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Intercomparison of biogeochemical sensors at ocean observatories

M Mowlem, S Hartman, S Harrison and K E Larkin

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Abstract:

Fundamental to integration of observing systems is an understanding of the current and future requirements of sensors to measure biogeochemical processes, particularly for sustained autonomous data recording.

Long term unattended operation of sensors is challenging for current biogeochemical sensor technology. This report gives an overview of the current state of the art in biogeochemical sensing. It reviews user experience of deployed systems and efforts made to provide quality assessment and control. Recommendations are made regarding what sensors should and should not be used in the immediate future for Eulerian observatories. Promising emerging technologies are summarised, and detailed methodological practices that should lead to improved technology and scientific data are outlined. Finally additional recommendations are made for raising the Technology Readiness Level (TRL) of biogeochemical sensor technology for this application.

Inter-comparison of biogeochemical sensors at ocean observatories

Matt Mowlem[1], Sue Hartman[2], Stephen Harrison[1], Kate E Larkin[2]

1 Forward

1.1 Terminology

Due to the conflicting use of terminology used to describe the performance of measurement systems, and biogeochemical sensors in particular, the terms used in this report are defined here. These definitions are drawn from [1, 2] and [3] and references therein.

Measurand:

Is the quantity intended to be measured.

Resolution:

Is the smallest change, in the value of a quantity being measured by a measuring system that causes a perceptible change in the corresponding indication.

In practical terms, for measurement systems with a quantised limit (e.g. a meter with a display of limited digits, or a ruler with finite graduation size) this is the size of the smallest unit on the scale.

For noise limited systems (i.e. the noise in the measurement is greater than the quantisation limit) the resolution is defined as the probability that the "true" value is within a specified range of the measured value to a measured degree of confidence. Typically the degree of confidence is 68%, and therefore the resolution is one standard deviation of the noise. Repeated measurements improve the resolution (as results may be averaged) as the square root of the number of samples. Therefore in time based systems it is standard practice to quote the noise limited resolution as a value per root hertz to enable comparison of systems of differing sample rate.

Precision:

Is the closeness of agreement between quantity values obtained by replicate measurements of a quantity, under specified conditions.

Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement. Again to allow comparison this can be quoted as a value per root hertz.

Accuracy
Is the closeness of agreement between a quantity value obtained by measurement and the true value of the measurands.

The value quoted is usually the difference between the mean of repeated measurements, and the true or reference value.

[1] Sensors Development Group, Underwater Systems Laboratory, National Oceanography Centre Southampton
[2] Ocean Biogeochemistry and Ecosystems National Oceanography Centre Southampton

Sensitivity

Is the quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured. E.g. nM/volt

Repeatability

Is the measurement precision under repeatability conditions of measurement, typically including the same measurement procedure, same operator, same measuring system, same operating conditions and same location, and replicated measurements over a short period of time

Reproducibility

Is the measurement precision under reproducibility conditions of measurement, typically including over different times, locations, operators, and measuring systems

Drift

Is the change in the indication of a measuring system, generally slow and continuous, related neither to a change in the quantity being measured nor to a change of an influence quantity

Limit of detection

Is the value of the measurands at which the probability of detection reaches a specified level of confidence. In practice this is usually taken as three times the standard deviation at blank concentrations.

Limit of quantification

Is the value of the measurands at which a quantification can be made to a specified level of confidence. In practice this is usually taken as ten times the standard deviation at blank concentrations.

Technology readiness level (TRL)

"The TRL approach ascribes descriptive phrases to the stages of technology readiness. As such it provides a consistent framework against which to assess current availability, and to help identify the work needed to raise the TRL to 8/9, in this case the level needed for long-term use in the deep ocean"[3]

Table 1 Technology readiness levels (from [3])

1	Basic principles of technology observed & reported
2	Technology Concept and/or Application Formulated
3	Analytical and Laboratory Studies to validate analytical predictions
4	Component and/or basic sub-system technology valid in lab environment
5	Component and/or basic sub-system technology valid in relevant environment
6	System/sub-system technology model or prototype demo in relevant environment
7	System technology prototype demo in an operational environment
8	System technology qualified through test & demonstration
9	System technology 'qualified' through successful mission operations

1.2 Nomenclature

ACT	Alliance for Coastal Technologies
ANIMATE	Atlantic Network of Interdisciplinary Moorings and Time-series for Europe
ARGO	A profiling float array http://www.argo.ucsd.edu/
BB3	basic blue 3
CDOM	chromophoric dissolved organic matter
CHARM	Channel Adaptive Re-locating Mooring
CTD	Conductivity Temperature and Depth
DI	Distilled
DIC	Dissolved Inorganic Carbon
DMS	Dimethyl Sulphide
DOM	Dissolved Organic Matter
DPA	Deep-sea Probe Analyzer
ESONET	The European Seafloor Observatory Network
ESP	Environmental Sample Processor
EU	European Union
Eur-Oceans	European network of excellence for OCean Ecosystems ANalysis
EuroSITES	European Deep ocean observatory Network
FRRF	Fast Repetition Rate Fluorometry
GNP	Gross National Product
HOT	Hawaii Ocean Time series
HPLC	High Pressure Liquid Chromatography
ISUS	In-Situ Ultraviolet Spectrometer
LED	Light Emitting Diode
LOD	Limit Of Detection
MARS	Monterey Accelerated Research System
MBARI	Monterey Bay Aquarium Research Institute
MODIS	Moderate Resolution Imaging Spectroradiometer
NEPTUNE	North-East Pacific Time-Series Undersea Networked Experiments

NERC	Natural Environment Research Council
NOAA	National Oceanic and Atmospheric Administration
NOC	National Oceanography Centre, Southampton
PAP	Porcupine Abyssal Plain (deployment site)
PAR	Photosynthetically Available Radiation
PP	Primary Production
RNA	Ribonucleic acid
SeaWiFS	Sea-viewing Wide Field-of-view Sensor
SOO	Ship Of Opportunity
TRL	Technology Readiness Level
UV	Ultra Violet
VENUS	Victoria Experimental Network Under the Sea

2 Introduction

2.1 Project Brief

EUR-OCEANS WP2.1 is tasked with integration of observing systems. Fundamental to this is an understanding of the current and future requirements of sensors to measure biogeochemical processes particularly for sustained autonomous data recording. A technologist with knowledge of ocean-going sensors was required to compile a report on the performance of biogeochemical sensors during long-term deployments. Through discussions with users this 2-4 month study will establish the present use and performance of sensors and make comparisons with other data bases such as ACT (Alliance for Coastal Technologies) identifying routes to increase TRL (Technology readiness Level) to meet user requirements. The results will be presented in a report to the EUR-OCEANS Steering Committee and will form the baseline for a sensor workshop coordinated through EUR-OCEANS in spring 2008

2.2 EurOceans long term monitoring programmes

The overall scientific objective of EUR-OCEANS is to develop models for assessing and forecasting the impacts of climate and anthropogenic forcing on food-web dynamics (structure, functioning, diversity and stability) of pelagic ecosystems in the open ocean. WP2.1 aims to integrate the existing European deep ocean observational capacity in order to produce a more reliable network and hence to foster model development and validation.
Crucial to these aims is the availability and quality of biogeochemical data. The practice with the EurOceans network, and in related observing networks (e.g. ANIMATE, M3A and EuroSITES) of long term unattended operation is challenging for current biogeochemical sensor technology.

2.3 Aims

This report gives an overview of the current state of the art in biogeochemical sensing, reviews user experience of deployed systems, summarises efforts to provide quality assessment and control, and makes recommendation for the use and development of technology for this application.

2.4 Summary of conclusions

Biogeochemical sensing in Eulerian applications is a logistical, technological, and scientific challenge and is at the edge of what is currently routinely achievable. To make advances in this field three areas need to be addressed. Firstly the technology readiness level of biogeochemical sensors needs to be raised. Secondly the long term drift, and performance

degradation of sensor systems with time needs to be evaluated and reduced. Thirdly the effects of biofouling must be mitigated

Many biogeochemical parameters cannot yet be characterised to the performance required by the science community. Though a number of promising technologies are in development a critical re-evaluation of the performance requirement, and priority measurands would enable more rapid progress.

Biogeochemical sensing is important in answering key scientific questions including the role of the ocean in global carbon cycles and climate change. A list of key biogeochemical parameters and required measurement performance is presented.

A review of commercial technologies identifies numerous devices, some of which meet the performance targets required for this application. These are oxygen optodes and the latest electrochemical oxygen sensors; reagent based nutrient analysers, a CO_2 sensor, and pH sensors.

Of the emerging technologies, microfluidic analysers are promising, as are: optodes for CO_2, pH, and methane; Fast Repetition Rate Fluorometry (FRRF) with additional reference measurements; *in situ* biomolecular analysers; and cytometers.

Biofouling remains a significant issue for most if not all sensors used in long term deployments. However, some success has been gained using copper and mechanical wipers in this context. The evaluation of sensor technologies concludes that oxygen sensors are now mature enough to be deployed routinely. Reagent based CO_2 sensors offer good long term performance, though they are currently large and have minor robustness and reliability issues, and reference measurements (for *in situ* calibration) are prone to error. Reagent based nutrient sensors offer high performance but remain at a low TRL. Spectrophotometer based nitrate sensors are promising, but currently exhibit too much drift and / or sensitivity to non nitrate associated optical changes to be considered accurate for long term applications. The practice of relating fluorometer measurements to chlorophyll concentration for long term deployments is extremely inaccurate, and should be discouraged, unless frequent calibrations are made using chlorophyll extraction (and analysis e.g. HPLC) to account for changes in community structure and physiology.

The activities of the Alliance for Coastal Technologies are reviewed. Whilst the ACT technology evaluations provide a useful resource, the performance target is not necessarily the same for coastal and oceanic Eulerian observatories. ACT does not specifically assess sensor drift, repeatability or reproducibility as required in Eulerian applications. This must be done independently by long term lab trials, and with testing in a range of water types.

To conclude recommendations are made regarding what sensors should and should not be used in the immediate future for Eulerian observatories. Promising emerging technologies are summarised, and detailed methodological practices that should lead to improved technology and scientific data are outlined. Finally additional recommendations for raising the TRL of biogeochemical sensor technology are made.

3 Biogeochemical sensing state of the art

3.1 Research and international context

The oceans play a crucial role in the prosperity and future of civilisation. They provide essential natural resources such as fish, minerals, offshore energy and a route for global transport of goods and resources. Natural biogeochemical cycles in the oceans, provide "ecosystem services" valued at US$19 trillion p.a., equivalent to the global GNP [4]. The oceans play a key role in climate regulation [5] arguably the most important environmental issue facing mankind [6].

In situ marine biogeochemical sensing is required to enable study of the biogeochemical processes, cycles and feedback mechanisms that enable and regulate the oceans role in climate and the availability of natural resources. This data is essential for the creation, testing

and validation of models of biogeochemical systems, which are required to enable prediction of the extent and effects of our changing environment.
Despite their global importance, the vast (1.3×10^9 km^3) oceans remain largely under-sampled (in both space and time). Key biogeochemical processes can exhibit variations of two orders of magnitude on hourly and metre scales [7] whereas current subsurface sampling with few isolated exceptions occurs on annual and kilometre scales. The oceans are opaque to electromagnetic radiation, which precludes the use of remote sensing beyond the surface. Water sampling is sparse, costly (~15k/ship/day), prone to contamination, unwanted processing and aging of the sample. *In situ* sensors and sensor networks have been identified by international consensus[8] as offering the solution to this under-sampling.
Eulerian observatories (see § 4) offer a number of advantages because they operate from a fixed location. This enables: large infrastructure which can support larger, more numerous, and more power hungry sensor payloads; the possibility of recovery / maintenance; and permanent telemetry links enabling real time data delivery.
Physical sensors exist for measurements of ocean temperature, pressure and salinity, and are rapidly reaching a mature stage in development. In contrast, biogeochemical sensors are in their infancy and are dominated by large macro (~0.5m^3), expensive (£10-100k) one-off devices requiring expert operation (and intervention) [9, 10]. It is this lack of biogeochemical sensor maturity that necessitates this study.

Long term *In situ* biogeochemical sensing in the marine environment is challenging. Not only is the environment hostile (e.g. remote, dark, corrosive, biologically active (resulting in biofouling), and characterised by a large range of temperature and pressure) but high levels of precision and long term performance are required. To complicate matters seawater is a complex and variable soup of chemical compounds and biological species. Many of these variable parameters interfere with measurement techniques that otherwise perform adequately in simpler solutions in the laboratory. To compound matters many of the parameters of interest are present in very small concentrations (e.g. nutrients are present in nM concentrations in open ocean oligotrophic waters). These low levels must not only be detected, but *quantified* with a high degree of confidence. Table 2 gives approximate ranges of the concentrations of selected chemical parameters of interest and indications of the resolution and accuracy currently achieved in the laboratory for estuarine, coastal and oceanic target environments. Sensing technologies must at least have limits of detection applicable to one of these environments, and resolution of at most 2% of the range of concentrations presented. Estuarine environments frequently present the highest concentrations, making sensor design for this application easier. Preferably sensors for a given parameter should be able to operate over the range of concentrations present in *all* target environments, *and* have resolution less than 2% of the smallest range. This challenging performance target requires careful transduction method selection – many published and commercialised techniques are orders of magnitude short of this target.

Table 2 Target analytes (Data from [11-17])

		Range	**Lab resolution**		**Range**	**Lab resolution**
Nitrate	Surface ocean Deep ocean	0.1 – 2.5 µM 2 – 40 µM	0.01 µM	Estuarine Coastal	10 – 400 µM 0.1 – 80 µM	0.1 µM
Nitrite	Surface ocean Deep ocean	0.1 – 200 nM 0.1 – 5 nM	0.1 nM	Estuarine Coastal	0.5 – 1.5 µM 0.1 – 2 µM	0.01 µM
Ammonia	Surface ocean Deep ocean	0.05 – 1.5 µM 0.02 – 0.05 µM	0.01 µM	Estuarine Coastal	0 – 600 µM 5 – 30 µM	0.1 µM

Phosphate	Surface ocean Deep ocean	0.02 – 0.20 μM 1 – 3.5 μM	0.001 μM	Estuarine Coastal	0.5 – 3 μM 0.02 – 1.5 μM	0.01 μM
Silicate	Surface ocean Deep ocean	0.1 – 3 μM 3 – 200 μM	0.01 μM	Estuarine Coastal	10 – 75 μM 0.1 – 35 μM	0.1 μM
DOC	Surface ocean Deep ocean	35 – 150 μM 4 – 75 μM	0.1 μM	Estuarine Coastal	200 – 2000 μM 60 – 200 μM	0.1 μM
DON	Surface ocean Deep ocean	4 – 10 μM 1 –2 μM	0.1 μM	Estuarine Coastal	15 – 160 μM 4 – 60 μM	0.1 μM
[3]**'dissolved' Fe**	Surface ocean Deep ocean	0.02 – 2.5 nM 0.4 – 1 nM	0.01 nM	Estuarine Coastal	0.7 – 1.5 μM 0.1 – 1 nM	0.01 μM
CH_4	Surface ocean Deep ocean	2.5 – 4 nM 0.5 – 4 nM	0.1 nM	Estuarine Coastal	0.01 – 1.4 μM 0.1 – 0.6 μM	0.01 μM
O_2	Surface ocean Deep ocean	120 – 190 μM 50 – 140 μM	1 μM	Estuarine Coastal	50 – 200 μM 30 – 100 μM	1 μM
pH	Surface ocean Deep ocean	7.60 – 8.00 7.40 – 7.55	0.001	Estuarine Coastal	3.00 – 6.50 7.70 – 8.15	0.001
TCO_2	Surface ocean Deep ocean	1900 – 2200 μM 2300 – 2400 μM	10 μM	Estuarine Coastal	1600 – 1900 μM 2000 – 2250 μM	10 μM

To accurately predict the roles of biogeochemical cycles in climate change and resource management, models are required that predict primary production (PP) and carbon sequestration and gas (e.g. CO_2 and Methane) flux. Chemical and Photosynthetically Available Radiation (PAR) data provides forcing and boundary conditions for these models, but to increase accuracy, data on biological function is also required.

PP may be measured directly using ^{14}C [18] oxygen isotope ratios [19] (and in addition oxygen/ Argon ratios[20]), and optical fluorescence [21] which though prone to error (see § 5) is possible *in situ*. Sequestration can be estimated from analysis of marine snow [22] or using measurements of water column chemistry [23]. Gas fluxes may be estimated from eddy correlation but these atmospheric measurements are beyond the scope of this work.

An alternative approach is to study organisms at the base of the marine productivity chain (i.e. Phytoplankton). Phytoplankton are central to marine biogeochemical processes, and cause harmful algal blooms effecting, the environment, tourism, and the marine economy. They are taxonomically and functionally diverse. They exist in a wide range of sizes (1 μm to 200 μm) and have important biogeochemical characteristics that vary taxonomically. Diatoms have hard siliceous shells (frustules) that make them heavy and so are likely to sediment rapidly, removing silicon and carbon from surface waters. Coccolithophores have high calcium content and so contribute to rapid dumping of organic matter and calcium into the oceans' interior. Prymnesiophytes generate dimethyl sulphide (DMS) a volatile gas involved in cloud formation; affecting the Earth's albedo; and hence climate control. There is further variation within species due to genotype and physiological and environmental factors. Therefore,

[3] 'Dissolved' Fe is generally measured rather than Fe(II) and Fe(III) directly, because of the unstability of Fe(II) in the oxic environment. 'Dissolved' Fe is an operationally defined parameter and is that which passes through a 0.2 or 0.4 μM filter

modelling the oceans' role in fuelling marine food webs and in controlling climate would benefit from measurement of phytoplankton taxonomy, and physiological function. Phytoplankton density ranges from extremely dilute (a few cells per litre) to in excess of millions of cells per litre in blooms. From a sensing perspective, a resolution in the order of ten cells would enable prediction of blooms and study of dynamics in biogeochemical cycles.

The list of desired parameters is extensive. In contrast the development of sensors to meet this need is laborious, and a significant research challenge. There are currently very few commercial or prototype systems available to measure even a small subset of this (incomplete) list. Therefore, prioritisation of targets, and coordinated (national and international) development is required. *Both* aspects should direct further developments in the field of sensor development and deployment.

There is no single analytical method that can provide measurement of all scientifically significant biogeochemical parameters to the required performance. Though many analytical methods (e.g. mass spectroscopy, Raman spectroscopy, Laser induced break down spectroscopy) offer multi-parameter sensing with mixed performance, it is currently unrealistic to propose their use on all but the largest and most regularly serviced of marine platforms, and thus their impact will be limited for the foreseeable future.

3.2 Commercial technologies

The most developed and miniaturised biogeochemical sensors suitable for use on long term observatories are electrochemical [24] and optical [25] oxygen sensors. The latter is particularly suitable for autonomous operation due to long term stability. Experience with these sensors in the oceanographic community is growing (see §5) with performance proving to be closely matched to initial assessments[26] [27]. These are perhaps the only biogeochemical sensors at TRL9. The documented disadvantages of the commercially available systems (Aanderaa) are the long rise time (poor frequency response) which is in the order of 25 seconds (though this is sufficient for most Eulerian applications) and the current lack of biofouling prevention.

Larger, more costly, and more complicated systems include an *in situ* UV spectrophotometer that measures nitrate [28] (ISUS) produced by Satlantic (see§5.1.4.2) which offers detection without reagents but is power hungry and relatively insensitive (~0.5μM, or 20% of open ocean maximum concentrations). Reliability and calibration problems have occurred suggesting a TRL of 8. A number of reagent based nutrient sensors are available commercially (e.g. SubChem [29], NAS-3X (EnviroTech LLC) and DPA (Systea)). Carbon dioxide sensors using a renewed reagent have been commercialised, and offer high accuracy long term operation [30]. Reagent based sensors produce high accuracy (e.g. 0.1μM nitrate resolution) measurements of a wide range of parameters (nitrate, nitrite, phosphate, ammonia, iron, and silicon), but are large (e.g. DPA is 10kg *excluding* reagents), expensive and complicated (and often temperamental) devices requiring expert operation and calibration (TRL 7-8). The maximum deployment duration of these large instruments is ~ six months (nutrients) or 1 year (CO_2). Recent developments in microfluidic technology have enabled a phosphate sensor with excellent reported performance (50nM resolution) [31].

A number of commercial electrochemical systems have been developed (e.g. by Unisense (O_2, H_2, H_2S, N_2O, pH and eH) e.g. [32], Seabird (O_2 and pH) [24], and Idronaut (trace metals (with a large system ~ 1m, 8kg)[33], O_2, and pH). Systems based on voltammetry for use in areas of high concentration (e.g. pore waters and hydrothermal vents)[34] have been commercialised, though performance is mixed (TRL 7) this technique offers the possibility of simultaneous determination of more than one chemical species. With the exception of the

Seabird O_2 sensor (2% drift in 1000hrs and hence TRL 8-9), little data is presented to demonstrate long term stability, though mitigation and reduction methods are in development, this issue and difficulty in obtaining high resolution, currently prevents use on many long term autonomous deployments.

Commercial systems for methane[35, 36] and carbon dioxide[37] measurement exist based on detection in the gas phase (hence requiring membranes and gas processing hardware) using both IR[35, 37] and semiconductor[36] (for methane) sensing (the latter suffers from lack of accuracy, and long recovery times [38]). The use of detection in the gas phase makes these devices somewhat complicated. For example sensor response times are extended (particularly for deep applications where a thicker inlet membrane must be used). Though these devices are reliable performance difficulties suggests a TRL of 7.

A number of systems for measurement of chlorophyll fluorescence exist (e.g. Chelsea Minitracka, Wetlabs FLNTU, and Turner Designs Cyclops). Despite the accuracy of these instruments in measuring the optical properties of seawater (TRL 9), prediction of chlorophyll concentration, and primary production from these parameters remains a challenge because of physiological and community structure variability in phytoplankton [39]. Fluorometers (e.g. Chelsea AquaTracka) have also been developed to track hydrocarbons and Gelbstoff. Commercial cytometer systems [40] though large (20kg) show promise for quantitative, and specific identification of marine microbes. Significant miniaturisation and robustness enhancement is required for many autonomous applications.

3.3 Emerging technologies

A number of research organisations are now involved in the development of *in situ* sensors. International workshops have tackled this issue [8] including meetings in the European context [41, 42]. A review and recent status evaluation is presented by[9].
Promising developments have been reported in the development of optical indicators for carbon dioxide[43, 44] and pH[45]. These fluorescence lifetime based indicators are akin to the oxygen sensors commercialised by Aanderaa. With further development these techniques could result in a similarly small and robust sensor (i.e. TRL 5-6)

Optical indicators offer the possibility of extremely compact sensors, with excellent performance. The development of sensing layers is the rate limiting step, though there are a number of encouraging developments. For example early results suggest that indicators for methane with a limit of detection at sub nM concentrations can be used with an extremely compact device[46] (TRL 5).

Whilst early *in situ* biosensors concentrated on particle counting and imaging [47], recent developments have allowed *in situ* cytometry[48] (Commercialised but TRL 7). Genomic and protenomic techniques borrowed from the biomedical industry are also allowing marine studies [49] and development of sensors such as the Environmental Sample Processor (ESP) [50] (TRL 7-8) which uses immobilised oligonucleotide molecular probes to indicate which species are present in a given bulk sample. Similar techniques have been employed in the lab funded by the European grant "GOCE-CT-2003-505491" though an *in situ* system is not yet developed (TRL 4-5). At University of Southern Florida (USF) *in situ* genetic amplification and measurement has been developed to allow characterisation of RNA signatures [51] (TRL 8).

Fluorometry has been used for decades to probe chlorophyll A and has recently been extended to include FRRF [52] that investigates the photosystem II for phytoplankton and enables

determination of the physiological state of these organisms (technology TRL 9 but for technique performance see also § 5.1.3).

Electrochemical sensors are the subject of ongoing research to improve performance and to extend the technique to new chemical parameters. For example recent efforts include the development of anodic stripping voltammetry[53], very low cost oxygen sensors[54] and silicate sensors[55] (TRL 6)

Spectrophotometry (particularly for the detection of nitrate [56]) has a long research history (TRL 8). However, with the progressive reduction in the emission wavelength of low cost Ultra Violet Light Emitting Diodes (UV LEDs), it is likely that in the near future devices of reduced power consumption, and size will be possible. Similar advances in optical detection and miniaturised spectroscopy may allow performance enhancement. One interesting possibility is the combination with fluorescence methods to determine Dissolved Organic Matter (DOM) independently which otherwise interfere with the measurement though clearly this is at an early stage of development (TRL 3-4).

The development of *in situ* marine sensors for a range of chemical analytes using reagent methods is ongoing, as current technology requires substantial change in size, robustness, and cost before many applications are possible. In addition no *in situ* method exists for measurement of many chemical species. Others are can be determined but not with sufficient precision. The use of well established reagent protocols enables high specificity and performance, but this is at expense of the engineering complexity of the devices. This can be reduced somewhat by the development of a modular parts library (an approach used by many developers including the National Oceanography Centre, Southampton (NOC)). For example most systems require modules for control electronics, optics, pumping etc which can be interchangeable. Devices have been reported for dissolved nutrients [57-59], and the trace metals (e.g. Mn [10, 60-62] and Fe [63]). With the exception of an osmotic iron sensor [64] none of these sensors have been deployed for long periods (TRL 7). Devices for the high accuracy determination of pH[65, 66] and carbonate chemistry[67] have been developed (particularly for underway applications), but *in situ* devices (e.g. [68]) remain at early stages of development (TRL 5).

Active research into miniaturised oceanographic systems using microfabrication is being undertaken. *In situ* reagent based chemical analysers and cytometer devices are in development at NOC, whilst a hand held biomolecular analyser is the subject of future collaborative research lead by Ikerlan. Monterey Bay Aquarium Research Institute (MBARI), USF, SubChem and Wetlabs have miniaturisation / microfabrication programmes though few functioning devices have been reported (TRL 3-4) and exception is the “Cycle” phosphate sensor with excellent reported performance (50nM resolution) [31]. There are numerous technical difficulties (e.g. biofouling, development of *in situ* optics and actuators) but microfabrication promises mass produced, robust devices with very small size and low power and reagent consumption motivating further research.

4 Long term deployments of biogeochemical sensors

There are a number of large, high profile mooring arrays and deep-sea observatories planned and in development and in operation in Europe (e.g. ESONet, EuroSITES, the Rapid array), North America (NEPTUNE, which consists MARS (US) and VENUS (Canada)) and Japan (H20). These long-term observing systems represent expenditure and committed funds in the order of hundreds of millions of dollars
[Orion - http://www.joiscience.org/ocean_observing/initiative;

Esonet- http://www.oceanlab.abdn.ac.uk/research/esonet.php].
Pioneering deployments of biogeochemical sensors for long term Eulerian observations have been achieved in the ANIMATE, and EurOceans programmes (oxygen, CO_2, fluorescence, nutrients). Biogeochemical sensors have also been used over extended periods in the Ferrybox programme. The experience of the technical and scientific teams involved forms an invaluable resource for both the assessment of technology, and for planning future deployments.
A summary of current biogeochemical sensor deployment on Eulerian observatories in Europe is shown in Table 3. A distillation of the experience and expertise from these sites is included in §5.3. Table 3 illustrates that Eulerian observatories are already making use of state of the art technologies for long term biogeochemical sensing i.e. Reagent based nutrient sensors, the SAMI-CO_2 sensor, oxygen sensors (both electrochemical and fluorescence lifetime based) and fluorometers.
The operating protocols developed for these deployments are comprehensive and robust. Drawing on best practice they recommend:

1. Review of real time data, using visual checking and algorithms to identify drifting moorings, inoperative sensors, and unrealistic values / rates of change[69, 70]
2. Site specific calibrations for fluorometers using sensor measurements and a) site and depth specific cultures [71], or b) bottle samples from a site specific profile
3. Detailed quality assurance procedures for reagent based nutrient sensors[72]
4. Calibration checking at the beginning and end of deployments with samples in close proximity to the sensors
5. Planned and opportunistic measurements in the vicinity of the deployment sites via sampling, proxies (e.g. mixed layer depth from ARGO float data (http://www.argo.ucsd.edu/)), and other sensors (e.g. with ships of opportunity, or research ships).

Further improvements could be made by:

1. Assessment of intrinsic sensor drift and the effects of biofouling by calibration of sensors before deployment, and shortly after recovery both before *and* after cleaning.
2. Development of a system to qualify sensors for long term operation *before* they are deployed on observatories consisting
    a. Pre deployment long term technology assessment both in the lab (to assess sensor drift) and in coastal test bed (to assess the effects of biofouling)
    b. Pre deployment performance evaluation (accuracy, precision, LOD)
3. The use of qualifying system to expand the list of sensors that can be deployed (e.g. other nutrients, methane)
4. Reduced maintenance intervals, and shorter deployments. The longest deployment should be shorter (~20%) than the expected duration of the sensor with the shortest valid operational life (to allow end point calibration)
5. Detailed tracking documents recording all aspects of maintenance, calibration, deployment and data recorded by each instrument – this is an invaluable resource for tracking design performance and therefore improving technology readiness level
6. The construction of real time data reporting systems for all biogeochemical sensors. This could for example enable retrieval and replacement of inoperative sensors shortly after deployment (thus saving the dataset) or reporting sensor degradation enabling retrieval before complete failure ensuring a post retrieval calibration is possible.
7. Use sensor redundancy where practical (and economic). For example retaining a spare before deployment would allow replacement of sensors that do not perform adequately on final calibration (often at the deployment site).
8. Wherever possible, the use of more frequent calibration. This is particularly imperative for Fluorometers as the relationship between fluorescence, and chlorophyll concentration is known to be extremely variable.

Table 3 Current state of the art in long-term biogeochemical sensor deployments in Europe. Shaded cells indicate real time data capability

Site	Lat/long	depth	Fluorescence (Chlorophyll)	Nitrate	POC	CO_2	Dissolved O_2	Turbidity	PAR
PAP (Porcupine Abyssal Plain)	49N, 16.5W	4800m	Hobi labs HS2, Wetlab FLNTUSB	NAS3, ISUS	yes	yes (SAMI)	no	no	no
ESTOC (Canary Islands)	29.04N, -15.15W	3670m	Wetlab FLNTUSB	NAS3	yes	yes (SAMI)	no	No	no
CIS (Central Irminger Sea)	59.4N, -39.4W	2800m	Wetlab FLNTUSB	NAS2	yes	yes (SAMI)	no	yes in Wetlab	no
CV (Cape Verde)	17.4N, -24.5W	3600m	Wetlab FLNTUSB	No	no	no	Aanderaa Optode	yes in Wetlab	no
Station M (Norwegian sea)	66N, 2E	weather ship	water bottle samples	Water bottle samples	no	no	Multiple depth samples	sea surface	no
DYFAMED (Ligurian Sea, http://www.obs-vlfr.fr/sodyf)	43.25N, 7.52E	2300m	Historical: Chelsea fluorometer (attached to CTD) Now: Wetlabs ECO-FLNTNS Pigments and Tchla HPLC	SAMPLES	200m,1000m) every 2 weeks	TCO_2, alkalinity SAMPLES	Winkler SAMPLES		no
M3A_Ligurian	43.79N, 9.16E	1300m	(36m) Wetlab ECO FLNTUS	No	no	no	yes (36m)	yes (36m)	no
	43.85 N 9.9 E	90 m							
M3A_Adriatic	41.28N, 17.66E	1050m	No Chlorophyll	No	yes	no			Yes
M3A_Cretan	35.4N, 24.59E	1050m	Wetlab Wet-Star (100m max) change to WETlabs ECO FLNTUA	NAS2 (100m max)	No	no	Yes	yes	yes

5 Performance evaluation of commercial sensors

5.1 Published evaluations

5.1.1 Oxygen sensors

In situ oxygen measurements commonly rely on the use of Clark type electrochemical sensors. Performance studies of various designs shown that the Clark electrode required frequent calibration (at least monthly) to obtain accurate data, presented stirring and pressure effects, cross sensitivity and contamination by hydrogen sulphide (Berntsson et al, 1997). Clark type sensors were used on a mooring at the Hawaii Ocean Time series (HOT) station from 1997 to 1998 where they were calibrated every month during periodic visits to maintain the high precision and accuracy needed for *in situ* deployment. Oxygen measurements within 0.5% accuracy were achieved during this field work [73]. Seabird SBE-43F oxygen Clark electrodes were deployed at the HOT station on several ARGO floats and their long term behaviour studied (Larson, 2003) and a calibration drift of 0.5% / year was recorded. However, the instrument still requires a pump thus draining on the batteries.

An alternative technology based on the use of selected substances to act as dynamic luminescence quenchers integrated into a sensor called optode may provide a more suitable method for direct *in situ* measurements of dissolved oxygen. A comprehensive evaluation of the optode technology for long term *in situ* measurements was carried out by Tengberg [26]. Up to 20 sensors for Aanderaa Data Instruments were tested for their calibration performance, cross-sensitivity and pressure hysteresis, sensitivity to biofouling and long term stability. These sensors have a measuring range of 0-500 µM, a resolution of 1 µM and an accuracy of 5 µM as well as an operating depth of up to 6000 m. The optodes were deployed during test periods lasting from a few days to 600 days on an ARGO float, and in different waters (estuarine, river, waste water, and ocean). Compared to the general behaviour of electrochemical sensors, the lifetime based optical technology was found superior in all aspect except for the fast response time (the Aanderaa optode has a t63 of 20s) that has been demonstrated by electrochemical microelectrodes (t90=0.1s). Fouling of the optode was slowed down by wrapping a beryllium-copper alloy net around the sensor. In heavy fouling environments (Chesapeake Bay and Gulf of Mexico), this method prolonged the service interval from approximately 7 to 10 days to 40 to 60 days. Long term stability data (1-2 year) were collected in low fouling environments (off Canada in Labrador Current). It is worth noting that cleaning the sensor will see it coming back to its original performances.

5.1.2 Carbon Dioxide sensors (SAMI-CO_2)

The most widely used *in situ* carbon dioxide sensor for oceanographic application is the SAMI-CO_2 sensor[30]. Some authors note that this sensor is difficult to operate. It is certainly large (~14kg in water), and must be calibrated by the manufacturers indicating a TRL of 7 or 8. Factory calibration may be advantageous, as it limits the expertise required from the user. In addition it enables a consistent standard. Reproducibility and repeatability are both good, and suggest operation calibration may be possible [74]. A miniaturised and robust version is in development though is not yet reported.

The sensing principle is based on a colorimetric (sulfonephthalein-type) pH indicator contained in a gas permeable membrane. The membrane is placed in direct contact with the seawater CO_2 and the reagent periodically renewed to improve accuracy.

Published experience with this sensor are good for long deployments (e.g. 16months [75]). However, the instrument is not intrinsically resistant to antifouling and this may cause local microenvironments affecting results. Users (see §5.3.2) have report success using copper based antifouling strategies.

5.1.3 Fluorometers

Fluorometers have a long history, and are produced by many manufacturers to a high standard. These are therefore at TRL 9.

Photosystem II in photosynthesising organisms exhibits fluorescence that is related to chlorophyll a concentration. In addition, excitation with repetitive flashes causes a time dependent fluorescence signature allows calculation of photosynthetic electron transfer rates from photochemical efficiency and light absorption measurements. Due to the simplicity of the measurement method, and the relative simplicity of the associated instrumentation, these phenomena are often used for *in situ* studies of marine organisms. However, there is widespread acceptance that these measurements record only the fluorescent properties of seawater (and communities therein) and *not* an unambiguous or accurate value for the concentration of chlorophyll a [21] or primary production [39, 76, 77]. Taxanomic, physiological, and environmental factors all contribute to the variability in estimates of chlorophyll concentration and productivity estimates. Accuracy may be improved by calibrating fluorometers *in situ*. However, this calibration will only hold as long as the environment, or phytoplankton community taxonomy, or physiology remains constant. This is unlikely to be true at different depths, different locations, and over extended time periods. For example Holm-Hansen et al[78] noted that separate calibrations coefficients were required for coastal *vs* pelagic waters (~60% difference) *and* that variation in Photosynthetic Available Radiation (PAR) had a marked effect (~factor of 5) requiring additional terms in the calibration equation. Even with these careful calibrations the peak to peak error of fluorescence derived chlorophyll concentrations was ~100% (133 samples). A similar view is expressed in ACT literature e.g. "several factors make *in situ* fluorescence monitoring of chlorophyll a semi-quantitative measure at best. Environmental conditions, photoplankton [sic] community composition, physiological status, cell morphology, irradiance history and the presence of interfering compounds all play a role in altering the relationship between fluorescence and the concentration of cholorophyll a….. Given that in vivo or *in situ* fluorometry is a relative measurement with no absolute 'true value'… accuracy in the measurement… cannot be determined directly "[79].

A similar problem is experienced when interpreting remote sensing data (i.e. the link between fluorescence and chlorophyll concentration, biomass, or productivity is at best indicative), but in addition, atmospheric effects, measurement errors in actinic light levels, surface films, turbidity, and variable depth all add significantly to the error. For example a comparative study of *in situ* and remotely sensed optical properties [80] "observed mean relative errors of 70.5%/-3.8% (SeaWiFS OC4v4), -21.4%/-49.3% (SeaWiFS Stumpf), 109.5%/13.4% (MODIS OC3m) and 0.5%/-48.9% (MODIS Stumpf) for Chl". These results are in line with the accepted error for such measurements [81] (accepted error in SeaWiFS Chlorophyll a determination ~60% (1σ) in areas of good agreement, and worse in areas such as the Southern Ocean) and [82] (uncertainty ~30%). This variability, and the change in fluorescence with depth suggest that the practice of calibrating fluorescence sensors with satellite data is unlikely to be beneficial.

5.1.4 Nutrients

5.1.4.1 Reagent based systems

Despite the widespread use of reagent based nutrient analysers for marine and environmental applications there is very little published material documenting their performance, or technology evaluations. However, systems such as the NAS2 and NAS3 carry onboard standards which are regularly analysed to provide quality control of the measurement data. Though not specifically published to enable performance evaluations this information can be used in this manner. A rudimentary analysis is included in §5.3.4.1.

An evaluation of a WetLabs *in situ* phosphate sensor has been published. This was deployed for 3 months on the Channel Adaptive Re-locating Mooring (CHARM) in the Santa Barbara Channel and showed no evidence of degradation [83]. Laboratory reagents were stored at room temperature for 3 months and showed no evidence of degradation when compared to freshly prepared reagents. Also the instrument compared well to water samples collected over a 2 month deployment which were then analysed using a traditional bench top system.

5.1.4.2 Spectrophotometers

Nitrate *in situ* UV spectrophotometer sensors have been deployed on long-term moorings in the ocean. For example, the MBARI sensor (ISUS commercialised by Satlantic) was deployed at the Bermuda Testbed Mooring at 80 and 200m depth [84]. But it was not possible to determine if anomalies in the resulting data were real or whether there was a sensor failure. More recently, Johnson and Colletti (2002)[28] carried out an in-depth study over a 6 month period comparing the ISUS nitrate in-situ measurements to on-board measurements carried out on collected seawater. The sensor measurements had a one standard deviation of the nitrate concentration of 0.5 μM and a limit of detection of 1.5 μM . Therefore the sensor would not be able to deal with the low nitrate concentrations in the oligotrophic open ocean (<300 nM). The sensor also had the potential of being left out on a mooring for over a year (sampling 5 times a day). However, after 4 months, the standard deviation of the measurements rapidly increased and the nitrate measurements became inconsistent. Also at low temperatures there was a bias in the measurements.

Following on from this work, the ISUS sensor was deployed at 1 m depth, 20 and 50 km offshore of Monterey Bay, California, with data telemetered back to the shore hourly [85]. The data was determined to be accurate to +/- 2 μM with a precision of 0.15 μM. However, the sensor experienced drift over time which resulted in negative nitrate measurements down to -2 μM, when the actually nitrate measurements in the ocean were near to zero. The total longest deployment duration was 973 days, with 640 days of data that passed quality control standards. However it is not clear whether intermittent maintenance of the sensor was carried out, as the site used is 50km offshore and the sensor deployed at 1m depth. The longest data set presented has continuous valid data for over seven months.

5.2 Intercomparison and laboratory evaluations

Laboratory evaluations of sensors are essential in determining the accuracy of stated performance (e.g. manufacturers data sheets). Typically the resolution, precision, accuracy, sensitivity, repeatability and reproducibility should be characterised, and quoted in standard units including the use of compensation for bandwidth where appropriate. (e.g. precision or noise limited resolution is quoted as μM/√Hz etc). During this work fluorometers (Wetlabs ECO FLNT and Hobi labs HS2) and an ISUS nitrate analyser were evaluated for these parameters. Reassuringly their short-term performance was in line with manufacturers data sheets.

In the long term deployment context long term reproducibility is crucial as this determines the accuracy of the sensor between pre-deployment and retrieval calibrations. This drift can be significant and this is the key performance variable limiting the confidence in returned long term data. Unfortunately it is the most difficult to assess, it requires operation of the sensor over a period at least in excess of the planned deployment duration and at a minimum intermittent testing with a high and low standard. Use of data from shorter evaluations may be possible, but drift etc will have to be extrapolated. Accelerated testing may be possible, for example, fluorescence lifetime sensors can experience photo-bleaching resulting in a degradation of the sensing element dependent on the number of measurements made. Reagent based systems suffer from reagent degradation, and degradation and contamination (and

hence temporal variability) of standards. This is difficult to asses in the lab, but may be predicted from stored standards and reagents. Accelerated testing may be useful in predicting performance of fluidic systems (e.g. pump and valve performance).
Unfortunately no long term testing was completed during this work due to the short testing period available. However the variability in fluorometer data was investigated, and data from previous intercomparisons analysed. Though revealing this data is no substitute for a comprehensive long term performance analysis. This should be completed on all sensors considered for long term deployments.

Figure 1 Variation between fluorometers instruments and with species: results from calibration *Isochrysis galbana* (IG) and *Chaetoceros ceratosporum* (CC) [86]Figure 1 demonstrates that fluorometer data is species dependent and that different fluorometers have different responses. This is to be expected as different fluorometers use different excitation and detection wavelengths, and have optics that will have different sensitivities to back scatter. In addition the fluorescence and scattering characteristics of phytoplankton is species dependant. If the response was a function of chlorophyll concentration only then a single linear relationship would be obtained. Clearly if species composition is changing as a function of time, independent sampling or measurement is required more frequently than any change to enable a quantitative measurement. To fulfil Nyquist criteria, the sampling should occur at twice (and preferably at least five times) the frequency of the species related fluorescence variation.

Figure 2 and Figure 3 illustrate water property or time dependence in spectrophotometer nitrate sensor calibrations. This is a significant result as either there is a time dependent drift (e.g. 5μM at low concentrations for sensor 59) or there is a dependence on other optical properties of the sample water. Both processes are likely as drift has been reported in the literature (§ 5.1.4.2) and the requirement for site specific calibrations have been reported by users (§ 5.3.4.2). Both errors cause concern for long term deployments. Drift if consistent may be characterised by long term testing (as above), but dependence on other optical properties of the water requires either:

1. that the sample waters optical properties do not change significantly[4] unless due to a nitrate signal only
2. that a reference measurement be made more frequently than any optical perturbations not associated with nitrate

In either case taking samples at regular intervals and measuring either nitrate, or the optical properties (or preferably both) would increase confidence in the sensors accuracy during long term deployments.

Figure 4 compares the calibration of a reagent based (NAS) and spectrophotometric (ISUS) nitrate sensors vs bottle samples analysed with an auto analyser. The NAS instrument produces a smaller low concentration offset, has higher precision, and is more sensitive. However, both instruments require calibration to improve accuracy prior to deployment. The NAS uncalibrated accuracy varies from ~0.3μM to 5.4μM, and the ISUS 4.5μM to 5.4μM in the 0-25μM range.

[4] i.e. producing an offset in nitrate readings less than the required accuracy

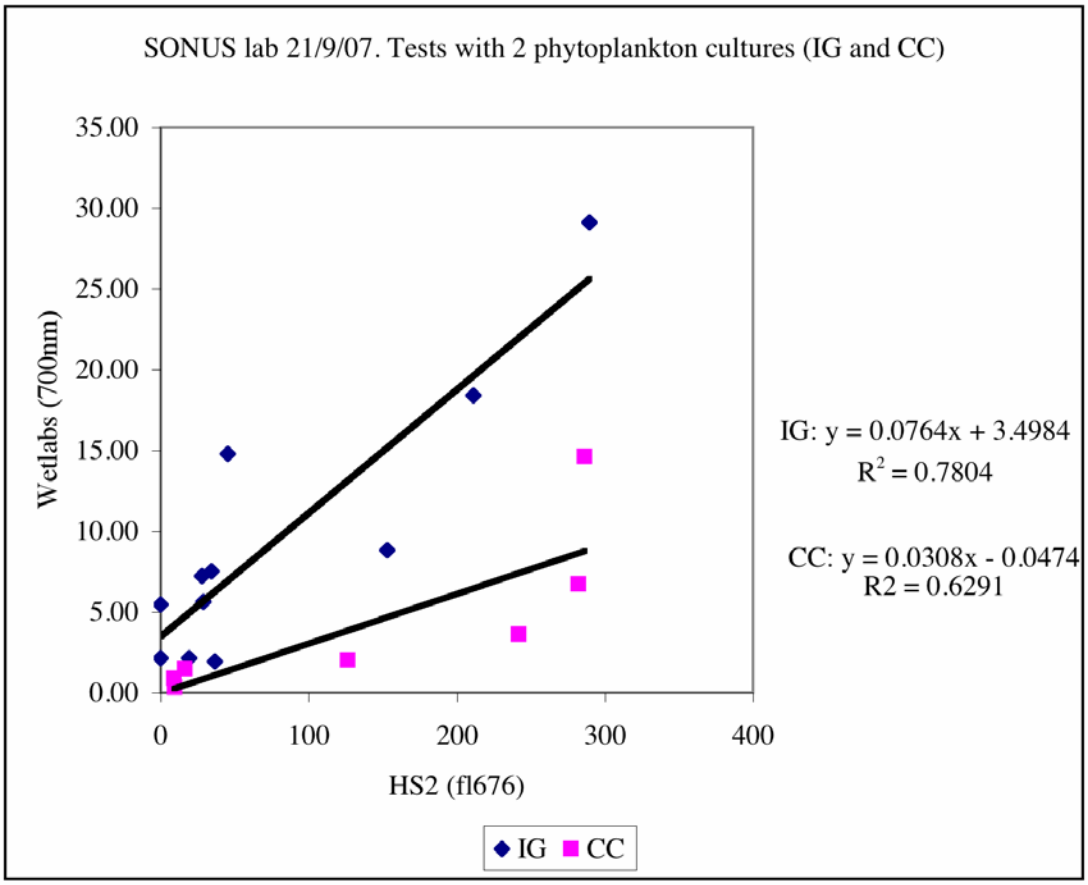

Figure 1 Variation between fluorometers instruments and with species: results from calibration *Isochrysis galbana* (IG) and *Chaetoceros ceratosporum* (CC) [86]

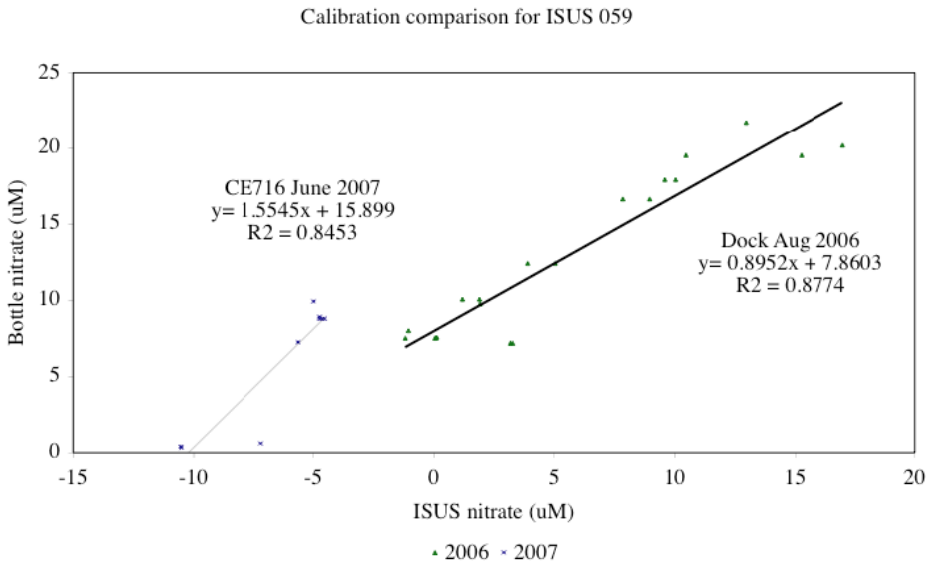

Figure 2 A comparison of calibrations in different waters for the ISUS S.N 059 nitrate [87]

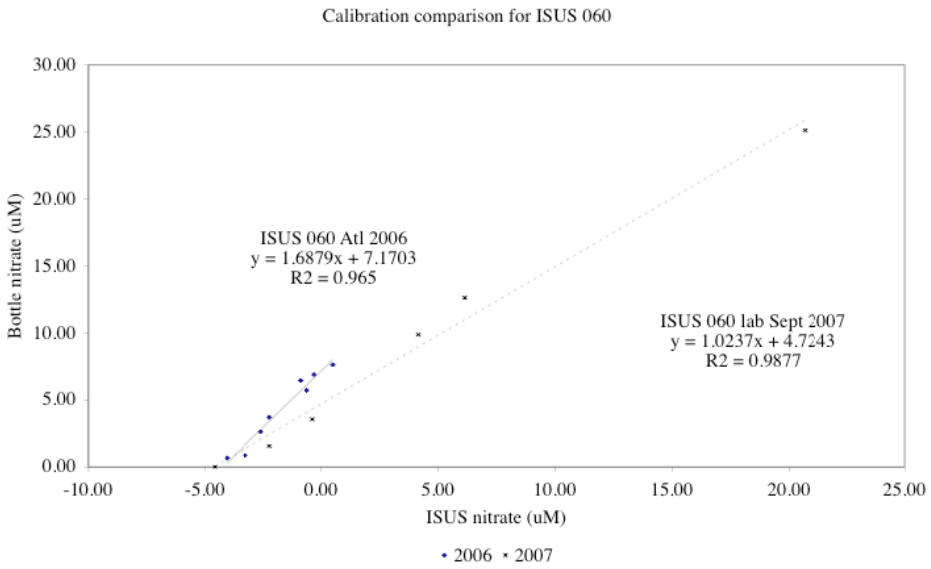

Figure 3 A comparison of calibrations in different waters for the ISUS S.N 060 nitrate [87]

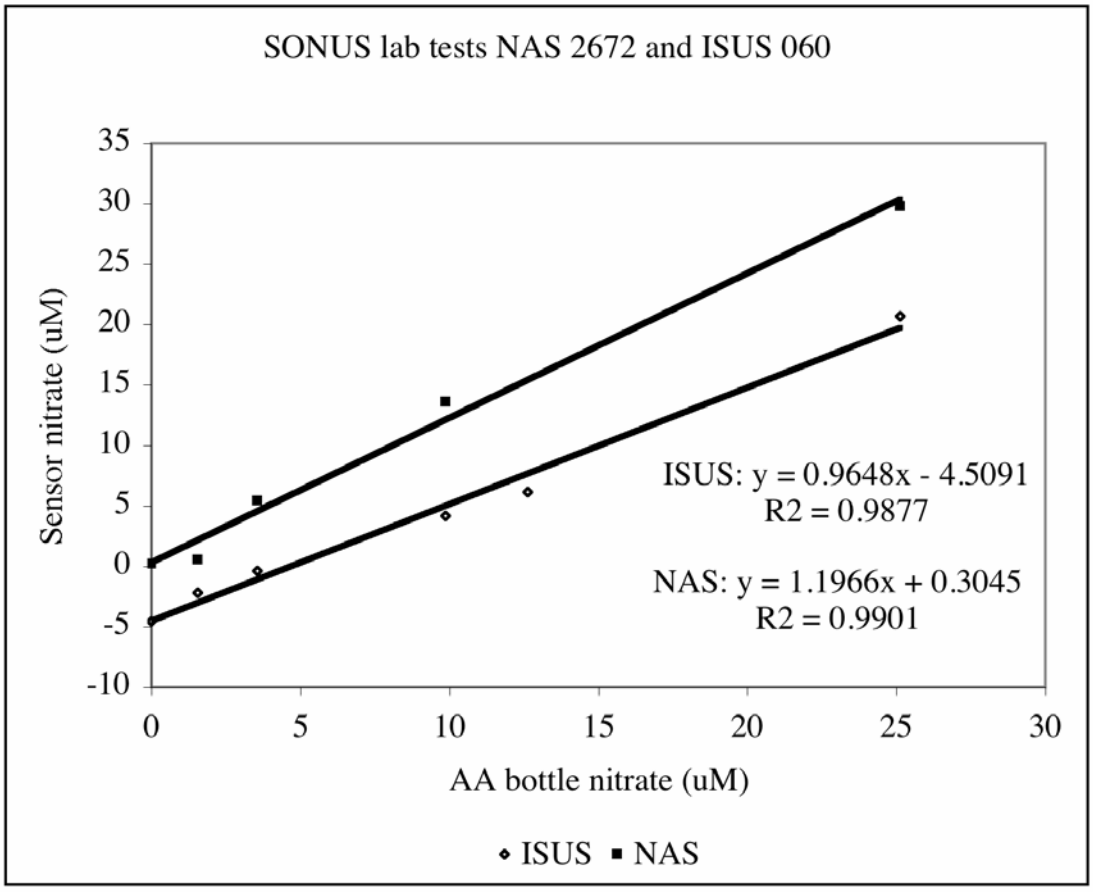

Figure 4 Intercomparison of spectrophotometric (ISUS) and reagent based (NAS) nitrate sensor [88]

5.3 Experience of long term Eulerian deployments

5.3.1 Oxygen sensors

Aanderaa optode oxygen sensor used by station M have shown good performance in line with manufacturers data sheet (precision <1μM, accuracy ~ 8μM). Plans to perform additional calibrations should allow improved accuracy, particularly at low temperatures experienced at this station.

Seabird 43 oxygen electrode sensors used at M3A_Ligurian exhibited performance in line with manufacturers specifications on deployment but suffered from biofouling, which in turn created a micro environment. This effect was reduced by reducing ambient light levels inside tubing supplying the sensor. Further biofouling mitigation techniques are likely to be effective in enabling longer term deployment.

Figure 5 and Figure 6 show calibration of a seabird 43 oxygen sensors at the DYFAMED site from CTD casts during 2006 and 2007. These calibrations are marginally worse than predicted by performance data given in the data sheets, but illustrate good performance of the senor in both instances.

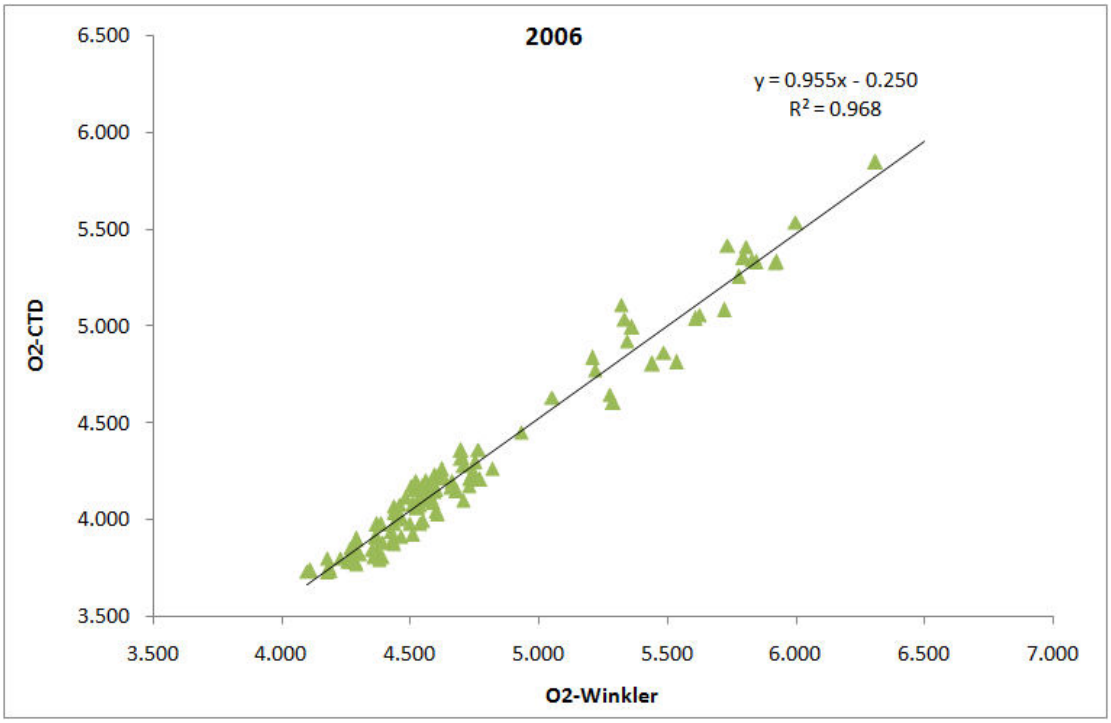

Figure 5 Oxygen sensor calibration DYFAMED 2006 (units ml/L) [89]

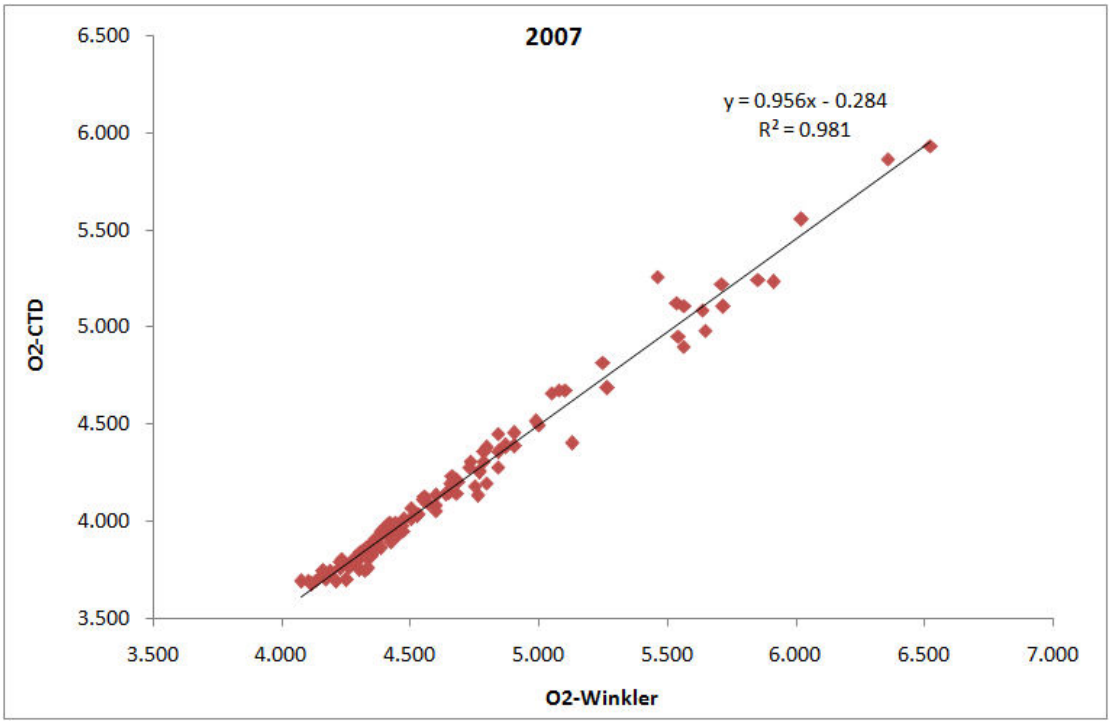

Figure 6 Oxygen sensor calibration DYFAMED 2007 (units ml/L) [89]

5.3.2 Carbon Dioxide sensors (SAMI-CO_2)

Carbon dioxide sensors operated successfully on the ANIMATE PAP moorings over three consecutive deployments (12 July 2003 -16 Nov. 2003, 17 Nov. 2003-16 June 2004, and 22 June 2004-18 July 2005). The sensor head was covered with a copper mesh to prevent biofouling on all deployments.

The SAMI-CO_2 sensors were calibrated by the manufacturer to the expected annual variability range in temperature and pCO_2 and should not require further calibration by the user side[30, 74]. However, post calibration of field data sometimes turns out to be necessary to account for problems with the initial calibration (possibly due to changes occurring during sensor shipment) or from drift.
Unfortunately post deployment calibration is problematic, not least due to the difficulty in achieving co-located samples, and because of sample contamination / degradation. This may introduce more error into the readings than if the sensor was not calibrated in the field, suggesting further that characterization of sensor stability and drift (in the lab and more easily services coastal deployments) is required. If the drift is known *a priori* then greater accuracy may be possible than by using field based intercalibrations. In these deployments pCO_2 values calculated from Dissolved Inorganic Carbon (DIC) and total alkalinity (A_T) (see 2.2) measured on samples from hydrocasts carried out at the mooring site can be employed to post-calibrate pCO_2 sensor readings whereas matching via temperature is preferable to pressure. This method was used whenever possible (PAP 2: start and end of deployment, PAP 3: start of deployment).

When possible, samples for DIC and A_T were taken from hydrocasts made at the PAP site before deployment or recovery of a mooring for post calibration of the SAMI-CO_2 sensors. These samples were measured onshore by extraction and subsequent coulometric titration of the evolved CO_2 for DIC [90] and by open-cell potentiometric seawater titration for A_T [91]. Accuracy of both DIC and A_T was assured by referencing against certified reference material (CRM) provided by Andrew Dickson (Scripps Institution of Oceanography, La Jolla,

California, USA).

Uncertainty in these comparisons arises from any sample contamination or degradation, spatial variability (surface water patchiness), internal wave action (vertical displacement of water properties) and temporal mismatch, i.e. in the timing of the hydrocast and the availability of stable sensor readings at depth. This method was not available at the end of the PAP4 deployment since the ***p*CO2 sensor started to malfunction about 4 months before recovery[92]**. At the end of the PAP 3/start of the PAP 4 deployment, DIC/A_T samples were taken and analyzed. Calculated pCO_2 values from these samples are deemed to be unreliable, however, as they show high scatter and yield values above 400 µatm which are far higher than expected values in this region during summer and are more than 100 µatm above the sensor values. The cause of this problem was not due to analytical problems but most likely improper poisoning.

Secondly, when this method failed, plausibility checks were made with underway pCO_2 measurements made on the 'Volunteer Observing Ship' M/V *Falstaff* when passing through the region. Unfortunately no such passage was available during the three consecutive mooring deployments. There are three cruises, however, which passed within 150-430 km of the PAP site either before (April and May 2003, data available via CarboOcean data portal: http://dataportal.carboocean.org/) or after the deployment series (April 2005, unpublished data of T.Steinhoff, IFM-GEOMAR, Kiel). Of these, the April 2005 cruise lends much credibility to the sensor data, which end about one month earlier but are in good agreement, especially when accounting for the seasonal cycle of climatological pCO_2 (Figure 7) [93].

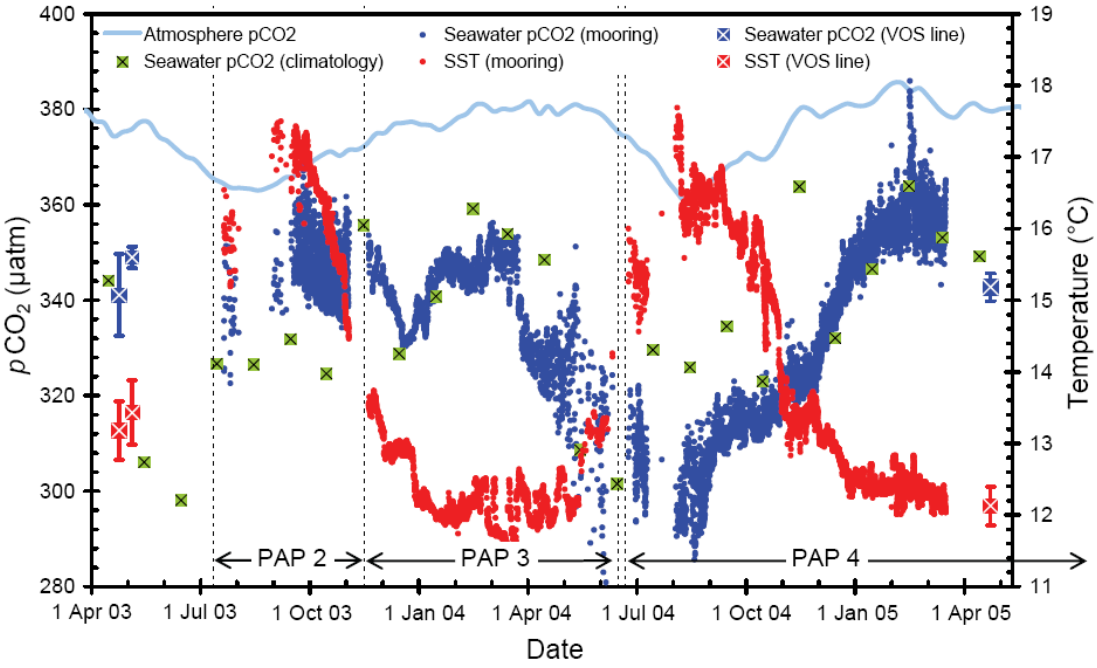

Figure 7 Time series of pCO_2 and temperature measured during three consecutive mooring deployments at the PAP site pCO_2, temperature data from a 'volunteer observing vessel' (VOS) plus corrected climatological pCO_2 of Takahashi *et al.*[93], atmospheric pCO_2 provided by GLOBALVIEW-CO_2. From [92]

Though difficult and prone to error, it appears that post-deployment calibration proved necessary at PAP 2. Here reference measurements suggest an offset of +47.5 µatm at the start of the deployment period, decreasing to +33.5 µatm at its end. The offset is large (~45% of the measured variation see Figure 7), though the drift is better (~15% of the measured variation). The sensor deployed at PAP 3 required an offset correction of 19 µatm at the

beginning. Due to lack of a calibration check at the end of the PAP 3 deployment this time-invariant offset was applied to the entire PAP 3 pCO_2 record. For the PAP 4 pCO_2 record no post-calibration is available. The fact that the PAP 4 record is nicely bracketed on both ends by the calibrated PAP 3 and VOS line data indicate that this sensor did not show a major offset or drift problem. Taking in account all sources of uncertainty, i.e. uncertainty in calculated pCO_2 due to errors in DIC and A_T as well as in the carbonic acid dissociation constants used, uncertainty from imperfect spatial and temporal match of discrete and VOS line data with the time-series record, the overall accuracy of pCO_2 data is likely not better than 5-10 μatm at a precision of about 1 μatm.

The 2-year pCO_2 time series analyzed in this study represents a significant effort by a multinational European consortium. With present sensor technology and reference measurement techniques, the generation of such long-term biogeochemical time series for the surface ocean remains a very demanding task, particularly because the instruments, platforms, and methods are still under development. Data losses due to failure or loss of instruments/moorings cannot always be avoided and data gaps for certain parameters at certain times limit the interpretation of the data. The 2-year record shown here represents that part of a 4-year effort for which the highest simultaneous data return was achieved.

5.3.3 Fluorometers

The experience of fluorometer deployment at the M3A site reflects the semi quantitative nature of this measurement. Petihakis *et al.*[71] minimise the error by performing site-specific calibrations using cultures of seawater collected from the deployment site. Without this technique factory calibrations produced results with an error of one order of magnitude. Culturing of the phytoplankton populations matching the community structure observed by *in situ* measurements is an elegant solution to interferences from species variation. However, the error induced by culturing (which may effect the physiology or change the relative proportion of species) remains undefined. In addition the species composition may change with time at the deployment site, suggesting that the instrument be recalibrated at regular intervals. Assessment of this error is only possible with a long term deployment where both samples and fluorometer readings are made concurrently. It is likely that this is time variant (and this may include non seasonal variation) making long term unattended operation problematic.
To address biofouling, copper tubing and bromide addition was tested indicating that copper tubing alone had the best biofouling preventative effect [71]. This is at variance with results for a transmissometer sensor where using both strategies in combination produced the best results. Despite these efforts biofouling remained an issue. One possible partial remedy would be to perform calibrations before deployment, post deployment, and post deployment after cleaning. This method was specified in procedural documentation, but unfortunately did not occur.
An illustration of the seasonal variation in the relationship between fluorescence and chlorophyll concentration is depicted in Figure 8. In 2003 calibrations using the profiling method were repeated in April and October. There is both poor agreement within a single profile ($R^2 \sim 0.6$, or 0.15μg (1σ) October) and significant offset (0.1μg at low sensor readings 0.5μg at the upper end). Neither is an entirely unexpected outcome as there is likely to be significant variation in the species composition, physiology, and environment with depth and with time, making a single calibration between fluorescence and chlorophyll illusive.

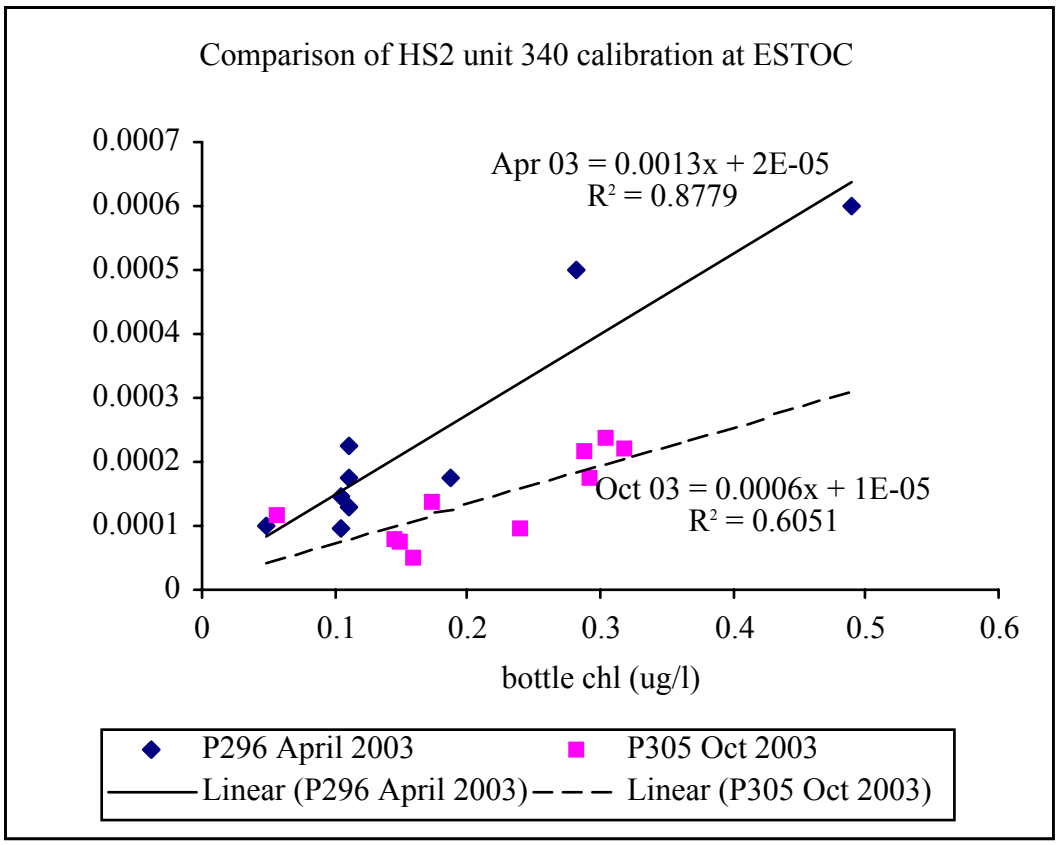

Figure 8 Seasonal variation in fluorometer calibrations at ESTOC from [94]

Illustration of the need for site-specific calibrations is shown in Figure 9, which clearly shows the dependence of fluorometer calibration coefficients on spatial location.

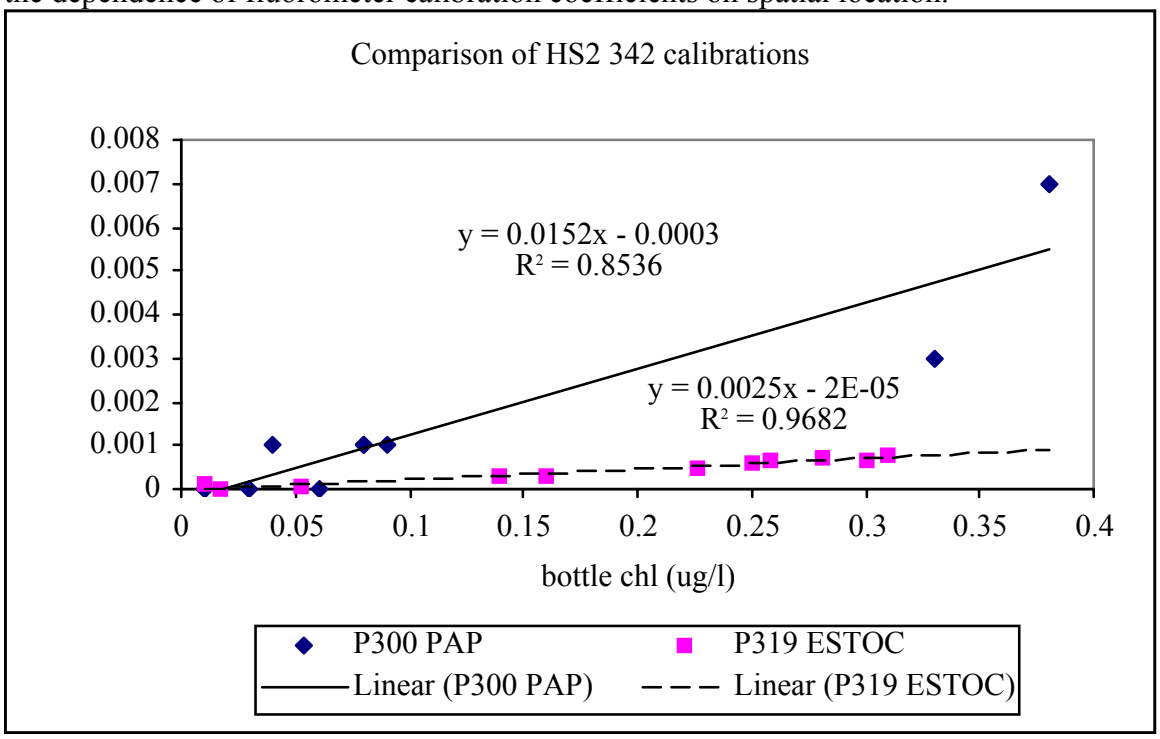

Figure 9 Illustration of spatial variation in fluorometer calibrations from [94]

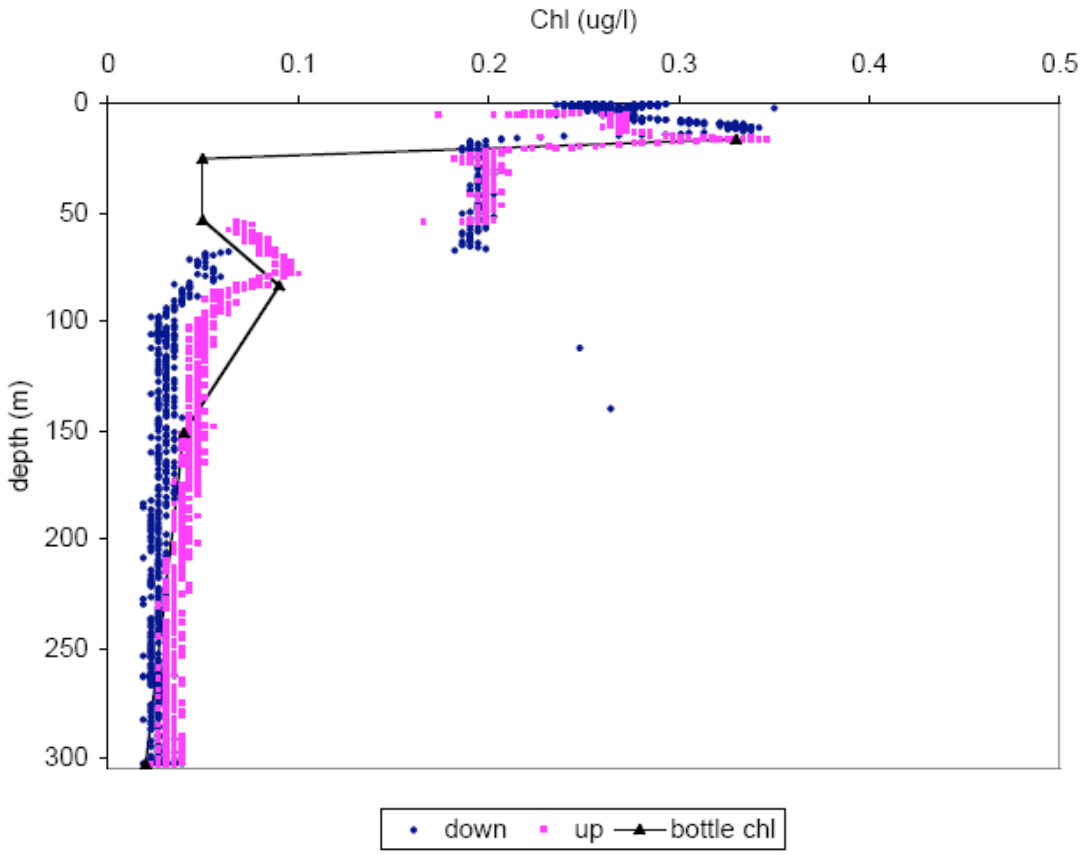

Figure 10 Calibration profile at CIS (FLNTUSB-268) dataStn 07 D309 (August 2007) from [95]

Fluorometer (Wetlabs FLNTUSB) calibrations at CIS were made *vs* bottle (Niskin) samples during profiling using a CTD frame. Figure 10 demonstrates a step change in fluorometer readings not seen in the bottle data indicating sensor error. There is also little data at intermediate concentrations reducing the confidence of the accuracy of the calibration across the range. This is a systematic problem with this calibration technique as the rate of change in [Chla] with depth is high at intermediate values making sampling inaccurate and difficult. A large gap in intermediate values is a feature of the majority of calibrations using this method (see Figure 12). However, the resultant data was used to generate a site-specific data, and subsequent profiles showed good agreement (see Figure 11). Good agreement is also shown in repeated calibrations at PAP from 2002-04 including in different seasons (maximum difference 0.08µg/L). An extended data set such as this leads to improved confidence that readings between calibrations at this site are accurate to within ~ 0.1µg/L mean or 0.2 µg/L for a single reading (10% and 20% full scale respectively). This is still short of the 5-10% accuracy required by users[96] . More regular calibrations would confirm the temporal variation in these coefficients.

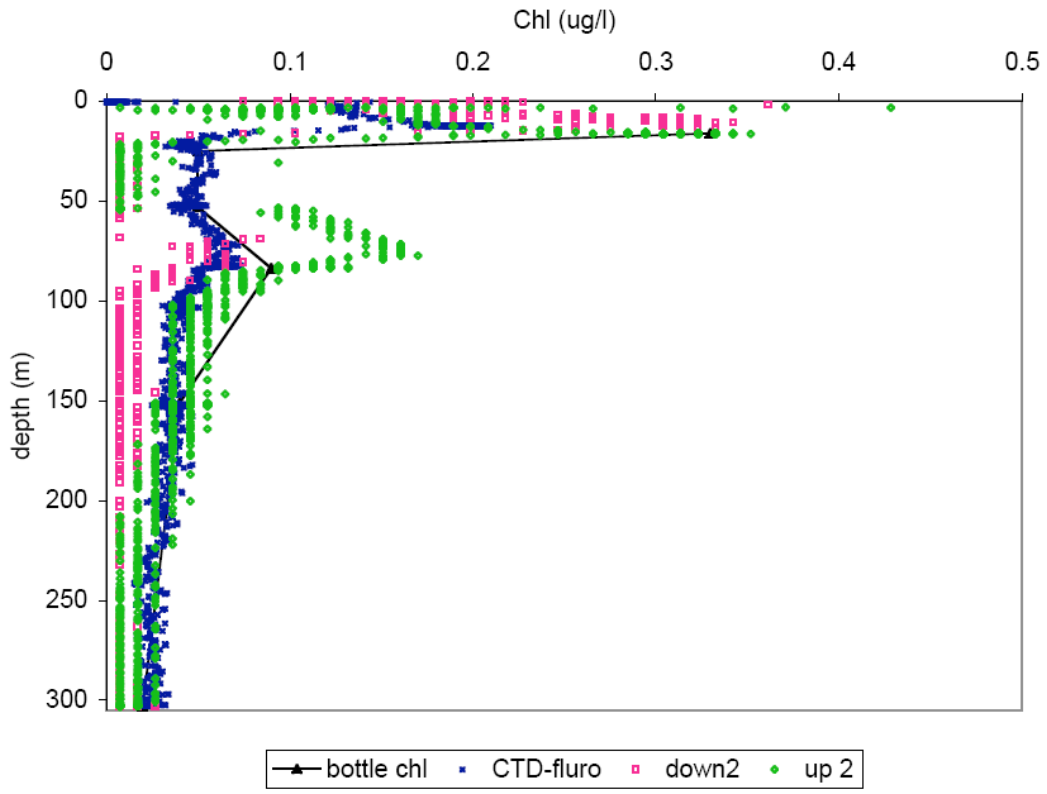

Figure 11 Post calibration profile CIS 2007 (before long term deployment) from [95]

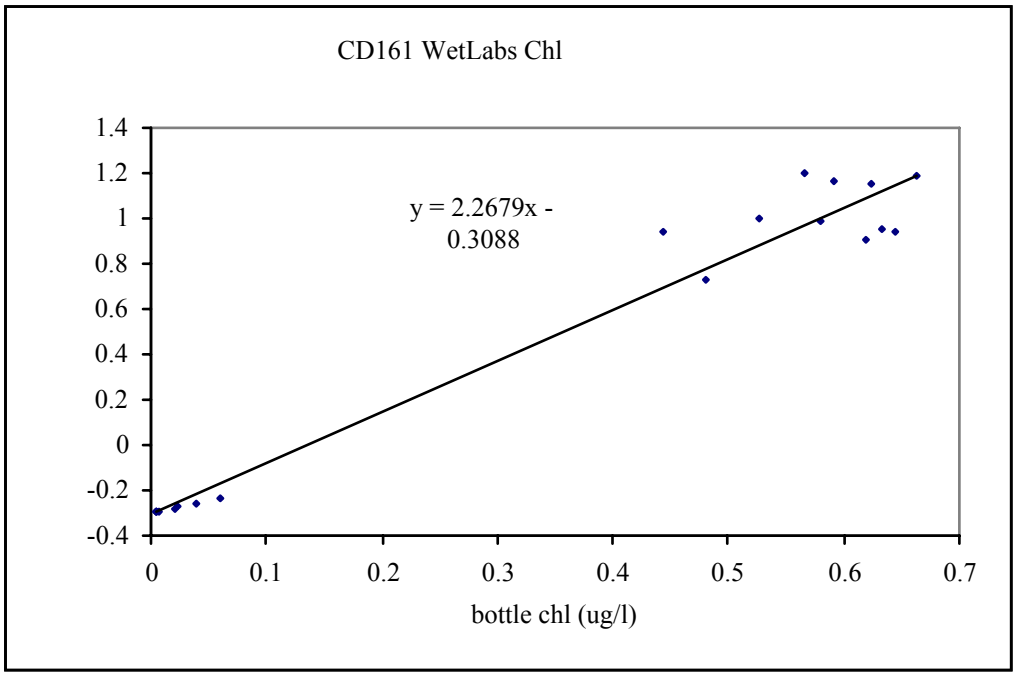

Figure 12 Wetlabs calibration at CIS on CD161 2004, note the lack of data at intermediate values (from [97])

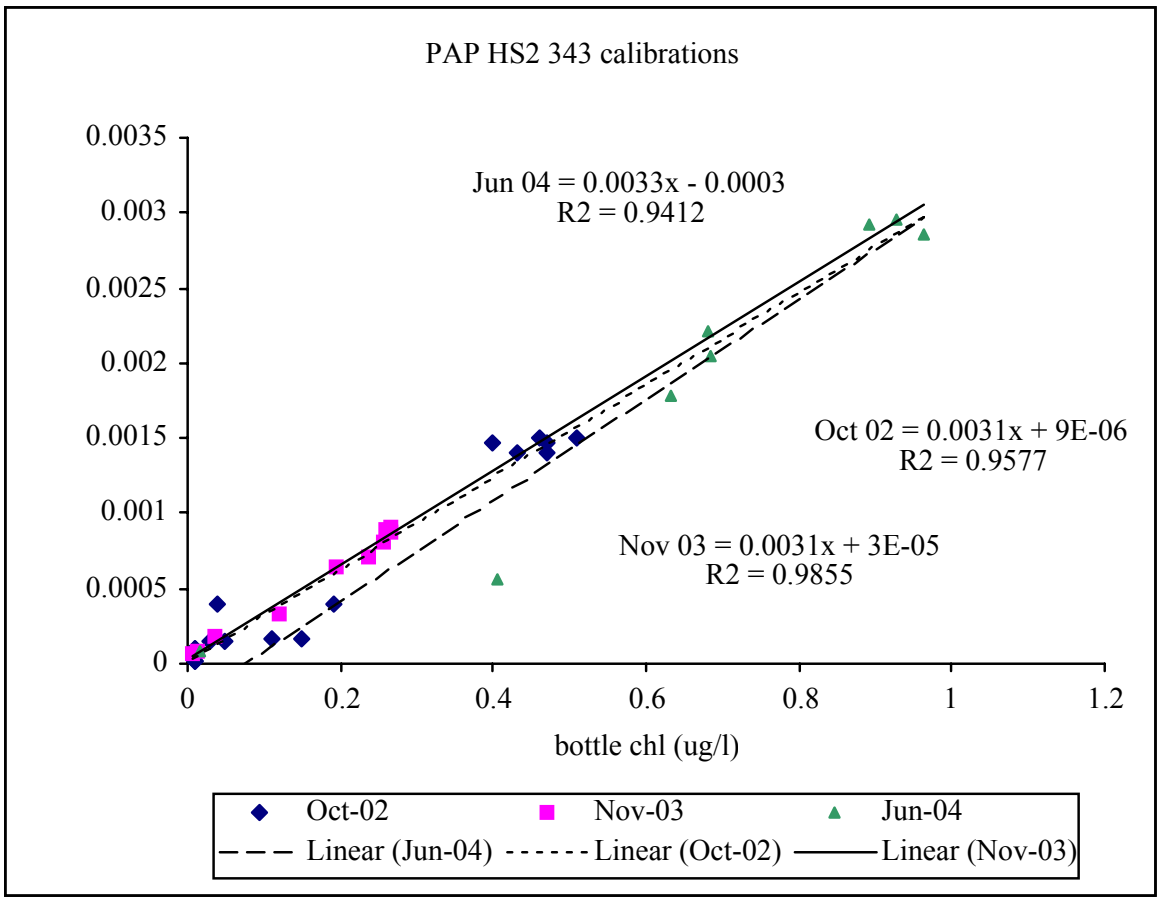

Figure 13 Example of consistent fluorometer calibrations at PAP from [94]

5.3.4 Nutrients

5.3.4.1 Reagent based systems

The experience of reagent based nutrient systems at the M3A site illustrates problems associated with devices not at TRL9. Data was gathered for 5 out of the 28 months of reported site operation[71]. Problems included fluidic errors (malfunctioning syringe) and flooding after factory service. A contributing factor was the lack of real time data providing no warning of malfunction. This lack of functionality is due to the design of the NAS analysers, again indicating a reduced TRL.

Despite problems with functionality and reliability the NAS analysers when operating correctly are sophisticated and high performance devices with excellent specificity and precision. Accuracy can also be extremely high due to the use of onboard standards (which can be analysed instead of samples to provide a reference), and the ability to measure the sample, standards and reagents independently to account for drifts and degradation in the optics, or the reagents. However, this technique relies on the maintenance of standards to a fixed value throughout the deployments. Though it is possible to estimate drift in standards by measurement of their concentration on retrieval (see [72]), performance is improved, and greater confidence in the results is obtained if degradation in the standards is prevented. Though it is common practice to inoculate standards (e.g. with Mercuric Chloride, or Chloroform), this has not always been done for deployments with Europe, and this greatly reduces the confidence in the measurements. Without inoculation, bacterial and phytoplankton growth may significantly alter nitrate levels in the standards, and hence degrades the accuracy of the sensor. It is important to note that the NAS system can make

~750 measurements before it needs recharging with reagent. The number of standard measurements is therefore limited, as is the temporal resolution for long term deployments.

An example of good data (from [72]) is shown in Figure 14 and Figure 15. Figure 14 shows absorption measurements of the standards (15 and 25μM), and the standards reacted with the colour-producing reagent. There is strong correlation between the absorption measured for both reacted standards (though variations equivalent to 1 μM are common) engendering confidence in the method. The unreacted standards are also consistently of low absorbance suggesting they remain uncontaminated. In Figure 15 a similar trend is seen in the unreacted sample, only a slow increase in absorbance is observed (consistent with a mild degradation of the optic cell e.g. caused by deposits from the coloured reagent product). The sample data in contrast has features as one would expect from environmental data. One interesting feature is the significant variability seen in the sampled data – and much of this is at a frequency equal to the sample frequency. This suggests that the system is undersampled in the temporal sense and that aliasing is occurring. To get a true picture of the variability of this system a higher sampling rate should be used.

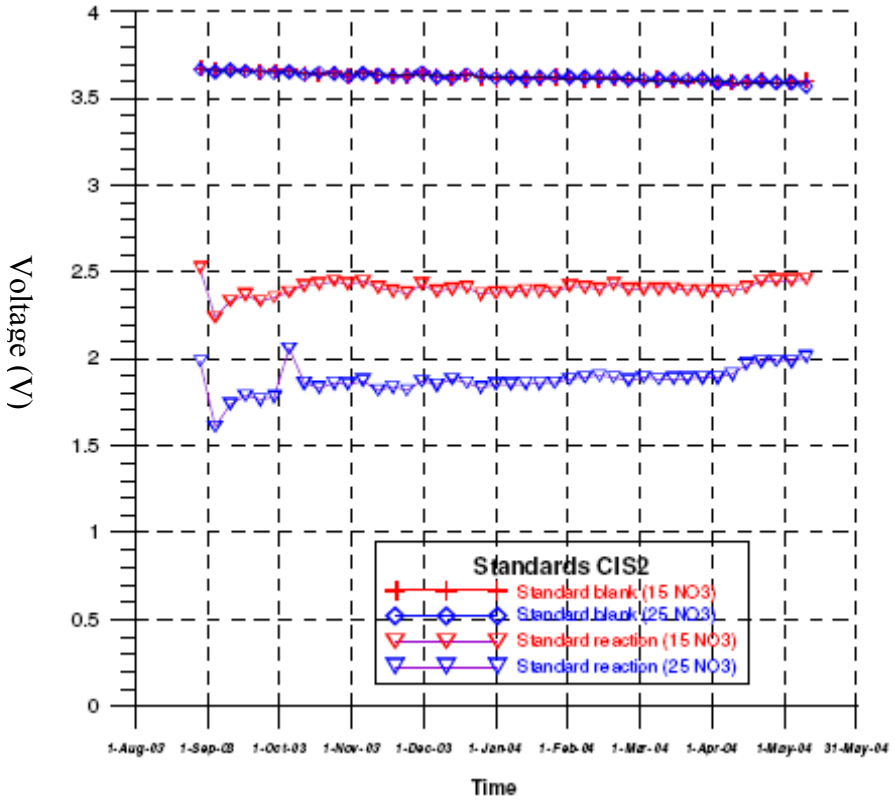

Figure 14 Blank and standard measurements during a 9 month deployment of NAS nitrate analyser (from [72])

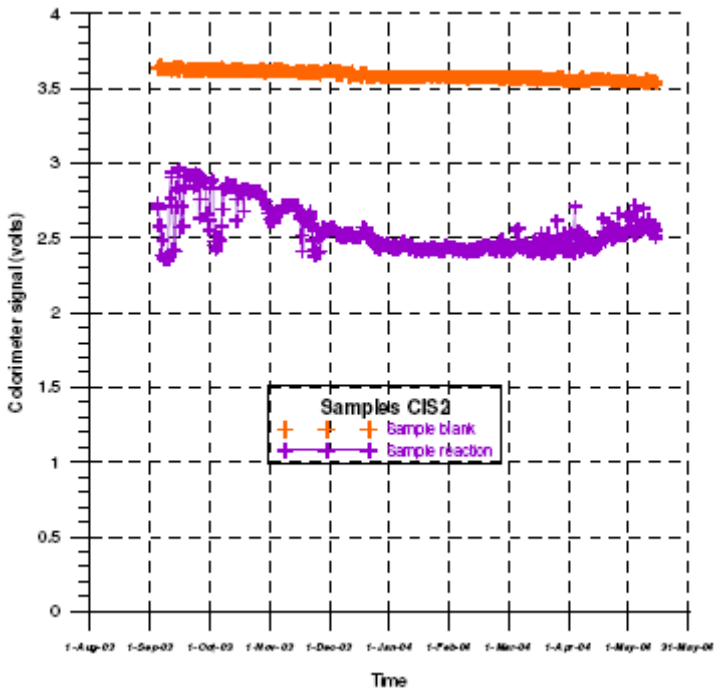

Figure 15 Reacted and unreacted sample measurements during a 9 month deployment of NAS nitrate analyser (from [72])

The experience of three deployments at the PAP site is more mixed with both good and bad data. One significant feature of these deployments is that the sensor packages experienced deep dives due to mooring instability in tidal flows; the sensors appear to be sensitive to such excursions again suggesting a lower TRL.

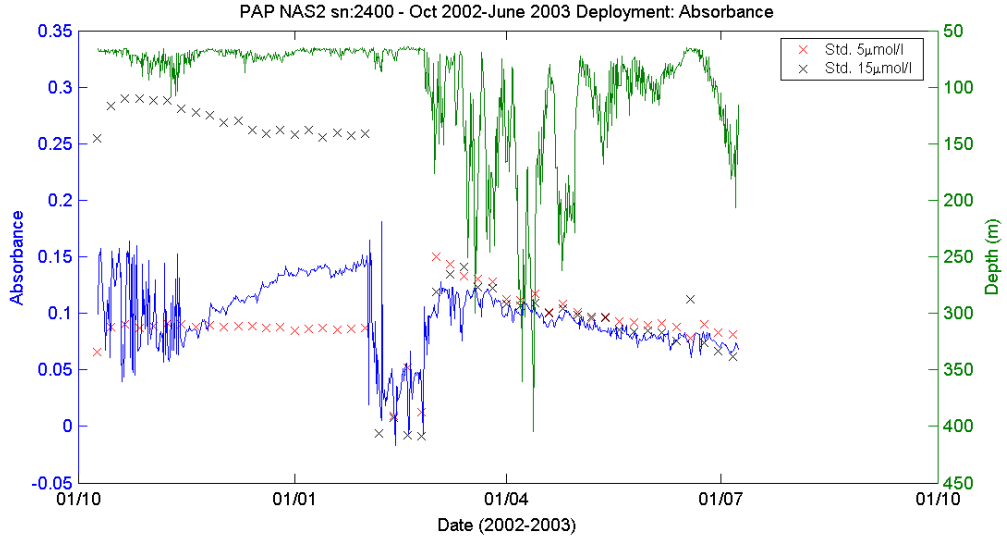

Figure 16 Performance of NAS nitrate analyser at PAP 10/02 - 6/03

Deployment (10/03 -10/04) produced good data for 4 months with believable values for the two standards being recorded. Note there is significant variability in the nitrate signal during the first month, indicating that a higher sampling rate may be beneficial. At four months an error occurs, it is difficult from the data to ascertain the exact cause, but the sudden onset rules out degradation of the samples or reagent. Initially it appears that there is a stuck valve or blocked reagent pipe as standards and sample all measure very low absorbance (little reaction if any has occurred). After approximately two weeks, the instrument experiences a depth excursion. This appears to clear the problem, and the sample signal appears to recover.

However, the low standard reads high absorbance, before settling, whilst the high standard never recovers. This casts serious doubt on any data obtained beyond month 4.

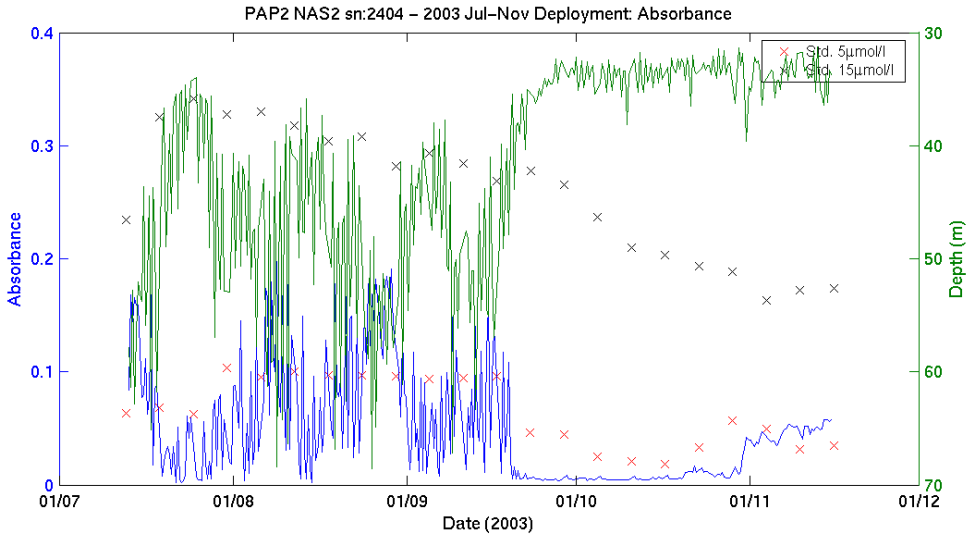

Figure 17 Performance of NAS2 nitrate analyser at PAP 6/03 – 11/03

Deployment 2 (Figure 18) shows a steady decrease in absorbance of the reacted standards over time, though there are a number of significant features. In addition the deployment is short, and the instrument is still operating when it is retrieved. This enables post retrieval calibration, greatly enhancing quality control and enabling standard degradation and sensor drift to be characterised. This gives confidence that the compensated data will be realistic. Again it is interesting to note the high frequency component in the sample signal suggesting the system is undersampled.

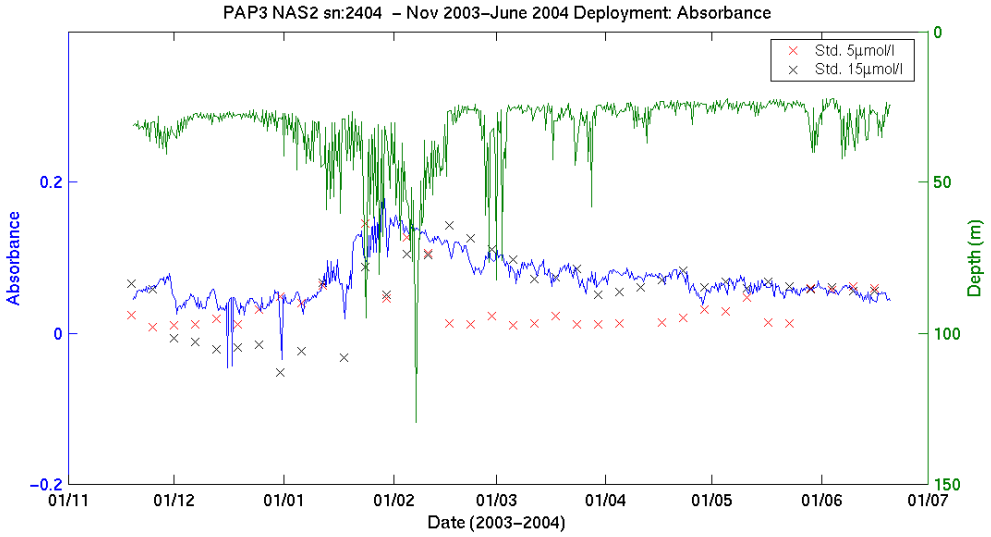

Figure 18 Performance of NAS2 nitrate analyser at PAP 11/03-06/04

Deployment 3 is disappointing from the outset. If real-time data were available, it is likely that (time and resource permitting) the instrument would have been recovered for investigation immediately after first deployment. Clearly the standards are not operating as required, and in the absence of this quality control data, it is difficult to have confidence that the instrument is operating correctly. In this circumstance additional standards from reference measurements and or sampling are required to interpret the data, or it must be abandoned. One could assume that only the high standard is incorrect, but this is difficult to confirm, especially as retrieval does not occur until approximately eight months after the problem is first apparent.

Despite these difficulties NAS analysers can provide high accuracy *in situ* data of nitrate over long time periods. With significant investment of time and expertise, it is possible to obtain long term measurements with these systems. This is demonstrated by the favourable comparison of NAS data with ship of opportunity nitrate data, and correlation with the position of the mixed layer depth observed using Argo floats (Figure 19 from [98])

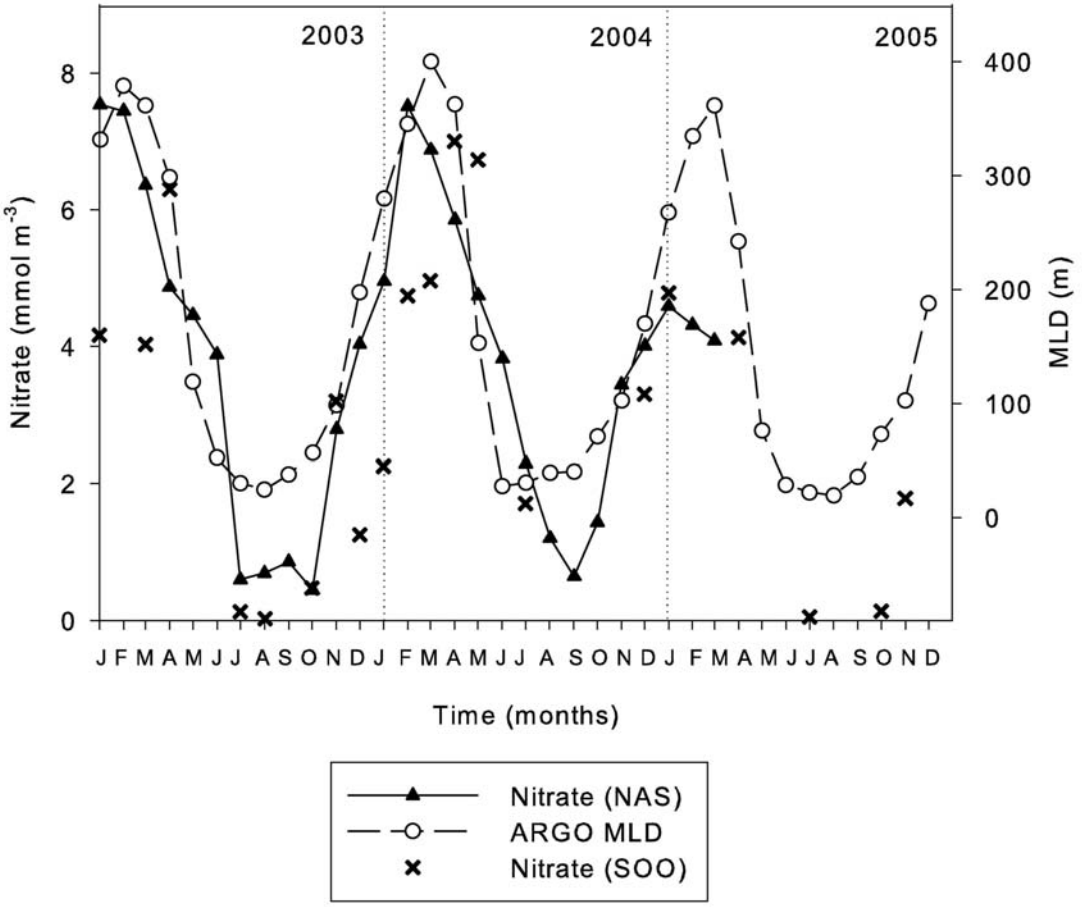

Figure 19 Three years of *in situ* sensor data compared with mixed layer depth (reported from ARGO) and samples from ships of opportunity [98]

5.3.4.2 Spectrophotometers

Some minor problems have been experienced with the Satlantic ISUS V2 by investigators at the PAP site. These include erroneous calibrations, and software interpretation indicating TRL8. The sensor performance is in line with the data sheet. In deployments LOD is 0.68µM (20 second average, therefore 3µM/√Hz). Precision of surface readings is of the order 0.4µM (90 second average, therefore 3.8µM/√Hz). Uncalibrated accuracy (3.5µM (at low concentrations) to 8.5µM) is improved by cast calibrations (-0.74µM to 0.94µM at low concentrations, -0.2 to 0.1µM otherwise). Cast specific calibrations suggest a temporal and possibly a spatial variation in calibrations.

5.4 The Alliance for Coastal Technologies (ACT)

The Alliance for Coastal Technologies (ACT) is a (National Oceanic and Atmospheric Administration) NOAA (US) funded partnership of research institutions, resource managers, and industry. Its remit is to develop and promote adoption of effective and reliable sensors and platforms for coastal applications. Implicit to this mission is the aim to provide the

Integrated Ocean Observing System (IOOS) with information required to enable reliable and cost-effective observing networks.

To fulfil this role ACT hosts a searchable data base of information pertaining to environmental technologies. It coordinates themed meetings targeting technology and application areas. In addition ACT has begun a comprehensive performance evaluation of commercially available biogeochemical sensor technology for coastal applications. This considerable body of work forms a useful resource for evaluation of current and future biogeochemical sensing capabilities for oceanic and Eulerian deployments and is therefore extremely relevant to this study. However, the performance target for coastal and Eulerian observatories is not the same. For example ACT technology evaluations do not emphasise drift, repeatability or reproducibility, which are crucial to Eulerian applications. In addition ACT tends not to publish direct quantitative performance comparison summaries, and this is what is required for Eulerian applications.

To date data has been published on Fluorometer based Chlorophyll a sensors, and oxygen sensors. Further tests of biogeochemical sensors are in progress. Data on testing of nutrient sensors will be the next to be published, and is therefore, unfortunately unavailable for this study.

5.4.1 Fluorometers

Between 2005 and 2006 ACT completed performance evaluations for eight *in situ* fluorescence sensors. All of the sensors tested are steady state fluorescence sensors (i.e. not FRRF instruments) and are designed to enable study of Chlorophyll a. It is interesting to note that none of the manufacturers claims that there is an unambiguous link between fluorescence and [Chla]. In contrast many of the manufacturers give very detailed explanations of how to account for variation due to environment and community structure using additional measurements (e.g Chla extraction and HPLC).
The ACT reports acknowledge that coastal users selected instruments on the basis of precision, accuracy, and detection limit. This is at odds with the variability induced by taxonomic, physiological and environmental factors Noting that this was in contradiction to the nature of the measurement, ACT opted to use laboratory monocultures (*Thalassiosira pseudonana*) and a surrogate (defined reference) for their performance evaluation of the technology. These test used basic blue 3 (BB3 (λ_{amax}= 654nm, λ_{emax}=661nm) [99]). Some additional tests were conducted with Rhodamine WT (λ_{amax}= 497nm, λ_{emax}=523nm). These surrogates have significantly different optical properties to chlorophyll (e.g. BB3 has low absorption 350-490nm) but offer sufficiently broad excitation and emission spectra to enable characterisation of the fluorometers tested. To calibrate the instruments with *Thalassiosira pseudonana* parallel sampling, chlorophyll extraction, and measurement with HPLC was performed.
The tests performed allow verification of sensor precision (noise limited), sensitivity to [Chla] in a monoculture of *Thalassiosira pseudonana* and an investigation of the robustness of the instruments in coastal deployments, including resistance to fouling. It is unfortunate that an assessment of instrument repeatability or reproducibility is absent. This information is not present as the only repeat calibrations occur before and after field deployment and hence sensor drift cannot be separated from any damage / influence due to biofouling, and subsequent cleaning. In addition these tests are only separated by four weeks, as compared to planned Eulerian deployments of 6 or 12 months
The results of these tests are summarised in Table 4. These tests indicated that instrument precision and performance was in line with that quoted by the manufacturers in their data sheets (DS). One possible discrepancy is the relatively large variation (5-13%) seen between repeated calibrations in the lab. Here a first calibration was undertaken (using BB3 as a

reference) to ascertain precision and sensor linearity. This calibration was repeated before adding coffee (to simulate Chromophoric Dissolved Organic Matter (CDOM)) and Formazin (to simulate turbidity) and exposure to light (to investigate sensitivity to PAR). It is not possible to trace the source of the differences in calibrations, but these may be due to experimental error.

The most significant results for long-term Eulerian applications are those relating to biofouling. The experiment placed examples of each sensor at eight coastal sites. Sensor performance was evaluated pre-deployment, post deployment, and post deployment after attempted cleaning. Performance was evaluated by taking measurements with a low blank (DI water) and two standard solutions contain 1) BB3 and 2) Rhodamine. Only the Wetlabs and YSI probes had significant antifouling protection (mechanical wipers and copper) and therefore exhibited the best resistance to fouling. The Wetlabs sensor exhibited the most resistance with virtually unaffected readings in all but one deployment.

Other significant findings include the high performance of the Chelsea spectrometers, and the promising utility of multi-wavelength analysis offered by the BBE Fluoropobe. The latter technique may yet allow compensation for variability induced by species present. Both require additional infrastructure to prevent biofouling.

Table 4 Summary of results from ACT fluorometer technology evaluations

Instrument	Precision		Sensitivity		Sensor drift		Biofouling		notes
	DS	ACT	DS	ACT	DS	ACT	DS	ACT (4 week test)	
BBE Moldaenke Flouroprobe 2	0.05µg/L	±2.16 to ±0.26 µg/L (±0.93 units)[5]	Not stated	1.619, 0.430, 1.087, 1.077, 3.461, 1.538 units/[Chla][6 7] (µg/L)	Not stated	12% between cals	Not stated	Not tested	Multi wavelength = reduces impact of species Max range 200µg/L PAR effect disputed (ACT error) CDOM + turbidity effects observed in multiwavelength analysis (as expected and required)[8]
Chelsea Aqua traka III	0.01µg/L	0.005µg/L at low [Chla] (±4.4mV)	Not stated	621mV/log([Chla]) (~1V/µg/L at low [Chla])[1]	Accuracy 0.02 or 3%	7% between cals	Not stated	Not resistant – up to 100 times reduction in sensitivity	LOD 0.018µg/L (ACT), 0.01 (DS) Max 100µg/L(DS) Turbidity increases offset (15%) CDOM + turbidity causes overestimate (15%) Unaffected by PAR
Chelsea mini tracka IIC	0.01µg/l	0.008 µg/l ±0.75mV	Not stated	83.7mV/[Chla] (µg/L)	Not stated	5% between cals	Not stated	Not resistant to biofouling	Range (DS) 0.03-100µg/ Turbidity no effect CDOM causes overestimate (<10%) Unaffected by PAR
Hydrolab DS5X Sonde	0.01ug/L	0.009 µg/l ±0.05mV	Not stated	5.4mV/[Chla] (µg/L)	Not stated	12% between cals	Not stated	battery failure = only one test (chesapaeke), offset ~10% in 4 weeks	LOD 0.018ug/L (ACT) Turbidity and CDOM increase offset CDOM + turbidity reduces sensitivity (7%) Unaffected by PAR
Turner Cyclops-7	0.01 µg/l (inferred from LOD)	0.06 µg/l ±4.22mV 50% lower @32ºC	Not stated	73mV/[Chla] (µg/L)	Not stated	13% between cals. 5 - 12 % in field	Not stated	Not resistant to biofouling	Same product as above, different packaging LOD 0.03µg/L (DS) 0.018µg/L (ACT) Max 500µg/L (DS) Turbidity and CDOM increase offset CDOM + turbidity reduces sensitivity (6%) Unaffected by PAR
turner SCUFA	0.007	0.16 µg/l	Not	1.480RFU/[Chla]	Not	12%	Not	10% to 100%	LOD 0.02µg/l (DS)

[5] Disputed by manufacturer (with some justification). Instrument not operating all the time increasing noise by factor ~5, plus bubbles in 15°C test, DS is therefore accurate.
[6] Species used: *Thalassiosira pseudonana*
[7] Instrument uses multiple excitation wavelengths, each giving different sensitivities
[8] NB descriptions of figure 5 in the performance verification statements for the BBE Fluoroprobe and YSI 6025 are tansposed between documents

	µg/l inferred from LOD	±0.235RFU	stated	(µg/L)	stated	between cals 4-17% in field	stated	(complete) loss in signal dependent on location	CDOM and turbidity double offset and produce underestimate (7%) Unaffected by PAR
Wetlabs ecoflntusb	Not stated	0.045µg/l ±1.13 counts	Not stated	25.06counts/[Chla] (µg/L)	Not stated	15% between cals. (~1%) in field	Not stated	Resistant (except Skidaway Isl (95% loss in signal))	LOD 0.01µg/L (DS) 0.018µg/L (ACT) Max 50µg/L (DS) Turbidity and CDOM increase offset CDOM + turbidity reduces sensitivity (6%) Unaffected by PAR
YSI 6025	0.1µg/	0.272ug/L	Not stated	0.483 counts/[Chla] (µg/L)	Not stated	15% between calls	Not stated	Some resistance (errors 3-600% increase)	Max 400µg/L (DS) Unaffected by PAR CDOM + turbidity reduces sensitivity (~7%)

5.4.2 Oxygen sensors

During 2004 ACT performed performance verifications for four oxygen sensors. This assessment enables determination of precision, accuracy and resistance to biofouling. In similarity with the fluorometers evaluations, no clear conclusions can be drawn regarding repeatability or reproducibility as the necessary data cannot be separated from the effects of biofouling and the deployment is too short (4 weeks). The results need some interpretation as a large number of Winkler titrations were used to provide reference oxygen values. This causes two effects, firstly this measurement is not without error (typical $1\sigma = 0.6\mu M$) and in addition, it is very difficult to create homogenous, or reproducible dissolved gas concentrations with open containers even in the lab. Therefore, there may be significant differences between the sample concentration and that encountered by the sensor. A more controlled calibration can be obtained using closed container [54].

The results confirm that the short term performance of the sensors is broadly in line with manufacturers data sheets (DS). However, none of the sensors performed well in coastal deployments with or without biofouling protection systems (BPS). However none had significant prevention strategies, and oxygen sensors are very sensitive to fouling as the creation of a local microenvironment greatly disturbs the oxygen concentration. It follows that the sensor can continue to operate without error, but record oxygen concentrations unrelated to the unperturbed environment. Independent evaluations of (particularly the fluorescence lifetime based) sensors over longer periods suggest that the sensors themselves can exhibit very low drift[26]. This suggests that urgent priority should be given to biofouling protection to enable their use in long term Eulerian applications.

Also of significance is the performance of these sensors with respect to the range of concentrations observed in the environment (see Table 2). Precision is less than 1% of the range, and accuracy 5-10% of the range.

Table 5 Summary of results from ACT oxygen sensor technology verifications (all values in μM)

Instrument	Precision[9]		Accuracy		Biofouling induced error		notes
	DS	ACT	DS	ACT[10]	DS	ACT (4 week test)	
Aanderaa optode	<1	0.6 +BPS[11] 1.6	8	~10	No standard protection	13-270 (no protection) 12-182 (BPS)	Optical fluorescence lifetime sensor
Greenspan D0300/DO1200	Not known	0.6 +BPS 1.6	9	~30	Copper cladding of absorption rod sample system	43-250 (no protection) 73-262 (BPS)	Galvanic (polarised) electrode
In-Situ Inc. Disolved oxygen RDO sensor	0.3	0.3 +BPS 0.3	3	~10	No standard protection	16-270 (no protection) 9-117 (BPS)	Optical fluorescence lifetime sensor
YSI inc rapid pulse oxygen sensor	0.3	0.3 +BPS 0.3	8	~10	No standard protection	12-260 (no protection) 18-226 (BPS)	Polagraphic electrochemical

[9] NB Winkler titrations used as the reference had a precision of 0.6μM

[10] NB ACT method is prone to error and these values should be taken with caution. For improved method see Sosna, M., *et al.*, Development of a reliable microelectrode dissolved oxygen sensor. Sensors and Actuators B-Chemical, 2007. 123(1): p. 344-351.

[11] Biofouling prevention system (in most cases a copper gauze or variant thereof)

6 Conclusions

Biogeochemical sensing in Eulerian applications is a logistical, technological, and scientific challenge and is at the edge of what is currently routinely achievable. A number of organisations across Europe, and globally, have (despite the difficulties) pioneered long term sensor deployments, and have produced significant data sets. To make similar or repeated undertakings easier, and more productive three areas need to be addressed. Firstly the technology readiness level of biogeochemical sensors needs to be raised particularly in terms of robustness, and failure prevention. Secondly the long-term drift, and performance degradation of sensor systems with time needs to be a) evaluated and b) reduced through engineering design and operational methodology. Thirdly the effects of biofouling must be mitigated, most likely through biofouling prevention strategies. All three areas require considerable development effort requiring significant funding. This should be addressed by a coordinated response from science funding agencies (e.g NERC, and EU), from the science community, from technology research and development groups, and from industry.

In addition many biogeochemical parameters cannot yet be characterised to the performance required by the science community. Though a number of promising technologies are in development a critical re-evaluation of the performance requirement, and priority measurands would enable more rapid progress, as would consistent funding of this area including through industrial collaboration.

This report reviews the research and international context of long term biogeochemical sensing and identifies its importance in answering key scientific question including the role of the ocean in global carbon cycles and climate change. A list of key biogeochemical parameters and required measurement performance has been identified though refinement of this list and relaxing of performance requirements where possible will speed the development of useful sensor technology. In summary sensors should be able to measure nutrients in the 1-50μM range, micronutrients in the nanomolar to low micromolar range, oxygen, and carbon dioxide at equilibrium concentrations and lower, methane and the carbonate system and pH in the nanomolar, micromolar, and mid pH ranges. A precision and accuracy of 2% of these ranges is usually sufficient. Performance parameters for biological measurements are less clear, but *in situ* measurement of primary production (with accuracy of 2% of range) and biomolecular analysis of 10 cells currently presents a challenging target.

A review of commercial technologies identifies numerous devices, some of which meet the performance targets required for this application. These are oxygen optodes and the latest electrochemical oxygen sensors; reagent based nutrient analysers, a CO_2 sensor, and pH sensors.

Of the emerging technologies, microfluidic analysers (for reagent based detection of nutrients and micronutrients) are promising, as are optodes for CO_2, pH, and methane. FRRF with additional reference measurements may yet enable unambiguous determination of primary production, and tantalising opportunities are afforded by *in situ* biomolecular analysers and cytometers.

A performance evaluation of commercial sensors based on published evaluations, laboratory evaluations and the experience gained through use in the field for long term deployments has been completed. This concludes that biofouling remains a significant issue for most if not all sensors used in long term deployments. However, some success has been gained using copper in this context. The evaluation of sensor technologies concludes that oxygen sensors are now mature enough to be deployed routinely. Reagent based CO_2 sensors offer good long term performance, though they are currently large and have minor robustness and reliability issues, and reference measurements (for *in situ* calibration) are prone to error. Further evaluation and collaboration with the manufacturers is recommended. Reagent based nutrient sensors remain at a low TRL, but when operating correctly produce high quality data, they remain the state of the art for nutrient sensing despite the difficulty of operation. Spectrophotometer based nitrate

sensors are promising, but currently exhibit too much drift and / or sensitivity to non nitrate associated optical changes to be considered accurate for long term applications. The use of Fluorometers for long term deployments is widespread, and they continue to provide useful information on growth rates of phytoplankton communities. However, the practice of relating their measurements to chlorophyll concentration for long term deployments is extremely inaccurate, and should be discouraged, unless frequent calibrations are made using chlorophyll extraction (and analysis e.g. HPLC) to account for changes in community structure and physiology.

The activities of the Alliance for Coastal technologies is reviewed. Whilst the ACT technology evaluations provide a useful resource, the performance target is not necessarily the same for coastal and oceanic Eulerian observatories. In addition a more direct quantitative comparison is required (e.g. by a table summarising performance parameters of each class of sensors). There are good reasons why ACT does not publish such a comparison but that is what the Eulerian observatory community needs. In addition, ACT does not specifically assess sensor drift, repeatability or reproducibility as required in Eulerian applications. This must be done by long term lab trials, and with testing in a range of water types. Biofouling should be measured separately by field deployments. Here a coastal deployment has advantages as fouling is more rapid allowing accelerated testing.

7 Recommendations

Recommendations are made regarding what sensors should and should not be used in the immediate future for Eulerian observatories. Promising emerging technologies are summarised, and detailed methodological practices that should lead to improved technology and scientific data are outlined. Finally additional recommendations for raising the TRL of biogeochemical sensor technology are made.

Recommended sensors

- Oxygen sensors: Aanderaa optode, Seabird 43
- Reagent based nutrient sensors; a critical evaluation of the best sensors is required, this may be supplied by ACT, but a bespoke analysis for long term Eurlerian applications is also required (i.e. to assess long term drift, repeatability, reproducibility and biofouling). Systems to evaluate are the NAS, Subchem, Wetlabs cycle P, and possibly DPA
- Reagent based carbon dioxide sensors (SAMI CO_2).
- pH – SAMI and Idronaut electrode sensors (the latter needs evaluation)

Sensors not currently recommended

- Fluorometer – unless only a relative measure of chlorophyll (e.g. timing of bloom) is required, or extensive sampling is available to give confidence that calibration is not time dependent.
- Spectrophotometric Nitrate sensors, a full performance audit is required to investigate drift / sensitivity to non nitrate optical changes

Suggested important emerging technologies

- Microfluidic reagent analysers
- Optical indicators e.g. for CO_2, pH and methane
- Cytometers
- Biomolecular analysers
- Primary production (FRRF together with reference measurements)

Methodological recommendations

- Best practice be adopted from current Eulerian observation practice including
    - Quality control and error analysis of real-time data

    - Site specific calibrations for fluorometers / FRRF preferably using site and depth specific cultures
    - Detailed quality assurance procedures for reagent based nutrient sensors[72]
    - Calibration checking at the beginning and end of deployments with samples in close proximity to the sensors
    - Planned and opportunistic reference measurements
- Assessment of intrinsic sensor drift and the effects of biofouling by calibration of sensors before deployment, and shortly after recovery both before *and* after cleaning. If sensor drift is known *a priori* before deployment, greater measurement accuracy may be possible, than if field calibrations were used (e.g. for CO_2 measurements).
- Development of a system to qualify sensors for long term operation *before* they are deployed on observatories consisting
    - Pre deployment long term technology assessment both in the lab (to assess sensor drift) and in coastal test bed (to assess the effects of biofouling)
    - Pre deployment performance evaluation (accuracy, precision, LOD)
- The use of qualifying system to expand the list of sensors that can be deployed (e.g. other nutrients, methane)
- Reduced maintenance intervals, and shorter deployments. The longest deployment should be shorter (~20%) than the expected duration of the sensor with the shortest valid operational life (to allow end point calibration)
- Detailed tracking documents recording all aspects of maintenance, calibration, deployment and data recorded by each instrument – this is an invaluable resource for tracking design performance and therefore improving technology readiness level
- The construction of real time data reporting systems for all biogeochemical sensors. This could for example enable retrieval and replacement of inoperative sensors shortly after deployment (thus saving the dataset) or reporting sensor degradation enabling retrieval before complete failure ensuring a post retrieval calibration is possible.
- Use sensor redundancy where practical (and economic). For example retaining a spare before deployment would allow replacement of sensors that do not perform adequately on final calibration (often at the deployment site).
- Wherever possible, the use of more frequent calibration. This is particularly imperative for Fluorometers / FRRF as the relationship between fluorescence, and chlorophyll concentration is known to be extremely variable.

Raising TRL

- Regular and far reaching reassessment of the key science questions and therefore which parameters are required to what resolution (to enable developers to focus their efforts)
- Accurate log keeping, and completion of sensor tracking documents to enable sensor performance and error analysis / debugging
- Greater collaboration with instrument manufacturers / developers
- Repeat and update this exercise (e.g. when ACT technology evaluation report on Nutrient sensors is available)
- Greater coordination and collaboration between the biogeochemical user, evaluator and developer community – perhaps by formation of a distinct body.

8 References:

1. **Currie, L.A., *Detection and quantification limits: basic concepts, international harmonization, and outstanding ("low-level") issues.* Applied Radiation and Isotopes, 2004. 61(2-3): p. 145-149.**
2. ***International vocabulary of basic and general terms in metrology (VIM) 3rd Edition*. 2004, International Organization for Standardization: Geneva.**
3. **Griffiths, G., *et al., Report on the Eulerian Workshop, Monaco (at IAEA and CIESM)*, in *EUR-OCEANS European Network of Excellence for Ocean Ecosystems Analysis*. 2006.**
4. **Costanza, R., *et al., The value of the world's ecosystem services and natural capital.* Nature, 1997. 387(6630): p. 253-260.**
5. **Watson, R.T., *Climate Change 2001: Synthesis Report. ,* in *The Third Assessment IPCC*. 2001, IPPC: Geneva. p. 184.**
6. **King, D., *Climate change: the science and the policy.* Journal of Applied Ecology, 2005. 42(5): p. 779-783.**
7. **Martin, A.P., *et al., Extreme spatial variability in marine picoplankton and its consequences for interpreting Eulerian time-series.* Biology Letters, 2005. 1(3): p. 366-369.**
8. **Gallager, S. and J. Whelan, *Report of the workshop on the next generation of in situ biological and chemical sensors in the ocean*. 2003, Woods Hole Oceanographic Institution: Massachusetts**
9. **Daly, K.L. and R.H. Byrne, *Chemical and biological sensors for time-series research: current status and new directions.* Marine Technology Society Journal, 2004. 38(2): p. 121-143.**
10. **Statham, P.J., *et al., Spatially Complex Distribution of Dissolved Manganese in a Fjord as Revealed by High-Resolution in Situ Sensing Using the Autonomous Underwater Vehicle Autosub.* Environ. Sci. Technol., 2005. 39(24): p. 9440-9445.**
11. **Dore, J.E. and D.M. Karl, *Nitrification in the euphotic zone as a source for nitrite, nitrate, and nitrous oxide at Station ALOHA.* Limnology and Oceanography, 1996. 41(8): p. 1619-1628.**
12. **Grunwald, M., *et al., A novel time-series station in the Wadden Sea (NW Germany): First results on continuous nutrient and methane measurements.* Marine Chemistry, 2007. 107(3): p. 411-421.**
13. **Uncles, R.J., P.E. Frickers, and C. Harris, *Dissolved nutrients in the Tweed Estuary, UK: inputs, distributions and effects of residence time.* Science of the Total Environment, 2003. 314: p. 727-736.**
14. **Libes, S.M., *An introduction to marine biogeochemistry*. 1992: John Wiley & sons.**
15. **Fujieki, L.A. *Hawaii Ocean Time-series Data Organization & Graphical System (HOT-DOGS©)*. 2008 [cited 2008 February]; Available from: http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html.**
16. **Fagan, K.E. and F.T. Mackenzie, *Air-sea CO_2 exchange in a subtropical estuarine-coral reef system, Kaneohe Bay, Oahu, Hawaii.* Marine Chemistry, 2007. 106(1-2): p. 174-191.**
17. **Bronk, D.A., A.H. Dennis, and A.C. Craig, *Dynamics of DON*, in *Biogeochemistry of Marine Dissolved Organic Matter*. 2002, Academic Press: San Diego. p. 153-247.**
18. **Steeman-Nielsen, E., *Measurement of production of organic matter in sea by means of carbon-14.* Nature, 1951. 267: p. 684-685.**

19. **Luz, B. and E. Barkan, *Assessment of oceanic productivity with the triple-isotope composition of dissolved oxygen.* Science, 2000. 288(5473): p. 2028-2031.**
20. **Reuer, M.K., *et al., New estimates of Southern Ocean biological production rates from O-2/Ar ratios and the triple isotope composition of O-2.* Deep-Sea Research Part I-Oceanographic Research Papers, 2007. 54(6): p. 951-974.**
21. **Falkowski, P. and D.A. Kiefer, *Chlorophyll-a Fluorescence in Phytoplankton - Relationship to Photosynthesis and Biomass.* Journal of Plankton Research, 1985. 7(5): p. 715-731.**
22. **Salter, I., *et al., Estimating carbon, silica and diatom export from a naturally fertilised phytoplankton bloom in the Southern Ocean using PELAGRA: A novel drifting sediment trap.* Deep-Sea Research Part Ii-Topical Studies in Oceanography, 2007. 54(18-20): p. 2233-2259.**
23. **Blain, S., *et al., Effect of natural iron fertilization on carbon sequestration in the Southern Ocean.* Nature, 2007. 446(7139): p. 1070-U1.**
24. **Carlson, J., *Development of an Optimized Dissolved Oxygen Sensor for Oceanographic Profiling.* International Ocean Systems, 2002. 6(5).**
25. **Demas, J.N., B.A. DeGraff, and P.B. Coleman, *Oxygen sensors based on luminescence quenching.* Analytical Chemistry, 1999. 71(23): p. 793A-800A.**
26. **Tengberg, A., *et al., Evaluation of a lifetime-based optode to measure oxygen in aquatic systems.* Limnology and Oceanography-Methods, 2006. 4: p. 7-17.**
27. **Tamburri, M., *Performance Verification Statement for the Aanderaa Instruments Inc. Dissolved Oxygen Optode 3830/3930/3835* in *UMCES Technical Report Series*. 2004, Alliance for Coastal Technologies.**
28. **Johnson, K.S. and L.J. Coletti, *In situ ultraviolet spectrophotometry for high resolution and long-term monitoring of nitrate, bromide and bisulfide in the ocean.* Deep-Sea Research Part I-Oceanographic Research Papers, 2002. 49(7): p. 1291-1305.**
29. **Hanson, A.K., *Cabled and autonomous submersible chemical analyzers for coastal observations.* Abstracts of Papers of the American Chemical Society, 2005. 229: p. U152-U153.**
30. **Degrandpre, M.D., *et al., In-Situ Measurements of Seawater Pco(2).* Limnology and Oceanography, 1995. 40(5): p. 969-975.**
31. ***"Cycle" Phosphate Sensor*. 2008 [cited 2008 February]; Available from: http://www.wetlabs.com/products/pub/specsheets/cyclessa.pdf.**
32. **Unisense, *Antioxidant - Electrochemical sensor and method for removal of false oxygen in an oxygen or dinitric oxide sensor*. 1997.**
33. **Tercier-Waeber, M.L., *et al., Submersible voltammetric probes for in situ real-time trace element measurements in surface water, groundwater and sediment-water interface.* Measurement Science & Technology, 1999. 10(12): p. 1202-1213.**
34. **Luther, G.W., *et al., Exploring the chemistry of diverse marine environments using in situ voltammetry.* Abstracts of Papers of the American Chemical Society, 2005. 229: p. U152-U152.**
35. ***CONTROS' HydroC™/ CO_2*. 2008 [cited 2008 February]; Available from: http://www.contros.eu/pdf/SpecSheet_HydroC-CO2_ENG.pdf.**
36. ***METS underwater methane sensor*. 2008 [cited 2008 February]; Available from: http://www.capsum.de/capsum_online/mets.pdf.**
37. ***CONTROS' HydroC™/ CH4*. 2008 [cited 2008 February]; Available from: http://www.contros.eu/pdf/SpecSheet_HydroC-CH4_ENG.pdf.**
38. **Faure, K., *et al., Methane seepage and its relation to slumping and gas hydrate at the Hikurangi margin, New Zealand.* New Zealand Journal of Geology and Geophysics, 2006. 49(4): p. 503-516.**

39. **Suggett, D.J., H.L. MacIntyre, and R.J. Geider, *Evaluation of biophysical and optical determinations of light absorption by photosystem II in phytoplankton.* Limnology and Oceanography-Methods, 2004. 2: p. 316-332.**
40. **Dubelaar, G.B.J., *et al.*, *Design and first results of CytoBuoy: A wireless flow cytometer for in situ analysis of marine and fresh waters.* Cytometry, 1999. 37(4): p. 247-254.**
41. **ECOPS. [cited; Available from: http://www.esf.org/publication/146/Marinescience.pdf.**
42. **aslo. [cited; Available from: http://www.neptune.washington.edu/pub/documents/Sensor.Workshop.web.doc.pdf.**
43. **Neurauter, G., I. Klimant, and O.S. Wolfbeis, *Microsecond lifetime-based optical carbon dioxide sensor using luminescence resonance energy transfer.* Analytica Chimica Acta, 1999. 382(1-2): p. 67-75.**
44. **Wolfbeis, O.S., *et al.*, *Fiber-optic fluorescence carbon dioxide sensor for environmental monitoring.* Mikrochimica Acta, 1998. 129(3-4): p. 181-188.**
45. **Schroder, C.R., B.M. Weidgans, and I. Klimant, *PH fluorosensors for use in marine systems.* Analyst, 2005. 130(6): p. 907-916.**
46. **Boulart, C., *et al. Development of a new in-situ methane sensor for deep-sea studies*. in *European Geosciences Union*. 2007.**
47. **Samson, S., Hopkins, T., Remson, A., Langebrake, L., Sutten, T. and Patten, J., *A system for high-reslution zooplankton imaging.* IEEE J Ocean Eng., 2001. 26: p. 671-676.**
48. **Burkill, P.H., *Flow cytometry & the rapid analysis of phytoplankton in marine waters.* Oceanology International, Conference Proceedings, 1998: p. 351-359.**
49. **Caron, D.A., *et al.*, *Protistan community structure: molecular approaches for answering ecological questions.* Hydrobiologia, 1999. 401: p. 215-227.**
50. **Greenfield, D.I., *et al.*, *Application of environmental sample processor (ESP) methodology for quantifying Pseudo-nitzschia australis using ribosomal RNA-targeted probes in sandwich and fluorescent in situ hybridization formats.* Limnology and Oceanography-Methods, 2006. 4: p. 426-435.**
51. **Patterson, S.S., *et al.*, *A nucleic acid sequence-based amplification assay for real-time detection of norovirus genogroup II.* Journal of Applied Microbiology, 2006. 101(4): p. 956-963.**
52. **Smyth, T.J., *et al.*, *A methodology to determine primary production and phytoplankton photosynthetic parameters from Fast Repetition Rate Fluorometry.* Journal of Plankton Research, 2004. 26(11): p. 1337-1350.**
53. **Taillefert, M., G.W. Luther, and D.B. Nuzzio, *The application of electrochemical tools for in situ measurements in aquatic systems.* Electroanalysis, 2000. 12(6): p. 401-412.**
54. **Sosna, M., *et al.*, *Development of a reliable microelectrode dissolved oxygen sensor.* Sensors and Actuators B-Chemical, 2007. 123(1): p. 344-351.**
55. **Lacombe, M., *et al.*, *Silicate deten-nination in sea water: Toward a reagentless electrochemical method.* Marine Chemistry, 2007. 106(3-4): p. 489-497.**
56. **Finch, M.S., D.J. Hydes, and C.H. Clayson, *A low power ultra violet spectrophotometer for measurement of nitrate in seawater: introduction, calibration and initial sea trials.* Analytica Chimica Acta, 1998. 377(2-3): p. 167-177.**
57. **Masserini, R.T. and K.A. Fanning, *A sensor package for the simultaneous determination of nanomolar concentrations of nitrute, nitrate and ammonia in seawater by fluorescence detection.* Marine Chemistry, 2000. 68: p. 323-333.**
58. **Vuillemin, R., *et al.*, *ANAIS: Autonomous nutrient analyzer in-situ.* Sea Technology, 1999. 40(4): p. 75-78.**

59. **Vuillemin, R., *et al. CHEMINI (CHEmical MINIaturised analyser) A new generation of in-situ chemical analysers for marine applications*. in *European Geosciences Union 2007*. 2007.**
60. **Okamura, K., *et al., Development of a deep-sea in situ Mn analyzer and its application for hydrothermal plume observation.* Marine Chemistry, 2001. 76: p. 17-26.**
61. **Chin, C.S., K.S. Johnson, and K.H. Coale, *Spectrophotometric determination of dissolved manganese in natural waters with 1-(2-pyridylazo)-2-napthol; application to analysis in situ in hydrothermal plumes.* Marine Chemistry, 1992. 37: p. 65-82.**
62. **Statham, P.J., *et al., Mapping the 3D spatial distribution of dissolved manganese in coastal waters using an in situ analyser and the autonomous underwater vehicle Autosub.* Underwater Technology, 2003. 25(3): p. 129-134.**
63. **Chin, C.S., *et al., In situ observations of dissolved iron and manganese in hydrothermal vent plumes, Juan de Fuca ridge.* journal of Geophysical Research, 1994. 99(B3): p. 4969-4984.**
64. **Chapin, T.P., H.W. Jannasch, and K.S. Johnson, *In-situ osmotic analyzer for the year-long continuous determination of Fe in hydrothermal systems.* Analytica Chimica Acta, 2002. 463: p. 265-274.**
65. **Bellerby, R.G.J., *et al., A high precision spectrophotometric method for on-line shipboard seawater pH measurements: the automated marine pH sensor (AMpS).* Talanta, 2002. 56(1): p. 61-69.**
66. **Liu, X.W., *et al., Spectrophotometric measurements of pH in-situ: Laboratory and field evaluations of instrumental performance.* Environmental Science & Technology, 2006. 40(16): p. 5036-5044.**
67. **Wang, Z.H.A., *et al., Simultaneous spectrophotometric flow-through measurements of pH, carbon dioxide fugacity, and total inorganic carbon in seawater.* Analytica Chimica Acta, 2007. 596(1): p. 23-36.**
68. **Nakano, Y., *et al., Simultaneous vertical measurements of in situ pH and CO_2 in the sea using spectrophotometric profilers.* Journal of Oceanography, 2006. 62(1): p. 71-81.**
69. **Villéon, L.P.d.l., *et al., In-situ real-time data quality control*, in *Marine EnviRonment and Security for the European Area*. 2005, 6th Framework Programme of the European Commission.**
70. ***Quality Control Procedures applied to Real-Time Data*. [cited 2007 November]; Available from: http://www.noc.soton.ac.uk/animate/data/Quality%20Control%20Procedures%20applied%20to%20Real.htm.**
71. **Petihakis, G., *et al., M3A system (2000-2005) - operation and maintenance.* Ocean Science, 2007. 3(1): p. 117-128.**
72. **Villagarcía, M.G., *Calibration of Biogeochemical data from sensors: Nitrate*, in *Atlantic Network of Interdisciplinary Moorings and Time series for Europe (ANIMATE)*. 2005, Instituto Canario de Ciencias Marinas (ICCM): Las Palmas, Spain. p. 1-17.**
73. **Emerson, S., *et al., In situ determination of oxygen and nitrogen dynamics in the upper ocean.* Deep Sea Research Part I: Oceanographic Research Papers, 2002. 49(5): p. 941-952.**
74. **DeGrandpre, M.D., M.M. Baehr, and T.R. Hammar, *Calibration-free optical chemical sensors.* Analytical Chemistry, 1999. 71(6): p. 1152-1159.**
75. **Kuss, J., *et al., Time-series of surface water CO_2 and oxygen measurements on a platform in the central Arkona Sea (Baltic Sea): Seasonality of uptake and release.* Marine Chemistry, 2006. 101(3-4): p. 220-232.**

76. **Corno, G., *et al.*, *Assessing primary production variability in the North Pacific subtropical gyre: A comparison of fast repetition rate fluorometry and C-14 measurements.* Journal of Phycology, 2006. 42(1): p. 51-60.**
77. **Suggett, D.J., *et al.*, *Fast repetition rate and pulse amplitude modulation chlorophyll a fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton.* European Journal of Phycology, 2003. 38(4): p. 371-384.**
78. **Holm-Hansen, O., A.F. Amos, and C.D. Hewes, *Reliability of estimating chlorophyll a concentrations in Antarctic waters by measurement of in situ chlorophyll a fluorescence.* Marine Ecology-Progress Series, 2000. 196: p. 103-110.**
79. **Tamburri, M., *Protocols for Verifying the Performance of In Situ Chlorophyll Fluorometers*. 2005, Alliance for Coastal Technologies.**
80. **Chang, G.C. and R.W. Gould, *Comparisons of optical properties of the coastal ocean derived from satellite ocean color and in situ measurements.* Optics Express, 2006. 14(22): p. 10149-10163.**
81. **Marrari, M., C. Hu, and K. Daly, *Validation of SeaWiFS chlorophyll a concentrations in the Southern Ocean: A revisit.* Remote Sensing of Environment, 2006. 105(4): p. 367-375.**
82. **Gregg, W.W., *Assimilation of SeaWiFS ocean chlorophyll data into a three-dimensional global ocean model.* Journal of Marine Systems, 2008. 69(3-4): p. 205-225.**
83. **Egli, P.J., *et al.*, *In situ Phosphate Measurements Aboard the Santa Barbara Channel CHARM Mooring.* http://www.wetlabs.com/products/cycle/cycleposter.pdf.**
84. **McNeil, J.D., *et al.*, *New chemical, bio-optical and physical observations of upper ocean response to the passage of a mesoscale eddy off Bermuda.* Journal of Geophysical Research-Oceans, 1999. 104(C7): p. 15537-15548.**
85. **Johnson, K.S., L.J. Coletti, and F.P. Chavez, *Diel nitrate cycles observed with in situ sensors predict monthly and annual new production.* Deep-Sea Research Part I-Oceanographic Research Papers, 2006. 53(3): p. 561-573.**
86. **Pers. Comm. Hartman, S. *Sensor tests in the SONUS laboratory*. 2008**
87. **Hartman, S., *ISUS calibration*. 2007, National Oceanography Centre Southampton.**
88. **Pers. Comm. Hartman, S. *sensors*. [email] 2007 December 22nd;**
89. **Pers. Comm. Coppola, L. *Sensors in Villefranche*. [email] 2007 October 9th;**
90. **Johnson, K.M., *et al.*, *Coulometric Total Carbon-Dioxide Analysis for Marine Studies - Maximizing the Performance of an Automated Gas Extraction System and Coulometric Detector.* Marine Chemistry, 1993. 44(2-4): p. 167-187.**
91. **Mintrop, L., *et al.*, *Alkalinity determination by potentiometry: Intercalibration using three different methods.* Ciencias Marinas, 2000. 26(1): p. 23-37.**
92. **Körtzinger, A., *et al.*, *The seasonal pCO_2 cycle at 49°N/16.5°W in the northeastern Atlantic Ocean and what it tells us about biological productivity.* . Journal of Geophysical Research, 2007. accepted.**
93. **Takahashi, T., *et al.*, *Global sea-air CO_2 flux based on climatological surface ocean pCO(2), and seasonal biological and temperature effects.* Deep-Sea Research Part Ii-Topical Studies in Oceanography, 2002. 49(9-10): p. 1601-1622.**
94. **Hartman, S., *Calibration of chlorophyll-fluorescence during ANIMATE*. 2006, National Oceanography Centre Southampton: Southampton.**
95. **Hartman, S., *Calibration of the WET Labs fluorometer at CIS (August 2006)*. 2007, National Oceanography Centre Southampton.**

96. ***Carbo-SOOS Questionnaire*****. [cited 2008 February]; Available from: http://www.clivar.org/organization/southern/expertgroup/SOOSfeedback/CarboSOOS.doc.**

97. **Hartman, S.,** ***Calibration of the WET Labs fluorometer at CIS (Sept 2004)*** **2005, National Oceanography Centre Southampton: Southampton.**

98. **Hartman, S.,** ***et al., Seasonal and inter-annual biogeochemical variations at PAP (49°N, 16.5°W) 2003-2005.*** **. Deep Sea Research II special issue, 2008. submitted.**

99. **Kopf, U. and J. Heinze,** ***2,7-Bis(Diethylamino)Phenazoxonium Chloride as a Quantum Counter for Emission Measurements between 240 and 700 Nm.*** **Analytical Chemistry, 1984. 56(11): p. 1931-1935.**