Nutrient preservation, analysis precision and quality control of an oceanographic database of inorganic nutrients, dissolved oxygen and chlorophyll *a* from the NW Mediterranean Sea

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SUMMARY: Oceanographic data from 28 cruises performed in the NW Mediterranean Sea between 1982 and 2003 and data from historical databases, amounting to more than 100000 data points, are used to define the quality control ranges at standard level depths of dissolved inorganic nutrients, dissolved oxygen and chlorophyll *a*. The quality of each of the key variables and the analytical precision are assessed. The results indicate that freezing is a valid preservation method for phosphate, nitrate, silicate and nitrite in samples from the NW Mediterranean Sea, though their relative error increases (6.3%, –1.6%, –2.5% and –11.4%, respectively). In contrast, freezing nutrient samples on the cruises gathered negatively affected the analytical precision and accuracy of the ammonium analysis. The analysis precision is also estimated using different approximations, the most realistic being the use of replicate samples from the same sampling bottle. Except for phosphate and dissolved oxygen, specific quality control ranges for mixed water columns, usually found in winter, have to be defined, since the surface concentrations are particularly high due to deep water mixing. The quality-controls described in this work are an important and useful tool for validating data and for detecting erroneous or anomalous data obtained in both historical and future works in the NW Mediterranean Sea.

Keywords: dissolved inorganic nutrients, dissolved oxygen, chlorophyll *a*, nutrient preservation, analysis precision, quality control, NW Mediterranean Sea.

RESUMEN: CONSERVACIÓN DE NUTRIENTES, PRECISIÓN DE LOS ANÁLISIS Y CONTROL DE CALIDAD DE UNA BASE DE DATOS OCEANOGRÁFICA CON NUTRIENTES INORGÁNICOS, OXÍGENO DISUELTO Y CLOROFILA *A* DEL MEDITERRÁNEO NOROCCIDENTAL. – Se han utilizado datos oceanográficos de 28 campañas realizadas entre 1982 y 2003 en el Mediterráneo noroccidental, así como de bases de datos históricas, para definir rangos de control de calidad en niveles de profundidad estándares para nutrientes inorgánicos, oxígeno disuelto y clorofila *a*. La calidad de cada una de estas variables clave, así como la precisión analítica, han sido evaluadas. Los resultados indican que la congelación es un método válido para conservar las concentraciones de fosfato, nitrato, silicato y nitrito en muestras procedentes del Mediterráneo noroccidental, aunque el error relativo del análisis incrementa (6.3%, –1.6%, –2.5% y –11.4% respectivamente). En cambio, en las campañas recopiladas la congelación de muestras de nutrientes ha afectado negativamente a la precisión y exactitud del análisis de amonio. La precisión de los análisis también ha sido estimada utilizando diferentes aproximaciones, siendo la más realista el uso de réplicas procedentes de la misma botella de muestreo. Excepto para fosfato y oxígeno disuelto, se han definido rangos de control de calidad específicos en condiciones de columnas de agua mezcladas, típicas de invierno, puesto que las concentraciones superficiales resultan particularmente elevadas debido a la mezcla con aguas profundas. Los rangos de control de calidad descritos en el presente trabajo son una herramienta útil e importante para validar datos oceanográficos, para detectar datos erróneos o anómalos, obtenidos en el pasado o futuros trabajos en el Mediterráneo noroccidental.

Palabras clave: nutrientes inorgánicos disueltos, oxígeno disuelto, clorofila *a*, conservación de nutrientes, precisión analítica, control de calidad, Mar Mediterráneo.

INTRODUCTION

Oceanographic databases are basic tools for preserving historical observations and for describing and tracking changes in ecosystems. Researchers constructing databases that include decades of oceanographic research should take into account that most probably there are very significant differences in the way the measurements were obtained. Such differences include changes in the analysis instruments, the sampling and the preservation procedures. Therefore, it is necessary to check whether data collected and processed using different methodologies and precisions can be combined. Subsequently, a quality-control (QC) for each variable should be applied in order to obtain a scientifically quality-controlled database. Since nowadays so much research is used to try to determine climate change or anthropogenic impacts, basic information about the analysis precision, limit of detection and other analytical characteristics is critical.

The study of the quality of oceanographic data gathered from different sources has been raised by others, and QC procedures have already been described for the whole oceans (Conkright *et al.*, 1994, 2000). Also, Fichaut *et al.* (1997) defined ranges of T, S and dissolved oxygen in our study area (region DS2 in MEDATLAS database), often with one single concentration range for the whole water column. However, there is no information about the ranges of concentration for nutrients and chlorophyll *a* (chl *a*) in the MEDATLAS database. In this article, our aim is to establish the ranges of concentration with depth for the main biochemical variables (nutrients, dissolved oxygen and chl *a*) in the NW Mediterranean Sea, the most productive area of the Mediterranean Sea. The oceanographic database gathered to accomplish this objective contains data from the almost annual oceanographic cruises performed since 1982 in the Catalan Sea, as well as historical data from MEDATLAS (Medar Group, 2002) and MATER (Mater Group, 2001) databases. The available oceanographic data include dissolved inorganic nutrients (phosphate, nitrate+nitrite (hereafter referred to as nitrate), nitrite, ammonium and silicate), dissolved oxygen and chl *a*, as well as temperature (T) and salinity (S).

The QC described in this work will be an important and useful tool for identifying erroneous or non-representative measurements of oceanographic data obtained in future studies in the NW Mediterranean Sea. Quality controlled data will provide a more accurate climatology of the NW Mediterranean Sea for the description of the distribution of nutrients, dissolved oxygen and chl *a* (Segura-Noguera *et al.*, submitted) for modeling the dynamics of the system and for studying long-term variations (e.g. seasonality or inter-annual trends, Segura-Noguera *et al.*, in preparation).

MATERIALS AND METHODS

Data source

Oceanographic data from 28 cruises performed in the NW Mediterranean Sea between 1982 and 2003, amounting to 1388 stations, were gathered (see Appendix 1 for more characteristics and instrumentation used on the cruises gathered). The information available for each station included date, position, sampling time and maximum depth. Information available for each water sample included depth (m), T (°C), S (practical salinity units), and density as σ-T (kg m^{-3}). Depending on the cruise, data of dissolved inorganic nutrients (µM), dissolved oxygen (ml l^{-1}) and chl *a* (µg l^{-1}) concentrations, were available.

In order to complement this information and to increase the pool of data for statistical analysis, data from the MEDATLAS (Medar Group, 2002) and MATER (Mater Group, 2001) databases were extracted using as limits for the extraction area the most extreme latitude and longitude of the cruises gathered (from 0 to 4.5°W and from 38.7 to 42.4°N, Fig. 1). MEDATLAS and MATER databases include data earlier than 1982, as indicated in Table 1, where the amount of data for each oceanographic variable is indicated. Among all the collected cruises, only data from the "Mater 95" cruise was included in both databases, and therefore it was checked that this cruise was not duplicated in the final working database. Data from stations with a bottom depth higher than 400 m (considered as "open sea," according to Medar Group, 2002) are also indicated in Table 1, because they will be used to define

TABLE 1. – Total data and data from open sea stations for each oceanographic variable, obtained from the cruises gathered ("cruises" including "Meso 95") and extracted from MEDATLAS and MATER Databases ("databases"). Open sea refers to stations with a bottom depth higher than 400 m.

	Range of years	Total data from:			Open sea data from:		
		Cruises	Databases	TOTAL	Cruises	Databases	TOTAL
Phosphate	1957 - 2003	8993	6466	15459	6179	3087	9266
Nitrate	1979 - 2003	13971	572	14543	9811	387	10198
Nitrite	1976 - 2003	12984	4903	17887	8954	2421	11375
Ammonium	1982 - 2003	4944	481	5425	3682	223	3905
Silicate	1970 - 2003	14013	5857	19870	9831	2844	12675
Oxygen	1910 - 2000	10247	8357	18604	7455	5394	12849
Chl *a*	1976 -2003	7201	3530	10731	5009	1446	6455
Total		72353	30166	102519	50921	15802	66723

TABLE 2. – Criteria used to define the stratification coefficient of the profiles according to the vertical stratification index at 100 m depth (VDSI100) calculated.

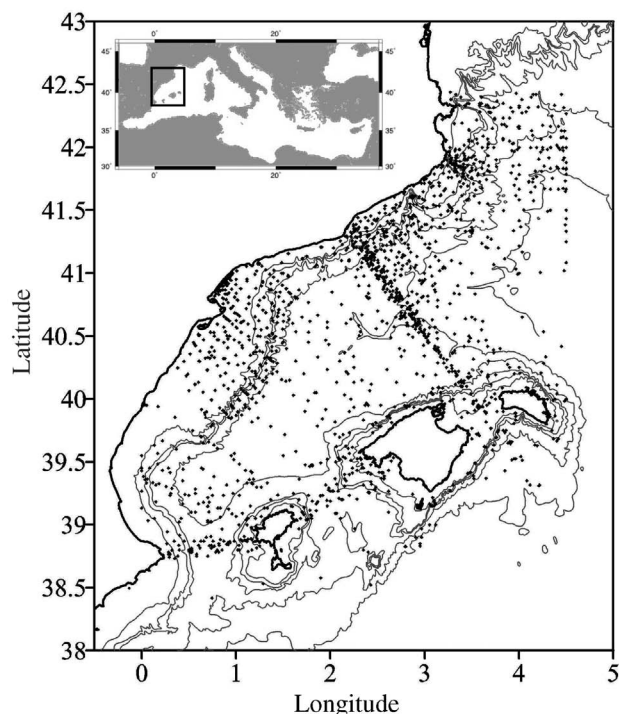| Stratification coefficient	VDSI100	Most representative season	Months in which this coefficient was found
1	≤ 5; surface T<15	Winter	November-April
2	(5, 30]	Spring	April-July
3	>30; surface T>24	Summer	June-September
4	(5, 30]	Autumn	September-December

FIG. 1. – Map of the Catalan Sea with the position of the oceanographic stations sampled during the cruises collected for this study. The inlet map shows the position of the Catalan Sea in the Mediterranean Sea.

the QC ranges for the NW Mediterranean Sea, following the procedures described in Conkright *et al.* (1994, 2000). The map of the NW Catalan Sea with all the stations sampled, including the data extracted from the MEDATLAS and MATER databases, is shown in Figure 1.

The regional limits for T and S described by the Medar Group (2002) were used to validate the quality of T and S data. As a result, less than 0.2% and 0.5% of the data, respectively, was found outside the limits. The most striking feature for these variables was a few low S values, which could be explained by their position close to the Ebro River Delta.

Analytical precision

The nutrient analysis precision is reported only for the "Fronts 89", "Fronts 90" and "Fronts 91" cruises (Varela and FRONTS Group, 1991). For the "Canyons" and "Caco" cruises, the precision of nutrient concentration was calculated during the analysis with Quality Control Charts (QCC) using internal standards

within the samples. No data about the precision for dissolved oxygen and chl *a* analysis were reported for all the data collected.

When the precision value was not reported, the analysis precision of all samples was estimated for each cruise and parameter, using two different methods. The first method was applied when there were different kinds of duplicated samples. In these cases the precision was estimated by calculating the standard deviation: (1) of subsamples (replicates) of the same sampling bottle, assuming that the bottle is a homogeneous sample; (2) of samples from different sampling bottles closed at the same depth, assuming that the water mass is a homogeneous sample; or (3) of consecutive analysis of the same sample in the autoanalyzer.

An alternative method for calculating the analysis precision is described by Garcia *et al.* (1998). These authors proposed calculating the regression line of the oceanographic variables concentration versus potential T (θ) and using samples that belong to the same water mass, which should be physically and chemically stable. Then, the precision is estimated as the standard deviation of the difference between the measured values and those expected from the regression. Among the different water masses present in the NW Mediterranean Sea, the most physically stable is Western Mediterranean Deep Water (WMDW, less than 1°C variation, Salat, 1995), which is found below 800 m depth. The concentration of nutrients in this water mass is fairly constant with time and depth, according to the results of previous studies (Béthoux and Copin-Montégut, 1986; Béthoux *et al.*, 1998, 2002; Moutin and Raimbault, 2002). However, there is not enough data from this water mass on all cruises. Thus, we used as an alternative the data sampled in Levantine Intermediate Water (LIW), located between 200 and 600 m depth, as well as in its core, characterized by a relative maximum of θ and an absolute maximum of S (Salat and Cruzado, 1981; Millot, 1999).

Besides assessing the analysis precision, we also compared these methodologies to check whether they can be effectively used to estimate this parameter.

Nutrient analysis

From 1982 to 2003, nutrient data were obtained by 7 analysts using 4 different autoanalyzer instruments. The nutrient analysis methodologies that were used are known only for the cruises gathered, but not for the data extracted from the databases. The analyses were based on the same general methodologies (phosphate,

Murphy and Riley 1962; silicate, Grasshoff 1964; Hansen and Koroleff 1999; nitrite, Bendschneider and Robinson 1952; nitrate, Hansen and Koroleff 1999; ammonium, Grasshoff 1970). Some adaptations and modifications for each autoanalyzer and analysis range were applied. However, it is commonly assumed that the quality of the measurements is often more dependent on the care during the sampling and analysis process than on the variations of a given methodology (Hager *et al.*, 1972). Ammonium data from 2003 cruises was obtained with a different methodology (Benesch and Mengelsdorf, 1972, cited by Riley *et al.*, 1975; Bower and Holm-Hansen, 1980), using sodium salicylate instead of phenol, to avoid the use of this toxic compound (a summary of the characteristics of the analyses can be found in Appendix 2). Nutrient concentrations are given in µmol l^{-1} and the range of density (σ_t) in the Catalan Sea is 25.13 kg m^{-3} (surface waters during summer) to 29.10 kg m^{-3} (corresponding to WMDW). Standards were always prepared at room temperature (20-25°C).

Another potential source of variability is the baseline used as a blank, which also varied during the study period. Two different baselines were used: (1) low nutrient seawater (LNSW), which is the most recommended because there is no difference in matrix between the sample and the blank (Hansen and Koroleff, 1999), and (2) artificial seawater (ASW), which in its simplest version is a solution of sodium chloride in double-distilled seawater, with the same salinity as the samples. When ASW is used as a baseline, it should be taken into account that even using the purest products (analytical grade), trace concentrations of elements of interest can be detected and must be quantified. During the analysis of surface water from summer cruises, using 38 g l^{-1} ASW as a blank, negative peaks were found. These are nutrient-depleted waters, the negative peaks being the result of contaminated ASW. This contamination was observed using different NaCl batches (Merck) and corresponds to at least 0.02-0.03 µM phosphate; 0.23-0.26 µM ammonium; 0.03-0.06 µM nitrite and 0.09-0.13 µM nitrate. No silicate contamination was detected. Moreover, double-distilled water can also contain nitrite ions, depending on the age of the water.

Nutrient preservation

Another major source of variability is how the samples are taken and processed. During the first cruises gathered, nutrient analyses were performed on board during the cruise, but from 1999 to 2003 nutrient samples were routinely frozen to be analyzed after the cruise (Appendix 2). When it is not possible to analyze immediately after sample collection, which would be the best option, freezing the samples is the most acceptable preservation method. However, the accuracy and precision of the analysis decreases (Stéfansson and Richards, 1963; Thayer, 1970; Strickland and Parsons,

1972; Riley *et al.*, 1975; Venrick and Hayward, 1985; Chapman and Mostert, 1990; Avanzino and Kennedy, 1993; Gordon *et al*, 1993; Valderrama, 1995; Dore *et al.*, 1996; Aminot and Kérouel, 1998; Kremling and Brügmann, 1999).

Many studies have been conducted to study the effect of freezing on nutrient samples, but no unequivocal conclusion has been reached. The variables studied in these works include the velocity of freezing or defreezing the samples, the material of the recipient, the previous filtration due to presence of plankton or suspended matter, the period of time during which the samples are kept frozen, the surface/volume ratio of the recipient and the salinity of the sample. Those studies show contradictory results regarding the effects of freezing and filtering the samples on phosphate and ammonium concentrations but seem consistent in the validity of preserving nitrate and silicate concentration, but only if the concentration of the latter does not exceed 60 µM. In summary, the conclusion is similar to that described by Venrick and Hayward (1985) in a bibliographic revision about preservation methods. Those authors concluded that the effectiveness of the procedure depends highly on the local biological and chemical characteristics of the water, as well as the methodology used. They therefore suggested that nutrient preservation should be considered as the last option, and if necessary each laboratory should determine the resulting error introduced.

As suggested by Venrick and Hayward (1985), we designed an experiment to test the effect of freezing samples from the NW Mediterranean Sea during a routine monthly sampling of the "Pudem" project. The results of this test will help us decide whether we can use the nutrient data that were frozen as a preservation method on the cruises gathered to build up the QC ranges of concentration for nutrients. Nutrient samples were taken from 6 different stations, at 2 different depths at each station (surface and bottom, the latter from 10 up to 40 m depth). Sampling tubes (polypropylene, 12 ml) were previously washed with 5% HCl for 24 hours, distilled water (3 washes) and finally double-distilled water. Samples were filtered through a 200-µm mesh to remove large zooplankton, and were preserved tightly closed in an icebox during the sampling time in an upright position. In the research centre 3 replicates of each station and depth were immediately analyzed, and 3 replicates were frozen in an upright position at -20ºC (the temperature at which preserved nutrient samples from the gathered cruises were frozen). Samples were stored in a freezer containing exclusively nutrient samples (no biological samples). Samples remained frozen for one week before analyses were conducted using an AA3 autoanalyzer (Bran+Luebbe). ASW was used as a baseline because this was the blank most used when nutrient samples from the cruises were frozen (Appendix 2).

During the experiment, additional precautions were taken to: (1) avoid water at the tip from reaching the

sample, for example by drying the exterior of the tube before the sample was homogenized and introduced into the sampler; (2) keep the sample exposed to the air as short a time as possible during the analysis; and (3) avoid touching the interior of the tube and tip with the fingers.

The range of nutrient values, as well as precision (standard deviation from QCC using standards) and detection limits of the analysis (calculated following Gomez-Taylor *et al.*, 2003) are the following: 0-0.5 μM, 3% and 0.006 μM for phosphate analysis; 0-3 μM, 1% and 0.015 μM for nitrate analysis; 0-0.5 μM, 4% and 0.004 μM for nitrite analysis; 0-3 μM, 3% and 0.020 μM for ammonium analysis; and 0-5 μM, 1% and 0.017 μM for silicate analysis.

The statistical analysis for the determination of differences between nutrient concentration of preserved and unpreserved samples was performed with the Statistica v6.0 software package.

Assuming that few samples were frozen during onboard analysis, due to technical problems or bad weather, we also compared the nutrient concentration of the cruises on which the nutrients were preserved with those on which the nutrients were analyzed on board (unpreserved). Because we could not expect that the handling precautions taken during the experiment described above were also taken during the cruises, this additional test would help us to decide whether historical data from preserved nutrient samples were accurate enough to use them to construct the QC ranges of concentration of key bio-chemical variables in the Catalan Sea.

Since there is no information in the MEDATLAS and MATER databases on whether a preservation method of the nutrient samples was used, these data were not included in this comparison. The possible differences between cruises in which nutrients were frozen or analyzed on board were also studied with a two-factor ANOVA statistical test using Statistica v6.0.

Dissolved oxygen analysis

The dissolved oxygen analysis methodology is one of the most robust from the analytical point of view, and has remained almost unmodified since its description, in the late 19th century (Winkler, 1888). The procedure for dissolved oxygen analysis can be found in detail in Hansen (1999). Basically, the oxygen present in a water sample oxidizes I^- ion to iodine, the amount of which will be determined by thiosulfate titration. The implementations of the methodology are mostly related to the automation of the final titration point, which also improves the precision of the analysis. The most important sources of error during the analysis are the oxygen present in reagents and in the blank (Culbertson *et al.*, 1991). The concentration obtained must be corrected according to the in situ water temperature. Most of the data of dissolved oxygen gathered from the NW Mediterranean Sea were

determined without automation of the final titration point. On the cruises "Pep 83", "Varimed 93", "Fans 1", "Fans 2", "Fans 3", "Hivern 99" and "Hivern 2000", a titration system with a platinum electrode was used (PEPS Group, 1986; Masó and PEPS Group, 1988; Varela and FRONTS Group, 1991; Masó and Varimed Group, 1995).

Chlorophyll *a* analysis

All the chl *a* concentration data collected were obtained using the methodology described in Yentsch and Menzel (1963). The most outstanding difference among the cruises gathered is the use of two different glass fibre filters; GF/C filters, with a 1.2 μm nominal pore diameter, were used during the cruises "Pep 82", "Pep 83" and "Pep 84", while GF/F filters, with a pore diameter of 0.7 μm, were used during the remaining cruises (PEPS Group, 1986; Masó and PEPS Group, 1988; Varela and FRONTS Group, 1991; Masó and Varimed Group, 1995). An inter-comparison experiment demonstrated that the amount of organic matter retained by the two filters was the same (Morán *et al.*, 1999).

The precision of the present method using natural populations shows a maximum variation of 15% for ten samples. The blank calibration of the fluorometer is constant for long periods, and instrument readings are repeatable within ±3% (Yentsch and Menzel, 1963). The limit of detection will depend upon the volume of seawater filtered and the sensitivity of the fluorometer. In general, the method is 5 to 10 times more sensitive than the spectrophotometric method, but it may be less accurate (Parsons *et al.*, 1984).

In the range of years studied, the fluorometer was usually calibrated once a year using a spectrophotometer and both commercial chl *a* (Sigma) and natural water samples from the study area. Natural samples were used to check whether there were interferences with chlorophyll *b* or *c*. The factors obtained with the two procedures were the same (L. Arin, personal communication), so there is no interference. Also, when the fluorometer was moved to an oceanographic ship to measure the chl *a* concentration on board, the calibration was repeated when the cruise had finished and the fluorometer was back in the laboratory.

On some cruises, just a few chl *a* samples were taken for the calibration of the CTD fluorescence probe. Chl *a* data obtained from a calibrated CTD fluorescence probe were not used in this study.

Quality Control

The QC was performed following Conkright *et al.* (1994, 2000) and Fichaut *et al.* (1997). Conkright *et al.* (1994) described a methodology to set QC for phosphate, nitrate and silicate using data from open oceanic waters. Unfortunately, because our region of study is surrounded by land and may be influenced by

continental inputs, it does not meet the criteria used by Conkright *et al.* (1994) for oceanic waters. Therefore, to identify areas far from the coast to be used to build the QC, we followed Fichaut *et al.* (1997), which adapted the definition of open sea for the Mediterranean Sea. On these bases, areas deeper than 400 m are considered as open sea. We also used the standard level depths (SLD) proposed for the Mediterranean Sea by Fichaut *et al.* (1997), which increase the resolution at the upper layers. The SLD are those depths that have been historically most sampled, and include data comprised between up to 25% of the distance with the upper SLD and up to 75% distance with the lower SLD.

The first step for the QC description for all the biochemical variables was the determination of the frequency distribution of the data for each parameter at the closest SLD. Following Conkright *et al.* (1994, 2000) the initial concentration ranges were defined at each SLD by those values with a frequency higher than 0.5%. However, due to the scarcity of available data of the region, this condition was changed; below 500 m depth the minimum frequency was set at 10% because the amount of data is scarcest; and above 500 m, where the amount of data is higher, the threshold frequency was raised to 5%. This criterion also allows us to better appraise the seasonal variation and proximity to the coast.

As it was observed that the concentration of nitrate and phosphate were fairly constant from 500 m depth to the bottom of the water column, and because the amount of data below this depth is scarce, we also merged all data below 500 m to set the deep initial concentration ranges of phosphate and nitrate. In the case of silicate, only data from 1400 m depth to bottom were averaged to set the deep initial concentration ranges of silicate, because the frequency distributions of SLD from 1400 m to the bottom were similar.

Chl *a* concentration depends on the phytoplankton concentration, which grows exponentially, so the concentration intervals defined for the study of the frequency distributions were exponentially distributed. Since nitrite concentration in the NW Mediterranean Sea is tightly coupled to phytoplankton dynamics (Estrada, 1999; Lomas and Lipshultz, 2006), the concentration intervals for designing the frequency distributions of this parameter were also established exponentially.

Finally, the mean and standard deviation were calculated with the data included in the initial ranges at each SLD, and were used to calculate the final ranges using the following criteria. For stations with more than 200 m bottom depth (open sea and slope stations), the final concentration ranges included 2 standard deviations above and below the mean at each SLD, instead of 3 standard deviations as described in Conkright *et al.* (1994, 2000). However, to the first 100 m depth data, final ranges were calculated as 3 standard deviations because this layer, which includes the DCM, is biological and physically more dynamic. For stations with less than 200 m

bottom depth (continental shelf and coastal stations), to include possible variations due to terrestrial influences, the final concentration ranges were set to three standard deviations below 100 m depth and 4 standard variations in the upper 100 m depth. These final concentration ranges constitute the QC for data from the NW Mediterranean Sea. Each value in the database was flagged with a "0" if it was included within the final concentration ranges, with a "1" if it was above the upper limit, and with a "2" if it was below the lower limit.

Using these criteria, it was observed that data from the photic layer corresponding to mixed waters in winter were mostly flagged with "1," except for phosphate and dissolved oxygen, so we concluded that the obtained overall ranges could not be applied to mixed winter surface waters. To resolve this problem, the degree of stratification was calculated for each individual station of each cruise with the following methodology. First, the 100 m depth vertical stratification index (VDSI100) was calculated following Estrada (1999)

$$\text{VDSI100} = \sum_{i=1}^{n} N_i^2 \Delta z_i$$

where z is depth, N_i is the Brunt-Väisälä frequency (s^{-1}) between z_{i-1} and z_i, and n=100. N was calculated as in Pond and Pickard (1983)

$$N^2 = g*[-(1/\rho)*(\delta\sigma_t/\delta z)]$$

where g is the gravity $(m\ s^{-2})$, ρ is the density of the water, and σ_t is the density as σ-T (units of both are Kg m^{-3}). As expected, the calculated VDSI100 values showed a strong seasonal variability (Fig. 2). Then, we designed a stratification index based on the observations in Figure 2. Stations with VDSI100 lower than 5 (winter conditions) were given a "1," whereas those with a VDSI100 higher than 30 (summer conditions) were given a "3". Stratification indices "2" and "4," which correspond to spring and autumn transition periods, were assigned following the criteria described in Table 2. Each collected profile was thus identified with a stratification index (from 1 to 4) and data from profiles corresponding to 1 were used to set the winter concentration ranges. New QCs for stations with a stratification index of 1 were defined following the same methodology. However, due to the low amount of data, the initial ranges were set using values with a frequency higher than 10% at all the SLD.

Finally, because VDSI100 is calculated from variations in density in the water column, which depends on the T and S, an additional study about the contribution of T and S to the stratification was performed using the following equations:

$$N^2 = N^2_T + N^2_S$$
$$N^2_T = g*[-(1/\rho)*(\delta\rho/\delta T)*(\delta T/\delta z)]$$
$$N^2_S = g*[(1/\rho)*(\delta\rho/\delta S)*(\delta S/\delta z)]$$

RESULTS

Analytical precision

The analytical precisions estimated with duplicated analyses and QCC during the cruises, and following Garcia *et al.* (1998), are shown in Tables 3 and 4. The precision is presented as the standard deviation (s) and as the coefficient of variation (CV in %) of the analysis, because these are the customary ways to report it. However, one needs to be aware that the CV depends on the sample concentration, so the resulting value is less stable than the standard deviation value. It is because of this that in deep waters, where the concentrations are high and constant (CV data not shown), the lowest CV for phosphate, nitrate, silicate and dissolved oxygen analyses are obtained using the methodology of Garcia *et al.* (1998). This explains why in the same cruise, lower (better) precision values were found when data from the WMDW were used, whereas higher (worse) precision values resulted when data only from the core of the LIW or all data in the LIW were used. This can also be observed in all the cruises studied, when the precisions obtained using the different water masses are compared (Table 4).

The overall mean precision calculated for inorganic nutrients and dissolved oxygen analysis is lower when duplicated samples and QCC are used (last row in Table 3) than when it is calculated with WMDW data, following Garcia *et al.* (1998) (last row in Table 4), except for nitrite, whose averages are similar.

The precisions calculated using duplicated samples from the same sampling bottle were unusually high for all nutrients in the "Meso 95" cruise and for silicate in the "Varimed 95" cruise. Removing these unusual

results, we obtained the following ranges and means of analysis precisions using data from Table 3: phosphate <0.01-0.04 μM, mean 0.02 μM; nitrate 0.03-0.16 μM, mean 0.11 μM; nitrite <0.01-0.02 μM, mean 0.02 μM; ammonium 0.04-0.43 μM, mean 0.14 μM; silicate 0.02-0.25 μM, mean 0.12 μM; and dissolved oxygen 0.01-0.21 ml l^{-1}, mean 0.11 ml l^{-1}.

Analysis precision for chl *a* analyses could only be estimated following Garcia *et al.* (1998); its range throughout the cruises studied using data from the WMDW was 0.01-0.03 μg l^{-1} (Table 4).

Nutrient preservation 1. Laboratory experiment

Measurements of preserved and unpreserved nutrient samples were significantly correlated for all the nutrients ($P<0.01$, Table 5). Changes in nutrient concentrations due to the preservation process were studied with a non-parametric ANOVA test for phosphate, nitrite and ammonium, whose data sets were not normally distributed (Kolmogorov-Smirnov test: $P<0.01$ for phosphate and $P<0.05$ for nitrite and ammonium), and a parametric ANOVA test for nitrate and silicate. The chl *a* concentration range of the samples was 0.63-0.89 μg l^{-1}. Table 5 shows the mean concentration of preserved and unpreserved samples of our experiment. Nutrient concentrations were not significantly affected by the freezing process except for nitrite, which had a concentration significantly lower in preserved samples ($P<0.05$). However, ammonium results showed the highest relative error (18.2%) and the highest standard deviation among frozen samples (0.23 μM)(Table 5).

The standard deviation among frozen samples was higher than that calculated in fresh samples (Table 5), as has already been observed (DeGobbis, 1973; Gordon *et*

TABLE 3. – Analysis precision calculated using different methods: reported (1, PEPS Group, 1986) and calculated using different samples collected at the same depth (2, without any information about the Niskin bottle number); different samples collected from the same sampling Niskin bottle (3); different samples collected from different Niskin bottles closed at the same depth (4); consecutive analysis of the same sample (5); and QCC using standards inserted among the samples (6). s, standard deviation (in μM for nutrients and ml l^{-1} for dissolved oxygen); CV, coefficient of variation (in %); n, amount of data. The mean (last row) was calculated with all the calculated precisions.

Cruise	Method	Phosphate			Nitrate			Nitrite			Ammonium			Silicate			Dissolved oxygen		
		s	CV	n	s	CV	n	s	CV	n	s	CV	n	s	CV	n	s	CV	n
Fronts 89, 90, 91	1		10			1			0.1			1			0.1				
Varimed 93 I	2				0.08	9	16	0.01	16	16				0.15	9	16	0.21	4	6
Varimed 93 II	2				0.08	7	43	0.02	11	43				0.17	12	41	0.18	3	10
Varimed 93 III	2				0.16	12	172	0.02	12	173				0.12	9	173	0.16	3	57
Varimed 95	3	<0.01	7	22	0.08	13	22	0.01	9	22				0.52	38	22			
Meso 95	3				0.65	30	6	0.07	14	6				0.44	18	6	0.30	5	6
Varimed 95	4	0.01	12	64	0.15	22	64	0.02	12	64				0.17	27	64			
Meso 95	4																0.07	2	4
Fans 1	4	0.03	31	68	0.06	18	68	<0.01	7	68				0.07	5	68	0.07	1	15
Fans 2	4	0.02	24	276	0.09	3	324	0.01	5	324				0.10	3	324	0.01	0	38
Fans 3	4	0.04	36	52	0.09	12	126	0.01	11	125				0.12	5	134	0.07	1	40
Hivern 1999	5	0.01	8	252	0.08	4	226	0.01	9	254	0.04	5	254	0.16	5	235			
Hivern 2000	5	0.01	3	220	0.05	1	216	0.01	9	220	0.08	12	220	0.09	2	195			
Canyons I	5	0.02	6	289	0.08	6	297	0.01	8	296	0.05	11	297	0.12	10	296			
Canyons II	5	<0.01	7	290	0.07	5	291	<0.01	7	291				0.02	2	290			
Canyons II - IV	6	0.03	6	92	0.10	5	110	0.02	5	104	0.43	16	34	0.25	5	92			
Caco 1, 2	6	0.01	3	90	0.03	2	84	0.01	5	83	0.09	6	89	0.02	1	90			
Mean		0.02	23	1715	0.11	9	2065	0.02	9	2089	0.14	11	894	0.12	8	2046	0.11	2	176

TABLE 4. – Analysis precision estimated for each cruise and oceanographic variable using the methodology described in Garcia *et al.* (1988) using data from WMDW, from the core of the LIW (*), and using all LIW data (**). The mean value was calculated using all the data at the WMDW. s, standard deviation (same units as Table 4, µg l^{-1} for chl *a*); n, amount of data.

Cruise	Phosphate		Nitrate		Nitrite		Ammonium		Silicate		Dissolved O$_2$		Chlorophyll *a*	
	s	n	s	n	s	n	s	n	s	n	s	n	s	n
PEP 82	0.01	5	0.14	7	0.00	8	0.01*	4	0.30	8	0.13	8	0.02*	10
PEP 83	0.07	12	0.54	12	0.01	12	0.03*	15	0.53	12	0.06	12	0.00	8
PEP 84	0.03	10	0.57	10	0.02	10	0.14*	4	0.64*	46	0.28	10	0.01*	9
Fronts-3-85	0.12	13	0.45	13	0.01	13			0.44	13	0.16	13	0.01	7
Fronts-6-85	0.06	31	0.73	31	0.09**	16			0.75	31	0.23	31	0.01	14
PEP 86			0.46	36	0.02**	33	0.14	35	0.24	37	0.21	37	0.03	9
Fronts 11-86			0.13	6	0.00	31	0.04	6	0.22	6	0.01	6		
PEP 87	0.05	34	0.47	33	0.02	37	0.14*	12	0.31	34	0.04	34	0.02	5
Fronts 89	0.03 *	15	0.33*	16			0.27*	16	0.41*	16	0.08*	17	0.08**	61
Fronts 90	0.04 *	8	0.03	4	0.01	6	0.30*	8	0.65	6	0.03	6	0.00	4
Fronts 91			0.31**	71					0.20**	3	0.07**	3		
Fronts 92	0.04 *	15	0.47*	16			0.13*	10	0.48*	16	0.17*	16	0.07*	15
Varimed 93 I			0.28	115	0.02	34			0.35	113	0.05	117		
Varimed 93 II			0.14	25	0.01	4			0.56	25	0.21	34		
Varimed 93 III			0.10	23	0.06	115			0.43	23	0.10	7		
Varimed 95	0.02	68	0.08	73	0.01*	18			0.26	73			0.05**	111
Meso 95			0.45	98	0.06	23			0.55	99	0.14*	50		
Fans 1	0.02	15	0.04	15	0.01	73			0.06	15	0.18	10	0.06**	77
Fans 2	0.01	23	0.09	23	0.00*	43			0.20	23	0.05	26	0.05**	94
Fans 3	0.05	10	0.23	24	0.01	15			0.34	9	0.06*	27	0.07**	122
Hivern 1999	0.07	24	0.84	10	0.00	24	0.22*	14	1.09	17	0.07*	4	0.01*	4
Hivern 2000	0.05	94	0.91	93	0.01	10	0.31	94	1.11	94	0.13	62	0.02	34
Canyons I	0.05*	15	0.79	20	0.02	24	0.44*	15	0.99	20			0.13**	62
Canyons II	0.02*	20	1.02*	20	0.02	94			0.15	20			0.03**	65
Canyons III	0.04	17	0.86	17	0.01	20			0.71	17			0.03**	99
Canyons IV	0.04*	9	0.65*	9	0.03	17			0.28	9			0.04**	34
Caco 1	0.03	4	0.47	4	0.01	4	0.21	16	0.47	4			0.04**	72
Caco 2	0.02	3	0.15	3	0.00	3	0.30	16	0.18	3			0.05**	76
Mean (WMDW)	0.04	339	0.39	680	0.02	577	0.20	142	0.46	655	0.12	413	0.01	81

TABLE 5. – Summary of the results of the experiment on the freezing effect on seawater nutrient concentration (µM). *P*-value for all the correlations <0.01. * indicates the existence of statistically significant differences (*P*<0.05) between the mean concentration of freshly analyzed samples and that of frozen samples. n=16.

	Phosphate	Nitrate	Nitrite	Ammonium	Silicate
Correlation coefficient	0.97	0.99	0.80	0.96	0.96
Mean fresh concentration	0.16	1.88	0.35	2.14	1.96
Mean frozen concentration	0.17	1.85	0.31 *	2.53	1.91
Difference	0.01	0.03	0.04	0.39	0.05
Relative error frozen samples	6.3%	-1.6%	-11.4%	18.2%	-2.5%
SD fresh samples (nM)	5	10	5	77	42
SD frozen samples (nM)	15	107	22	227	118

al., 1993; Dore *et al.*, 1996). The standard deviation of freshly analyzed samples was similar to that calculated from QCC using standards, except for ammonium and silicate which had higher standard deviations (about 4 and 2.5 times higher respectively).

Nutrient preservation 2. Comparison of cruise samples: on board analysis versus frozen nutrient samples

The study of the existence of statistically significant differences between the concentration of preserved and unpreserved nutrient samples is difficult. Because the concentration of nutrients changes with depth and season, the water column nutrient concentration can be different. We therefore applied a factorial ANOVA test to the data, the factors being the preservation of the sample, the SLD, and the degree of stratification of the water column. The results showed that there were statistically significant differences in the concentration of nutrients between previously frozen samples and unpreserved ones (*P*>0.05). However a general pattern could not be found, because there were no differences at all the SLDs; when there were differences, sometimes the concentration was higher and sometimes it was lower.

Because the proportion of samples at each SLD is similar for cruises on which nutrient samples were frozen and those on which nutrient samples were analyzed on board, we estimated the relative error due to the freezing process in the same way as in the laboratory experiment. Table 6 shows the mean concentration of samples preserved and unpreserved for the cruises under study, as well as the relative error as a result of the preservation of the nutrient samples.

TABLE 6. – Summary of the results on the freezing effect on seawater nutrient concentration (μM) using the data of the cruises collected. *P*-value for all adjusted r^2<0.01. * indicates the existence of statistically significant differences (*P*<0.01) between the mean concentration of freshly (on board) analyzed samples and frozen nutrient samples. Nitrate average from frozen samples does not include 19 values higher than 15 μM from the "Hivern 1999" cruise.

	Phosphate	Nitrate	Nitrite	Ammonium	Silicate
Adjusted R²	0.51	0.80	0.18	0.15	0.78
Mean fresh concentration	0.18	3.06	0.13	0.48	2.91
Number of fresh samples	6065	10856	9865	2562	10897
Mean frozen concentration	0.19 *	3.23 *	0.10 *	0.96 *	2.97
Number of frozen samples	2928	3097	3119	2382	3116
Difference fresh-frozen	0.01	0.17	0.03	0.48	0.06
Relative error of frozen samples	6.2%	5.8%	-21.5%	102.1%	1.8%

Several trends were found that agree with the results of the laboratory experiment. First, the concentration of nitrite decreased, while the concentration of phosphate and ammonium increased. Second, the magnitude of the error was lower for silicate and nitrate, the same for phosphate, and higher for nitrite and ammonium.

For phosphate, the difference of mean concentrations was the same as that calculated in the laboratory experiment, and was similar to the mean analysis precision (Table 3, last row). In addition, the relative error was also the same (6%, Tables 5 and 6). For ammonium, the mean concentration of preserved samples was twice that of unpreserved ones (relative error 102%). The mean concentration of nitrite in preserved samples was 0.03 μM lower than the mean concentration in unpreserved samples, the same as in the laboratory experiment. However, as the mean value was lower, the relative error was higher (21.5%).

For silicate, differences between the mean concentration of preserved and unpreserved samples were not statistically significant. The differences between means and the relative error were similar to those found in the laboratory experiment (0.05-0.06 μM and 2% respectively), but positive (Tables 5 and 6).

Preserved nitrate samples also behaved contrary to what was found in the laboratory experiment: the mean concentration increased in 0.3 μM, which represented a relative error of 9.2%. However, nitrate concentrations of the "Hivern 1999" cruise, on which nutrient samples were preserved, contained data higher than 15 μM that could not be found in any of the other cruises. After they had been removed from the analysis, the mean nitrate concentration from preserved samples was 3.23 μM, and the relative error was lowered to 5.8% (n=3097).

Due to the high error found when ammonium samples are preserved, comparing data from the cruises and from the results of the laboratory experiment, data of ammonium concentration from preserved samples were not used to define the QC concentration ranges of ammonium in the NW Mediterranean Sea.

Water column stratification

According to our results, the contribution of the T to the stratification of the water column is higher than the contribution of S (75% and 25%, respectively).

These results are consistent with previous studies that state that the vertical stratification is controlled by the T (Velásquez, 1997). During spring and summer, the upper layer is warmed and a mixed layer is formed. This mixed layer is broken up in autumn and winter because of convective processes (vertical mixing), due to an increase in the wind intensity as well as storms (Salat, 1996; Send *et al.*, 1999). During winter, the T of the whole water column is around 12°C, and even thermal inversion can be observed (Salat, 1996). Therefore, when the water mass was highly mixed, the T contribution to the stratification decreased, but it was still higher than the S contribution. While highest surface S values were found in winter, in general this variable does not show such evident changes throughout the year as T. The highest S values in winter are a consequence of the evaporation due to the effect of strong winds, and due to mixing with deeper waters that are saltier than the surface ones (Salat, 1996). Only the S contribution was important to the vertical stratification at stations close to the coast and to the Ebro Delta.
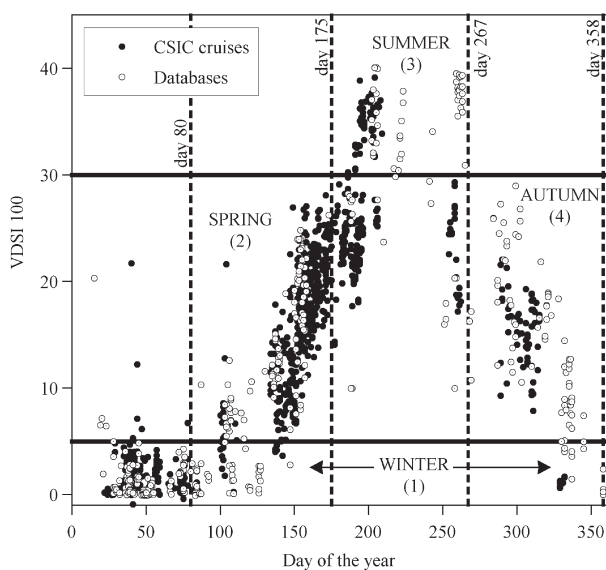

FIG. 2. – Variation throughout the year of the vertical stratification index at 100 m. CSIC Cruises, data from the cruises gathered for this study; Databases, data from MEDATLAS and MATER databases. Between brackets, stratification index that represents each season of the year.

TABLE 7. – Phosphate concentration (µM) ranges in the NW Mediterranean Sea. SLD, Standard Level Depth; LR, Lower Range; UR, Upper Range; Coast and continental shelf stations have a bottom depth less than 200 m. Slope and open sea stations have a bottom depth larger than 200 m. n, amount of data within the range; % n range, proportion of the total amount of data included in the range at each SLD.

| SLD | Slope and open sea | | Coast and continental shelf | | n range | % n range |
	LR	UR	LR	UR		
0	0.00	0.30	0.00	0.37	357	90.2
5	0.00	0.30	0.00	0.37	449	93.5
10	0.00	0.31	0.00	0.39	298	87.9
20	0.00	0.30	0.00	0.37	472	90.1
30	0.00	0.29	0.00	0.36	498	92.6
40	0.00	0.30	0.00	0.37	497	90.5
50	0.00	0.29	0.00	0.36	486	92.6
60	0.00	0.34	0.00	0.41	407	90.4
80	0.00	0.36	0.00	0.44	896	89.3
100	0.00	0.42	0.00	0.51	809	89.8
120	0.06	0.43	0.00	0.52	300	90.6
160	0.06	0.44	0.00	0.54	368	89.3
200	0.10	0.49	0.03	0.58	527	89.5
250	0.13	0.50			195	88.6
300	0.17	0.52			289	79.8
400	0.28	0.51			339	73.4
500	0.30	0.52			205	67.4
600	0.32	0.52			191	79.9
800	0.31	0.52			196	81.3
1000	0.30	0.50			128	71.1
1200	0.29	0.49			37	82.2
1400	0.32	0.50			16	55.2
1600	0.31	0.50			49	83.1
1800	0.30	0.49			22	91.7
2000	0.29	0.48			39	90.7
2500	0.31	0.50			21	100.0
≥ 500	0.31	0.51			904	76.3
Total					8091	87.3

TABLE 8. – Nitrate concentration (µM) ranges in the NW Mediterranean Sea in stratified water columns (stratification coefficient = 2, 3 and 4). Headers as in Table 7.

| SLD | Slope and open sea | | Coast and continental shelf | | n range | % n range |
	LR	UR	LR	UR		
0	0.00	0.76	0.00	0.95	234	93.2
5	0.00	0.85	0.00	1.07	472	84.3
10	0.00	0.78	0.00	0.98	342	94.5
20	0.00	0.83	0.00	1.04	512	88.0
30	0.00	0.86	0.00	1.07	483	88.8
40	0.00	1.36	0.00	1.71	529	81.1
50	0.00	1.98	0.00	2.49	457	82.2
60	0.00	4.25	0.00	5.29	550	82.5
80	0.00	7.64	0.00	9.31	1106	91.4
100	0.13	7.95	0.00	9.26	852	84.6
120	2.86	7.74	1.64	8.95	334	85.9
160	3.15	8.26	1.87	9.53	344	84.7
200	3.50	8.96	2.13	10.32	520	85.4
250	5.13	9.17			153	84.1
300	5.41	9.79			351	86.9
400	6.87	9.68			362	79.6
500	7.83	9.59			270	72.4
600	8.16	9.37			138	61.3
800	8.17	9.40			196	74.2
1000	8.14	9.33			164	66.9
1200	8.19	9.16			35	70.0
1400	7.92	9.27			27	81.8
1600	8.01	9.44			57	68.7
1800	8.06	928			20	76.9
2000	8.05	9.32			35	79.5
2500	8.00	9.07			17	89.5
≥ 500	8.03	9.43			959	70.4
Total					8560	83.9

The stratification of the water column starts at the beginning of April, and highly stratified stations can already be observed at the beginning of July (Fig. 2). The stratification lasts between 4 and 5 months from the start of its formation (around the 100th day of the year) to the beginning of its disappearance (around the 250th day), the same length as described for the thermocline presence in the Mediterranean Sea (Duarte *et al.*, 1999).

QC concentration ranges

Phosphate concentration in open sea surface waters (0-50 m) of the NW Mediterranean Sea ranged between 0.0 and 0.3 µM (Table 7). From 50 to 400-500 m depth, the concentration increased monotonically towards the maximum ranges measured in the NW Mediterranean Sea. The concentration range from 400-500 m depth to the bottom of the basin was between 0.3 and 0.5 µM. In coastal and continental shelf waters, concentration ranges followed the same pattern, except that the upper limit was 0.1 µM higher, and the lower one was 0.0 µM at all the SLDs.

On the continental shelf, phosphate data above the described ranges were located in front of the Ebro Delta, as well as in front of the city of Barcelona. At open sea stations, there was no clear pattern of localization of values above and below the phosphate concentration ranges found.

At stratified stations, nitrate concentration ranges in open sea surface waters above 30 m depth in the NW Mediterranean Sea were between 0.0 and around 0.8 µM (Table 8). From 40 to 300 m the concentration increased constantly and the ranges became narrower. Below 500 m, nitrate concentration ranges showed little variation, between 8.0 and 9.4 µM. In coastal and continental shelf waters, the upper limit of the nitrate concentration range increased up to 1.0 µM at the surface and up to 10.3 µM at 200 m depth.

In mixed water columns, surface nitrate concentration ranges were wider than those described above, and nitrate concentrations were between 0.0 and 3.5 µM (Table 9). Nitrate concentration ranges in deep waters were similar to those described for the rest of the year. Nitrate concentration ranges in mixed water column stations in coastal and continental shelf surface waters (0–30 m depth) were from 0.0 to 4-4.5 µM. At 200 m depth, the concentration ranges were between 1.3 and 7.8 µM (Table 9).

Coastal and continental shelf stations with nitrate data above the ranges were located near the Ebro Delta. Outside the continental shelf in front of the city of Tarragona (see Fig. 1), where the continental shelf widens, data from several cruises ("Fronts 89", "Meso 95", "Fans 1", "Fans 2", "Fans 3", "Caco 1" and "Caco 2") were below the described ranges at all depths.

TABLE 9. – Nitrate concentration (µM) ranges in the NW Mediterranean Sea in mixed water columns (stratification coefficient = 1). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	3.58	0.00	4.48	49	100.0
5	0.00	3.48	0.00	4.25	104	79.4
10	0.00	3.31	0.00	4.12	48	96.0
20	0.00	3.31	0.00	4.09	101	80.8
30	0.00	3.70	0.00	4.50	71	93.4
40	0.00	4.36	0.00	5.25	122	79.7
50	0.00	3.94	0.00	4.78	40	97.6
60	0.00	4.91	0.00	5.81	104	81.3
80	0.00	5.48	0.00	6.51	146	82.5
100	0.00	5.99	0.00	7.01	122	72.2
120	0.80	5.78	0.00	7.03	19	65.5
160	1.69	6.17	0.57	7.30	59	72.8
200	2.41	6.70	1.34	7.78	77	59.7
250	3.69	7.24			21	60.0
300	4.80	9.06			57	59.4
400	6.56	9.49			84	74.3
500	7.68	9.27			44	51.2
600	7.55	9.15			17	40.5
800	8.29	9.10			27	51.9
1000	8.17	8.92			28	43.8
1200	8.32	8.85			12	75.0
1400	8.10	8.69			5	62.5
1600	8.00	8.82			9	40.9
1800						
2000	8.01	8.93			4	36.4
2500	7.96	9.14			4	66.7
≥ 500	7.90	9.13			150	48.4
Total					1374	72.6

TABLE 10. – Nitrite concentration (µM) ranges in the NW Mediterranean Sea in stratified water columns (stratification coefficient = 2, 3 and 4). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	0.19	0.00	0.25	370	96.6
5	0.00	0.22	0.00	0.27	591	94.6
10	0.00	0.18	0.00	0.23	359	96.8
20	0.00	0.21	0.00	0.26	576	93.2
30	0.00	0.23	0.00	0.29	628	94.4
40	0.00	0.30	0.00	0.37	639	95.8
50	0.00	0.42	0.00	0.53	653	97.3
60	0.00	0.47	0.00	0.59	575	96.5
80	0.00	0.42	0.00	0.52	1210	93.9
100	0.00	0.31	0.00	0.38	1039	93.6
120	0.00	0.21	0.00	0.28	399	95.2
160	0.00	0.16	0.00	0.21	475	94.2
200	0.00	0.16	0.00	0.21	605	89.4
250	0.00	0.09			246	89.1
300	0.00	0.09			376	83.0
400	0.00	0.09			443	87.5
500	0.00	0.10			337	78.4
600	0.00	0.08			230	79.0
800	0.00	0.08			243	77.4
1000	0.00	0.08			183	71.8
1200	0.00	0.08			42	80.8
1400	0.00	0.09			22	66.7
1600	0.00	0.08			60	73.2
1800	0.00	0.07			17	65.4
2000	0.00	0.08			40	90.9
2500	0.00	0.07			16	84.2
≥ 500	0.00	0.08			1164	75.3
Total					10374	91.2

TABLE 11. – Nitrite concentration (µM) ranges in the NW Mediterranean Sea in mixed water columns (stratification coefficient = 1). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	0.38	0.00	0.47	45	91.8
5	0.00	0.31	0.00	0.38	137	95.8
10	0.00	0.50	0.00	0.62	48	100.0
20	0.00	0.46	0.00	0.56	116	93.5
30	0.00	0.46	0.00	0.56	62	84.9
40	0.00	0.45	0.00	0.53	129	83.8
50	0.00	0.59	0.00	0.69	34	82.9
60	0.00	0.51	0.00	0.61	101	84.9
80	0.00	0.38	0.00	0.45	127	73.0
100	0.00	0.36	0.00	0.43	140	82.8
120	0.00	0.33	0.00	0.42	30	90.9
160	0.00	0.26	0.00	0.33	85	94.4
200	0.00	0.28	0.00	0.37	99	78.0
250	0.00	0.11			42	87.5
300	0.00	0.10			90	87.4
400	0.00	0.07			95	79.8
500	0.00	0.08			87	87.9
600	0.00	0.07			39	79.6
800	0.00	0.07			49	83.1
1000	0.00	0.06			54	84.4
1200	0.00	0.03			16	100.0
1400	0.00	0.05			5	100.0
1600	0.00	0.05			17	77.3
1800	0.00	0.07			3	75.0
2000	0.00	0.06			9	81.8
2500	0.00	0.05			4	66.7
≥ 500	0.00	0.06			271	80.9
Total					1663	85.3

Nitrite surface concentration ranges at open sea stratified stations, between 0 and 30 m depth, were between 0.0 and 0.2 µM (Table 10). There was a maximum of nitrite concentration between 60 and 80 m depth with a range of concentration of 0.0 to 0.5 µM. Below 250 m depth the concentration range was constant, from 0.0 to less than 0.1 µM. In coastal and continental shelf areas the surface concentration ranged from 0.0 to 0.3 µM and the maximum upper limit was 0.6 µM (Table 10).

In mixed water columns, open ocean surface nitrite concentration ranges were between 0.0 and 0.4-0.5 µM (Table 11). The maximum concentration was located at 50 m depth, where the upper limit range reached 0.6 µM. The deep nitrite concentration range was the same as during the rest of the year, from 0.0 to less than 0.1 µM. At coastal and continental shelf stations, the surface ranges increased up to 0.4-0.5 µM, and the maximum upper limit concentration was 0.7 µM.

Nitrite data above the ranges from stations on the continental shelf were situated mainly in front of the Ebro Delta and in front of the cities of Barcelona and Palma (Fig. 1). At open sea stations, data above the ranges were observed at stations located on submarine canyons.

At open sea stratified stations, ammonium concentration ranges from the surface to 30 m depth were between 0 and 0.8-1.0 µM (Table 12). Below 30 to 100 m depth the ranges became narrower, from 0.0 to 0.5

TABLE 12. – Ammonium concentration (µM) ranges in the NW Mediterranean Sea in stratified water columns (stratification coefficient = 2, 3 and 4). Below 500 m depth, data was gathered only from stations with stratification index 2, so they could not be representative for other stratification conditions. Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	0.81	0.00	1.00	101	73.7
5	0.00	0.51	0.00	0.63	61	100.0
10	0.00	1.00	0.00	1.23	114	82.6
20	0.00	0.88	0.00	1.08	107	77.5
30	0.00	0.95	0.00	1.16	116	79.5
40	0.00	0.79	0.00	0.97	100	65.4
50	0.00	0.72	0.00	0.88	142	75.5
60	0.00	0.67	0.00	0.83	139	73.2
80	0.00	0.69	0.00	0.84	250	72.9
100	0.00	0.70	0.00	0.87	181	74.8
120	0.00	0.52	0.00	0.68	79	73.8
160	0.00	0.50	0.00	0.66	59	80.8
200	0.00	0.63	0.00	0.81	69	64.5
250	0.00	1.01			21	100.0
300	0.00	1.07			38	70.4
400	0.00	0.92			46	85.2
500	0.04	1.09			29	64.4
600	0.00	1.36			6	100.0
800	0.00	1.19			12	92.3
1000	0.04	1.13			13	100.0
1200						
1400	0.00	1.40			5	100.0
1600	0.00	1.34			7	100.0
1800	0.87	1.06			2	100.0
2000					1	100.0
2500						
Total					1698	75.7

TABLE 13. – Ammonium concentration (µM) ranges in the NW Mediterranean Sea in mixed water columns (stratification coefficient = 1). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.02	1.22	0.00	1.51	12	92.3
5						
10	0.04	1.11	0.00	1.37	13	86.7
20	0.15	1.30	0.00	1.59	14	93.3
30	0.00	2.03	0.00	2.56	14	100.0
40	0.05	2.05	0.00	2.55	13	86.7
50	0.04	2.05	0.00	2.55	16	100.0
60	0.00	2.18	0.00	2.82	11	91.7
80	0.00	1.94	0.00	2.45	19	95.0
100	0.00	2.17	0.00	2.75	16	94.1
120	0.42	1.39	0.00	1.88	11	91.7
160	0.53	1.18	0.00	1.50	4	80.0
200	0.26	1.24	0.00	1.74	12	85.7
250						
300	0.71	1.77			4	80.0
400	0.21	1.43			10	100.0
500	0.70	1.61			3	100.0
600						
800						
1000						
1200						
1400						
1600						
1800						
2000						
2500						
Total					172	92.5

µM. From 250 to 500 m depth ammonium concentration ranges became wider again, and below 500 m the concentrations ranged from 0.0 to 1.0-1.4 µM. At coastal and continental shelf stations, surface concentrations ranged from 0.0 to 1.0-1.2 µM. From 40 to 200 m depth, the upper limit range decreased to 0.6 µM.

In mixed water columns, the ammonium surface concentration ranges were more variable, and ran from 0.0-0.1 µM to 1.0-2.0 µM (Table 13). Ammonium concentration in mixed water columns for coastal and continental shelf stations ranged from 0.0 to 1.1-1.2 µM in surface waters and up to 2.5-2.9 µM between 30 and 80 m depth.

High ammonium concentrations in deep waters are unexpected in an oxic environment like the NW Mediterranean Sea, and the origin of such high values remains unclear. Also, the low ammonium data makes it difficult to extrapolate, from the ranges of concentration, any feature in the vertical distribution of ammonium. Ammonium data above the ranges were located mainly in the upper 200 m depth, and were spread over the continental shelf. At the open sea, data above the ranges were also highly scattered.

Surface (30-40 m depth) silicate concentrations at open-sea stratified stations of the NW Mediterranean Sea ranged from 0.0 to 2.5 µM (Table 14). From surface, silicate concentration ranges steadily increased to reach values of between 7.7 and 10.4 µM below 1400

TABLE 14. – Silicate concentration (µM) ranges in the NW Mediterranean Sea in stratified water columns (stratification coefficient = 2, 3 and 4). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	2.58	0.00	3.15	422	94.6
5	0.00	2.45	0.00	2.99	581	88.4
10	0.00	2.59	0.00	3.17	435	94.8
20	0.00	2.79	0.00	3.39	631	92.1
30	0.00	2.67	0.00	3.22	699	91.4
40	0.00	2.91	0.00	3.51	640	85.1
50	0.00	3.37	0.00	4.06	731	94.7
60	0.00	4.19	0.00	5.02	610	90.6
80	0.00	4.57	0.00	5.40	1330	92.3
100	0.16	5.15	0.00	5.98	1131	92.6
120	1.43	5.15	0.59	5.98	438	92.6
160	1.64	5.29	0.73	6.20	491	93.3
200	2.17	6.03	1.20	6.99	680	87.5
250	3.07	6.43			250	86.2
300	4.17	7.36			391	78.2
400	4.75	8.36			505	89.2
500	5.75	9.09			395	84.0
600	5.76	8.90			255	84.2
800	6.54	9.49			246	72.6
1000	6.48	10.49			218	80.7
1200	7.10	10.65			35	61.4
1400	7.43	10.85			24	63.2
1600	7.63	10.47			59	64.1
1800	8.04	10.51			23	74.2
2000	7.92	10.01			37	72.5
2500	7.87	10.22			19	90.5
≥ 1400	7.74	10.41			162	52.9
Total					11276	89.0

TABLE 15. – Silicate concentration (µM) ranges in the NW Mediterranean Sea in mixed water columns (stratification coefficient = 1). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	4.22	0.00	5.00	59	93.7
5	0.00	4.78	0.00	5.73	108	76.1
10	0.00	3.99	0.00	4.72	60	95.2
20	0.00	3.83	0.00	4.54	113	80.7
30	0.00	5.14	0.00	6.08	87	96.7
40	0.00	5.33	0.00	6.36	143	86.7
50	0.00	4.24	0.00	4.96	48	84.2
60	0.00	5.20	0.00	6.05	95	74.8
80	0.00	5.22	0.00	6.13	144	73.8
100	0.00	5.78	0.00	6.80	151	80.3
120	0.81	4.86	0.00	5.87	37	84.1
160	1.26	4.90	0.35	5.81	68	70.8
200	2.77	5.64	2.05	6.36	80	54.4
250	3.40	5.77			31	60.8
300	4.34	6.74			48	42.1
400	4.96	8.83			86	66.7
500	5.46	9.19			67	61.5
600	5.26	8.89			32	61.5
800	6.91	8.84			27	41.5
1000	6.79	9.09			33	47.8
1200	7.54	9.25			2	10.5
1400	7.67	10.27			7	70.0
1600	8.36	10.65			11	44.0
1800	8.88	10.73			4	57.1
2000	8.20	11.12			6	42.9
2500	8.21	10.89			6	75.0
≥ 1400	8.35	10.73			32	50.0
Total					1553	70.9

TABLE 16. – Dissolved oxygen (ml l⁻¹) ranges in the NW Mediterranean Sea. Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	4.22	6.63	3.82	7.03	532	96.9
5	4.20	6.60	3.80	7.00	476	95.2
10	4.50	6.52	4.16	6.86	523	92.1
20	4.58	6.60	4.24	6.94	623	89.6
30	4.73	6.77	4.39	7.11	710	92.1
40	4.78	6.77	4.45	7.10	505	91.2
50	4.68	6.75	4.33	7.10	688	91.6
60	4.50	6.55	4.15	6.89	483	93.6
80	4.46	6.23	4.16	6.53	1079	89.0
100	4.48	5.99	4.18	6.27	999	91.7
120	4.48	5.67	4.18	5.97	417	91.9
160	4.32	5.61	4.00	5.93	525	95.1
200	4.23	5.43	3.93	5.73	700	91.7
250	4.08	5.34			390	95.4
300	4.02	5.17			553	94.0
400	3.92	5.03			565	94.3
500	3.92	4.89			501	92.3
600	3.98	4.93			316	92.1
800	4.05	4.86			411	96.0
1000	4.25	4.85			294	90.5
1200	4.28	4.82			136	88.3
1400	4.33	4.81			109	95.6
1600	4.40	4.82			128	94.1
1800	4.40	4.87			72	94.7
2000	4.46	4.80			86	94.5
2500	4.44	4.78			68	98.6
Total					11889	92.5

TABLE 17. – Chl *a* concentration (µg l⁻¹) ranges in the NW Mediterranean Sea in stratified water columns (stratification coefficient = 2, 3 and 4). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	0.83	0.00	1.03	385	90.8
5	0.00	1.05	0.00	1.31	294	88.6
10	0.00	1.08	0.00	1.36	331	94.3
20	0.00	1.74	0.00	2.17	414	96.1
30	0.00	1.36	0.00	1.71	520	97.2
40	0.00	1.78	0.00	2.19	478	94.8
50	0.00	1.70	0.00	2.09	508	93.7
60	0.00	1.70	0.00	2.07	429	94.7
80	0.00	1.29	0.00	1.57	932	92.5
100	0.00	0.92	0.00	1.13	688	83.2
120	0.00	0.35	0.00	0.45	242	96.4
160	0.00	0.26	0.00	0.35	234	94.4
200	0.00	0.16	0.00	0.21	271	91.2
250	0.00	0.18			53	86.9
300	0.00	0.10			39	73.6
400	0.00	0.05			67	91.8
500	0.00	0.05			24	77.4
600	0.00	0.05			10	83.3
800	0.00	0.05			5	62.5
1000	0.00	0.03			5	45.5
1200	0.00	0.00			1	100.0
1400						
1600	0.00	0.00			1	100.0
1800						
2000						
2500						
Total					5931	91.9

m. At coastal and continental shelf stations, silicate in surface waters ranged from 0.0 to 3.0-3.2 µM. The lower limit was 0.0 µM from surface to 100 m depth, increasing up to 1.2 µM at 200 m depth. The upper limit range increased steadily from the surface, reaching values of 7.0 µM at 200 m depth.

In mixed water columns, silicate concentration ranges in the open ocean in the first 100 m depth were wider than the ranges at stratified stations, from 0.0 to 5.8 µM. From 120 m they increased linearly with depth to reach maximum values of 5.5-9.2 µM at 500 m depth (Table 15). Winter ranges in deep waters were similar to those of the rest of the year (8.4-10.7 µM). For coastal and continental shelf stations, upper limit ranges were between 4.5 and 6.0 µM and did not show a clear pattern. The lower limit was 0.0 µM from surface waters to 120 m depth, increasing to 2.0 µM at 200 m depth.

Silicate data above the described concentration ranges were located in front of the Ebro Delta and in front of the city of Palma, both on the continental shelf and slope. In the open sea area, values below the range were mostly found inside submarine canyons.

Open sea surface dissolved oxygen concentration ranges in the NW Mediterranean Sea were between 4.2 and 6.6 ml l⁻¹ (Table 16). Dissolved oxygen concentration increased with depth up to 40 m, where the concentration range was 4.8 to 6.8 ml l⁻¹. Below this depth, the ranges decreased to 400-500 m depth, where a lower limit of 3.9 ml l⁻¹ was found. Finally, from 500

TABLE 18. – Chl *a* concentration (µg l⁻¹) ranges in the NW Mediterranean Sea in mixed water columns (stratification coefficient = 1). Headers as in Table 7.

SLD	Slope and open sea		Coast and continental shelf		n range	% n range
	LR	UR	LR	UR		
0	0.00	2.01	0.00	2.31	41	66.1
5	0.00	1.78	0.00	2.12	91	77.8
10	0.00	2.15	0.00	2.53	54	79.4
20	0.00	2.29	0.00	2.73	110	85.3
30	0.00	2.14	0.00	2.56	69	81.2
40	0.00	2.06	0.00	2.46	128	82.1
50	0.00	2.20	0.00	2.60	49	81.7
60	0.00	1.10	0.00	1.31	78	70.3
80	0.00	0.99	0.00	1.18	125	71.0
100	0.00	0.77	0.00	0.92	106	69.7
120	0.00	0.42	0.00	0.53	20	60.6
160	0.00	0.39	0.00	0.51	61	84.7
200	0.00	0.41	0.00	0.53	78	83.9
250	0.00	0.31			21	84.0
300	0.00	0.21			17	60.7
400	0.00	0.06			17	89.5
500	0.00	0.10			7	70.0
600	0.00	0.05			6	75.0
800					0	0.0
1000					0	0.0
1200						
1400						
1600						
1800						
2000						
2500					0	0.0
Total					1078	76.6

m to the bottom, concentration increased slightly with depth to reach values of 4.4-4.8 ml l⁻¹. Concentration ranges at coastal and continental shelf stations were from 4.2-7.0 ml l⁻¹ in surface waters to 3.9-5.7 ml l⁻¹ at 200 m depth. The maximum upper limit found was 7.1 ml l⁻¹. This value is close to the upper limit proposed in the MEDATLAS database (7.0 ml l⁻¹, Medar Group, 2002). The few dissolved oxygen data above the ranges encountered on the continental shelf were located in front of the Ebro Delta and the city of Barcelona.

Surface chl *a* concentration ranges in open sea stratified waters from the NW Mediterranean Sea were around 0.0 and 1.0 µg l⁻¹ (Table 17). The lower limit was 0.0 µg l⁻¹ for all the SLDs. The maximum values were found between 40 and 60 m depth (the usual depth of the DCM in the NW Mediterranean Sea), where the upper limit concentration ranges were 1.7-1.8 µg l⁻¹. Below 200 m, the observed chl *a* concentration range

was between 0.0 and 0.05 µg l⁻¹, although there were very few available data. At coastal and continental shelf stations, the upper limit of the surface concentration ranges increased from 1.0 to 1.7-2.2 µg l⁻¹ at the DCM. Below this, the upper limit decreased with depth to reach 0.21µg l⁻¹ at 200 m.

In mixed water columns, the chl *a* concentration ranges in surface open seawaters rose to 2.3 µg l⁻¹ (Table 18). The concentration then decreased steadily with depth from 20 to 120 m, where values were 0.0-0.4 µg l⁻¹. In deep waters, concentration ranges were similar to those described for the rest of the year. At coastal and continental shelf stations, the winter upper limit for chl *a* concentration ranges was higher than 2.0 µg l⁻¹ from surface waters to 50 m depth. The maximum value found was 2.7 µg l⁻¹. At 200 m depth, the chl *a* upper limit range was 0.5 µg l⁻¹. The highest chl *a* concentrations were found in front of the Ebro Delta, in front of the cities of Barcelona and Palma and inside some submarine canyons.

The proportion of data within the quality control ranges for each variable from the cruises gathered, as well as from the historical databases, is shown in Table 19, which also shows the proportions of data outside the ranges from open sea versus coast and continental shelf stations, and from preserved samples versus samples analyzed on board.

Using the data included within the QC ranges of concentration described in the present paper, vertical profiles for the different oceanographic variables have been obtained and are described elsewhere (Segura-Noguera *et al.*, submitted).

DISCUSSION

Analytical precision

It would be desirable for all the analytical data reported elsewhere to also incorporate information about some basic parameters, such as the limit of detection and the analysis precision. This was not the case with most of the data gathered, so we estimated the analytical precision using different methodologies, according to the data available for each cruise.

Our results indicate that standard deviations estimated following Garcia *et al.* (1998) were usually higher than those calculated from repeated analyses

TABLE 19. – Proportion (%) of the data gathered that is found within and outside the quality control ranges defined in this study.

	Within the QC Ranges		Outside the QC Ranges			
	Cruises	Databases	Open Sea and Slope	Coast and Cont. Shelf	Samples analyzed on board	Frozen samples
Phosphate	91.9	93.4	7.0	1.1	9.8	8.0
Nitrate	90.8	84.4	8.6	0.6	7.6	22.7
Nitrite	91.4	97.1	7.1	1.4	11.9	6.4
Ammonium	73.5	57.2	17.1	9.3	17.1	34.6
Silicate	94.4	96.4	5.2	0.4	5.2	10.8
Dissolved oxygen	95.2	94.9	4.6	0.2		
Chlorophyll *a*	94.5	96.2	5.3	6.0		

of the same sample or from standards, because they included a component of natural variability. This component was even more evident when we compared the precision estimated using data in the different water masses: better precision values were obtained using data in the WMDW, which is the most stable water mass, physically and chemically. Worse values were obtained using data from the core of the LIW or using all data sampled in LIW. This is because this water type (the core of the LIW) and water mass (the whole LIW) can be present in the NW Mediterranean Sea under different degrees of modification after its formation in the eastern Mediterranean Sea or other areas of the Mediterranean Sea (Emelianov *et al.*, 2006); it is physically less stable than WMDW. Furthermore, at the interval of 200-600 m depth where the LIW is detected, the remineralization and dissolution processes are still active, so nutrient and dissolved oxygen concentrations are not constant. Thus, if the precision must be estimated following Garcia *et al.* (1998), we recommend using data from WMDW but not from the other water masses of the NW Mediterranean Sea.

Better precisions were obtained with the other methods used to estimate the analytical precision (Table 3). Again, a component of natural variability can be observed when the precision is calculated from different bottles of the same water mass, rather than when it is calculated from different samples from the same Niskin bottle, as can be observed in the "Varimed 95" cruise (Table 3). Another factor that could have an influence on the variation of the estimated analytical precision is the analyst (Hager *et al.*, 1972).

Finally, traceability is another chemical parameter that should be taken into account in studies about the quality of the data. Even if the precision and the limit of detection of an analysis are acceptable, a systematic error can be present (e.g. by using a contaminated baseline or reagents). In this case, all the analyses will be deviated from the real value in the same way. The standards from which the calibration line is calculated are the most important source of systematic errors in nutrient analysis (Gordon *et al.*, 1993). The best way to know whether the results obtained are true is to use certified reference material (Riu *et al.*, 2002), but the participation in intercalibration experiments also gives an idea of how exact the laboratory analyses are. The only traceable nutrient values are those provided by the Department of Aquatic Biogeochemistry (CEAB-CSIC, analysts 2 and 3 in Appendix 2), which participated in an interlaboratory experiment and demonstrated a high accuracy in the analysis (Aminot and Kirkwood, 1995).

Nutrient preservation

According to Chapman and Mostert (1990), freezing is a suitable method for nutrient preservation if the study can support errors as high as 10%, for example, in studies dealing with anthropogenic contamination.

However, for open deep sea water studies that require higher precision or studies of nutrient limitation in euphotic layers where concentrations are low, nutrient analyses should be done immediately.

Our laboratory experiment results indicate that freezing is a valid preservation method that did not significantly affect the concentration of phosphate, nitrate and silicate in samples coming from historical cruises in the NW Mediterranean Sea. The analysis errors calculated for preserved samples are 6.3% for phosphate, 1.6% for nitrate and 2.5% for silicate. These results are consistent with the results from the comparison of preserved and unpreserved nutrient samples from cruises in the NW Mediterranean Sea and with those from studies performed at other locations (Stéfansson and Richards, 1963; Strickland and Parsons, 1972; Riley *et al.* 1975; Chapman and Mostert, 1990; Avanzino and Kennedy, 1993; Valderrama, 1995; Dore *et al.*, 1996; Krom *et al.*, 2005).

By contrast, nitrite and ammonium concentrations in samples from the NW Mediterranean Sea are significantly altered by freezing, as has been observed previously in other regions (Carpenter and McCarthy, 1975; Eppley *et al.*, 1977). The relative errors are higher than those found for the other nutrients: 11.4% for nitrite analysis and 18.2% for ammonium analysis. However, the decrease in nitrite concentration due to freezing (0.04 μM) is similar to the baseline contamination estimated from the use of ASW made with NaCl diluted in double-distilled water (0.03-0.06 μM). Thus, it is not clear whether the different concentrations found for preserved and unpreserved nitrite samples are the consequence of both preservation procedure and differences in the nitrite concentration in ASW. It is also possible that nitrite had been quickly oxidized to nitrate during the realization of the experiment or during the handling of the cruise samples.

The increase in ammonium concentration (0.39 μM) is higher than the contamination introduced by the use of ASW (0.23-0.26 μM) and close to the upper limit of the precision calculated (0.04-0.43 μM—this last parameter calculated for cruises on which nutrient samples were frozen, as can be observed in Table 3). It is evident that the ammonium concentration was affected by the analysis baseline used, as well as by freezing of the samples. The error in the analysis is much higher when it is calculated with data from the cruises (102%, Table 6). Moreover, the ammonium data gathered that has been frozen previous to analysis showed the highest percentage (35%) of data outside the resulting QC ranges of concentration among the studied variables (Table 19). Thus, we do not recommend using historical ammonium data from samples that have been preserved by freezing, because both the accuracy and the precision of the analysis are significantly affected. If nutrient samples need to be frozen, it is important, both for ammonium and nitrite analyses, to establish which part of the error is due to the use of ASW and/ or distilled water as a baseline, and which is caused

by the freezing process, including the handling of the samples. For a revision of storage, pre-treatment of the samples and the risk of contamination during analysis, it is recommended to read Aminot *et al.* (2009).

The results of the comparison of preserved and unpreserved samples from cruises were used only as a reference. This is because it is not realistic to assume that the precautions taken in the laboratory experiment during the sampling process and later freezing and analysis, described above, were followed in all cruises. According to Aminot and Kérouel (1998), the main disadvantage of freezing nutrient samples is that they should be continuously kept frozen until the moment of the analysis, so there is always a risk of losing the samples if the equipment fails. The transfer of the frozen samples from the ship to the laboratory on land is particularly critical, as the samples can be partially melted down if transfer takes too long. It was not possible to check whether this has happened during the manipulation of preserved samples on any of the cruises of this study.

Usually during a cruise on which the samples were analyzed on board, some samples had to be preserved when there were malfunctions of the autoanalyzer, or when it was not possible to work because of the bad weather. Therefore, it is likely that not all the samples that we treated as analyzed on board should be in that group. It was thus predictable that the resulting concentration errors would be higher than those calculated in the laboratory experiment, as in fact happened. As a consequence, the errors shown in Table 6 can be considered as the highest ones that can be obtained when nutrient samples are frozen during the course of an oceanographic cruise.

Part of the increase in nutrient, except nitrite, found for cruises on which the nutrients were preserved could be reflecting the proportion of cruises on which the water column was mixed (stratification index 1). The number of cruises on which samples were frozen was 4 out of 10, but the number of those cruises on which samples were analyzed on board was only 4 out of 18. Note that the highest surface values of all nutrients were found when the water column was mixed and that most of the samples were commonly taken in the upper 100 m.

It is also possible that the higher mean concentrations of phosphate, nitrate and ammonium calculated in preserved samples compared with unpreserved ones—observed both in the laboratory experiment and in the comparison of cruise samples—were the result of breaking cells during the freezing process, reinforcing the hypothesis of Carpenter and McCarthy (1975).

QC concentration ranges

In this study, ranges of concentration of key oceanographic variables in the NW Mediterranean Sea are reported. The QC ranges for oceanographic data in this area were obtained following the methodology proposed by Conkright *et al.* (1994, 2000). However, one of the steps proposed by those authors was skipped. This step consisted in the interpolation of the data whose depth was not coincident with an SLD, in order to obtain the value in the SLD. This step was skipped because of the segmentation of the profiles available in the NW Mediterranean Sea, which usually only cover up to 12 of the 26 SLD considered by Fichaut *et al.* (1997). Moreover, the sampling was concentrated mainly in surface waters; the amount of data below 120 m decreased in number with increasing depth (see Tables 9-21). It should also be taken into account that the SLDs considered by Fichaut *et al.* (1997) for the Mediterranean Sea and used in the present study, include more SLDs in surface waters (0-120 m depth, an area of high variation and low concentrations for most of the variables studied) than the SLDs used by Conkright *et al.* (1994, 2000). While the resolution in surface waters should be improved, more SLDs involve fewer data at each depth. Therefore, it was considered that the interpolation, together with the relatively low amount of data on hand compared to those gathered by Conkright *et al.* (1994, 2000), would result in an increase in the uncertainty instead of improving the profile.

Also, some QCs can observe false maximums and minimums at 100 m depth, which are the consequence of using different standard deviations to define the concentration ranges.

Seasonal variations in surface nutrient concentration and in chl *a* concentration emerged clearly during the development of QCs, when most of the winter data except for phosphate and dissolved oxygen data were found outside the concentration ranges, as it is known that vertical mixing is related to an increase in nutrients and chl *a* at the surface. Consequently, QCs were developed separately for those data coming from mixed water column stations (winter conditions alone) and from stratified stations. Unfortunately, these QCs for winter conditions alone should be taken with precaution because the amount of data used to establish them is sometimes very low. The scarcity of data is observed with variations in the standard deviation between consecutive SLDs, which leads to abrupt variations in the amplitude of the ranges.

Finally, due to the possible error introduced by the freezing of ammonium samples, only ammonium data from onboard analysis were used to define the QC for this nutrient in the NW Mediterranean Sea. Unfortunately, below 500 m depth only ammonium data from spring are available, and it is not known how representative ammonium spring concentrations of deep waters are throughout the whole year. We therefore recommend the use of the QC for ammonium samples only from the surface to 500 m depth.

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APPENDIX 1. – Cruise name, date, number of stations, oceanographic vessel and instrumentation used of the cruises gathered in the present study. Source: I, PEPS Group, 1986; II, Masó and PEPS Group, 1988; III, Varela and FRONTS Group, 1991; IV, Masó and Varimed Group, 1995.

Cruise	Date	Number of stations	Oceanographic Ship	CTD	Thermometer	Salinometer	Pressure	Source
Pep-82	11–30 July 1982	49	Garcia del Cid		reversible	induction	line	I
Pep-83	30 June-17 July 1983	83	Garcia del Cid	Neil Brown	sensor	sensor	sensor	I
Pep-84	15–30 May 1984	53	Garcia del Cid		reversible	induction	line	I
Fronts-3-85	15–26 March 1985	17	Garcia del Cid	Neil Brown (part)	reversible	induction	line	II
Fronts-6-85	1–6 June 1985	23	Garcia del Cid		reversible	induction	line	II
Pep-86	13–24 June 1986	39	Garcia del Cid		reversible	induction	line	II
Fronts-11-86	7–12 November 1986	15	Garcia del Cid		reversible	induction	line	II
Pep-87	23–30 May 1987	20	Garcia del Cid		reversible	induction	line	II
Fronts 89	13–22 May 1989	28	Garcia del Cid	Sea-Bird SEB 19 (some)	reversible	induction	line	III
Fronts 90	9–21 February 1990	30	Garcia del Cid	Neil Brown Mark-III (part)	reversible	conductivity	line	III
Fronts 91	10–21 April 1991	37	Garcia del Cid	Neil Brown Mark-III (part 1) Seabird SBE 25 (part 2)	sensor	sensor	sensor	III
Fronts 92	15 Oct.-4 Nov. 1992	27	Garcia del Cid	Seabird SBE 25	sensor	sensor	sensor	IV
Varimed 93 I	1–8 June 1993	60	Hespérides	Neil-Brown Mark V	sensor	sensor	sensor	IV
Varimed 93 II	10–16, 23–29 June 1993	21	Hespérides	Neil-Brown Mark V	sensor	sensor	sensor	IV
Varimed 93 III	18–22 June 1993	41	Hespérides	Neil-Brown Mark V	sensor	sensor	sensor	IV
Varimed 95	2–14 June 1995	32	Hespérides	Neil-Brown Mark V	sensor	sensor	sensor	M. Estrada
Meso 95	31 May-23 June 1995	176	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	A. Cruzado
Fans 1	1–11 November 1996	53	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	L. Arin
Fans 2	4–14 February 1997	105	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	L. Arin
Fans 3	7–16 July 1997	110	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	L. Arin
Hivern 99	20 Feb.-15 March 1999	47	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	M. Estrada
Hivern 2000	21 Jan.-11 Feb. 2000	50	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	M. Estrada
Canyons I	13–19 March 2001	32	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	M. Emelianov
Canyons II	24–31 May 2001	44	Garcia del Cid	Neil-Brown Mark-III	sensor	sensor	sensor	M. Emelianov
Canyons III	10–15 July 2001	24	Garcia del Cid	Sea-bird SBE 25 / Idronaut	sensor	sensor	sensor	M. Emelianov
Canyons IV	26–29 November 2001	14	Garcia del Cid	Neil Brown Mark-III	sensor	sensor	sensor	M. Emelianov
Caco 1	18–25 July 2003	67	Garcia del Cid	Neil Brown Mark-III 1138	sensor	sensor	sensor	J. Salat
Caco 2	11–20 September 2003	65	Garcia del Cid	Neil Brown Mark-III 1138	sensor	sensor	sensor	J. Salat

APPENDIX 2. – Nutrient analysis characteristics for the data gathered from cruises in the NW Mediterranean Sea. Methodologies: S & P 72, Strickland and Parsons, 1972; W 81, Whitledge et al., 1981; C 89, Cruzado, 1989; G 83, Grasshoff et al., 1983; B+L 99, Bran+Luebbe, 1999; LNSW, low nutrient seawater; DW, distilled water; ASW, artificial seawater, usually 37 - 38 g l^{-1} sodium chloride. I to IV, same as Table 1.

Cruise	Autoanalyzer	Methodology	Base line	Preservation	Analyst	Source
Pep-82	Technicon	S & P, 72	LNSW, DW	no	1	I
Pep-83	Technicon	S & P, 72	LNSW, DW	no	1	I
Pep-84	Technicon	S & P, 72	LNSW, DW	no	1	I
Fronts-3-85	Technicon	S & P, 72	LNSW, DW	no	1	II
Fronts-6-85	Technicon	S & P, 72	LNSW, DW	freezing	1	II
Pep-86	Technicon	W, 81	LNSW	no	2	II
Fronts11-86	Technicon	W, 81	LNSW, DW	freezing	1	II
Pep-87	Technicon	W, 81	?	no	?	II
Fronts 89	Skalar	W, 81; C, 89	LNSW	no	2	III
Fronts 90	Skalar	W, 81; C, 89	LNSW	no	2	III
Fronts 91	Skalar	W, 81; C, 89	LNSW	no	2	III
Fronts 92	Skalar	W, 81; C, 89	LNSW	no	2	IV
Varimed 93 I	Skalar	W, 81; C, 89	LNSW	no	2, 3	IV
Varimed 93 II	Skalar	W, 81; C, 89	LNSW	no	2, 3	IV
Varimed 93 III	Skalar	W, 81; C, 89	LNSW	no	2, 3	IV
Varimed 95	Skalar	W, 81; C, 89	LNSW	no	2	M. Estrada
Meso 95	Technicon	W, 81; C, 89	LNSW	no	4	A. Cruzado
Fans 1	Skalar	W, 81; C, 89	LNSW	no	4	L. Arin
Fans 2	Skalar	W, 81; C, 89	LNSW	no	4	L. Arin
Fans 3	Skalar	W, 81; C, 89	LNSW	no	4	L. Arin
Hivern 99	Evolution-II, Alliance	G, 83	ASW	freezing	5, 6	M. Estrada
Hivern 2000	Evolution-II, Alliance	G, 83	ASW	freezing	5	M. Estrada
Canyons I	Evolution-II, Alliance	G, 83	ASW	freezing	7	M. Segura
Canyons II	AA3, Bran + Luebbe	B+L, 99	ASW	freezing	7	M. Segura
Canyons III	AA3, Bran + Luebbe	B+L, 99	ASW	freezing	7	M. Segura
Canyons IV	AA3, Bran + Luebbe	B+L, 99	ASW	freezing	7	M. Segura
Caco 1	AA3, Bran + Luebbe	B+L, 99	ASW	freezing	7	M. Segura
Caco 2	AA3, Bran + Luebbe	B+L, 99	ASW	freezing	7	M. Segura