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.

A cura di: Marina Lipizer

Con la collaborazione di:
C. De Vittor, C. F. Falconi, C. Comici, M. Kralj, M. Giani

Direttore della Sezione di Oceanografia
Dott. Paola Del Negro

Relazione OGS 2012/86 OCE 3 BIPPA dd. 18/10/2012

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 ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$, $Si\text{-}SiO_2$), 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:

Variable	Period
Dissolved oxygen (O_2)	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 ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$, SiO_2)	Monthly since August 1996; irregularly sinc 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:

Variable	Period
Total chlorophyll *a* and phaeopigment concentration	Monthly from April 1986 to July 1990
Total and size fractionated chlorophyll *a* 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:

Variable	Period
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-*a* determined by spectrophotometry until 1990
1	Total chlorophyll-*a* determined by spectrofluorometry since 1998
1	pre-filtered on 20 µm mesh to obtain nanophytoplankton chl-*a*
0.5	pre-filtered on 2 µm to obtain picophytoplankton chl-*a*

- **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 nm	June 2007 – December 2009	Ex. 435 nm; Em 668
Jasco FP-6500	January 2009 - now	Ex. 435 nm; Em 668 nm Intercomaparison 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^{-1} and 0.068 µg L^{-1} 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^{-1}, standard deviation: 0.01 µg L^{-1}) for chlorophyll *a*, and 0.7% (mean: 1.47 µg L^{-1}, standard deviation: 0.01 µg L^{-1}) 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^{-1}) to 0.5 (at chlorophyll-*a* concentration 0.14 µg L^{-1}).

Participation to QUASIMEME Quality Assurance exercise in June and October 2011: satisfactory result at chlorophyll-*a* concentration of 6.28 µg L^{-1} (Exercise 930 - R65 Chlorophyll-*a* in Seawater: April-Aug 2011); at chlorophyll-*a* concentration of 7.52, 1.54 and 4.55 µg L^{-1} (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).

Bottle used	Period	Notes
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

Instrument used	Period	Notes
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 µM; standard deviation: 0.35 µM).

Inorganic nutrients:

Before 1998 samples for inorganic nutrient analysis have been filtered on Millipore HA (47 mm diameter; porosity 0.45 µm), afterwards on Whatmann GF/F glass fiber filters, collected in acid washed polyethylene vials rinsed with seawater and immediately frozen (-20°C) until laboratory analysis. Inorganic nutrients ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$, $Si\text{-}SiO_2$) 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.

Instrument used	Period Notes
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 µM, 0.016 µM, 0.040 µM, 0.0015 µM and 0.010 µM, 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_4 (mean: 0.33 µM-N, standard deviation: 0.04 µM-N); 10% for NO_2 (mean: 0.02 µM-N, standard deviation: 0.002 µM-N); 50% for NO3 at limit of detection (mean: 0.02 µM-N, standard deviation: 0.01 µM-N); 2.5%

for PO_4 (mean: 0.04 µM-P, standard deviation: 0.001 µM-P); 0.1% for SiO_2 (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_4 (mean: 1.12 µM-N, standard deviation: 0.04 µM-N); 1% for NO_2 (mean: 0.25 µM-N, standard deviation: 0.002 µM-N); 1% for NO3 (mean: 60.57 µM-N, standard deviation: 0.46 µM-N); 5% for PO_4 (mean: 0.30 µM-P, standard deviation: 0.02 µM-P); 1% for SiO_2 (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 – ($N\text{-}NH_4$+$N\text{-}NO_2$+$N\text{-}NO_3$) and DOP = TDP – $P\text{-}PO_4$.

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).

Instrument used	Period
Shimadzu TOC 5000A	Nov. 1998- Feb. 2007
Shimadzu TOC – V_{CSH}	May 2007 - now

Limit of detection: : 0.33 µM (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 µM.
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).

Filtered Vol.(l)	Instrument used	Period
1	Perkin Elmer 2400 CHNS/O	Nov. 1998- Feb. 2006
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 µM-N and 0.11 µM-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 µM-N; standard deviation: 3.02 µM-N) and 4% for POC (mean: 359.06 µM-C; standard deviation: 14.03 µM-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_4 after combustion at 450°C and extraction in 1 N HCl (24h), according to Solorzano and Sharp (1980).

Filtered Vol. (l)	Period	Notes
1	Nov. 1998 - now	

The detection limit for PP, defined as twice the standard deviation of the blank, was 0.003 µM-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 µM-P; standard deviation: 0.004 µM-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 (NO_3 vs 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_2) concentration, while the distribution is asymmetrical in the case of all nutrients ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$, $Si\text{-}SiO_2$), 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, Mahalonobis 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 $N\text{-}NO_3$, $P\text{-}PO_4$ and SiO_2; 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_3 and high PO_4, 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_3 and low PO_4) 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 μM of $P\text{-}PO_4$ and 0.4 μM 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.

Literature cited:

Aminot A. and Kerouel R. 2004 Dissolved organic carbon, nitrogen and phosphorus in the N-E Atlantic and the N-W Mediterranean with particular reference to non-refractory fractions and degradation. Deep Sea Res. I, 51,1975-1999.

Armbruster D.A. and Pry T. 2008 Limit of Blank, Limit of Detection and Limit of Quantitation. Clin Biochem Rev, 29, 49-52.

Armstrong F. A. J., Williams P. M. and Strickland J. D. H. 1966 Photo-oxidation of organic matter in seawater by ultraviolet radiation, analytical and other applications. Nature, 211 481-483.

Ben-Gal I. 2005 Outlier detection. In: Maimon O. and Rockach L. (Eds.9 Data Mining and knowledge discovery handbook: a complete guide for practitioners and researchers. Kluwer Academic Publishers. Pp.1-15.

Boldrin A., Giani M., Langone L., Margiotta F., Povero P., Saggiomo V. 2010 CARBONIO ORGANICO ED AZOTO TOTALE PARTICELLATI (POC E PTN) In: Metodologie di studio del Plancton marino. edito da G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, C. Totti Manuali e Linee Guida 56/2010;101-110.

Cauwet G. 1994 HTCO method for dissolved organic carbon analysis in influence of catalyst on blank estimation. Mar Chem 47: 55-64.

Cauwet G. 1999 Determination of dissolved organic carbon and nitrogen by high temperature combustion. In: Grasshoff K., Kremling K., Erhard M. (eds.), Methods of Seawater analysis.Wiley-VCH, Weinheim (Germany), :408-420.

Chapman A. D. 2005 Principles of Data Quality, version 1.0. Report for the Global Biodiversity Information Facility, Copenhagen.

Church M.J., Ducklow H. W., Karl D.M. 2002 Multiyear increase in dissolved organic matter inventories at Station ALOHA in the Northern Pacific Subtropical Gyre. Limnol. Oc., 47, 1-10.

Consorzio per la gestione del Laboratorio di Biologia Marina di Trieste 1990 Osservatorio dell'Alto Adriatico Rgioni Veneto e Friuli-Venezia Giulia Repubbliche di Slovenia e Croazia. Campagna scientifica di ricerca e di monitoraggio sullo stato chimico, fisico e biologico delle acque dell'Alto Adriatico, in relazione al fenomeno di formazione degli ammassi gelatinosi. Technical Report.

Cozzi S., Margiotta F., Saggiomo V. 2010 AZOTO E FOSFORO TOTALI E TOTALI DISCIOLTI In: Metodologie di studio del Plancton marino. edito da G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, C. Totti Manuali e Linee Guida 56/2010; 87 – 100.

Cozzi S., Falconi C., Comici C., Čermelj B., Kovac N., Turk V., Giani M. 2012 Recent evolution of river discharges in the Gulf of Trieste and their potential response to climate changes and anthropogenic pressure. Estuarine and Coastal Shelf Sciences, doi:10.1016/j.ecss.2012.03.005

De Vittor C., Paoli A., Fonda Umani S., 2008 Dissolved organic carbon variability in a shallow coastal marine system (Gulf of Trieste, northern Adriatic Sea). Estuarine, Coastal and Shelf Science, 78, 280 – 290.

Fonda Umani S., Franco P., Ghirardelli E., Malej A. 1990 Ouline of oceanography and the plankton of the Adriatic Sea. In: The Adriatic Sea, 25th MBS Symposium, Ferrara 1990.

First IODE Workshop on Quality Control of Chemical Oceanographic Data Collections. Workshop Report No.228; IOC Project Office for IODE, 2010.

Giani M., De Vittor C., Savelli F., Santinelli C. 2010 DETERMINAZIONE DEL CARBONIO ORGANICO DISCIOLTO NELL'ACQUA MARINA In: Metodologie di studio del Plancton marino. edito da G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, C. Totti Manuali e Linee Guida 56/2010; 81 – 86.

Golimowski J., Golimowska K., 1996 UV-photooxidation as pretreatment step in inorganic analysis of environmental samples. Anal. Chim. Acta. 86, 63 – 68.

Grasshoff K. 1983 Determination of oxygen. In: Methods of Seawater Analysis. Edited by Grasshoff K., Ehrhardt M., Kremling K., Verlag Chemie, Weinheim, 1983, pp. 61 – 72.

Grasshoff K. 1983 Determination of nutrients. In: Grasshoff, K., M. Ehrhardt and K. Kremling. (Eds.), Methods of Seawater Analysis. Verlag Chemie. pp.125-187.

Greenberg A.E., Clesceri L.S., Eaton A. D. 1992 Standard methods for the examination of water and wastewater . APHA, AWWA, WEF, Ed. A.E. Greenberg, L.S. Clesceri, A. D. Eaton, 18° Edition, 1030 Data quality, 1-9 – 1-13.

Guadayol O., Peters F., Marrasè C., Gasol J.M., Roldan C., Berdalet E., Massana R., Sabata A. 2009 Episodic meteorological and nutrient-load events as drivers of coastal planktonic ecosystem dynamics: a time-series analysis. MEPS, 381, 139-155.

Holm-Hansen O., Lorenzen C.J., Holmes R. W.,. Strickland J.D.H 1965 Fluorometric Determination of Chlorophyll J. Cons. int. Explor. Mer. 30(1): 3-15.

IODE Group of Experts on Biological and Chemical Data Management and Exchange Practices (GE-BICH). IO C Project Office for IODE, 2011.

Koroleff F. 1983 Total and organic nitrogen. In: Grasshoff, K.; Ehrhardt, M.; Kremiling, K.; Methods of Seawater Analysis, 2nd ed., Verlag Chemie: Kiel, 162-173.

Krom M.D., Woodward E.M.S., Herut B., Kress N., Carbo P., Mantoura R.F.C., Spyres G., Thigstad T.F., Wassmann P., Wexel-Riser C., Kitidis V., Law C.S., Zodiatis G., 2005 Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: results from a phosphorus starved system. Deep Sea Res. II. 52 (22-23), 2879-2896.

Lipizer M., Cossarini G., Falconi C., Solidoro C., Fonda Umani S. 2011 Impact of different forcing factors on N:P balance in a semi-enclosed bay: the Gulf of Trieste (North Adriatic Sea). Continental Shelf Research, 31 (16), 1651-1662.

Lorenzen C.J. and Jeffrey S.W., 1980 Determination of chlorophyll in seawater. UNESCO, Technical Papers in Marine Science (No. 35), 1-20.

Malačič V., Viezzoli D., Cushman Roisin B., 2000 Tidal dynamics in the northern Adriatic Sea. Journal of Geophysical Research. 105 (C11), 26265-26280.

Marini M., Jones B. H., Campanelli A., Grilli F., Lee C. M. 2008 Seasonal variability and Po river plume influence on biochemical properties along western

Adriatic coast. Journal of Geophysical Research, 113, C05S90, doi:10.1029/2007JC004370.

Milani L., Cabrini M., Fonda Umani S., Honsell G. 1991 Environmental parameters (temperature, salinity, dissolved oxygen, chlorophyll a and phaeopigments) in a station in the Gulf of Trieste from March 1986 to September 1988: Data Report, Nova Thalassia, 9, 97-145.

Mozetič P., Fonda Umani S., Kamburska L., 2002 Plankton variability in the Gulf of Trieste (Northern Adriatic). Arch. Oceanogr. Limnol. 23, 7-19.

Murphy J. and Riley J. P. 1962 A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27, 30.

Outdot C., Gerard R., Morin P. 1988 Precise shipboard determination of dissolved oxygen (Winkler procedure) for productivity studies with commerical system. Limnol. Oceanogr., 33 :146-150.

Riebesell U., Fabry V. J., Hansson L. & Gattuso J.-P. (Eds.), 2010 Guide to best practices for ocean acidification research and data reporting, 260 p. Luxembourg: Publications Office of the European Union.

Saggiomo V., Catalano G., Corato F., Ribera D'Alcalà M. 2010 METODI AUTOMATICI DI ANALISI DEI NUTRIENTI In: Metodologie di studio del Plancton marino. edito da G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, C. Totti Manuali e Linee Guida 56/2010; pp.55-80.

Saggiomo V., Catalano G., Ribera d'Alcalà M. 2010 OSSIGENO DISCIOLTO In: Metodologie di studio del Plancton marino. edito da G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, C. Totti Manuali e Linee Guida 56/2010; 21 – 32.

Sharp J. H. 1974 Improved analysis for particulate organic carbon and nitrogen from seawater. Limnology and Oceanography, 19, 984-989.

Solorzano L. and Sharp J.H. 1980 Determination of Total Dissolved Phosphorus and Particulate Phosphorus in Natural Waters. Limnology and Oceanography, Vol. 25, No. 4 : 754-758.

Strickland J.D.H. and Parsons T.R.; 1972 *A practical handbook of seawater analysis*, Bull. Fish. Res. Board Can. 167, 2nd ed., Ottawa, Ontario, 310 pp.

Strickland J. D. H. and Parsons T. R. 1972 A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, 167 pp.

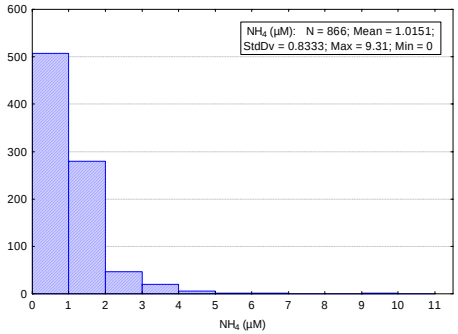
Walsh T. W. 1989 Total dissolved nitrogen in seawater: A new high temperature combustion method and a comparison to photo-oxidation. Marine Chemistry, 26, 295-311.

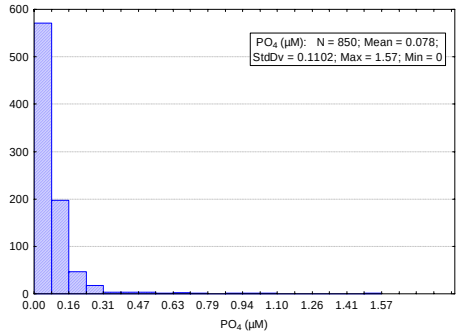
Winkler L.W. 1888 Die Bestimmung des im Wasser gelosten Sauerstoffes. Chem. Ber., 21, :2843-2855.

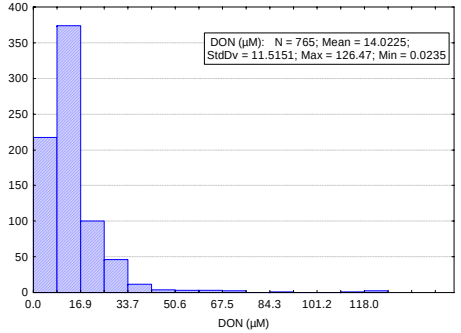
Zavodnik D. 1983 400 of the Adriatic marine science. Thalassia Jugosl., 10 (1-4), 405-429.

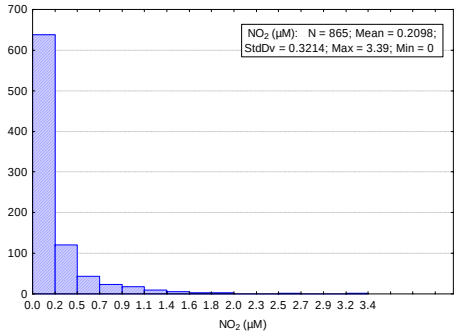
Annex 1:

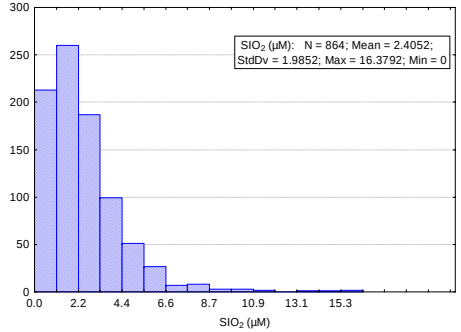
Frequency distribution of hydrological and biogeochemical data measured in LTER station C1 from 11/1998 to 12/2009. Descriptive statistics of the dataset is reported on the plot.

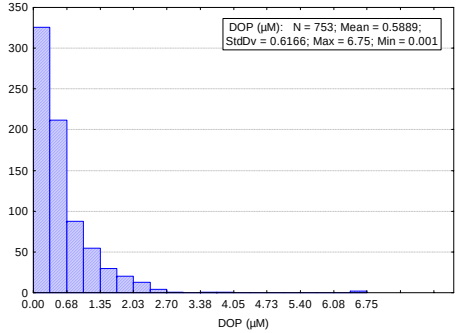


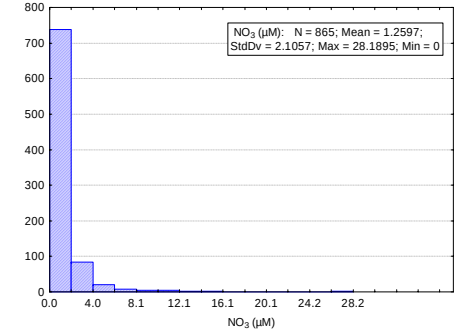


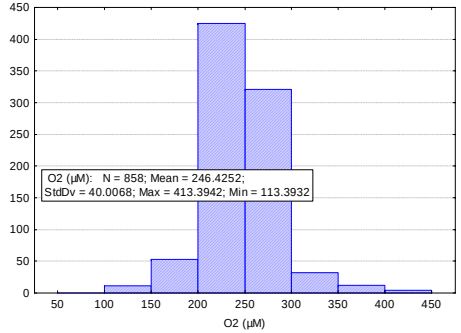


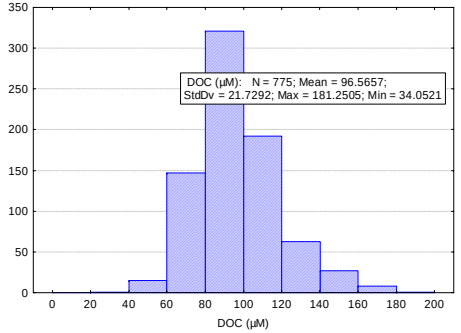






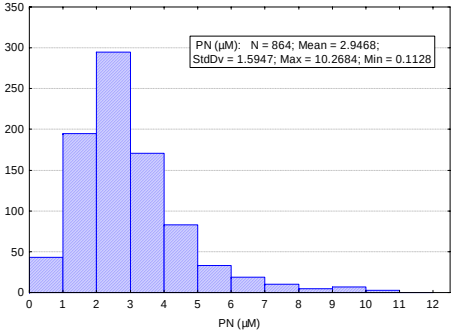


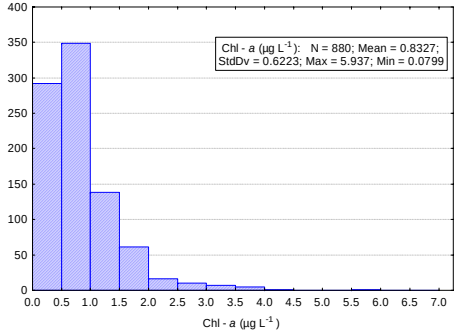


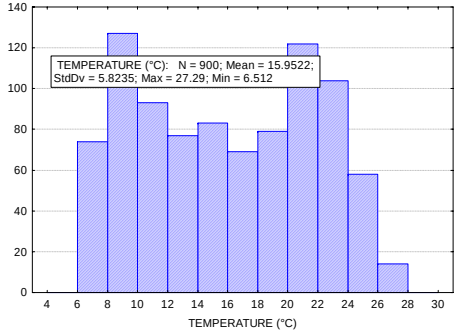


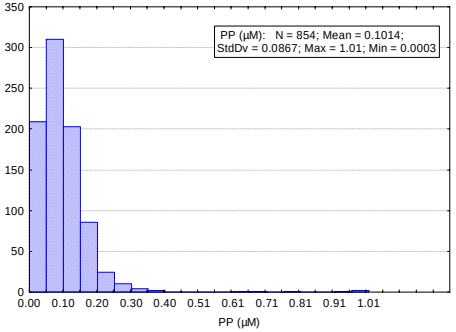
Annex 1:

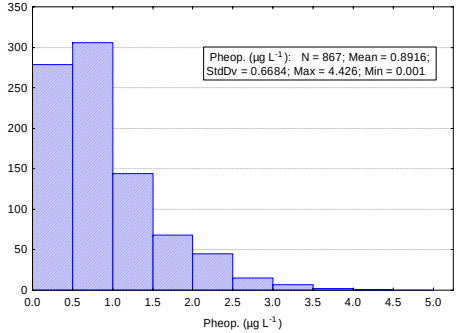
Frequency distribution of hydrological and biogeochemical data measured in LTER station C1 from 11/1998 to 12/2009. Descriptive statistics of the dataset is reported on the plot.

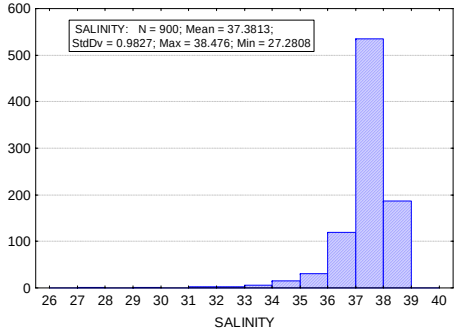


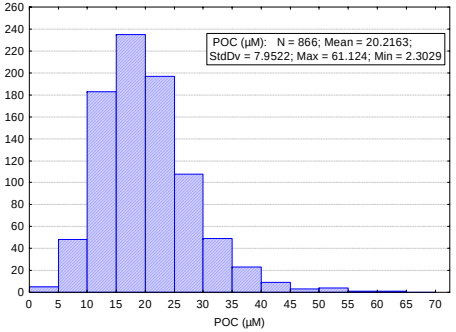






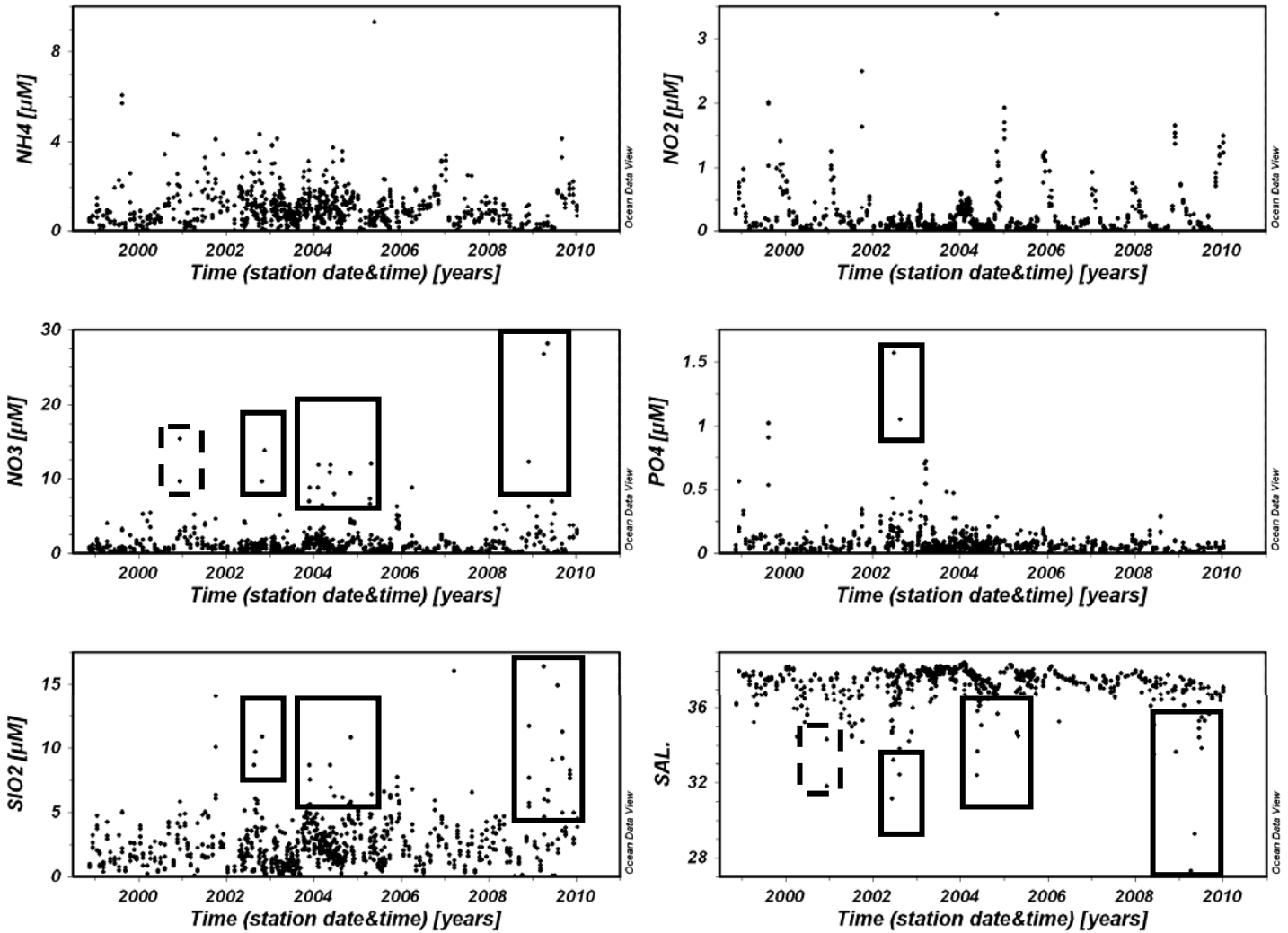






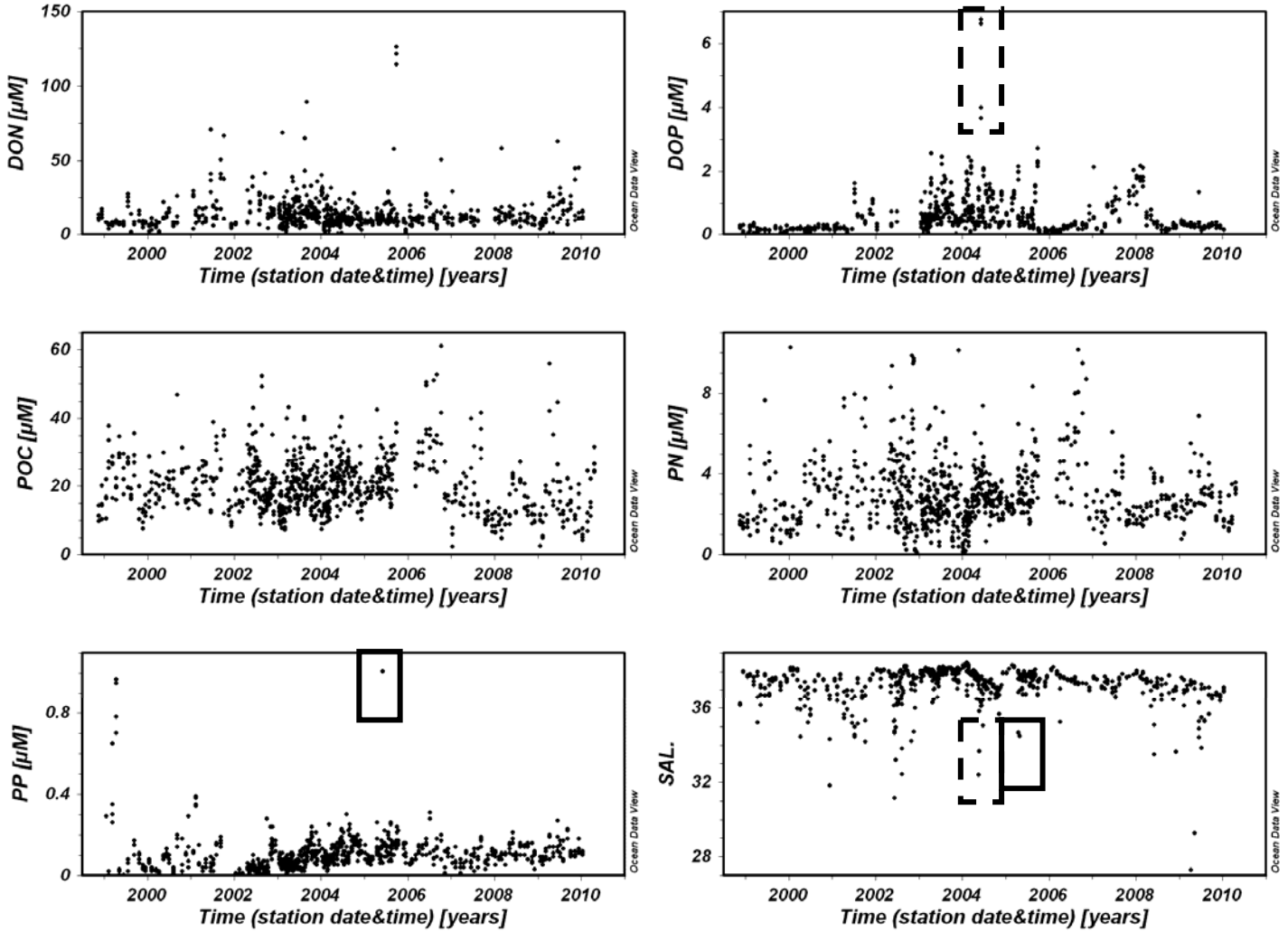
Annex 2:

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



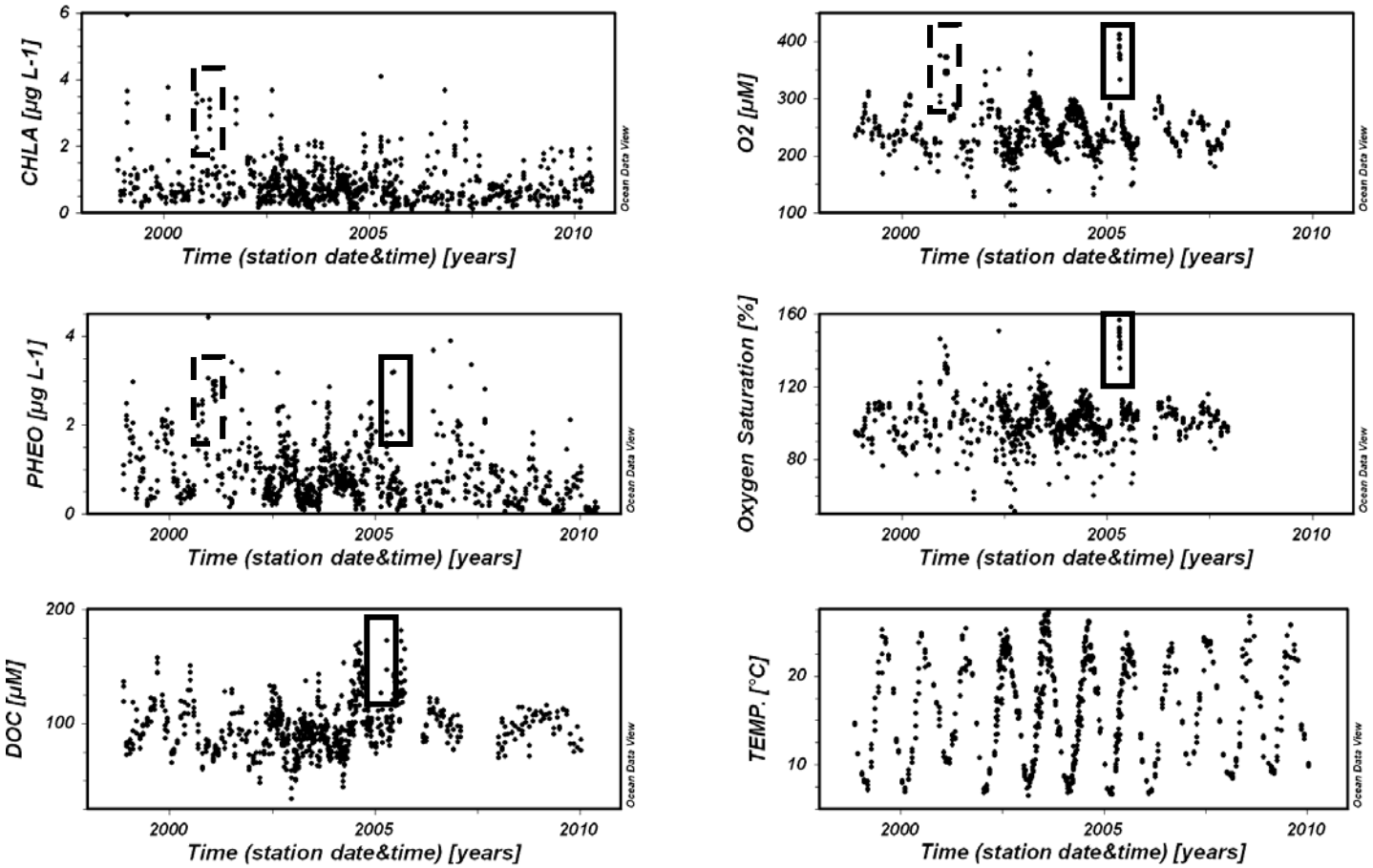
Annex 2:

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



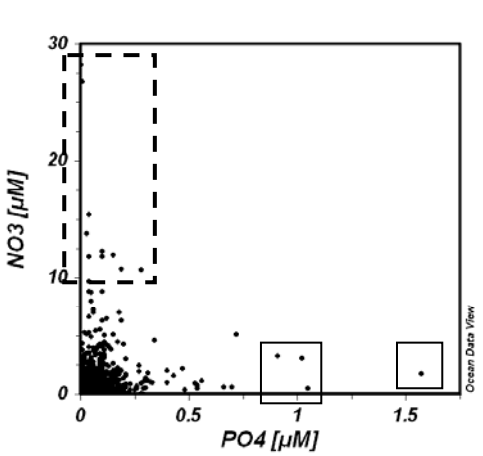
Annex 2:

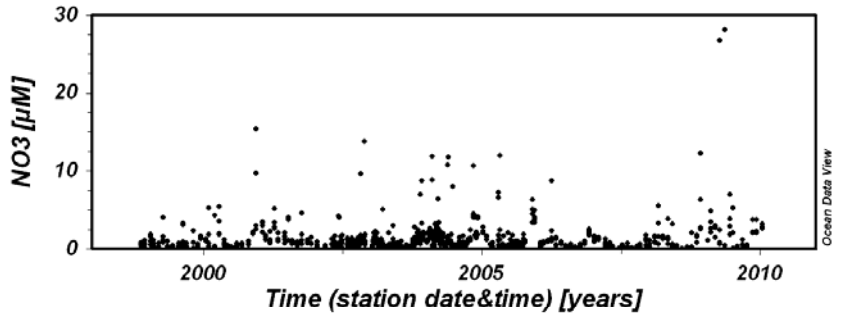
Time series of biogeochemical properties measured in LTER station C1 from 11/1998 to 12/2009.

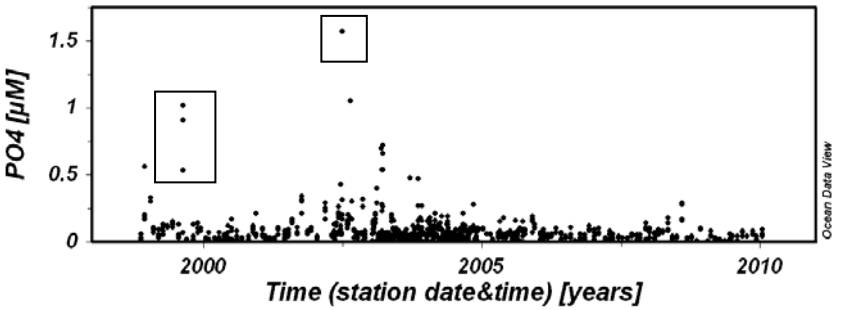


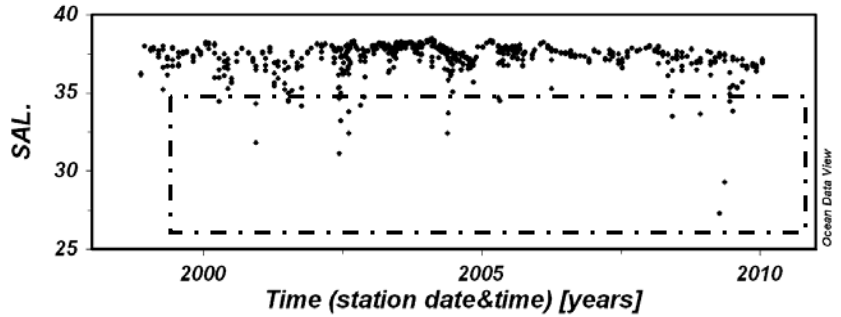
Annex 3:

Property-property plots used to verify data consistency.



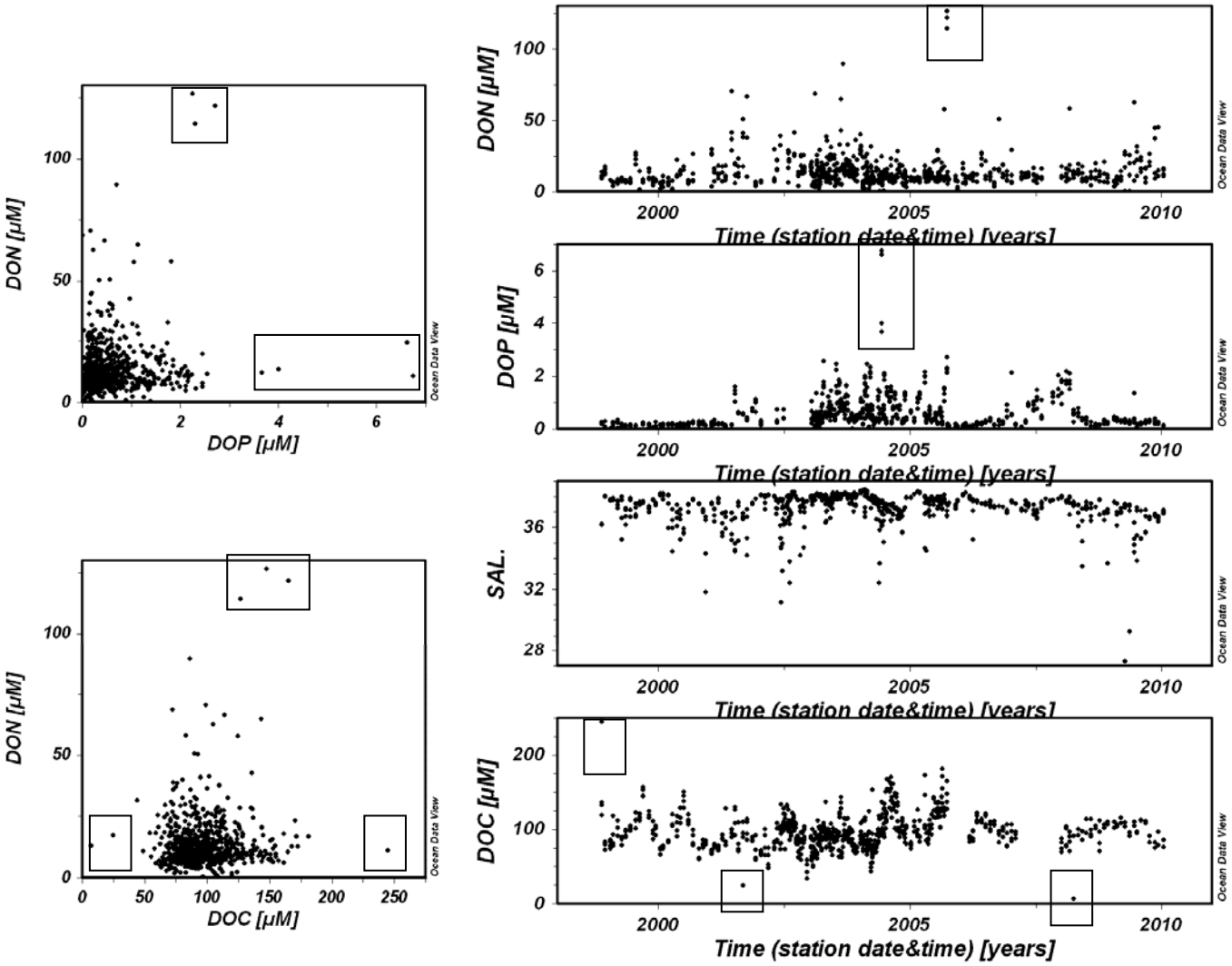






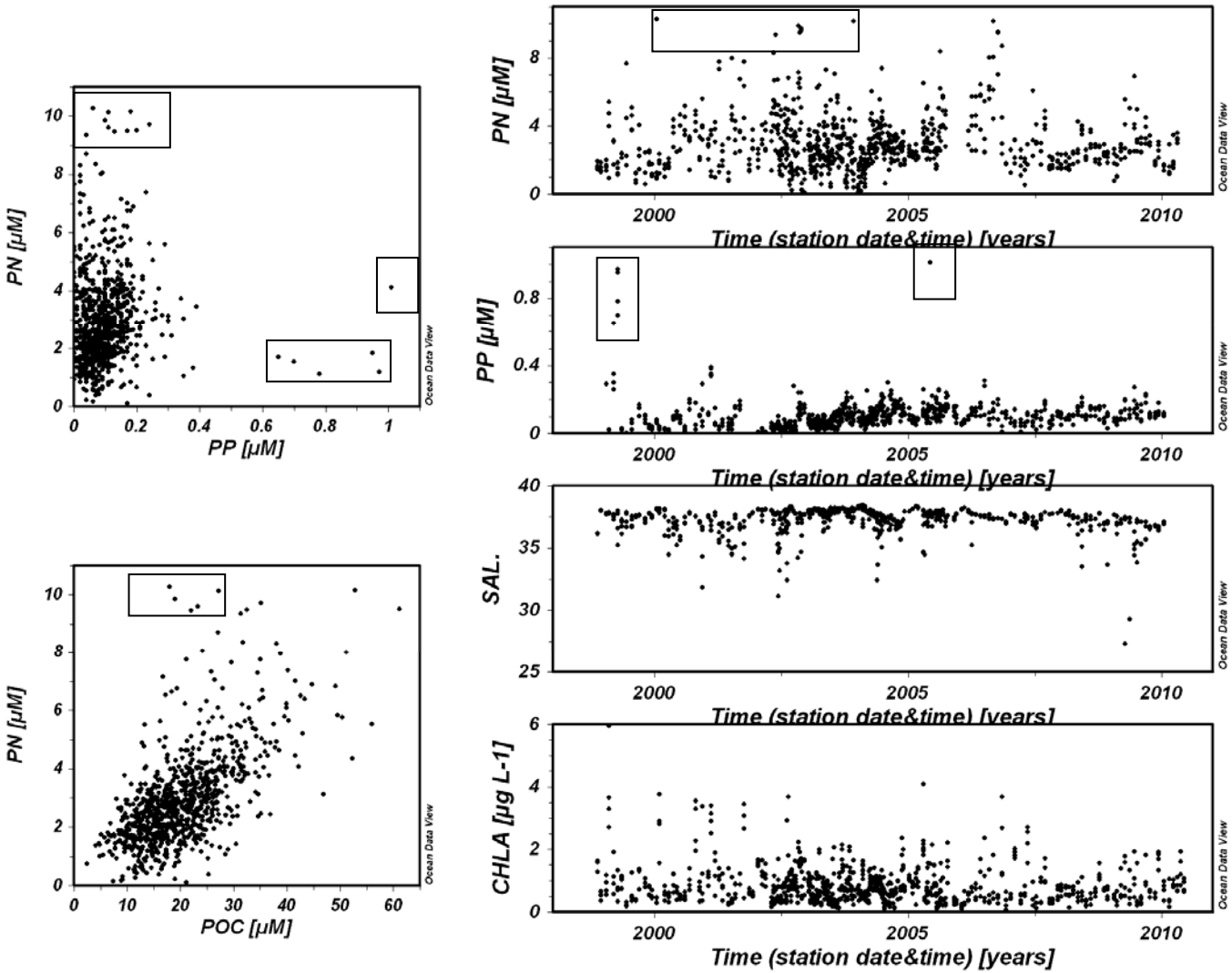
Annex 3:

Property-property plots used to verify data consistency.



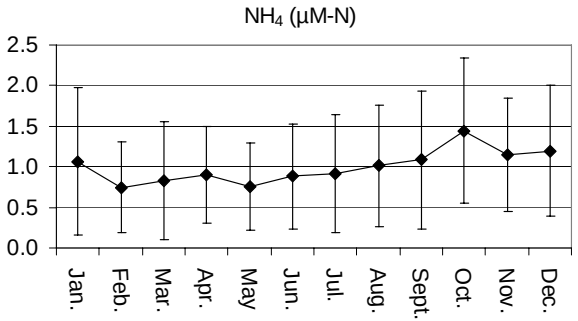
Annex 3:

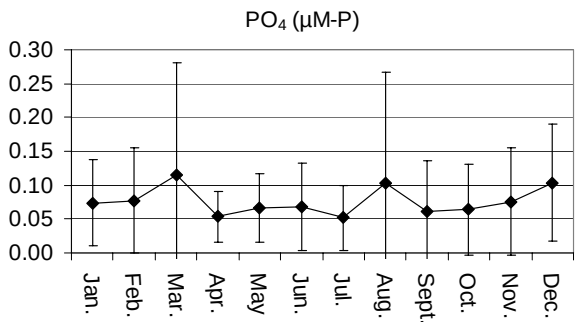
Property-property plots used to verify data consistency.

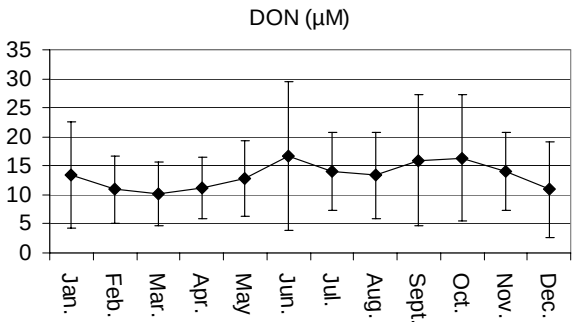


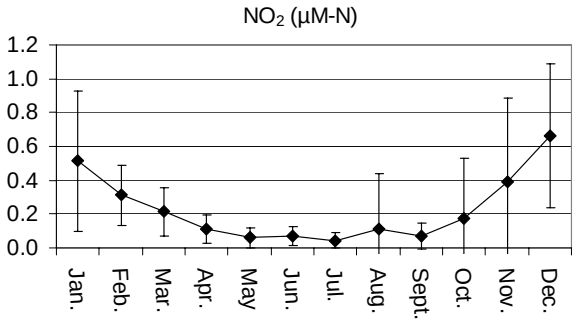
Annex 4:

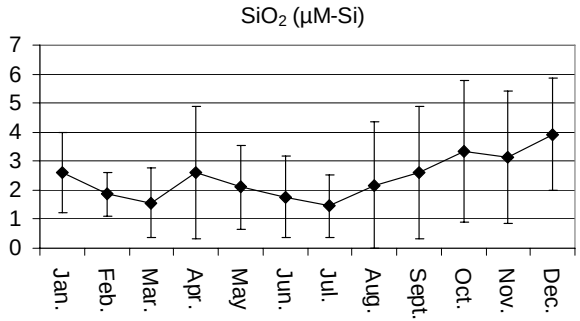
Climatology of biogeochemical properties measured at LTER station C1 from 11/1998 to 12/2009.

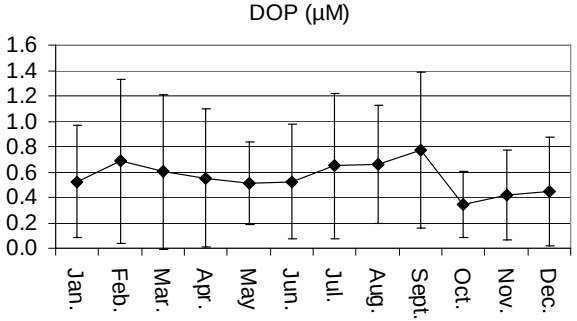


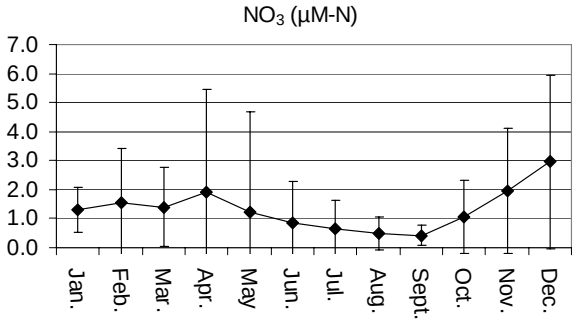


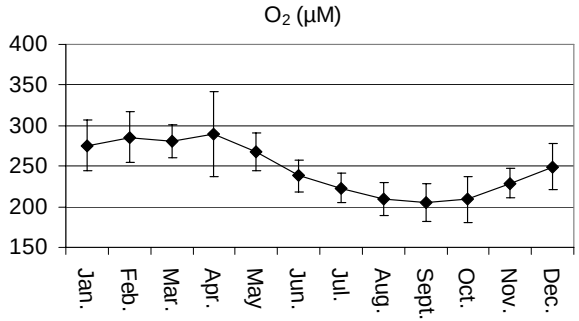


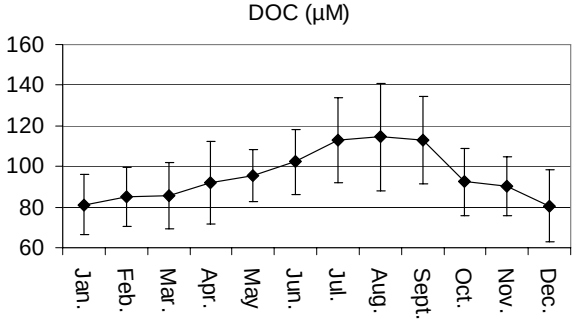












Annex 4:

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