Nitrate drawdown during a shelf sea spring bloom revealed using a novel microfluidic in-situ chemical sensor deployed within an autonomous underwater glider

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Abstract

Here we describe, for the first time, the use of a miniaturized Lab-on-Chip (LoC) nutrient sensor deployed within an autonomous underwater vehicles (AUV; Kongsberg Seaglider) to collect high-resolution nitrate (nitrate + nitrite) data in a highly dynamic shelf environment. Seasonally stratified temperate shelf seas act as important carbon sinks, where primary production is controlled by the availability of nutrients such as nitrate. Spring phytoplankton blooms reduce nitrate concentrations to below the limit of detection over short time scales. Diapycnal mixing of nitrate replete bottom layer water can result in increased primary production. These mixing events can happen on temporal scales from hours to days, with current sampling methods unable to capture high frequency events, leading to an underestimation of nutrient supply and hence carbon drawdown. We highlight firstly, an excellent

agreement between the LoC and shipboard nitrate + nitrite measurements ($r^2 = 0.98$ n = 11). Secondly, the ability of LoC and AUV deployments to accurately captured simultaneous biogeochemical and physical parameters at an enhanced resolution, on both spatial and temporal scales, improving our understanding of biogeochemical cycles within the dynamic temperate shelf sea environments.

Introduction

Seasonally stratified temperate shelf seas act as important global carbon sinks through the continental shelf pump mechanism.[1–3] Despite the shelf seas relatively small size (8 % of global ocean area), they actually account for 15 – 30 % of total oceanic primary production[4] and have an average carbon fixation rate per unit area ~ 2.5 times greater than the open ocean.[5] The interplay between light, nutrients and mixing are key drivers of primary production. A well-studied temperate shelf system, the Celtic Sea shows a clear seasonal cycle whereby initial light and nutrient conditions are ideally suited to support the onset of the spring bloom.[6] This results in the rapid drawdown of nitrate (NO_3^-) from ~ 6 - 8 μM to below the limit of detection (< 0.1 μM) (e.g., see refs [7,8]). Post-bloom, new primary production is limited to the sub-surface chlorophyll maximum (SCM) where fluxes of NO_3^- into the thermocline fuels new production.[9]

In the central Celtic Sea, shear generated turbulence[10–12] and wind-driven oscillations[13] are central mechanisms in driving diapycnal mixing of NO_3^- up into the nutrient deplete surface mixed layer. Wind-driven shear occurs in episodic short-lived spikes (0.5- 1 hr) and has the potential to have a large impact on NO_3^- fluxes with observations indicating that this flux can be up to 4 times greater than when no shear was observed. [10,12,14]

At present, it is difficult to effectively sample at the high resolution required to capture these key short-term mixing events.[11,15] Combined with limited winter data, this can lead to incorrect seasonal estimates of NO_3^- fluxes that are key to primary production and carbon fixation. Chemical *in-situ* sensors can provide high-resolution data necessary to resolve biogeochemical processes occurring in shelf seas.[16,17] Wet-chemical analyzers, centered on microfluidic Lab-on-Chip (LoC) technology, are at the leading edge of advancements for chemical *in-situ* nutrient measurements.[18] Due to their compactness, low resource use and analytical performance comparable to laboratory-based methods, LoC nutrient sensors are well suited to high-resolution float, glider and mooring deployments. Autonomous underwater gliders can provide an economic and efficient observation platform to resolve mesoscale and submesoscale structures allowing for high-resolution sampling of biogeochemical parameters such as NO_3^- in shelf seas[19,20].

Here we demonstrate the power of coupling the LoC nutrient sensor into a Seaglider to obtain continuous *in-situ* high temporal and spatial resolution NO_3^- + NO_2^- (hereafter defined as ΣNO_x) measurements over the duration of the spring bloom in April 2015. This enabled short-term mixing events key to establishing the spring bloom and its subsequent decline to be observed.

Experimental

A single LoC ΣNO_x sensor (Ocean Technology and Engineering Group, National Oceanography Center, Southampton, UK) was integrated within the science bay of a Kongsberg Seaglider (Ogive fairing) and deployed from the *R.S.S Discovery* in the Celtic Sea, as part of the NERC funded Shelf Sea Biogeochemistry program in April 2015. In addition, a second LoC ΣNO_x sensor was mounted on a Conductivity-

Temperature-Depth (CTD) rosette to enable direct comparison of its measurements to concentrations of ΣNO_x from seawater samples collected *in-situ* at the same depth and time. Both the Seaglider deployment and discrete ship-based CTD samples were collected from the Central Celtic Sea site (CCS; Figure 1) Located ~137 miles off the Cornish coast, UK (49°24.134'N, 8°36.248'W), with a water column depth of ~ 145 meters. The Seaglider with integrated LoC ΣNO_x sensor was deployed for 21 days (4th-25th April 2015) and completed 776 dives within 10 km of the CCS station. A total of 24 CTD casts within < 22 km of CCS were also conducted. The rosette package consisted of a Seabird 911 plus CTD and 24-way Niskin bottle system, which was used to collected discrete seawater samples. Chlorophyll *a (*hereafter Chl-*a*) was measured on a pre-calibrated (spinach extract, Sigma Aldrich) fluorometer (Turner Design Trilogy). Water samples collected from the Niskin bottles was analyzed onboard for NO_3^- + NO_2^- using a segmented-flow autoanalyser (Bran & Luebbe) following the colorimetric procedures of Woodward and Rees (2001).[21]

The LoC ΣNO_x sensor is composed of a three-layer PMMA chip which contains precision-milled microchannels (150 μm wide, 300 μm deep), mixers and optical components consisting of LEDs (525 nm, Avago Technologies, USA) and photodiodes. Syringe pump, valves and electronics are mounted on the chip, which was encased in a mineral oil-filled housing (PVC, 12 cm diameter, 30 cm height) with an internally fitted pressure-compensating bladder. The LoC ΣNO_x sensor uses colourimetric detection, using the Griess assay [22] where NO_3^- is reduced to NO_2^- using an off-chip copper activated cadmium column to enable ΣNO_x to be determined. A detection limit of 20 nM and linear range of up to 350 μM have been demonstrated in laboratory settings.[23] The LoC ΣNO_x sensor relies on a standard measurement and a blank measurement to determine the concentration of the sample. All reagents

(Griess, imidazole buffer), standard and blank solutions were stored in externally attached gas impermeable 150 mL Flexboy bags (Sartorius, UK) and the waste was collected into a 500 ml Flexboy bag. The LoC ΣNO_x sensor, reagents and standards used in this study have previously been described in detail by Beaton *et al.*,[23,24] where it was deployed in a dynamic estuarine environment. More recently, the LoC ΣNO_x sensor was deployed on a benthic lander in the Mauritian oxygen minimum zone to examine cross-shelf transport of NO_3^- rich waters,[25] and in glacial meltwaters rivers draining the Greenland Ice Sheet.[26]

For deployments on the CTD, the LoC ΣNO_x sensor was programmed in a continuous mode and performed a repeating measurement sequence of artificial seawater blank, sample, and NO_3^- standard (3 μM), until the CTD rosette was recovered to 5 m where the power was turned off. The operation of the LoC sensor was as follows. The seawater sample was drawn into the sensor through a 0.45 μm MILLEX-HP filter unit (Millipore). Both the filtered seawater sample and imidazole buffer were pushed simultaneously through a 0.46 m serpentine mixer (used to aid mixing) before moving through an off-chip cadmium column. Greiss reagent was added to the resultant buffered reduced seawater sample and then pushed through the 25 mm absorption measurement cell, where the pink-coloured azo dye developed. Absorbance was calculated by comparing the optical intensity measured by the photodiode after a 100 second reaction wait time for each measurement. Each step involved 7 flushes prior to the measurement to minimize sample carryover. Photodiode data was recorded at 1 Hz and the average of the last 10 readings of each wait stage was used to calculate absorbance, according to the Beer-Lambert law. Concentrations were calculated by comparing the absorbance of each sample to that of the subsequent standard measurement. This results in one blank-corrected sample measurement and standard every 17 minutes during CTD deployments.

For the Seaglider deployments, the LoC ΣNO_x sensor was programmed to obtain a minimum of one artificial seawater blank and a standard measurement (6.5 µM NO_3^-) at the beginning and end of each dive. After the first measurement of blank and standard, continuous sample measurements took place on both the descent and ascent until the glider was at 10 m. Over the period of sampling (21 days) this resulted in 312 and 199 artificial seawater blanks and standards, which was sufficient to determine both the ΣNO_x concentration and any drift associated with either the artificial seawater blank and/or the NO_3^- standard.

The LoC ΣNO_x sensor was integrated into the wet bay of the Seaglider and connected by cable directly into one of the glider serial ports. The Seaglider software uses a CNF file that contains the configuration for each on-board instrument and a CMD file that provides mission parameters. The CNF file enables communication between the seaglider and the LoC ΣNO_x sensor. The LoC ΣNO_x sensor is set to 'logger' in the CNF file, which enables the glider to send a number of commands. These commands allow the Seaglider to send and receive data to and from the sensor. Some of the key commands are 'clock-set' used only at the start of each dive but enables the sensor to store any time offset between glider and sensor, 'Status' which sends the sensor depth every 5 seconds along with 3 trigger values and 'download' sent at the end of each dive requesting the sensor to send both ascent and descent data files of processed ΣNO_x values. During deployments the CMD file is typically transmitted to the Seaglider by satellite and includes three trigger values that can be passed to the LoC ΣNO_x sensor using the 'status' command. These triggers are used by the sensor to modify sensor behavior at different depths. Primarily these are used to ensure the LoC ΣNO_x sensor does not take samples on the surface and risk the intake of air, and to aid with additional blank and standard measurements.

The Seaglider is a buoyancy driven autonomous underwater vehicle capable of multi-month deployments collecting high-resolution profiles to 1000 m with a maximum travel range of 4,600 km.[27] Bilateral communication between the Seaglider and base station, through an Iridium satellite connection, allowed dive configurations to be modified once deployed. Data was transmitted back to shore during deployments to assess the performance of the LoC ΣNO_x sensor. In addition to the LoC ΣNO_x sensor within the science bay, the Seaglider measured conductivity & temperature (non-pumped Sea-Bird SBE13 CT Sail, Seabird Electronics), pressure (Pain Electronics) and fluorescence, turbidity and optical backscatter (Triplet Ecopuck, Wet Labs). Conductivity, temperature and pressure were collected at a frequency of 1 Hz during deployment with all dive profiles lying within 4 km of CCS (Fig. 1). Temperature and conductivity were extracted and processed using the UEA Glider Toolbox.[28] These routines apply manufacturer calibrations, correct for thermal inertia following the methods of Garau *et al.,*[29] remove spikes and anomalous data, and draw upon a flight model similar to that described by Frajka-Williams *et al.*[30] Four CTD casts, taken within 1.6 km of the glider, were used to calibrate the temperature and salinity. Manufacturer calibrations were initially applied to data from the Wetlabs Triplet for coloured dissolved organic matter (CDOM), backscatter & fluorescence by subtracting the instrument blank and applying a scaling factor. Calibration to convert fluorescence to Chl-*a* is based on the sensor's response to a cultured diatom, *Thalassiosira weissflogii* at a known Chl-*a* concentration.[31,32]

Results and Discussion

The ability of the LoC ΣNO_x sensor to accurately determine ΣNO_x was assessed in two ways: (i) by comparison of the LoC ΣNO_x sensor mounted on the stainless steel rosette with discrete water samples collected at the same time and (ii) by comparing profiles obtained from the LoC ΣNO_x sensor deployed within the glider with discrete water samples collected from the CTD on the same day at CCS. The LoC ΣNO_x sensor, along with battery and reagents, was a similar size to the 20L Niskin bottle and was mounted in place of a single Niskin bottle on the frame. Once below 5 meters, a pressure sensor on the battery activated the power to the LoC ΣNO_x sensor. The CTD rosette was held at three depths (45, 50 & 90 meters) for at least 90 minutes to allow for triplicate LoC ΣNO_x sensor measurements at the prescribed depth, each bracketed by a blank and standard measurement. During this time period, two Niskin bottles were fired, one within 5 minutes of the first LoC ΣNO_x sensor measurement and the second within 5 minutes of the last LoC ΣNO_x sensor measurement. Water was collected from these discrete bottle firings for determination of $NO_3^- + NO_2^-$ concentrations using the segmented flow autoanlyzer in the ship-based laboratory. Figure 2a indicates the excellent agreement between sensor measurements and analysis of discrete water samples with a correlation of $r^2 = > 0.99$ ($n = 9$; $p = < 0.001$). The estimated analytical uncertainty of LoC ΣNO_x sensor was calculated from two times the standard deviation of the absorbance value of the deployed standard over the three deployments and was 0.14 µM ($n = 10$). This analytical uncertainty is higher than reported values for traditional segmented flow autoanlyzer analysis of ΣNO_x (0.03 - 0.07 µM)[33] but lower than previous LoC ΣNO_x deployments (0.4 - 1 µM).[25]

LoC ΣNO_x data, collected from within the glider, was compared with $NO_3^- + NO_2^-$ values from 24 CTD profiles collected at CCS throughout the 21 day deployment. Unlike the previous assessment of analytical uncertainty, data from the CTD profiles were not collected at the same time (1 – 10 hr window) or depth range (± 3 meters) and all samples were collected in a dynamic shelf sea system. Figure 2b however, shows the excellent agreement the LOC ΣNO_x data and the discrete water sample measurements over the 21 day period, with a correlation of $r^2 = > 0.98$ ($n = 51$; $p = < 0.001$). The average estimated analytical uncertainty for the LoC ΣNO_x sensor during this period was 0.19 µM (n = 142) similar to the 0.14 µM (n = 10) for the CTD NO_3^- profiles.

Initial Seaglider dives (4th to 9th of April) were configured in a standard flight mode, whereby the Seaglider adjusts its pitch and buoyancy to maintain a uniform glide slope and decent and ascent speed.[34] The LoC ΣNO_x sensor was switched on at the beginning of each dive and completed a blank and standard measurement followed by continuous measurements. The LoC ΣNO_x sensor acquired depth information directly from the Seaglider, and using a depth trigger at 10 meters the sensor recognized the Seaglider was diving and after completing its current blank or standard measurement would undertake continuous sample measurements. Triggers were also used to take advantage of extra time at the beginning, apogee and end of dives to undertake extra blank and standard measurements.

Figure 3a shows that when the Seaglider was operated in the standard dive mode, the LoC ΣNO_x sensor carried out 5 sample measurements per 120 m dive with a total dive time of 30 ± 8 minutes. Moreover, these measurements were always in the same depth ranges within the water column due to the relatively shallow water column (~ 145 meters) and sensor operation timings. Figure 3b shows the excellent agreement between the LoC ΣNO_x sensor and traditional CTD segmented flow autoanlyzer measurements, for one single CTD cast at 02:06 am and sixteen dives by the Seaglider from 00:18 to 09:25 am on the 6th of April 2015. However, the temporal and spatial resolution of ΣNO_x data (over the period of the day within the surface layer and across boundaries such as the nitricline) would not be sufficient to investigate the depletion of ΣNO_x as the spring bloom develops. As the glider can be controlled remotely, to increase the distribution of measurements by the LoC ΣNO_x sensor throughout the water column, (in particular across the nitricline), a second dive methodology - termed a 'loiter' dive - was employed. After the Seaglider has reached its maximum depth for that particular dive and started its ascent, for 30 mins the ascent angle was lowered and the glider 'loitered', thus increasing the resolution of measurements within the water column (Fig. 3a). Selected maximum target depths (90, 60, 40 and 25 meters) were used to control the maximum dive depth and ensured a higher number of measurements in areas of interest (Fig. 3c). Loiter dives to 90 meters doubled the amount of measurements made compared to the previous standard dives. Figure 3c shows a comparison between ΣNO_x concentrations from two CTD casts at 02:06 and 08:22 am and LoC ΣNO_x concentrations from seven 'loiter' dives from 11:19 am to 16:25 pm on the 15th of April 2015. Once more, good agreement between the LoC ΣNO_x sensor and traditional segmented flow autoanlyzer measurements of CTD discrete samples was observed throughout the whole water column during this 14-hour period. To ascertain the ability of the LoC ΣNO_x sensor to make comparable measurements to the segmented flow autoanlyzer, over an extended time period (4th to 25th April, 2015), we compared the measurements within the bottom layer at 60 -120 m where little changes in ΣNO_x were observed. Excellent agreement between both the segmented flow autoanlyzer (6.86 ± 0.09 μM; n = 22) and LoC ΣNO_x sensor (6.86 ± 0.16 μM; n = 120) was observed. We have demonstrated the ΣNO_x concentrations measured from the LoC ΣNO_x sensor are comparable to those of the shipboard measurements analyzed on a segment flow autoanlyzer. Moreover, this shows that accurate measurements can be obtained from the LoC ΣNO_x wet chemical sensor over 21 days in a dynamic shelf environment.

On-board calibration with artificial seawater blanks and NO_3^- standard for each dive enables both the monitoring of instrument performance and stability over long-term deployments. To ensure maximum efficiency of the cadmium column, where NO_3^- is reduced to NO_2^- a flow rate of 150 µl/min was chosen.[23] However, it is important to monitor any drift in the efficiency of the cadmium column over time as this may impact on the ΣNO_x concentrations. In this study, a decrease in absorbance values was observed, likely due to the gradual reduction in efficiency of the cadmium column over time. As sample concentrations are calculated from their associated blank and standard measurements, where the ratio of the absorbance of the sample and standard are determined, any drift caused by the decreasing reduction efficiency of the cadmium column is compensated for. Our results demonstrate that any decrease in absorbance values observed did not impact on the accurate determination of ΣNO_x from the LoC as shown by the excellent agreement with traditional autoanlyzer ΣNO_x method from discrete water samples collected throughout the 21-day deployment.

The data set presented here was collected during the spring phytoplankton bloom, a period during which integrated net productivity becomes greater than integrated losses and phytoplankton biomass accumluates in surface waters.[35] Over the 21-day deployment the LoC ΣNOx sensor was able to accurately capture the large drawdown of ΣNOx within the surface layer due to the onset of the spring bloom (Figure 4). Concentrations decreased from 5.74 µM (4th) to 1.42 µM (25th), whilst bottom layer NO_3^- concentrations remained constant (6.86 ± 0.16 µM), as observed in previous studies within the Celtic Sea .[36,37]

At the start of the deployment (4th – 6th April 2015), a small 0.8 μM difference between near surface (20-40 m) and bottom water (60-80m) ΣNO_x concentrations was observed. During this time, surface Chl-*a* concentrations were relatively low (1.8 mg m^{-3}), but much higher (by 1.5 mg m^{-3}) that those typically observed during the winter in the area.[38,39] This suggests that some phytoplankton growth had already occurred prior to deployment of the glider.

Between the 4th and 6th April the ΣNO_x concentration at 20-40 m remained constant. Any changes in the near surface (< 20 m) water however, where you might expect the largest draw down in NO_3^-, were not resolved since the standard dive pattern used during this early period did not result in near surface LoC ΣNO_x measurements being made. Nevertheless, it is clear from Figure 4 that a large drawdown of 3 μM ΣNO_x occurred between the 4th and 11th of April 2015 (< 40 m Coincidently, surface water Chl-*a* increased from 1.8 mg m^{-3} to 3.7 mg m^{-3} indicative of phytoplankton growth and the onset of the spring bloom. Changing the dive configuration to 'loiter' dives on the 11th of April increased the resolution of ΣNOx surface data.

Just as the temperature sensor resolves the gradual deepening and warming of the surface mixed layer, the LoC ΣNOx sensor resolves the coincident deepening of the nitricline and draw down of ΣNOx above it. Between the 4th and 25th April surface waters warm by > 1°C and a 40 m deep thermocline is established. During this time there is a 4.2 μM drawdown of ΣNOx and an increase in Chl-a from a background of 1.8 mg $m-^{3}$ to 4 - 6.8 mg m^{-3}.

By the end of the Seaglider deployment (25th) and a two-layer water column had developed with a warm, nutrient depleted, 40 m surface layer overlying colder, nutrient rich bottom waters. Previous studies suggest that phytoplankton growth starts to become ΣNO_x limited when concentrations fall below 1 μM.[40] The low surface water ΣNO_x concentrations (1.4 μM) measured during the end of the deployment suggest that this was close to happening. This is supported by a coincident decrease in Chl-*a* (to < 2 mg m^{-3}) towards the end of the deployment. Ship-based observations show that it was not until the 28th that surface water concentration were below the limit of detection of 0.1 μM.[41]

This study has demonstrated for the first time that it is possible to accurately measure ΣNO_x over long-term deployments using a wet chemical nutrient sensor deployed within an autonomous vehicle. This enables an increase in observations of ΣNO_x dynamics in temperate shelf seas during key transitional events (e.g. the onset of stratification and the spring bloom, convective overturning and the autumn bloom) and across fine-scale vertical and horizontal features (e.g. tidal mixing fronts, sub-surface chlorophyll maximum). At present, wet chemical biogeochemical sensors do not have the vertical resolution capabilities of the commonly used physical and optical sensors such as temperature, conductivity and fluorescence. This resolution could be further improved by decreasing the time taken between measurements. By increasing flow rate, decreasing color development time, decreasing the number of flushes and increasing N.E.D(napthylethylenediamine dihydrochloride) concentration and reaction temperature, an increase measurement resolution could be achieved, but at the expense of measurement sensitivity.[23,42]

For long-term deployment of wet chemical sensors, reduced resource consumption (power and reagents) and compact size are the main advantages of microfluidic systems. Deployment of sensors on moorings, where solar and wind power are available, negates power constraints. However, for autonomous underwater vehicles power becomes the greatest limiting resource.[43] During the 21-day deployment, the LoC ΣNO_x sensor (version 3.2) had a low power consumption of 1.5 W. This was only marginally higher than the other standard sensor packages on the Seaglider (0.9 W and 0.25 W for the Wetlabs ECO Triplet and SBE pumped payload CTD respectively), but lower than other wet chemical and UV absorption systems (e.g. ISUS V3, Satlantic, USA; NitraVis, YSI, USA; SubChemPak, SubChem Systems, USA). A single dive of the LoC ΣNO_x sensor consumed 2.5 ml of Griess reagent, 2.5 ml of buffer solution and 0.21 ml of standard and blank solution achieving 1 blank and standard measurement and ~ 10 samples on a dive to 120 meters. This would enable a total of 400 dives to be made and 4000 sample measurements. During this study, the LoC sampled at time periods when the *R.S.S Discovery* was also sampling at CCS to enable a direct comparison between the two types of measurements over a long-term deployment of the LoC. This resulted in accurate LoC ΣNO_x determined over 21 days. With a sampling strategy focused on achieving the maximum amount of measurements, a profile consisting of 10 sample measurements could be undertaken every ~ 40 minutes, allowing for increased observations of episodic and transient events unable to be observed by discrete sampling.

The temporal and spatial variability of biogeochemical processes has been successfully measured through the use of remote sensing, time series moorings and ship-based methods, but these, to an extent, fall short in resolving the dynamic temporal and spatial elements with long-term endurance in a low cost package. From this, there has been a strong call for the development of biogeochemical sensors to be deployed, on stationary and mobile platforms, to provide *in situ* measurements as part of sensor networks aimed at providing the long term monitoring within a low cost package.[17,19] The LoC ΣNO_x sensor deployed within the Seaglider in this study clearly demonstrates that nitrate & nitrite can be accurately determined over monthly timescales due to the sensor's low resource use, small size and *in-situ* calibration abilities. Moreover as deployed within a glider with other physical and biochemical data (e.g. CTD and the Wetlabs Triplet sensor measurements), it provided a powerful tool for resolving dynamic biogeochemical processes within a dynamic shelf system.

Acknowledgements

The authors would like to thank the captain and crew of the RSS Discovery. This project was funded by UK Natural Environmental Research Council through the Sensors on Gliders program (NE/J020184/1) to MCL and (NE/K001701/1) to JEH. The field deployment took place under the Shelf Sea Biogeochemistry program with funding to MCL from NERC (NE/K001779/1). Funding from the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement n°614141 (SenseOCEAN) contributed to the research leading to these results. We would also like to thank David White, Alvaro Lorenzo Lopez, Sam Ward, James Burris and Stephen Woodward of the Marine Autonomous and Robotics Systems group, and David Owsianka and Adrian Nightingale of the Ocean Technology and Engineering group at the National Oceanography Centre, UK.

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