships visit a significant fraction of the ocean on a yearly bases with high frequency, although activity is less in the southern hemisphere.

The OceanScope Vision is phrased as follows:

'In partnership with the maritime industries we will develop an integrated approach to observation of the global ocean on a regular and long-term basis as an essential component of, and contribution to, the Global Ocean Observing System (GOOS). This activity, 'OceanScope' will equip commercial ships with fully automated unattended instrumentation to accurately measure and report upon the currents and the physical, chemical and biological characteristics of the water column throughout the world ocean. The freely distributed data generated will be a fundamental resource for understanding the climatic state and health of our planet.'

OceanScope will, if they can organize the funds, start with a 5 year pilot in the North Atlantic Ocean. Denise Smythe-Wright is partner of OceanScope and this program also mentions the use of active fluorometers in order to obtain photosynthetic information. PROTOOL will now make this possible, and a PROTOOL-II should be considered in the context of OceanScope, and can advance the impact of OceanScope significantly. If PROTOOL can be adopted in OceanScope, and if OceanScope can be realized, it will revolutionize our knowledge of phytoplankton primary production and obtain data allowing a much better estimate of the activity of biological pump, and hence make IPCC assessments of global change far more accurate.

There remains one item unresolved however: from our high resolution PROTOOL measurements in both time and space on the north-east Atlantic and the Baltic Sea it became obvious that large daily fluctuations in photosynthetic activity exist. A next PROTOOL should also focus further miniaturization so that we can perform high resolution measurements on buoys, gliders etc so that this phenomenon can be better understood and be accurately modeled. In addition additional information is needed to improve the accuracy of our algorithms to predict the quantum requirement for C-fixation.

To summarize: although PROTOOL was a relatively small consortium, it made significance advancements in both the development of autonomous measuring devices to measure photosynthetic activity of phytoplankton as well as scientific breakthroughs in measuring methodology and understanding of quantum requirements. But a follow up is needed to complete our work.

List of Websites:

http://www.protool-project.eu