Final Degree Work

Photochemical Transformation of Dissolved Organic Matter to Dissolved Inorganic Carbon

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List of abbreviations

Alk	Total Alkalinity
C_{ant}	Anthropogenic carbon
CDOM	Chromophoric/coloured Dissolved Organic Matter
$CDOM_\lambda$	CDOM absorption coefficient at wavelength λ (m^{-1})
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
CRM	Certified Reference Material
C_{TEP}	Concentration of TEP ($\mu g\ X_{eq}\ L^{-1}$)
dDIC/dt	Photoproduction rate of DIC ($\mu mol\ L^{-1}\ h^{-1}$)
DI water	De-ionized water
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EPS	Exopolymers
HCO_3^-	Bicarbonate ion
PML	Plymouth Marine Laboratory
SDICS	Secondary DIC standard
TEP	Transparent exopolymers

Abstract

Coloured dissolved organic matter (CDOM) and dissolved inorganic carbon (DIC) photoproduction rate (dDIC/dt) were measured at two marine time series stations (coastal station L4; 50.25°N, 4.22°W and open-shelf station E1; 50.03°N, 4.37°W) and three rivers (river Dart, 50.59°N, 3.91°W; river Erme, 50.38°N, 3.92°W; and river Plym; 50.46°N, 4.04°W) all located in South-West England. Sampling was done during February-April 2016, when eleven samples were analysed. Additional data collected previously, during May-June 2015, was incorporated into the data analysis presented here. This project observed a correlation between CDOM and dDIC/dt during both periods (p-value = 8.32×10^{-3} (2015) and 2.39×10^{-5} (2016)). Both datasets show an understudied relationship, the seasonal variability of both CDOM and dDIC/dt. Different slopes were observed in their correlations ($0.0340 \pm 0.0055 \mu$mol m L^{-1} h^{-1} (2015) and $0.0939 \pm 0.0118 \mu$mol m L^{-1} h^{-1} (2016)), seasonal variation being the most probable cause. However, the slope for oceanic water during the 2016 period ($0.0302 \pm 0.0108 \mu$mol m L^{-1} h^{-1}) was in the range of the slope for all the data during the 2015 period. This would suggest that major changes in CDOM and dDIC/dt are only observed in fluvial water, although compositional changes between the different water types may also influence this result.
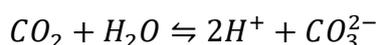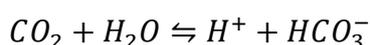
Introduction

Around 420×10^{12} g of terrestrial organic carbon are transported by rivers to the oceans every year (Schlesinger and Melack, 1981; Schlünz and Schneider, 2000). Approximately 10% of the organic carbon is buried into the sediments, the rest is oxidised in the water column, including photo-oxidation in the photic zone (Schlünz and Schneider, 2000).

Coloured dissolved organic matter (CDOM) refers to the fraction of dissolved organic carbon (DOC) that absorbs light (Rochelle-Newall and Fisher, 2002). Kieber, McDaniel, and Mopper (1989) suggested for the first time that photochemical oxidation of CDOM was a sink of DOC. The first photochemical oxidation product from DOC studied was carbon monoxide (CO) (Erickson, 1989; Swinnerton et al., 1970). In recent years, the photoproduction of dissolved inorganic carbon expressed as dDIC/dt has started to be studied, when researchers found out that dissolved inorganic carbon (DIC) is produced from CDOM at about twenty times the rate of CO production. This makes it the fastest produced inorganic carbon product (Johannessen, 2000; Miller and Zepp, 1995). The efficiency of this process depends on the source of CDOM and on the irradiation history (Johannessen et al., 2007).

DIC is the sum of three compounds: bicarbonate ion (HCO_3^-), carbonate ion (CO_3^{2-}), and carbon dioxide (CO_2). Therefore, it is calculated by the addition of the concentrations of these three compounds, whose proportion depends on the pH (Berner, 1965).

$$DIC = [HCO_3^-] + [CO_3^{2-}] + [CO_2]$$

$$CO_2 + H_2O \leftrightharpoons H^+ + HCO_3^-$$

$$CO_2 + H_2O \leftrightharpoons 2H^+ + CO_3^{2-}$$

Models predict values of photochemical production of DIC of around 10^{14}-10^{15} moles of DIC per year, which is in the same range of orders of magnitude as the sequestration of DIC by new production (10^{15} moles DIC per year (Johannessen et al., 2007)).

Since the early 1990s, scientists have studied the increase in the DOC in the United Kingdom's freshwater (Freeman et al., 2001; Tranvick and Jansson, 2002; Worrall and Burt, 2004). A 65% increase in DOC through a 12 year period was reported by Freeman et al. (2001). It has been suggested that increase in DOC is temperature related (Freeman et al., 2001). However, others have indicated that DOC is hydrologically affected (Tranvick and Jansson, 2002). More recently, it has been implied that DOC increases are caused by the collaboration of numerous processes such as enzymatic latch mechanisms triggered by temperature changes, with an important impact of droughts (Worrall and Burt, 2004).

Since CDOM is a fraction of DOC, an increase in DOC may ultimately produce an increase in DIC photoproduction, a direct photochemical product. This DIC increase will approach the DIC/Alk ratio closer to 1 in many areas, therefore reducing the buffer capacity of oceans to take up carbon dioxide (CO_2) (Revelle and Suess, 1957), and more importantly anthropogenic CO_2 (C_{ant}). The ocean's role in carbon sequestration is of paramount importance. It has a major function as a sink of C_{ant}, annually capturing around a quarter of the emitted gas (Guallart et al., 2015; Halloran et al., 2015; LeQuéré et al., 2014). Therefore, this work provides new data and input to modellers and future researches who work with C-cycling.

The main objective of this work was to study the hypothesis that dDIC/dt is controlled by CDOM. The consequences of the verification hypothesis will have application in furthering the understanding of C-cycling, and an improvement in the understanding of current changes in riverine DOC concentrations.

Methods

Sample locations

Plymouth Marine Laboratory (PML) systematically analyses two stations in the Western English Channel; the same two oceanic stations were selected.
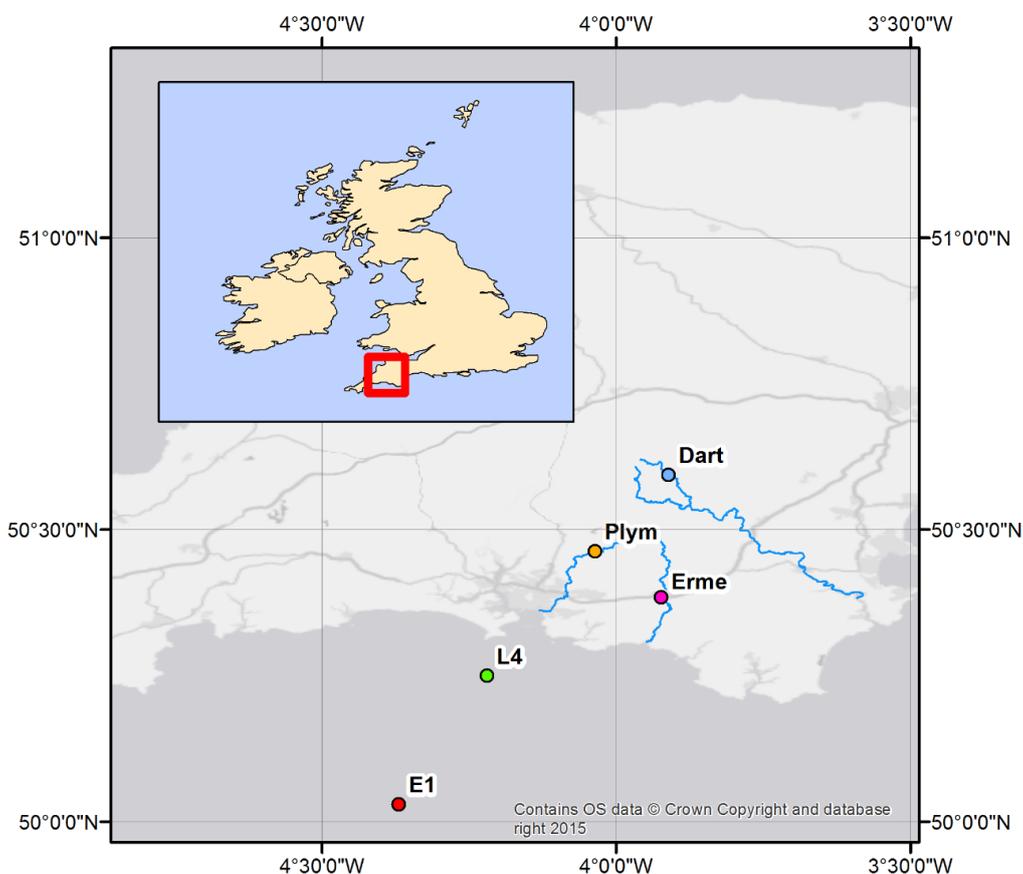

Figure 1. Location of the sampled stations. L4 is located approximately 12 km off the coast of Plymouth and has been sampled since 1988. E1 is positioned 38 km from Plymouth and has been sampled since 1903.

The first one is a coastal station sampled weekly aboard the *RV Plymouth Quest*, named L4 (50.25°N, 4.22°W; Figure 1). The second station is an open-shelf station named E1 (50.03°N, 4.37°W; Figure 1). Sampling in this station is carried out on a monthly basis aboard the same vessel (Kitidis et al., 2012; Smyth et al., 2010).

Three major rivers close to Plymouth are the Dart (50.59°N, 3.91°W), the Erme (50.38°N, 3.92°W), and the Plym (50.46°N, 4.04°W). Consequently, these three rivers were selected in order to obtain photochemical transformation rates of DIC at different CDOM concentrations.

Water sample collection and storage

A 20L carboy was used to collect the water. Once the sample was collected, it was left in the dark in order to minimize phototransformation. Water from the rivers was taken the morning the samples were analysed. At the selected locations, water was collected from the middle of the river at a shallow depth (~10cm) in the interest of not getting sediments from turbulent waters. The carboy was then transported to the lab and the water was filtered as indicated below.

Water collected from the costal ocean stations was gathered by the crew members from the *RV PLYMOUTH QUEST*. It was collected in the morning from the vessel's surface water intake (~2.5m depth), and transferred directly to a 20L carboy. Upon its arrival at the PML at midday, the filtration took place.

$HgCl_2$ was not used to preserve samples since it is photoreactive and would have affected the results. Consequently, the experiment started the same day during the late afternoon, within 12 hours of sample collection.

DIC stripping efficiency and irradiation

Each sample was gravity filtered through a 0.7µm pore size, 142mm diameter Whatman filter, followed by a 0.2µm Akropak 1000 filter to remove particles and microbes on the same day the sample was collected, as indicated by Kitidis et al. (2011). Previous research showed that >99% of the microbial population was removed (Kitidis et al., 2011). The filtrate was transferred to a dilute HCl-washed 5L glass Erlenmeyer flask

The photochemically produced DIC was very low after 48h (Johannessen, 2000), between 1-5µmol L^{-1}, compared to 1900-2000µmol of DIC in the studied seawater, and around 100µmol for river water. Consequently, the existing DIC from the sample had to be stripped. As indicated previously (Johannessen, 2000; Johannessen and Miller, 2001) the pH of the sample should be decreased to 3-4. A pH of 3.5 was selected for this work. At this pH, the seawater carbonate products are converted into CO_2 as shown by a Bjerrum plot (Figure 2). About 10mL of 1M HCl was used to acidify 5L of sample.
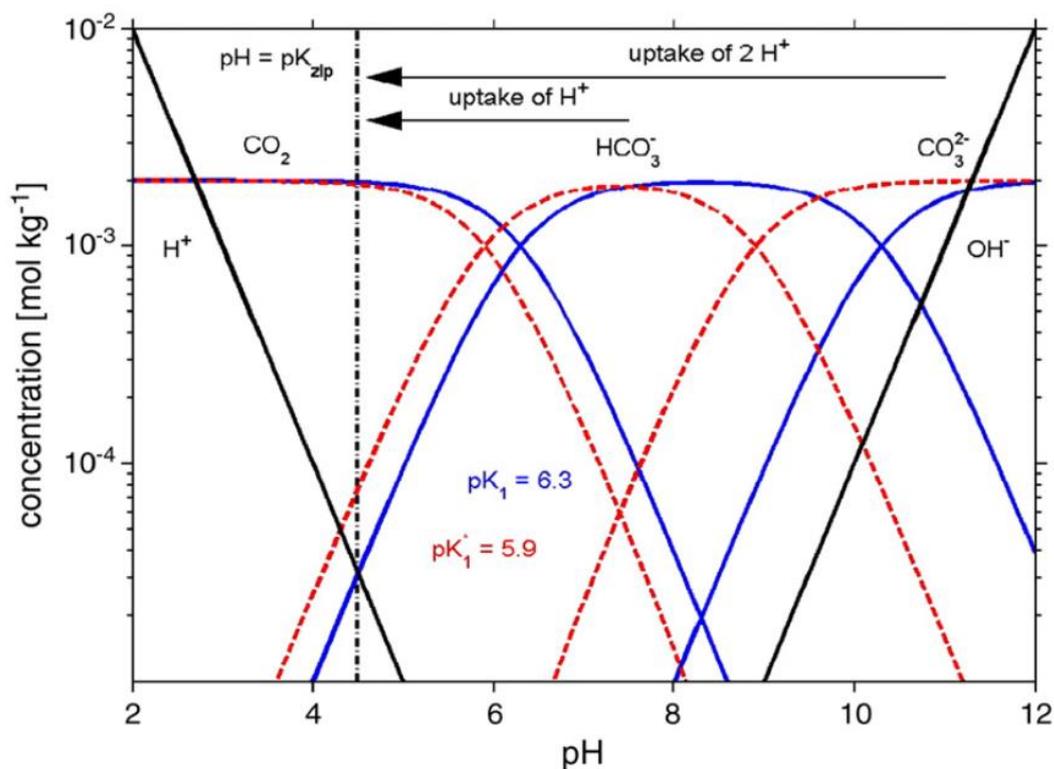
Figure 2. Relationship between carbon species (CO_2, HCO_3^-, CO_3^{2-}) and pH, a Bjerrum plot (Wolf-Gladrow et al., 2007). The desired pH of the acidified sample was between 3 and 4 in order to convert the carbonate species into CO_2, which could be purged.
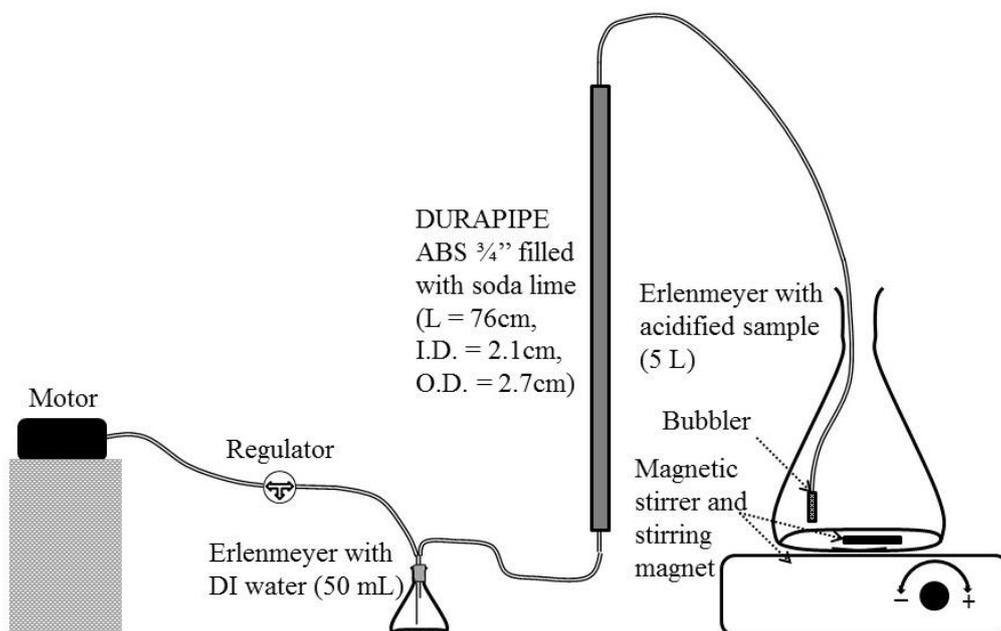
Figure 3. Custom-made DIC stripper.

After acidifying the water, CO_2-free air was bubbled using a custom-made DIC stripper (Figure 3). It consisted of a small air pump (maximum speed of 3L min^{-1}) at its lowest setting, plus a regulator to decrease the air flow even further. The air got moisturised in a small Erlenmeyer in order to increase the CO_2 capture efficiency of the soda lime. The

moist air with CO_2 went through a 2.1cm wide PVC tube (DURAPIPE ABS) packed with soda lime in order to remove the CO_2, leaving the desired CO_2-free air. This air was then bubbled into the acidified sample through a bubbler. The bubbler would produce small bubbles increasing the surface area of CO_2-free air. The objective of the stirrer was to homogenize the sample while the stripping took place.

Additionally, the PVC tube was stored horizontally and the soda lime pellets were compacted in order to reduce channelling, increasing the systems durability and efficiency. Next, the sample was continually stirred in order to homogenize the acidified sample. Finally, the bubbler used in the sample produced small bubbles in pursuance of increasing the surface area. In order to increase the efficiency of the system several characteristics were applied. In the first place, the air was bubbled into water to increase the moisture. Secondly, the flow was limited; increasing the time it spent in contact with the soda lime and thus dwell time. Thirdly, a 76cm long PVC tube was used as the soda lime storage, further increasing the dwell time.
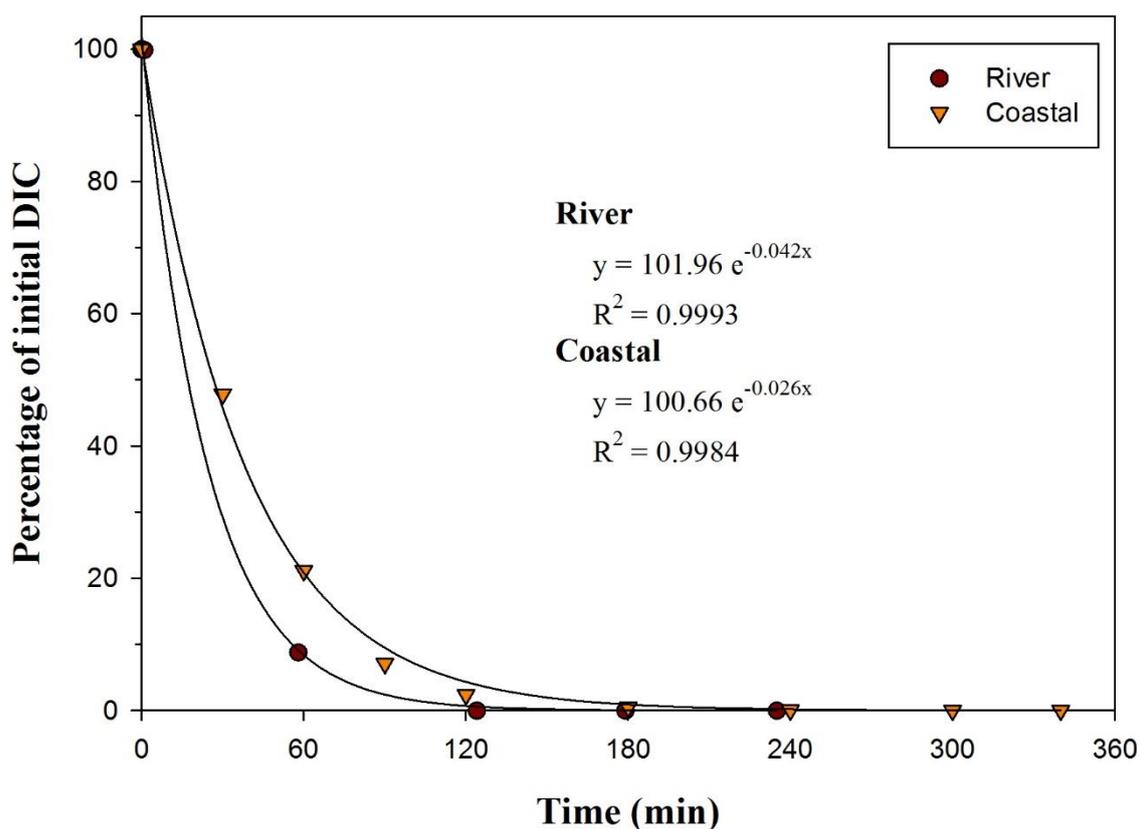
Figure 4. Efficiency of the CO_2 scrubber in river and coastal water. After four hours (240 min), the DIC had reached its minimum in both types of water.

Since the DIC had to be stripped before each experiment, an appropriate time was used for all the experiments. After analysing the efficiency of the CO_2 scrubber, 4h was used as the desired time, since it eliminated >99.5% of the DIC for coastal and river samples. The DIC stripping follows an exponential decay and leaving it for more than 4h did not

produce significant changes (Figure 4). Furthermore, less than 4h was not considered optimal, since the standard deviation of the measurements was higher, increasing the uncertainty of the data.

After striping the DIC and buffering the pH to the initial value using sodium hydroxide (NaOH), the samples were bottled into six borosilicate and eight quartz bottles, used in the following manner. Three borosilicate bottles were analysed instantly to obtain the remaining DIC (named initial sample). The three other borosilicate bottles were used for the samples irradiated with visible light only since borosilicate blocks UV wavelengths. Four quartz bottles were used to obtain values for DIC photoproduction after being irradiated with UV and visible light. The last four quartz bottles were wrapped in at least three layers of aluminium foil and acted as dark control samples.
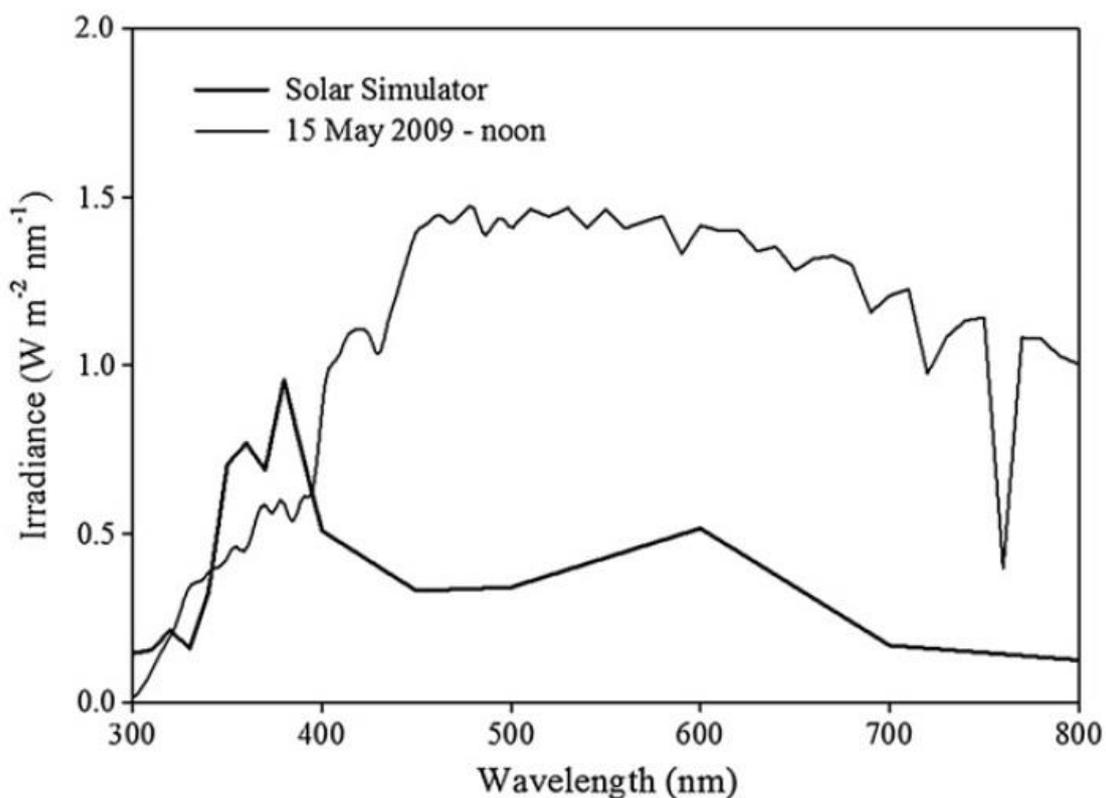
Figure 5. Irradiance spectrum of natural light at 20°N 18°W and the solar simulator (Kitidis et al., 2011).

Since three bottles were analysed initially, only eleven bottles (three borosilicate, eight quartz) were inserted in the solar simulator. The three bulbs in the solar simulator produced an irradiance of $4.4 Wm^2$ UVB (290–320nm), $49.1 Wm^2$ UVA (320–400nm), and $159.1 Wm^2$ PAR (400–800nm) (Kitidis et al., 2014; Kitidis et al., 2011) (Figure 5).

Samples were left in the solar simulator for 48h. In order to remove the heat produced by the lights used in the solar simulator, which raised the temperature of the samples, a water cooling system was used. The temperature ranged between 23°C at the beginning of each

experiment to 25.6°C after about 6h, and remained at that temperature until the end of each experiment. This temperature was similar to previous experiments (Groeneveld et al., 2015). Due to the slight increase in temperature, some samples developed bubbles and were not used for DIC measurements.

DIC measurements

DIC was measured using a Dissolved Inorganic Carbon Analyzer (Model AS-C3; Apollo SciTech, Bogart, GA, USA). This apparatus works by adding 1mL of an acidic solution (10% H_3PO_4 + 10% NaCl), which shifts the DIC equilibrium to CO_2 gas. This gas is removed by sparging with pure nitrogen (N_2) through a drying and cooling system to remove water vapour. The dried CO_2 gas was then analysed in a LICOR LI-7000 CO_2 Analyser (a differential, nondispersive, infrared gas analyser) (Findlay et al., 2013).

The equipment was left to cool for half an hour, to a temperature between 1 and 5°C before the calibration was started. Five point calibrations were done using volumes of 0.1, 0.2, 0.65, 0.75 and 0.85mL. Triplicate measurements of the standard/sample had to be within a 0.1% error, and each sample was analysed a maximum of 10 times. Calibrations were done both with certified reference materials (CRM; provided by A. Dickson, Scripps Institute of Oceanography, San Diego, California) and secondary DIC standards (SDICS).

CDOM measurements

Measurements of CDOM were done by spectrophotometry. A 50mm quartz cuvette was used, and measurements were calibrated against Milli-Q ultrapure DI water. The spectrophotometers varied throughout the project, as more user-friendly or precise spectrophotometers became available. All spectrophotometers were precise in the 280-800nm range. Initially a manual and single beam spectrophotometer was used, the Ultrospec II. The process was very time consuming and as a result, a dual beam scanning spectrophotometer was used instead, initially the Perkin Elmer Lambda 800, later the Perkin Elmer Lambda 35. For the scanning spectrophotometers, the slit size used was 1nm, the speed was 120nm/min, and the ranges studied were 270-800nm. The CDOM spectra were done for all the samples, including the pre-filtered sample, the sample after filtering, the sample post-buffering (also defined as initial sample), the sample that had been irradiated with UV+visible light, that with visible light only, and the dark control sample.

CDOM absorption coefficients ($CDOM_\lambda$; m^{-1}) were calculated from (Kitidis et al., 2008):

$$CDOM_\lambda = \frac{2.303 \times A_\lambda}{l}$$

The spectral slope ($S_{290-350}$) has previously been used to identify sample provenance and photochemical transformation (Kitidis et al., 2014). In this work a simplified version, the $CDOM_{300}/CDOM_{350}$ ratio has been calculated, and used in the same manner.

Transparent Exopolymers (TEP)

Polysaccharides are an important labile component of DOC (Passow, 2002). Many aquatic organisms, such as phytoplankton and bacteria, produce extracellular polysaccharides known as exopolymers (EPS). A fraction of these can be found as single particles instead of cell coatings or dissolved slimes (Alldredge et al., 1993; Passow, 2002). Furthermore, they can not only aggregate, but can also be collected by filtration (Alldredge et al., 1993; Passow and Alldredge, 1995). These EPS are known as transparent exopolymers (TEP).

Many methodologies have been found throughout the existing bibliography. Some authors have used dynamic laser scattering spectroscopy (Chin et al., 1998) measuring diameters, while others have used a coulter counter (Zhang et al., 2013). The methodology used in this work followed the instructions from Meng and Liu (2016) and Passow and Alldredge (1995).

The sample was gravity filtered through a 0.7µm filter, followed by a 0.1µm Akropak 1000 filter and it was left to settle for 30min. About 30min after filtration, Chin et al. (1998) described by dynamic laser scattering the presence of a polydisperse collection of assembled polymer gels ranging in size from 200nm to 1µm, which would imply that TEP was being reformed. Following this time, the samples were filtered through a series of 47mm diameter polycarbonate filters with pore sizes of 0.1µm, 0.2µm and 0.4µm (Whatman, UK), at a constant vacuum of 0.2bar (Figure 6A). The TEP remaining in the filters was stained with 1mL of alcian blue solution (Figure 6B). The alcian blue solution consists of a concentration of 0.02% of alcian blue 8 GX (Sigma, USA) in acetic acid (Meng and Liu, 2016). A 0.06% acetic acid was used to obtain a final solution of pH 2.5, accordingly, 60mL 1M acetic acid was used to prepare 100mL of the solution.

The alcian blue solution was left in the filter for 3s, after which the pump was restarted. Milli-Q water was then filtered through the filters to remove the excess alcian blue (Figure 6C). The filters were then inserted into HCl-washed, DI water cleaned beakers containing 10mL of 80% H_2SO_4 (Meng and Liu, 2016; Passow and Alldredge, 1995). The filters were left to dissolve for a period of 2h. During this period, they were stirred between three to five times (Figure 6D). Finally, the absorptivity was measured (Passow and Alldredge, 1995) in a 50mm cuvette (Figure 6E).
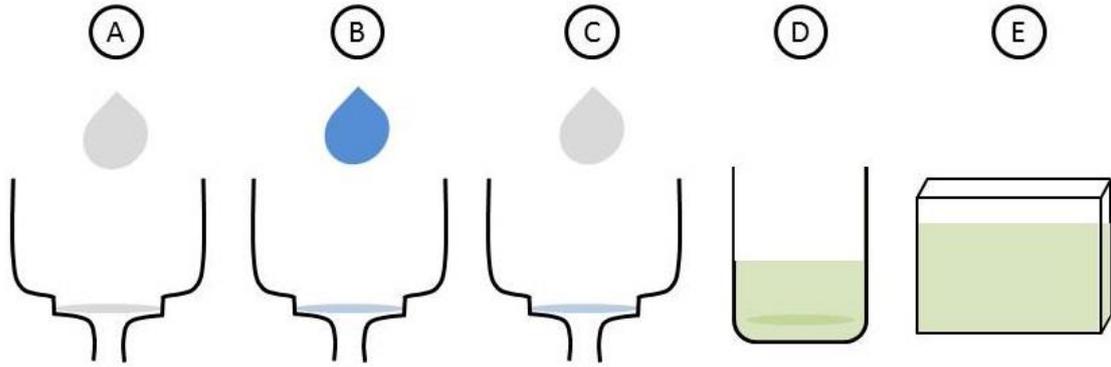
Figure 6. TEP process. A) Addition of sample, B) Addition of alcian blue, C) Rinsing with DI water, D) Dyed filter in 80% H2SO4, E) Dyed H2SO4 in the cuvette.

We used the spectrophotometer Perkin Elmer Lambda 35, and measured the absorptivity at 787nm. This wavelength is used because it is the maximum absorption of alcian blue in 80% H_2SO_4 (Alldredge et al., 1993; Passow and Alldredge, 1995). There is a linear correlation between the measured absorptivity and the concentration of TEP, as long as the filters used do not get clogged.

TEP concentration (C_{TEP}), is measured in µg of gum xanthan equivalents per litre (µg X_{eq} L^{-1}). It is determined from

$$C_{TEP} = (E_{787} - C_{787}) \times (V_f)^{-1} \times f_x$$

where E_{787} is the absorbance of the sample, C_{787} is the absorbance of the blank, V_f is the volume of sample filtered in litres and f_x is the calibration factor in micrograms. Filter blanks, which vary with filter type and stain batch, are blank filters that have been stained. These blanks are always subtracted from the absorption of the sample.

The calibration factor is determined according to

$$f_x = W \times [(est_{787} - C_{787}) \times V_{st}^{-1}]^{-1}$$

where W is the dry weight of the standard (µg L^{-1}), est_{787} is the absorbance of the standard, and V_{st} is the volume filtered for staining. The calibration factor has to be calculated during each experiment, since the dye content of the solution varies as it ages. This is due to re-aggregation of dye particles. The calibration standard is prepared by mixing ~15mg of gum xanthan into 200mL of distilled water. The solution is then grinded with a tissue grinder a few times to break gel-like particles (Passow and Alldredge, 1995). Dry weights from the calibration standards are calculated by filtering precise volumes, between 2 and 5mL into dried pre-weighted filters. Alcian blue stainable particles are measured the same way samples are calculated, but using smaller volumes, between 1 and 2mL.

Results

A calibration of the DIC analyser had to be done every day samples were measured, normally a CRM would be used. Since CRMs have to be certified and transported from the USA, and each bottle can only be used once, scientists have started to produce their own standards which they then correct to a CRM (Feely et al., 2008). This has only been done in recent years, therefore, there is no existing protocol or information about how to properly prepare secondary standards.

Part of the Final Degree Work (FDW) was spent creating a reliable SDICS production method. Over 20L of surface seawater were collected, followed by filtering through a 0.2μm followed by a 0.1μm Akropak 1000 filter and poisoning the water with mercuric chloride ($HgCl_2$) (Dickson et al., 2007; Feely et al., 2008; Findlay et al., 2013; Loucaides et al., 2012). Samples were exposed for an hour to air whilst being stirred, in order to reach a DIC concentration closer to equilibrium. This water was then bottled into 125mL borosilicate bottles, whose stoppers had been previously greased with Apiezon L Grease, in order to block any gas transfer (Dickson et al., 2007; Feely et al., 2008).

Four SDICS were calibrated against a CRM for each batch of secondary standards, and the values for DIC, Total Alkalinity and salinity were taken. A week later, a SDICS was compared to another SDICS, in order to observe if there were changes between different bottles. Finally, a month later, another four SDICS were calibrated against a new CRM. In total, ten SDICS for each batch were used to find out if the SDICSs were consistent and did not vary over time. Two batches were made, the first one being twenty bottles and the second one forty bottles. No significant changes (values inside the standard error) were observed in each batch. The CRMs used for the inter-calibrations belonged to Batches numbers 140 and 152 (A. Dickson, SIO).

CDOM was measured before filtering, after re-buffering the pH following the DIC stripping and in some cases after irradiation. Changes in CDOM were observed in all the different steps, although differences between samples in the same conditions were not visibly significant.
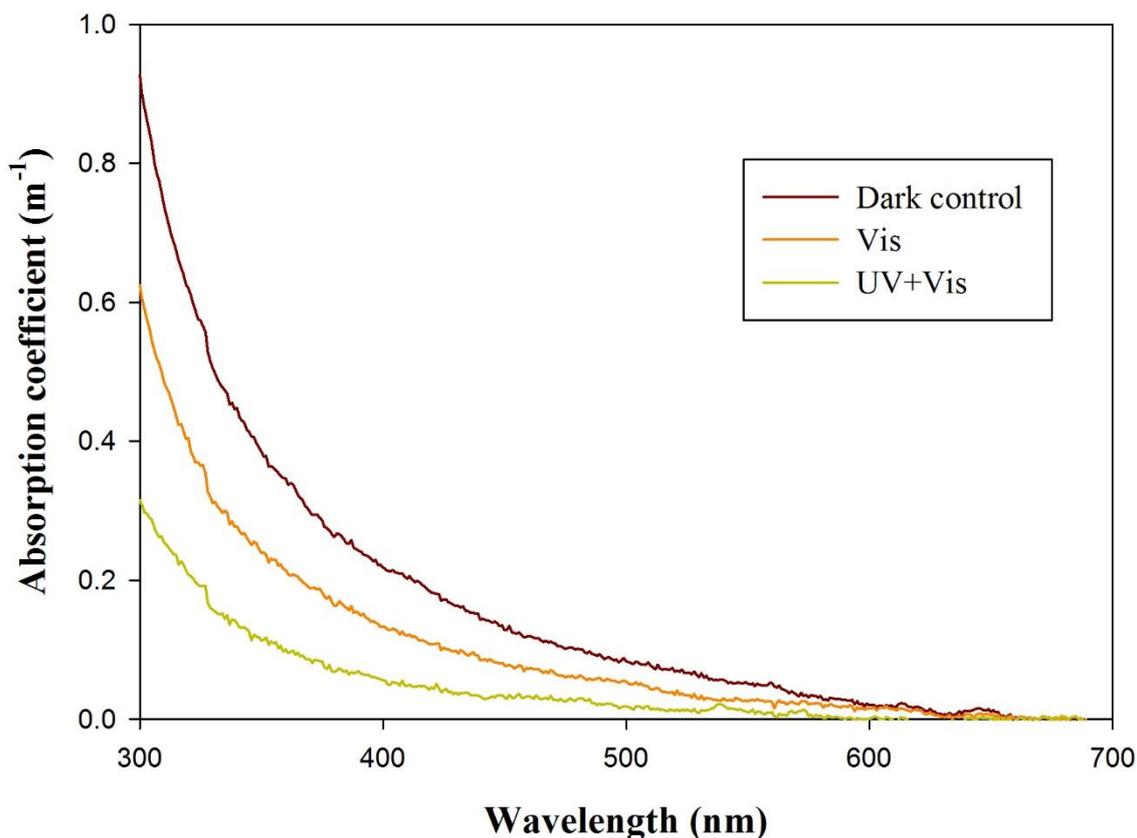
Figure 7. Absorption coefficients for dark control (Dark control), visible irradiation (Vis) and UV plus visible irradiation (UV+Vis)

Absorption coefficients did not vary much for the sample before and after stripping the DIC, and between the initial sample and the dark control. Major changes were observed between the dark control, the visible irradiation and the UV plus visible irradiation samples. The dark control and the initial sample had the same absorption coefficients due to the lack of CDOM phototransformation. As observed in Figure 7, absorption coefficients varied through the experiment decreasing as phototransformation occurred. The higher the phototransformation, the lower the absorption coefficient, i.e. CDOM lost its colour. This process is called CDOM photobleaching. Concurrently, DIC photoproduction was observed (Figure 8).

DIC for each sample was measured in four different conditions, measuring four bottles for each condition, and a graph similar to Figure 8 was created for each sample. The slope of the line connected from the initial value to the UV+visible data points represents the dDIC/dt of the sample. The data proved that the dark control values were within the standard deviation of the initial samples, and the other data points did not intersect with each other, showing observable data ranges depending on the spectral irradiance.
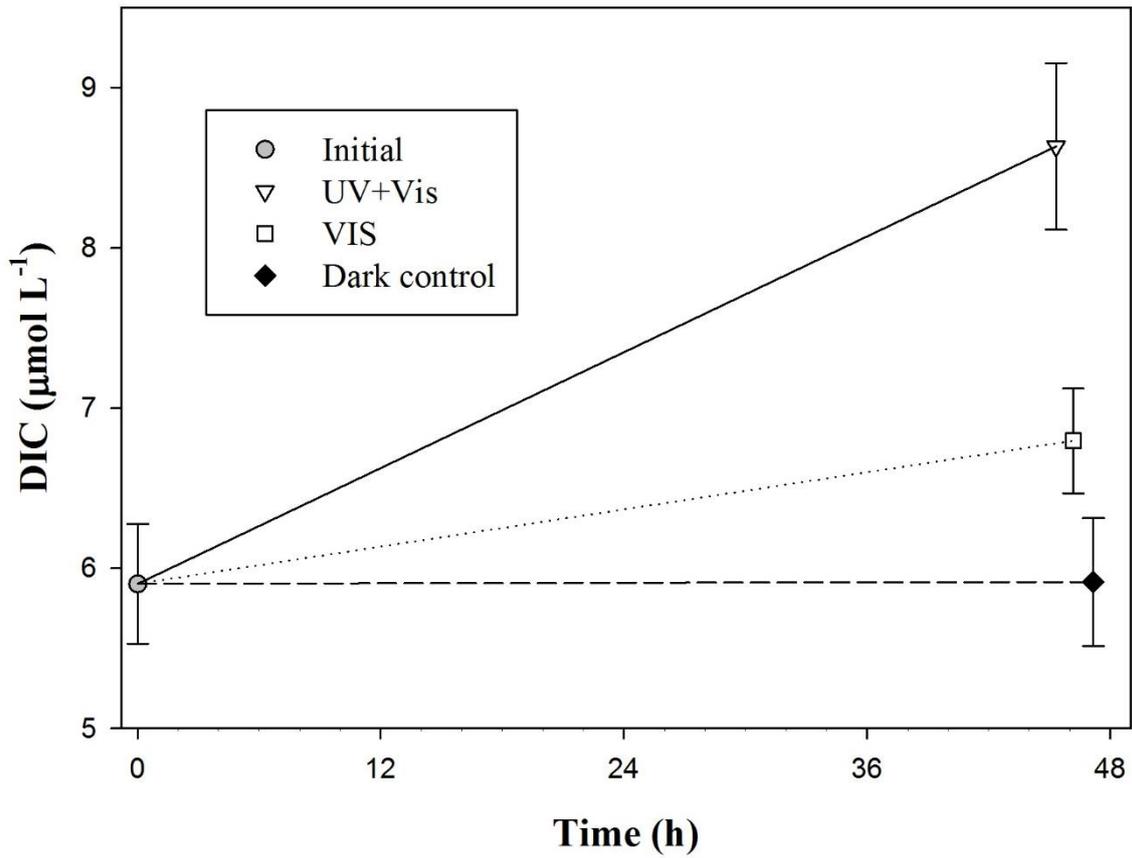
Figure 8. DIC photoproduction for one representative experiment. Values include their standard deviation (in the coastal station L4).
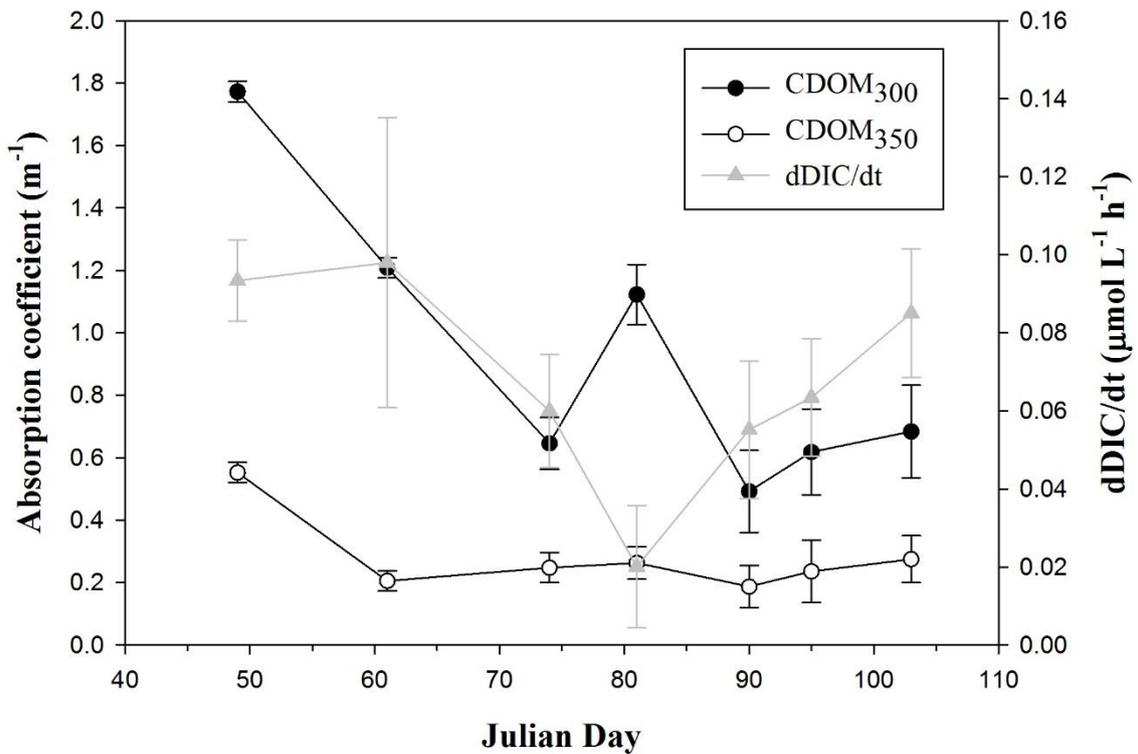

Figure 9. Temporal variation of CDOM and dDIC/dt throughout the experiments at L4. Error bars show the standard deviation.

CDOM and dDIC/dt for station L4 during Feb-Apr 2016 were plotted versus the Julian Day (being noon of 01/01/2016 day number 1.5; Figure 9) in order to show any temporal variation. Weather conditions were observed and written down the days previous to the sampling, in case there was any anomaly which would affect the data. Figure 9 shows the two CDOM measurements at different wavelengths which correlated with a p-value = 0.0319 (n = 7). A significant correlation between CDOM and dDIC/dt was not obtained (p-value > 0.05; n = 7) for L4 station during Feb-Apr 2016. It was observed that on day 81, $CDOM_{300}$ increased noticeably and the dDIC/dt decreased in the same manner. The previous and later points show an observable positive lineal correlation, although not significant, between these two types of measurements. This outlier has been related to changes due to the weather, since over the previous days (78-80), a cold front with high wind speeds and heavy rain showers passed through the sampling station, which likely affected the organic matter composition and may explain this outlier. Therefore, the lack of correlation between CDOM and dDIC/dt may be due to both the low number of data points and the weather changes.
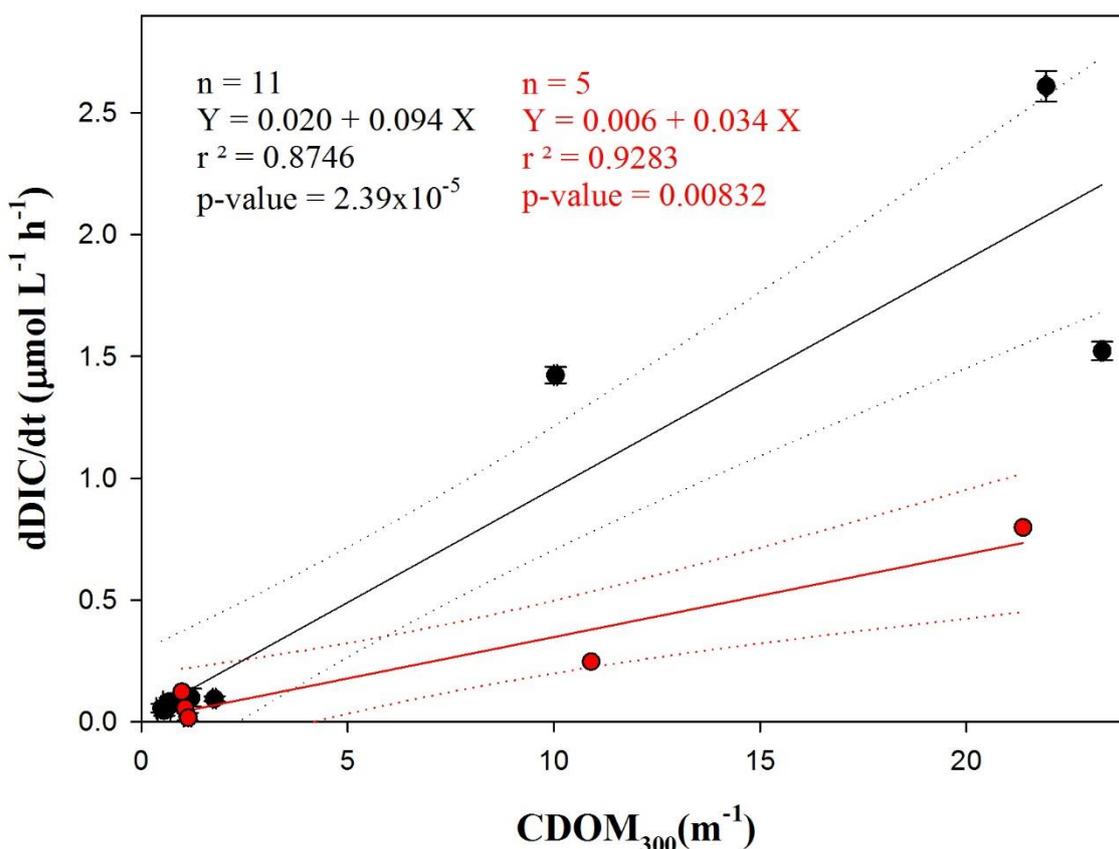

Figure 10. dDIC/dt correlation with $CDOM_{300}$ for all the experiments carried out during this project. In red values obtained during May-Jun 2015, standard deviations were not available (by K. Coombes). In black values obtained during Feb-Apr 2016, error bars show the standard deviation.

Rates were computed and plotted versus the CDOM in order to confirm the correlations between both variables (Figure 10). This graph contains data obtained by K. Coombes (unpublished data) in L4, river Dart and river Plym during May and June 2015 (plotted in red). It also contains all the data acquired between February and April 2016 (plotted in black). CDOM absorption coefficients values between $0.55m^{-1}$ and $1.8m^{-1}$ correspond to samples from L4 and E1, and absorption coefficients of over $5m^{-1}$ were obtained for riverine samples.

Both slopes show a positive significant correlation between CDOM and dDIC/dt, the May-Jun 2015 slope was $0.0340\pm0.0055\mu mol\ m\ L^{-1}\ h^{-1}$, while the slope from Feb-Apr 2016 increased to $0.0939\pm0.0118\mu mol\ m\ L^{-1}\ h^{-1}$.
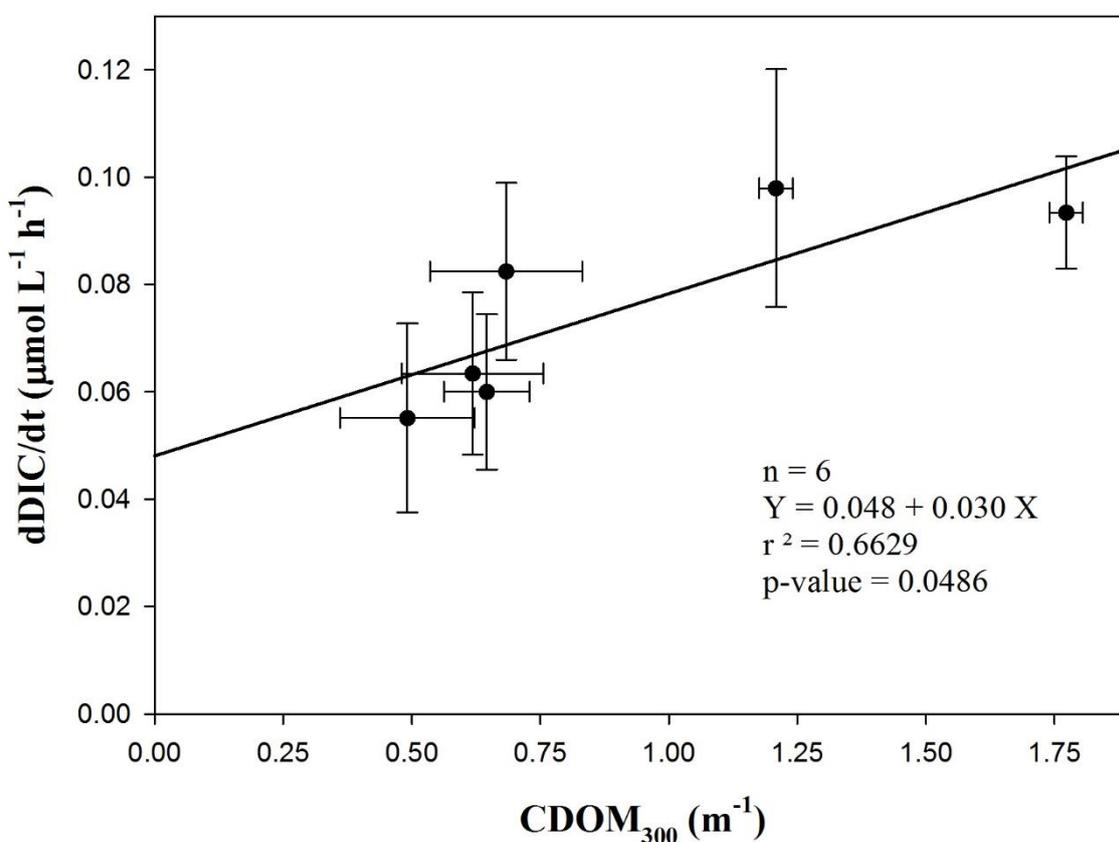
Figure 11. dDIC/dt correlation with $CDOM_{300}$ for the costal station L4 for the period Feb-Apr 2016, horizontal and vertical error bars show their individual standard deviation.

During Feb-Apr 2016, data in the coastal station L4 alone, showed a significant correlation between CDOM and the photochemically produced DIC (Figure 11). A characteristic of this data is that the slope obtained was $0.0302\pm0.0108\mu mol\ m\ L^{-1}\ h^{-1}$, which is similar to that for May-Jun 2015 data (Figure 10).
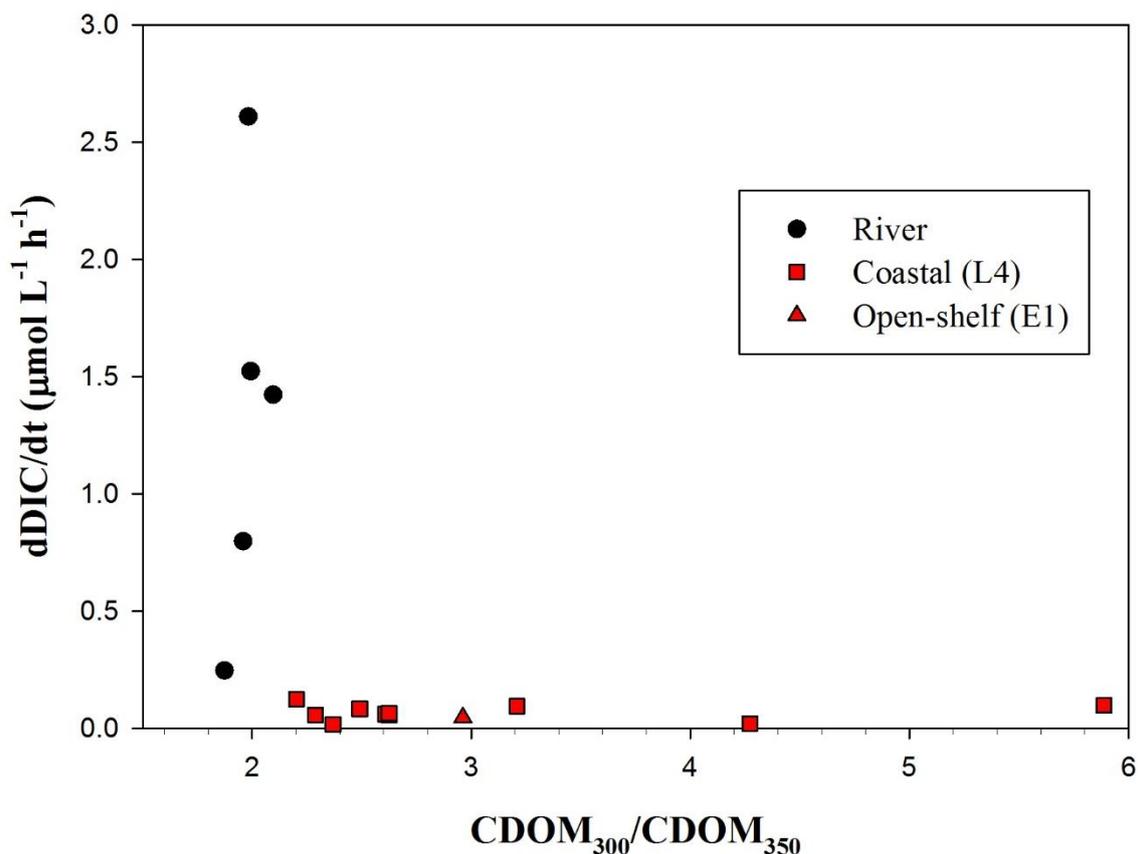
Figure 12. CDOM ratio vs. DIC photochemical production rate. The spatial distribution of the data plot shows two different water types.

The dDIC/dt varies significantly with the water type analysed. Figure 12, shows that the water type can also be characterised by the $CDOM_{300}/CDOM_{350}$ ratio. Ratio values >2 correspond to seawater (coastal, red squares; and open-shelf, red triangle), and ratio values <2 with riverine water, following an asymptote when plotted against the DIC photochemical production rate.

Discussion

DIC stripping to calculate the photochemically produced DIC is not an optimal method. Firstly, acidification of the sample will cause changes in at least the chemical properties of the sampled seawater. Secondly, bubbling of CO_2-free air will not only strip the CO_2, but other volatile compounds and possibly modify the photoproduction rate. Despite these problems, it is currently the most favourable method to obtain these type of measurements, since the photoproduction rates are too low compared to initial DIC values (Johannessen, 2000; Johannessen and Miller, 2001; Johannessen et al., 2007; Kitidis et al., 2009). Therefore, this method of obtaining values is considered an acceptable compromise by the scientific community.

Meteorological conditions should be considered as they increased the variability of the dataset and created outliers affecting the general trend (Figure 9). The abnormal

conditions affect CDOM and dDIC/dt, even producing opposite changes, as in day 81, where an increase in CDOM was not correlated with a high dDIC/dt, as expected from the other data and previous studies (Groeneveld et al., 2015), but with a decrease. Therefore, understanding the controls of DIC photoproduction will help understand natural variability.

CDOM was photochemically consumed throughout the experiment producing with concomitant production of DIC. Consequently, the CDOM spectra varied through the experiment. There were small changes (< 7%) between the original sample and the initial sample (after the DIC stripping had taken place and the sample had been buffered). The initial sample and dark control sample did not vary significantly since photoproduction did not occur in the dark control. The main differences were observed between the initial sample and the samples that had been irradiated, being the UV+visible the sample with the biggest change as the highest irradiation took place in this sample.

Photochemical production of DIC showed a significant variation in the DIC produced depending on the spectral irradiance. UV+visible irradiation had a dDIC/dt 35.3±9.7% higher than visible irradiation for rivers. In marine water, UV+visible dDIC/dts were between 83% to 535% higher than dDIC/dt with only visible irradiation. Therefore, reported data of dDIC/dt produced should consider the origin of the samples as well as the type of irradiated light used, and consequently, special care should be taken when deciding which container types are irradiated, since they will affect the phototransformation of CDOM to DIC.

Figure 10 shows the significant correlation between CDOM and dDIC/dt (p-values = 8.32×10^{-3} (2015) and 2.39×10^{-5} (2016)), confirming the initial hypothesis that there is a significant correlation between the content of CDOM in water (at least in riverine and coastal water) and the dDIC/dt. It also produces a new question that has not been investigated before except for Groeneveld et al. (2015). The data obtained shows there is an important seasonal change in the slope of dDIC/dt versus CDOM, around three fold (from $0.0340\pm0.0055\mu$mol m L^{-1} h^{-1} (2015), to $0.0939\pm0.0118\mu$mol m L^{-1} h^{-1} (2016)). This characteristic is observed in the change in slope (Figure 10). Groeneveld et al. (2015), obtained data from June to November 2014 in a lake in Sweden producing a model for a three year period and observed that the rate was not three fold as obtained from our data, but as much as five times higher. Other models have been produced with measurements which do not consider time variations, and consequently they are not able to describe the real dDIC/dt throughout a whole year, since overestimation or underestimation would be achieved.

Unexpectedly, coastal samples from February-April 2016 have the same slope as samples from May-June 2015 ($0.0302\pm0.0108\mu$mol m L^{-1} h^{-1} and $0.0340\pm0.0055\mu$mol m L^{-1} h^{-1}

respectively). These results imply that seasonal variability is not that important in coastal regions as it is in fluvial waters, although further research should be done in the subject, and a new project will be started in the following years to study this characteristic.

The relationship between the $CDOM_{300}/CDOM_{350}$ ratio (Figure 12) with the dDIC/dt gives very important information. High ratios (>2) were obtained for oceanic waters, while lower ratios were obtained in riverine waters (<2), characterising the sample provenance (Kitidis et al., 2014). Furthermore, we can discern three different water types with a ratio >2. Coastal waters had ratios between 2 and 2.8. Open-shelf water consisted of a ratio of 2.96. Water brought to L4 during the storms had a ratio above 3.2.

CDOM values can be obtained in a matter of minutes, while DIC experiments are carried over a three-day period. As a result, experiments with low CDOM ratios, corresponding with more riverine water could be irradiated for a shorter period of time, producing faster and equally valuable dDIC/dt results.

The results obtained in these experiments may show that the dDIC/dt is higher for riverine water, but if photochemical production is considered throughout the entire water column instead of the surface production, the photochemically produced DIC in oceanic waters would be higher. CDOM is thought to be the main absorber of UV radiation in all areas of the ocean, so equations can be used to get an approximation of the light attenuation coefficient (k_∞; m^{-1}) (Johannessen and Miller, 2001; Preisendorfer, 1976).

$$k_\infty = \frac{4}{3} \, CDOM_{350}$$

During 2016, dDIC/dts were ~29 times higher in riverine water, but the light attenuation coefficient was ~34 times higher. Consequently, the photic region was deeper for coastal water, which would lead to higher DIC photoproduction when integrated over the water column. The differences for 2015 were higher (~8 and ~18 respectively), so the contrast between the DIC photoproduced in coastal and riverine water was bigger.

Many factors can affect dDIC/dt. An increase in DIC photoproduction is correlated with an increase in the CDOM aromatic content, an increase in iron concentration, or with a decrease in pH (Brinkmann et al., 2003; Gao and Zepp, 1998; Groeneveld et al., 2015; Miller and Zepp, 1995). Photobleaching, CDOM phototransformation to less coloured and aromatic compounds, could be an important factor in the seasonal scale, which would explain the decrease in the slope of Figure 10 (Brinkmann et al., 2003; Groeneveld et al., 2015). This change is due to CDOM becoming less photoreactive, as the more photoreactive CDOM transforms.
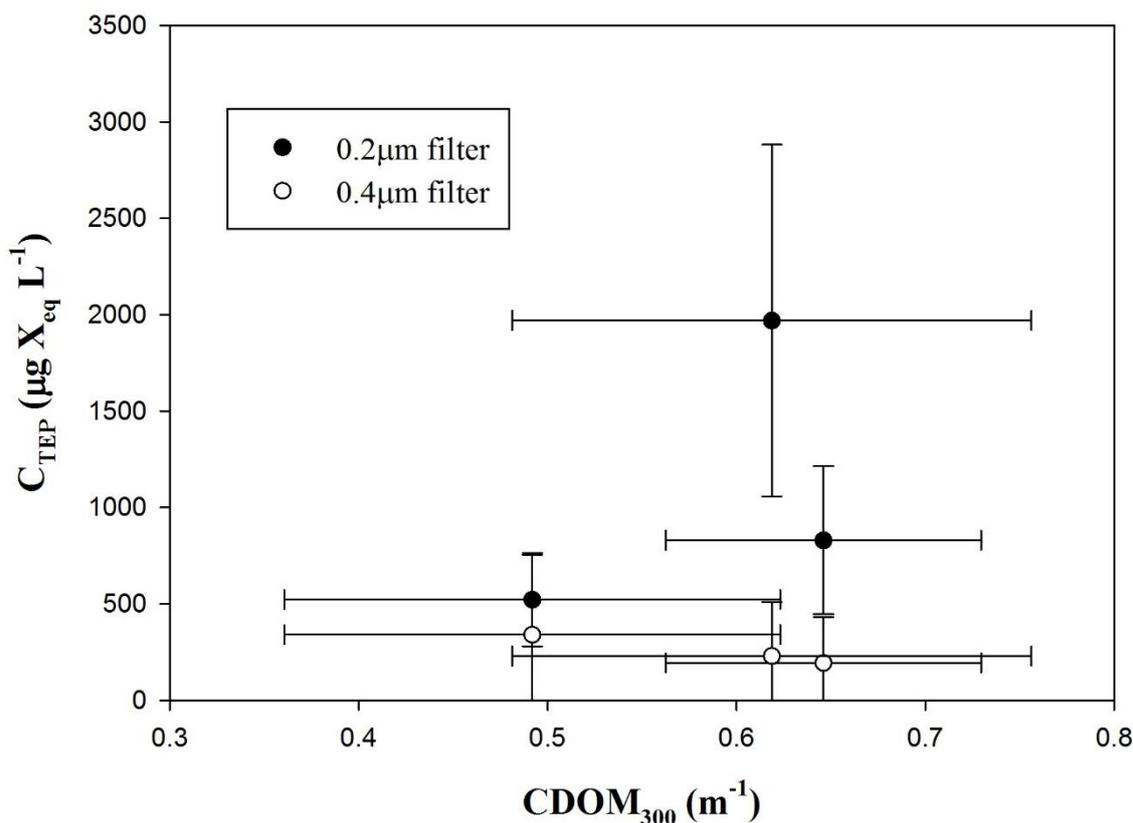
Figure 13. C_{TEP} vs $CDOM_{300}$ correlation using 0.2 and 0.4μm filters.

This work, tried to correlate CDOM with the amount of TEP (Figure 13). However, the method use was not sensitive enough. Furthermore, the time constraint resulted in the collection of a small amount of data (n = 2, 3, 3 for 0.1μm, 0.2μm, 0.4μm respectively). Consequently, no significant results were acquired, and further work on the subject is necessary. The importance of obtaining more data is that TEP are considered discrete pools of high nutrient concentration that can be colonized by microorganisms (Verdugo and Santschi, 2010). A positive correlation between DOC and TEP exists (Mari et al., 2007). This correlation may affect the CDOM-dDIC/dt correlation, since in theory TEP and DIC photoproduction will compete for the available CDOM in the water.

Conclusions

This project has contributed to the understanding of DIC photoproduction with a great amount of data. Due to the significant correlation between CDOM and dDIC/dt, the initial hypothesis that DIC photoproduction is controlled by CDOM has been confirmed for coastal and riverine water. This linear positive correlation, with an important seasonal variability, will help produce more realistic models and predictions. dDIC/dt experiments require high maintenance equipment and extended periods of time to prepare and analyse and extensive lab work, while CDOM measurements are fast, inexpensive and can be carried out in research cruises, where only a spectrophotometer is needed. Therefore, if the correlation is understood and seasonal patterns predicted, models for DIC

==photoproduction== can easily be produced from existing and future CDOM datasets. However, seasonal variability is not the only factor affecting the CDOM dDIC/dt relation. Data obtained after storms show their impact in the results. Additionally, dDIC/dt of UV+visible and visible only irradiation has shown the impact the spectral irradiance has in the different water types. As a result, scientists should consider the use of quartz flasks when doing experiments. The use of UV absorbing flasks or tubes should not be accepted since the results will not be able to represent the real process, as they constrain an important fraction of the phototransformation of CDOM.

Two distinct relationships at different periods of time between CDOM and the dDIC/dt have been observed. The most probable explanation for this phenomenon is seasonal variability (Groeneveld et al., 2015). It is an important factor that should be considered in future research since it will significantly influence any model or conclusion generated from the data if a long period of data collection is not considered.

Experiments with TEP did not provide the expected significant correlation (Mari et al., 2007; Verdugo and Santschi, 2010), so the competing effect of both processes was not studied. More experiments should be carried out, and the possibility of finding another method to calculate TEP should be considered.

Acknowledgements

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Appendix

Detailed description of the activities done during the FDW

The Final Degree Work (FDW) was carried out conjointly with the External Practical Work of the Degree of Marine Science at the Universidad de Las Palmas de Gran Canaria. The work was done under the supervision of Dr. V. Kitidis at Plymouth Marine Laboratory (PML).

The activities carried out during the FDW were:

- Bibliographic search. The initial knowledge was obtained from Hansell and Carlson (2014). Further knowledge was obtained from electronic bibliographic resources such as *SCOPUS* (thanks to the ULPGC accreditation), *Web of Science, Google Scholar* and the *National Marine Biological Library* (www.nmbl.org).
- Equipment preparation, including a new, custom-built, efficient and inexpensive CO_2 stripping mechanism.
- Preparation of stable secondary DIC standards (SDICS).
- Data gathering and analysis. Explanations have been given to the reasoning behind the characteristics of the data gathered, contributing to furthering the knowledge. Three data types were gathered:
  - o DIC measurements.
  - o CDOM measurements.
  - o TEP measurements.
- Final work presentation at PML.
- FDW write-up. After analysing the data, the FDW was written to present the knowledge gathered throughout the project.

GRADO EN CIENCIAS DEL MAR. ASIGNATURA: 40632 – Trabajo Fin de Título

DOCUMENTO 1: DEFINICIÓN DE ACTIVIDADES Y TEMPORALIZACIÓN

Datos del TFT

Título: Photochemical Transformation of Dissolved Organic Matter to Dissolved Inorganic Carbon	
Periodo de duración. Desde (dd/mm): 18/01	hasta (dd/mm): 21/06

Datos alumno:

Alumno: David González Santana	e-mail: david.gonzalez135@alu.ulpgc.es
Beca:	☒ Si, Indicar: Erasmus + Prácticas ☐No

Datos tutor/es:

Grupo/Centro/empresa: Facultad de Ciencias del Mar	
Tutor: Melchor González Dávila	e-mail: melchor.gonzalez@ulpgc.es
Grupo/Centro/empresa: Facultad de Ciencias del Mar	
Tutor: Milagros Rico Santos	e-mail: milagros.ricosantos@ulpgc.es

I. Actividades propuestas.

Las actividades que se proponen para que realice el alumno durante sus prácticas de empresa son:

1	Meetings.
2	Reading of existing literature and bibliography. Producing an accurate research proposal.
3	Set up of method and equipment.
4	DIC measurements.
5	CDOM measurements.
6	TEP measurements.
7	Data processing.
8	FDW write-up.

II. Cronograma (ver nota aclaratoria):

Actividad	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	HP	HNP
1																	10	0
2																	5	45
3																	10	5
4																	30	0
5																	40	50
6																	15	0
7																	10	30
8																	0	50
															Total		120	180

En Las palmas de Gran Canaria, a 16 de marzo de 2016

Firma alumno

Firma tutor del TFT

Nota:
- Las semanas se cuentan desde la primera semana del periodo de las prácticas hasta la última y pueden ser menos de 16.
- Sombrear las celdas de las semanas en las que se vaya a trabajar la actividad indicada, que pueden ser o no consecutivas.
- En las últimas columnas indicar el nº de horas presenciales (HP) y no presenciales (HNP) por actividad. La suma total debe ser la indicada.
- El alumno debe enviar este documento al tutor del TFT tras la primera reunión de planificación.

Training received

The training received during the FDW will produce a long term benefit in my education and future work and research experiences:

- Increase of efficiency with the use of reference management programs in particular *EndNote* and *Mendeley*.
- Increase of safety consideration thanks to the preparation of Control of Hazardous to Health (COSHH) forms.
- Hands on training in lab work related to photochemistry.
  - Use of DIC analyser (Model AS-C3; Apollo SciTech, Bogart, GA, USA)
  - Single beam spectrophotometer (Ultrospec II), dual beam spectrophotometers (Lambda 35 and Lambda 800; Perkin Elmer).
- *Microsoft Excel* What-If Analysis, Goal Seek.
- *SigmaPlot*. Statistical and graphic software used for data processing and data presentation.
- *ArcGIS*. A geographic information system (GIS) for working with maps and geographic information, used during the map production and the sampling selection.
- Pipette training courses. The student was able to participate in a 3h course by *Alpha Laboratories* to improve pipetting ergonomics, techniques and maintenance.
- *PlyMSEF* Annual Student Conference 2016. During the stay at the PML, the student was able to participate in the conference, gaining further knowledge in the field, obtaining ideas to further improve his experiments and facilitate his networking.

Level of integration and implication in the organization

The student has had a significant integration in the group and PML organization. He has accompanied the Penlee Point Atmospheric Observatory (PPAO) group in one of their routine equipment inspection runs. He has also joined the monthly Marine Biogeochemistry and Ocean Observations Science Group Meetings gaining inside knowledge of the researcher's interests and issues. Finally, he was considered for an Antarctic Cruise during December 2016 - January 2017, which he had to reject due to time conflict with his future masters studies. His understanding and autonomous work has made him a valuable asset at PML, helping other researchers instruct their new students in the use of the different lab equipment. Overall, the FDW has been a productive time for networking, enabling future work experiences and collaborations.

Most significant aspects related to the development of the FDW

The FDW has been the perfect opportunity to implement most of the knowledge acquired during the degree, while also providing ample opportunities to seek future interests in the Marine Oceanography research field.

Personal evaluation of the skills obtained during the FDW

Many skills have been acquired or improved during the FDW. Some of the most important have been data gathering techniques and analysis. The opportunity has granted the student time to observe scientists in their field permitting first-hand experience in many fields overlapping the same interests. He has gained further knowledge and improved awareness in work related topics. English oral and communication skills have improved by utilising English as the main communication language at work. Communication with other scientists and a final oral presentation showing his results has also helped in these necessary skills. Work ethics have been ingrained on the student during the FDW, which will help future experiences. Group work skills have also been improved, since schedules to use the different equipment had to be considered, and the lab benches had to be shared.