



# Istituto Nazionale di Oceanografia e di Geofisica Sperimentale

SEZIONE DI OCEANOGRAFIA

Long Term Ecological Research (LTER) site in the  
Gulf of Trieste – C1 station.  
Inventory of sampling and analytical methods and  
quality control of biogeochemical data.

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# Long Term Ecological Research (LTER) site in the Gulf of Trieste – C1 station – Inventory of sampling and analytical methods and quality control of biogeochemical data.

## Introduction

The time series station C1 (Latitude 45°42'2.99"N Longitude 13°42'36.00"E) in the Gulf of Trieste, in the northernmost part of the Adriatic Sea, is part of the Italian North Adriatic Long Term Ecological Research (LTER) site (<http://www.lteritalia.it/siti.htm>). Station C1 is located at the outer border of the Natural Marine Reserve of Miramare (NMRM), a marine protected area managed by WWF – Italy, at 0.2 Km from the coast and with a bottom depth of 17 m. The Gulf of Trieste is a semi-enclosed basin, located in the northernmost and shallowest part of the Adriatic Sea, with a maximum depth of 26 m (Mozetic *et al.*, 2002). The hydrodynamics and the trophic conditions of the basin are characterized by very large inter-annual, seasonal and short term variability due to the peculiar tidal regime of the area (Malacic *et al.*, 2000), to the alternation between winter mixing and summer stratification processes of the water column, to the strong north-easterly winds (Bora), to the variable freshwater contributions mainly from the Isonzo river (Cozzi *et al.*, 2012), to the ingression of the Eastern Adriatic Current and to biological interactions (Lipizer *et al.*, 2011).

Since the mid 1970s in the time series station C1 a wide range of physical, chemical and biological parameters have been measured (Table 1); during the relatively long time series of observations, however, sampling frequency, parameters measured, as well as sampling and analytical protocols have been, to some extent, modified during the years.

The purposes of this report are (1) to make an inventory of analytical protocols and instruments used for biogeochemical analysis since the beginning of data acquisition, (2) to define a quality control protocol common for the whole biogeochemical dataset and (3) to provide a quality checked dataset, which is a pre-requisite for time series analysis.

## History of the time series

The northern part of the Adriatic Sea is one of the most studied seas; the first scientific information dates back to at least 400 years ago (Zavodnik, 1983; Fonda Umani *et al.*, 1990).

In the Gulf of Trieste, Northern Adriatic Sea, marine biological studies started long ago and it was in Trieste that the first biological research station in the Adriatic Sea, the Zoological Station of Trieste, was founded in 1875. During the First World War biological research was interrupted and the Zoological Station was closed. Only in

the 1960s, thanks to the enthusiasm of the late Prof. Elvezio Ghirardelli of the University of Trieste, marine research in Trieste was resumed.

The first “organized” and regular biological observations at station C1 date back to the early 1970s when the study of the net-zooplankton community of the Gulf of Trieste was initiated thanks to the far-sightedness of Prof. Mario Specchi of the University of Trieste, who clearly recognised the importance of continuous biological observations. However, a regular monthly sampling for hydrological, chemical as well as biological (phytoplankton and zooplankton in several size classes) analysis only began in 1986. Since 1994, thanks to several EU-Interreg founded projects, to the support of the regional authority (Regione Friuli Venezia Giulia) and to the strong interest of the Director of the Laboratory of Marine Biology of Trieste (LBM), Prof. Serena Fonda Umani, the set of measured parameters progressively increased, including also smaller size classes such as nanoplankton, picoplankton, viruses and several physiological processes. Since 2002, the study was further extended to the benthic environment and a dedicated data-bank was created to store data collected since 1986. The time series station C1, initiated by the University of Trieste, was carried on thereafter by the Laboratory of Marine Biology of Trieste since December 1979 and, since October 2005, by the Department of Biological Oceanography (BIO) of the Istituto Nazionale di Oceanografia e Geofisica Sperimentale (OGS) of Trieste. Since 2006 the time series station C1 has been formally included in the Italian network of long term ecological research sites (LTER-Italy) as part of the northern Adriatic LTER site.

## Description of the dataset

The biogeochemical dataset considered in this report consists in data of chlorophyll-*a*, phaeopigments, dissolved oxygen, nutrients (N-NH<sub>4</sub>, N-NO<sub>2</sub>, N-NO<sub>3</sub>, P-PO<sub>4</sub>, Si-SiO<sub>2</sub>), dissolved organic matter (dissolved organic nitrogen – DON, phosphorus – DOP and carbon – DOC), particulate matter (particulate nitrogen – PN, phosphorus – PP and particulate organic carbon – POC) collected in the time-series station C1, on a monthly basis at four discrete depths (0.5, 5, 10 and 15 meters) since November 1998. Some data are available also for the period 1986 – 1990, however the temporal distribution is not uniform.

Table 1: Complete list of the measured parameters, duration and frequency of sampling in the water column in the LTER site in the Gulf of Trieste.

### Physical Measurements:

Variable	Period
hydrographic profiles (temperature, salinity, density)	Monthly since March 1986
biogeochemical profiles (fluorescence, pH, dissolved oxygen)	Monthly since February 1991
optical profiles (solar light attenuation, PAR Irradiance)	Monthly since November 1998

## Chemical Measurements:

<b>Variable</b>	<b>Period</b>
Dissolved oxygen (O <sub>2</sub> )	Monthly from May 1986 – December 1990 and from October 1998
Dissolved organic carbon (DOC)	Monthly since October 1998
Total Dissolved nitrogen (TDN)	Monthly since October 1998
Total Dissolved phosphorus (TDP)	Monthly since October 1998
Inorganic nutrients (N-NH <sub>4</sub> , N-NO <sub>2</sub> , N-NO <sub>3</sub> , P-PO <sub>4</sub> , SiO <sub>2</sub> )	Monthly since August 1996; irregularly since 1986
Particulate organic carbon (POC)	Monthly since October 1998
Particulate nitrogen (PN)	Monthly since October 1998
Particulate phosphorus (PP)	Monthly since November 1998

## Biomass and biodiversity Measurements:

<b>Variable</b>	<b>Period</b>
Total chlorophyll <i>a</i> and phaeopigment concentration	Monthly from April 1986 to July 1990
Total and size fractionated chlorophyll <i>a</i> concentration	Monthly since November 1998
Total and size fractionated phaeopigment concentration	Monthly since November 1998
Virus abundance	Monthly from October 1998 to December 2009
Picoplankton abundance	Monthly since July 1993
Nanoplankton abundance	Monthly since April 1995
Microphytoplankton abundance	Monthly since March 1986
Microzooplankton abundance	Monthly from March 1986 to December 1990 and since 1998
Mesozooplankton abundance and Carbon content	Monthly from March 1986; in the Gulf of Trieste also from April 1970 to December 1980

## Biological processes:

<b>Variable</b>	<b>Period</b>
Primary production	Monthly from October 1998 – February 2007
Plankton respiration	Monthly since October 1998
Bacterial secondary production	Monthly since October 1998
Esoenzymatic activities	Monthly from October 1998 to October 2005

## Methods

In order to provide an accurate and reliable dataset, an inventory of the sampling, storage and analytical protocols used since the beginning of regular data acquisition has been achieved and the dataset has been analysed in order to identify possible discrepancies or anomalies.

The report is divided in two parts, the first dedicated to the inventory of protocol/instrument/major changes occurred since the beginning of regular sampling, and the second is focused on the definition of a protocol for Quality Control Check based on the outcomes from the First IODE Workshop on Quality Control of Chemical Oceanographic Data Collections (2010).

## Inventory of sampling and analytical methods

The review of the field and analytical protocols, and of the instruments used for the analysis of biogeochemical parameters is presented in the following tables which contain also indications of any protocol or instrument change occurred since the beginning of the time-series, and of any intercomparison experiments. Although data collected earlier than November 1998 will not be analysed in this report as very few of them are at the moment organized in the database, the review of the sampling and analytical protocols includes also information on procedures used since the beginning of the regular data acquisition in the time series station. This information may be useful when comparing older data and most is derived from technical reports resulting from several monitoring programs (see for example Consorzio per la gestione del Laboratorio di Biologia Marina di Trieste, 1990).

### Chlorophyll-*a*:

Concentrations of chlorophyll-*a* and phaeopigments have been measured by spectrophotometry from April 1986 to July 1990 and by spectrofluorometry from October 1998 onwards, after particulate sample concentration by vacuum filtration and extraction in 90% acetone according to Holm – Hansen et al. (1965) and Lorenzen and Jeffrey (1980). Size fractionation was initiated in October 1998 in order to assess the relative contribution of the pico- (<2 µm), nano- (>2 µm and < 20 µm) and microphytoplankton (> 20 µm) size classes. During the period 1986 – 1990 samples were filtered on Millipore filters (47 mm diameter, porosity 0.8 µm) (Milani et al., 1991); after November 1998 samples were filtered on Whatmann GF/F filters (diameter 47 mm, nominal porosity 0.7 µm) which were stored frozen (-20°C) until laboratory analysis.

Chlorophyll-*a* concentration due to microphytoplankton is assessed subtracting the value of the sample filtered on plankton net (mesh size 20 µm) from the chlorophyll-*a* concentration of the whole sample. Chlorophyll-*a* concentration due to nanoplankton is assessed subtracting the value of the sample pre-filtered on 2 µm porosity, from the value of the sample filtered on 20 µm. Chlorophyll-*a* concentration due to picoplankton is assessed on the sample pre-filtered on 2 µm porosity (Millipore Isopore Membrane, nominal porosity 2.0 µm, TTTP).

Microphytoplankton = whole sample – (20 µm-filtered)  
 Nanophytoplankton = (20 µm-filtered) – (2 µm-filtered)  
 Picophytoplankton = 2 µm-filtered

Filtered Vol. (l)	Notes
2	Total chlorophyll- <i>a</i> determined by spectrophotometry until 1990
1	Total chlorophyll- <i>a</i> determined by spectrofluorometry since 1998
1	pre-filtered on 20 µm mesh to obtain nanophytoplankton chl- <i>a</i>
0.5	pre-filtered on 2 µm to obtain picophytoplankton chl- <i>a</i>

- **Spectrophotometry**

Instrument used	Period	Notes
Lambda 2 Perkin Elmer UV/VIS	April 1986 - July 1990	

- **Spectrofluorometry:**

Instrument used	Period	Notes
Perkin Elmer LS50B	November 1998- May 2007	Ex. 450 nm; Em 670 nm
Shimadzu	June 2007 – December 2009	Ex. 435 nm; Em 668 nm
Jasco FP-6500	January 2009 - now	Ex. 435 nm; Em 668 nm Intercomparision Shimadzu-Jasco in January 2009

Limit of detection: defined as twice the standard deviation of 3 blank filters treated in the same way as samples: 0.001 µg L<sup>-1</sup> and 0.068 µg L<sup>-1</sup> for chlorophyll-*a* and phaeopigments, respectively.

Method precision, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected at 10 meters depth at the C1-LTER station: 0.5% (mean: 2.10 µg L<sup>-1</sup>, standard deviation: 0.01 µg L<sup>-1</sup>) for chlorophyll *a*, and 0.7% (mean: 1.47 µg L<sup>-1</sup>, standard deviation: 0.01 µg L<sup>-1</sup>) for phaeopigments.

Instrument precision tested on the repeated analysis of the same sample: CV% ranges from 0.09 (at chlorophyll-*a* concentration 4.34 µg L<sup>-1</sup>) to 0.5 (at chlorophyll-*a* concentration 0.14 µg L<sup>-1</sup>).

Participation to QUASIMEME Quality Assurance exercise in June and October 2011: satisfactory result at chlorophyll-*a* concentration of 6.28 µg L<sup>-1</sup> (Exercise 930 - R65 Chlorophyll-*a* in Seawater: April-Aug 2011); at chlorophyll-*a* concentration of 7.52, 1.54 and 4.55 µg L<sup>-1</sup> (Exercise 950 - R67 Chlorophyll-*a* in Seawater: October 2011 – January 2012).

## Dissolved oxygen:

Dissolved oxygen is determined according the method first described by Winkler (1888), and, since 1998, using a potentiometric end-point titration procedure as described in Outdot et al. (1988).

<b>Bottle used</b>	<b>Period</b>	<b>Notes</b>
Volume = 300 ml	November 1998- May 2007	subsamples of Vol=50ml were analysed
Pre-calibrated bottles of Volume = 50 -65 ml	April 2006 – now	the whole sample is analysed

<b>Instrument used</b>	<b>Period</b>	<b>Notes</b>
Metrohom 665 Dosimat	1990	
Mettler DL21	November 1998- May 2007	
Mettler Toledo G20 2010 - now		

Method precision, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected at 10 meters depth at the C1-LTER station was 0.12% (mean: 280.07  $\mu\text{M}$ ; standard deviation: 0.35  $\mu\text{M}$ ).

## Inorganic nutrients:

Before 1998 samples for inorganic nutrient analysis have been filtered on Millipore HA (47 mm diameter; porosity 0.45  $\mu\text{m}$ ), afterwards on Whatmann GF/F glass fiber filters, collected in acid washed polyethylene vials rinsed with seawater and immediately frozen ( $-20^{\circ}\text{C}$ ) until laboratory analysis. Inorganic nutrients (N-NH<sub>4</sub>, N-NO<sub>2</sub>, N-NO<sub>3</sub>, P-PO<sub>4</sub>, Si-SiO<sub>2</sub>) have been analyzed using a spectrophotometer until June 1990 according to Strickland & Parson (1972) and using a segmented flow autoanalyzer according to the standard methods described by Grasshoff (1983), Murphy and Riley (1962) and Strickland & Parsons (1972) after July 1992.

<b>Instrument used</b>	<b>Period</b>	<b>Notes</b>
Perkin Elmer Lambda 2	1986 - 1990	
Alliance Integral Segmented Flow Analyzer	1992 - 2001	
BRAN-LUEBBE Autoanalyzer 3	2001 - today	

The detection limit for phosphate, silicate, ammonium, nitrite and nitrate were 0.020  $\mu\text{M}$ , 0.016  $\mu\text{M}$ , 0.040  $\mu\text{M}$ , 0.0015  $\mu\text{M}$  and 0.010  $\mu\text{M}$ , respectively.

Method precision at low concentrations, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected at 10 meters depth at the C1-LTER station was: 12% for NH<sub>4</sub> (mean: 0.33  $\mu\text{M-N}$ , standard deviation: 0.04  $\mu\text{M-N}$ ); 10% for NO<sub>2</sub> (mean: 0.02  $\mu\text{M-N}$ , standard deviation: 0.002  $\mu\text{M-N}$ ); 50% for NO<sub>3</sub> at limit of detection (mean: 0.02  $\mu\text{M-N}$ , standard deviation: 0.01  $\mu\text{M-N}$ ); 2.5%

for PO<sub>4</sub> (mean: 0.04 μM-P, standard deviation: 0.001 μM-P); 0.1% for SiO<sub>2</sub> (mean: 2.50 μM-Si, standard deviation: 0.003 μM-Si).

Method precision at high concentrations, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected in the Isonzo river: 3% for NH<sub>4</sub> (mean: 1.12 μM-N, standard deviation: 0.04 μM-N); 1% for NO<sub>2</sub> (mean: 0.25 μM-N, standard deviation: 0.002 μM-N); 1% for NO<sub>3</sub> (mean: 60.57 μM-N, standard deviation: 0.46 μM-N); 5% for PO<sub>4</sub> (mean: 0.30 μM-P, standard deviation: 0.02 μM-P); 1% for SiO<sub>2</sub> (mean: 25.46 μM-Si, standard deviation: 0.33 μM-Si).

## **Total dissolved nitrogen and phosphorus:**

As in the case of inorganic nutrients, samples for total dissolved nitrogen (TDN) and phosphorus (TDP) analysis have been filtered on Whatmann GF/F glass fiber filters, collected in acid washed polyethylene or polypropylene vials rinsed with seawater and immediately frozen (-20°C) until laboratory analysis. From 1998 to August 2011, total dissolved nitrogen and phosphorus were determined after quantitative conversion to inorganic N and P by exposure to UV radiation with addition of hydrogen peroxide to ensure oxidation (Armstrong et al., 1966; Golimomowski and Golimowska, 1996) and subsequent analysis for total dissolved nitrogen and phosphorus. After August 2011, oxydation has been achieved by Persulfate wet-oxidation according to Koroleff (1983).

From TDN and TDP, dissolved organic nitrogen (DON) and phosphorus (DOP) are computed from the relationship:  $DON = TDN - (N-NH_4 + N-NO_2 + N-NO_3)$  and  $DOP = TDP - P-PO_4$ .

### **Instrument used**

### **Period**

Alliance Integral Segmented Flow Analyzer	1992 - 2001
BRAN-LUEBBE Autoanalyzer 3	2001 - today

Method precision, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected in the Isonzo river: 1% for TDN (mean: 85.06 μM-N, standard deviation: 0.67 μM-N); 8% for TDP (mean: 0.32 μM-P, standard deviation: 0.02 μM-P).

Instrument precision tested on the repeated analysis of the same sample: for TDP, CV% for 5 replicates is 1.12% (mean: 0.179 μM; standard deviation: 0.002 μM).

## **Dissolved organic carbon:**

Dissolved organic carbon (DOC) has been determined on samples filtered using a glass syringe (50 ml capacity) fitted with a 3-way valve, on glass fibre filters (Whatmann GF/F pre-combusted at 480°C for 4 hours and acidified, 1N HCl). The filtered samples were stored frozen (-20°C) in glass vials treated with chromic mixture and precombusted at 480°C for 4 hours until analysis by high-temperature oxidation (HTCO) according to Cauwet (1994; 1999). Before analysis, samples were acidified (pH < 2) with 6N HCl solution and purged with high-purity oxygen bubbling (De Vittor et al, 2008).



<b>Instrument used</b>	<b>Period</b>
Shimadzu TOC 5000A	Nov. 1998- Feb. 2007
Shimadzu TOC – V <sub>CSH</sub>	May 2007 - now

Limit of detection: : 0.33  $\mu\text{M}$  (instrumental – with high sensitivity measurement kit); defined from measurement of certificated low carbon water (University of Miami) treated in the same way as samples: 1-2  $\mu\text{M}$ .

Tests on replicate field sampling resulted in CV% between 1.5% and 4%.

Instrument precision tested on the repeated analysis of a minimum of three samples: CV% lower than 2%.

DOC results are periodically referenced against the international community of DOC analysts by using consensus reference material (CRM – University of Miami).

### **Particulate organic carbon and particulate nitrogen:**

Particulate organic carbon (POC) and particulate nitrogen (PN) collected on glass fiber filters (Whatmann GF/F pre-combusted at 450°C for 4 hours; 25 mm diameter) are measured with an elemental analyzer after high-temperature combustion according to Sharp (1974).

<b>Filtered Vol.(l)</b>	<b>Instrument used</b>	<b>Period</b>
1 2006	Perkin Elmer 2400 CHNS/O	Nov. 1998- Feb.
0.25		March 2006 - now
0.25	Costech Elemental Combustion Syst.	March 2007 - now

The detection limit for POC and PN, defined as twice the standard deviation of three blank filters, were 0.08  $\mu\text{M-N}$  and 0.11  $\mu\text{M-C}$  respectively.

Method precision, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected at 10 meters depth at the C1-LTER station was 7% per PN (mean: 43.07  $\mu\text{M-N}$ ; standard deviation: 3.02  $\mu\text{M-N}$ ) and 4% for POC (mean: 359.06  $\mu\text{M-C}$ ; standard deviation: 14.03  $\mu\text{M-C}$ ).

### **Particulate phosphorus:**

Particulate phosphorus (PP) collected on filters (Whatmann GF/F pre-combusted at 450°C for 4 hours), is measured colorimetrically as P-PO<sub>4</sub> after combustion at 450°C and extraction in 1 N HCl (24h), according to Solorzano and Sharp (1980).

<b>Filtered Vol. (l)</b>	<b>Period</b>	<b>Notes</b>
1	Nov. 1998 - now	

The detection limit for PP, defined as twice the standard deviation of the blank, was 0.003  $\mu\text{M-P}$ .

Method precision, tested on replicate sampling: coefficient of variation (CV%) on 3 replicate samples collected in the river Isonzo was 8% (mean: 0.06  $\mu\text{M-P}$ ; standard deviation: 0.004  $\mu\text{M-P}$ ).

## Quality control analysis steps

In order to provide accurate and reliable data for the long-term study of ecological systems in the Gulf of Trieste, Northern Adriatic Sea, and to allow the comparison with data from other LTER sites, the recommendations to establish quality control/quality assurance (QC/QA) of biogeochemical data resulting from the First IODE Workshop on Quality Control of Chemical Oceanographic Data Collections (2010) have been followed.

With the purpose of assessing precision and accuracy of measurements, on a regular basis, analytical and field precision measurements are carried out, based on the analysis of replicate samples and, with the aim of testing the accuracy, the laboratory participates to international intercalibration tests (QUASIMEME). Analytical precision is assessed with the repeated analysis of a single sample and is a measure of the analytical method precision; field replication is the analysis of two or more samples taken from a single sampling bottle and is a measure of the variance due to sub-sampling, storage and natural within sample variability.

As limit of detection (LOD), determined by a statistical approach, we considered two times the standard deviation of the blank (Armbruster and Pry, 2008; Greenberg, Clesceri and Eaton, 1992).

With the purpose of checking the quality of historical and of currently acquired data, a protocol for Quality Control analysis is here proposed, based on the minimum recommended Quality Control Checks proposed during the first IODE Workshop (2010):

1. Data range checks
2. Excessive gradient
3. Excessive spike
4. No gradient

In order to follow the recommendations, the following steps are here proposed to investigate the distribution of the data and to identify possible discrepancies in the time-series:

1. Analysis of the frequency distribution of the data
2. Visual inspection and comparison of the time series distribution of several parameters
3. Property-property plots ( $\text{NO}_3$  vs  $\text{PO}_4$ , PN vs PP vs POC; DON vs DOP vs DOC)
4. Analysis of the climatology to derive the natural range of variability
5. Comparison of new data with climatology
6. Quality flag attribution (Table 2)

Table 2: Quality control flags scheme adopted (IODE GE-BICH wiki) GE-BICH QC:

Code	Description	Test criteria
1	Good	Passed all documented required QC tests
2	Quality not evaluated	Used for data when no QC test performed or the information on quality is not available
3	Questionable/suspect	Failed non-critical documented metric or subjective test(s)
4	Bad	Failed critical documented QC test(s) or as assigned by the data producer
9	Missing data	Used as place holder when data are missing

The analysis of frequency distribution of the data and, in particular, the deviation from the normal distribution allows to identify the overall pattern in the dataset. The frequency distribution of the data of C1 LTER station and the descriptive statistics for biogeochemical parameters, as well as for hydrological data are presented in the Annex 1.

The histograms of biogeochemical data display a quasi-normal distribution in the case of particulate and dissolved organic carbon (POC, DOC), particulate nitrogen (PN) and dissolved oxygen (O<sub>2</sub>) concentration, while the distribution is asymmetrical in the case of all nutrients (N-NH<sub>4</sub>, N-NO<sub>2</sub>, N-NO<sub>3</sub>, P-PO<sub>4</sub>, Si-SiO<sub>2</sub>), dissolved organic nitrogen and phosphorus (DON, DOP), particulate phosphorus (PP), chlorophyll-*a* and phaeopigments. In these cases, the extremely asymmetrical distribution of data and the large distance from the mean values may indicate the presence of outliers or unreliable data. However, although outliers are often considered as error or noise, they may carry important information. In fact, the definition of an outlier often depends on hidden assumptions regarding the data structure (Ben-Gal, 2005) and the kind of data. The typical statistical methods to detect outliers are generally based on the distance of suspicious data from the mean or median of the data, taking into account the standard deviation or the inter-quartile range as a measure of the dispersion of the data (distance-based methods) (e.g. Dixon Q-test, Chebyshev distance, Mahalanobis distance,...). However, a mere statistical approach may, in cases of highly variable conditions, not be appropriate.

In fact, special care must be dedicated to data obtained in coastal and estuarine areas, where environmental variability may be extremely large. In particular, in areas influenced by river and coastal runoff nutrient concentrations may vary of an order of magnitude and "apparent outliers" may, on the other hand, reveal particular environmental conditions. In this case, the histogram of salinity data is here presented to compare the frequency distribution of low salinity with that of nutrient data, as the increase in inorganic nutrients, and sometimes also in particulate and dissolved organic matter, is often associated with continental inputs which bring about a parallel decrease in salinity. The histogram shows that cases of strong dilutions are not common in the dataset, but may cause a significant decrease in salinity (minimum salinity: 27.28). These cases may be responsible for the large deviations in nutrient concentrations from the most common situations.

To verify this hypothesis, time series of salinity, dissolved inorganic and organic matter and particulate phosphorus are displayed in the plots in Annex 2.

The comparison allows to recognise apparent "outliers" in nutrient distribution associated with large dilutions (marked cases in the salinity time series) and to identify outliers which are not associated with salinity decreases. The boxes contain cases of large dilutions and associated peaks in N-NO<sub>3</sub>, P-PO<sub>4</sub> and SiO<sub>2</sub>; some peaks in nutrient and dissolved organic matter distributions are, however, not directly related to low salinity episodes.

Furthermore, as in many cases, multivariable observations can not be detected as outliers when each variable is considered independently, outlier detection is possible only when multivariate analysis is performed and interaction among different variables are compared within class of data.

To further investigate possible discrepancies in the data, property-property plots of key parameters which usually present similar dynamic are considered, in order to reveal inconsistent data (Annex 3). Groups of data marked in the plots with solid boxes (low NO<sub>3</sub> and high PO<sub>4</sub>, low PN high PP, low PP and high PN) deviate significantly from the common patterns, do not present similar patterns in other key parameters and are, consequently, flagged as "3 (questionable)". On the other hand, data within dashed boxes (high NO<sub>3</sub> and low PO<sub>4</sub>) seem typical of low salinity waters and are flagged as "1 (good)".

After the Quality Control Check, the dataset has been re-processed to calculate the climatology which will be useful to compare newly collected data and to facilitate routine Quality Control. The climatology (represented as monthly average and standard deviation) of biogeochemical and hydrological data for the period November 1998 – December 2009 is presented in Annex 4.

As a first step in Quality Control check, comparison of new data with historic data range of variability will allow to regard as "good" all data inside the historic envelope and as "questionable" data outside this range. However, the not normal frequency distribution of the biogeochemical data in this area requires the comparison with other parameters, *in primis* with salinity, according to the proposed protocol, in order to assess the quality of the data.

If the origin of the discrepancy of the data remains unidentifiable, the data are flagged "questionable" if the values are outside the 95% confidence interval (greater than 2 standard deviations from the historical mean), and "good" if within this error envelope. If a source for the discrepancy is discovered the data are flagged "bad".

As a final remark, most nutrient and dissolved organic matter data derive from analysis on frozen samples. Although it is recommendable to analyse samples short after sampling, the debate on data quality of nutrient data deriving from frozen samples is still open ("Freezing of non-acidified samples of seawater has already been shown to be a reliable preservation technique up to 5-6 months (Walsh, 1989; Williams et al., 1993; Tupas et al., 1994)" in: Aminot and Kerouel, 2004) and the protocol of storing frozen samples is widely accepted and used in several time-series stations (HOTS, BATS,...) as well as cited in many articles (to cite a few: Guadayol et al., 2009; Marini et al., 2008; Church et al., 2002). However, some

authors (Krom et al., 2005) state that at ambient concentrations below 0.02  $\mu\text{M}$  of P- $\text{PO}_4$  and 0.4  $\mu\text{M}$  of N- $\text{NO}_3$  data derived from frozen samples may not be reliable. On the basis of these studies, when calculating derived variables such as, for example, stoichiometric ratios, it is recommendable to remove from the analysis values calculated from data lower to detection limit, which in our case for phosphate coincides with the values recognised by Krom et al., 2005 as "giving unacceptable error".

## **Recommendations for regular Quality Control:**

- Short term data Quality Control check every 6 – 12 months
- Intermediate Quality Control check: participation to intercomparison experiments and comparison with certified standards every couple of years.

## **List of Annexes:**

Annex 1: Frequency distribution of hydrological and biogeochemical data measured in LTER station C1 from 11/1998 to 12/2009. Descriptive statistics of the dataset is reported on the plot.

Annex 2: Time series of biogeochemical properties measured in LTER station C1 from 11/1998 to 12/2009.

Annex 3: Property-property plots used to verify data consistency.

Annex 4: Climatology of biogeochemical properties measured at LTER station C1 from 11/1998 to 12/2009.

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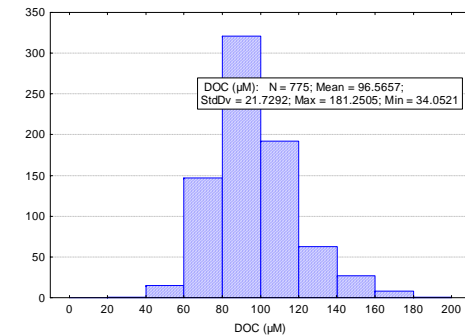
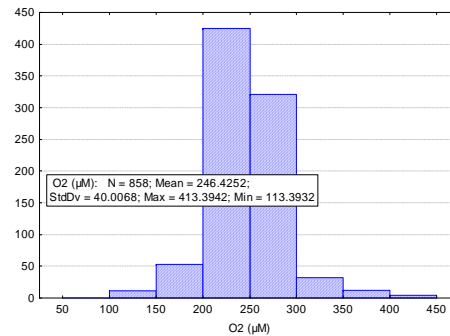
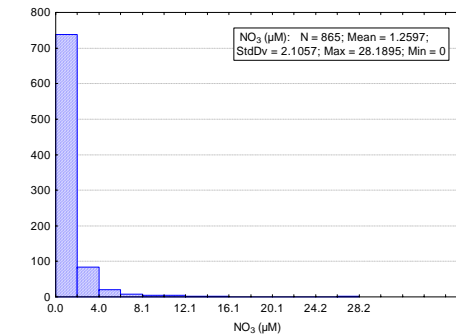
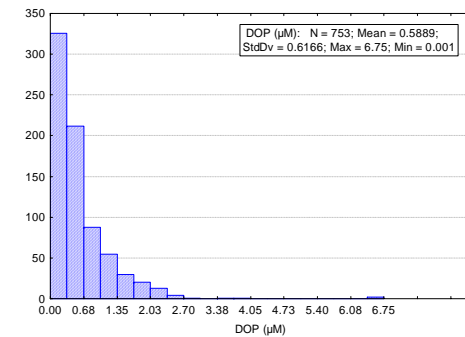
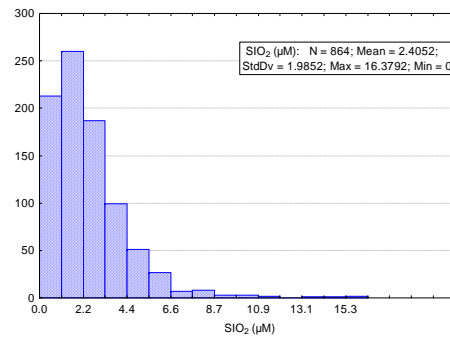
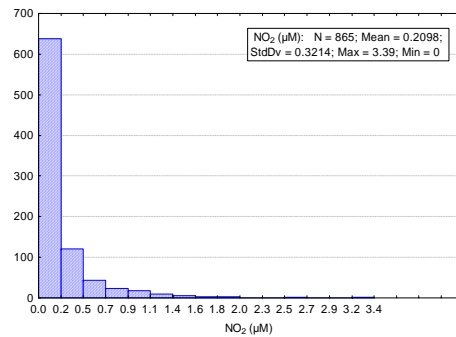
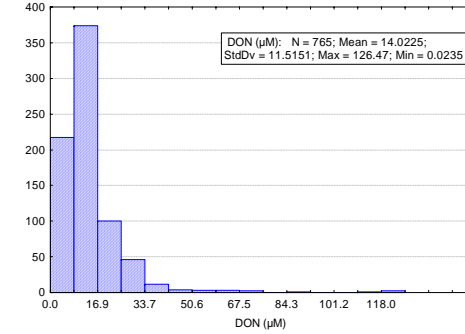
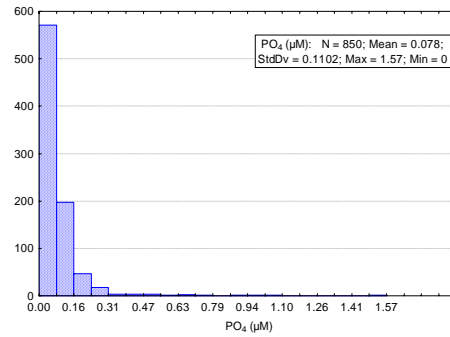
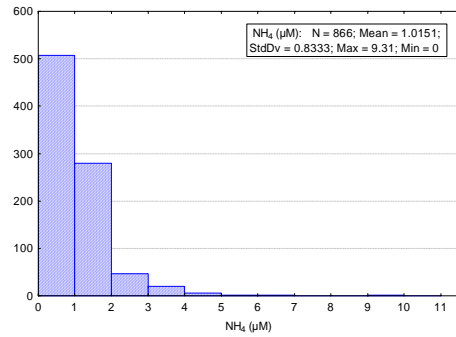
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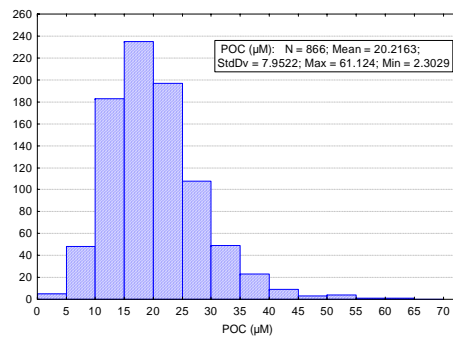
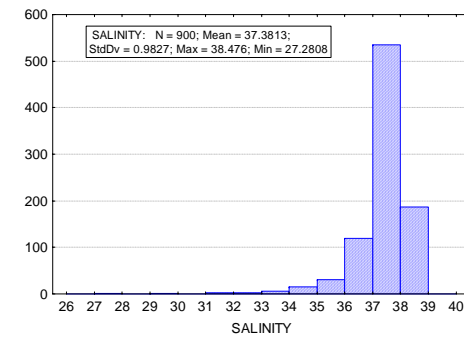
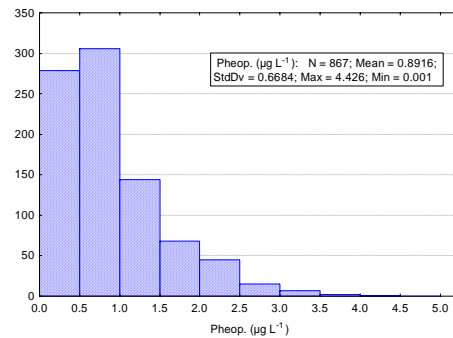
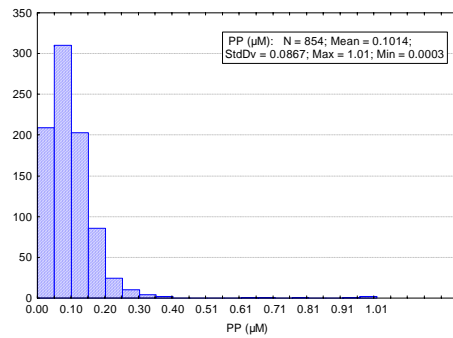
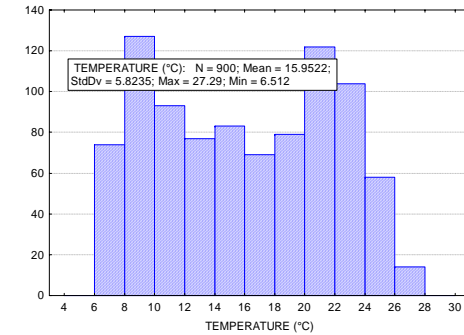
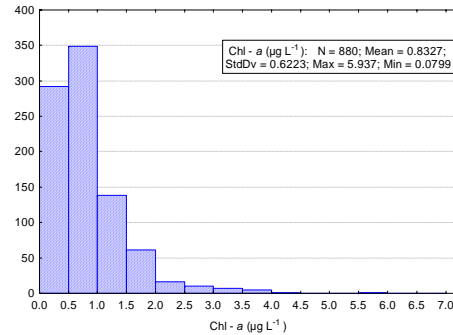
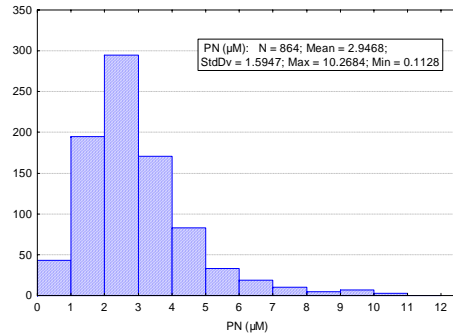
## Annex 1:

Frequency distribution of hydrological and biogeochemical data measured in LTER station C1 from 11/1998 to 12/2009. Descriptive statistics of the dataset is reported on the plot.



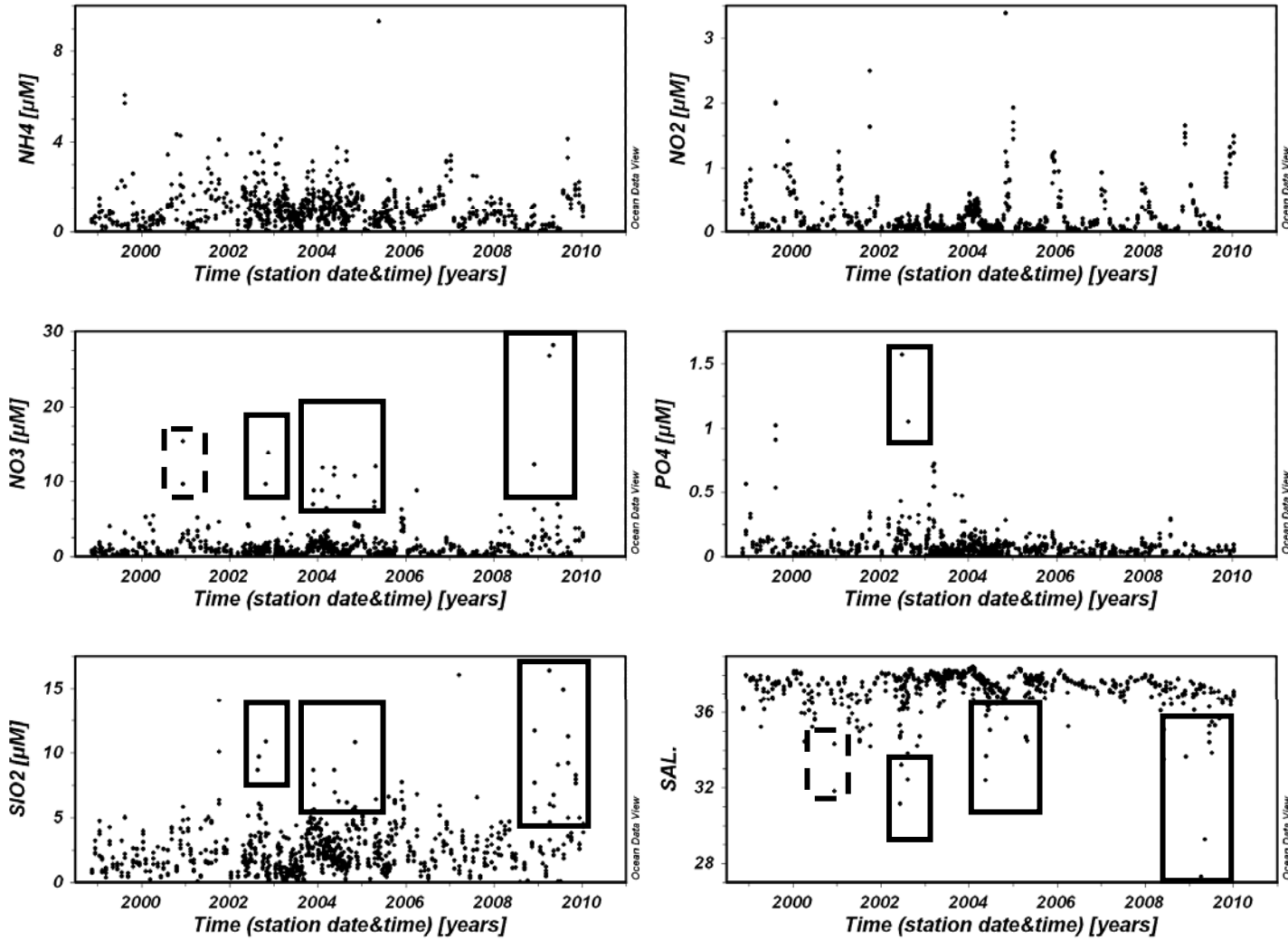
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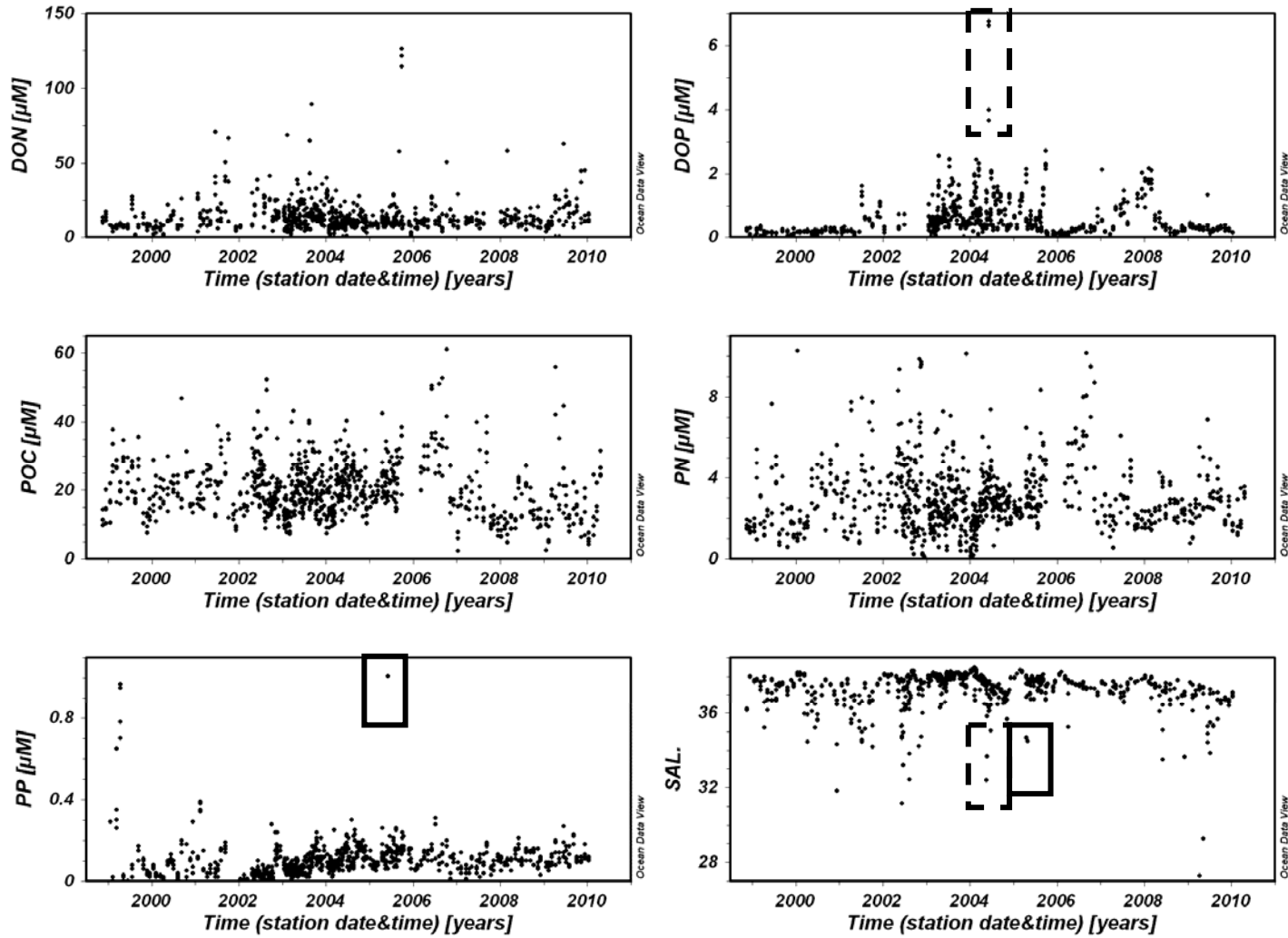
## Annex 2:

Time series of biogeochemical properties measured in LTER station C1 from 11/1998 to 12/2009.



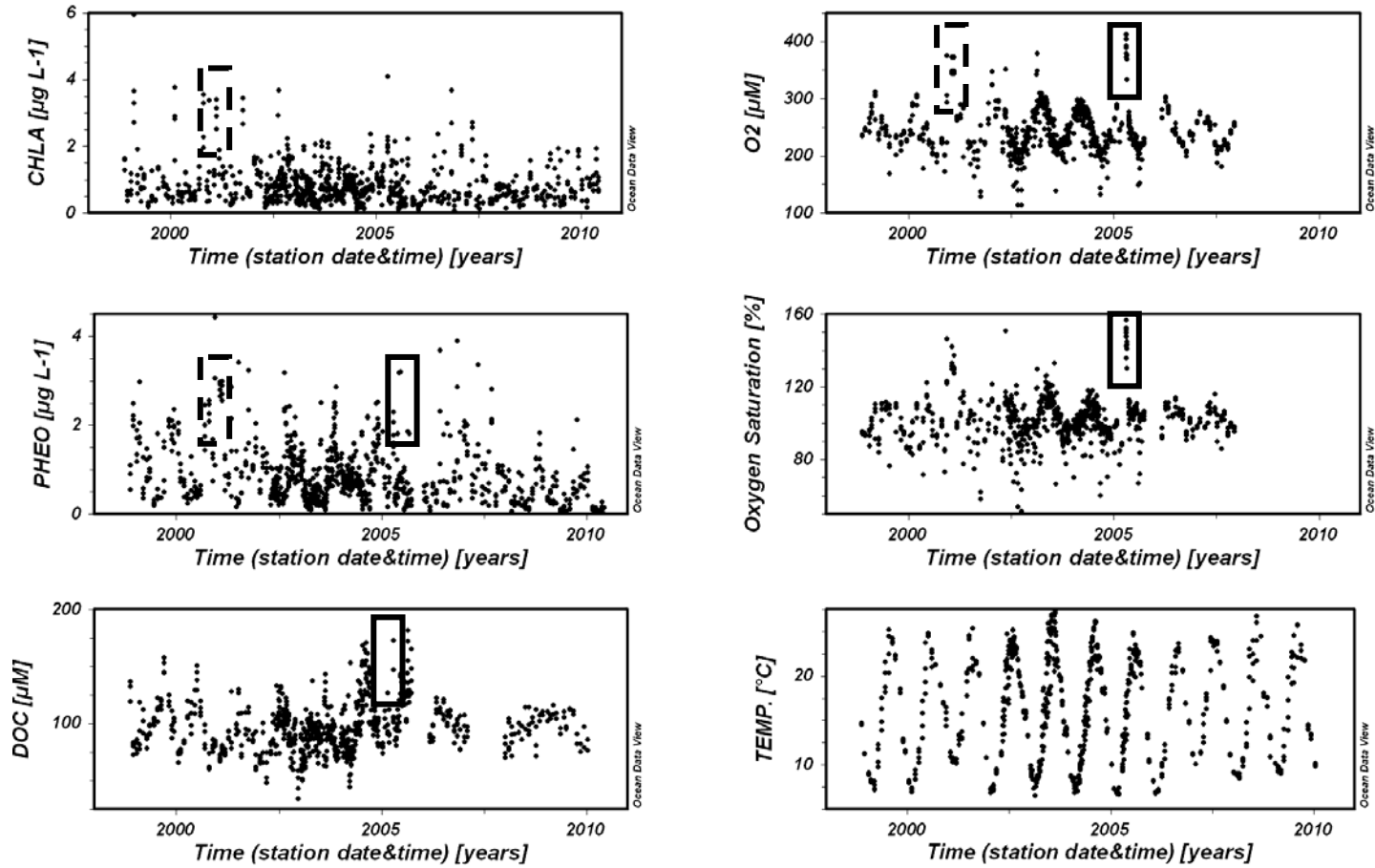
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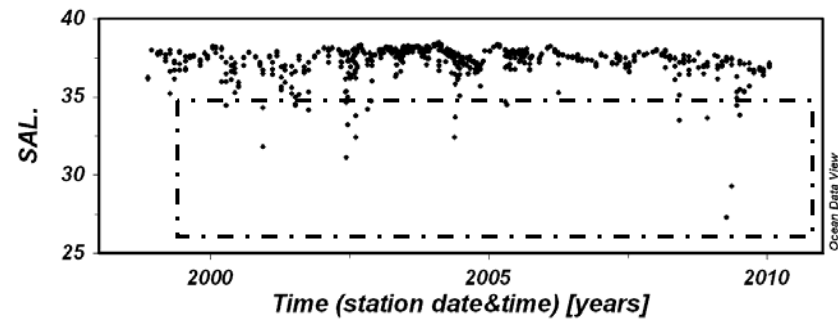
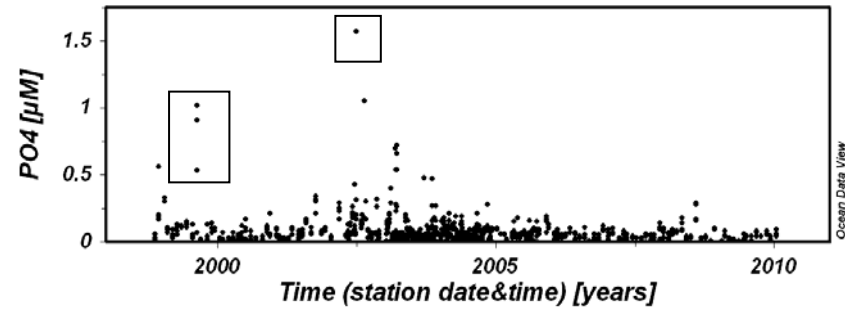
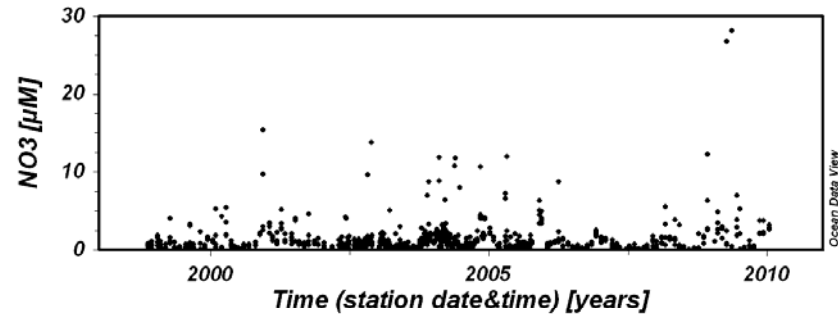
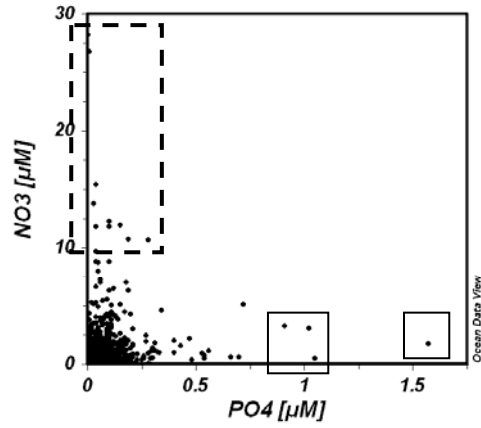
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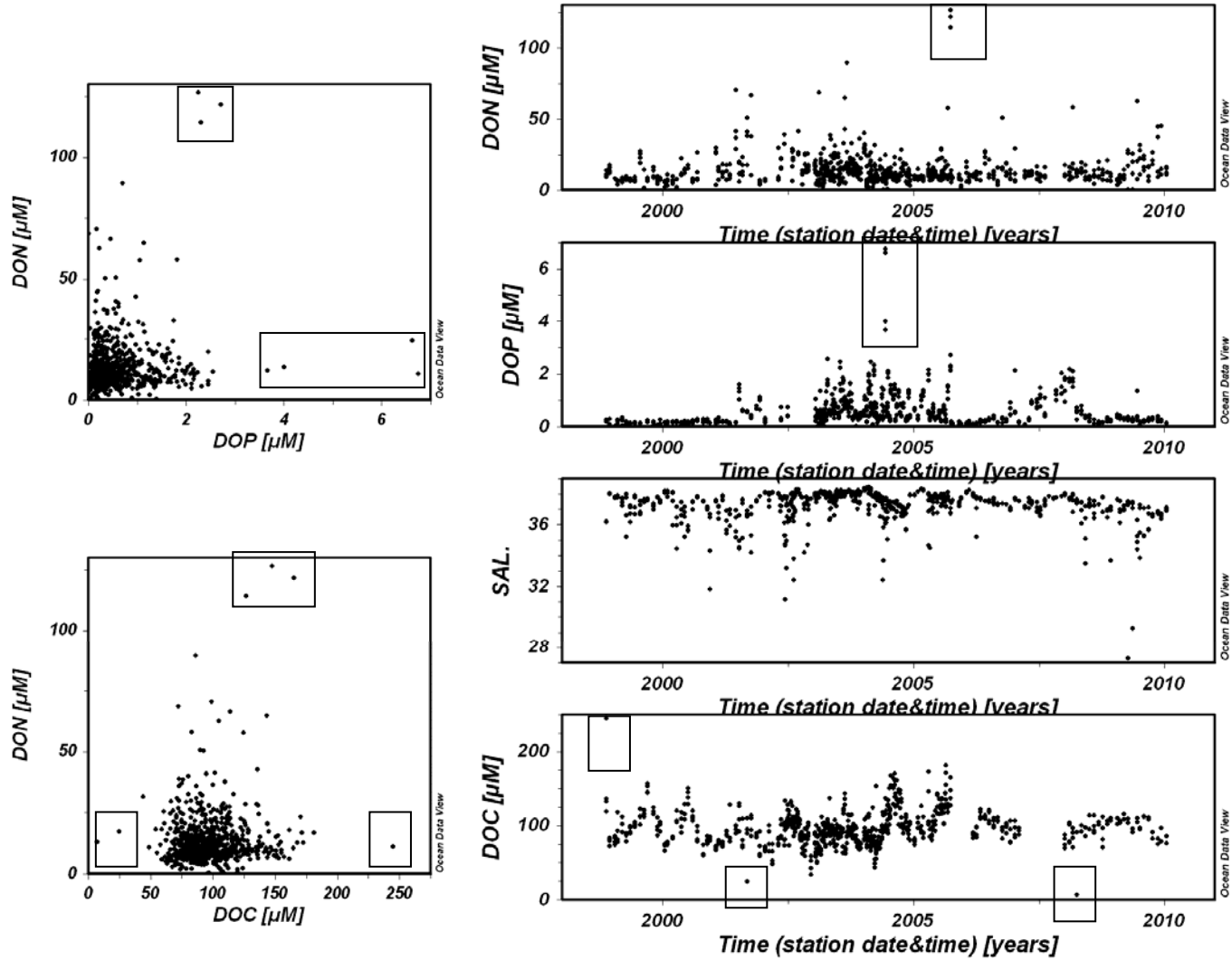
### Annex 3:

Property-property plots used to verify data consistency.



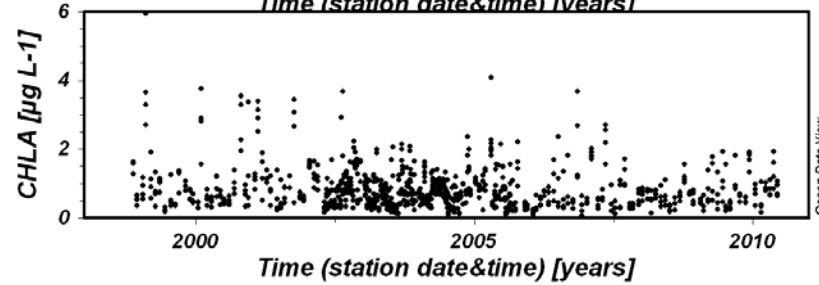
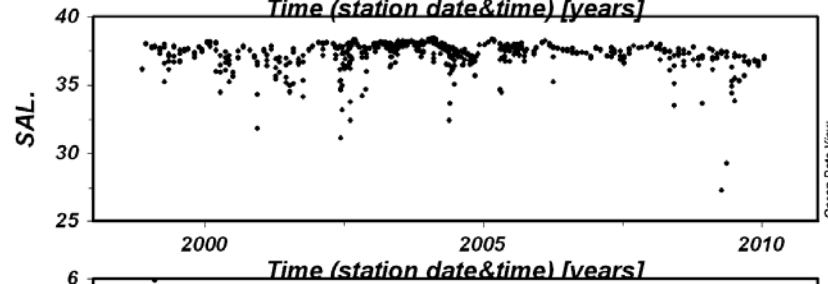
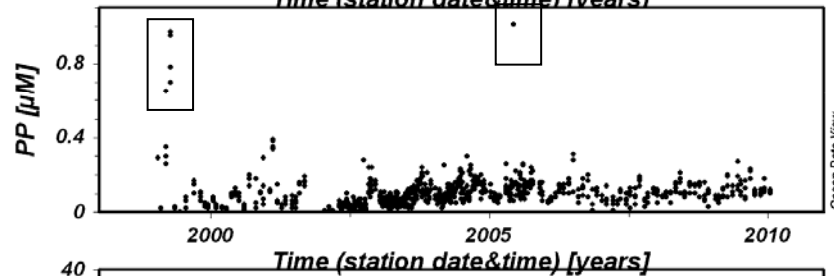
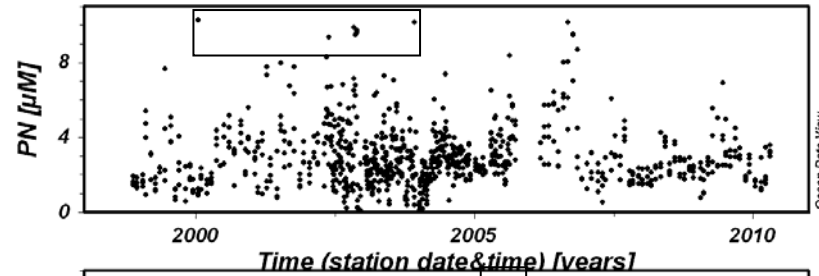
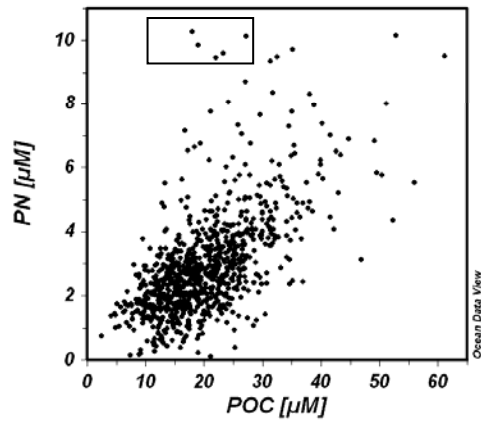
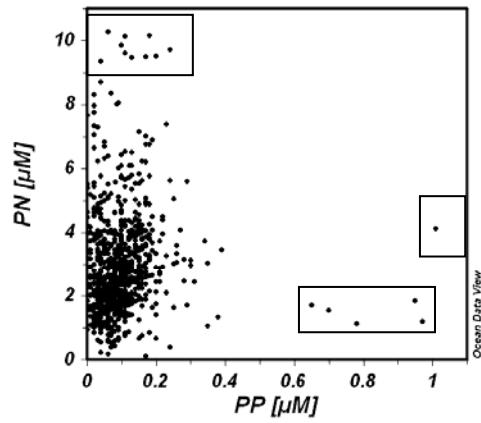
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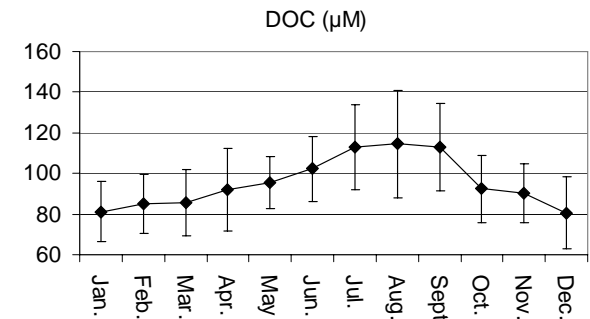
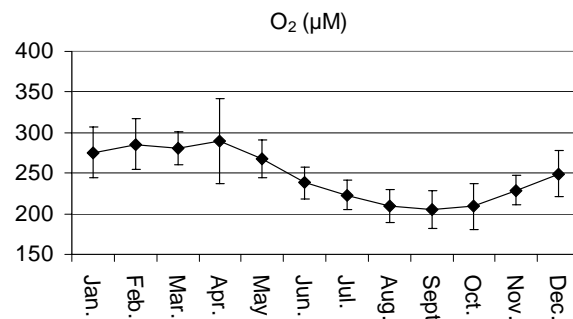
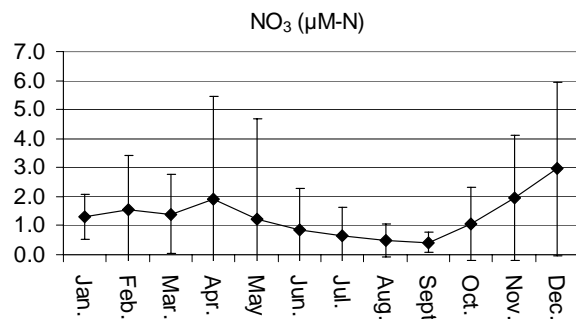
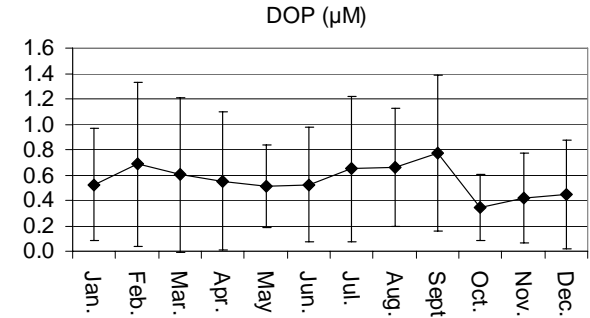
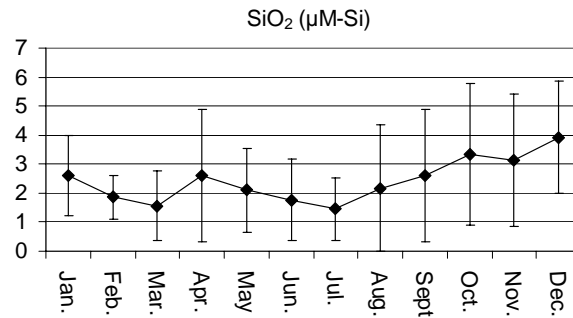
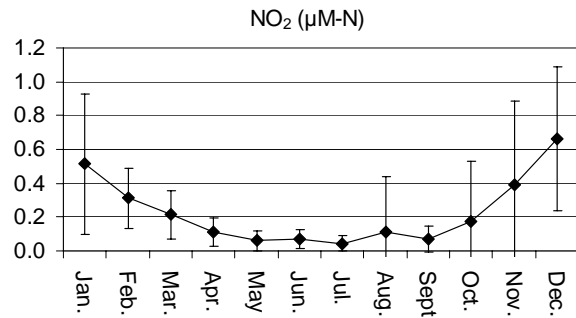
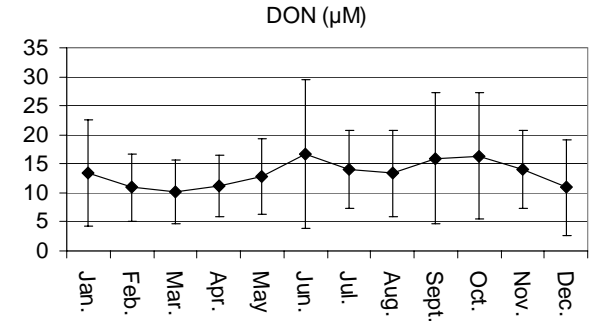
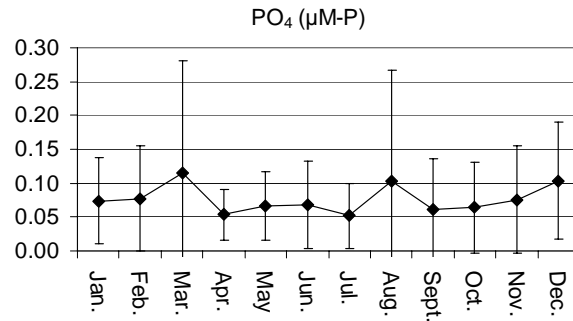
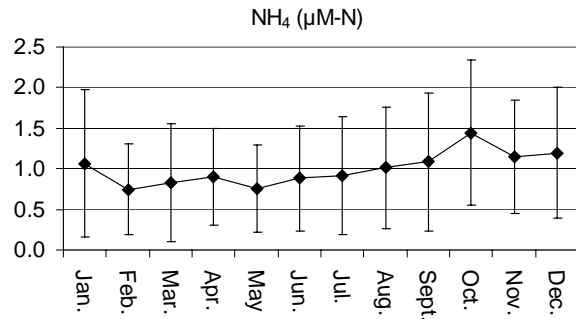
Property-property plots used to verify data consistency.





## Annex 4:

Climatology of biogeochemical properties measured at LTER station C1 from 11/1998 to 12/2009.



## Annex 4:

Climatology of biogeochemical properties measured at LTER station C1 from 11/1998 to 12/2009.

