14

In Situ Primary Production Measurements as an Analytical Support to Remote Sensing - An Experimental Approach to Standardize the ^{14}C Incorporation Technique

Tamara Cibic and Damiano Virgilio*
Dipartimento di Oceanografia Biologica,
Istituto Nazionale di Oceanografia e Geofisica Sperimentale (OGS), Trieste,
**Current address: Regional Environmental Protection Agency-FVG, Palmanova,*
Italy

1. Introduction

Primary production measurement in marine waters is one of the most important tools to understand the ecosystem functioning and the transport of inorganic/organic matter through the food web. Direct measurement of the driving process, that is photosynthesis, in this dynamic system was first achieved using the Winkler technique for estimating dissolved oxygen concentrations, introduced by Gaarder and Gran in 1927 (Williams et al., 2002). Nowadays the light-dark bottle oxygen technique is considered not sensitive enough and the rate had poor accuracy and precision (Marra, 2002).

The introduction of the ^{14}C technique changed the study of productivity (Marra, 2002). The year 1952 marked the end of a century-long struggle to develop a method to determine oceanic primary productivity with precision, accuracy and efficiency (Barber & Hilting, 2002). Thousands of measurements of marine phytoplankton productivity have been made at discrete locations throughout the world's oceans since the introduction of the radiolabelled carbon uptake method in 1952 (Steemann-Nielsen, 1952). Although numerous, these discrete primary productivity measurements only provide information for infinitesimally small points over the oceans' surfaces. Scaling these discrete measurements to global projections by means of satellite-based estimates requires mathematical models. Clearly, although much of the discrepancy between modelled and measured production results from limitations of the models, some of the disagreement is also due to methodological differences in ^{14}C measurements and errors in the ^{14}C data (Behrenfeld & Falkowski, 1997). To compare modelled primary production with that obtained *in situ*, a high quality database of ^{14}C measurements is required. Calculating accurate primary production estimates over large areas is a primary step for ecosystem models charged with the task of assessing trophic dynamics. Reliable estimates of primary production are also necessary for multiple other applications, including quantifying the flux of carbon dioxide, assessing export production and estimating production of climate-active gases such as dimethyl sulphide. Estimating accurate primary production on global scales is also essential to understanding the consequences of climate change on phytoplankton growth (Friedrichs et al., 2009).

The ^{14}C method is relatively simple. A known amount of ^{14}C-CO_2 is added to bottles containing the water samples and after an incubation time the organic carbon is frequently separated from the remaining inorganic carbon by filtration (Peterson, 1980). Several debates have arisen from the comparison of the results obtained with the ^{14}C method and the Winkler method, the latter largely used for primary production estimates before 1952 (Peterson, 1980). In 1972 Schindler and co-workers modified the ^{14}C method proposed 20 years earlier by Steemann-Nielsen. The authors described a method in which the samples were not filtered, in order to avoid the rupture of cells during filtration and loss during drying of filters that can still cause underestimation of primary production (Theodórsson & Bjarnason, 1975). The problems encountered in filtering and the somewhat arbitrary nature of the separation between dissolved and particulate matter led to the development of the acidification and bubbling procedure (Peterson, 1980). Unfortunately, this technique does not permit to assess the two fractions of primary production labelled with ^{14}C-CO_2: POC (Particulate Organic Carbon, corresponding to the ^{14}C retained in the autotrophic cells) and DOC (Dissolved Organic Carbon, released from the autotrophic cells). Therefore this technique is not useful to understand the transformation of the organic matter along the trophic levels, in particular the link between autotrophic production and other levels of the marine food web. In most of the references regarding primary production in the water column, the method usually applied to stop the process and remove the excess labelled ^{14}C was filtration and subsequent acidification with HCl (Table 1).

HCl	sample volume	reference
filters wetted with 1N	50 ml (filter)	Babin et al., 1994
concentrated acid fumes	75 ml (filter for POC production)	Cermeño et al., 2006
100 µl of 18.5% acid	5 ml (filtrated for DOC production)	
filters rinsed twice with 1N	50 ml (filter)	Hewson et al., 2001
1 ml, 2M (filters stored for 2-3 days at -20°C before acidification and counting)	100-500 ml (filter) for primary production estimate	Karl et al., 1998
0.5 ml, 6N	1 ml (total sample)	Lewis & Smith, 1983
200 µl, 0.1N	50 ml (filter)	Mangoni et al,. 2008
concentrated acid fumes	5 ml (filter for POC production)	Marañón et al., 2004
100 µl of 50% acid	5 ml (filtrated for DOC production)	
100 µl of 50% acid	5 ml (total sample for TOC production)	
500 µl, 0.5N	320 (?) ml (filter)	Moutin & Raimbault, 2002
2 drops of 5N	not available	O'Donohue & Dennison, 1997
0.5 ml, 6N	5 ml (filtrated for extracellular release)	Pugnetti et al., 2005
0.5 ml, 6N	5 ml (total primary productivity)	
fumes of concentrated acid	75 ml (filter)	Teira et al., 2005

Table 1. Summary of currently in use primary production filtration and acidification methods. POC = particulate organic carbon; DOC = dissolved organic carbon.

Many authors use filters to estimate primary production, without considering the filtrated fraction, which corresponds to the exudate release, nor the total sample. Moreover, different HCl concentrations are used in the reported papers. When samples are filtered, filters can be wetted or rinsed with acid (Babin et al., 1994; Hewson et al., 2001) or, in more recent studies, exposed to concentrated acid fumes (Cermeño et al., 2006; Marañón et al., 2004; Teira et al., 2005). The added HCl concentration ranged from 0.1N (Mangoni et al., 2008) to 6N (Lewis & Smith, 1983; Pugnetti et al., 2005). Considering different acid volumes added to different

sample volumes also the final acid concentrations were very diverse among the studies. Although the ^{14}C technique has now been used in more than 35 000 oceanographic experiments (del Giorgio & Williams, 2005), from the review of the earlier and recent literature, we evidenced that different methods are still used to stop the photosynthetic activity, and that highly variable concentrations of HCl are added to remove the excess labelled bicarbonate which was not assimilated by the microalgae. In order to standardize this step of the ^{14}C method, we designed a series of experiments, using both water samples and a mix culture of microalgal taxa which represent a coastal phytoplankton community. The first aim of our study was to define which HCl concentration, with an equal volume, among 0.1N, 0.2N, 0.5N, 1N, 2N and 5N was sufficient both to remove the excess labelled bicarbonate and to kill the cells but without damaging their structures with a consequent loss of assimilated ^{14}C. Secondly, using both chlorophyll *a* red autofluorescence and motility of flagellates and diatoms as proxies of microalgal viability, we tested if different acid concentrations were sufficient to stop the microalgal photosynthetic activity.

2. Materials and methods

2.1 Water sampling

On 18th October 2006, 29th January 2007 and 4th September 2008 five litres of seawater was collected at 5 m depth by a Niskin bottle about 10 km far from the coast in the Gulf of Trieste (northern Adriatic Sea, Italy). In each cruise seawater temperature and chlorophyll *a* (chl *a*) were recorded by a Multiparameter Probe Ocean Seven 316 Idronaut. In laboratory the sampled water was immediately transferred to translucent polycarbonate carboy (Nalgene) and kept at *in situ* temperature (20°C in October, 10°C in January, 20°C in September) and light conditions ranging from 100 to 200 µmol photons s^{-1} m^{-2}, according to the sampling day. Flushing by an air pump kept the water at oxygen saturation. Water was exposed to a 14:10 h light:dark cycle.

2.2 Experimental design

The next morning (10.00-11.00 a.m.) 1.320 l of sampled seawater was transferred to an Erlenmeyer flask and kept in the darkness for 30 minutes to reduce the photosynthetic activity. Subsequently, 99 µCi (3.66 MBq) of $NaH^{14}CO_3$ (DHI, Denmark) was added into the flask. The sample was gently stirred and then 6 subsamples of 220 ml were transferred to six Erlenmeyer flasks. Three of these were incubated at *in situ* conditions, while the other three were wrapped up in aluminium foils to maintain them in darkness and incubated together with the light ones at *in situ* temperature for 2 hours. Inoculum and subsampling were performed at very low light to avoid the beginning of the photosynthetic activity before incubation. After incubation each 220 ml replicate was stored in dark and cold conditions (ice was used to keep temperature close to 0°C) until filtration. From each of 220 ml replicates, 6 aliquots of 25 ml were filtered using a Millipore vacuum filtration manifold (with 12 wells) through polycarbonate 0.2 µm filters applying a low vacuum pressure in order to avoid cell damage. The 6 filters were put into 6 ml plastic scintillation vials (Perkin Elmer); 5 ml of the 6 filtrated samples was collected and put into 20 ml glass scintillation vials (Perkin Elmer) to assess the rate of phytoplankton exudate release. Finally, six 5 ml aliquots of the total sample, drawn from the 220 ml replicate, were directly put into 20 ml glass scintillation vials. To each final sample series (6 filters + 6 filtrated samples + 6 total samples) obtained from the 220 ml replicate, 200 µl of HCl at progressively higher

concentrations (0.1N-0.2N-0.5N-1N-2N-5N which correspond to final HCl concentrations of 0.004N-0.008N-0.02N-0.04N-0.08N-0.2N, respectively, for filtrated and total samples) was added and left under hood overnight to remove the labelled bicarbonate which was not assimilated by the microalgae. 5 ml of Filter Count scintillation cocktail (Perkin Elmer) was added to filters, while 10 ml of Ultima Gold XR (Perkin Elmer) was added both to filtrated and not filtrated samples. Disintegrations per minute (DPM) were measured twice by a QuantaSmart TRI-CARB 2900 TR Liquid Scintillation Analyzer (Packard BioScience, USA) including quenching correction, obtained using internal standards.

2.3 Statistical analysis

Student's t-test was applied to test for significant differences between pairs of experiments considering different acid normalities for filters, filtrated and total samples. Only data obtained from the first reading was used for the t-test.

2.4 Effects of HCl on the planktonic microalgal community

In order to test if the added acid concentrations were sufficient to stop the microalgal photosynthetic activity, another experiment was carried out. A mix of six microalgae, representing the local phytoplankton community, was prepared, simulating the proportion among the groups which is characteristic of a resuspended coastal water mass. We chose: *Chaetoceros socialis* as a typical planktonic diatom, *Cylindrotheca fusiformis* as a tychopelagic diatom, *Paralia sulcata* as a common benthic diatom, *Gymnodinium* sp. representing naked dinoflagellates, *Lingulodinium polyedrum* representing thecate dinoflagellates and an undetermined Cryptophycea as a typical small phytoflagellate (Table 2). We stored the mix culture for 24 h at 15°C and 12:12 light:dark photoperiod (50 µmol photons s^{-1} m^{-2}). The next day 7 aliquots of 5 ml were transferred into 20 ml scintillation glass vials. The following protocol was performed: one replicate was used as a control, while to the other six 200 µl of HCl at increasing concentrations (the same of the previous experiments) was added. Then, we performed a litmus test (Merck), estimating the pH in each sample. We left the samples under hood overnight to simulate the primary production protocol as described above. The next day a few drops of each sample were observed under an inverted microscope (Leica DMI 3000B) using both phase contrast and epifluorescence, under a blue filter set (BP450-490 nm) at different magnifications (from 200 to 400X). As proxies of viability we considered both chlorophyll *a* red autofluorescence and motility of flagellates and of the tychopelagic diatom. The motility of planktonic (*Chaetoceros socialis*) and benthic (*Paralia sulcata*) diatoms was not taken into account because these two taxa do not have specific structures allowing movement.

Taxon	Representing group	Presence
Chaetoceros socialis	Planktonic diatom	abundant
Cylindrotheca fusiformis	Tychopelagic diatom	abundant
Paralia sulcata	Benthic diatom	rare
Gymnodinium sp.	Naked dinoflagellate	rare
Lingoludinium polyedrum	Thecate dinoflagellate	rare
undet. Cryptophycea	Small phytoflagellate	the most abundant

Table 2. Composition of the mix culture artificially created in the laboratory to simulate a natural phytoplankton community of coastal waters.

2.5 Recovery experiment

During microscopic observations we observed several shades of red autofluorescence. Since we could not be sure that a pale orange colour was a sign of a not viable cell, we carried out another test. We believed that some cells could be still viable after a mild acid treatment and thus able to photosynthesize, especially during the period elapsing between the addition of the acid and the addition of the scintillation liquid. We tried to demonstrate that after a mild acid treatment some microalgae were still photosynthetically active. Consequently, we restored their maintenance conditions looking at the microalgal growth after two weeks. Therefore, the residual aliquots (>4 ml) of a few selected treatments (without acid, 0.1N, 0.2N and 5N) from the first experiment (mix culture) were transferred into 100 ml Erlenmeyer flasks which were filled with F/2 medium to a final volume of 50 ml. The four flasks were kept at 15°C and 12:12 light:dark photoperiod until microscopic observations.

3. Results and discussion

3.1 *In situ* primary production as an analytical support to remote sensing

Frequently the problem with the model efficiency is data limited by the amount of representative *in situ* measurements. As more *in situ* data become available they can be exploited within most of the existing complex formulations. When asked what is needed to improve model performance, all model developers coincide in requesting more data (Carr et al., 2006). When comparing primary production measurements with those from different studies, it is difficult to decipher whether differences are due to inter-annual changes, the spatial location of the stations sampled in each region, the frequency over which the measurements were taken or the measurement protocol used (Tilstone et al., 2009). Often *in situ* primary production data are associated with a level of uncertainty and need to be regarded as ranges rather than as exact values (Friedrichs et al., 2009). Another problem with *in situ* measurements is that ship resources cannot solve low-frequency spatial and temporal variability, much less make direct observations of mesoscale variability beyond isolated snapshots. The chronic undersampling of ship-based estimates of global primary production requires significant extrapolations, making it essentially impossible to quantify basin-scale variability from *in situ* measurements (Carr et al., 2006). Fortunately, satellite provide a solution. Sensors that measure ocean colour are presently used to estimate chlorophyll concentration in the upper ocean (Carr et al., 2006). The simplest models estimate time and depth-integrated production as a function of surface chlorophyll. However, surface chlorophyll explains only 30-40% of the variance in primary production at the scale of a single station (Hyde et al., 2008). To go from biomass, a pool, to photosynthesis, a rate, a time dependent variable is needed (Carr et al., 2006). Recently, a series of round-robin experiments were carried out to evaluate and compare models which estimate primary productivity from ocean colour (Campbell et al., 2002, as cited in Tilstone et al., 2009). In these experiments, *in situ* measurements of carbon uptake were used to predict depth-integrated primary production based on information accessible via remote sensing. According to the authors, there is no way to quantify model performance without comparing the output to *in situ* data (Carr et al., 2006). For any model, a vital element of model skill is the ability to reproduce *in situ* observations; in the case of primary production models, measurements of primary production. If observations are representative and the data have undergone careful quality control, firm conclusions can be reached regarding the environmental conditions that challenge model skill. These challenging conditions, in turn,

can be taken into account by model developers and end-users to improve model formulation and/or application. As a consequence, improving primary production estimates will increase the skill of global models (Friedrichs et al., 2009).

3.2 Different fixatives and HCl concentrations in phytoplankton primary production

Results of the phytoplankton primary production experiments are presented in separate figures as filter, filtrated and total fractions. In the graphs each bar represents one biological replicate, while standard deviations result from 2 scintillation counts (from the Quanta-Smart scintillation analyzer). In Figure 1 are shown filter DPM values of the three experiments. In the second experiment some problems took place during filtration, i.e. the process occurred too quickly probably due to not proper positioning of the following filters: 1L0.1N, 2L0.1N, 2L0.5N, 1L1N, 2L1N, 1L5N and 2L5N, therefore leading to an underestimate of DPM values.

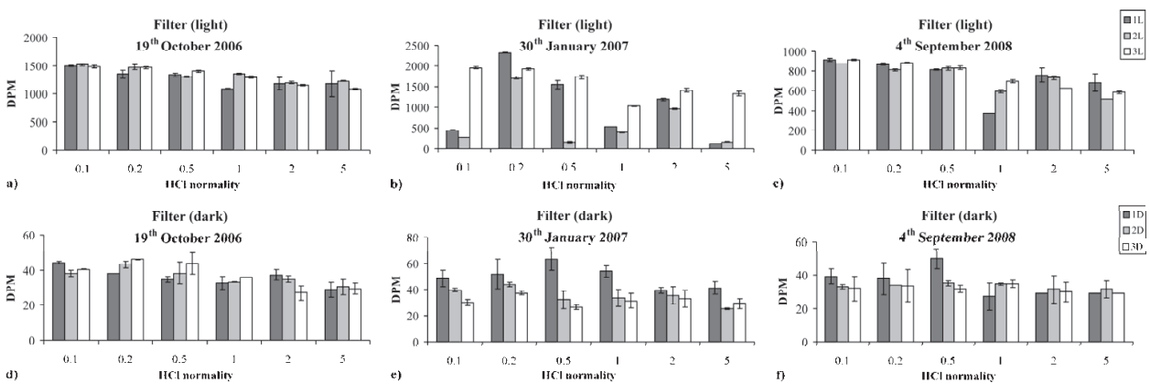


Fig. 1. Filter DPM values of light and dark replicates resulted from the three experiments. Each bar represents the mean of 2 scintillation counts for each biological replicate. For each experiment a different y-axis scale was applied to better highlight the difference between acid treatments.

In the same experiment we observed that DPM values of the first dark replicate were higher than those of the second dark replicate, which were in turn higher than the third one (except for the 5N treatment). Not considering the above mentioned replicates, we noted that both light and dark DPM values of the second experiment were higher than those of the first and third ones. Higher values were probably due to a higher chl *a* content (2.62 μg l^{-1}) measured at 5 m depth on 30th January 2007 if compared to the chl *a* content estimated on the other two sampling dates (0.88 and 0.52 μg l^{-1} in 2006 and 2008, respectively). Focusing only on the first and last experiments, we observed a decrease in DPM values going from 0.1N to 5N treatment, both in dark, but especially in light replicates. Considering the average of the three light biological replicates, there was a consistent reduction in DPM values in correspondence with progressively stronger acid treatments. In detail, the DPM decrease between 0.1N and 0.2N treatments, expressed as percentage, was very similar: 5.99% and 5.33% in the first and last experiment, respectively. This percentage of difference was higher comparing the 0.1N acid treatment to the 2N one: 19.18% and 20.02% in the first and last experiment, respectively. Finally, the percentage decrease between 0.1N and 5N treatments reached 31.17% in the third experiment.

In Figure 2a-f are shown DPM values of filtrated samples of the three experiments. In the second experiment higher DPM values were registered due to the previously reported filtration issue. We infer that some of the labelled phytoplankton ended up in the vial placed underneath the corresponding filter without being retained by the filter. In fact there is a relationship between high DPM values of the filtrated samples and low DPM values of the corresponding filters (1L0.1N, 2L0.1N, 2L0.5N, 1L1N, 2L1N, 1L5N and 2L5N). Unlike the filters, where light values were 1 or 2 orders of magnitude higher than dark values, in the filtrated samples their difference was markedly lower. Since the volume of the filtrated sample was 5 times lower than that of the filter sample, DPM values obtained from the filtrated samples were generally 1 or 2 orders of magnitude lower than those obtained from the filters. When DPM values are very low, the biological variability among replicates is amplified. This variability was noted particularly in light and dark samples of the first experiment.

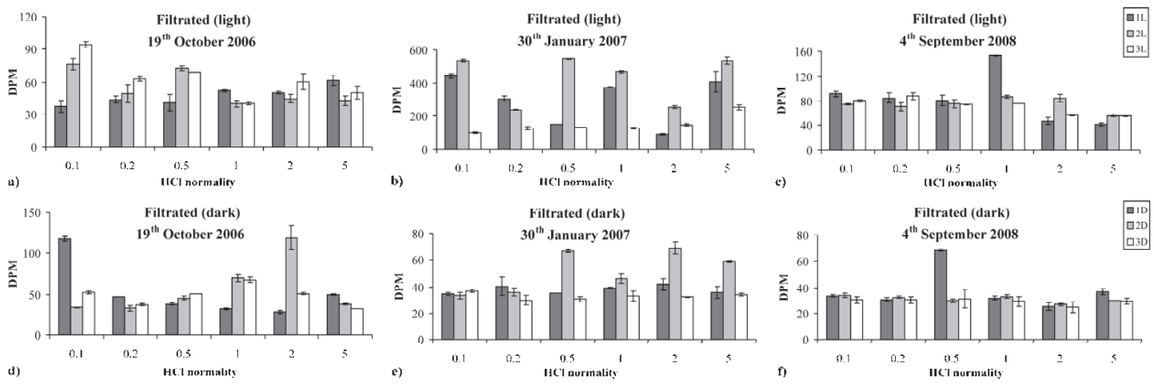


Fig. 2. DPM of light and dark filtrated fractions resulted from the three experiments. Each bar represents the mean of 2 scintillation counts for each biological replicate. For each experiment a different y-axis scale was applied to better highlight the difference between acid treatments.

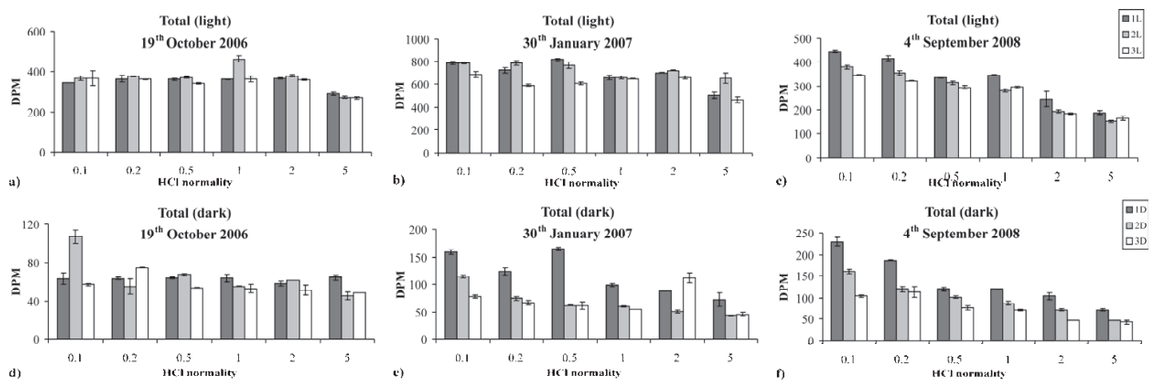


Fig. 3. DPM of light and dark total samples resulted from the three experiments. Each bar represents the mean of 2 scintillation counts for each biological replicate. For each experiment a different y-axis scale was applied to better highlight the difference between acid treatments.

In Figure 3a-f are shown DPM values from total samples of the three experiments. Due to higher chl *a* content DPM light values from the second experiment were twice as high than those obtained in the other two experiments. On the contrary, DPM dark values, which were always lower than the corresponding DPM light values, were progressively higher from the first to the last experiment. However, the decrease in DPM values, going from 0.1N to 5N treatment, was mostly evident in the third experiment, both for light and dark replicates.

While the average reduction of light DPM values between the 0.1N and 0.2N treatments was identical in the second and third experiments (6.62% and 6.61%, respectively), the percentage decrease in other pairs of treatments (0.1N *vs* 0.5N; 0.1N *vs* 1N; 0.1N *vs* 2N; 0.1N *vs* 5N) was much higher in the last experiment, reaching even 56.4% when the 0.1N and 5N treatments were compared.

3.3 Statistical analysis

The t-test applied to dark samples of the three experiments highlighted only a few statistically significant differences between pairs of the tested acid normalities, e. g. for filters in the first experiment and the filtrated fraction in the last one, probably because DPM values were comparable to the background noise of the instrument. Except for the last experiment, performed in September 2008, the t-test did not show any differences between pairs of the tested acid normalities when performed on filtrated fractions, either (Table 3). In detail, in the first light-experiment the 2N acid treatment on filters was significantly different from the 0.1N, 0.2N and 0.5N ones, while the 5N treatment of the total light fraction was significantly diverse from almost all the other treatments. Similarly, in the same dark-experiments only the 5N acid treatment performed on filters was significantly different from the 0.1N, 0.2N and 0.5N ones. The most significant differences between pairs of acid treatments were obtained in the last experiment for the total light fraction.

	1st experiment (light)			1st experiment (dark)			2nd experiment (light)			2nd experiment (dark)			3rd experiment (light)			3rd experiment (dark)		
	filters	filtrated	total	filters	filtrated	total	filters	filtrated	total	filters	filtrated	total	filters	filtrated	total	filters	filtrated	total
0.1N *vs* 0.2N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.1N *vs* 0.5N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.49*	n.s.	n.s.	n.s.	n.s.	n.s.
0.1N *vs* 1N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.1N *vs* 2N	7.90**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	5.36**	n.s.	5.09*	n.s.
0.1N *vs* 5N	n.s.	n.s.	5.50*	4.14*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.19*	4.51*	7.27**	n.s.	n.s.	n.s.
0.2N *vs* 0.5N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.2N *vs* 1N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	5.03**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.2N *vs* 2N	3.13*	n.s.	n.s.	n.s.	n.s.	n.s.	3.79*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.72*	n.s.	4.75*	n.s.
0.2N *vs* 5N	n.s.	n.s.	11.87***	4.61*	n.s.	n.s.	3.44*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	3.17*	6.63*	n.s.	n.s.	n.s.
0.5N *vs* 1N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.5N *vs* 2N	3.04*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.77*	n.s.	n.s.	n.s.
0.5N *vs* 5N	n.s.	n.s.	6.48**	3.26*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.68*	8.98***	n.s.	n.s.	2.90*
1N *vs* 2N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	3.61*	n.s.	4.85*	n.s.
1N *vs* 5N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	6.03**	n.s.	n.s.	n.s.
2N *vs* 5N	n.s.	n.s.	10.65***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	- 3.12*	n.s.

Table 3. Student's t-test applied to pairs of treatments (acid normalities); n.s. = not significant; *** p ≤0.001; ** p≤0.01; * p≤0.05

3.4 Effects of HCl on the planktonic microalgal community

The litmus test performed on the planktonic community revealed that the addition of 200 µl of HCl 0.1N was sufficient to decrease the pH value from 8 to 4 (Table 4, Fig. 4).

pH value						
Without acid	**0.1N**	**0.2N**	**0.5N**	**1N**	**2N**	**5N**
8	4	2 - 3	2	1 - 2	1	0 - 1

Table 4. Litmus test performed on the mix culture (5 ml) in order to test the pH value at increasing acid normalities.

The further increase of HCl normality gradually lowered the pH value down to zero. Since HCl is added to remove the excess labelled C, it is important to know the pH of the acidified sample. In fact, if HCl is not sufficiently concentrated, pH will not reach the value which is necessary to shift the equilibrium towards the CO_2 fraction and consequently the removal of the labelled bicarbonate from the system. Only a pH < 5 ensures a complete shift towards the CO_2 fraction (Libes, 1992). In water samples the 0.1N HCl treatment was enough to lower pH below this value. On the other hand, if the acid is too aggressive it can damage the cell membrane, causing the loss of an undetermined quantity of assimilated ^{14}C and therefore leading to an underestimate of DPM values.

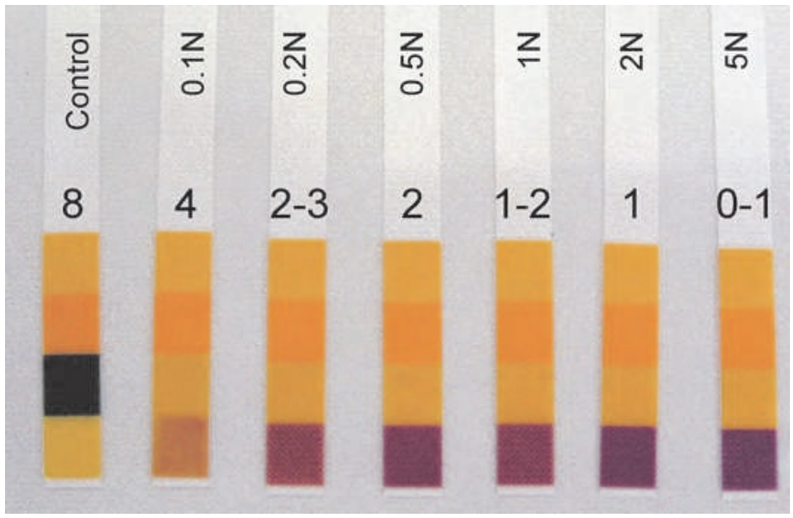


Fig. 4. Litmus test performed on the mix culture (5 ml) in order to test the pH value at increasing acid normalities.

The macroscopic observation of the acidified mix culture samples revealed that while the control was brownish red coloured (mostly due to diatom pigments), 0.1N, 0.2N and 0.5N treatments were greenish. We hypothesize that in the first three mild treatments HCl degraded the accessory pigments, i.e. fucoxanthin, typical of diatoms and responsible for the brownish red colouring, but not chlorophyll *a*. Samples treated with an acid concentration from 1N up to 5N lost their colouring.

Another experiment on the planktonic community was performed where both motility of flagellates and of the tychopelagic diatom and chlorophyll *a* fluorescence were used as proxies of cell viability. Motility was observed only in the control, since from the 0.1N treatment onward all cells were motionless (Table 5). From the microscopic observations in contrast phase we noticed that from the 0.1N treatment onward some frustules of *Cylindrotheca fusiformis* appeared deformed and thinner than in the control (Fig. 5). In the same samples, all *Gymnodinium* sp. cells showed detachment of their thecal membranes.

	Chlorophyll *a* status							Motility						
	not acidified	0.1N	0.2N	0.5N	1N	2N	5N	not acidified	0.1N	0.2N	0.5N	1N	2N	5N
Planktonic diatoms	+++	+	0	0	0	0	0	—	—	—	—	—	—	—
Benthic (centric) diatoms	+++	+++	+++/++	++	++	++	++/+	—	—	—	—	—	—	—
Tychopelagic diatoms	+++	+++/+ (*)	+	+	+/0	0	0	++	0	0	0	0	0	0
Naked dinoflagellates	+++	+	+	0	0	0	0	+	0	0	0	0	0	0
Thecate dinoflagellates	+++	+++	+++	+++	+++	++/+	+	+	0	0	0	0	0	0
Phytoflagellates	+++	+	0	0	0	0	0	++	0	0	0	0	0	0

Table 5. Viability test on the mix culture at increasing acid normalities using both cell motility and chl *a* fluorescence as proxies. Chl *a* status: +++, intense red; ++, orange; + pink; 0, not fluorescent. Motility: ++, good motility; +, reduced motility; —, naturally not motile; 0, induced non-motility by the acid. (*) half of the cells with viable chl *a*, the other half with degraded chl *a*.

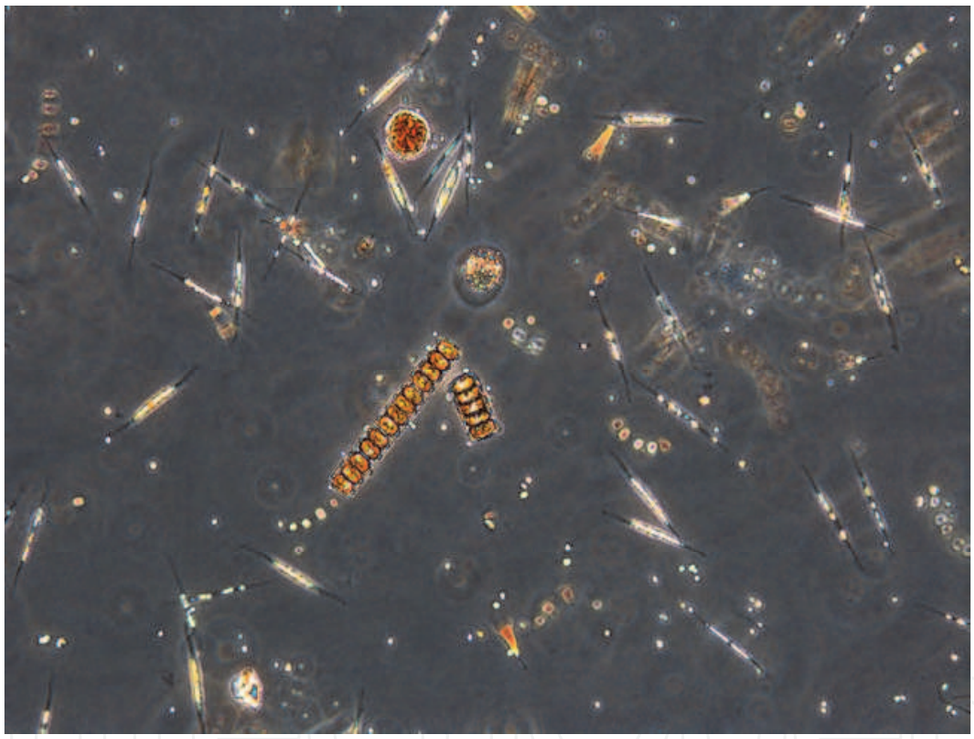


Fig. 5. Micrograph of the mix culture treated with 0.1N HCl observed under an inverted microscope at 200X magnification using phase contrast.

Analysing samples in epifluorescence, we noticed that in the control all cells appeared red coloured. In the 0.1N treatment a different response was observed according to the taxon: the planktonic diatom, the naked dinoflagellate and the small phytoflagellate showed a less intense pigmentation (with respect to the control); half of the observed tychopelagic diatom cells was still red coloured, while the other half became faded. Only the benthic diatom and the thecate dinoflagellate were still bright red (Fig. 6).

In the 0.2N treatment the planktonic diatom and the small phytoflagellate did not show fluorescence any more, whereas the tychopelagic diatom and the naked dinoflagellate emitted a faint fluorescence.

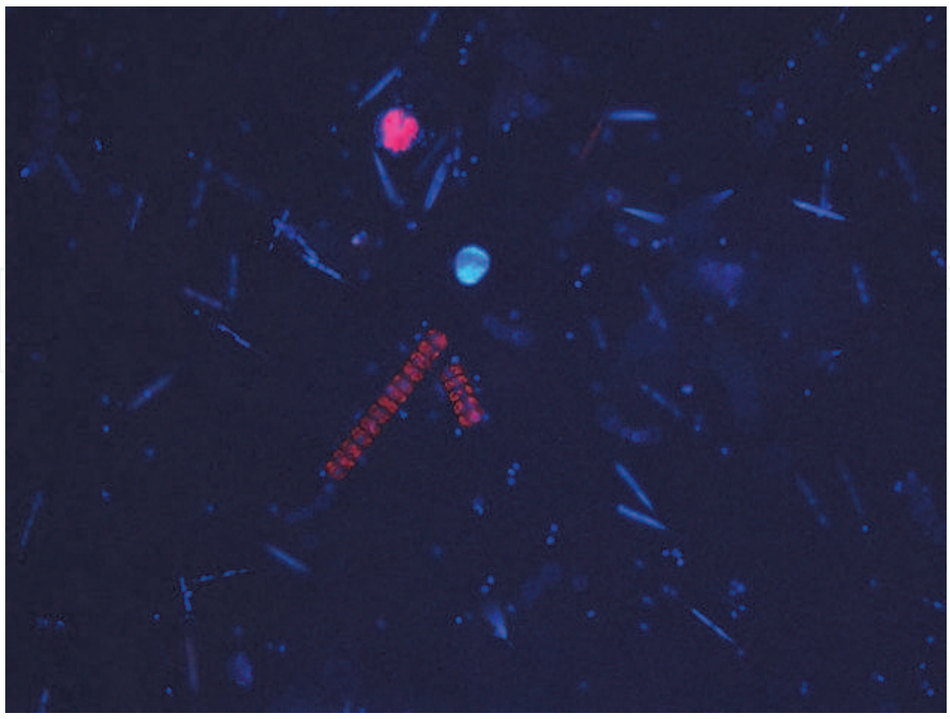


Fig. 6. Micrograph of the mix culture treated with 0.1N HCl observed under an inverted microscope at 200X magnification using epifluorescence.

Most of benthic diatom cells turned from red to orange, while only thecate dinoflagellate cells kept a bright red colour. Unlike the 0.2N treatment, the 0.5N sample presented naked dinoflagellate cells which lost their fluorescence and benthic diatom cells which were definitively orange. The only alteration in the next treatment (1N) was represented by tychopelagic diatoms which became pale. In the 2N treatment tychopelagic diatoms were not fluorescent any more, while thecate dinoflagellate cells turned their fluorescence from intense red to faded orange. In the last treatment (5N) benthic diatoms turned to faded orange, whereas thecate dinoflagellates emitted a faint fluorescence.

The microscopic observation of the recovered mix culture of the not acidified treatment revealed that some taxa were still alive and motile. The undetermined Cryptophycea was very active and abundant. *Cylindrotheca fusiformis* completely colonized the bottom of the flask, whereas *Lingulodinium polyedrum* was still motile but not abundant. We believe that both *Gymnodinium* sp. and *Chaetoceros* sp. did not grow due to interspecific competition, while *Paralia sulcata* was probably inhibited by the high temperature and irradiance since this species prefers low temperature and dim light (McQuoid & Nordberg, 2003). The recovered culture of the 0.1N treatment showed a similar scenario with the exception of the undetermined Cryptophycea which disappeared from the flask, confirming its immediate reaction to acid addition. In the recovered culture of the 0.2N treatment we observed the empty frustules of *Cylindrotheca fusiformis* as well as completely pale and not motile *Lingulodinium* cells. In this treatment a *Paralia sulcata* colony (15 cells ca.) was encountered; most of the cells were still pigmented. In the recovered culture of the 5N treatment none of the microalgae was observed.

Considering the results of the litmus test and the microalgal viability test together, some considerations can be made. If the pH value is not sufficiently low, the inorganic ^{14}C cannot

be completely removed from the system and the cells remain still metabolically active. Consequently, the microalgae are able to continue both to uptake inorganic carbon and to consume the assimilated ^{14}C during the period elapsing between the addition of the acid and the addition of the scintillation liquid. Moreover, if the pH value is sufficiently low to completely remove the inorganic ^{14}C, but not to kill all the cells, primary production could be underestimated. In fact, with a complete depletion of inorganic carbon (pH <5), the cells cannot uptake it any more, but they could continue to consume the already assimilated ^{14}C. This could occur treating the not filtered water samples with a mild acid.
Therefore we reckon that the acid concentration is crucial: only a sufficiently strong acid allows the complete removal of the inorganic carbon and at the same time the killing of all microalgal cells. In fact, when mild acid treatments were used, some taxa seemed to be not affected by the acid, as demonstrated by both our viability test and our recovery experiment. For example, the benthic diatom *Paralia sulcata* maintained a bright autofluorescence at lower pH values than other taxa. Benthic microalgae have a thicker and more silicified frustule compared to planktonic forms, which probably prevents the acid from penetrating into the cell. This can be of particular interest when the phytoplankton community is characterized by the presence of benthic diatoms; those communities are typical of shallow coastal water masses rather than deeper waters.

4. Conclusion

The aim of this study was to define which HCl concentration, with an equal volume, among 0.1N, 0.2N, 0.5N, 1N, 2N and 5N (which correspond to final HCl concentrations of 0.004N-0.008N-0.02N-0.04N-0.08N-0.2N, respectively, for 5 ml filtrated and total samples) was sufficient both to remove the excess labelled bicarbonate and to kill the cells but without damaging their structures and therefore leading to loss of assimilated ^{14}C. The litmus test demonstrated that the use of a mild acid (0.1N) does decrease the pH from 8 to 4. However, the results obtained from our viability test and recovery experiment suggest that some microalgal taxa in the not filtrated samples could remain still photosynthetically active after a too mild acid treatment. Therefore, the use of a slightly more concentrated acid (0.2N) is recommended. In our experiments this acid concentration led to on average 6% lower DPM values if compared to the 0.1N treatment, both for filters and total samples. Nevertheless, we believe that this small loss is acceptable in exchange for greater sureness of having killed the majority of the microalgal cells. In contrast, it is very likely that the 5N treatment damages the cell membrane with the consequent loss of assimilated ^{14}C, leading to an underestimate of DPM values up to 31% for filters and 56% for total samples if compared to the mildest acid treatment. Our experiments were carried out using oligotrophic water (Fonda Umani et al., 2004) from the Gulf of Trieste. In the next future we are going to carry out another set of experiments on mesotrophic and eutrophic waters, applying the same chloridric acid concentrations, to test if similar results are achieved.

5. Acknowledgment

We thank Daniela Fornasaro and Benedetta Guardiani for helping us during the experiments. We are also grateful to Oriana Blasutto for water sampling and Massimo Celio for multiparameter probe data. Additional thanks go to Marina Cabrini and Paola Del Negro for the help they have provided in relation to this work. Finally, we thank Alfred Beran for providing us with microalgal cultures.

6. References

Babin, M.; Morel, A. & Gagnon, R. (1994). An incubator designed for extensive and sensitive measurements of phytoplankton photosynthetic parameters. *Limnology and Oceanography*, Vol.39, No.3, pp. 694-702

Barber, R.T. & Hilting, A.K. (2002). Carbon Assimilation in Marine and Freshwater Ecosystems, In: *Phytoplankton Productivity*, P.J. le B. Williams; D.N. Thomas & C.S. Reynolds, (Eds.), 16-43, Blackwell Science, ISBN0-632-05711-4, Oxford, UK

Behrenfeld, M.J. & Falkowski, P.G. (1997). Photosynthetic rates derived from satellite-based chlorophyll concentration. *Limnology and Oceanography*, Vol.42, No.1, pp. 1-20

Carr, M,E.; Friedrichs, M.A.M.; Schmeltz, M.; Aita, M.N.; Antoine, D.; Arrigo, K.R.; Asanuma, I.; Aumont, O.; Barber, R.; Behrenfeld, M. et al. (2006). A comparison of global estimates of marine primary production from ocean color. *Deep-Sea Research II*, Vol.53, pp. 741-770

Cermeño, P.; Marañón, E.; Pérez, V.; Serret, P.; Fernández, E. & Castro, C.G. (2006). Phytoplankton size structure and primary production in a highly dynamic coastal ecosystem (Ría de Vigo, NW-Spain): Seasonal and short-time scale variability. *Estuarine Coastal and Shelf Science*, Vol.67, pp. 251-266

Fonda Umani, S.; Beran, A.; Parlato, S.; Virgilio, D.; Zollet, T.; De Olazabal, A.; Lazzarini, B. & Cabrini, M. (2004). Noctiluca scintillans MACARTNEY in the Northern Adriatic Sea: long-term dynamics, relationships with temperature and eutrophication, and role in the food web. *Journal of Plankton Research*, Vol.26, pp. 545-561

Friedrichs, M.A.M.; Carr, M.E.; Barber, R.T.; Scardi, M.; Antoine, D.; Armstrong, R.A.; Asanuma, I.; Behrenfeld, M.J.; Buitenhuis, E.T.; Chai, F. et al. (2009). Assessing the uncertainties of model estimates of primary productivity in the tropical Pacific Ocean. *Journal of Marine Systems*, Vol.76, pp. 113-133

del Giorgio, P.A. & Williams, P.J. le B. (2005). *Respiration in Aquatic Ecosystems*, Oxford University Press, ISBN 0-19-852709-8, Oxford, UK

Hewson, I.; O'Neil, J.M.; Heil, C.A.; Bratbak, G. & Dennison, W. C. (2001) Effects of concentrated viral communities on photosynthesis and community composition of co-occurring benthic microalgae and phytoplankton. *Aquatic Microbial Ecology*, Vol.25, pp. 1-10

Hyde, K.J.W.; O'Reilly, J.E. & Oviatt, C.A. (2008). Evaluation and application of satellite primary production models in Massachusetts Bay. *Continental Shelf Research*, Vol.28, pp. 1340-1351

Karl, D.M.; Hebel, D.V. & Björkman, K. (1998). The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean. *Limnology and Oceanography*, Vol.43, pp. 1270-1286

Lewis, M.R. & Smith, J.C. (1983). A small volume, short-incubation-time method for measurement of photosynthesis as a function of incident irradiance. *Marine Ecology Progress Series*, Vol.13, pp. 99-102

Libes, S. M. (1992). *An Introduction to Marine Biogeochemistry*. Wiley & Sons, Inc. ISBN 978-0-12-088530-5, New York, U.S.A.

Mangoni, O.; Modigh, M.; Mozetič, P.; Bergamasco, A.; Rivaro, P. & Saggiomo, V. (2008). Structure and photosynthetic properties of phytoplankton assemblages in a highly dynamic system, the Northern Adriatic Sea. *Estuarine Coastal and Shelf Science,* Vol.77, No.4, pp. 633-644

Marañón, E.; Cermeño, P.; Fernández, E.; Rodríguez, J. & Zabala, L. (2004). Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic system. *Limnology and Oceanography*, Vol.49, pp. 1652-1666

Marra, J. (2002). Approaches to the Measurement of Plankton Production, In: *Phytoplankton Productivity*, P.J. le B. Williams; D.N. Thomas & C.S. Reynolds, (Eds.), 78-108, Blackwell Science, ISBN0-632-05711-4, Oxford, UK

McQuoid, M.R. & Nordberg, K. (2003). The diatom *Paralia sulcata* as an environmental indicator species in coastal sediments. *Estuarine Coastal and Shelf Science*, Vol.56, pp. 339-354

Moutin, T. & Raimbault, P. (2002). Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996 (MINOS cruise). *Journal of Marine Systems*, Vol. 33-34, pp. 273-288

O'Donohue, M.J.H. & Dennison, W.C. (1997). Phytoplankton productivity response to nutrient concentrations, light availability and temperature along an Australian estuarine gradient. *Estuaries*, Vol.20, No.3, pp. 521-533

Peterson, B.J. (1980). Aquatic primary productivity and the ^{14}C-CO_2 method: A history of the productivity problem. *Annual Review of Ecology and Systematics*, Vol.11, pp. 359-385

Pugnetti, A.; Armeni, M.; Camatti, E.; Crevatin, E.; Dell'Anno, A.; Del Negro, P.; Milandri, A.; Socal, G.; Fonda Umani, S. & Danovaro, R. (2005). Imbalance between phytoplankton production and bacterial carbon demand in relation to mucilage formation in the Northern Adriatic Sea. *Science of the Total Environment*, Vol.353, pp. 162-177

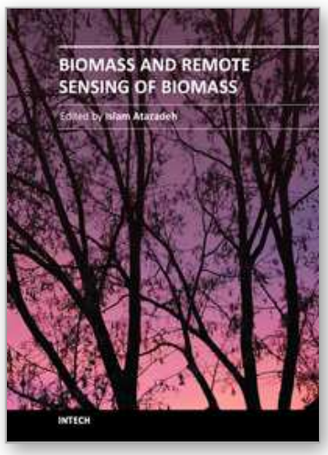
Steemann-Nielsen, E. (1952). The use of radioactive carbon (14C) for measuring organic production in the sea. *Journal du Conseil Permanent International pour l'Exploration de la Mer.,* Vol.16, pp. 117-140

Teira, E.; Mouriño, B.; Marañón, E.; Pérez, V.; Pazó, M. J.; Serret, P.; de Armas, D.; Escánez, J.; Woodwaard, E.M.S. & Fernández, E. (2005). Variability of chlorophyll and primary production in the Eastern North Atlantic Subtropical Gyre: potential factors affecting phytoplankton activity. *Deep-Sea Research Part I*, Vol.52, pp. 569-588

Theodórsson, P. & Bjarnason, J.O. (1975). The acid-bubbling method for primary productivity measurements modified and tested. *Limnology and Oceanography*, Vol.20, pp. 1018-1019

Tilstone, G.; Smyth, T.; Poulton, A. & Huston, R. (2009). Measured and remotely sensed estimates of primary production in the Atlantic Ocean from 1998 to 2005. *Deep-Sea Research II*, Vol.56, pp. 918-930

Williams, P.J. le B.; Thomas, D.N. & Reynolds, C.S. (2002). *Phytoplankton Productivity*, Blackwell Science, ISBN0-632-05711-4, Oxford, UK



Biomass and Remote Sensing of Biomass
Edited by Dr. Islam Atazadeh

ISBN 978-953-307-490-0
Hard cover, 262 pages
Publisher InTech
Published online 06, September, 2011
Published in print edition September, 2011

Generally, the term biomass is used for all materials originating from photosynthesis. However, biomass can equally apply to animals. Conservation and management of biomass is very important. There are various ways and methods for biomass evaluation. One of these methods is remote sensing. Remote sensing provides information about biomass, but also about biodiversity and environmental factors estimation over a wide area. The great potential of remote sensing has received considerable attention over the last few decades in many different areas in biological sciences including nutrient status assessment, weed abundance, deforestation, glacial features in Arctic and Antarctic regions, depth sounding of coastal and ocean depths, and density mapping. The salient features of the book include:
Several aspects of biomass study and survey
Use of remote sensing for evaluation of biomass
Evaluation of carbon storage in ecosystems
Evaluation of primary productivity through case studies

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Tamara Cibic and Damiano Virgilio (2011). In Situ Primary Production Measurements as an Analytical Support to Remote Sensing - An Experimental Approach to Standardize the 14C Incorporation Technique, Biomass and Remote Sensing of Biomass, Dr. Islam Atazadeh (Ed.), ISBN: 978-953-307-490-0, InTech, Available from: http://www.intechopen.com/books/biomass-and-remote-sensing-of-biomass/in-situ-primary-production-measurements-as-an-analytical-support-to-remote-sensing-an-experimental-a

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen