ICES COOPERATIVE RESEARCH REPORT SERIES

THE FIFTH ICES INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEAWATER

by

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ANNEXES

1. INTRODUCTION

In these increasingly cost-conscious times, tax-payers are entitled to ask whether their national environmental research and monitoring programmes are delivering value for money, and it is understandable and right that they should. More people than ever are now inclined to enquire as to the quality of the results of chemical analysis before using them for their intended purpose, and 'Quality Assurance' is the phrase on everyone's lips.

It is no secret that marine chemists have generated their share of random numbers in the past. That said, some of the techniques in use in environmental analysis twenty years ago were not sufficiently well-developed for their intended task ; for example, the role of contamination and its influence on these techniques was not fully appreciated. Nevertheless, today, despite much-improved instrumentation and facilities, problems still can and do occur.

The key to solving problems is first to recognise them, and it is in this context that intercomparison and intercalibration (I/C) exercises have an important role to play.

The 1988 report of the ICES Marine Chemistry Working Group (MCWG) recommended that an intercomparison exercise for the determination of nutrients in seawater should be undertaken in 1989/90 and that consideration should be given to two further exercises at approximately four-year intervals.

This recommendation was approved by the Council at its 1988 Statutory Meeting (C. Res. 1988/4:10) and invitations to participate were issued by the ICES General Secretary to all member-countries, to the Oslo and Paris Commissions, and to the Baltic Marine Environment Protection Commission (Helsinki Commission).

The 1989/90 exercise was conducted on behalf of the MCWG by Don Kirkwood, Alain Aminot and Matti Perttilä, and the report was published in 1991 as ICES Cooperative Research Report No. 174. The authors designated it the 'Fourth Intercomparison Exercise for Nutrients in Sea Water (NUTS I/C 4) on the basis that, by their reckoning, there had been three previous events involving marine research institutes from ICES member-countries, in which the chemical analysis of seawater, and particularly nutrients, had been the central theme.

NUTS I/C 4 did not confine itself to ICES member-countries ; in the short time there was available to publicise the exercise, considerable interest was shown by laboratories in non-ICES countries, and a total of 68 laboratories in 22 countries took part.

A brief history of the four ICES nutrients I/C exercises to date can be found in annex I (which contains all the information, etc. that was sent to participants).

2. PARTICIPATION IN NUTS I/C 5

The year 1990 marked the twenty-fifth anniversary of the first of these I/C exercises, and the above-mentioned summary entitled '25 Years of ICES Nutrients I/Cs' was widely circulated amongst ICES member-countries and beyond, soon after the completion of NUTS I/C 4 in an effort to ensure that NUTS I/C 5 would include as many laboratories as possible. These did not have to be in ICES member-countries ; it was sufficient that they were involved in the

determination of nutrients in seawater and had an interest in participating in an exercise that was to be organised on similar lines to NUTS I/C 4.

During 1992, plans were being made to launch 'QUASIMEME' - Quality Assurance of Information for Marine Environmental Monitoring in Europe. This is a quality assurance initiative funded by the European Commission and managed on its behalf by D. E. Wells of the SOAFD (formerly DAFS) Marine Laboratory, Aberdeen, UK. QUASIMEME established a Quality Assurance Steering Group, understandably drawing heavily on the ICES Marine Chemistry Working Group for its membership. As QUASIMEME's aim is to complement rather than compete with ICES MCWG initiatives, participation in ICES NUTS I/C 5 was made a precondition for 'nutrients-laboratories' joining the QUASIMEME project, and this resulted in a last-minute influx of additional participants just before the distribution of samples (end of November 1992).

A total of 142 sets of samples were distributed in 31 countries. Results were returned by 132 laboratories, 61 of which had participated in NUTS I/C 4, and 56 of which were participating in QUASIMEME.
This made I/C 5 approximately double the size of I/C 4, which was the target the organisers had set themselves.

Annex II lists the participating laboratories in country-alphabetical order, then North to South within each country. QUASIMEME participants are indicated with 'Q', and I/C 4 participants are indicated with their I/C 4 laboratory number.

3. THE FORMAT OF NUTS I/C 5

Several significant changes have been introduced since NUTS I/C 4.

3.1. Analytical requirements

At the planning stage of I/C 4, statisticians insisted we should ask for replicate analyses for each determinand in each sample in order to obtain information on within-laboratory variability ; consequently, we asked for two replicates, separated by at least 24 hours.

Many laboratories supplied what we asked, but others were evidently content to treat adjacent auto-analyser 'clone' peaks as replicates, although we could argue that, at best, they are nothing more than a continuous instrumental signal regularly interrupted by a wash. Users of manual techniques, with some justification, might insist that colour developed in two separate aliquots of sample, measured against a single calibration curve, all part of the same batch or 'analytical event', represents replicate analyses.

The effect of day-to-day calibration bias was what we really wanted to investigate, but our failure to find a suitable definition for the term 'replicate', equally acceptable to both auto- and manual-users, and to which we could be completely sure they would conform, prevented us from obtaining valid data.

We now believe that it was unrealistic to expect to obtain information of this kind and that the data quoted in I/C 4 for intra-laboratory variance are under-estimates, biased by the effects of a variety of mis-interpretations of the term 'replicate'.

This time the issue has been avoided and we asked simply "what is the phosphate content of this sample ?", which is, after all, the question asked of the analyst when a sample of water is brought aboard ship. We wanted the analyst's best estimate, irrespective of how it was obtained.

The error associated with this concentration is, of course, important, but it is the concern of each individual laboratory and is best arrived at by long-term in-house quality control procedures, well outside the scope of a simple intercomparison exercise.

3.2. Sample logistics

In I/C 4, laboratories indicating that they used manual analytical methods were sent double quantities of samples.

Much effort goes into the preparation and control of sample materials for an exercise on this scale, and there is a limit to what is practicable given that IFREMER has made no charge for these services.

Methods of sample preparation for I/C 5 (fully described in annex III) imposed constraints which resulted in there being only two relevant determinands per sample (nitrate and nitrite in one series, and ammonia and phosphate in another series).

This and the fact that replicate analyses were not required, led us to conclude that 150 ml of each sample should be sufficient even for those laboratories using manual methods, consequently all participants received an identical package.

3.3. Selection of determinands

The 'determinands of primary interest' in I/C 4 were (nitrate + nitrite) and phosphate, but participants were encouraged to supply data for nitrite, ammonium, silicate, total-N and total-P if any of these were measured routinely in their laboratories.

For I/C 5 the intention was to increase the number of nutrients covered and special efforts have been made to include nitrite and ammonia, both reputed to present preservation problems due to their ease of oxidation to nitrate.

After some preliminary experiments at IFREMER, including autoclaving, gave satisfactory results, we decided to introduce these in I/C 5 on an experimental basis as this had not been attempted before.

Due to constraints imposed by the autoclaving process, the sample bottles chosen were necessarily glass in preference to any other material, despite the fact that gradual dissolution of glass by the sample causes a significant increase in silicate and a very slight increase in phosphate concentration. As all samples were distributed in glass bottles, the determination of silicate has thereby been excluded from I/C 5.

3.4. Analytical methodology

As part of I/C 4, a detailed review of participants' methods for the determination of phosphate was undertaken. The main purpose of this was to assess the potential of each participant's method for susceptibility to colorimetric bias (in the determination of phosphate) from unnaturally high silicate concentrations in the test samples, due to significant dissolution of the glass container-bottles.

The review was included in the I/C 4 report, and although bias from this source was shown not to be a serious general problem, some useful information came to light.

The review provided ample evidence that individual workers, authors, and equipment suppliers are capable of making apparently arbitrary (and possibly unintentional) changes to their own and to each others' methods, sufficient to cause substantial divergence from the manual methods on which they claim to be based. Conclusions drawn from chemical interference studies on the original methods may be rendered invalid by such changes, and readers of the report were urged to examine their methods closely to assess how well these adhered to the conditions specified by the parent manual method.

3.5. Anonymity/openness

This is one aspect that has definitely not changed since I/C 4.

All laboratories are identified, full results are listed, and any reader can link each and every laboratory with its set of results.

In both I/C 4 and I/C 5, intending participants were made aware that the reports would be published on this basis, and we remain convinced that our insistence on complete openness has been a positive influence towards the improvement of quality control procedures in general.

4. THE SAMPLES

In I/C 4, one of the samples used was a totally natural oceanic water that was simply bottled directly from 30-litre Niskin samplers and received no treatment to ensure its stability. It proved to be highly satisfactory, but as we have no access to further sources (of demonstrated stability, and covering a useful concentration range), we rely on 'slightly-artificial' samples that at least started life as natural seawater.

4.1. Preparation

Annex III contains details of the methods used for preparation and control of the samples, derived from those used in I/C 4, but these are also summarised briefly here.

A large volume of natural seawater, low in nutrients, is spiked with known concentrations of nutrient salts. A large number of bottles are filled, capped, then sterilised as a single batch by heat treatment in an autoclave. Some pH adjustment to the bulk solution is necessary to prevent precipitation during the heat treatment. Nitrate and nitrite concentrations apparently remain unchanged by the process, and significant increases in ammonia and phosphate, attributable to hydrolysis of naturally present N- and P-containing compounds, although detectable, are consistent and cause no problems of variability (see annex III).

4.2. Concentrations

From the participants' point of view, at the time of analysis the samples were uncompromised reference materials, but we can now divulge the ***assigned values*** for the concentrations from a knowledge of the method of their preparation.

This time there are no 'blanks'. There are three concentration levels for each determinand (low, medium, high) and a greater range is covered than in I/C 4.

Sample	Level	Nitrate	Nitrite	Ammonia	Phosphate
1	Medium	9.98	0.505	-	-
2	Low	1.33	0.143	-	-
3	High	26.03	1.406	-	-
4	Low	-	-	0.34	0.08
5	High	-	-	4.86	1.85
6	Medium	-	-	1.83	0.495

(all concentrations are expressed in micromoles per litre)

5. RESPONSE

Samples were sent to the 142 laboratories which had confirmed their intention to participate. These 'confirmed' laboratories undertook to submit results or return the samples intact before the deadline.

Samples were returned by 5 laboratories, results submitted by 132 laboratories, consequently there were 5 defaulters. Table 1 summarizes all the information relevant to samples and participants' responses for the inorganic nutrients.

In addition to the four determinands of primary interest, participants were invited to supply results for any others that were 'routine' in their laboratories :

- 8 laboratories submitted results for Total-N,
- 6 laboratories submitted results for Total-P.

Table 1

Summary of response from participants.

Nutrient	Level	Sample number	Number of damaged samples		Number of results			
			Replaced	Unclaimed	Received	Out of given range	> x [1] or < x	Statistically treated [2]
Nitrate + nitrite	Low	2	0	-	129	-	3	127 [3]
	Medium	1	3	3	126	-	-	127 [3]
	High	3	2	-	129	-	1	129 [3]
Nitrite	Low	2	0	-	125	-	7	118
	Medium	1	3	3	122	-	-	122
	High	3	2	-	125	3	-	122
Nitrate	Low	2	0	-	125	-	3	122
	Medium	1	3	3	122	-	-	122
	High	3	2	-	125	-	1	124
Ammonia	Low	4	1	-	106	-	15	91
	Medium	6	1	1	105	-	2	103
	High	5	2	-	106	1	-	105
Phosphate	Low	4	1	-	131	1	16	114
	Medium	6	1	1	130	1	1	128
	High	5	2	-	131	3	1	127

(1) Within the given range.

(2) See paragraph 6.1.

(3) One additional result computed by summing separated nitrate and nitrite results.

6. STATISTICAL TREATMENT

6.1. Consensus means and standard deviations

A primary purpose of the application of statistical techniques to the results is to assess how well they agree, as a whole, with the assigned values.

Given that all of the determinand concentrations in I/C 5 are well removed from the detection limits of the analytical techniques, we consider relatively simple statistical treatment to be adequate to describe the data sets, consequently we have followed the guidelines proposed by the ICES MCWG (Tenerife 9-14 March, 1992) after the recommendations of Berman (1992). Berman suggests the successive application of a t-test at the 95 per cent confidence level to remove outliers and isolate a population approximating to a Normal distribution, then to characterise the performance of this homogeneous group in terms of mean and standard deviation. The test was applied until a stable mean was reached, assuming then a Normal distribution (see annex V).

Before applying any statistical treatment we first had to consider how best to treat the few 'less than' and 'greater than' results that were submitted. As the approximate concentration ranges were stated, and as none of the concentrations were uncomfortably near the detection limits of most currently used techniques, we considered such results to be of poor quality and chose to exclude them rather than, for example, include '< x' as 'x', or 'x/2' as is sometimes suggested.

6.2. Z-scores

'Z-scores' are now widely used to evaluate the performance of laboratories.

For a single result on one sample, Z is defined as follows :

$$Z = \frac{x_i - X}{s}$$

where x_i is the result submitted by laboratory (i),
X is the concentration assigned to the sample,
s is the consensus standard deviation.

In effect, Z is the expression of bias in units of standard deviation. Biases 'normalised' in this way are consequently comparable numbers which can be summed or meaned to obtain an indication of the overall performance of each laboratory.

The Z-scores in this exercise are entirely relative as the consensus standard deviations were derived from the data, rather than using target values fixed in advance.

A crude estimate of the mean accuracy of a laboratory's results for a given nutrient is obtained from the mean of the absolute values of the Z-scores for the three levels of concentration (the three samples). For example, for phosphate :

$$Z_{PO_4} = \frac{|Z_{PO_4(4)}| + |Z_{PO_4(5)}| + |Z_{PO_4(6)}|}{3}.$$

It follows that the overall Z-score for a laboratory is given by the combined Z-scores according to $Z_n = (\Sigma Z_i)/n$, where 'i' refers to individual determinands and n to the number of determinands.

In practice, two overall Z-scores produce useful information, Z_3 combining nitrate, nitrite and phosphate (123 laboratories) and Z_4 including ammonia (100 laboratories) :

$$Z_3 = \frac{Z_{NO_3} + Z_{NO_2} + Z_{PO_4}}{3} \qquad \text{and} \qquad Z_4 = \frac{Z_{NO_3} + Z_{NO_2} + Z_{PO_4} + Z_{NH_4}}{4}.$$

No Z_n was calculated where results for fewer than three determinands were submitted.

It should be noted that 'less than' and 'greater than' results have been excluded from the Z-score calculation, and this has the effect of improving Z-scores to some extent.

6.3. Estimate of the random, proportional and constant errors of individual laboratories

Deviations from the true value, generally refered to as uncertainties or errors, are of several types, and can be classified as follows :

- **random errors** : these cause dispersion (imprecision) of the measurements ;

- **systematic errors** : these cause biases, *i.e.* inaccurate results and may be of two types :
  - **proportional** (relative), dependent on analyte concentration ;
  - **constant** (absolute), independent of concentration.

Random errors are inherent in every method but their magnitude may be increased by lack of attention to important details of procedure such as reaction conditions, temperature, etc. Proportional errors are generally caused by faulty calibration technique, while constant errors mainly originate from mis-definition of the blank. It should be noted that matrix (salt) effects may cause proportional or constant errors, or both.

In order to assess the various types or errors of the laboratories in this exercise, the linear regression method has been applied (Massart *et al.*, 1988). Plotting the results of each laboratory against the assigned values, a straight regression line should be obtained.

Let us consider the effects of the different kinds of errors.

The **random errors** lead to a scatter of the points around the least-squares fitted line. An estimate of the mean random error is obtained from the calculation of the standard deviation of the estimate of y on x, $s_{y/x}$, according to :

$$s^2_{y/x} = \frac{\sum (y_i - \hat{y}_i)^2}{n-2}$$

where y_i is the concentration measured for sample i,
$\hat{y}_i$ is the concentration calculated by the regression for sample i,
and $n - 2$ represents the degree of freedom.

In the present case, only three samples were distributed, therefore n - 2 = 1, and although the power of this test may seem poor, very relevant information can be extracted.

The **proportional error** leads to a change in slope so that the difference between slope and unity gives an estimate of that type of error. Finally, the **constant error** is obtained by the value of the intercept. Consequently, as stated by Massart *et al.* (1988), "the study of the regression therefore leads to estimates of the three types of error (random, proportional and constant), which enables one to conclude that least-squares analysis is potentially the most useful statistical technique for the comparison of two methods".

In this treatment results reported as 'greater than' have been removed while '< x' have been included as 'x' in order to enable regression calculation. The few laboratories which reported 'less than' results are invited to re-examine their own data by plotting them against the assigned values.

We anticipate that the identification of individual types of error in this way should be a great help to laboratories in their efforts to improve their techniques.

7. RESULTS

7.1. Raw results

Full results as reported by the participants are listed in annex IV, table IV.1. The distributions of the original full sets are shown on figures 1 to 5. It should be noted that concentrations submitted as '< x' are plotted as 'x'.

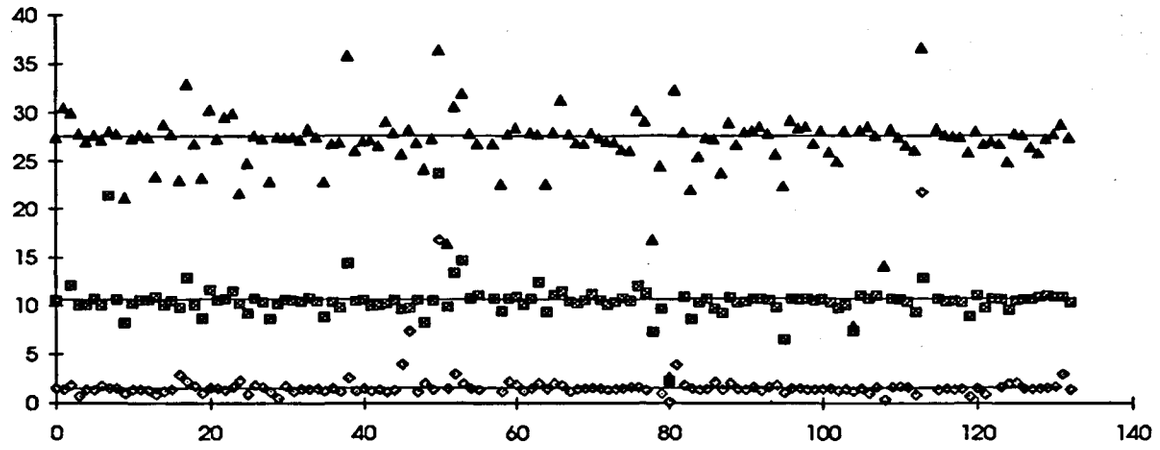

Figure 1 : Nitrate + nitrite results : concentrations (µmol/l) versus laboratory number. The lines represent assigned values.

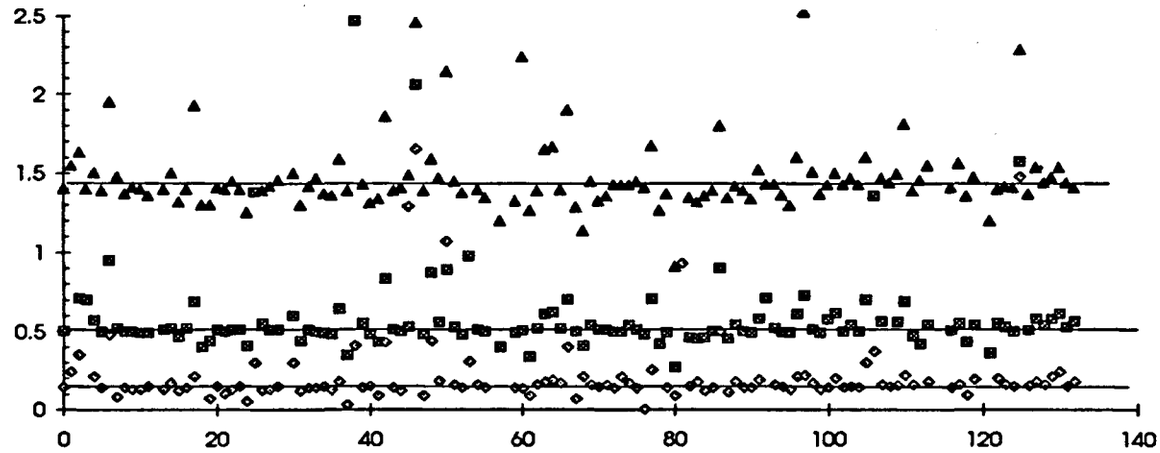

Figure 2 : Nitrite results : concentrations (µmol/l) versus laboratory number. The lines represent assigned values.

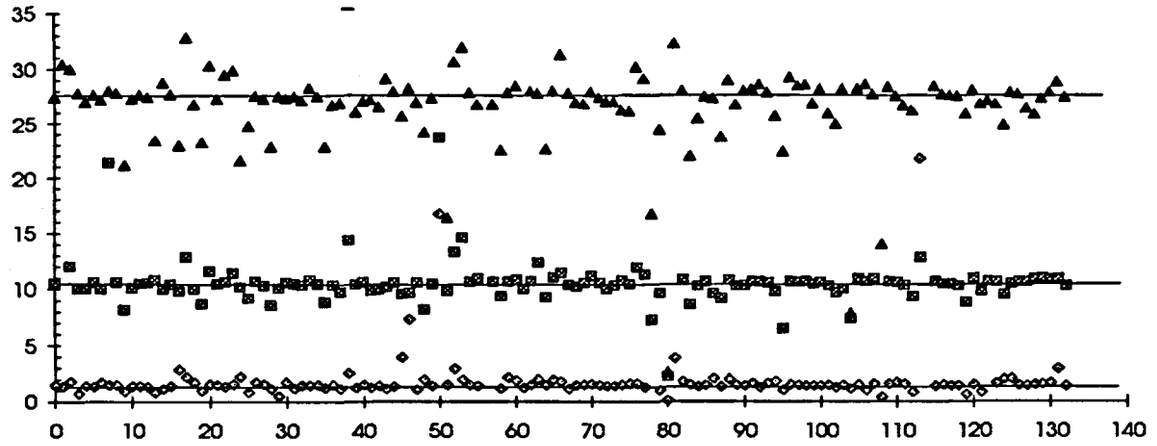

Figure 3 : Nitrate results : concentrations (µmol/l) versus laboratory number. The lines represent assigned values.

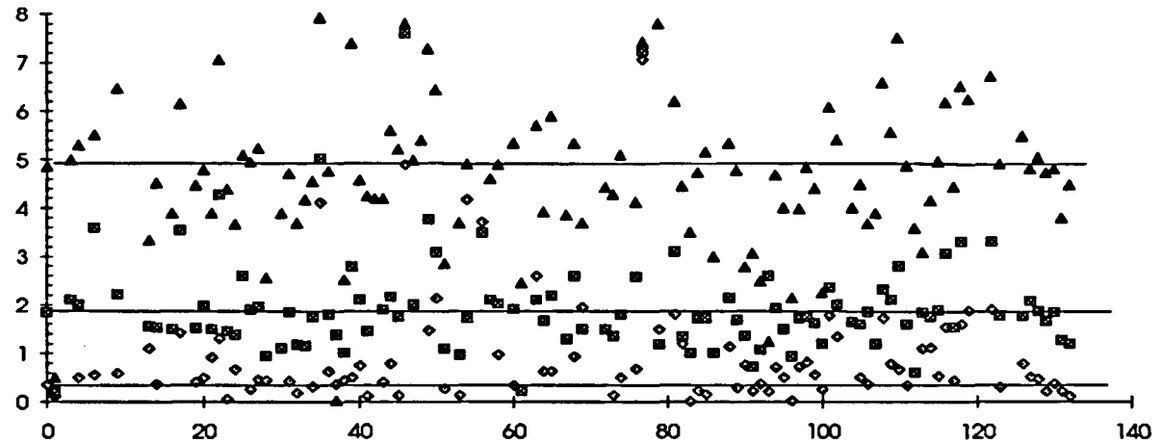

Figure 4 : Ammonia results : concentrations (µmol/l) versus laboratory number. The lines represent assigned values.

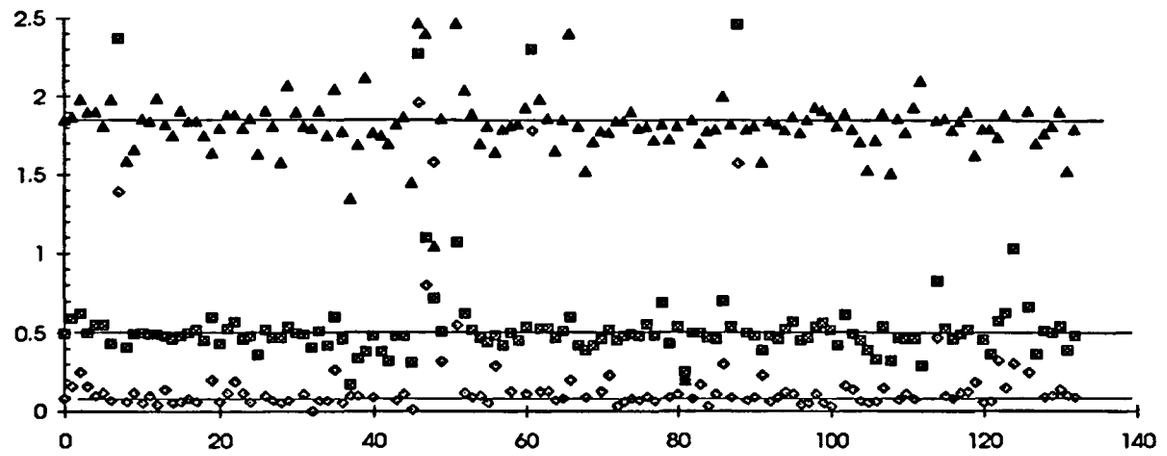

Figure 5 : Phosphate results : concentrations (µmol/l) versus laboratory number. The lines represent assigned values.

7.2. Statistical data

Raw means and standard deviations are summarized in table 2.

Table 2

Raw means and standard deviations obtained from the full set of results (in micromoles per litre).

Nutrient	Low			Medium			High		
	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.
Nitrate + nitrite	127	**1.84**	2.35	127	**10.55**	2.00	129	**26.80**	4.06
Nitrite	118	**0.22**	0.25	122	**0.59**	0.28	122	**1.49**	0.27
Nitrate	122	**1.63**	2.31	122	**9.96**	2.00	124	**25.16**	4.03
Ammonia	91	**0.92**	1.12	103	**1.98**	1.10	105	**4.66**	1.45
Phosphate	114	**0.19**	0.33	128	**0.56**	0.35	127	**1.82**	0.26

Application of successive rejections at 95 % confidence level (see paragraph 6.1 and annex V) leads to the isolation of sets of consistent laboratories, hence to consensus means and standard deviations for each determinand. Table 3 summarizes the consensus data in comparison with the assigned values.

Table 3

Consensus means and standard deviations compared with assigned concentrations.

Nutrient	Sample number	Assigned	(s.d.)	Consensus				
				mean	s.d.	r.s.d. %	n	(n %)
Nitrate	1	**10.48**	(0.05)	**10.52**	0.30	2.9	87	(69)
+	2	**1.47**	(0.01)	**1.45**	0.27	19	110	(87)
nitrite	3	**27.43**	(0.05)	**27.50**	0.80	2.9	92	(71)
	1	**0.505**	(0.003)	**0.511**	0.049	9.6	98	(80)
Nitrite	2	**0.143**	(0.001)	**0.157**	0.048	31	104	(88)
	3	**1.406**	(0.010)	**1.413**	0.071	5.0	99	(88)
	1	**9.98**	(0.05)	**10.04**	0.22	2.2	72	(59)
Nitrate	2	**1.33**	(0.01)	**1.27**	0.32	25	114	(93)
	3	**26.03**	(0.06)	**26.04**	0.83	3.2	98	(79)
	4	**0.34**	(0.02)	**0.43**	0.24	56	66	(73)
Ammonia	5	**4.86**	(0.03)	**4.60**	0.99	22	92	(88)
	6	**1.83**	(0.03)	**1.64**	0.37	23	80	(78)
	4	**0.08**	(0.01)	**0.090**	0.036	40	91	(80)
Phosphate	5	**1.85**	(0.02)	**1.830**	0.053	2.9	87	(69)
	6	**0.495**	(0.02)	**0.487**	0.078	16	118	(92)

Each of the fifteen consensus concentrations was derived from the results of around one hundred laboratories ; we therefore maintain that the high level of agreement between consensus and assigned values serves to validate *a posteriori* the methods of sample preparation and assignment of concentrations.

The I/C 5 results shows that the precision of the determination of the nutrients decreases in the order :

nitrate (or nitrate + nitrite) >> nitrite > phosphate >> ammonia,

with standard deviations of about 3 % for nitrate, 5-15 % for nitrite and phosphate and 20-25 % for ammonia. These figures refer to medium and high levels only, those levels which are typical of winter coastal waters with continental inputs.

The nitrate determination appears especially satisfactory. In the I/C 4 intercomparison, standard deviations of 4-5 % were recorded within a reduced set of laboratories similar in proportion to the present set but with a lower concentration range (Kirkwood *et al.*, 1991). This determinand is consequently correctly measured by a large majority of the laboratories. Note that the standard deviations of nitrate may be lower than the standard deviations of nitrate + nitrite since they are not derived from exactly the same population of laboratories.

Phosphate, the other nutrient of primary interest in I/C 4 gives similar results in both intercomparisons, with improved precision at the higher concentration level in I/C 5.

Ammonia and nitrite were not present in significant concentrations in I/C 4 therefore I/C 5 is the first worldwide intercomparison exercise to include these determinands in seawater.

Surprisingly, nitrite exhibits relatively poor precision (6-10 %) having regard to the sensitivity and simplicity of the procedure, and given that it is the basis of the nitrate determination. This may be attributed to contamination problems and instability of standards, both of these aspects being generally underestimated by analysts.

Ammonia results reveal that the nutrients-measuring community, as a whole, has a particular problem with this determinand, which confirms the general opinion of most analysts.

7.3. Z-scores

Z-scores, computed according to the method described in paragraph 6.2, are reported in table 4 for each determinand. Combined scores are computed when at least three determinands have been determined, *i.e.* Z_3 for nitrate + nitrite + phosphate and Z_4 with ammonia additionally.

Note. The Z-scoring system used in this ICES report is an expression of a laboratory's errors in units of consensus standard deviation, after rejection of outliers. It serves only to rank the performance of a laboratory relative to its peers, as in a league table. In the QUASIMEME report, a laboratory's Z-scores can be expected to be numerically different from those in this report because the basis of their calculation is different. In QUASIMEME, Z-scores are laboratory errors expressed in units of 'maximum allowable error targets' that were pre-set by the organisers, and not in any way derived from the data. In effect, the Z-scoring system used by QUASIMEME serves two purposes. It ranks the relative performance of laboratories (as does the ICES Z-scoring system) but in addition to this, it determines whether or not laboratories have achieved a pre-set target level of performance.

Table 4. Determinand Z-scores and combined Z-scores (see text).

Lab No.	$Z_{NO_{3+2}}$	Z_{NO_2}	Z_{NO_3}	Z_{NH_4}	Z_{PO_4}	Z_3	Z_4
1	2.1	1.8	2.1	3.3	1.3	1.7	2.1
2	3.2	3.7	2.9		2.9	3.2	
3	1.6	2.0	1.8	0.4	1.1	1.6	1.3
4	0.8	1.3	1.3	0.5	0.7	1.1	0.9
5	0.5	0.1	0.5		0.9	0.5	
6	0.9	7.4	1.8	2.1	1.2	3.4	3.1
7	12	0.8	17		25	14	
8	0.3	0.2	0.4		2.2	0.9	
9	5.9	0.1	6.6	1.2	1.6	2.8	2.4
10	0.6	0.2	0.7		0.4	0.4	
11	0.2	0.3	0.2		0.3	0.3	
12	0.3				1.3		
13	2.8	0.1	2.8	1.8	0.8	1.2	1.4
14	1.5	0.6	1.6	0.4	1.1	1.1	0.9
15	0.3	0.7	0.2		0.6	0.5	
16	4.3	0.1	4.3	0.9	0.1	1.5	1.4
17	5.8	3.6	6.0	3.5	0.4	3.3	3.4
18	1.1	1.7	1.2		1.2	1.4	
19	4.3	1.3	4.7	0.5	2.9	3.0	2.3
20	2.5	0.1	2.9	0.4	0.8	1.3	1.0
21	0.1	0.4	0.2	1.4	0.7	0.4	0.7
22	1.2	0.3	1.2	4.3	1.5	1.0	1.8
23	2.2	0.1	2.5	0.9	0.8	1.1	1.1
24	3.7	1.9	3.4	1.3	0.3	1.9	1.7
25	3.2	17	6.2	1.2	2.9	8.6	6.8
26	0.7	0.5	0.7	0.2	0.7	0.6	0.5
27	0.4	0.2	0.5	0.4	0.5	0.4	0.4
28	4.4	0.3	5.0	1.7	2.1	2.5	2.3
29	1.6				1.7		
30	0.4	2.1	0.2	1.5	0.5	0.9	1.1
31	0.4	1.0	0.5	0.2	0.6	0.7	0.5
32	0.2	0.1	0.3	1.2	1.4	0.6	0.7
33	0.7	0.3	0.8	1.3	0.5	0.5	0.7
34	0.1	0.3	0.1	0.2	1.0	0.5	0.4
35	3.9	0.5	4.4	9.2	3.4	2.8	4.4
36	0.4	1.8	0.7	0.4	0.9	1.1	1.0
37	1.4	1.9	1.2	2.0	4.7	2.6	2.5
38	9.2	24	6.2	1.7	1.9	11	8.5
39	0.9	0.4	1.0	1.9	3.3	1.6	1.7
40	0.3	0.6	0.4	0.9	0.7	0.5	0.6
41	1.1	1.1	1.1	0.8	1.7	1.3	1.2
42	1.0	5.9	2.1	0.7	2.5	3.5	2.8
43	1.4	0.1	1.4	0.4	0.3	0.6	0.6
44	0.5	0.2	0.5	1.2	0.5	0.4	0.6
45	4.8	8.4	3.5	0.5	4.0	5.3	4.1
46	8.5	25	8.2	12	29	21	18.6
47	0.8	0.6	0.7	0.3	12.7	4.7	3.6
48	4.4	5.2	5.5	0.5	20	10	7.8
49	0.1	0.9	0.2	4.1	2.3	1.1	1.9
50	37	12	38	4.2			
51	5.3	0.4	5.4	1.4	11	5.5	4.5
52	6.4	0.3	7.3		2.1	3.2	
53	7.1	9.0	7.2	1.4	0.5	5.5	4.5
54	0.5	0.2	0.5	5.4	1.2	0.6	1.8
55	0.9	0.3	1.1		0.8	0.7	
56				8.4	3.3		
57	0.8	2.2	1.0	0.5	1.0	1.4	1.2
58	3.5			1.1	0.7		
59	1.3	0.4	1.3		0.6	0.8	
60	1.3	3.1	1.1	0.2	1.0	1.7	1.3
61	1.2	2.0	1.0	2.4	35	13	10.1
62	0.6	0.3	0.6		1.4	0.8	
63	2.9	1.9	3.3	3.7	0.7	1.9	2.4
64	3.3	2.1	4.0	0.8	1.5	2.5	2.1
65	1.5	0.3	1.6	1.1	0.1	0.7	0.8
66	3.2	4.9	2.7		5.0	4.2	

Lab No.	$Z_{NO_{3+2}}$	Z_{NO_2}	Z_{NO_3}	Z_{NH_4}	Z_{PO_4}	Z_3	Z_4
67	0.6	1.0	0.5	1.2	0.9	0.8	0.9
68	0.5	2.1	0.4	1.7	2.6	1.7	1.7
69	0.4	0.5	0.3	2.9	1.8	0.9	1.4
70	1.1	0.4	1.4		1.1	0.9	
71	0.2	0.3	0.2		2.0	0.8	
72	0.7	0.1	0.9	0.7	0.7	0.6	0.6
73	0.3	0.6	0.4	0.9	0.3	0.4	0.6
74	0.9	0.5	1.0	0.3	0.3	0.6	0.5
75	0.8	0.2	0.7		0.5	0.5	
76	3.0	1.2	3.7	1.4	0.7	1.9	1.7
77	1.8	3.1	1.8	15	1.0	1.9	5.2
78	12	1.6	13		1.5	5.5	
79	2.7	0.3	2.9	3.2	1.1	1.4	1.9
80	21	3.8	23		0.7	9.3	
81	7.5	75	7.0	3.6	12	32	25
82	1.1	0.6	1.3	1.8	0.0	0.6	0.9
83	4.3	0.9	4.9	1.7	1.8	2.5	2.3
84	1.2	0.6	1.1	0.3	1.0	0.9	0.8
85	0.3	0.1	0.4	0.5	0.8	0.4	0.4
86	1.7	6.6	2.3	2.1	3.9	4.2	3.7
87	3.0	0.8	3.3		0.5	1.5	
88	1.7	0.5	1.6	1.6	29	10	8
89	0.5	0.1	0.4	0.2	0.5	0.3	0.3
90	0.4	0.4	0.5	1.7	0.4	0.4	0.7
91	0.8	1.3	0.6	1.8	3.5	1.8	1.8
92	1.0	2.2	0.7	1.5	0.3	1.1	1.2
93	0.5	0.3	0.5	2.1	0.4	0.4	0.8
94	1.9	0.2	2.0	0.7	0.9	1.1	1.0
95	7.0	0.6	8.4	0.8	0.7	3.2	2.6
96	1.0	1.9	0.8	2.2	1.1	1.3	1.5
97	0.6	6.2	0.2	0.9	0.4	2.3	1.9
98	0.8	0.6	0.8	0.7	1.0	0.8	0.8
99	0.4	0.3	0.4	0.6	0.9	0.5	0.6
100	0.5	0.5	0.5	1.6	0.7	0.6	0.8
101	0.8	1.5	1.1	2.9	0.9	1.2	1.6
102	2.1	0.1	2.4	1.7	1.6	1.3	1.4
103	0.8	0.5	1.0		1.0	0.8	
104	12	0.1	13	0.7	1.2	4.7	3.7
105	0.9	3.1	0.8	0.6	2.7	2.2	1.8
106	1.3	12	1.7	0.4	1.7	5.1	3.9
107	0.9	0.7	0.8	1.3	1.1	0.9	1.0
108	10.4	0.3	9.8	2.9	4.3	4.8	4.3
109	0.8	0.8	0.7	1.1	0.3	0.6	0.7
110	0.5	3.3	0.3	2.2	0.9	1.5	1.7
111	0.5	0.4	0.5	0.2	0.7	0.5	0.5
112	2.5	1.2	2.6	2.3	3.7	2.5	2.4
113	31	1.0	28	1.7			
114				1.4	5.1		
115	0.8			0.3	0.4		
116	0.2	0.0	0.2	3.2	0.6	0.3	1.0
117	0.2	1.0	0.1	0.5	0.5	0.5	0.5
118	0.3	1.0	0.2	3.6	0.9	0.7	1.4
119	3.4	0.9	4.1	3.9	3.6	2.8	3.1
120	0.9				0.8		
121	1.8	2.6	1.6		1.1	1.8	
122	0.6	0.7	0.6	4.1	3.2	1.5	2.2
123	0.7	0.3	0.6	0.1	1.4	0.8	0.6
124	2.7	0.1	2.9		15	6.1	
125	0.9	20	2.6		86	36	
126	0.3	0.2	0.4	0.9	2.6	1.1	1.0
127	0.7	1.3	0.7	0.5	2.3	1.4	1.2
128	1.2	0.5	1.3	0.3	0.7	0.8	0.7
129	0.7	1.2	0.7	0.4	0.5	0.8	0.7
130	0.9	1.9	0.7	0.1	1.1	1.2	0.9
131	2.9	0.3	1.4	1.0	2.7	1.5	1.4
132	0.2	0.6	0.4	1.0	0.5	0.5	0.6

It may be instructive to examine the number of laboratories with Z-scores ≤ 1. For one determinand and one concentration it corresponds to the range : mean ± one standard deviation. In an ideal Normal distribution, this range contains 68 % of the observations. For the present fifteen determinations, the percentages of $|Z| \leq 1$ (related to the consistent sets) lie between 62 % and 74 % with a mean value of 69 % which confirms the validity of the applied treatment to the distribution of these results.

The number of laboratories with determinand $Z \leq 1$ and combined $Z \leq 1$ has been compared with the number of participants in each category (table 5). Between 42 % (ammonia) and 60 % (nitrite) of all participants exhibit determinand $Z \leq 1$. These figures are obviously below the theoretical 68 % since they are related to the full population and not to the reduced consistent set of laboratories.

Table 5

Number of laboratories with determinand Z-scores and combined Z-scores less than or equal to 1.

Z-scores	Total number of laboratories	$\|Z\| \leq 1$	
		Number of laboratories	% total
$Z_{NO_{3+2}}$	130	64	49
Z_{NO_2}	125	75	60
Z_{NO_3}	125	58	46
Z_{NH_4}	106	45	42
Z_{PO_4}	130	62	48
Z_3	123	49	40
Z_4	100	37	37

Considering the combined Z-scores, Z_3 and Z_4, it can be seen that a smaller proportion of the laboratories exhibits $Z_n \leq 1$ because of inconsistency in laboratory performances for different determinands.

A number of laboratories (31) have shown consistently good performance throughout the range of nutrients (excluding ammonia). These have *simultaneous* $Z_{NO_3} \leq 1$, $Z_{NO_2} \leq 1$ and $Z_{PO_4} \leq 1$. They are, in ascending Z_3 order : 116, 11, 89, 27, 44, 21, 93, 90, 10, 73, 85, 75, 34, 5, 132, 111, 15, 40, 117, 99, 33, 100, 109, 72, 74, 26, 31, 118, 67, 98, 103.

Among the above laboratories and including ammonia, a core group (15) have four Z-scores ≤ 1. They are, in ascending Z_4 order :

89, 27, 34, 85, 111, 73, 117, 26, 74, 99, 31, 72, 40, 132, 98.

7.4. Estimation of individual errors

As mentioned in paragraph 6.3, individual laboratory errors have been estimated by regression analysis. They are summarized in annex IV, table IV.2, which contains the following information :

- the standard-deviation (µmol/l), equivalent to the mean random error (repeatability) within the range of concentration ;

- the slope shift (in percent), equivalent to the proportional error accompanied by its standard-error (se) ;

- the intercept (µmol/l), equivalent to the constant error, accompanied by its standard-error.

For each laboratory, the data are presented on two lines, the first containing the laboratory errors, the second the standard errors of these errors.

The use of table IV.2 may be illustrated by the following examples.

Laboratory 9, nitrate :
- low standard deviation : ± 0.18 µmol/l ;
- significant proportional error : + 22 ± 0.9 % ;
- negligible constant error : - 0.1 ± 0.2 µmol/l.

This laboratory should focus on its calibration procedure.

Laboratory 2, nitrite :
- low standard deviation : ± 0.01 µmol/l ;
- low proportional error : + 1.5 ± 0.6 % ;
- significant constant error : + 0.20 ± 0.01 µmol/l.

This laboratory should focus on its blank determination procedure.

Laboratory 6, ammonia :
- high standard deviation : ± 1.13 µmol/l.

This laboratory should focus firstly on the random error sources. It may have proportional and/or constant errors but they are presently concealed by random errors.

7.4.1. Random errors

The frequency distribution of random errors is shown in figure 6.

From these data, it is interesting to extract the standard deviation obtained by a certain proportion of the participants in order to identify some achievable within-laboratory repeatability.

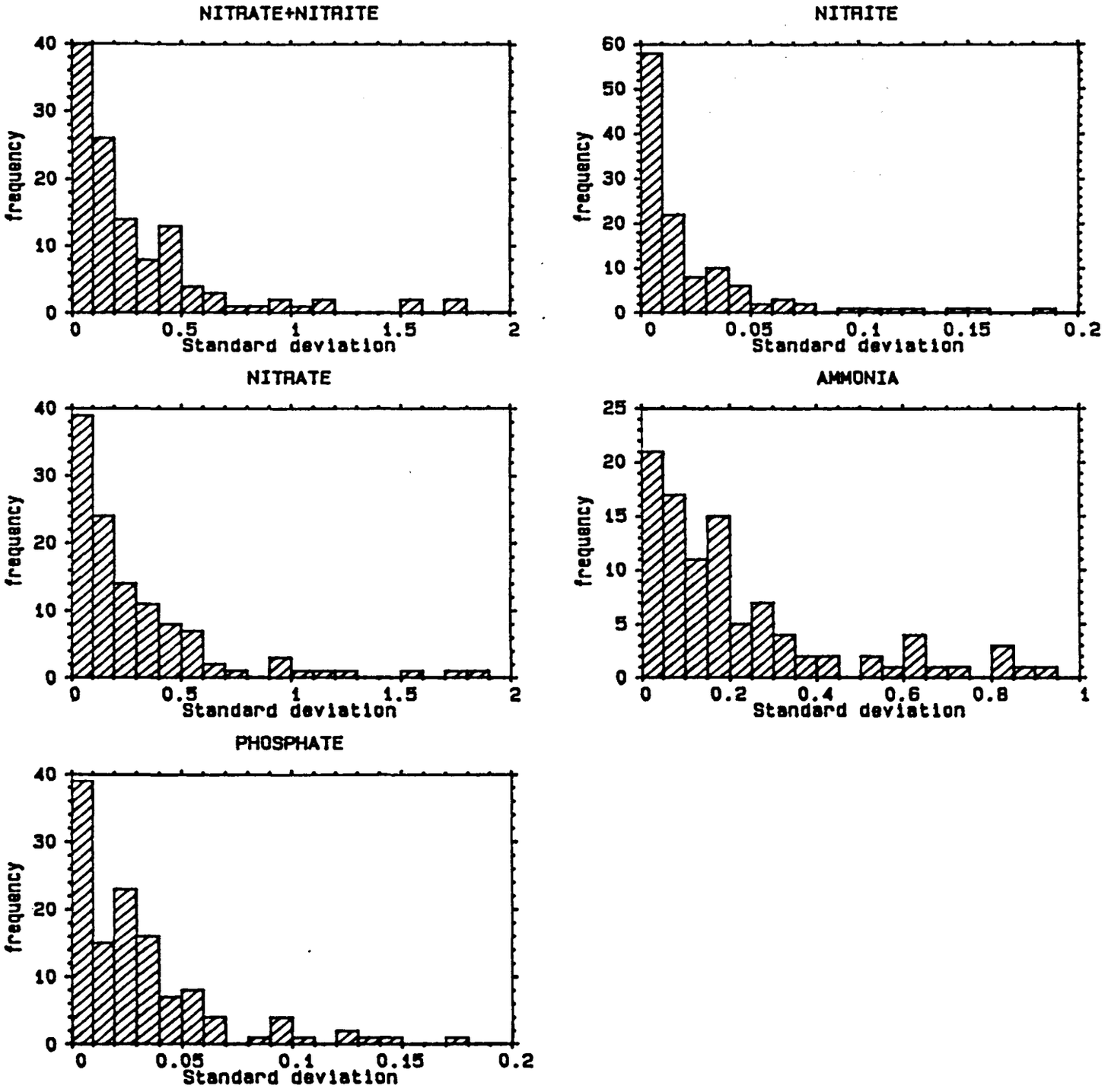

Figure 6 : Frequency distribution of individual standard deviations.

The standard deviation obtained by a two-to-one majority of participants (67 %) is considered achievable by every analyst under normal conditions. These standard deviations are :

- nitrate + nitrite (and nitrate) : 0.35 µmol/l,
- nitrite : 0.025 µmol/l,
- ammonia : 0.25 µmol/l,
- phosphate : 0.04 µmol/l.

The following laboratories, with standard deviations of double the above values (or greater), have a serious problem of analytical repeatability that should be given urgent attention :

- nitrate + nitrite (and nitrate) : 7, 13, 24, 45, 46, 48, 51, 53, 63, 80, 95, 104, 113, 122 ;
- nitrite : 3, 26, 37, 38, 45, 46, 48, 50, 60, 61, 66, 67, 68, 73, 76, 78, 97, 100, 106, 121 ;
- ammonia : 6, 22, 25, 37, 42, 46, 48, 54, 56, 61, 63, 69, 76, 79, 82, 86, 93, 101, 102, 108, 112 ;
- phosphate : 7, 37, 38, 39, 42, 46, 48, 49, 56, 66, 78, 88, 91, 106, 112, 121, 125.

7.4.2. Proportional errors

The frequency distribution of proportional errors is shown on figure 7.

No marked positive or negative tendency is shown in the histograms. There is some evidence for a small negative trend for ammonia and phosphate.

For ammonia, the range of proportional error is almost twice that of the other nutrients. Attention is drawn towards a group of laboratories with errors around - 50 % and + 50 %, suggesting calibration/computation errors arising from the use of ammonium sulfate (two 'ammonias' per molecule) as a standard.

Additionally, it may be useful to identify laboratories with proportional errors greater than 10 % (in absolute value), this percentage corresponding to an error of one order of magnitude smaller than the concentration to be determined.

However the significance of the computed proportional errors (PE) is affected by random errors, consequently they should not be considered without their associated standard-error (se). For simplicity, laboratories were identified at the 84 % confidence level which means :

$$|PE| - se \geq 10\ \%.$$

(Note that $|PE| \geq 10$ % corresponds to the 50 % confidence level, *i.e.* one chance in two that the error exceeds 10 %).

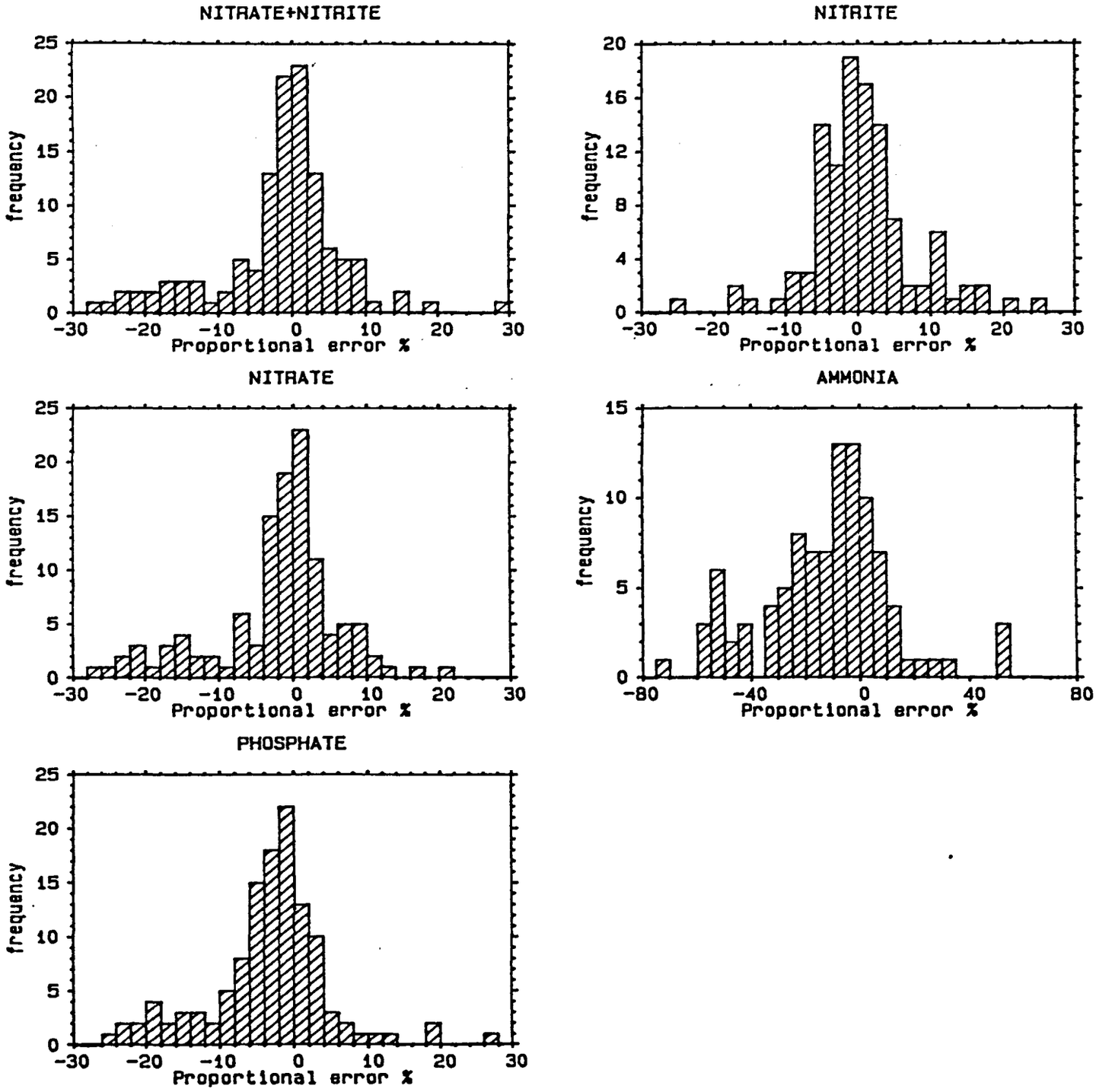

Figure 7 : Frequency distribution of individual proportional errors.

The laboratory numbers are as follows :

- nitrate + nitrite and nitrate (21 labs) : 9, 16, 17, 19, 20, 24, 25, 28, 35, 38, 50, 51, 58, 64, 66, 78, 80, 83, 87, 104, 124 ;
- nitrite (22 labs) : 3, 6, 17, 25, 38, 42, 46, 53, 60, 63, 64, 66, 68, 80, 92, 96, 97, 106, 110, 112, 117, 125 ;
- ammonia (43 labs) : 1, 9, 13, 16, 21, 24, 28, 32, 37, 38, 39, 40, 43, 45, 48, 49, 51, 53, 56, 57, 61, 64, 65, 67, 69, 77, 83, 86, 90, 91, 92, 93, 95, 96, 97, 100, 104, 106, 107, 110, 113, 114, 131 ;
- phosphate (23 labs) : 8, 9, 19, 28, 29, 37, 39, 45, 46, 48, 56, 66, 68, 78, 81, 83, 91, 105, 108, 114, 122, 124, 131.

While no more than 17-18 % of errors greater than 10 % is found in nitrate, nitrite and phosphate, this percentage exceeds 40 % in ammonia.

Bearing in mind that the discriminating percentage (10 %) is purely arbitrary and has no statistical basis, the procedure is intended only to help laboratories identify the nature of their major error. Nevertheless, it is evident that a significant part of the spread of ammonia data originates from proportional errors, and these are the type of error most readily identified and corrected.

7.4.3. Constant errors

As shown by the frequency distribution of constant errors (fig. 8), nitrate and nitrite exhibit only a slight tendency towards positive constant errors, mainly attributable to a few large errors. Positive errors are more evident for phosphate and especially for ammonia.

Constant errors originate mainly from misdefinition of the blank, a source of error which appears to be underestimated or ignored by many participants.

It is noticeable that the determinands with positive constant errors exhibit negative proportional errors. In some cases these effects may counteract to produce artificially accurate results (and good Z-scores).

As for random errors, the range of constant errors in which the majority of the participants (67 %) lies, indicates what can be considered achievable. They are :

- nitrate + nitrite (and nitrate) : ± 0.35 µmol/l,
- nitrite : ± 0.05 µmol/l,
- ammonia : ± 0.5 µmol/l,
- phosphate : ± 0.06 µmol/l.

Considering the precision normally expressed in typical nutrients results, these figures (excepting nitrate) are far from negligible, given that constant errors, in most cases, have well known origins. Particular attention should therefore be paid to blank correction procedures especially when normal seawater concentrations are being determined.

Ammonia deserves special mention in this context ; it has the widest range of constant errors, and yet it has the narrowest natural concentration range in coastal and oceanic waters.

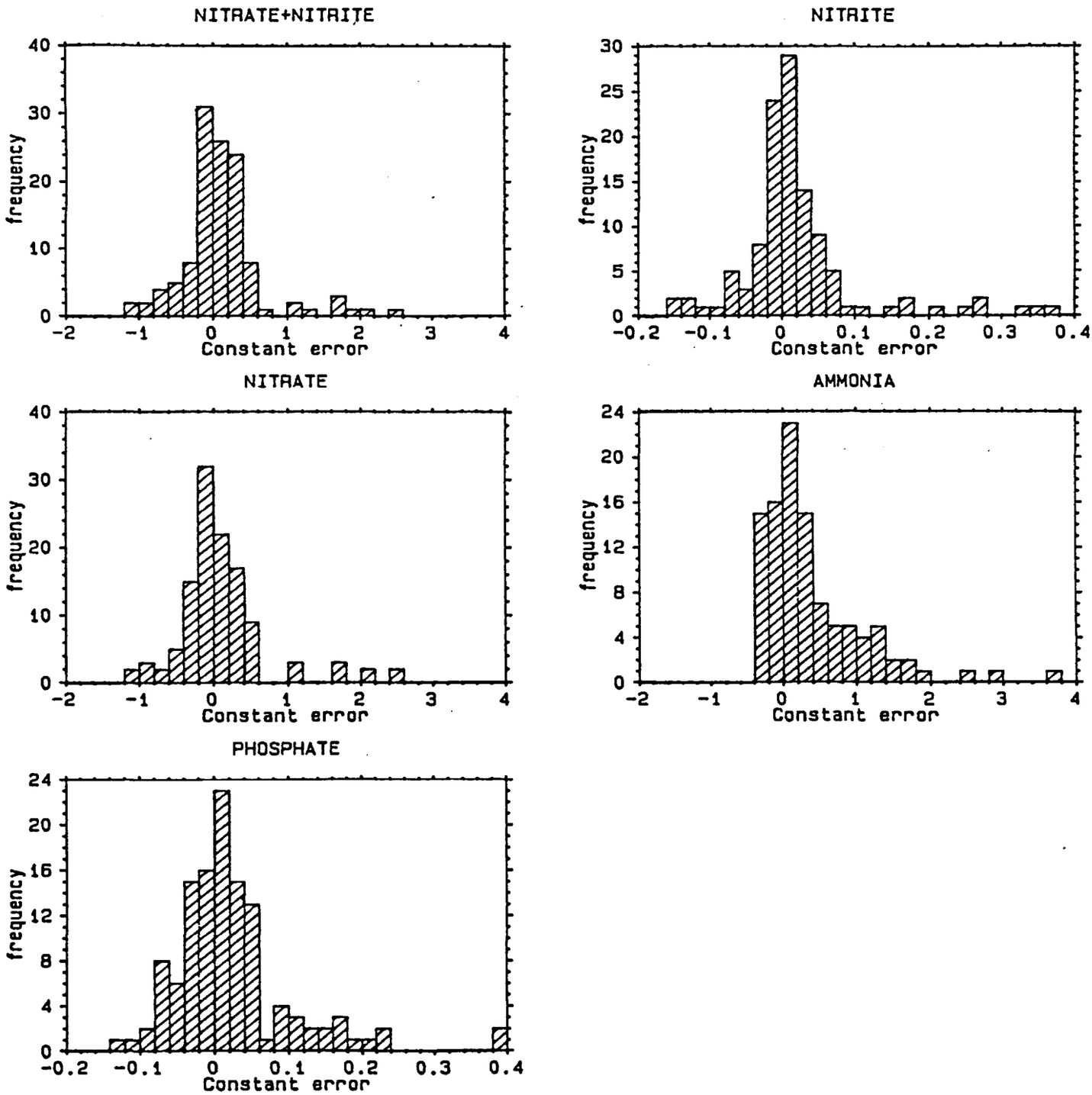

Figure 8 : Frequency distribution of individual constant errors.

7.5. Total N and total P results

A few laboratories sent results for total nitrogen (TN) and total phosphorus (TP). They are summarized in table 6 and plotted on figure 9.

No values have been *assigned* to the concentrations of these determinands.

Table 6

Raw results for total nitrogen and total phosphorus (µmol/l N or P).

Lab No.	Total nitrogen						Total phosphorus					
	1	2	3	4	5	6	1	2	3	4	5	6
19	18.0	11.3	34.8	-	-	-	-	-	-	-	-	-
20	25.6	16.2	41.4	-	-	-	-	-	-	0.54	2.10	0.87
32	18.37	7.91	33.26	8.58	12.79	9.16	0.09	0.15	0.16	0.10	1.88	0.39
69	-	-	-	-	-	-	-	-	-	0.19	1.84	0.55
72	-	-	-	7.10	11.4	7.71	-	-	-	-	-	-
76	39.107	29.330	49.728	34.885	38.441	32.886	-	-	-	-	-	-
91	18.64	8.21	30.07	7.07	11.57	9.36	0.29	0.23	0.13	0.26	2.03	0.65
100	20.0	8.57	31.43	-	-	-	-	-	-	0.969	2.20	0.840
132	16.67	7.76	30.44	7.85	12.18	8.71	0.22	0.23	0.22	0.23	1.88	0.60

Table 7

Statistics for total nitrogen and total phosphorus.

Nutrient	Sample	Full set			Reduced set		
		number of labs	mean (µmol/l)	s.d. (µmol/l)	number of labs	mean (µmol/l)	s.d. (µmol/l)
Total N	1	7	22.3	7.9	5	18.3	1.2
	2	7	12.8	7.9	5	8.8	1.5
	3	7	35.9	7.2	5	32.0	2.0
	4	5	13.1	12.2	4	7.7	0.7
	5	5	17.3	11.8	4	12.0	0.6
	6	5	13.6	10.8	4	8.7	0.7
Total P	1	3	0.20	0.10	-	-	-
	2	3	0.20	0.05	-	-	-
	3	3	0.17	0.05	-	-	-
	4	6	0.38	0.32	4	0.20	0.07
	5	6	1.99	0.14	-	-	-
	6	6	0.65	0.18	-	-	-

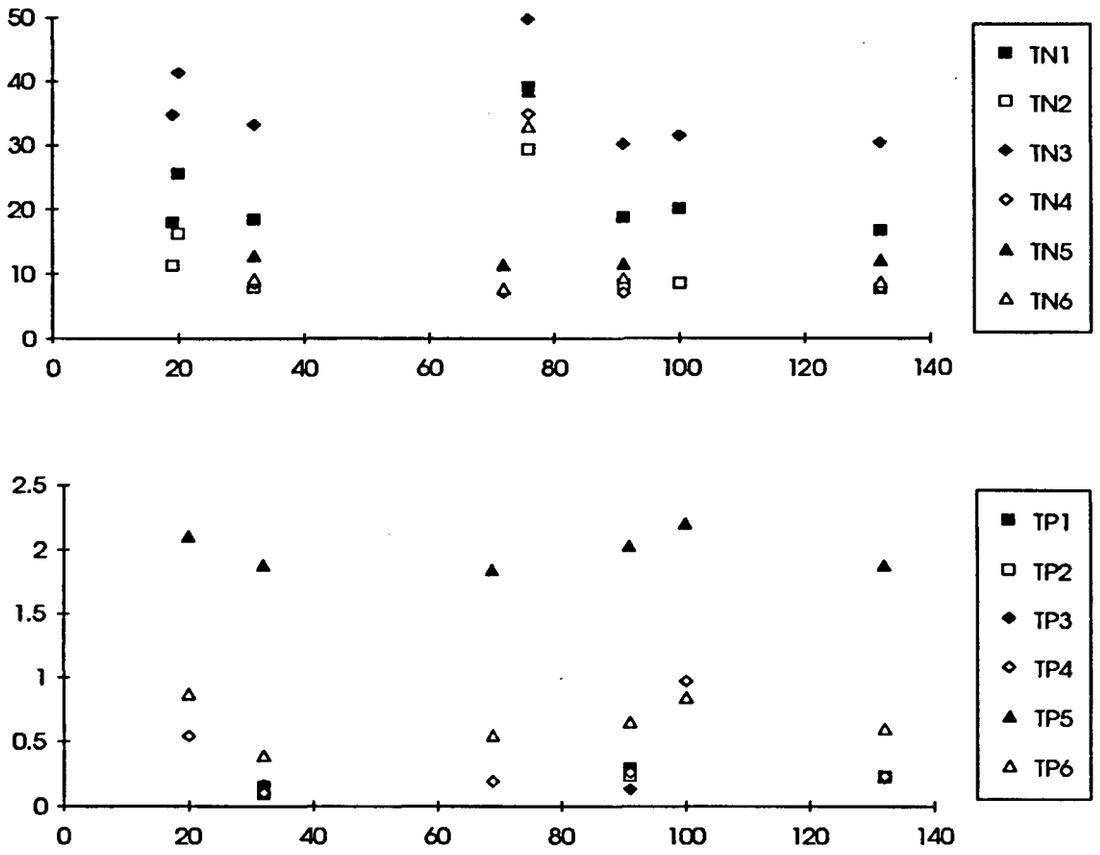

Figure 9 : Total nitrogen (upper) and total phosphorus (lower) results. Concentrations (µmol/l) versus laboratory number.

Statistics are summarized in table 7. No sophisticated treatment was applied, therefore the reduced set data (consensus data) were obtained after removing obvious outliers such as laboratories 20 and 76 in TN (all samples) and laboratory 20 and 100 in TP, sample 4. This removal corresponds to roughly 95 % confidence level rejection as for the other nutrients.

Plotting laboratory results versus consensus means (fig. 10) shows that most of the differences between laboratories are of a constant type.

Total N. Laboratory 76 exhibits all kinds of errors (random, proportional and constant) and needs to improve its entire technique. For all other participants, the differences in slope range between - 7 to + 9 % however, variation between laboratories are not consistent with that calculated for nitrate + nitrite. Consequently, the differences may originate from differences in the oxidative capacity of reagents.

Ignoring laboratory 76, all intercepts lie in a range of ± 1 µmol/l except for laboratory 20 with + 6.3 µmol/l. Although minor for most participants, all these differences are attributable to blank misdefinition.

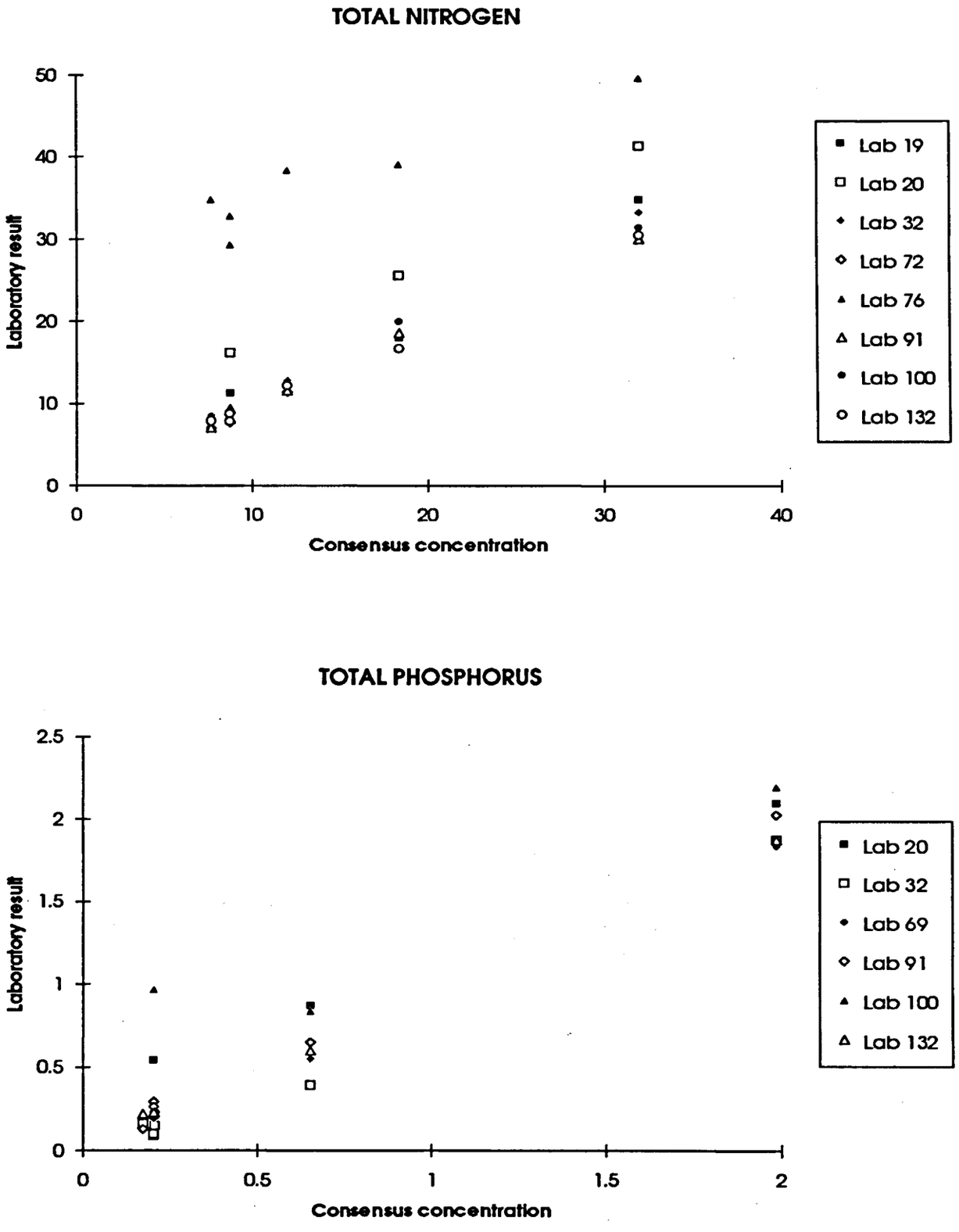

Figure 10 : Plot of laboratory results against consensus concentrations (µmol/l).

Total P. As for TN, differences in TP between laboratories are mostly attributable to constant differences (fig. 10). Laboratory 100 seems to have contaminated sample 4 while laboratory 20 has a high positive constant error associated with a negative proportional error. An inconsistent result is produced by laboratory 32 for sample 6 with TP < phosphate (- 0.02 µmol/l). However this laboratory exhibits a significant negative intercept of - 0.09 µmol/l originating from its phosphate determination. Excepting laboratories 20 and 100, all proportional differences remain within a few percent, a surprisingly narrow range compared with the phosphate proportional errors of these laboratories which are in a range of about 30 %.

From a knowledge of their preparation methods, samples 1, 2 and 3 are effectively replicates for phosphate and TP. This may give laboratories a way of estimating their internal repeatability.

8. SUMMARY AND DISCUSSION

In this exercise, no attempt was made to evaluate separately the results from various analytical methods or techniques (manual and automatic) as was done in I/C 4. This chapter summarizes the results for each type of determinand with particular attention to specific sources of error. Where appropriate, reference to technical points pertaining to automatic techniques has been made.

8.1. Nitrate + nitrite and nitrate

The determination of nitrate in seawater almost invariably involves reduction by copperised cadmium and subsequent measurement as nitrite. For this reason, the sum 'nitrate + nitrite' is considered a single determinand and is treated separately from nitrate. Nitrate is obtained by subtraction of nitrite (determined independently) and may therefore exhibit specific precision and accuracy differing from that of nitrate + nitrite. It is important that the efficiency of the nitrate to nitrite reduction should be maintained as close to 100 % as possible, and should preferably not fall below 95 %. When a column produces a low yield, substantial errors may be produced when mixed (nitrate + nitrite) standards and samples have significantly different nitrate/nitrite ratios. A recent paper by Garside (1993) deals with this problem in detail and gives typical examples.

The present exercise shows no significant difference in precision between nitrate + nitrite and nitrate alone, the reason being that nitrite concentrations are almost one order of magnitude lower than corresponding nitrate concentrations.

Within a consistent set of two thirds (or more) of the laboratories, a reproducibility of 3 % with an absolute value of 0.2-0.3 µmol/l, is achieved. This precision, slightly better than in I/C 4, is in good agreement with the precision (3-4 %) obtained in previous intercomparison exercises (Koroleff and Palmork, 1972 ; Grasshoff, 1977). Nitrate appears therefore to have reached a stable overall reproducibility level.

The examination of individual errors shows that for most laboratories, random errors are acceptable and that proportional and constant errors are equally distributed positive and negative. These observations linked to the fact that assigned and consensus concentrations are in excellent agreement lead to the conclusion that nitrate is correctly determined by a majority of laboratories.

Laboratories with the largest errors are invited to focus on each kind of error, bearing in mind that human skin is a significant source of contamination (Kérouel and Aminot, 1987), *i.e.* of random errors, as well as nitric vapors in the laboratory (as produced by some digestion processes).

Although concentrated stock standard solutions of individual nutrients are said to be stable indefinitely (Riley *et al.*, 1972 ; Strickland and Parsons, 1972), it is good practice to renew them at least every year, remembering to check the new against the old, before discarding the old. Working standards should be prepared daily and renewed in the case of signal drift. They should be prepared in the same matrix as the samples, *i.e.* in low nutrient seawater (LNSW). Incorrect standard solutions and matrix effects generate proportional errors. Constant errors are usually attributable to blank problems. The blank is produced by the presence of nitrate and nitrite in reagents (mainly in ammonium chloride). It should be determined using freshly drawn high quality demineralised water. In effect, distilled water (and all stored water) absorbs nitrogenous compounds from the atmosphere, the reduced forms being susceptible to eventual oxidation to nitrite and nitrate.

8.2. Nitrite

In the marine environment, apart from exceptional cases, the determination of nitrite is included in the determination of nitrate since nitrite is not often separately measured because of its low contribution to the nitrogen pool (generally one order of magnitude lower than that of nitrate).

Given that the method for the determination of nitrite is very sensitive and chemically uncomplicated, a relative standard deviation of 6-10 % appears rather high.

In concentration, the standard deviation is equal to or greater than about 0.05 µmol/l which is greater than that obtained in previous intercomparisons, *i.e.* 0.01 to 0.04 µmol/l within the same range of concentration (Koroleff and Palmork, 1972 ; Grasshoff, 1977).

Contrary to nitrate, contamination from skin is not significant (Kérouel and Aminot, 1987), however stock standards are not claimed to be stable for more than a few weeks or months (Riley *et al.*, 1972 ; Strickland and Parsons, 1972) ; all the more reason for working standards to be regarded as particularly unstable.

The relatively small 'within' standard deviation of a majority of the participants (0.025 µmol/l) indicates that random errors are not a major source of inaccuracy, which is in agreement with the known sources of error for this determinand. Given the low concentrations generally present in seawater, great attention should be paid to the blank determination using high quality demineralised water. With automatic equipment, positive constant errors are to be expected, due to the optical system generating a refractive index blank when the matrix composition (salinity) changes.

8.3. Ammonia

The dispersion of the ammonia results shows the particular difficulty encountered by analysts in the accurate determination of this nutrient, characterized by relative standard deviations greater than 20 % and absolute values exceeding ± 0.2 µmol/l. In the exercise reported by Grasshoff (1977) standard deviations of 0.09-0.16 µmol/l are reported for ammonia spikes of 1.2-3.7 µmol/l

(4-14 %). However, natural unspiked waters, with average concentrations of 0.2-0.4 µmol/l, produced standard deviations of 0.12-0.26 µmol/l.

The precision of the ammonia determination stated by Koroleff (1969, 1983a) is close to ± 5 %. At a level of 3 µmol/l, Riley *et al.* (1972) reported ± 4 % (0.12 µmol/l) and Solorzano (1969) ± 0.07 µmol/l (2.3 %). Considering such values as within laboratory repeatability they may be compared with the standard deviations of 0.02-0.03 µmol/l obtained from replicate analysis during the Second Baltic Intercalibration Workshop (Koroleff, 1983b).

These observations show that the major part of the difference between laboratories is attributable to constant and proportional errors. The same conclusion can be drawn from the present exercise despite the relatively large range of errors of every type.

It is worth restating that the ammonia determination is highly susceptible to skin and atmospheric contamination (Kérouel and Aminot, 1987), the main sources being the general background of atmospheric ammonia and amines (particularly in urban laboratories), the analyst in person, and the presence of volatile chemicals. As a consequence, a few recommendations may be made : sample bottles should be stored in a clean environment, opened only when necessary, aliquots (as large as possible) must not be pipetted by mouth and should be treated immediately.

Participants' attention is drawn to some additional important details. High quality demineralised water is the only acceptable 'pure water' suitable for use in this determination. It should be freshly prepared and used as soon as it has been drawn from the deioniser equipment. Blanks and standards should be used immediately after preparation, discarded soon afterwards, and renewed as required.

In the widely used indophenol blue method, certain reagents are known to be unstable, and should be stored cold and frequently renewed. Various versions of the method are described in the literature and some laboratory modifications are unsuitable for the determination of ammonia in the full range of seawater salinities (0-38 PSS). The matrix effect is significant, not necessarily linear, and should be determined by each analyst.

In the present exercise the tendency to produce negative proportional errors is assumed to be a matrix effect rather than a calibration problem. This negative effect may also explain the difference between consensus and assigned concentrations.

In automatic methods, reaction conditions may differ from those used in manual methods since the medium is heated to accelerate colour development. Refractive index blanks are also generated by colorimeters' optics and flowcells.

Strict application of blank and standard procedures are vital for a successful ammonia determination. Every potential source of ammonia in the analytical environment, in reagents and 'pure' water should be identified and kept in mind at every stage of the procedure, as well as the instability of working standards and samples due to biological activity.

8.4. Phosphate

The results of the present intercomparison are very similar to those of the previous one (NUTS I/C 4). The standard deviations are in the same range as for earlier exercises (Koroleff and Palmork, 1972 ; Grasshoff, 1977), *i.e.* 0.03 to 0.09 µmol/l for concentrations up to 3 µmol/l.

These figures should be compared with the laboratory repeatability of 0.02-0.03 µmol/l stated by Riley *et al.* (1972) and Strickland and Parsons (1972) and less than 0.04 µmol/l for a majority of laboratories in the present exercise.

The determination of phosphate was specially addressed in I/C 4 with particular attention to the origin of deviations from the mean and to biases caused by automatic methods. Participants can find a full treatment in the corresponding report (Kirkwood *et al.*, 1991).

Additional information from the individual errors estimation shows a tendency towards negative proportional errors. Since there is no salt effect on colour intensity in Murphy and Riley's (1962) method, this may originate from inconsistent changes to their basic procedure.

Contamination from skin may significantly affect seawater concentrations in autoanalyser cups (Kérouel and Aminot, 1987).

Analysts' attention is drawn to the fact that some methods for the determination of nitrite (hence nitrate) specify the use of phosphoric acid rather than hydrochloric, as was used in the original Bendscheider and Robinson (1952) procedure. The use of phosphoric acid in this context is a potentially serious source of contamination in the determination of phosphate and should be avoided.

9. COORDINATORS' FINAL REMARKS

A) The concurrence of I/C 5 and QUASIMEME produced an unforeseen advantage. The I/C 4 report contains our opinions on the most probable sources of error in the results. This time we have had the benefit of direct personal contact with, and feedback from the nutrients analyst of each of the 56 QUASIMEME laboratories that submitted results. At QUASIMEME Workshop III in Portugal (October 1993), seminars and discussion sessions were devoted specifically to the results and problems of these laboratories and we now know their precise nature. We have no reason to suspect that the problems of the 76 non-QUASIMEME laboratories were any different from those of the 56.

B) The results have been scrutinised for the four highest and four lowest values for each determinand-sample combination and the laboratories responsible for these 'extreme values' (EV) have been ranked according to the number of EVs each produced (four determinands, three samples, four high and four low for each, produces 96 EVs). Eleven laboratories have 3 or more EVs against their names and although this treatment has no statistical basis, there must be some justification for describing these labs (8 % of the total) as the group whose performance appears to be most in need of improvement.

   Applying the same criteria to the I/C 4 results produces a similar sized group (9 % of the total), amounting to 6 laboratories (The same process applied to I/C 3 would undoubtedly produce its group, but they would be anonymous).

   The point is that in I/C 4 and I/C 5 we know exactly who these labs are, and there is a clear correlation between the production of EVs and participation in previous exercises of this kind.

   1. None of the 11 labs identified in I/C 5 had participated in I/C 4.
   2. None of the 6 labs identified in I/C 4 had participated in I/C 3.

Evidently 'novice' laboratories are likely to be the worst performers, and while this is no less than would be expected, it shows the value of participation, as they have no way of knowing how good or bad their analytical chemistry is until they have participated.

If they produce poor results in an I/C, then they have learned something useful from their participation, and poor performance can be remedied once recognised.

If they produce good results in their first I/C, so much the better, and not only have they proved it to themselves, but the whole nutrients world knows they have done it.

C) Once more, we wish to record our disapproval of the way some participants express their results. The precision and sensitivity implied by a result containing five or six significant-looking digits is totally unrealistic in colorimetric analysis, and can only serve to mislead.

D) Section 5 mentions the fact that there were five defaulters, *i.e.* laboratories that accepted samples but returned neither results nor unused samples. In early correspondence and in the information that accompanied the samples, participants were made aware that they could expect to attract some criticism if they defaulted in this way. We suspect their reasons for default are either inertia, or they chose to retain the samples for their own non-ICES purposes. We remind laboratories that as participation in ICES NUTS I/Cs is free of charge, they are expected to comply with the rules of the game.

E) Inspection of the identities of laboratories listed in 7.4 as having serious errors, while showing a few surprises (labs which did well in I/C 4), shows that the majority are newcomers to ICES exercises and probably have little or no experience of intercomparison work for nutrients ; we suspect they were unprepared for an exercise of this kind. Early correspondence with intending participants advised them strongly to read the I/C 4 report to give them some indication of what might be expected of them, but we know of cases where such correspondence reaches the laboratory but not the analyst. We also know that in some organizations, nutrients are thought to be 'easy' and are entrusted to inadequately trained staff.

F) Inspection of the identities of laboratories listed in 7.3 as having produced high-quality results shows that the great majority of these also did well in, or at least participated in I/C 4. While this comes as no surprise, it is worth noting that one laboratory (85) with no past history in ICES or any other intercomparison work known to the coordinators, has produced particularly good results. It can be done !

ACKNOWLEDGEMENTS

The coordinators acknowledge the contribution of Roger Kérouel for data management and treatment and of Marie-Pierre Le Bris for typing the manuscript.

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ANNEX I

Compilation of information and instructions sent to participants

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague

Fifth Intercomparison Exercise for Nutrients in Seawater "NUTS I/C 5"

This letter is to let you know that you have not been forgotten, and that the organisation of NUTS I/C 5 is proceeding as planned.

Progress in sample preparation has enabled us to bring forward the expected date for sample distribution from early 1993 to late 1992 but the deadline for reporting results will remain unchanged.

The list of provisional participants now stands at over 100 and it looks likely that this exercise will be the largest ever for nutrients in seawater.

You will be aware that there is no charge for the samples, but as the cost of packaging and postage is quite considerable, we ask you now to confirm your intentions so that we may avoid sending samples unnecessarily to laboratories which are not in a position to participate.

The enclosed reply card should be used to confirm your participation, and the following points should be clearly understood.

1. If you do not return the card you will not receive any samples.

2. If the card is not returned within 30 days, we will assume that you may not have received this letter, or that the card has gone astray. We will send a further copy of this letter and card.

3. We will acknowledge receipt of your card promptly. If you do not receive an acknowledgement within 20 days please contact us in case your card has gone astray.

4. A returned card confirming your wish to participate commits your laboratory to analysing the samples and submitting results before the reporting deadline, or returning the samples intact before the reporting deadline, if for any reason you are unable to analyse them. (Any laboratory which accepts samples, retains them and fails to submit results before the deadline can expect to attract criticism in the Report.)

Also enclosed is a short note entitled "ICES Nutrients I/Cs - The First 25 Years". We hope it will be of some interest to you. (NUTS I/C 5 will not differ substantially from the NUTS I/C 4 format.)

Once more we remind you, return the card if you want to receive samples.

Further details of deadlines etc. will follow after your participation has been confirmed.

Best wishes.

Alain Aminot and Don Kirkwood

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Fifth Intercomparison Exercise for Nutrients in Seawater "NUTS I/C 5"

I have received your letter of and
now return this card to confirm my intention to participate

Signature

Date

Annex I

ICES NUTRIENTS I/Cs - THE FIRST 25 YEARS

The **first** Intercalibration (to include nutrients) was an entirely Baltic affair in June 1965 when three research vessels met by private agreement in Copenhagen.

'Aranda'	IMR, Helsinki
'Hermann Wattenberg'	Institut fur Meereskunde, Kiel
'Skagerak'	Royal Fishery Board, Gothenburg

Each ship contributed freshly collected bulk samples to the experiment and these were sub-sampled and analysed on board each of the three participating ships on the same day.

Oxygen, salinity, chlorinity, alkalinity and phosphate were determined.

Folke Koroleff compiled the report (ref 1) which contained contributions from himself, Stig Fonselius and Klaus Grasshoff, and it was presented at the ICES 53rd Statutory Meeting in Rome in October 1965.

Such was the success of this venture, these three campaigned via the Hydrography Committee for a continuation of intercalibration work under a proposal entitled "Intercalibration and Standardisation of Chemical Methods'.

The **second** exercise, in 1966 under the auspices of the newly formed ICES Working Group on the Intercalibration of Chemical Methods, while still predominantly a Baltic initiative, consisted of two parts, Leningrad, during the 5th Conference of Baltic Oceanographers, and Copenhagen, at the ICES 54th Statutory Meeting.

Part I Leningrad (May 1966)

The participating research vessels were

'Alkor'	Institut fur Meereskunde, Kiel
'Okeanograf'	IMR, Leningrad
'Prof Otto Krammel'	Institut fur Meereskunde, Warnemünde
'Skagerak'	Fisheries Board of Sweden, Gothenburg

RVs delivered bulk samples which were sub-sampled and analysed almost immediately for oxygen, salinity, chlorinity pH and phosphate.

Part II Copenhagen (September 1966)

The list of interested parties continued to grow, and in addition to Baltic countries, Norway and UK were now represented.

Once more, RVs delivered bulk samples and the various participants analysed samples simultaneously in Copenhagen. As for Part I (Leningrad) and in the previous year's exercise (Copenhagen 1965) the determinands of primary interest were oxygen, salinity and chlorinity, but, in addition to phosphate, this time nitrate, nitrite and silicate were included.

The final report edited by Grasshoff (ref 2) makes no mention of nitrate nor nitrite but some of those who were present are now prepared to confess that these results were "too terrible to be included'! To be fair to those involved, 1966 was early days for heterogeneous cadmium-based nitrate/nitrite reduction techniques and some of the associated problems were presumably not fully appreciated at the time.

Evidently nitrate had some way to go to achieve the reliability and ease of operation of the Murphy & Riley (1962) phosphate technique but it is worth noting that intercomparison work on phosphate so far had consisted of simultaneous analysis of freshly obtained sub-samples by a small number of highly competent workers, in close contact with each other exchanging calibration solutions, ideas, technical details, etc.

Subsequent to the Copenhagen trial, Jones and Folkard undertook a detailed laboratory examination of the individual methods used by the participants, and in their contribution (ref 3) to Grasshoff's report they were pleased to announce "There seems to be no need for any further intercalibration in the determination of inorganic phosphate by this method.'

Clearly this happy state of affairs could not last, and it didn't. Along came the auto-analyser!

The **third** exercise was organised by the ICES Working Group on Chemical Analysis of Seawater under the joint auspices of ICES and SCOR and its title, "The International Intercalibration Exercise for Nutrient Methods", shows that it set out to be an ambitious project.

There was a distribution of samples in 1969/70 and 45 laboratories from 20 countries submitted results, but it was to be 1977 before the final report (ref 4), Cooperative Research Report No. 67, was published.

The time had come to study "nutrients" separately from oxygen, salinity, chlorinity and pH, but aware of the problems arising from the instability of natural seawater samples, the organisers (Koroleff, Palmork, Ulltang and Gieskes) chose to use standard solutions which were prepared and distributed by the Sagami Chemical Research Center, Japan.

In this exercise participants performed the analyses in their own laboratories but despite being supplied (knowingly) with appropriate blank solutions for each determination, the overall accuracy, particularly for phosphate and nitrate, was disappointing.

The report concludes "As methods did not diverge much, it is clear that variations must be sought primarily in the standardization procedures. The results will also aid participants in re-evaluating their analytical procedures by comparison of their methods with those that appear most satisfactory from this exercise'.

The names of the participating laboratories were listed, as were the tables of results, but there was no way of linking these together. Hindsight suggests that this may have been counter-productive ; we now suspect that there is no greater incentive for a laboratory to improve its performance than the knowledge that its peer-laboratories throughout the world are all aware that it is producing poor quality data.

Various "workshop" and multi-ship events following the ICES-SCOR exercise included nutrient studies but it was to be many years later (1988) before the ICES Marine Chemistry Working Group produced volunteers (Kirkwood, Aminot and Perttilä) to organise the next large scale I/C, designated the **fourth**. "NUTS I/C 4" did not set out to be world-wide, beginning only with laboratories in ICES member countries, but others who got to hear about it were not turned away.

The fourth differed from the third in three important respects:-

a) The test samples were natural or near-natural seawater rather than standard solutions. (Strictly speaking, this made the exercise an Intercomparison rather than an Intercalibration.)

b) Participants were unaware that "blank" samples were included.

c) Anonymity was abolished. Participants were made aware from the outset that the final report would list identities of laboratories, results, and a means for any reader to connect these.

69 laboratories from 22 countries submitted results, and thanks in some measure to the "fax" the final 83 page report (ref 5) was in the hands of participants within two years of the distribution of samples. Statistical treatment identified 58 laboratories consistent in phosphate, 51 consistent in nitrate, and 48 consistent in both phosphate and nitrate, including a group of 12 whose results were especially close to the consensus concentrations.

Due to the generally perceived need for more and better quality control in analytical measurement, the Marine Chemistry Working Group has plans for further exercises at approximately four-year intervals and the organisers now feel it is time to go truly world-wide again. The **fifth** exercise "NUTS I/C 5" or in strict ICES parlance "5/NT/SW", will begin with a distribution of samples in 1993 and the intention is to include every laboratory anywhere that measures nutrients in seawater. There will be no charge for the samples but intending participants will be at a definite disadvantage if they haven't first read ICES Cooperative Research Report No. 174 (the NUTS I/C 4 Report).

The provisional list of participants now stands at 110.

References

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2. Report on Intercalibration Measurements, Leningrad 24-28 May 1966, Copenhagen, September 1966, UNESCO Technical Papers in Marine Science, No. 9, 114pp.
3. Report on the analysis of phosphate at the ICES intercalibration trials of chemical methods held at Copenhagen 1966. ICES CM 1967/C20.
4. The International Intercalibration Exercise for Nutrient Methods, ICES Cooperative Research Report No. 67 (1977), 44pp.
5. Fourth Intercomparison Exercise for Nutrients in Sea Water, ICES Cooperative Research Report No. 174, (1991), 83pp.

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague

Fifth Intercomparison Exercise for Nutrients in Seawater "NUTS I/C 5"

Thank you for returning the card confirming your wish to participate in this exercise.

Samples will be sent to you from IFREMER, in November 1992.

With the samples you will receive information on salinity, approximate concentration ranges for nutrients, and full instructions on results reporting procedures and deadlines.

At the same time as these samples are posted, you will also be sent a separate letter letting you know that the samples are on their way.

Best wishes

Alain Aminot and Don Kirkwood

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague

Fifth Intercomparison Exercise for Nutrients in Seawater "NUTS I/C 5"

More than 30 days have passed since you were sent information on this exercise, including a post-card that you were required to return in order to confirm your wish to participate.

Just in case the information did not reach you for some reason, we enclose with this letter all of the information that you were sent on 5 August.

Sample materials are in limited supply and at the moment there are six laboratories on a reserve-list ready to participate if any laboratories drop out.

If we do not receive a reply from you within 30 days of the date of this letter we will assume you no longer wish to participate and your laboratory will be replaced by one of those on the reserve-list.

If you do not wish to participate we would appreciate notification rather than default ; we can then be sure that our letters have been reaching you.

Remember, return the card if you want to receive samples.

Yours sincerely

Alain Aminot and Don Kirkwood

Enc.

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague

ICES Fifth Intercomparison Exercise for Nutrients in Seawater "NUTS I/C 5"

This letter is to let you know that your package of samples will be sent from IFREMER, Brest around the end of November, or early December.

Enclosed is a copy of the instructions that will accompany the samples and you should take the opportunity to ensure that whoever will analyse the samples, reads and understands these instructions fully.

Your results should be sent to Alain Aminot at IFREMER, Centre de Brest, BP70, 29280 Plouzané France and should reach him before the end of April 1993.

Receipt of your results will be promptly acknowledged by the organisers.

If you have not received your samples within what you would consider to be an acceptable postal transit time from France, or if there is some problem with the samples, please contact Alain Aminot, preferably by fax, 98224548 (phone 98224466).

Yours sincerely

A Aminot and D Kirkwood

ICES FIFTH INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEAWATER "NUTS I/C 5"

IMPORTANT INSTRUCTIONS TO BE READ IN FULL AS SOON AS RECEIVED, AND BEFORE OPENING ANY SAMPLES

1. Package contents

- Your package contains 6 bottles intended for the following determinations :
  Nitrate and nitrite : 3 bottles numbered 1, 2 and 3 (red labels).
  Phosphate and ammonia : 3 bottles numbered 4, 5 and 6 (yellow labels).

2. Preservation of samples

- No preservatives have been added.
- *DO NOT OPEN ANY BOTTLES BEFORE YOU ARE COMPLETELY READY FOR THE ANALYSIS* ; when opened, their sterility will be lost and their concentrations compromised.
- Store samples in darkness at room temperature (acceptable range 15-20 °C).

3. Analysis

- The two parameters in each bottle should be determined on the same day.
- If the two parameters are not determined simultaneously, re-seal the bottle carefully immediately after first use and store in a refrigerator during the interval between the two determinations (do not freeze).

Additional information relevant to the analysis

- The samples should be analysed without filtration.
- Salinities are 35.3 ± 0.1 except for sample 5 which is 34.8.
- Concentrations can be assumed to be in the following ranges : nitrate < 40 (µmol/l), nitrite < 3, phosphate < 3, ammonium < 8.
- In order to minimise the dissolution of glass by the contained sample, avoid delaying the analysis of samples 4, 5 and 6 beyond a few months. This should ensure that colorimetric interference from silicate in the determination of phosphate will be negligible.

4. Reporting of results (to Alain Aminot, at IFREMER, Brest)

- Report concentrations in micromoles per litre on the attached results sheet.
- Note the dates of receipt and analysis of the samples.
- Report only one value for each parameter for each sample.

5. Additional determinations

- Participants are welcome to supply results for total-N and total-P if these determinations are routine in their laboratory.
- Please use the reverse side of the report form for any additional information of this kind, or any comments or suggestions you may wish to make.

Annex I

ICES FIFTH INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEA WATER 'NUTS I/C 5'

RESULTS REPORT FORM

LABORATORY : _____

_____

_____

_____

DATE OF RECEIPT OF SAMPLES : _____

DATE(S) OF ANALYSIS : sample 1 _____

sample 2 _____

sample 3 _____

sample 4 _____

sample 5 _____

sample 6 _____

Results in micromoles per litre

	Sample 1	Sample 2	Sample 3
NO3 + NO2			
NO2			
NO3 (by subtraction)			

	Sample 4	Sample 5	Sample 6
PO4			
NH4			

THESE RESULTS SHOULD BE SENT TO :

Alain Aminot
IFREMER, Centre de Brest,
BP70 29280 Plouzane
FRANCE

Annex I

Plouzané, 1993

O/Ref. : DEL/CMCN/012/AA/93

Object : **ICES Fifth Intercomparison exercise for nutrients in seawater "NUTS I/C 5"**

Dear Colleague,

We are pleased to acknowledge receipt of your NUTS I/C 5 results. These are now in our computer awaiting further treatment.

To ensure these have been no transcription errors etc., we now invite you to check that the results attributed to you by our computer are identical to those you submitted :

lab	sample	NO3 + NO2	NO2	NO3	sample	PO4	NH4
	1				4		
	2				5		
	3				6		

Please let us know, without delay, if there are any discrepancies.

Thank you.

Yours sincerely.

A. AMINOT

Annex I

22 April 1993

Dear Colleague

ICES FIFTH INTERCOMPARISON EXERCISE FOR NUTRIENTS, "NUTS I/C 5"

We have not yet received results from your laboratory for the fifth ICES Intercomparison Exercise for nutrients in seawater.

You may recall that the letter which accompanied the samples in November/December 1992 requested that results should be submitted before the end of April 1993. We considered this a generous deadline and look forward to receiving your results in the next few days.

If you have not yet analysed these samples ***please let us know whether you intend to analyse them*** and we will wait a little longer before commencing our statistical analysis of the results.

If you do no intend to submit results you are expected to return the samples intact to IFREMER.

Failure to submit results or return the samples will expose your laboratory to some criticism in the Report of this exercise ; you may recall that these were conditions of participation.

Yours sincerely

A Aminot and D Kirkwood

PS If you have sent your results very recently, please disregard this letter.

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague

ICES Fifth Intercomparison Exercise for Nutrients in Seawater (NUTS I/C5)

This letter is to let you know that NUTS I/C5 has now concluded.

A full report on this exercise is expected to be considered by the ICES Marine Chemistry Working Group at its forthcoming meeting in Brest in February 1994, and publication by ICES as a Cooperative Research Report is anticipated as soon afterwards as is practical.

Meanwhile, the enclosed histograms will allow you to make a preliminary approximate assessment of your laboratory's performance.

As for NUTS I/C4, the NUTS I/C5 report will contain:-

a) a list of participating laboratories

b) their results, in full

c) statistical treatment and discussion

d) details of methods used for sample preparation

Please be aware that ***you*** are the only person in your institute/organisation who has received this package of information ; if there are others who wish or need to be informed, we are relying entirely on ***you*** to do so.

As our listed participant, ***you*** can expect to receive one free copy of the final ICES Report. Further copies may be purchased from ICES if required.

We take this opportunity to thank you for your participation and assure you that your laboratory will be automatically included in the mailing list for any further exercises of this kind. (NUTS I/C6 should be around 1996/7).

Yours sincerely

Alain Aminot and Don Kirkwood

ANNEX II

List of participating laboratories

ICES (fifth) INTERCOMPARISON EXERCISE FOR NUTRIENTS "NUTS I/C 5"

LIST OF PARTICIPANTS

1			CNICT-CNP, Puerto Madryn	ARGENTINA
2			CSIRO North Beach, WA	AUSTRALIA
3			Water Board, West Ryde, NSW	"
4			EPA, Lidcombe, NSW	"
5			CSIRO, Hobart, Tas.	"
6	Q	27	MVLB-Math. Model NS, Oostende	BELGIUM
7	Q	26	Univ. Libre, Bruxelles	"
8		77	BBSR, Ferry Reach	BERMUDA
9			Univ. BC, Vancouver, BC	CANADA
10		82	IOS Sidney, BC (A)	"
11			" " (B)	"
12		72	BIO, Dartmouth, NS	"
13			SIO-SOA, Hanzhou	CHINA
14	Q		WQI, Hørsholm	DENMARK
15	Q	1	DIFMR, Charlottenlund	"
16	Q	2	NERI, Charlottenlund	"
17		31	IEE-TT Univ., Tallinn	ESTONIA
18		30	EMI, Tallinn	"
19		53	HS, Tórshavn	FAROE ISLANDS
20	Q	32	FIMR, Helsinki	FINLAND
21	Q		NBWERL, Helsinki	"
22	Q		LF-A, Dunkerque	FRANCE (11)
23	Q		IPL, Gravelines	"
24	Q		IFREMER, Boulogne	"
25	Q	47	INTECHMER, Cherbourg	"
26	Q	39	LMR, Rouen	"
27		60	Univ. BO, Brest	"
28	Q	61	LM, Brest	"
29		57	IFREMER, Nantes	"
30		63	IEEB, Bordeaux	"
31			IFREMER, Sète	"
32	Q		Univ. A-M, Marseille	"
33	Q	8	LWKS-H, Kiel	GERMANY (18)
34		7	IfM, Univ. Kiel	"
35	Q		SAUN, Stralsund	"
36	Q	4	IfO, Warnemünde	"
37			Univ. Rostock	"
38			BAH-MH, Helgoland	"
39			NLfO-FK, Norderney	"
40		10	Univ. Hamburg	"
41			Bran & Luebbe, Hamburg	"
42			UHAU, Hamburg	"
43	Q		BSH, Hamburg	"

44		13	Alfred Wegner IPM, Bremerhaven	GERMANY (cont)
45			ZFKM, Wilhelmshaven	"
46			GKSS-FG, Geesthacht	"
47			Univ. Oldenburg	"
48			Fed. IH, Berlin	"
49	Q		NLO, Hildesheim	"
50	Q		BfG, Koblenz	"
51	Q		NCMR, Athens	GREECE
52	Q		Univ. Athens	"
53	Q		IMB, Iraklion, Crete	"
54	Q	64	MRI, Reykjavik	ICELAND
55	Q		FRC, Dublin	IRELAND
56	Q	51	Dublin Corp., Dublin	"
57		49	ESU, Trinity College, Dublin	"
58	Q	55	Univ. College, Galway	"
59	Q		EOLAS, Shannon	"
60	Q		ENEA, La Spezia	ITALY
61			ICRS, Rome	"
62			JAMSTEC, Yokosuka	JAPAN
63			MMC-HA, Riga	LATVIA
64			LMRL, Klaipeda	LITHUANIA
65	Q	21	NIOZ, Texel	NETHERLANDS
66		22	TNO-AMRL, Den Helder	"
67	Q	25	Rijks. - TWD, Middelburg	"
68	Q	24	NIOO-CEMO, Yerseke	"
69			AA&A, Auckland	NEW ZEALAND
70	Q	23	IMR, Bergen	NORWAY
71			Univ. Bergen	"
72		17	NIWR, Oslo (A)	"
73	Q		NIWR " (B)	"
74			SBSF, Hisøy	"
75		15	SFI, Gdynia	POLAND
76		14	IMWM, Gdynia	"
77	Q		Univ. Aveiro	PORTUGAL
78	Q		DGQA-CIA, Lisboa	"
79	Q	68	IH, Lisboa	"
80			Univ. Qatar, Doha	QATAR
81			CSIR, Congella, Natal	SOUTH AFRICA
82	Q		AZTI-SIO, Pedernales	SPAIN (8)
83	Q	66	IEO, Coruña	"
84			IIM-CSIC, Vigo	"
85			CEAB-CSIC, Blanes	"

86	Q		LCT-CONTOX, Madrid	SPAIN (cont)
87	Q	65	IEO, Palma-Mallorca	"
88	Q		DGITFAP, Huelva	"
89	Q	71	IEO, Tenerife	"
90	Q	36	Univ. Umeå, Hörnefors	SWEDEN (13)
91			KML, Uppsala	"
92		19	IAER, Solna	"
93	Q	20	ASKO Lab., Univ. Stockholm	"
94		18	ABH, Bromma	"
95	Q		SMHI, Norrkoping	"
96		12	KML, Uddevalla	"
97	Q	11	RSAS, Fiskebäckskil	"
98	Q	5	SMHI, Göteborg	"
99		6	Univ. Göteborg	"
100			KML, Halmstad	"
101			KML, Helsingborg	"
102		3	VBB, Malmö	"
103		70	MET Univ., Içel	TURKEY
104		38	Highland RPB, Dingwall	UK (22)
105	Q	33	SOAFD, Aberdeen	"
106		42	SMBA, Oban	"
107	Q	35	Forth RPB, Edinburgh	"
108	Q	40	Clyde RPB, Glasgow	"
109			NW-NRA, Carlisle	"
110			DANI, Belfast	"
111	Q		DED-ISC Lisburn	"
112			Univ. Liverpool, Port Erin, IOM	"
113			Univ. Liverpool	"
114			MBCC, Bangor	"
115		29	Univ. EA, Norwich	"
116			Anglian-NRA, Peterborough	"
117	Q	28	MAFF, Lowestoft	"
118	Q	45	Welsh-NRA, Llanelli	"
119			Wallace-Evans, Bridgend	"
120			IOS, Wormley	"
121			Univ. Southampton	"
122			SW-NRA, Exeter	"
123		54	PML, Plymouth (A)	"
124	Q		" (B)	"
125			Univ. Plymouth	"
126		83	OS Univ., Corvallis, OR	USA (7)
127			Univ. NH, Durham, NH	"
128			Univ. RI, Narragansett, RI	"
129		76	Univ. Maryland, Solomons, MD	"
130	Q		Texas A & M Univ., College Station, TX	"
131		78	NOAA, Miami, FL	"
132		84	Univ. H, Manoa, Honolulu, HI	"

QUASIMEME participants (56) are indicated by "Q" in column 2.
NUTS I/C4 participants (61) are indicated by their I/C4 Laboratory Number in column 3.

ANNEX III

Preparation and control of sample materials

Preparation and testing of reference material for the ICES fifth intercomparison exercise for nutrients in sea water

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CONTENTS

1. INTRODUCTION

Intercomparisons are important tools for quality assurance of chemical analysis. They rely on the distribution of Reference Material, *i.e.* a homogeneous and stable material, similar in type to the sample to be analysed (Taylor, 1983). The ICES fourth intercomparison exercise for nutrients in sea water used two types of reference material with significant nutrients concentrations : a naturally stable deep sea water and a coastal sea water stabilized by autoclaving (Kirkwood *et al.*, 1991 ; Aminot and Kérouel, 1991). The aim of the fourth exercise being to check only nitrate and phosphate determination performances, natural untreated waters were convenient. Indeed these determinands are the final products of the mineralisation oxidation steps in which nitrogen and phosphorus are involved and therefore are stable in oxic sea water. However, ammonia and nitrite are unstable under such conditions since they can be transformed by nitrifying bacteria into more oxidized compounds.

Consequently, the only way of conducting an exercise involving nitrate, nitrite, ammonia and phosphate was to produce sterile reference material. The fifth ICES intercomparison exercise for nutrients in sea water was therefore based on the production of autoclaved samples according to the method previously described (Aminot and Kérouel, 1991), but in addition to the former preparation scheme, the objective was to obtain a reference material to which concentrations could be assigned, independent of the statistical evaluation extracted from the participants' results.

This paper describes, in detail, how the material was prepared and how the values were assigned, and presents the results of homogeneity and stability testing.

2. GENERAL DESCRIPTION OF THE PROCEDURE

2.1. PREPARATION OF THE MATERIAL

2.1.1. CONCENTRATION LEVELS

The aim was to offer three concentration levels for nutrients, covering the range of normal concentrations in temperate European coastal waters. For simplicity, samples were produced in which all nutrients were at the same nominal level, low, intermediate or high according to their concentrations.

It is, however, hardly practical to expect to obtain samples of natural sea water high and low in nutrients, simultaneously.

To overcome this difficulty, it is preferable to obtain a bulk sample of water many months before the exercise and to let the nutrients reach low levels due to the action of phytoplankton under laboratory light and temperature conditions. This low nutrients sea water can then be enriched, if necessary, to the desired level using concentrated nutrient salt solutions. This method was used for the preparation of the present samples.

2.1.2. VOLUME OF SAMPLES AND NUTRIENT GROUPING

The decision to send two bottles of each water instead of one was for preservation reasons. In most laboratories, all four nutrients are not generally determined together, therefore if only one sample were available for the four nutrients, the question of storage of the sample between the various determinations would become important. With two bottles per sample, no problem should normally be encountered.

Grouping nitrate and nitrite together seemed the best compromise, since they are determined using the same reagents. Ammonia and phosphate remained consequently for the second bottle. Two bottles containing about 140 ml each appeared convenient even for laboratories using only manual methods.

2.1.3. STABILISATION OF THE SAMPLES

Nutrients are known to be very unstable species in sea water samples since they are taken up and/or released by the living organisms present in the water. To stabilize the samples, large organisms are first removed by filtration and the reminder killed or inhibited.

We have chosen to filter the water through glass fibre filters (Whatman GF/C) having about 1 µm pore size. The water was then heat sterilized (120 °C, 20 min) without addition of preservative, as some may have adverse effects on the subsequent determination of nutrients. The natural matrix is preserved almost unchanged.

The samples were sterilized in a 200 l chamber autoclave (LEQUEUX) that could treat all the bottles containing the same sample (*i.e.* prepared from the same bulk of water) in a single batch.

2.1.4. STORAGE OF THE SAMPLES

Normally the autoclaved samples are stable at ambient temperature as shown previously for nitrate and phosphate. However, recent work (Aminot *et al.*, 1992) has shown that the dissolution of glass into sea water can lead to an increase in the phosphate content of the samples. Since the dissolution rate of glass is drastically decreased at low temperature, the samples for phosphate (and therefore for ammonia which is associated with it in the same bottle) were stored at 5 °C for the first storage period (4 months) before the bottles were sent to the participants. Then all samples were stored at around 20 °C. The participants were advised how they should store the samples.

The bottles chosen to contain the samples (one unique type of bottle for all nutrients) are the same as those previously used in the Nuts I/C 4 exercise. They have proved satisfactory, especially concerning their closure. The bottles, in plain glass, are closed with a one-piece polypropylene screwcap without any additional insert. Inserts are generally a source of random contamination from manual handling when removed and re-placed with insufficient care (no gloves or special tools used). With these caps, the seal is obtained through two thin lips moulded inside the cap which act as a joint.

2.2. ANALYTICAL CONSIDERATIONS

2.2.1. TESTING OF HOMOGENEITY AND STABILITY

Many tests are required in order to follow step by step the preparation of the samples and to verify their homogeneity and stability.

Test samples are drawn before and after spiking (when spiking is required) to compare the added concentration with that expected.

During the preparation of the first series of samples, the intersample homogeneity was checked before autoclaving in order to verify the efficiency of the mixing method and to avoid, if unsuccessful, sterilizing a bad lot. Tests verified efficient mixing and this step was subsequently omitted.

Homogeneity and stability were checked immediately after autoclaving (one day), at four months *i.e.* just before sending the samples and at 11-12 months *i.e.* after or close to the end of the analysis period for the participants.

Additionally, untreated samples were analysed to evaluate the behaviour of the samples when the remaining biomass is not killed.

2.2.2. STANDARDIZATION

In order to verify the stability of samples over a long period of time, a high degree of repeatability must be reached over that period of time. Usually this is the role ascribed to reference material, since such material is assumed to be stable over the intented period of time. Presently the problem is reversed as it must be demonstrated that the prepared material can actually be considered a reference material. Additionally the aim is to assign concentration values to the samples.

The problem could have been solved by using Sagami standards as reference material, however, none are available for ammonia. Additionally, these standards are not prepared in sea water and hence are not reference material in the accepted meaning of the definition (Taylor, 1983).

Our standard solutions were prepared according to the following rules :

1. New concentrated standards in Milli-Q water are prepared for each checking series ; these standards are prepared using recent lots of crystalline nutrient salts of the highest degree of purity accompanied by an analysis certificate.Working standards are made using nutrient depleted sea water spiked with concentrated solutions.

2. Cross-controls with other standardization solutions (commercial and otherwise), including the previously prepared concentrated solutions are performed.

Previous work (Aminot and Kérouel, 1991) has shown these methods to be highly satisfactory.

In addition, sample concentrations are always closely bracketted with working standards frequently run during the analysis of a series.

2.2.3. BLANKS

An important point in the analysis of low concentrations is the determination of the blank(s). Usually the main contribution is the reagent blank, originating from the presence of traces of the determinand as an impurity in reagents. The determination of the reagent blank has to be performed using a medium which is as close as possible to the samples in composition but does not contain the determinand at detectable concentrations. Distilled or demineralized water usually can satisfy this condition depending on the nutrient concerned. However, as always for trace analysis, the purity of these waters are of the greatest importance, but they generally must be assumed to be of satisfactory purity since it is particularly difficult, if not impossible, to determine the actual concentration of traces in waters of high purity.

In the present work, Milli-Q water (Millipore) was the reference as "zero concentration" water for the determination of blanks. In automated segmented continuous flow analysis (SCFA), Milli-Q water is also used as the baseline water. Additionally, freshly drawn off Milli-Q water is analysed at regular intervals, within the series of samples, to check the "zero level".

In SCFA, the curvature of the flowcell generates an additional signal due to refraction when the sample matrix differs from that of the baseline. This is the case with sea water analysed against Milli-Q water. As our instrument does not automatically correct this effect, it has been separately determined and

subtracted from the sample signal as the "refractive index blank (RIB)", according to the procedures described by Tréguer and Le Corre (1975).

2.2.4. SIGNAL RECORDING AND TREATMENT

The output signal from the colorimeters are recorded on a paper chart recorder and simultaneously on a computer, via a 10 V/12 bits electronic device. The signal is sampled at 20 Hertz and treated to obtain an average every second for recording. The stability (or resolution) is about 1 mV (full scale ± 5 000 mV).

Software has been developed at IFREMER for the treatment of the recorded signal. This software is not fully automatic : peaks are individually examined before acceptance so that account can be taken of any noise on the plateaus.

3. OPERATIONAL

3.1. PREPARATION AND TESTING SCHEME

Flow charts for the preparation and testing of the reference material are shown in figures 1 and 2.

It must be remembered that the determinands are grouped two by two in the same bottle (nitrate + nitrite and phosphate + ammonia). Therefore, each concentration level requires two separate preparations, one for each group of nutrients. This also applies to the homogeneity and stability testing ; nitrate and nitrite determinations are run separately from phosphate and ammonia. This allowed more attention to be focussed on the preparation of standards or spiking solution for only two nutrients at any one time.

3.2. CONTAMINATION PRECAUTIONS : HANDLING AND CLEANING

The bulk water is stored in large (100 l) polyethylene carboys in which nutrients depletion proceeds. The depleted water is then filtered by gravity using Whatman GF/C glass fibre filters fitted in an on-line teflon Millipore filter-holder. The filtered water is collected in another 100 l carboy (B) and then subsampled into a third 60 l polypropylene carboy (C) for spiking and bottling. All equipment receives great attention against contamination risks and is consequently carefully handled (with disposable latex gloves).

3.2.1. FILTRATION DEVICE AND CARBOYS

The filter holder is thoroughly washed with Milli-Q water, stored free from dust and rinsed again with Milli-Q water before being assembled. It is then rinsed with several litres of the sea water to be filtered before the working bulk of water is collected.

Carboys B and C are cleaned using 10 l demineralised water acidified with H_2SO_4 (0.5 mol/l). Entire container inner walls are left in contact with the acid solution for at least one hour by placing the carboy in an appropriate position. After draining, the carboys are rinsed three times with 1 l ordinary demineralised water then three times with Milli-Q water. Caps and taps are rinsed using a wash-bottle containing Milli-Q water. After cleaning, a check is performed by rinsing the carboys with a small volume

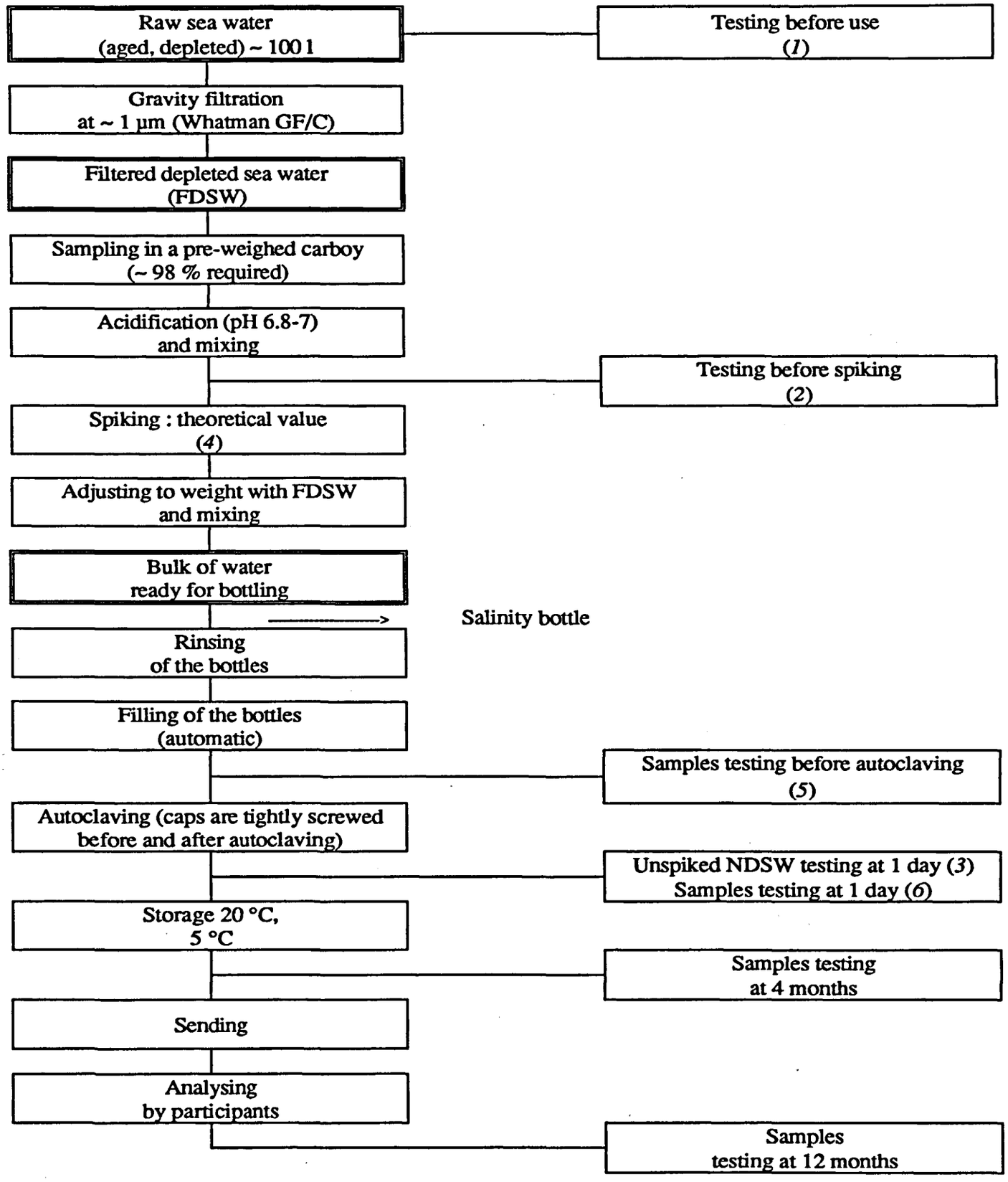

Figure 1 : **FLOW CHART for the preparation of reference material for nutrients.**
Numbers in italics, in parenthesis, refer to the "code" numbers in tables IX, A to E.

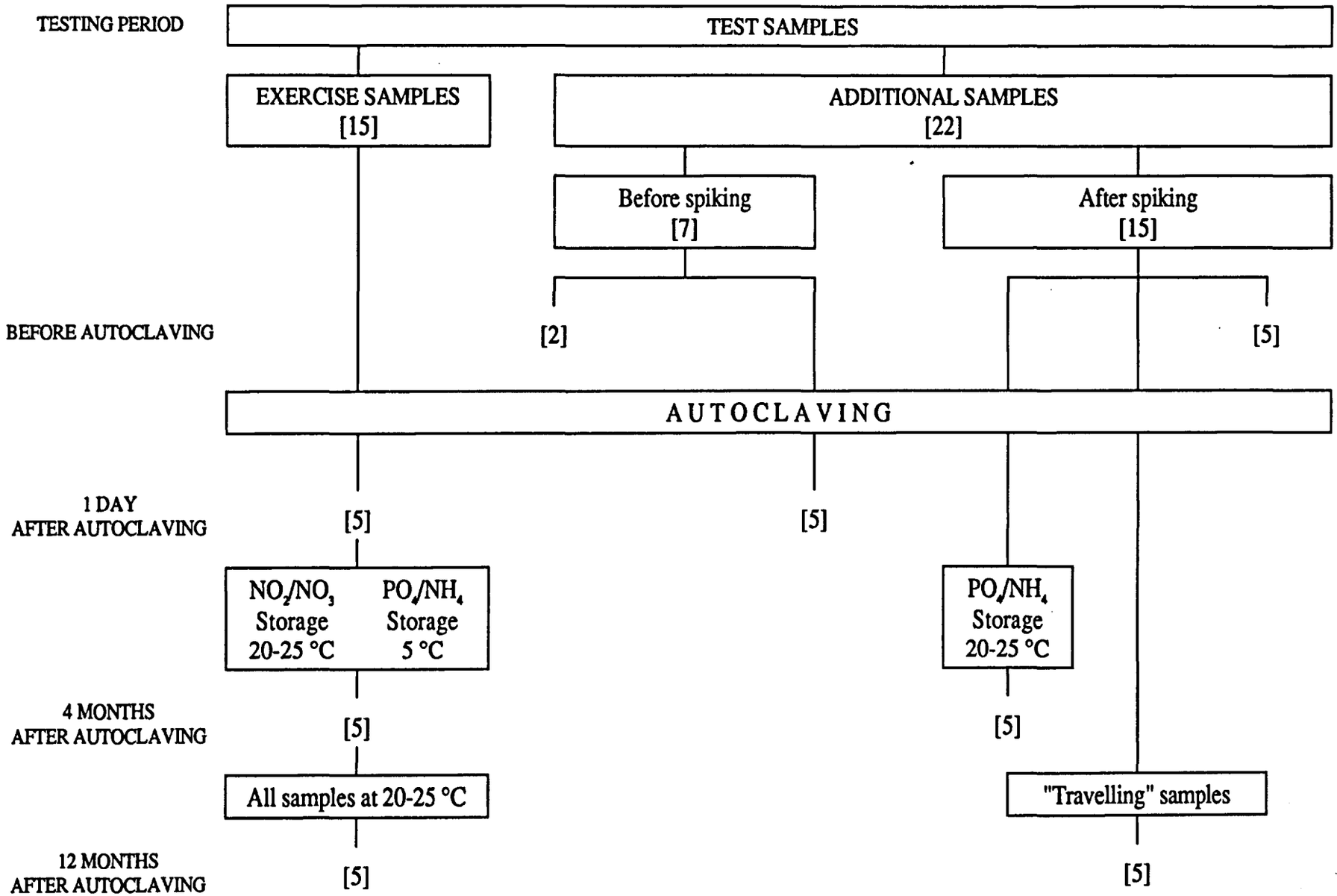

Figure 2 : **Flow chart for testing of reference material for nutrients.**
Minimum numbers of samples are in brackets.

of Milli-Q water (0.5 % of the carboy capacity), then determining the nutrients in that water. The nitrate and nitrite concentrations found (≤ 0.02 µmol/l) would have been insufficient to contaminate the depleted sea water.

Taking account of the satisfactory results obtained with nitrate and nitrite, the same cleaning procedure was applied before the preparation of the samples for phosphate and ammonia. However no check was undertaken since the concentration of these two determinands before spiking was not expected to be extremely low.

3.2.2. SAMPLE BOTTLES

A sample bottle is a two-piece device : the container (the bottle) itself and the closure. The bottles, in plain glass, and the closures, in polypropylene, were cleaned separately before use.

Before commencing the preparation of reference material, a series of bottles was washed in a washing machine fed with demineralised water (SADON Cartridge System) and using a phosphate free detergent (Neodisher UW). Following this cleaning procedure, the possibility of filling the bottles with sample without additional rinsing was anticipated. However the residual nutrients were not as low as expected, especially nitrite, and various tests were undertaken to identify the origin of the problem. It was concluded that atmospheric contamination was responsible for the residual nutrients. Bottles should not be left open too long after washing, even inside the washing-machine. A quick, single rinse with demineralised water was found sufficient for cleaning new bottles, and these should be immediately capped for storage before use.

However, a rinse with a small volume of sample water immediately before filling was found to be necessary.

3.2.3. ACID CONTRIBUTION

Hydrochloric acid is used to prevent precipitation of phosphate observed during autoclaving. Two lots of acid were checked after dilution with Milli-Q water at concentrations three times that required for the preparation of the reference material. At normal added quantities, nitrate, nitrite, ammonia and phosphate remain at undetectable levels, respectively < 0.01 µmol/l, < 0.001 µmol/l, < 0.01 µmol/l and < 0.002 µmol/l.

3.3. SPIKING PROCEDURE

3.3.1. PREPARATION OF CONCENTRATED SOLUTIONS FOR SPIKING

In order to obtain the required concentrations of nutrients in the samples, the water is spiked with known quantities of nutrients concentrated solutions. These concentrates are prepared using the following crystallised salts from J.T. Baker, the purity of which is guaranteed by certificates of analysis (see in annex) :

Salt	Given purity (%)	Purity factor (p)
- Potassium nitrate	99.3	1.007
- Sodium nitrite	> 98 (assumed 99)	1.010
- Ammonium sulfate	99.1	1.009
- Potassium dihydrogen phosphate	100.0	1.000

Before use the salts are oven-dried for about 2 hours at 105 °C in small glass vessels. Then the salts are stored in a desiccator until use within a few days. In case of re-use later, the drying process is repeated.

The concentrated solutions are prepared by weighing the salts and the water, instead of using volumetric glassware. The concentrations are calculated for a solution in Milli-Q water at 20 °C assuming the density of the solution is that of pure water. The theoretical mass of salt (m_t) is determined for a certain concentration (C_c) and a certain volume (v) of solution to be prepared, taking account of the correction factor (p) for the purity of the salts. Then the salt is accurately weighed to a mass close to the theoretical, and the corresponding water mass is calculated and added.

Example : to prepare 1 litre of 5 mmol/l nitrate solution

- Theoretical salt mass (g) :

$$m_t = M_{KNO_3} \times p \times C_c \times v = 101.11 \times 1.007 \times 0.005 \times 1 = 0.50911 \text{ g}$$

- Actual weighed salt mass : $m_s = 0.50059$ g

- Mass of water to be added (g) :

$$M_w = \left(d_w^{20} - d_a^{t,P}\right) \times v \times (m_s/m_t) = 997 \times 1 \times \frac{0.50059}{0.50911} = 980.32 \text{g}$$

where d_w^{20} is the density of pure water at 20 °C (998.23 g/l), $d_a^{t,P}$ is the density of air at the time of weighing (1.1 to 1.3 g/l in the ranges t = 20 ± 5 °C and P = 1 013 ± 50 hPa). The density factor $\left(d_w^{20} - d_a^{t,P}\right)$ will be rounded to 997 g/l for all conditions, which does not introduce errors greater than a few hundredths of a per cent (therefore negligible).

Table I

Preparation of spiking solutions.

Nutrient	Salt	Molar mass (g)	Required concentr. (mmol/l)	Expected vol. of solution (l)	Required salt mass m_t (g)	Weighed salt mass m_s (g)	Theoret. wat. mass M_w (kg)	Actual water mass (kg)
Nitrate	KNO_3	101.11	25.00	0.5	1.2728	1.2952	0.50727	0.50726
Nitrite	$NaNO_2$	69.00	2.000	1.0	0.13939	0.14165	1.01320	1.01319
Ammonia	$(NH_4)_2SO_4$	132.14	2.000	1.0	0.13334	0.12961	0.96911	0.96909
Phosphate*	KH_2PO_4	136.09	2.000	1.0	0.27355	0.26705	0.97821	0.97912

* For phosphate the exact purity (100.0 %) was obtained later and the purity factor used first was 99.5. Correcting for this factor and the water mass difference (theoretical/actual), the concentration of the phosphate spiking solution is actually 2.0082 mmol/l instead of 2.000.

The concentrated solutions are prepared in the storage bottle itself. The salts are weighed in small plastic or glass weighing boats on an electronic balance (Sartorius 2004MP, 0.01 mg resolution). They are transfered with caution into the pre-weighed bottle (4 000 g Sartorius 1364MP electronic balance, 0.01 g resolution) using a wash-bottle and then the rest of water is added up to the required quantity (balance is zeroed with empty bottle on, hence total weight equals water plus salt weights). The bottle is tightly capped and the salts dissolved by shaking.

These concentrated solutions are, as stated, accurate at 20 °C if expressed in mass or mole per volume and their use in the range 15-25 °C would introduce a maximum difference of ± 0.1 % in concentration. The weighing data are summarized in table I.

3.3.2. USE OF CONCENTRATED SOLUTIONS FOR SPIKING

Spiking the nutrients depleted sea water is done by weighing both the sea water and the concentrated nutrient solution. Indeed, the volume of sample to be prepared is highly variable and no volumetric glassware exists for quantities of 30-60 l.

The sea water was weighed in a 60 l polypropylene carboy using a 61 kg Sartorius electronic balance, model F61S (1 g readability ; ± 1 g linearity ; ± 0.5 g reproducibility). The concentrates were weighed in small polyethylene bottles (30-50 ml) carefully rinsed, dried and stored in a desiccator before use. A 400 g Sartorius electronic balance 1265MP with a resolution of 0.001 g was used for these concentrates.

The masses of nutrient concentrate and of sea water were calculated as follows.

For a given nutrient, the concentrate concentration is C_c and the expected added concentration in sea water is C_s. To prepare a volume V of sample, the mass of concentrate to be weighed (in g) is :

$$m_c = 997 \times (C_s / C_c) \times V .$$

The factor 997 is the density of the concentrate (g/l), assumed to be the same than that of pure water, and corrected for air boyancy (see § 3.3.1).

The mass of sea water to be weighed is given by :

$$M_{sw} = V_{sw} \times \left(d_{sw}^{20} - d_a^{t,P} \right)$$

where d_{sw}^{20} is the density of sea water (salinity ~ 35 PSS) at 20 °C and $d_a^{t,P}$ is the density of air at the time of weighing (1.2 ± 0.1 g/l, see § 3.3.1).

The density of sea water at 20 °C and 35 PSS, according to Cox *et al.* (1970), is 1 024.8 g/l, hence the density factor $\left(d_{sw}^{20} - d_a^{t,P} \right)$ is (1 024.8 – 1.2) = 1 023.6. As the salinity was expected in the range 34.5-35 PSS, the rounded value 1 023 was used. Further determinations gave 34.9 to 35.4 PSS which leads to a slight underestimation of the density by 0.04-0.10 %. Hence the actual concentration are higher than expected by the same factors (although they may be considered negligible).

Once the mass is determined, about 98 % of the sea water is introduced in the weighing carboy. Then the concentrate is weighed in the plastic bottle, and the plastic bottle is emptied into the carboy containing the sea water. The bottle is rinsed at least five times by filling it with sea water withdrawn from the bulk and then poured into the weighing carboy. The remaining of sea water is then added up to the required mass. Table II summarizes the solutions, masses and volumes involved in the preparation of the samples.

Table II

Preparation of the sea water samples for bottling.

Sample number	Nutrient	Conc. level	Concentrate conc.	Required added conc.	Final vol. of SW	Mass of conc. sol.		Mass of sea water	
						theoretical	actual	theoretical	actual
			(mmol/l)	(µmol/l)	(l)	(g)	(g)	(kg)	(kg)
1	Nitrate	interm.	25.00	10.00	33	13.160	13.162	33.759	33.759
	Nitrite	interm.	2.000	0.500		8.225	8.224		
2	Nitrate	low	25.00	1.30	42	2.177	2.178	42.966	42.972
	Nitrite	low	2.000	0.14		2.931	2.936		
3	Nitrate	high	25.00	26.0	30	31.106	31.106	30.690	30.694
	Nitrite	high	2.000	1.40		20.937	20.930		
4	Ammonia	low							
	Phosphate	low	-	0	-	-	-	-	-
5	Ammonia	high	2.000	4.50	32	71.784	71.786	32.736	32.732
	Phosphate	high	2.008	1.80		28.596	28.602		
6	Ammonia	interm.	2.000	1.50	40	29.910	29.912	40.920	40.920
	Phosphate	interm.	2.008	0.45		8.936	8.941		

3.4. DETERMINATION OF NUTRIENT CONCENTRATIONS

3.4.1. CALIBRATION

Calibration procedures require the preparation of two kinds of nutrients solutions : the concentrated solutions and the working solution obtained by their dilution. The preparation of both solutions is described below, followed by the use and comparison of standards.

3.4.1.1. Concentrated solutions

Concentrated solutions are prepared in the same way as the spiking solutions and using the same salts (§ 3.3.1). However, they were prepared by another analyst and their concentrations are different from the spiking solutions (table III).

Table III

Example of preparation of concentrated solutions for calibration of nutrients.
This set was prepared for the first round of sample checkings.

Nutrient	Salt	Molar mass (g)	Required concentr. (mmol/l)	Expected vol. of solution (l)	Required salt mass m_t (g)	Weighed salt mass m_s (g)	Theoret. wat. mass M_w (kg)	Actual water mass (kg)
Nitrate	KNO_3	101.11	5.000	1	509.11	500.59	0.98032	0.98033
Nitrite	$NaNO_2$	69.00	5.000	1	348.48	347.38	0.99385	0.99395
Ammonia	$(NH_4)_2SO_4$	132.14	1.000	2	133.34	131.83	1.97142	1.97255
Phosphate	KH_2PO_4	136.09	0.500	2	136.09	122.04	1.78814	1.78824

3.4.1.2. Working solutions

Working solutions are obtained by dilution of the concentrated solutions with nutrient depleted sea water using volumetric glassware and pipette. The volumetric equipment was checked before use.

The preparation of working solutions by weighing was tested and abandoned since it was found to be less reliable. This was attributed to excessive handling of small aliquots due to the high dilution factor and the necessary limited volume of working solution to be prepared. The only exception was the preparation of an intermediate solution for nitrite. In this case, to avoid a too large dilution of the concentrated solution in one step, a secondary concentrated solution was first prepared by a ten-fold dilution of the 5 mmol/l nitrite primary standard.

3.4.1.3. Volumetric tools checkings

Automatic pipettes are now currently used in laboratories. Previous work has shown that there is no risk of adsorption of phosphate on the plastic tip (Kérouel and Aminot, 1990). For the present work an electronic pipette Biohit Proline of 1 ml capacity was used.

The pipette was checked gravimetrically on several occasions during the exercise. Results are summarized in table IV. The pipette was always set in direct mode using a new tip each time (only one pipetting with one tip). Milli-Q water was used for the checks.

The results show that the pipette achieves a repeatability of around ± 2 µl with a mean bias of - 0.4 µl on the whole range. Separate examination of results at 500 µl and 1 000 µl setting gives biases of - 1.7 µl and + 0.9 µl respectively on four determinations. Taking account of the fact that standardization curves average the biases and that most standards are prepared by dispensing volumes between 700 and 1 000 µl, it can be considered that the pipette does not introduce biases greater than around 0.1 %.

Table IV

Checking of the electronic pipette.

Date of checking	Pipette setting (µl)	Water temperature (°C)	Correction factor	Weight (mg)	Volume (µl)	Difference from setting (µl)
23/06/92	500	25.5	1.0041	498.5	500.5	+ 0.5
	1 000	25.5	1.0041	997.8	1001.9	+ 1.9
15/09/92	500	23.2	1.0036	495.4	497.2	- 2.8
	500	23.2	1.0036	495.0	496.8	- 3.2
	750	23.2	1.0036	747.6	750.3	+ 0.3
	1 000	23.2	1.0036	999.7	1003.2	+ 3.2
	1 000	23.2	1.0036	996.3	999.9	- 0.1
	1 000	23.2	1.0036	995.1	998.7	- 1.3
14/10/92	250	22.1	1.0033	247.9	248.7	- 1.3
	500	22.1	1.0033	497.0	498.7	- 1.3
mean ± stand. dev.	all					- 0.4 ± 2.0
	500					- 1.7 ± 1.7
	1 000					+ 0.9 ± 2

The dilution of the concentrated solutions were done using class A volumetric flasks of various capacities (100 ml to 1 000 ml). Their accuracy, which is normally within ± 0.1 % (100 ml) to ± 0.04 % (1 000 ml) of nominal volume, was also checked in previous work and found to be in agreement with the stated accuracy (table V).

Table V

Checking of volumetric flasks.

Nominal volume (ml)	Water temperature (°C)	Correction factor	Weight (g)	Volume (ml)	Relative difference (%)
100	21.5	1.0032	99.812	100.13	+ 0.13
200	21.5	1.0032	199.341	199.98	- 0.01
250	21.5	1.0032	249.207	250.00	0.00
500	21.5	1.0032	498.62	500.22	+ 0.04
500	21.5	1.0032	498.52	500.11	+ 0.02

3.4.1.4. Use and comparison of calibration solutions

New concentrated calibration solutions are prepared at each testing step. Where commercial concentrates were available they were compared with our laboratory solutions. At each testing step, the concentrated standards prepared for the former step were measured using the new standards. The new

standard is considered at each step as the reference. The results are summarized in table VI. They show that the differences between standards are all within about ± 1 % of the reference.

Table VI

Comparison of concentrated solutions of nutrients used for testing the reference material samples.
All values are relative to the new concentrate prepared at each step.
Extra concentrates are also tested.

Step (date)	Concentrate	NITRATE means diff. %	NITRATE RSD % (n)	NITRITE means diff. %	NITRITE RSD % (n)	AMMONIA means diff. %	AMMONIA RSD % (n)	PHOSPHATE means diff. %	PHOSPHATE RSD % (n)
	Standard 1	ref.	0.2 (3)	ref.	0.5 (4)	ref.	0.3 (2)	ref.	0.1 (2)
1 (May/June 92)	Spiking solution	- 0.2	0.1 (3)	+ 0.7	0.1 (4)	- 0.5	0.4 (5)	- 1.0	0.1 (5)
	Dilut-it BAKER	-	-	-	-	- 0.5	0.1 (2)	+ 1.1	0.1 (2)
	Standard 2	ref.	0.1 (4)	ref.	0.1 (4)	ref.	0.4 (3)	ref.	0.1 (3)
2 (Sept./Oct. 92)	Standard 1	+ 0.5	0.1 (4)	0.0	0.2 (4)	- 0.4	0.1 (2)	0.0	0.2 (3)
	Standard 3	ref.	0.4 (5)	ref.	0.2 (5)	ref.	0.1 (5)	ref.	0.1 (5)
3 (May 93)	Standard 2	0.0	0.9 (4)	- 1.0	0.1 (4)	- 0.6	0.1 (5)	0.1	0.1 (5)
	Standard 1	- 0.2	0.6 (2)	- 0.3	< 0.1 (2)	- 0.5	0.1 (2)	0.0	< 0.1 (2)

3.4.2. ANALYTICAL PROCEDURE

3.4.2.1. Methods of determination

The classical colorimetric methods as described by Strickland and Parsons (1972) are used following their adaptation to the Autoanalyzer II Technicon by Tréguer and Le Corre (1975). The only modifications concern first the strict application of Murphy and Riley's reagents (1962) for phosphate (half the concentration used by Tréguer and Le Corre), and secondly the injection of citrate before soda (instead of a mixed reagent) for the determination of ammonia. In the ammonia method, a slight pH effect has been recorded (1.5 % decrease per pH unit decrease in sea water samples) and correction made accordingly.

The performance of the methods from replicates of standards is summarized in table VII.

Table VII

Performance of the automatic methods used for the determination of nutrients. Examples of the repeatability of various standards at each testing period during the exercise.
The figures correspond to a unique preparation of each working standard which is measured at several occasions during the series of sample analysis. Values are raw (*i.e.* uncorrected for blank) data in millivolts. Electronic amplification may vary from one period to another hence lead to variable signal intensity for similar concentrations.

Testing period	Data	Nitrate		Nitrite			Ammonia		Phosphate	
1 day	Level (µmol/l)	1.1	27	0.2	0.6	1.5	1.2	2.2	0.6	1.9
	n	4	8	7	8	9	6	5	4	5
	mean (mV)	273	2 560	411	1 345	2 049	920	1 608	996	3 272
	SD (mV)	3.3	5.8	0.9	2.4	4.0	3.3	8.5	1.7	1.3
	RSD (%)	1.2	0.2	0.2	0.2	0.2	0.4	0.5	0.2	0.04
4 months	Level (µmol/l)	10	27	0.14	0.6	1.5	1.7	4.7	0.4	1.9
	n	3	5	5	6	5	5	4	5	7
	mean (mV)	3 738	3 169	411	1 610	2 004	1 251	3 422	575	3 219
	SD (mV)	1	2.6	0.9	0.8	1.9	1.1	5.4	1.1	2.7
	RSD (%)	0.03	0.08	0.2	0.05	0.09	0.09	0.2	0.2	0.08
12 months	Level (µmol/l)	9.5	10.5	0.1	0.5	1.4	1.5	4.5	0.25	1.9
	n	3	3	3	6	5	6	4	4	5
	mean (mV)	3333	3710	417	1622	2036	1249	2786	549	2641
	SD (mV)	8.4	6.6	1.5	2.9	1.9	7.2	9.6	2.2	3.0
	RSD (%)	0.3	0.2	0.4	0.2	0.1	0.6	0.3	0.4	0.1

3.4.2.2. Blanks

The importance of blanks has been indicated in § 2.6.2. The most important in SCFA is the system blank mainly due to refractive index changes between the fresh water baseline and salt water samples. In addition, the necessity of adding wetting agents to the reagents for hydraulic reasons is sometimes the cause of a slight turbidity in sea water, a phenomenon integrated in the system blank usually called Refractive Index Blank (RIB).

The determination of this blank is done by running a normal analysis but replacing one indispensable reagent by distilled water. The reagent replaced is at a very low concentration, so that the medium for the determination of RIB is as close as possible to the normal reaction medium, except that no color can develop.

Since the blank variability controls the detection limit, we have extracted from our recordings series of system blanks for nitrate, nitrite, ammonia and phosphate. These results are summarized in table VIII.

Table VIII

System blanks (RIB) as determined with their Autoanalyzer II Technicon by the authors. The blanks are converted into their equivalent in micromole per litre of the corresponding nutrients. Salinities of the RM waters are the following : RM1 35.44 (PSS), RM2 35.34, RM3 35.45, RM4 35.34, RM5 34.85, RM6 35.25.

Nutrient	RM sample number	Date in 1992	Blanks (µmol/l) n	mean	S.D.	Limit of detection (= 3 × SD) nmol/l
Nitrate	1	15 May	7	0.014	0.002	6
	2	20 May	7	0.020	0.007	21
	3	21 May	11	0.012	0.003	9
Nitrite	1	15 May	7	0.043	< 0.0001	< 1
	2	20 May	11	0.043	0.0008	2.5
	3	21 May	13	0.040	0.0005	1.5
Ammonia	4	16 June	5	0.232	0.0019	6
	5	17 June	5	0.234	0.0027	8
	6	18 June	6	0.229	0.0035	10
Phosphate	4	16 June	5	0.087	0.0008	2.5
	5	17 June	5	0.086	0.0009	2.5
	6	18 June	10	0.088	0.0008	2.5

It can be seen that the detection limits are around a few nanomoles per litre for all parameters. However the detection limit may vary from one batch to another by a factor of two. These differences seem to originate from variation in the hydraulic behaviour of the system (due to ageing of tubing for instance).

However, the very low standard deviation of the system blank must not mask the fact that these blanks may be systematically biased compared with the true blank. Indeed, their determination is performed with all but one indispensable reagent to prevent color development. The medium being not exactly the same as during the reaction, it may not be excluded that, in some circumstances, slight differences can exist between the actual and the measured blanks.

Despite the fact that there is probably no simple way to ascertain whether the system blank is biased or not, a long experience in the field, covering the analysis of a large selection of nutrient depleted sea waters (NDSW), allows us to have strong conviction that our blank determinations are correct at least for nitrate, nitrite and phosphate. Indeed, frequent results are stated as zero at the given precision of the blank (table VIII) and no significant negative values are ever encountered.

In the case of ammonia, the greater complexity of the reagent mixture and the higher value of the system blank contribute to more uncertainty. Contrary to other methods, the system blank may vary from series to series without any rational explanation. Additionally, almost all NDSW exhibit low but measurable ammonia concentrations (presently 0.06-0.08 µmol/l) while nitrite and nitrate are at undetectable levels. Although questionable this behaviour cannot however be considered as the proof of a blank error. During the present exercise, 53 ammonia system blanks have been performed at various periods from May until November 1992. Their value is 0.233 ± 0.005 µmol/l. Another series of 46 blanks was performed from 1st to 9th of June 1993 lead to 0.207 ± 0.005 µmol/l. Given this satisfying stability on a one year working period, the blank was considered unbiased.

4. RESULTS

4.1. PREPARATION CHECKING

The results presented here concern the preparation of the material, *i.e.* concentrations before and after spiking, and before and after autoclaving. Added concentrations measured before and after autoclaving are compared to the theoretical. All these figures are summarized in table IX, A to E. Additionally, initial concentrations that can be assigned to the samples immediately after their preparation are given, since they are determined from the above values (see § 5).

The results show good agreement, between expected and measured added concentrations (table IX, A to E). In nitrate, nitrite and phosphate, the differences remain lower than or close to one percent for the intermediate and high level or at the limit of the analytical possibilities for the low level (*i.e.* ≤ 0.002 µmol/l in nitrite and ≤ 0.03 µmol/l in nitrate ; no spike at low level in phosphate). In ammonia, differences between measured and expected concentration reach 2-3 % which may be considered satisfactory for this determinand. Autoclaving has no significant effect on the added concentrations of nitrate and nitrite, although it seems to have a slight positive effect on ammonia and a slight negative effect on phosphate.

The spiking concentrates and the concentrated standard solution used for determining the nutrient concentrations are prepared using the same dry salts, therefore, the comparability between measured and expected concentrations is not biased by impurities in the chemicals.

4.2. HOMOGENEITY AND STABILITY TESTING

The results of homogeneity and stability testing are summarized in table X.

4.2.1. HOMOGENEITY

The homogeneity is expressed by the standard deviation (s) and the relative standard deviation (RSD in %). It should be noted that they are significantly lower than the stated precision of the methods and that they do not exceed 0.5 % at all levels for nitrite and nitrate. For ammonia and phosphate the RSD is < 0.7 % at high level, < 1.7 % at intermediate level and < 5 % at low level. At the low level, the RSD must be considered particularly satisfactory since it corresponds to s ≤ 0.015 in ammonia and s = 0.003 µmol/l in phosphate. It is self-evident that the storage has no adverse effect on the variability of concentrations, so no F-test for comparison of variance is applied.

Table IX A

Concentration of nutrients in the samples : measured and expected values in micromole per litre. Code numbers refer to numbers in italics in figure 1.

CODE	Reference	LOW LEVEL		INTERMEDIATE LEVEL		HIGH LEVEL	
		NITRATE + NITRITE					
		MEASURED CONCENTRATIONS					
		mean ± s.d.	(n)	mean ± s.d.	(n)	mean ± s.d.	(n)
1	Raw NDSW	0.00 ± 0.007	(2)	0.03 ± 0.04	(2)	-	
	NOT AUTOCLAVED SW						
2	Prepared NDSW	0.00 ± 0.007	(5)	0.00 ± 0.004	(5)	0.03 ± (0)	(2)
5	Sample	1.41 ± 0.01	(8)	10.44 ± 0.03	(10)	-	
	AUTOCLAVED SW						
3	Prepared NDSW	0.02 ± 0.008	(5)	0.00 ± 0.005	(5)	0.02 ± 0.004	(5)
6	Sample	1.44 ± 0.002	(5)	10.42 ± 0.03	(5)	27.27 ± 0.02	(12)
	AUTOCLAVING EFFECT						
(3 - 2)	Prepared NDSW	+ 0.02		0.00		- 0.01	
(6 - 5)	Sample	+ 0.03		- 0.02		*	
	ADDED CONCENTRATIONS						
4	THEORETICAL	1.44		10.50		27.40	
	FROM MEASURES						
(5 - 2)	• Before autoclaving	1.41		10.44		-	
(5 - 2) - 4	Difference	- 0.03		- 0.06		-	
	(Relat. diff. %)	(- 2)		(- 0.6)		-	
(6 - 3)	• After autoclaving	1.42		10.42		27.25	
(6 - 3) - 4	Difference	- 0.02		- 0.08		- 0.15	
	(Relat. diff. %)	(- 1.4)		(- 0.8)		(0.5)	
	INITIAL ASSIGNED CONCENTRATIONS						
4 + 2 + (6 - 5)	Spike + initial conc. + autoclaving effect	1.47		10.48		27.43	

* The concentration before autoclaving being not determined, the autoclaving effect is assumed to be 0.00 µmol/l, the mean of the five other effects.

Table IX B

Concentration of nutrients in the samples : measured and expected values in micromole per litre. Code numbers refer to numbers in italics in figure 1.

CODE	Reference	LOW LEVEL		INTERMEDIATE LEVEL		HIGH LEVEL	
		NITRITE					
		MEASURED CONCENTRATIONS					
		mean ± s.d.	(n)	mean ± s.d.	(n)	mean ± s.d.	(n)
1	Raw NDSW	0.003 ± (0)	(2)	0.001 ± (0)	(2)	-	
	NOT AUTOCLAVED SW						
2	Prepared NDSW	0.001 ± (0)	(5)	0.001 ± (0)	(5)	0.003 ± (0)	(2)
5	Sample	0.140 ± 0.0008	(8)	0.494 ± 0.001	(10)	-	
	AUTOCLAVED SW						
3	Prepared NDSW	0.004 ± (0)	(5)	0.004 ± 0.0004	(5)	0.005 ± (0)	(5)
6	Sample	0.142 ± 0.0004	(5)	0.498 ± 0.001	(5)	1.389 ± 0.005	(12)
	AUTOCLAVING EFFECT						
(3 - 2)	Prepared NDSW	+ 0.003		+ 0.003		+ 0.002	
(6 - 5)	Sample	+ 0.002		+ 0.004		*	
	ADDED CONCENTRATIONS						
4	THEORETICAL	0.140		0.500		1.400	
	FROM MEASURES						
(5 - 2)	• Before autoclaving	0.139		0.493		-	
(5 - 2) - 4	Difference	- 0.001		- 0.007		-	
	(Relat. diff. %)	(- 1)		(- 1.4)		-	
(6 - 3)	• After autoclaving	0.138		0.494		1.384	
(6 - 3) - 4	Difference	- 0.002		- 0.006		- 0.016	
	(Relat. diff. %)	(- 1.4)		(- 1.2)		(- 1.1)	
	INITIAL ASSIGNED CONCENTRATIONS						
4 + 2 + (6 - 5)	Spike + initial conc. + autoclaving effect	0.143		0.505		1.406	

* The concentration before autoclaving being not determined, the autoclaving effect is assumed to be + 0.003 µmol/l, the mean of the five other effects.

Table IX C

Concentration of nutrients in the samples : measured and expected values in micromole per litre. Code numbers refer to numbers in italics in figure 1.

CODE	Reference	LOW LEVEL		INTERMEDIATE LEVEL		HIGH LEVEL	
		NITRATE (by subtraction)					
		MEASURED CONCENTRATIONS					
		mean ± s.d.	(n)	mean ± s.d.	(n)	mean ± s.d.	(n)
1	Raw NDSW	0.00 ± 0.007	(2)	0.03 ± 0.04	(2)	-	
	NOT AUTOCLAVED SW						
2	Prepared NDSW	0.00 ± 0.007	(5)	0.00 ± 0.004	(5)	0.03 ± (0)	
5	Sample	1.27 ± 0.01	(8)	9.95 ± 0.03	(10)	-	
	AUTOCLAVED SW						
3	Prepared NDSW	0.01 ± 0.008	(5)	0.00 ± 0.006	(5)	0.02 ± 0.004	(5)
6	Sample	1.29 ± 0.003	(5)	9.92 ± 0.03	(5)	25.88 ± 0.02	(12)
	AUTOCLAVING EFFECT						
(3 - 2)	Prepared NDSW	+ 0.01		0.00		- 0.01	
(6 - 5)	Sample	+ 0.02		- 0.03		(0.00)	
		ADDED CONCENTRATIONS					
4	THEORETICAL	1.30		10.0		26.0	
	FROM MEASURES						
(5 - 2)	• Before autoclaving	1.27		9.95		-	
(5 - 2) - 4	Difference	- 0.03		- 0.05		-	
	(Relat. diff. %)	(- 2.3)		(- 0.5)		-	
(6 - 3)	• After autoclaving	1.28		9.92		25.86	
(6 - 3) - 4	Difference	- 0.02		- 0.08		- 0.04	
	(Relat. diff. %)	(- 1.5)		(- 0.8)		(- 0.2)	
		INITIAL ASSIGNED CONCENTRATIONS					
4 + 2 + (6 - 5)	Spike + initial conc. + autoclaving effect	1.32		9.97		26.03	

Table IX D

Concentration of nutrients in the samples : measured and expected values in micromole per litre. Code numbers refer to numbers in italics in figure 1.

CODE	Reference	LOW LEVEL		INTERMEDIATE LEVEL		HIGH LEVEL	
		AMMONIA					
		MEASURED CONCENTRATIONS					
		mean ± s.d.	(n)	mean ± s.d.	(n)	mean ± s.d.	(n)
1	Raw NDSW	-		-		-	
	NOT AUTOCLAVED SW						
2	Prepared NDSW	0.13 ± 0.006	(3)	0.07 ± 0.006	(3)	0.08 ± (0)	(3)
5	Sample			1.52 ± 0.03	(5)	4.57 ± 0.008	(5)
	AUTOCLAVED SW						
3	Prepared NDSW	0.34 ± 0.01	(7)	0.28 ± (0)	(3)	0.25 ± 0.006	(3)
6	Sample			1.78 ± 0.01	(7)	4.85 ± 0.02	(10)
	AUTOCLAVING EFFECT						
(3 - 2)	Prepared NDSW	+ 0.21		+ 0.21		+ 0.17	
(6 - 5)	Sample			+ 0.26		+ 0.28	
		ADDED CONCENTRATIONS					
4	THEORETICAL	0.00		1.50		4.50	
	FROM MEASURES						
(5 - 2)	• Before autoclaving	-		1.45		4.49	
(5 - 2) - 4	Difference			- 0.05		- 0.01	
	(Relat. diff. %)			(- 3)		(- 0.2)	
(6 - 3)	• After autoclaving	-		1.50		4.60	
(6 - 3) - 4	Difference			0.00		+ 0.10	
	(Relat. diff. %)			(0.0)		(+ 2.2)	
		INITIAL ASSIGNED CONCENTRATIONS					
4 + 2 + (6 - 5)	Spike + initial conc. + autoclaving effect	0.34		1.83		4.86	

Table IX E

Concentration of nutrients in the samples : measured and expected values in micromole per litre. Code numbers refer to numbers in italics in figure 1.

CODE	Reference	LOW LEVEL		INTERMEDIATE LEVEL		HIGH LEVEL	
		PHOSPHATE					
		MEASURED CONCENTRATIONS					
		mean ± s.d.	(n)	mean ± s.d.	(n)	mean ± s.d.	(n)
1	Raw NDSW	-		-		-	
	NOT AUTOCLAVED SW						
2	Prepared NDSW	0.043 ± 0.001	(3)	0.009 ± 0.0006	(3)	0.026 ± 0.001	(4)
5	Sample			0.460 ± 0.002	(5)	1.827 ± 0.001	(5)
	AUTOCLAVED SW						
3	Prepared NDSW	0.072 ± 0.003	(7)	0.030 ± 0.003	(3)	0.040 ± 0.004	(3)
6	Sample			0.473 ± 0.007	(6)	1.830 ± 0.011	(10)
	AUTOCLAVING EFFECT						
(3 - 2)	Prepared NDSW	+ 0.029		+ 0.021		+ 0.014	
(6 - 5)	Sample			+ 0.013		+ 0.003	
		ADDED CONCENTRATIONS					
4	THEORETICAL	0.00		0.450		1.800	
	FROM MEASURES						
(5 - 2)	• Before autoclaving	-		0.451		1.801	
(5 - 2) - 4	Difference			+ 0.001		+ 0.001	
	(Relat. diff. %)			(+ 0.2)		(+ 0.05)	
(6 - 3)	• After autoclaving	-		0.443		1.790	
(6 - 3) - 4	Difference			- 0.007		- 0.010	
	(Relat. diff. %)			(- 1.4)		(- 0.6)	
		INITIAL ASSIGNED CONCENTRATIONS					
4 + 2 + (6 - 5)	Spike + initial conc. + autoclaving effect	0.072		0.472		1.829	

Table X

Results of homogeneity and stability testing.

Level	Time after autoclaving	n	Mean (µmol/l)	Range (µmol/l)	SD (µmol/l)	RSD (%)
			NITRATE + NITRITE			
LOW	1day	5	**1.44**	1.43 - 1.44	0.002	0.1
	4 months	5	**1.42**	1.41 - 1.43	0.006	0.4
	12 months	9	**1.44**	1.43 - 1.45	0.007	0.5
INTERMEDIATE	1 day	5	**10.42**	10.38 - 10.45	0.03	0.3
	4 months	5	**10.42**	10.39 - 10.46	0.03	0.3
	12 months	10	**10.44**	10.41 - 10.47	0.02	0.2
HIGH	1 day	12	**27.27**	27.23 - 27.32	0.02	0.07
	4 months	5	**27.36**	27.30 - 27.41	0.04	0.15
	12 months	10	**27.42**	27.36 - 27.49	0.04	0.15
			NITRITE			
LOW	1day	5	**0.142**	0.142 - 0.143	0.0004	0.3
	4 months	5	**0.144**	0.143 - 0.144	0.0005	0.3
	12 months	9	**0.145**	0.145 - 0.147	0.0007	0.5
INTERMEDIATE	1 day	5	**0.498**	0.496 - 0.499	0.001	0.2
	4 months	5	**0.506**	0.505 - 0.507	0.001	0.2
	12 months	10	**0.503**	0.502 - 0.507	0.002	0.4
HIGH	1 day	12	**1.389**	1.379 - 1.395	0.005	0.4
	4 months	5	**1.410**	1.409 - 1.413	0.002	0.1
	12 months	10	**1.404**	1.393 - 1.408	0.005	0.4
			NITRATE (by subtraction)			
LOW	1day	5	**1.29**	1.29 - 1.30	0.003	0.2
	4 months	5	**1.28**	1.27 - 1.29	0.006	0.5
	12 months	9	**1.29**	1.28 - 1.30	0.007	0.5
INTERMEDIATE	1 day	5	**9.92**	9.88 - 9.95	0.03	0.3
	4 months	5	**9.92**	9.88 - 9.96	0.03	0.3
	12 months	10	**9.93**	9.91 - 9.97	0.02	0.2
HIGH	1 day	12	**25.88**	25.84 - 25.93	0.02	0.07
	4 months	5	**25.95**	25.89 - 26.00	0.04	0.15
	12 months	10	**26.02**	25.95 - 26.09	0.04	0.15

Table X (continued)

Results of homogeneity and stability testing.

Level	Time after autoclaving	n	Mean (µmol/l)	Range (µmol/l)	SD (µmol/l)	RSD (%)
			AMMONIA			
LOW	1day	7	**0.34**	0.32 - 0.36	0.015	4
	4 months	5	**0.31**	0.29 - 0.32	0.014	5
	12 months	5	**0.33**	0.31 - 0.34	0.011	3
INTERMEDIATE	1 day	7	**1.78**	1.76 - 1.80	0.012	0.7
	4 months	5	**1.75**	1.74 - 1.76	0.008	0.5
	12 months	5	**1.73**	1.71 - 1.76	0.018	1.0
HIGH	1 day	10	**4.85**	4.83 - 4.88	0.02	0.4
	4 months	11	**4.81**	4.78 - 4.88	0.03	0.6
	12 months	10	**4.78**	4.75 - 4.81	0.02	0.4
			PHOSPHATE			
LOW	1day	7	**0.072**	0.070 - 0.076	0.003	4
	4 months	5	**0.074**	0.071 - 0.078	0.003	4
	12 months	5	**0.088**	0.085 - 0.091	0.002	3
INTERMEDIATE	1 day	6	**0.473**	0.461 - 0.479	0.007	1.5
	4 months	5	**0.467**	0.454 - 0.475	0.008	1.7
	12 months	5	**0.488**	0.481 - 0.494	0.005	1.0
HIGH	1 day	10	**1.830**	1.808 - 1.844	0.011	0.6
	4 months	5	**1.817**	1.812 - 1.819	0.003	0.2
	7 months	8	**1.824**	1.802 - 1.836	0.013	0.7
	12 months	10	**1.857**	1.845 - 1.871	0.009	0.5

4.2.2. STABILITY

The stability can be evaluated by the variation of the average concentrations of the test samples (table X) measured in strictly identical conditions, *i.e.* new concentrated standards from the same dried salts at each testing period and use of the same volumetric equipment. It should be noted that these data include the normal analytical long term repeatability.

Nitrate and nitrite. For nitrate + nitrite (hence nitrate), the differences in means do not exceed 0.02 µmol/l or 0.5 % over the 12 months of testing. No trend should be deduced from these figures since they remain within the normal analytical long term repeatability. On the contrary they demonstrate the high degree of stability for nitrate in the autoclaved samples. For nitrite, the differences are 0.003 µmol/l, 0.008 µmol/l (1.6 %) and 0.021 µmol/l (1.5 %) at the low, intermediate and high level respectively. No obvious trend can be observed, the differences remaining within the analytical repeatability.

Ammonia. For ammonia the maximum differences of means are 0.03 µmol/l, 0.05 µmol/l (3 %) and 0.07 µmol/l (1.5 %) at low, intermediate and high level respectively. Although a negative drift seems to be observed (except at low level), it should be interpreted cautiously given that the magnitude of the differences may be considered surprisingly small according to the difficulties inherent in the determination of ammonia (see the intercomparison results). For the purpose and the duration of the present exercise, we think it reasonable to consider that the storage does not induce any effect upon the ammonia concentrations.

Phosphate. For phosphate, a possible contribution from the dissolution of the bottle wall was expected since previously demonstrated (Aminot *et al.*, 1992). Hence, the samples were stored at 5 °C before the sending to participants, in order to minimize dissolution of glass. They were then maintained at about 20 °C, the ambient temperature, as suggested to the participants. The results confirm the effect of phosphorus dissolution at 20 °C : systematic increase at all levels between 4 months and 12 months of storage (a mean increase of 40 µmol/l in silicate proves the dissolution of the glass from the bottle). The first step at 5 °C seems to have efficiently stabilised the phosphate concentrations. Taking the mean of the concentrations at 1 day and 4 months as the starting value, increases due to glass contributions during the following 8 months are :

- 0.015 µmol/l at low level, *i.e.* 0.002 µmol/l per month ;
- 0.018 µmol/l at intermediate level, *i.e.* 0.002 µmol/l per month ;
- 0.033 µmol/l at high level, *i.e.* 0.004 µmol/l per month.

4.2.3. SHIPMENT EFFECTS

The above tests for stability and homogeneity were performed with samples stored in our laboratory during the entire storage period. Since the samples were to travel by air throughout the world, adverse effects could be suspected due to storage conditions modifications with a possible increase or decrease of the sample temperature (possible freezing), and shaking of the samples. To obtain information about such modifications, special intercomparison packages were sent fo five ICES participants in different countries in the world who were asked to send them back as soon as received and without opening the boxes. We are grateful to these participants and thank them for their helpful contribution :

- M. Perttilä, FIMR, Helsinki, Finland ;
- J. Olafsson, MRI, Reykjavik, Iceland ;
- D. J. Mackey, CSIRO, Hobart, Australia ;
- D.M. Karl, UHM, Hawaii, USA ;
- F.A. Whitney, IOS, Sidney, Canada.

Other confirmed participants who finally were unable to analyse the samples in time sent also them back. All returned samples were analysed for ammonia.

Once returned to our laboratory, the samples were stored as for the other test samples and analysed together with them 12 months after preparation.

It must be pointed out that special devices placed in the packages together with the samples proved that no sample froze during transit (note that it took place in winter in northern countries).

The results (table XI) show that nitrate and nitrite present no obvious systematic difference in concentration between samples kept in the laboratory and "traveller" samples. A comparison using a t-test detects a significant difference (confidence level 95 %) only for nitrate + nitrite (hence nitrate) at the intermediate level. However that difference does not exceed 0.03 µmol/l (0.3 %) which can be considered analytically insignificant. As a consequence, both sets of nitrate and nitrite results at the 12 months checking time have been pooled to obtained the values in table X.

For phosphate a slight positive shipment effect is shown by the test. This effect is an increase of concentrations and standard deviations. It is attributed to an increase of the dissolution rate of glass into the sea water samples induced mainly by the shaking of samples during carriage (the mean silicate concentration increases by about 40 µmol/l compared with sedentary samples). Surprisingly the magnitude of the increase is not the same at all concentration levels. This might be explained by slight differences in the sea water used to prepare the material and in their final pH after autoclaving. At low and high levels the increases (0.006 and 0.003 µmol/l respectively) are statistically insignificant at the 95 % confidence level. At the intermediate level the increase of 0.03 µmol/l is significant. However it results from two journeys although the samples analysed by participants travelled only once. A mean shipment effect of + 0.015 µmol/l of phosphate must be applied to the intermediate level.

Table XI

Comparison of nutrient concentrations (µmol/l) in samples kept in the laboratory and samples having travelled to various countries.
Storage time 12 months.

Level	Sample type	n	Mean	Range	SD
		NITRATE + NITRITE			
LOW	sedentary	5	1.43	1.43 - 1.44	0.004
	traveller	4	1.44	1.43 - 1.45	0.008
INTERMEDIATE	sedentary	5	10.42	10.41 - 10.44	0.01
	traveller	5	10.45	10.44 - 10.47	0.01
HIGH	sedentary	5	27.43	27.36 - 27.49	0.05
	traveller	5	27.40	27.36 - 27.42	0.03
		NITRITE			
LOW	sedentary	5	0.145	0.145 - 0.145	(0)
	traveller	4	0.146	0.145 - 0.147	0.001
INTERMEDIATE	sedentary	5	0.503	0.502 - 0.503	0.0006
	traveller	5	0.504	0.502 - 0.507	0.002
HIGH	sedentary	5	1.403	1.393 - 1.407	0.006
	traveller	5	1.404	1.399 - 1.408	0.004
		NITRATE			
LOW	sedentary	5	1.29	1.28 - 1.29	0.004
	traveller	4	1.29	1.28 - 1.30	0.007
INTERMEDIATE	sedentary	5	9.92	9.91 - 9.94	0.01
	traveller	5	9.95	9.94 - 9.97	0.01
HIGH	sedentary	5	26.03	25.97 - 26.09	0.05
	traveller	5	26.00	25.95 - 26.02	0.03

Table XI (continued)

Comparison of nutrient concentrations (µmol/l) in samples kept in the laboratory and samples having travelled to various countries.
Storage time 12 months.

Level	Sample type	n	Mean	Range	SD
		AMMONIA*			
LOW	sedentary	5	0.33	0.31 - 0.34	0.011
	traveller	11	0.37	0.32 - 0.64	0.09
		[10	0.34	0.32 - 0.37	0.020]
INTERMEDIATE	sedentary	5	1.73	1.71 - 1.76	0.018
	traveller	11	1.76	1.41 - 1.99	0.14
		[8	1.75	1.73 - 1.80	0.024]
HIGH	sedentary	10	4.78	4.75 - 4.81	0.02
	traveller	11	4.78	4.73 - 4.93	0.05
		[10	4.76	4.73 - 4.80	0.02]
		PHOSPHATE			
LOW	sedentary	5	0.088	0.085 - 0.091	0.002
	traveller	4	0.094	0.089 - 0.101	0.005
INTERMEDIATE	sedentary	5	0.488	0.481 - 0.494	0.005
	traveller	8	0.518	0.507 - 0.528	0.009
HIGH	sedentary	10	1.857	1.845 - 1.871	0.009
	traveller	7	1.860	1.844 - 1.874	0.013

* In brackets : values obtained after rejection of outlying values (see table XII).

For ammonia the results are more complex. It can be seen (table XI) that some discrepancy does exist between travelling and sedentary samples. A few outlying results are found and this need to be more closely examined. When outlying values are removed the results found in travelling samples are not distinct from those of sedentary samples, in mean as well as in standard deviation. Now let us examine all data in detail (table XII).

The places from where samples have been returned are divided into two groups. In group 1 are the people to whom an additional package had been sent in order that they return it intact. These packages were not opened by the participants and were sent back shortly to our laboratory. In group 2 are the confirmed participants who sent back their samples because they were unable to analyse them. These packages have been opened by the participants and stored for months in their laboratories before being returned.

Table XII

Ammonia results in all travelling samples.

Return from	Level low	intermediate	high
GROUP 1			
Canada	0.38	*1.41*	4.75
Australia	0.36	1.73	4.77
Finland	0.36	1.74	4.75
Hawaii	0.37	1.74	4.76
Iceland	(0.81)*	1.73	4.74
GROUP 2			
Sweden	0.36	1.80	*4.93*
Great-Britain	0.34	*1.93*	4.78
Malaysia	0.36	1.77	-
Germany	0.33	1.74	4.73
Spain	*0.64*	*1.99*	4.80
Ireland	0.32	1.74	4.75
France (Bordeaux)	0.32	-	4.78
Mean**	0.34	1.75	4.76

* Excluded (cap broken).

** Group 1 + Group 2 without outlying results (italics).

It is obvious from this test that the samples of group 1 exhibit no increase in concentration (apart from one bottle with a broken cap), all samples with concentrations higher than expected belonging to group 2. The concentration increases are not quite randomly distributed. the samples returned from Spain and Sweden exhibit systematically high concentrations at all three levels which suggests a contamination from the atmosphere through the plastic cap. Such possibility has been demonstrated in our laboratory by submitting test samples to an ammonia enriched atmosphere (in a box containing a few millilitres of concentrated ammonia solution).

This test points out clearly that storage conditions of the sample bottles is one of the major factors for obtaining reliable data in ammonia. The present exercise cannot therefore discriminate between storage conditions and analytical capabilities.

In addition to the increase in ammonia, one outlying value is lower than expected. Since there is no obvious explanation for it, it is thought that an accidental closure failure (as suggested by a close examination of the cap) lead to a bacterial development responsible for ammonia consumption. Among the 128 autoclaved test samples analysed for ammonia, only one exhibited such a phenomenon. Consequently the risk for a sample sent to the participants seems very low, but in the case of a suspect result this possibility should be mentioned.

5. ASSIGNED CONCENTRATIONS

The assignment of nutrients concentrations to the samples requires some comments. We point out that the samples are prepared using nutrient depleted sea waters (NDSW). These waters, obtained by storing natural low-nutrient surface sea water in polyethylene carboys in the laboratory, contain very low, if not undetectable, nutrients concentrations. The final concentrations in the samples are obtained by adding a precisely known amount of concentrated nutrient salts solutions to these waters then autoclaving. The concentration in a sample is the sum of five components :

1) initial concentration in the NDSW,
2) added concentration using concentrates,
3) autoclaving effect,
4) storage (ageing) effect,
5) shipment effect.

Point one, the initial concentration in the NDSW, is a very low but **measured** quantity. Its reliability depends on our ability to measure low levels, *i.e.* on our blank procedure thus on our detection limit. Table VIII shows that the detection limits are low enough for the determination of the levels in the NDSW with sufficient reliability for the aims of the project. Any potential bias originating from standardisation can only have an insignificant effect on the low concentration of these waters. Only errors in system blanks evaluation could be incriminated (see § 3.4.2.2).

Point two is a **theoretical** added quantity. It is obtained using only balances as measuring instruments a way which is assumed to introduce only undetectable errors in the concentrations. Only two sources of error are identified at this stage : the purity of the original dried salts and potential losses either during the addition step or by physical and biochemical processes in the carboy before bottling.

Point three, the autoclaving effect, is a low but **measured** quantity. Depending on the nutrient concerned, autoclaving has from almost undetectable (nitrate, nitrite) to very measurable effects attributed to hydrolysis (ammonia) or to a combination of hydrolysis, glass leachage and hypothetical precipitation (phosphate). The overall effect is determined by comparing the concentrations before and after autoclaving. Since the autoclaving effect is obtained by difference on samples measured simultaneously with the same standardization curve, its value is assumed to be free of significant errors. Possible artefacts could only come from slight variations in concentration between preparation and measurement for non autoclaved samples.

As the starting point, the "initial" assigned concentrations were determined from the data corresponding to the above points one to three and are hence presented in table IX, A to E.

In order to obtain the practical assigned concentrations, the storage, or ageing, effect and the shipment effect should be evaluated. Both are **measured** quantities obtained from the stability tests (see paragraph 4).

In the case of nitrate, nitrite and ammonia, it has been shown that no significant drift could be detected in the samples whatever their concentration level and whether they had travelled or not. Consequently, their assigned concentrations, within the limits of the exercise, are those initially assigned (table IX, A, B and C).

Considering phosphate, the problem is complicated by the dissolution from glass into the sample. Summing storage and shipment effects lead to express rigorously the concentrations as a function of Δt, the number of months after October 1992, as follows :

low level : $(0.072 + 0.002\ \Delta t)$ µmol/l ;
intermediate level : $(0.472 + 0.002\ \Delta t + 0.015)$ µmol/l ;
high level : $(1.829 + 0.004\ \Delta t)$ µmol/l.

Averaging throughout the period will give :

low level : 0.080 ± 0.008 µmol/l ;
intermediate level : 0.495 ± 0.008 µmol/l ;
high level : 1.845 ± 0.017 µmol/l.

One of the major questions when assigning concentrations to samples is *what is the confidence interval of the assigned concentrations ?* The above discussion has shown that the main part of the intermediate concentration and high levels comes from the added nutrient concentrate. The purity of the salts used to prepare the concentrates is therefore an important factor. The purity of the salts is certified by the manufacturer and given with one decimal figure, which should assume, in the absence of any other information, a ± 0.1 % accuracy. The only exception is sodium nitrite, the purity of which is given as > 98 %. Since we assumed it is equal to 99 %, the nitrite concentrations have therefore an uncertainty of ± 1 %. For the calculation of total standard deviations, this value has been considered as the confidence interval at 95 % probability which implies a relative standard deviation of ± 0.5 %.

The total standard deviation attributed to the assigned values has been obtained by summing the variances of all contributions as follows :

$$s^2_{total} = s^2_{initial} + s^2_{spike} + s^2_{autoclaving} + s^2_{storage} + s^2_{shipment} .$$

All these contributions have been determined and the measured ones can be found in paragraph 4 (note that $s^2_{autoclaving} = s^2_{before\ autoclaving} + s^2_{after\ autoclaving}$). We point out that storage and shipment have no detectable effect on the variability except for phosphate.

From these considerations, the following assigned concentrations (in micromoles per litre) with 95 % confidence intervals are attributed to the samples in nitrate, nitrite and ammonia :

	Low	Intermediate	High
Nitrate + nitrite	1.47 ± 0.02	10.48 ± 0.10	27.43 ± 0.10
Nitrite	0.143 ± 0.002	0.505 ± 0.006	1.406 ± 0.020
Ammonia	0.34 ± 0.03	1.83 ± 0.06	4.86 ± 0.05

For phosphate, the variability originating from the storage drift is pooled with the other sources of variability which lead to the following assigned concentrations and 95 % confidence intervals :

	Low	Intermediate	High
Phosphate	0.08 ± 0.02	0.495 ± 0.03	1.85 ± 0.04

6. COMPARISON OF ESTIMATION FROM VARIOUS ORIGINS

Three sources of estimation of the nutrient concentrations in the reference material are available : the assigned concentrations (see paragraph 5), the concentrations measured in the test samples in our laboratory throughout the duration of the exercise (see paragraph 4) and the means calculated from the participants' results.

The measured concentrations are obtained by pooling all determinations from the test samples at the various testing periods. A shipment effect of 0.015 µmol/l has been added at the intermediate level in phosphate in accordance with our findings.

The means from the participants' results have been calculated (according to Berman 1992) after rejection of outliers by successive application of a *t*-test at the 95 % confidence level until an approximatively Normal distribution is obtained (stable mean and rejection of around 5 % of the data).

The results are summarized in table XIII with ranges at the 95 % confidence level.

The agreement between the three estimations may be considered highly satisfactory, even in the case of ammonia despite the wide standard deviation. The agreement between the statistical results and the assigned concentrations may also be interpreted as an additionnal confirmation of the samples' stability.

Table XIII

Comparison of the sample concentrations obtained from three separate sources.

	Concentrations and intervals at the 95 % confidence level (± 2 s.d.)					
	assigned		measured		from participant results	
NITRATE + NITRITE						
low	1.47 ± 0.02		1.43 ± 0.02	[19]	1.45 ± 0.54	[110]
intermediate	10.48 ± 0.10		10.43 ± 0.05	[20]	10.52 ± 0.60	[87]
high	27.43 ± 0.10		27.34 ± 0.16	[27]	27.50 ± 1.60	[92]
NITRITE						
low	0.143 ± 0.002		0.144 ± 0.003	[19]	0.157 ± 0.095	[104]
intermediate	0.505 ± 0.006		0.503 ± 0.007	[20]	0.511 ± 0.100	[98]
high	1.406 ± 0.020		1.400 ± 0.019	[27]	1.41 ± 0.14	[99]
AMMONIA						
low	0.34 ± 0.03		0.32 ± 0.04	[15]	0.43 ± 0.48	[66]
intermediate	1.83 ± 0.06		1.76 ± 0.05	[17]	1.64 ± 0.74	[80]
high	4.86 ± 0.05		4.81 ± 0.08	[31]	4.60 ± 2.0	[92]
PHOSPHATE						
low	0.08 ± 0.02		0.08 ± 0.02	[15]	0.09 ± 0.07	[91]
intermediate	0.495 ± 0.03		0.49 ± 0.02	[16]	0.49 ± 0.16	[118]
high	1.85 ± 0.04		1.835 ± 0.04	[33]	1.83 ± 0.11	[87]

Number of observations in brackets.

7. SUMMARY AND CONCLUSION

The aim of this work was to produce and test reference material for nutrients in sea water in order to perform intercomparison exercises. The first production of this type of material was for the ICES fourth intercomparison exercise (Kirkwood *et al.*, 1991) and involved only nitrate and phosphate. This time nitrite and ammonia have been included in order to cover more widely the field of nutrient determinations. As previously, autoclaving was used as the preservation method. This method requires the use of materials that are resistant to temperature and pressure effects therefore glass was chosen despite its known solubility in sea water which is the origin of a leachage of phosphate.

A very large series of samples was used to test stability in order that the participants could be given guarantees about the preservation of the samples.

Additionally, in this exercise, the assignment of concentration to the samples was done in order to detect a possible bias in methodologies and to try to avoid the difficulty of extracting the "true" concentrations from the results.

The results of the tests have shown the high degree of stability of the nitrogenous nutrients in the samples, those compounds having no interaction with the bottle material. Phosphate exhibits a slight drift but the tests allow its determination with sufficient accuracy for the purpose of the exercice.

Three sources of concentration estimations have been compared : the assigned concentrations, the concentrations measured in our laboratory and the consensus of concentrations extracted from the participants' results. All three agree very closely, showing that there is no systematic bias due to sample preservation problems.

It can be concluded that the reference material for nutrients in sea water prepared for the present exercise met the expected requirements.

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ANNEX IV

Results submitted by participating laboratories

Table IV.1

Raw results for inorganic nutrients (µmol/l).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	4 Low	5 High	6 Medium	4 Low	5 High	6 Medium
1	DS	1.35	30.44	DS	0.24	1.55	DS	1.11	28.89	0.11	0.50	0.19	0.16	1.87	0.59
2	12.0	1.8	30.0	0.71	0.35	1.63	11.2	1.4	28.4	ND	ND	ND	0.25	1.98	0.62
3	10.0	0.7	27.8	0.7	< 0.7	1.4	9.3	0.7	26.4	< 0.7	5.0	2.1	0.16	1.9	0.5
4	10	1.4	27	0.57	0.21	1.5	9.5	1.2	25	0.50	5.3	2.00	0.10	1.9	0.55
5	10.62	1.32	27.7	0.50	0.14	1.39	10.12	1.18	26.3	ND	ND	ND	0.12	1.81	0.55
6	10.03	1.71	27.24	0.95	0.48	1.95	9.08	1.23	25.29	0.559	5.51	3.60	0.07	1.98	0.43
7	21.4	1.5	28.1	0.52	0.08	1.48	20.8	1.4	26.5	ND	ND	ND	1.39	2.62	2.37
8	10.60	1.50	27.85	0.50	0.14	1.37	10.10	1.36	26.48	ND	ND	ND	0.06	1.59	0.41
9	8.16	0.91	21.18	0.50	0.13	1.41	7.66	0.78	19.77	0.58	6.48	2.22	0.12	1.66	0.49
10	10.1	1.4	27.3	0.49	0.13	1.40	9.6	1.3	25.9	ND	ND	ND	0.05	1.86	0.50
11	10.5	1.4	27.7	0.49	0.15	1.36	10.0	1.25	26.3	ND	ND	ND	0.10	1.84	0.49
12	10.540	1.318	27.468	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.041	1.989	0.489
13	10.81	0.81	23.44	0.51	0.13	1.40	10.30	0.67	22.04	1.10	3.34	1.56	0.14	1.82	0.48
14	10.00	1.14	28.80	0.52	0.17	1.50	9.48	0.97	27.30	0.36	4.52	1.53	0.05	1.75	0.46
15	10.44	1.39	27.76	0.47	0.12	1.32	9.97	1.27	26.44	ND	ND	ND	0.06	1.91	0.48
16	9.8	2.87	23.0	0.52	0.14	1.40	9.28	2.73	21.6	< 0.3	3.9	1.5	0.08	1.84	0.50
17	12.87	2.21	32.9	0.69	0.21	1.93	12.18	2.0	30.97	1.43	6.16	3.54	0.06	1.84	0.52
18	10.0	1.7	26.8	0.4	< 0.1	1.3	9.6	1.7	25.5	ND	ND	ND	< 0.05	1.75	0.45
19	8.72	0.96	23.3	0.44	0.07	1.30	8.28	0.89	22.0	0.40	4.48	1.52	0.20	1.64	0.60
20	11.59	1.55	30.33	0.51	0.15	1.41	11.08	1.40	28.92	0.49	4.79	1.98	0.06	1.80	0.43
21	10.5	1.5	27.3	0.5	0.1	1.4	10.0	1.4	25.9	0.91	3.9	1.5	0.12	1.88	0.53
22	10.63	1.32	29.55	0.51	0.13	1.45	10.12	1.19	28.1	1.30	7.07	4.28	0.19	1.88	0.57
23	11.43	1.57	29.90	0.51	0.15	1.40	10.92	1.42	28.50	0.05	4.39	1.45	0.12	1.80	0.46
24	10.22	2.26	21.64	0.41	0.05	1.25	9.81	2.21	20.39	0.67	3.67	1.39	0.06	1.86	0.48
25	9.22	0.86	24.82	1.38	0.30	4.07	7.8	0.6	20.8	< 0.2	5.1	2.6	< 0.02	1.63	0.36
26	10.73	1.76	27.60	0.55	0.125	1.395	10.18	1.635	26.205	0.25	4.95	1.90	0.10	1.91	0.52
27	10.3	1.6	27.3	0.51	0.13	1.42	9.8	1.5	25.9	0.45	5.24	1.97	0.07	1.81	0.47

Table IV.1 (continued)

Raw results for inorganic nutrients (µmol/l).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	1	2	3	1	2	3	1	2	3	4	5	6	4	5	6
	Medium	Low	High	Medium	Low	High	Medium	Low	High	Low	High	Medium	Low	High	Medium
28	8.63	1.12	22.90	0.51	0.15	1.46	8.12	0.97	21.44	0.44	2.56	0.94	0.05	1.58	0.47
29	10.1	0.5	27.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	2.07	0.54
30	10.6	1.7	27.4	0.6	0.3	1.5	10.0	1.4	25.9	< 0.2	3.9	1.1	< 0.1	1.9	0.5
31	10.50	1.17	27.50	0.44	0.12	1.30	10.06	1.05	26.20	0.42	4.72	1.85	0.11	1.81	0.49
32	10.39	1.45	27.22	0.51	0.14	1.42	9.88	1.31	25.80	0.18	3.70	1.18	0.00	1.80	0.41
33	10.8	1.45	28.3	0.50	0.14	1.47	10.3	1.31	26.8	< 0.1	4.17	1.15	0.07	1.91	0.51
34	10.45	1.51	27.51	0.49	0.15	1.37	9.96	1.36	26.14	0.31	4.55	1.75	0.07	1.75	0.42
35	8.896	1.218	22.932	0.479	0.125	1.357	8.417	1.093	21.575	4.116	7.933	5.022	0.264	2.049	0.604
36	10.38	1.51	26.78	0.64	0.18	1.59	9.74	1.33	25.19	0.62	4.76	1.80	0.05	1.78	0.46
37	9.79	1.14	26.94	0.35	0.03	1.39	9.44	1.11	25.55	0.35	0.03	1.39	0.10	1.35	0.17
38	14.39	2.55	35.84	2.47	0.41	3.84	11.92	2.14	32.00	0.44	2.52	1.02	0.10	1.69	0.34
39	10.42	1.22	26.09	0.55	0.14	1.43	9.87	1.08	24.66	0.50	7.40	2.78	< 0.05	2.12	0.38
40	10.60	1.51	27.05	0.48	0.15	1.31	10.12	1.36	25.75	0.74	4.57	2.10	0.09	1.77	0.48
41	9.91	1.19	27.2	0.43	0.09	1.34	9.48	1.10	25.86	0.12	4.25	1.46	< 0.01	1.75	0.38
42	10.0	1.40	26.6	0.83	0.43	1.86	9.17	0.97	24.7	< 1	4.2	< 1	< 0.3	1.7	0.32
43	10.22	1.12	29.10	0.51	0.14	1.39	9.71	0.98	27.71	0.4	4.2	1.9	0.07	1.82	0.48
44	10.60	1.32	27.95	0.50	0.12	1.41	10.10	1.20	26.54	0.78	5.61	2.17	0.11	1.87	0.48
45	9.62	3.97	25.71	0.53	1.29	1.49	9.09	2.68	24.22	0.13	5.21	1.75	0.01	1.45	0.31
46	9.72	7.36	28.26	2.06	1.65	2.46	7.66	5.71	25.8	4.9	7.8	7.6	1.96	2.47	2.27
47	10.60	1.11	26.97	0.48	0.09	1.39	10.11	1.03	25.59	< 0.5	5	2	0.8	2.4	1.1
48	8.23	1.94	24.20	0.87	0.44	1.59	7.36	1.50	22.61	< 3	5.40	< 3	1.58	1.05	0.72
49	10.49	1.41	27.34	0.557	0.184	1.47	9.93	1.23	25.87	1.47	7.29	3.77	0.319	1.86	0.507
50	23.74	16.78	36.41	0.89	1.07	2.14	22.85	15.71	34.27	2.14	6.43	3.09	< 0.4	< 0.4	< 0.4
51	9.88	1.51	16.43	0.53	0.16	1.45	9.35	1.35	14.98	0.27	2.86	1.09	0.55	2.47	1.07
52	13.38	2.96	30.68	0.48	0.14	1.38	12.90	2.82	29.30	ND	ND	ND	0.12	2.04	0.62
53	14.6	1.95	32.02	0.98	0.31	2.67	13.62	1.64	29.35	0.14	3.70	0.97	0.09	1.89	0.52
54	10.7	1.50	27.9	0.51	0.16	1.40	10.2	1.34	26.5	4.17	4.91	1.72	0.10	1.70	0.47

Table IV.1 (continued)

Raw results for inorganic nutrients (µmol/l).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	4 Low	5 High	6 Medium	4 Low	5 High	6 Medium
55	11.0	1.4	26.8	0.50	0.14	1.35	10.5	1.26	25.45	ND	ND	ND	0.05	1.81	0.44
56	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.709	11.387	3.495	0.2906	1.6468	0.4843
57	10.7	< 1.0	26.8	0.4	< 0.05	1.2	10.3	< 1.0	25.6	< 1.4	4.6	2.1	< 0.10	1.79	0.42
58	9.45	1.17	22.61	ND	ND	ND	ND	ND	ND	0.97	4.90	2.03	0.13	1.81	0.50
59	10.72	2.15	27.87	0.49	0.14	1.33	10.23	2.01	26.54	ND	ND	ND	< 0.03	1.82	0.45
60	10.870	1.855	28.470	0.508	0.138	2.240	10.362	1.717	26.230	0.331	5.336	1.906	0.114	1.925	0.538
61	10.05	1.20	> 14.30	0.34	0.09	1.27	9.71	1.11	> 13.03	0.23	2.46	0.22	1.78	> 3.20	2.30
62	10.75	1.50	28.01	0.52	0.16	1.39	10.23	1.34	26.62	ND	ND	ND	0.13	1.98	0.53
63	12.4	2.0	27.8	0.61	0.18	1.65	11.8	1.8	26.1	2.6	5.7	2.1	0.13	1.86	0.53
64	9.35	1.45	22.65	0.62	0.19	1.67	8.73	1.26	20.98	0.62	3.92	1.67	0.07	1.65	0.47
65	11.08	1.95	28.0	0.52	0.17	1.40	10.56	1.78	26.6	0.63	5.89	2.19	0.08	1.85	0.51
66	11.5	1.8	31.3	0.7	0.4	1.9	10.8	1.4	29.4	ND	ND	ND	0.2	2.4	0.6
67	10.43	1.14	27.79	0.50	0.07	1.29	9.93	1.07	26.5	< 0.01	3.86	1.29	< 0.01	1.81	0.42
68	10.26	1.44	26.96	0.41	0.21	1.14	9.85	1.23	25.82	0.93	5.33	2.59	0.09	1.52	0.39
69	10.6	1.50	26.86	0.54	0.16	1.45	10.03	1.34	25.41	1.95	3.70	1.49	< 0.13	1.71	0.42
70	11.21	1.55	27.93	0.51	0.15	1.33	10.7	1.4	26.6	ND	ND	ND	0.13	1.78	0.46
71	10.61	1.48	27.41	0.51	0.16	1.36	10.10	1.32	26.05	ND	ND	ND	0.23	1.77	0.52
72	10.07	1.36	27.06	0.50	0.14	1.43	9.57	1.22	25.63	< 0.36	4.43	1.50	0.03	1.84	0.45
73	10.4	1.43	27.0	0.50	0.21	1.43	9.9	1.22	25.6	0.14	4.29	1.36	0.06	1.84	0.48
74	10.8	1.5	26.3	0.54	0.17	1.43	10.3	1.3	24.9	0.5	5.1	1.8	0.08	1.90	0.49
75	10.53	1.61	26.16	0.51	0.14	1.45	9.96	1.44	24.71	ND	ND	ND	0.07	1.80	0.48
76	12.012	1.618	30.152	0.482	0.000	1.411	11.530	1.618	28.741	0.672	4.123	2.582	0.092	1.805	0.556
77	11.277	1.315	29.131	0.702	0.253	1.673	10.575	1.062	27.458	7.054	7.429	7.237	0.065	1.724	0.483
78	7.3	< 3	16.8	0.42	< 0.25	1.27	6.9	< 3.0	15.5	ND	ND	ND	< 0.50	1.82	0.69
79	9.7	0.97	24.5	0.49	0.14	1.37	9.2	0.83	23.1	1.5	7.8	1.2	0.09	1.73	0.43
80	2.23	0.09	2.65	0.27	0.09	0.91	1.96	0.00	1.74	ND	ND	ND	0.11	1.81	0.54
81	DS	3.9	32.3	DS	0.93	13.6	DS	2.97	18.7	1.8	6.2	3.1	0.2	0.2	0.25

Table IV.1 (continued)

Raw results for inorganic nutrients (µmol/l).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	4 Low	5 High	6 Medium	4 Low	5 High	6 Medium
82	10.90	1.80	28.05	0.46	0.15	1.35	10.44	1.65	26.70	1.20	4.45	1.35	0.08	1.85	0.50
83	8.65	1.50	22.00	0.45	0.18	1.32	8.20	1.32	20.68	0.0	3.5	1.0	0.17	1.7	0.5
84	10.3	1.35	25.5	0.46	0.12	1.36	9.85	1.23	24.1	0.22	4.73	1.72	0.03	1.78	0.47
85	10.73	1.45	27.48	0.50	0.14	1.39	10.23	1.31	26.09	0.15	5.15	1.73	0.11	1.79	0.46
86	9.7	2.1	27.3	0.9	0.5	1.8	8.8	1.6	25.5	< 1	3	1	0.3	2.0	0.7
87	9.25	1.35	23.79	0.45	0.11	1.35	8.8	1.24	22.44	ND	ND	ND	0.09	1.82	0.54
88	10.85	2.00	28.94	0.54	0.18	1.42	10.31	1.82	27.52	1.14	5.32	2.15	1.57	2.96	2.46
89	10.35	1.45	26.75	0.50	0.14	1.39	9.9	1.3	25.4	0.29	4.76	1.69	0.07	1.79	0.50
90	10.39	1.39	28.01	0.49	0.14	1.34	9.90	1.25	26.67	0.75	2.79	1.36	0.09	1.81	0.48
91	10.71	1.64	28.21	0.58	0.19	1.52	10.13	1.45	26.69	0.21	3.07	0.71	0.23	1.58	0.39
92	10.7	1.25	28.6	0.71	< 0.36	1.43	10.0	1.07	27.1	0.36	2.50	1.07	0.06	1.84	0.48
93	10.61	1.61	27.90	0.52	0.16	1.43	10.1	1.45	26.5	0.20	1.25	2.59	0.09	1.82	0.46
94	9.821	1.802	25.690	0.497	0.148	1.364	9.324	1.654	24.326	0.703	4.686	1.927	0.125	1.789	0.521
95	6.55	1.01	22.4	0.49	0.13	1.30	6.06	0.88	21.1	0.50	4.00	1.49	0.11	1.87	0.57
96	10.71	1.51	29.2	0.61	0.21	1.6	10.1	1.3	27.6	0.01	2.14	0.93	0.039	1.77	0.45
97	10.64	1.47	28.44	0.73	0.22	2.53	9.91	1.25	25.91	0.71	3.99	1.72	0.05	1.85	0.47
98	10.70	1.39	28.57	0.51	0.17	1.51	10.19	1.22	27.06	0.81	4.84	1.75	0.11	1.93	0.54
99	10.52	1.40	26.83	0.49	0.13	1.37	10.03	1.27	25.46	0.55	4.40	1.62	0.05	1.91	0.56
100	10.64	1.43	28.14	0.572	0.143	1.43	10.07	1.29	26.71	0.25	2.26	1.20	0.032	1.87	0.517
101	10.3	1.48	25.9	0.614	0.200	1.50	9.71	1.28	24.4	1.78	6.07	2.36	< 0.06	1.81	0.420
102	9.71	1.26	25.0	0.500	0.143	1.43	9.21	1.12	23.6	1.34	5.40	2.00	0.165	1.89	0.616
103	10.06	1.44	28.11	0.54	0.15	1.47	9.52	1.29	26.64	ND	ND	ND	0.14	1.79	0.49
104	7.429	1.143	7.929	0.500	0.143	1.429	6.929	1.000	6.500	< 0.21	4.000	1.643	0.065	1.710	0.452
105	11.0	1.5	28.2	0.7	0.3	1.6	10.3	1.2	26.6	0.5	4.5	1.6	0.05	1.53	0.39
106	10.71	0.98	28.58	1.36	0.37	2.63	9.35	0.61	25.95	0.35	3.68	1.85	0.06	1.72	0.33
107	11.0	1.6	27.7	0.56	0.16	1.47	10.4	1.4	26.2	< 0.1	3.9	1.2	0.15	1.89	0.54
108	DS	0.37	14.09	DS	0.15	1.44	DS	0.22	12.65	1.73	6.57	2.32	< 0.02	1.51	0.32

Table IV.1 (continued)

Raw results for inorganic nutrients (µmol/l).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	1 Medium	2 Low	3 High	4 Low	5 High	6 Medium	4 Low	5 High	6 Medium
109	10.734	1.567	28.34	0.558	0.154	1.496	10.17	1.413	26.84	0.78	5.55	2.11	0.071	1.86	0.47
110	10.65	1.69	27.52	0.69	0.22	1.81	9.96	1.47	25.71	0.67	7.50	2.80	0.11	1.77	0.46
111	10.4	1.59	26.7	0.47	0.158	1.39	9.93	1.432	25.31	0.33	4.88	1.60	0.074	1.93	0.46
112	9.4	0.84	26.2	0.42	< 0.01	1.46	8.98	0.84	24.7	< 0.3	3.6	0.60	< 0.1	2.1	0.29
113	12.88	21.72	36.61	0.54	0.18	1.55	12.34	21.54	35.06	1.09	3.08	1.84	ND	ND	ND
114	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.109	4.160	1.752	0.469	1.843	0.829
115	10.7	1.37	28.4	ND	ND	ND	ND	ND	ND	0.52	4.96	1.89	0.10	1.86	0.53
116	10.4518	1.4933	27.7253	0.5076	0.1448	1.4108	9.9442	1.3485	26.3145	1.5400	6.1629	3.0647	0.0817	1.7878	0.4607
117	10.5	1.4	27.6	0.55	0.16	1.56	9.95	1.24	26.04	0.43	4.44	1.54	0.12	1.84	0.49
118	10.3	1.42	27.5	0.43	0.092	1.36	9.87	1.33	26.14	1.6	6.5	3.3	0.13	1.9	0.52
119	8.90	0.615	25.9	0.54	0.195	1.48	8.36	0.42	24.4	1.87	6.235	DS	0.184	1.625	DS
120	11.0	1.5	28.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	1.79	0.45
121	9.8	0.8	26.8	0.36	< 0.15	1.2	9.4	0.8	25.6	ND	ND	ND	0.06	1.79	0.36
122	10.7	< 7	27.1	0.55	0.20	1.40	10.15	< 7	25.7	1.9	6.7	3.3	0.32	1.74	0.57
123	10.65	1.64	26.82	0.53	0.16	1.42	10.12	1.48	25.41	0.30	4.91	1.78	0.15	1.88	0.62
124	9.57	2.00	24.90	0.50	0.15	1.41	9.07	1.85	23.49	ND	ND	ND	0.30	3.58	1.03
125	10.48	2.05	27.82	1.57	1.48	2.29	8.91	0.57	25.53	ND	ND	ND	3.5	7.0	5.7
126	10.634	1.476	27.720	0.507	0.152	1.370	10.127	1.324	26.350	0.781	5.469	1.765	0.248	1.904	0.662
127	10.65	1.42	26.45	0.58	0.18	1.54	10.06	1.24	24.91	0.51	4.81	2.06	< 0.04	1.70	0.36
128	10.94	1.50	25.84	0.54	0.16	1.44	10.40	1.34	24.40	0.46	5.04	1.86	0.09	1.76	0.51
129	11.0	1.53	27.3	0.58	0.21	1.48	10.4	1.32	25.8	0.20	4.73	1.66	0.10	1.81	0.50
130	10.90	1.68	27.85	0.61	0.24	1.54	10.29	1.44	26.31	0.36	4.81	1.84	0.14	1.90	0.54
131	10.91	2.95	28.79	0.53	0.15	1.44	10.38	1.10	27.35	0.21	3.80	1.27	0.10	1.52	0.39
132	10.37	1.38	27.44	0.56	0.18	1.41	9.81	1.20	26.03	0.11	4.48	1.19	0.09	1.79	0.48

REMARKS ON PARTICIPANTS' RESPONSE

- Laboratories 92 and 112 sent results in obviously wrong units and were asked to re-submit them in the correct units.

- Laboratories 20, 45 and 75 sent several results for each determinand in each sample. They were asked to decide for themselves which result they considered to be correct.

- Laboratory 2 did not calculate nitrate by subtraction of its nitrate + nitrite and nitrite data.

- Laboratories 46, 70 and 78 gave nitrate and nitrite results but not the sum.

PARTICIPANTS' REMARKS

- Laboratories 64, 75 and 76 mentioned that the volume of the samples was insufficient for replicates using manual analysis.

- Laboratory 126 mentioned considerable moisture in the plastic bags containing samples 4 and 5 indicating possible leakage.

- Laboratory 12 mentioned salt crystals between the cap and the bottle of sample 4.

- Laboratory 60 rechecked its nitrite data after receipt of the raw histograms concluding the exercise and found a mis-measurement of the peak height in sample 3.

DEFAULTERS

The following laboratories returned neither results nor unused samples :

- F.I., Univ. C., Winnipeg, Canada ;
- L.A.C., A. Univ., Thessaloniki, Greece ;
- SOEST, Univ. H., Honolulu, USA ;
- I.B.M., CNR, Venezia, Italy ;
- INIP, Lisboa, Portugal.

Table IV.2

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
1	-	-	-	-	-	-	-	-	-	0.04	91	0.06	0.02	-3.9	0.10
		-	-		-	-		-	-		*1.2*	*0.04*		*1.7*	*0.02*
2	0.33	8.3	0.4	0.01	1.5	0.20	0.28	9.0	0.1	-	-	-	0.03	-1.6	0.15
		1.8	*0.3*		*0.6*	*0.01*		*1.6*	*0.3*		-	-		*2.1*	*0.02*
3	0.08	4.5	-0.9	0.16	40	0.52	0.32	4.4	-0.9	0.01	-4.8	0.37	0.05	-0.5	0.05
		0.5	*0.1*		*17*	*0.15*		*1.8*	*0.3*		*0.4*	*0.01*		*4.1*	*0.04*
4	0.23	-1.2	-0.2	0.01	2.3	0.06	0.03	-3.6	-0.1	0.07	6.6	0.10	0.02	1.2	0.03
		1.2	*0.2*		*0.8*	*0.01*		*0.2*	*0.0*		*2.0*	*0.06*		*1.7*	*0.02*
5	0.12	1.5	-0.1	0.00	-1.1	0.00	0.11	1.6	-0.1	-	-	-	0.03	-5.1	0.06
		0.6	*0.1*		*0.1*	*0.00*		*0.6*	*0.1*		-	-		*2.0*	*0.02*
6	0.43	-1.2	0.0	0.04	15	0.34	0.46	-2.1	-0.3	1.13	2.7	0.82	0.07	9.5	-0.06
		2.3	*0.4*		*4.2*	*0.04*		*2.6*	*0.4*		*35*	*1.04*		*5.2*	*0.06*
7	8.58	-5.7	4.6	0.03	10	-0.06	8.54	-6.7	4.6	-	-	-	0.54	43	1.67
		46	*7.8*		*3.3*	*0.03*		*48*	*7.8*		-	-		*41*	*0.46*
8	0.04	1.5	0.0	0.01	-2.8	0.00	0.05	1.7	0.0	-	-	-	0.01	13	-0.01
		0.2	*0.0*		*0.6*	*0.01*		*0.3*	*0.0*		-	-		*0.5*	*0.01*
9	0.17	22	-0.1	0.00	1.3	-0.01	0.18	23	-0.1	0.24	32	0.00	0.01	13	0.05
		0.9	*0.2*		*0.3*	*0.00*		*1.0*	*0.2*		*7.5*	*0.23*		*0.5*	*0.01*
10	0.23	0.0	-0.2	0.00	0.6	-0.02	0.25	-0.2	-0.2	-	-	-	0.02	1.8	-0.02
		1.2	*0.2*		*0.3*	*0.00*		*1.4*	*0.2*		-	-		*1.5*	*0.02*
11	0.02	1.3	-0.1	0.01	-4.1	0.01	0.02	1.4	-0.1	-	-	-	0.01	-1.4	0.01
		0.1	*0.0*		*0.6*	*0.01*		*0.1*	*0.0*		-	-		*1.1*	*0.01*
12	0.12	0.6	-0.1	-	-	-	-	-	-	-	-	-	0.01	10	-0.05
		0.6	*0.1*		-	-		-	-		-	-		*0.5*	*0.01*
13	1.73	14	0.5	0.01	0.2	-0.01	1.73	15	0.4	0.22	49	0.81	0.04	-4.1	0.04
		9.3	*1.6*		*1.4*	*0.01*		*9.7*	*1.6*		*6.8*	*0.21*		*3.2*	*0.04*
14	0.60	7.1	-0.7	0.02	6.0	0.00	0.57	7.2	-0.8	0.16	-7.0	-0.04	0.01	-4.2	-0.02
		3.2	*0.5*		*2.7*	*0.02*		*3.2*	*0.5*		*5.0*	*0.15*		*0.7*	*0.01*
15	0.08	1.7	-0.1	0.00	-5.1	-0.01	0.09	2.0	-0.1	-	-	-	0.01	4.8	-0.03
		0.4	*0.1*		*0.5*	*0.00*		*0.5*	*0.1*		-	-		*0.8*	*0.01*
16	0.05	22	1.7	0.01	-0.6	0.01	0.05	24	1.7	0.01	20	0.04	0.01	-0.7	0.00
		0.2	*0.0*		*1.6*	*0.01*		*0.3*	*0.0*		*0.3*	*0.01*		*0.4*	*0.00*
17	0.01	18	0.5	0.01	36	0.01	0.03	17	0.5	0.44	2.0	1.32	0.03	-0.2	0.00
		0.0	*0.0*		*1.1*	*0.01*		*0.2*	*0.0*		*14*	*0.41*		*2.5*	*0.03*
18	0.33	-3.0	0.1	0.03	-4.1	-0.06	0.35	-3.3	0.2	-	-	-	0.00	-4.0	-0.03
		1.8	*0.3*		*3.8*	*0.03*		*2.0*	*0.3*		-	-		*0.1*	*0.00*
19	0.01	14	-0.3	0.01	-3.0	-0.06	0.00	15	-0.2	0.18	-8.6	-0.01	0.05	20	0.16
		0.0	*0.0*		*1.5*	*0.01*		*0.0*	*0.0*		*5.5*	*0.17*		*3.7*	*0.04*

Table IV.2 (continued)

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
20	0.04	11	-0.1	0.00	-0.2	0.01	0.03	11	-0.1	0.06	-5.2	0.20	0.03	-1.0	-0.04
		0.2	*0.0*		*0.1*	*0.00*		*0.2*	*0.0*		*1.8*	*0.05*		*2.3*	*0.03*
21	0.04	-0.7	0.1	0.02	2.3	-0.03	0.02	-0.8	0.1	0.32	32	0.51	0.00	-0.5	0.04
		0.2	*0.0*		*2.4*	*0.02*		*0.1*	*0.0*		*9.7*	*0.29*		*0.2*	*0.00*
22	0.39	9.1	-0.5	0.00	4.5	-0.02	0.40	9.3	-0.5	0.86	22	1.35	0.01	-4.2	0.11
		2.1	*0.4*		*0.1*	*0.00*		*2.2*	*0.4*		*27*	*0.80*		*1.0*	*0.01*
23	0.02	9.1	0.0	0.00	-1.1	0.01	0.01	9.6	0.0	0.02	-3.8	-0.29	0.04	-4.1	0.02
		0.1	*0.0*		*0.1*	*0.00*		*0.1*	*0.0*		*0.8*	*0.02*		*3.2*	*0.04*
24	0.99	26	1.7	0.01	-5.3	-0.08	0.99	27	1.8	0.22	32	0.32	0.00	1.7	-0.02
		5.3	*0.9*		*1.4*	*0.01*		*5.6*	*0.9*		*6.6*	*0.20*		*0.1*	*0.00*
25	0.04	-7.7	-0.5	0.00	199	-0.13	0.10	18	-0.4	0.63	4.6	0.18	0.03	-8.4	-0.07
		0.2	*0.0*		*0.0*	*0.00*		*0.6*	*0.1*		*19*	*0.58*		*2.2*	*0.02*
26	0.00	-0.5	0.3	0.05	-0.7	0.01	0.05	-0.5	0.3	0.08	3.5	-0.06	0.00	2.3	0.02
		0.0	*0.0*		*5.3*	*0.05*		*0.3*	*0.0*		*2.5*	*0.07*		*0.3*	*0.00*
27	0.18	-0.8	0.0	0.01	1.9	-0.01	0.20	-1.0	0.1	0.05	6.3	0.06	0.01	-1.5	-0.01
		0.9	*0.2*		*0.9*	*0.01*		*1.1*	*0.2*		*1.5*	*0.04*		*0.5*	*0.01*
28	0.04	16	-0.1	0.01	4.0	-0.01	0.01	17	-0.1	0.16	52	0.19	0.05	15	0.01
		0.2	*0.0*		*1.3*	*0.01*		*0.1*	*0.0*		*4.9*	*0.15*		*3.7*	*0.04*
29	0.18	3.8	-0.9	-	-	-	-	-	-	-	-	-	0.00	13	-0.02
		1.0	*0.2*		-	-		-	-		-	-		*0.1*	*0.00*
30	0.02	-1.0	0.2	0.03	-4.1	0.14	0.02	-0.8	0.1	0.26	17	-0.22	0.02	2.1	0.01
		0.1	*0.0*		*3.8*	*0.03*		*0.1*	*0.0*		*7.9*	*0.24*		*1.3*	*0.01*
31	0.15	1.3	-0.2	0.01	-6.2	-0.02	0.16	1.7	-0.2	0.01	-4.9	0.10	0.01	-3.6	0.02
		0.8	*0.1*		*1.6*	*0.01*		*0.9*	*0.1*		*0.3*	*0.01*		*1.1*	*0.01*
32	0.00	-0.7	0.0	0.00	1.3	0.00	0.01	-0.8	0.0	0.13	21	-0.16	0.01	1.9	-0.09
		0.0	*0.0*		*0.3*	*0.00*		*0.0*	*0.0*		*3.9*	*0.12*		*0.7*	*0.01*
33	0.03	3.4	-0.1	0.02	5.8	-0.02	0.05	3.1	0.0	0.23	-8.5	-0.34	0.01	3.8	-0.01
		0.1	*0.0*		*1.8*	*0.02*		*0.3*	*0.0*		*7.2*	*0.22*		*0.5*	*0.01*
34	0.07	0.2	0.0	0.01	-3.2	0.01	0.06	0.4	0.0	0.03	-6.4	0.01	0.03	-4.3	-0.03
		0.4	*0.1*		*0.8*	*0.01*		*0.4*	*0.1*		*1.0*	*0.03*		*2.6*	*0.03*
35	0.11	16	0.0	0.00	-2.5	-0.01	0.12	17	0.1	0.28	14	3.67	0.06	2.3	0.15
		0.6	*0.1*		*0.1*	*0.00*		*0.7*	*0.1*		*8.7*	*0.26*		*4.7*	*0.05*
36	0.08	-2.7	0.1	0.04	10	0.05	0.04	-3.4	0.1	0.15	-7.5	0.23	0.00	-2.3	-0.03
		0.4	*0.1*		*4.8*	*0.04*		*0.2*	*0.0*		*4.5*	*0.14*		*0.3*	*0.00*
37	0.24	-0.4	-0.5	0.06	9.2	-0.16	0.18	-0.9	-0.3	0.92	113	0.89	0.17	25	-0.06
		1.3	*0.2*		*6.0*	*0.05*		*1.0*	*0.2*		*28*	*0.85*		*13*	*0.15*
38	0.23	28	0.8	0.85	149	0.54	0.54	21	0.2	0.08	53	0.24	0.10	-7.7	-0.04
		1.2	*0.2*		*93*	*0.80*		*3.1*	*0.5*		*2.6*	*0.08*		*7.9*	*0.09*

Table IV.2 (continued)

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
39	0.46	-4.6	0.1	0.03	1.3	0.01	0.43	-5.0	0.0	0.00	53	-0.02	0.12	20	-0.12
		2.5	*0.4*		*3.5*	*0.03*		*2.4*	*0.4*		*0.1*	*0.00*		*9.3*	*0.10*
40	0.18	-1.8	0.2	0.00	-8.1	0.02	0.18	-1.4	0.1	0.08	16	0.50	0.00	-5.0	0.01
		1.0	*0.2*		*0.2*	*0.00*		*1.0*	*0.2*		*2.4*	*0.07*		*0.2*	*0.00*
41	0.25	0.4	-0.4	0.01	-0.6	-0.06	0.23	0.5	-0.4	0.02	-8.5	-0.20	0.03	-1.0	-0.09
		1.3	*0.2*		*1.6*	*0.01*		*1.3*	*0.2*		*0.5*	*0.02*		*2.3*	*0.03*
42	0.12	-2.8	-0.1	0.01	13	0.26	0.09	-3.8	-0.4	0.85	24	0.29	0.24	15	0.09
		0.6	*0.1*		*0.9*	*0.01*		*0.5*	*0.1*		*26*	*0.78*		*18*	*0.20*
43	0.49	8.2	-0.7	0.01	-1.3	0.00	0.51	8.7	-0.7	0.20	17	0.22	0.00	-1.1	-0.01
		2.6	*0.4*		*1.0*	*0.01*		*2.9*	*0.5*		*6.1*	*0.18*		*0.0*	*0.00*
44	0.03	2.6	-0.2	0.01	1.9	-0.02	0.02	2.6	-0.2	0.16	7.8	0.33	0.03	0.2	0.01
		0.2	*0.0*		*0.9*	*0.01*		*0.1*	*0.0*		*5.0*	*0.15*		*2.5*	*0.03*
45	1.52	15	1.9	0.65	67	0.88	0.91	12	1.0	0.04	13	-0.28	0.03	18	-0.07
		8.2	*1.4*		*70*	*0.61*		*5.1*	*0.8*		*1.3*	*0.04*		*2.2*	*0.02*
46	3.93	16	4.1	0.14	40	1.64	4.09	15	2.4	1.40	44	5.46	0.15	75	2.03
		21	*3.6*		*15*	*0.13*		*23*	*3.7*		*43*	*1.29*		*11*	*0.13*
47	0.41	-0.8	-0.1	0.01	2.6	-0.05	0.39	-0.9	-0.1	0.01	-0.5	0.17	0.06	-8.2	0.69
		2.2	*0.4*		*1.5*	*0.01*		*2.2*	*0.4*		*0.4*	*0.01*		*4.5*	*0.05*
48	1.15	13	0.1	0.08	11	0.36	1.23	13	-0.3	0.63	43	2.47	0.57	116	1.25
		6.2	*1.1*		*8.7*	*0.07*		*7.0*	*1.1*		*19*	*0.58*		*44*	*0.49*
49	0.06	-0.2	0.0	0.00	1.7	0.04	0.06	-0.3	-0.1	0.31	27	1.20	0.14	-9.8	0.17
		0.3	*0.1*		*0.4*	*0.00*		*0.3*	*0.1*		*9.4*	*0.28*		*10*	*0.11*
50	0.12	24	15.7	0.39	-4.9	0.72	0.52	25	15.0	0.37	-2.8	1.61	-	-	-
		0.6	*0.1*		*42*	*0.36*		*2.9*	*0.5*		*11*	*0.34*		-	-
51	2.57	45	2.0	0.00	2.1	0.01	2.60	47	2.0	0.03	43	0.06	0.05	7.2	0.50
		14	*2.3*		*0.0*	*0.00*		*15*	*2.4*		*0.8*	*0.02*		*4.2*	*0.05*
52	0.64	6.2	1.7	0.01	-1.5	-0.01	0.65	6.6	1.7	-	-	-	0.04	7.6	0.06
		3.4	*0.6*		*1.3*	*0.01*		*3.7*	*0.6*		-	-		*3.0*	*0.03*
53	1.78	14	1.2	0.01	87	0.04	1.83	10	1.1	0.28	20	-0.28	0.01	1.5	0.01
		9.5	*1.6*		*0.6*	*0.00*		*10*	*1.7*		*8.4*	*0.25*		*0.5*	*0.01*
54	0.03	1.7	0.0	0.00	-1.7	0.02	0.04	1.8	0.0	2.16	71	2.91	0.00	-9.5	0.03
		0.2	*0.0*		*0.5*	*0.00*		*0.2*	*0.0*		*66*	*1.99*		*0.3*	*0.00*
55	0.63	-2.8	0.3	0.01	-4.5	0.01	0.62	-2.7	0.3	-	-	-	0.02	-0.2	-0.04
		3.4	*0.6*		*1.1*	*0.01*		*3.5*	*0.6*		-	-		*1.4*	*0.01*
56	-	-	-	-	-	-	-	-	-	2.20	83	1.91	0.10	21	0.17
		-	-		-	-		-	-		*68*	*2.03*		*7.4*	*0.08*
57	0.60	-1.2	-0.1	0.02	-9.4	-0.07	0.55	-0.9	0.0	0.28	27	1.00	0.06	-3.1	-0.01
		3.2	*0.5*		*1.8*	*0.02*		*3.1*	*0.5*		*8.7*	*0.26*		*4.5*	*0.05*

Table IV.2 (continued)

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
58	0.67	18	0.3	-	-	-	-	-	-	0.19	12	0.57	0.02	-4.6	0.04
		3.6	*0.6*		-	-		-	-		*5.8*	*0.17*		*1.4*	*0.02*
59	0.29	-0.7	0.5	0.01	-6.0	0.01	0.30	-0.4	0.5	-	-	-	0.00	1.1	-0.05
		1.5	*0.3*		*0.8*	*0.01*		*1.7*	*0.3*		-	-		*0.0*	*0.00*
60	0.18	2.7	0.3	0.18	71	-0.21	0.05	-0.8	0.4	0.06	11	-0.08	0.00	2.3	0.03
		1.0	*0.2*		*20*	*0.17*		*0.3*	*0.0*		*1.8*	*0.06*		*0.0*	*0.00*
61	-	-	-	0.07	-4.7	-0.09	-	-	-	0.60	47	-0.27	-	-	-
		-	-		*7.6*	*0.07*		-	-		*18*	*0.55*		-	-
62	0.04	2.1	0.0	0.01	-2.8	0.02	0.03	2.3	0.0	-	-	-	0.03	5.1	0.03
		0.2	*0.0*		*0.6*	*0.01*		*0.2*	*0.0*		-	-		*2.0*	*0.02*
63	1.16	-1.7	1.2	0.01	16	0.02	1.20	-2.8	1.1	1.22	24	1.69	0.00	-2.2	0.05
		6.2	*1.1*		*0.7*	*0.01*		*6.8*	*1.1*		*37*	*1.12*		*0.3*	*0.00*
64	0.44	19	0.5	0.00	17	0.03	0.45	21	0.4	0.03	27	0.35	0.02	11	0.01
		2.3	*0.4*		*0.5*	*0.00*		*2.6*	*0.4*		*0.9*	*0.03*		*1.8*	*0.02*
65	0.07	0.3	0.5	0.00	-2.6	0.03	0.07	0.4	0.5	0.14	17	0.16	0.01	-0.3	0.01
		0.4	*0.1*		*0.2*	*0.00*		*0.4*	*0.1*		*4.3*	*0.13*		*0.9*	*0.01*
66	0.43	14	-0.1	0.10	22	0.17	0.33	14	-0.3	-	-	-	0.09	26	0.04
		2.3	*0.4*		*11*	*0.10*		*1.8*	*0.3*		-	-		*6.9*	*0.08*
67	0.03	2.6	-0.4	0.06	-5.1	-0.03	0.04	3.0	-0.3	0.01	15	-0.27	0.01	1.9	-0.08
		0.2	*0.0*		*6.9*	*0.06*		*0.2*	*0.0*		*0.3*	*0.01*		*0.7*	*0.01*
68	0.03	-1.7	0.0	0.05	25	0.07	0.01	-0.5	-0.1	0.17	-3.7	0.69	0.03	19	0.01
		0.2	*0.0*		*5.7*	*0.05*		*0.0*	*0.0*		*5.2*	*0.15*		*2.1*	*0.02*
69	0.24	-2.5	0.2	0.01	1.9	0.02	0.21	-2.8	0.2	0.83	56	1.36	0.06	-9.3	0.02
		1.3	*0.2*		*0.9*	*0.01*		*1.2*	*0.2*		*26*	*0.77*		*4.8*	*0.05*
70	0.41	1.2	0.3	0.02	-7.0	0.03	0.38	1.7	0.2	-	-	-	0.04	-5.7	0.03
		2.2	*0.4*		*1.9*	*0.02*		*2.2*	*0.3*		-	-		*3.4*	*0.04*
71	0.10	-0.2	0.1	0.00	-5.1	0.03	0.10	0.0	0.0	-	-	-	0.06	12	0.13
		0.6	*0.1*		*0.5*	*0.00*		*0.5*	*0.1*		-	-		*4.2*	*0.05*
72	0.17	-0.8	-0.2	0.01	2.3	-0.01	0.16	-1.0	-0.2	0.16	-9.0	-0.04	0.00	2.3	-0.05
		0.9	*0.2*		*0.8*	*0.01*		*0.9*	*0.1*		*5.0*	*0.15*		*0.3*	*0.00*
73	0.08	-1.6	0.0	0.05	-2.1	0.04	0.11	-1.4	0.0	0.12	-7.5	-0.24	0.00	0.5	-0.02
		0.4	*0.1*		*5.1*	*0.04*		*0.6*	*0.1*		*3.6*	*0.11*		*0.2*	*0.00*
74	0.56	-5.0	0.4	0.01	-0.4	0.03	0.59	-5.0	0.3	0.17	2.8	0.06	0.01	3.1	-0.01
		3.0	*0.5*		*0.8*	*0.01*		*3.3*	*0.5*		*5.3*	*0.16*		*1.0*	*0.01*
75	0.32	-5.7	0.4	0.00	3.8	-0.01	0.30	-6.1	0.3	-	-	-	0.00	-2.3	-0.01
		1.7	*0.3*		*0.5*	*0.00*		*1.7*	*0.3*		-	-		*0.3*	*0.00*
76	0.39	9.5	0.2	0.06	10	-0.12	0.33	9.5	0.3	0.62	27	0.76	0.05	-4.4	0.04
		2.1	*0.4*		*6.7*	*0.06*		*1.9*	*0.3*		*19*	*0.57*		*3.7*	*0.04*

Table IV.2 (continued)

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
77	0.25	6.9	-0.1	0.03	12	0.11	0.22	6.7	-0.2	0.05	92	7.05	0.02	-6.8	0.00
		1.3	*0.2*		*3.6*	*0.03*		*1.2*	*0.2*		*1.5*	*0.04*		*1.7*	*0.02*
78	-	-	-	0.10	17	0.08	0.38	49	2.1	-	-	-	0.09	23	0.38
		-	-		*11*	*0.09*		*2.2*	*0.3*		-	-		*7.1*	*0.08*
79	0.45	-9.8	-0.1	0.00	-2.6	0.00	0.46	10	-0.1	1.90	51	-0.04	0.03	-6.5	-0.01
		2.4	*0.4*		*0.2*	*0.00*		*2.6*	*0.4*		*58*	*1.76*		*2.7*	*0.03*
80	1.01	91	0.5	0.04	34	-0.03	1.09	94	0.5	-	-	-	0.02	-4.5	0.05
		5.4	*0.9*		*4.7*	*0.04*		*6.1*	*1.0*		-	-		*1.9*	*0.02*
81	-	-	-	-	-	-	-	-	-	0.12	-1.9	1.40	0.04	101	0.22
		-	-		-	-		-	-		*3.7*	*0.11*		*3.0*	*0.03*
82	0.01	1.1	0.3	0.03	-4.3	0.00	0.01	1.4	0.3	0.74	24	0.54	0.00	-0.1	0.00
		0.0	*0.0*		*2.9*	*0.03*		*0.1*	*0.0*		*23*	*0.68*		*0.3*	*0.00*
83	0.03	21	0.4	0.04	-8.5	0.02	0.08	22	0.3	0.12	22	-0.33	0.02	13	0.09
		0.2	*0.0*		*4.9*	*0.04*		*0.5*	*0.1*		*3.8*	*0.11*		*1.7*	*0.02*
84	0.46	-7.4	0.2	0.01	-1.5	-0.03	0.49	-7.9	0.3	0.01	-0.3	-0.11	0.02	-1.7	-0.03
		2.5	*0.4*		*1.3*	*0.01*		*2.8*	*0.4*		*0.3*	*0.01*		*1.8*	*0.02*
85	0.20	0.1	0.1	0.00	-1.1	0.00	0.19	0.1	0.1	0.05	11	-0.26	0.03	-4.3	0.01
		1.1	*0.2*		*0.1*	*0.00*		*1.1*	*0.2*		*1.7*	*0.05*		*2.6*	*0.03*
86	0.92	-2.1	0.2	0.02	2.3	0.37	0.94	-2.3	-0.2	0.53	53	0.56	0.00	-4.0	0.22
		4.9	*0.8*		*2.4*	*0.02*		*5.3*	*0.9*		*16*	*0.49*		*0.1*	*0.00*
87	0.09	14	0.1	0.01	-1.5	-0.04	0.11	14	0.2	-	-	-	0.03	-3.1	0.03
		0.5	*0.1*		*1.3*	*0.01*		*0.6*	*0.1*		-	-		*2.6*	*0.03*
88	0.40	4.2	0.3	0.00	-1.9	0.04	0.41	4.4	0.2	0.29	-5.7	0.66	0.44	32	1.78
		2.2	*0.4*		*0.4*	*0.00*		*2.3*	*0.4*		*9.0*	*0.27*		*34*	*0.37*
89	0.10	-2.6	0.1	0.00	-1.1	0.00	0.13	-2.6	0.1	0.06	-0.8	-0.08	0.02	-3.3	0.01
		0.5	*0.1*		*0.1*	*0.00*		*0.7*	*0.1*		*1.8*	*0.05*		*1.6*	*0.02*
90	0.19	2.7	-0.2	0.00	-5.1	0.01	0.20	3.1	-0.2	0.05	55	0.57	0.01	-2.6	0.01
		1.0	*0.2*		*0.5*	*0.00*		*1.1*	*0.2*		*1.5*	*0.05*		*0.8*	*0.01*
91	0.12	2.5	0.1	0.01	5.1	0.04	0.13	2.3	0.0	0.35	35	-0.20	0.12	21	0.09
		0.7	*0.1*		*0.8*	*0.01*		*0.7*	*0.1*		*11*	*0.33*		*9.3*	*0.10*
92	0.03	5.4	-0.3	0.03	16	0.26	0.15	5.5	-0.4	0.00	53	0.20	0.00	0.5	-0.02
		0.2	*0.0*		*3.7*	*0.03*		*0.8*	*0.1*		*0.1*	*0.00*		*0.2*	*0.00*
93	0.10	1.4	0.1	0.00	0.6	0.01	0.10	1.5	0.0	1.64	87	1.03	0.03	-1.6	-0.01
		0.5	*0.1*		*0.3*	*0.00*		*0.6*	*0.1*		*50*	*1.51*		*2.1*	*0.02*
94	0.22	-7.8	0.3	0.00	-3.7	0.01	0.22	-8.0	0.3	0.07	11	0.36	0.00	-6.1	0.05
		1.2	*0.2*		*0.0*	*0.00*		*1.2*	*0.2*		*2.2*	*0.07*		*0.3*	*0.00*
95	1.51	16	-1.0	0.02	-7.9	0.01	1.53	17	-1.0	0.13	22	0.16	0.04	-1.4	0.05
		8.1	*1.4*		*2.1*	*0.02*		*8.6*	*1.4*		*4.0*	*0.12*		*2.8*	*0.03*

Table IV.2 (continued)

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
96	0.33	7.0	-0.2	0.00	10	0.05	0.33	6.8	-0.3	0.17	54	-0.05	0.00	-2.3	-0.04
		1.8	*0.3*		*0.1*	*0.00*		*1.9*	*0.3*		*5.4*	*0.16*		*0.3*	*0.00*
97	0.15	4.0	-0.1	0.12	86	-0.11	0.02	-0.2	-0.1	0.06	27	0.43	0.00	1.7	-0.03
		0.8	*0.1*		*13*	*0.11*		*0.1*	*0.0*		*1.8*	*0.05*		*0.1*	*0.00*
98	0.10	4.8	-0.2	0.03	7.0	0.00	0.06	4.7	-0.2	0.31	-9.0	0.33	0.00	2.8	0.03
		0.5	*0.1*		*3.8*	*0.03*		*0.4*	*0.1*		*9.6*	*0.29*		*0.2*	*0.00*
99	0.24	-2.3	0.1	0.00	-1.9	-0.01	0.23	-2.3	0.1	0.16	14	0.17	0.06	3.7	0.00
		1.3	*0.2*		*0.4*	*0.00*		*1.3*	*0.2*		*4.9*	*0.15*		*4.4*	*0.05*
100	0.05	2.9	-0.1	0.05	0.6	0.03	0.10	3.0	-0.1	0.23	57	0.23	0.04	2.9	-0.03
		0.3	*0.0*		*5.2*	*0.04*		*0.6*	*0.1*		*7.1*	*0.21*		*3.2*	*0.04*
101	0.28	-6.2	0.2	0.03	2.1	0.07	0.27	-6.7	0.2	0.67	-1.1	1.08	0.04	-0.2	-0.04
		1.5	*0.3*		*3.6*	*0.03*		*1.5*	*0.2*		*21*	*0.62*		*3.0*	*0.03*
102	0.17	-8.7	0.0	0.01	2.2	-0.01	0.17	-9.2	0.0	0.54	-6.9	0.73	0.04	-3.4	0.11
		0.9	*0.2*		*1.0*	*0.01*		*1.0*	*0.2*		*17*	*0.50*		*2.8*	*0.03*
103	0.51	3.2	-0.3	0.01	4.3	0.01	0.52	3.1	-0.4	-	-	-	0.03	-6.1	0.05
		2.7	*0.5*		*1.0*	*0.01*		*2.9*	*0.5*		-	-		*2.2*	*0.02*
104	3.16	77	2.5	0.01	2.1	-0.01	3.22	81	2.4	0.15	17	0.01	0.00	-7.1	-0.01
		17	*2.9*		*1.0*	*0.01*		*18*	*2.9*		*4.5*	*0.14*		*0.1*	*0.00*
105	0.19	2.7	0.1	0.02	2.3	0.17	0.16	2.7	-0.1	0.18	10	0.10	0.01	16	-0.02
		1.0	*0.2*		*2.4*	*0.02*		*0.9*	*0.1*		*5.4*	*0.16*		*0.4*	*0.00*
106	0.12	6.2	-0.5	0.27	72	0.28	0.11	2.7	-0.8	0.32	28	0.28	0.09	-4.0	-0.07
		0.7	*0.1*		*29*	*0.26*		*0.6*	*0.1*		*9.9*	*0.30*		*7.1*	*0.08*
107	0.27	0.3	0.3	0.02	3.2	0.02	0.25	0.2	0.2	0.12	15	-0.25	0.01	-1.4	0.06
		1.5	*0.2*		*2.1*	*0.02*		*1.4*	*0.2*		*3.8*	*0.11*		*1.1*	*0.01*
108	-	-	-	-	-	-	-	-	-	0.81	12	0.92	0.04	15	-0.07
		-	-		-	-		-	-		*25*	*0.74*		*2.9*	*0.03*
109	0.10	3.2	0.0	0.02	5.8	0.01	0.12	3.1	0.0	0.19	6.7	0.31	0.02	1.4	-0.02
		0.5	*0.1*		*1.7*	*0.01*		*0.7*	*0.1*		*6.0*	*0.18*		*1.2*	*0.01*
110	0.00	-0.5	0.2	0.01	26	0.05	0.00	-1.9	0.2	0.10	52	0.10	0.03	-5.5	0.02
		0.0	*0.0*		*1.2*	*0.01*		*0.0*	*0.0*		*3.0*	*0.09*		*2.3*	*0.03*
111	0.08	-3.3	0.2	0.03	-1.6	0.00	0.11	-3.4	0.2	0.18	1.8	-0.11	0.04	5.8	-0.03
		0.4	*0.1*		*3.5*	*0.03*		*0.6*	*0.1*		*5.7*	*0.17*		*2.9*	*0.03*
112	0.19	-2.1	-0.7	0.00	15	-0.16	0.17	-3.2	-0.5	0.63	23	-0.30	0.22	18	-0.12
		1.0	*0.2*		*0.5*	*0.00*		*1.0*	*0.2*		*19*	*0.58*		*17*	*0.18*
113	11.26	32	14.8	0.03	9.2	0.01	11.21	34	14.8	0.08	56	0.98	-	-	-
		60	*10.3*		*2.8*	*0.02*		*63*	*10.2*		*2.3*	*0.07*		-	-
114	-	-	-	-	-	-	-	-	-	0.29	31	0.72	0.03	23	0.43
		-	-		-	-		-	-		*8.9*	*0.27*		*2.3*	*0.02*

Table IV.2 (continued)

Regression analysis data for individual laboratories.
s.d. = standard deviation (µmol/l) ; prop. = proportional error (%) ;
const. = constant error (µmol/l) ; *se* = standard error).

Lab No.	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.	s.d.	prop.	const.
		se	*se*		*se*	*se*		*se*	*se*		*se*	*se*		*se*	*se*
115	0.04	4.2	-0.2	-	-	-	-	-	-	0.08	-1.3	0.14	0.01	-0.9	0.03
		0.2	*0.0*		-	-		-	-		*2.3*	*0.07*		*1.0*	*0.01*
116	0.12	1.2	-0.1	0.00	0.2	0.00	0.12	1.2	-0.1	0.00	2.3	1.19	0.02	-3.2	-0.01
		0.6	*0.1*		*0.0*	*0.00*		*0.7*	*0.1*		*0.0*	*0.00*		*1.3*	*0.01*
117	0.01	0.9	-0.1	0.01	11	0.00	0.02	0.4	-0.1	0.17	10	0.03	0.03	-2.2	0.03
		0.0	*0.0*		*1.0*	*0.01*		*0.1*	*0.0*		*5.2*	*0.16*		*2.0*	*0.02*
118	0.14	0.6	-0.1	0.02	0.9	-0.06	0.12	0.6	-0.1	0.07	8.0	1.27	0.02	0.5	0.04
		0.7	*0.1*		*2.2*	*0.02*		*0.7*	*0.1*		*2.1*	*0.06*		*1.5*	*0.02*
119	0.39	-2.2	-1.0	0.02	2.2	0.04	0.37	-2.6	-1.1	-	-	-	-	-	-
		2.1	*0.4*		*2.0*	*0.02*		*2.1*	*0.3*		-	-		-	-
120	0.22	2.3	0.1	-	-	-	-	-	-	-	-	-	0.01	-1.5	-0.03
		1.2	*0.2*		-	-		-	-		-	-		*0.5*	*0.01*
121	0.02	0.2	-0.7	0.07	15	-0.01	0.07	0.5	-0.6	-	-	-	0.08	-0.3	-0.07
		0.1	*0.0*		*7.8*	*0.07*		*0.4*	*0.1*		-	-		*6.3*	*0.07*
122	2.63	20	4.4	0.00	-5.1	0.07	2.73	22	4.5	0.15	7.1	1.46	0.06	18	0.22
		14	*2.4*		*0.5*	*0.00*		*15*	*2.5*		*4.5*	*0.13*		*4.9*	*0.05*
123	0.22	-3.2	0.3	0.01	-0.4	0.02	0.21	-3.3	0.3	0.03	2.2	-0.06	0.05	-3.4	0.10
		1.2	*0.2*		*0.8*	*0.01*		*1.2*	*0.2*		*1.0*	*0.03*		*3.8*	*0.04*
124	0.30	11	0.5	0.01	0.0	0.00	0.29	12	0.5	-	-	-	0.03	86	0.13
		1.6	*0.3*		*1.0*	*0.01*		*1.6*	*0.3*		-	-		*2.3*	*0.03*
125	0.41	-0.3	0.4	0.11	33	1.32	0.32	1.4	-0.9	-	-	-	1.08	73	4.01
		2.2	*0.4*		*12*	*0.11*		*1.8*	*0.3*		-	-		*82*	*0.91*
126	0.04	1.1	0.0	0.00	-3.7	0.02	0.03	1.3	0.0	0.45	6.4	0.18	0.02	-6.9	0.19
		0.2	*0.0*		*0.5*	*0.00*		*0.2*	*0.0*		*14*	*0.41*		*1.5*	*0.02*
127	0.44	-4.0	0.2	0.01	7.5	0.03	0.43	-4.6	0.2	0.11	-5.5	0.25	0.05	-4.9	-0.07
		2.3	*0.4*		*0.9*	*0.01*		*2.4*	*0.4*		*3.3*	*0.10*		*4.1*	*0.05*
128	0.80	-7.0	0.6	0.01	1.1	0.02	0.79	-7.4	0.5	0.09	1.9	0.07	0.02	-6.2	0.03
		4.3	*0.7*		*1.1*	*0.01*		*4.5*	*0.7*		*2.7*	*0.08*		*1.7*	*0.02*
129	0.42	-1.1	0.3	0.00	0.4	0.07	0.41	-1.3	0.2	0.03	0.4	-0.16	0.00	-3.4	0.02
		2.3	*0.4*		*0.5*	*0.00*		*2.3*	*0.4*		*0.8*	*0.02*		*0.1*	*0.00*
130	0.11	0.7	0.3	0.00	3.0	0.09	0.11	0.6	0.2	0.01	-1.6	0.03	0.01	-0.3	0.05
		0.6	*0.1*		*0.2*	*0.00*		*0.6*	*0.1*		*0.3*	*0.01*		*0.8*	*0.01*
131	0.81	0.3	1.0	0.01	1.9	0.01	0.07	6.2	-0.3	0.10	20	-0.12	0.03	19	0.02
		4.3	*0.7*		*0.9*	*0.01*		*0.4*	*0.1*		*3.0*	*0.09*		*2.6*	*0.03*
132	0.04	0.4	-0.1	0.02	-3.2	0.05	0.07	0.6	-0.2	0.29	-1.6	-0.38	0.01	-3.8	0.01
		0.2	*0.0*		*2.4*	*0.02*		*0.4*	*0.1*		*8.9*	*0.27*		*0.5*	*0.01*

ANNEX V

Consensus data determination

Variation of the mean versus the number of successive rejection tests at the 95 % confidence level.
Arrows indicate retained means.