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REPORT ON THE RESULTS OF THE FIFTH ICES INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEA WATER

by

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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 analyses before using them for their intended purpose, and 'Quality Assurance' is a 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 greatly improved instrumentation and facilities, problems still can and do occur. The key to solving problems is first to recognize 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 sea water should be undertaken in 1989/1990 and that consideration should be given to conducting 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 in the exercise were issued by the ICES General Secretary to all ICES Member Countries, to the Oslo and Paris Commissions, and to the Baltic Marine Environment Protection Commission (Helsinki Commission).

The 1989/1990 exercise was conducted on behalf of the MCWG by Don Kirkwood (MAFF, Lowestoft), Alain Aminot (IFREMER, Brest) and Matti Perttilä (FIMR, Helsinki), and the report on the results 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 sea water, and particularly nutrients, had been the central theme.

Participation in NUTS I/C 4 did not confine itself to ICES Member Countries; in the short time available for publicizing 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 intercomparison exercises carried out to date can be found in Annex 1 (which also contains all the information, etc., that was sent to participants in the present exercise).

2 PARTICIPATION IN THE FIFTH ICES INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEA WATER

The year 1990 marked the twenty-fifth anniversary of the first of this series of ICES intercomparison exercises, and the above-mentioned summary in Annex 1, entitled 'ICES Nutrients I/Cs—The First 25 Years', was widely circulated amongst ICES Member Countries and beyond soon after the completion of NUTS I/C 4, in an effort to ensure that the next exercise, the Fifth ICES Intercomparison Exercise for Nutrients in Sea Water (NUTS I/C 5), would include as many laboratories as possible. Participating laboratories did not have to be in ICES Member Countries; it was sufficient that they were involved in the determination of nutrients in sea water and had an interest in participating in an exercise that was to be organized on similar lines to NUTS I/C 4.

Plans were being made in 1992 to launch the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) programme. QUASIMEME 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, 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 to join the QUASIMEME project, and this resulted in a last-minute influx of additional participants just before the distribution of samples at the end of November 1992.

A total of 142 sets of samples was distributed to laboratories 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 NUTS I/C 5 approximately twice the size of NUTS I/C 4, which was the target the organizers had hoped to achieve.

Annex 2 lists the participating laboratories in alphabetical order by country, then by geographical location (north to south) within each country. QUASIMEME participants are indicated with a 'Q', and NUTS I/C 4 participants are noted by their NUTS I/C 4 laboratory number.

3 THE FORMAT OF NUTS I/C 5

Several significant changes have been introduced since NUTS I/C 4. These are indicated in the sub-sections below.

3.1 Analytical Requirements

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

Many laboratories supplied what was requested, 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 NUTS I/C 4 for intra-laboratory variance are underestimates, biased by the effects of a variety of misinterpretations of the term 'replicate'.

In NUTS I/C 5, this issue has been avoided and we simply asked "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 NUTS 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 NUTS I/C 5 (fully described in Annex 3) imposed constraints which resulted in there being only two relevant determinands per sample (nitrate and nitrite in one series; 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 of samples.

3.3 Selection of Determinands

The determinands of primary interest in NUTS 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 NUTS I/C 5 the intention was to increase the number of nutrients covered and special efforts were made to include nitrite and ammonia, both reputed to present preservation problems due to their ease of oxidation to nitrate. After some preliminary experiments (including autoclaving) at IFREMER yielded satisfactory results, it was decided to introduce nitrite and ammonia in NUTS 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 concentrations. As all samples were distributed in glass bottles, the determination of silicate has thereby been excluded from NUTS I/C 5.

3.4 Analytical Methodology

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

The review was included in the NUTS 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 NUTS I/C 4 report were

urged to examine their methods closely to assess how well they adhered to the conditions specified by the parent manual method.

3.5 Anonymity/Openness

Openness is one aspect that has definitely not changed since NUTS 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 NUTS I/C 4 and NUTS I/C 5, prospective participants were informed that the reports on the results of these exercises would be published on this basis, and we remain convinced that our insistence on complete openness has had a positive influence on the improvement of quality control procedures in general.

4 THE SAMPLES

In NUTS 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 there was no access to additional seawater sources of demonstrated stability covering a useful concentration range, 'slightly artificial' samples were used that at least started life as natural sea water.

4.1 Preparation

Annex 3 contains details of the methods used for the preparation and control of the samples, derived from those employed in NUTS I/C 4. These methods are summarized briefly below.

A large volume of natural sea water, low in nutrients, was spiked with known concentrations of nutrient salts. A large number of bottles were filled, capped, then sterilized 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 3).

4.2 Concentrations

From the participants' point of view, at the time of analysis the samples were uncompromised reference materials. From a knowledge of the method of sample preparation ***assigned values*** were attributed for the concentrations of the nutrients (see Table 4.2).

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

5 RESPONSE

Samples were sent to the 142 laboratories which had confirmed their intention to participate. These laboratories undertook to submit results or return the samples intact before the deadline. Samples were returned by five laboratories, and results were submitted by 132 laboratories; consequently, there were five defaulters. Table 5.1 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 other determinands that were analysed on a routine basis in their laboratories. On this basis, eight laboratories submitted results for total N, amd six laboratories submitted results for total P.

Table 4.2. Assigned concentrations of the nutrients in the intercomparison samples.

Sample	Level	Nitrate µmol l^{-1}	Nitrite µmol l^{-1}	Ammonia µmol l^{-1}	Phosphate µmol l^{-1}
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

Table 5.1. Summary of responses 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 Section 6.1, below.
(3) One additional result computed by summing separate 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 NUTS 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 % confidence level to remove outliers and to isolate a population approximating a normal distribution, then to characterize the performance of this homogeneous group in terms of mean and standard deviation. The test was applied until a stable mean was reached, then assuming a normal distribution (see Annex 5).

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 'normalized' 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:

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

It follows that the overall Z-score for a laboratory is given by the combined Z-scores according to:

$$Z_n = (\sum Z_i)/n$$

where 'i' refers to individual determinands and 'n' to the number of determinands.

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

$$Z_3 = \frac{Z_{NO_3} + Z_{NO_2} + Z_{PO_4}}{3}$$

and

$$Z_4 = \frac{Z_{NO_3} + Z_{NO_2} + Z_{PO_4} + Z_{NH_4}}{4}.$$

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 calculations, 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 referred 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 misdefinition 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 of 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 degrees 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 4, Table A4.1. The distributions of the original full sets are shown in Figures 7.1.1 to 7.1.5. It should be noted that concentrations submitted as '< x' are plotted as 'x'.

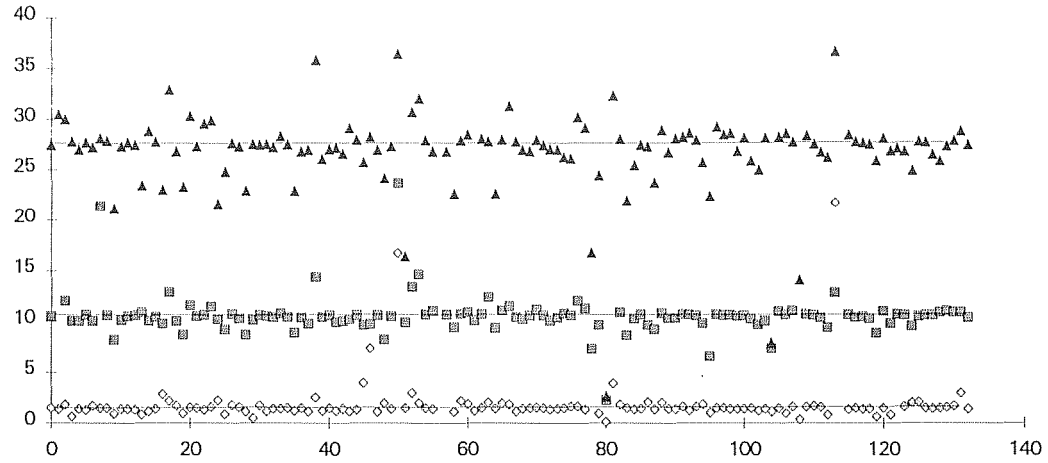


Figure 7.1.1. Nitrate + nitrite results: concentrations (µmol/l) *versus* laboratory number. The lines represent assigned values.

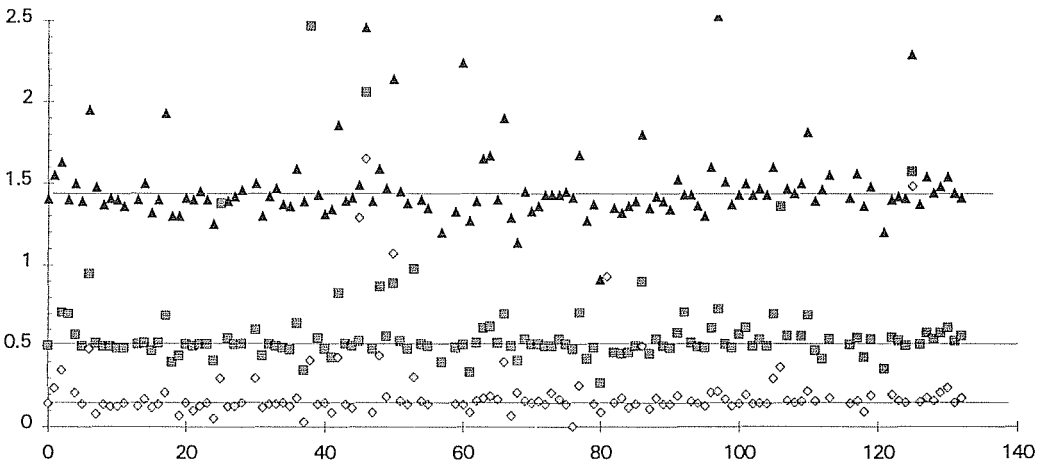


Figure 7.1.2. Nitrite results: concentrations (µmol/l) *versus* laboratory number. The lines represent assigned values.

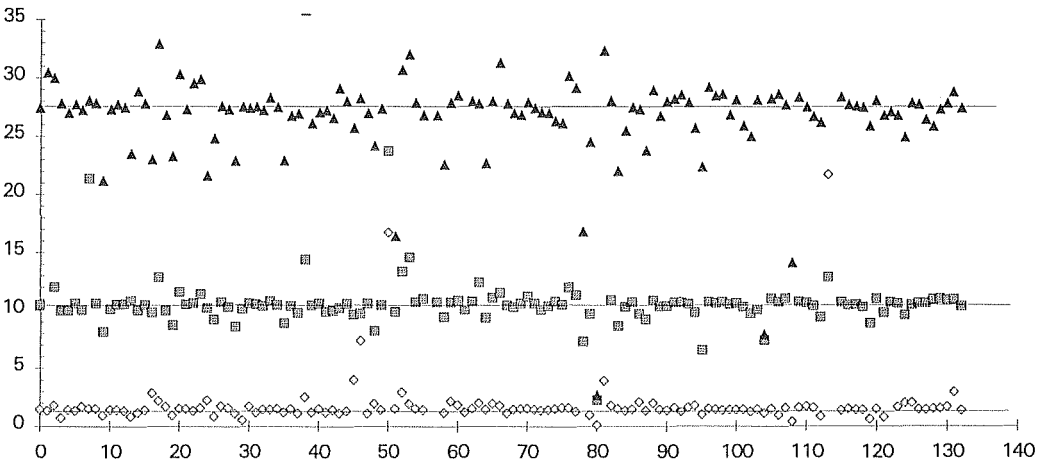


Figure 7.1.3. Nitrate results: concentrations (µmol/l) *versus* laboratory number. The lines represent assigned values.

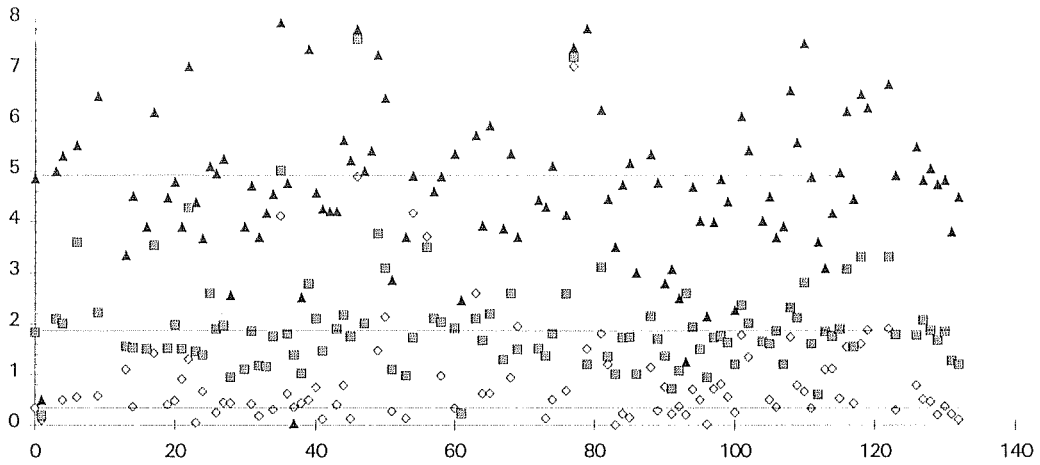


Figure 7.1.4. Ammonia results: concentrations (µmol/l) *versus* laboratory number. The lines represent assigned values.

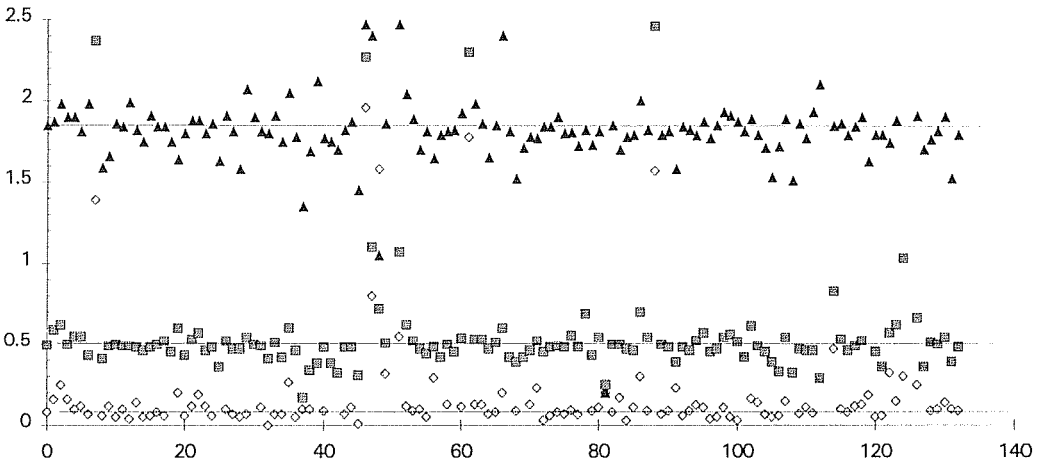


Figure 7.1.5. Phosphate results: concentrations (µmol/l) *versus* laboratory number. The lines represent assigned values.

7.2 Statistical data

Raw means and standard deviations are summarized in Table 7.2.1. Application of successive rejections at the 95 % confidence level (see Section 6.1, above, and Annex 5) leads to the isolation of sets of consistent laboratories, hence to consensus means and standard deviations for each determinand. Table 7.2.2 summarizes the consensus data in comparison with the assigned values. 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 NUTS I/C 5 results show 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, i.e., those levels which are typical of winter coastal waters with continental inputs.

The nitrate determination appears especially satisfactory. In the NUTS 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 NUTS I/C 4, gives similar results in both

intercomparisons, with improved precision at the higher concentration level in NUTS I/C 5.

Ammonia and nitrite were not present in significant concentrations in NUTS I/C 4, therefore NUTS I/C 5 is the first worldwide intercomparison exercise to include analyses of these determinands in sea water.

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, above, are reported in Table 7.3.1 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 here 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 organizers, 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.*

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 treatment applied to the distribution of these results.

Table 7.2.1. Raw means and standard deviations obtained from the full set of results (in micromoles per litre).

	Low			Medium			High		
Nutrient	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

Table 7.2.2. Consensus means and standard deviations compared with assigned concentrations.

Nutrient	**Sample number**	**Assigned** (µmol/l)	**(s.d.)** (µmol/l)	**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)

Table 7.3.1 Determinand Z-scores and combined Z-scores.

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
7	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

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 7.3.2). 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 7.3.2. Number of laboratories with determinand Z-scores and combined Z-scores less than or equal to 1.

Z-scores	Total # of labs	$\|Z\| \leq 1$	
		# of labs	% 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 laboratories 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: Lab. Nos. 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: Lab. Nos. 89, 27, 34, 85, 111, 73, 117, 26, 74, 99, 31, 72, 40, 132, 98.

7.4 Estimation of Individual Errors

As mentioned in Section 6.3, above, individual laboratory errors have been estimated by regression analysis. They are summarized in Annex 4, Table A4.2, which contains the following information:

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

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

3) 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 A4.2 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 7.4.1. 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.

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 twice 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 in Figure 7.4.2. No marked positive or negative tendency is shown in the histograms. There is some evidence for a small negative trend for both ammonia and phosphate. For ammonia, the range of proportional error is almost twice that of the other nutrients. Attention is drawn to a group of laboratories with errors around –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 %.)

Laboratories with proportional errors greater than 10 % are listed in Table 7.4.2.

While no more than 17–18 % of errors greater than 10 % is found for nitrate, nitrite, and phosphate, this percentage exceeds 40 % for 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 (Figure 7.4.3), 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 sea water concentrations are being determined.

Table 7.4.2. Laboratories with proportional errors > 10 %.

Determinand	Laboratory Number
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

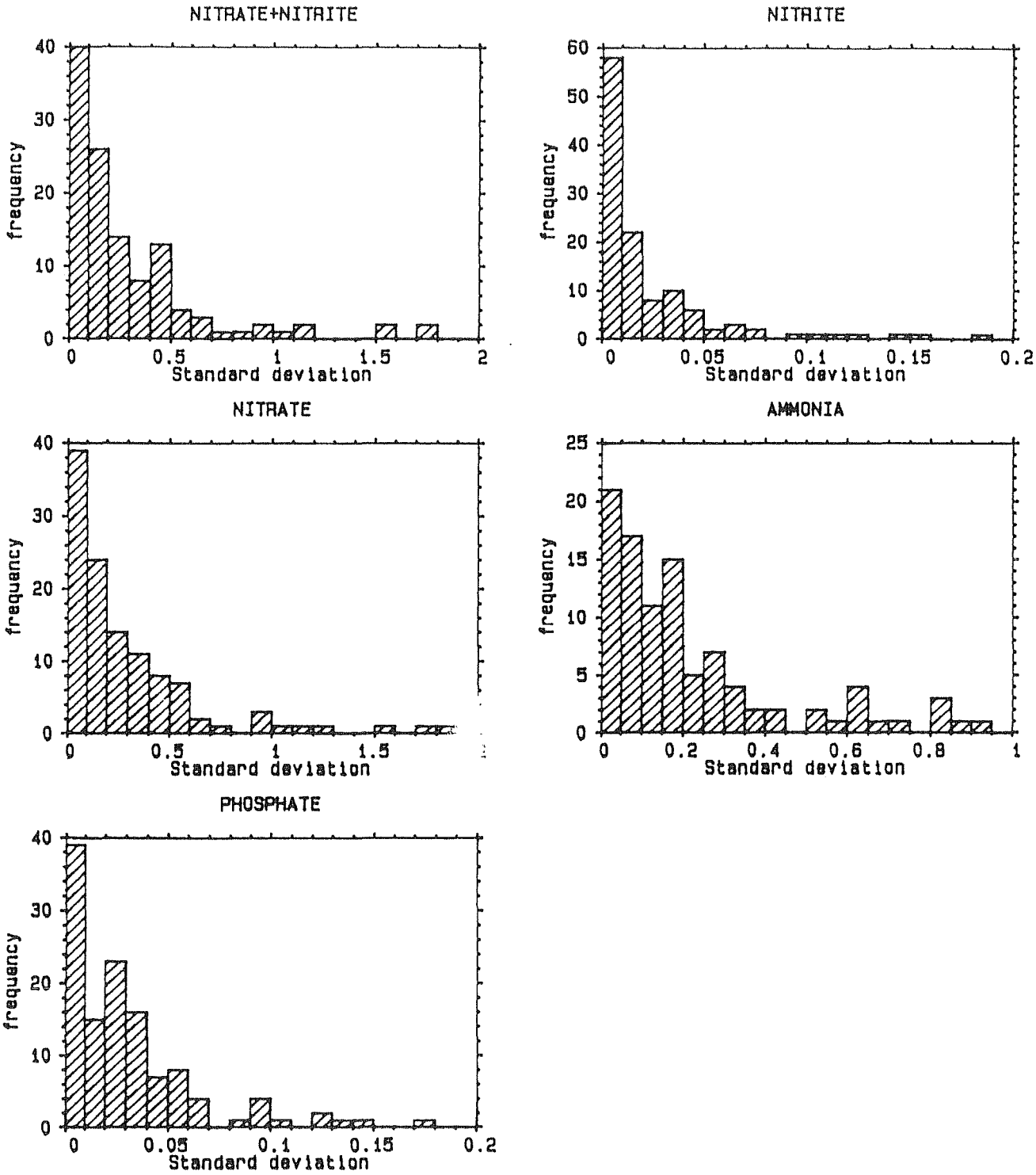


Figure 7.4.1. Frequency distribution of individual standard deviations.

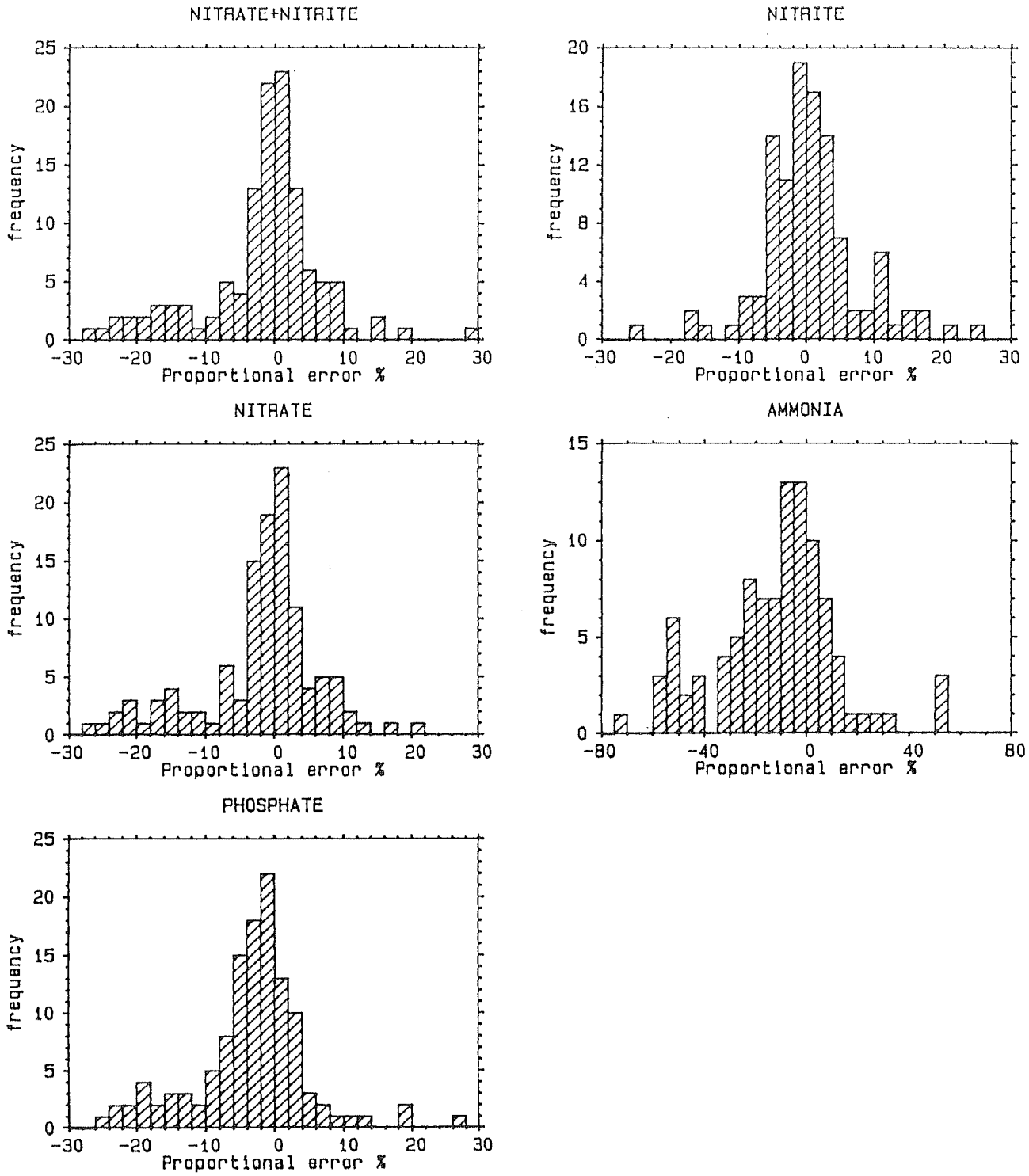


Figure 7.4.2. Frequency distribution of individual proportional errors.

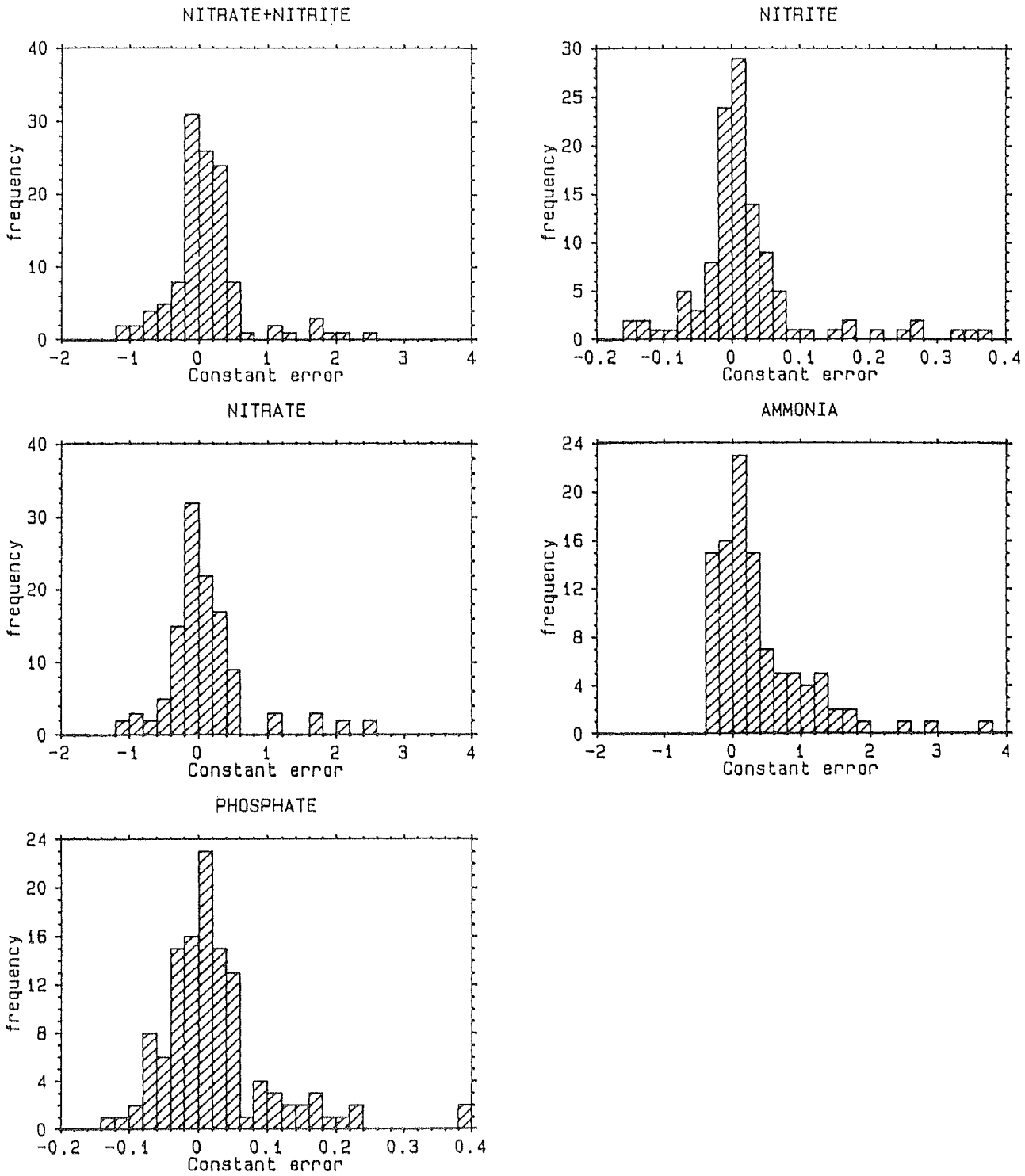


Figure 7.4.3. Frequency distribution of individual constant errors.

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.

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 7.5.1 and plotted in Figure 7.5.1. No values have been assigned to the concentrations of these determinands.

Statistics are summarized in Table 7.5.2. 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 laboratories 20 and 100 in TP (sample 4). This removal corresponds to roughtly 95 % confidence level rejection, as for the other nutrients.

Plotting laboratory resuts versus consensus means (Figure 7.5.2) shows that most of the differences betweeen laboratories are of a constant type.

Total N

Laboratory 76 exhibits all types 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, variations 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 Laboratory 20 with +6.3 µmol/l. Although minor for most participants, all these differences are attributable to blank misdefinition.

Total P

As for TN, differences in TP between laboratories are mostly attributable to constant differences (Figure 7.5.2). 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. Except for 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 at a level of approximately 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.

Table.7.5.1. 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 7.5.2. 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	-	-	-

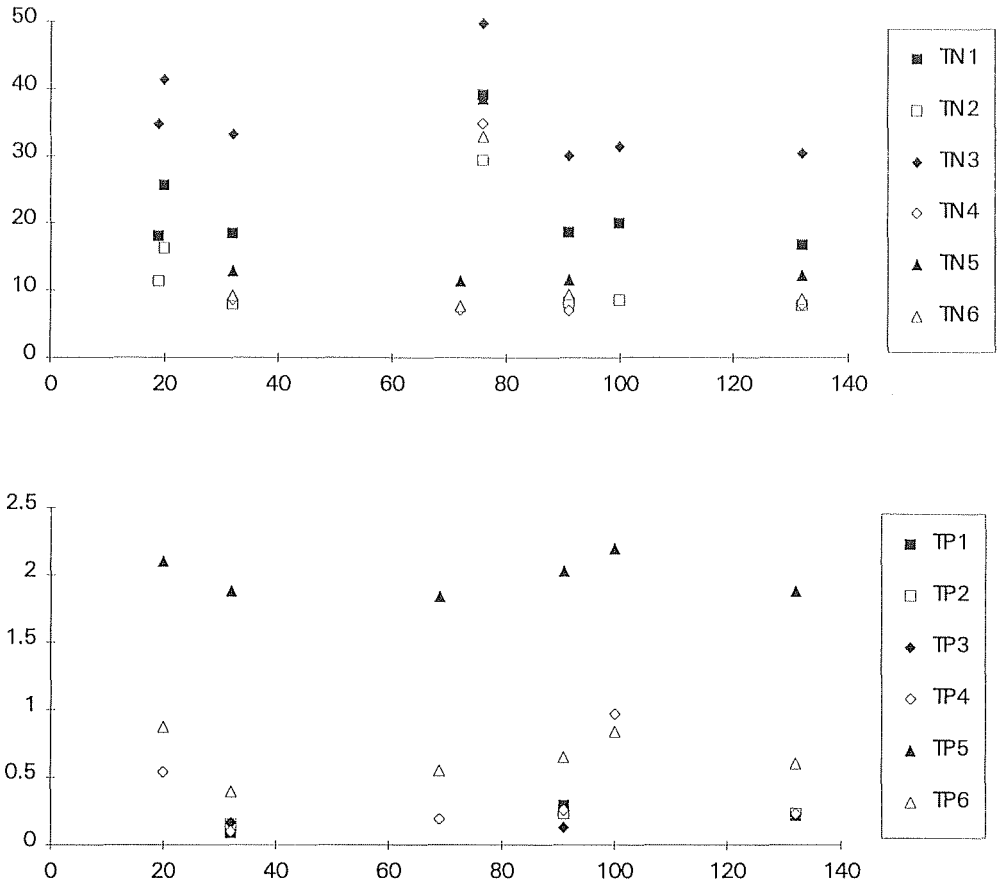


Figure 7.5.1. Total nitrogen (upper) and total phosphorus (lower) results. Concentrations (µmol/l) *versus* laboratory number.

8 SUMMARY AND DISCUSSION

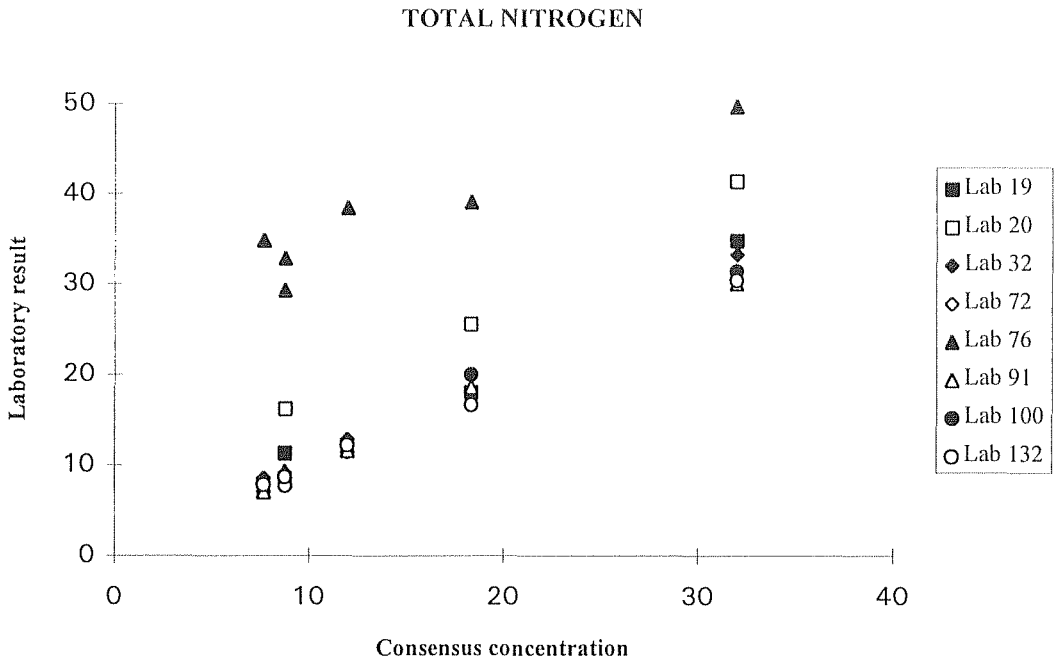
In this exercise, no attempt was made to evaluate separately the results from various analytical methods or techniques (manual and automatic) as was done in NUTS I/C 4. This section summarizes the results for each type of determinand with particular attention to specific sources of error. Where appropriate,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

8.1 Nitrate + Nitrite and Nitrate

The determination of nitrate in sea water almost invariably involves reduction by copperized 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 that in NUTS I/C 4, is in good agreement with the precision (3–4 %) obtained in previous intercomparison exercises (Koroleff and Palmork, 1972; Grasshoff, 1977). Therefore, the determination of nitrate appears to have reached a stable overall reproducibility level.



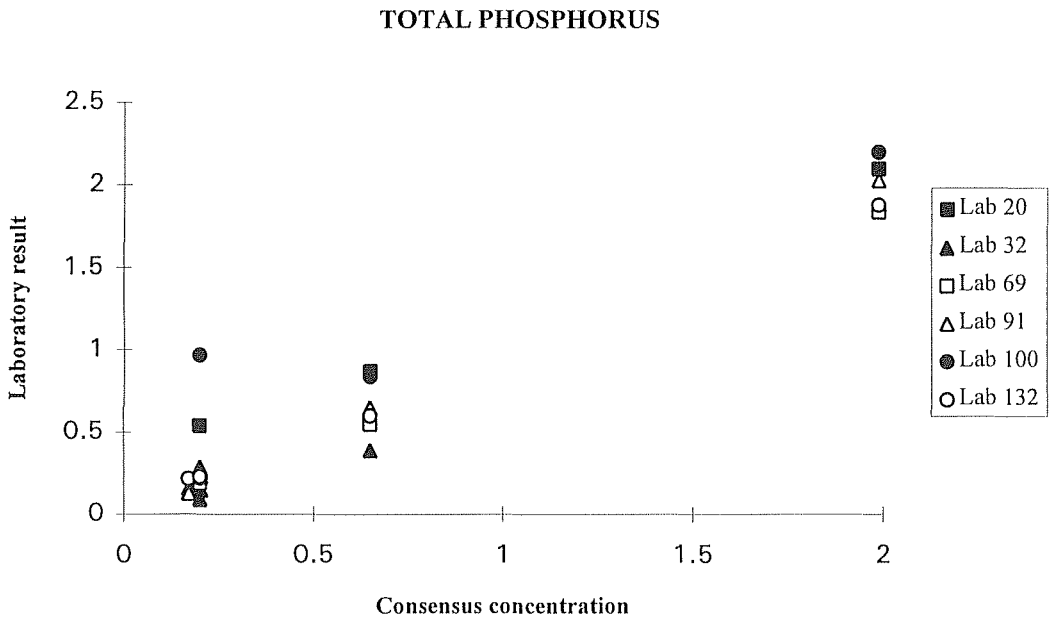


Figure 7.5.2. Plot of laboratory results against consensus concentrations (µmol/l).

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, in conjunction with 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 once per year, remembering to check the new standard solution against the old, before discarding the old standard. 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 sea water (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 demineralized 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 %, as obtained in this exercise, 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 concentrations (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). This is 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 sea water, great attention should be paid to the blank determination using high-quality demineralized 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 were 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) reported ±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 himself, 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 demineralized 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 deionizer 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 sea water 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 the optics and flowcells of the colorimeters.

Strict application of blank and standard procedures is vital for a successful ammonia determination. Every potential source of ammonia in the analytical environment, in reagents, and in '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 exercise are very similar to those of the previous exercise (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); they are less than 0.04 µmol/l for a majority of laboratories in the present exercise.

The determination of phosphate was specially addressed in NUTS 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 report on the results of that exercise (Kirkwood *et al.*, 1991).

Additional information from the individual errors estimations 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 sea water 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 acid, as was employed 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 FINAL REMARKS

Section 5 of this report 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 that their reasons for default are either inertia, or that they chose to retain the samples for their own purposes. We remind laboratories that, as participation in ICES NUTS I/C is free of charge, they are expected to comply with the rules of the game.

Inspection of the identities of laboratories listed in Section 7.4 as having serious errors, while showing a few surprises (laboratories which did well in NUTS 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 that they were unprepared for an exercise of this kind. Early correspondence with intending participants advised them strongly to read the NUTS I/C 4 report (Kirkwood *et al.*, 1991) 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 their determination is entrusted to inadequately trained staff.

The concurrence of NUTS I/C 5 and QUASIMEME produced an unforeseen advantage. The NUTS I/C 4 report (Kirkwood *et al.*, 1991) 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 the 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 QUASIMEME laboratories.

The results have been scrutinized for the four highest and four lowest values for each determinand/sample combination and the laboratories responsible for these 'extreme values' (EVs) 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 three or more EVs and although this treatment has no statistical basis, there must be some justification for describing these laboratories (8 % of the total) as the group whose performance appears to be in most need of improvement.

Applying the same criteria to the NUTS I/C 4 results produces a similar sized group (9 % of the total), amounting to six laboratories. (The same process applied to NUTS I/C 3 results would undoubtedly produce its group also, but these laboratories have not been identified.) The point is that in NUTS I/C 4 and NUTS I/C 5 we know exactly who these laboratories are, and there is a clear correlation between the production of EVs and non-participation in previous exercises of this kind. This is borne out by the fact that:

- none of the eleven laboratories identified in NUTS I/C 5 had participated in NUTS I/C 4; and
- none of the six laboratories identified in NUTS I/C 4 had participated in NUTS I/C 3.

Evidently 'novice' laboratories are likely to be the worst performers, and while this is no less than would be expected, it illustrates the value of participation in intercomparison exercises, as laboratories have no way of knowing how good or bad their analytical chemistry is until they have participated. If laboratories produce poor results in an intercomparison exercise, then they have learned something useful from their participation, and poor performance can be remedied once recognized. If laboratories produce good results in their first intercomparison exercise, so much the better, and not only have they proved it to themselves, but the whole community of nutrients analysts knows that they have done so.

Once more, we wish to record our dissatisfaction with the way some participants expressed their results. The precision and sensitivity implied by a result containing five or six significant digits is totally unrealistic in colorimetric analysis, and can only serve to mislead.

Inspection of the identities of laboratories listed in Section 7.3 as having produced high quality results shows that the great majority of them also did well, or at least participated, in NUTS I/C 4. While this comes as no surprise, it is worth noting that 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!

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ANNEX 1

COMPILATION OF INFORMATION AND INSTRUCTIONS SENT TO PARTICIPANTS

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague,

Fifth Intercomparison Exercise for Nutrients in Sea Water "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 sea water.

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 Sea Water "NUTS I/C 5"

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

Signature

Date

ICES NUTRIENTS INTERCOMPARISONS—THE FIRST 25 YEARS

First Exercise

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'	Institute of Marine Research (IMR), Helsinki
'Hermann Wattenberg'	Institut für Meereskunde, Kiel
'Skagerak'	Royal Fishery Board, Gothenburg

Each ship contributed freshly collected bulk samples to the experiment, which 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 (UNESCO, 1965), which contained contributions from himself, Stig Fonselius, and Klaus Grasshoff. The report was presented at the 53rd ICES Statutory Meeting in Rome in October 1965.

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

Second Exercise

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

Part I Leningrad (May 1966)

The participating research vessels were:

'Alkor'	Institut für Meereskunde, Kiel
'Okeanograf'	Institute of Marine Research, Leningrad
'Prof Otto Krammel'	Institut für Meereskunde, Warnemünde
'Skagerak'	Fisheries Board of Sweden, Gothenburg

Research vessels 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 the UK were now represented. Once more, research vessels delivered bulk samples and the various participants analysed samples simultaneously in Copenhagen. As in 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 (UNESCO, 1966) makes no mention of nitrate or 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 an early time for heterogeneous cadmium-based nitrate/nitrite reduction techniques and some of the associated problems were presumably not fully appreciated at the time.

Evidently nitrate analysis had some way to go to achieve the reliability and ease of operation of the Murphy and 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 (ICES, 1966) undertook a detailed laboratory examination of the individual methods used by the participants and in their contribution to Grasshoff's report, in which they announced, "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 and did not last. Along came the auto-analyser!

Third Exercise

The third exercise was organized by the ICES Working Group on Chemical Analysis of Sea Water under the joint auspices of ICES and SCOR and its official title, "The International Intercalibration Exercise for Nutrient Methods2, shows that it set out to be an ambitious project.

Samples were distributed in 1969/1970 and 45 laboratories from 20 countries submitted results, but the final report on the results of the exercise was not published for several years (ICES, 1977).

The time had come to study "nutrients" separately from oxygen, salinity, chlorinity, and pH, but with the awareness of problems arising from the instability of natural seawater samples, the organizers (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 it was not possible to link them 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 peer laboratories throughout the world are aware that it is producing poor quality data.

Fourth Exercise

Various "workshop" and multi-ship events following the ICES/SCOR exercise included nutrient studies, but it was many years later (1988) before the ICES Marine Chemistry Working Group produced volunteers (Kirkwood, Aminot, and Perttilä) to organize the next large-scale intercalibration exercise, designated "NUTS I/C 4". This exercise did not set out to be world-wide, beginning only with laboratories in ICES Member Countries, but other laboratories who were interested in participating were not turned away.

The fourth exercise differed from the third exercise in three important respects.

1) The test samples were natural or near-natural sea water rather than standard solutions. (Strictly speaking, this made the exercise an intercomparison rather than an intercalibration.)

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

3) 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 the them.

Sixty-nine laboratories from 22 countries submitted results and, thanks in some measure to the telefax machine, the final 83-page report (Kirkwood *et al.*, 1991) was in the hands of participants within two years of the distribution of samples. Statistical treatment identified 58 laboratories consistent in phosphate analyses, 51 consistent in nitrate analyses, and 48 consistent in both phosphate and nitrate analyses, 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 organizers now

feel it is time to go truly world-wide again. The fifth exercise "NUTS I/C 5", or in strict ICES parlance "5/NUT/SW", will begin with a distribution of samples in 1993 and the intention is to include every laboratory anywhere that measures nutrients in sea water. There will be no charge for the samples but intending participants will be at a definite disadvantage if they have not first read ICES *Cooperative Research Report* No. 174 (the report on the results of NUTS I/C 4).

The provisional list of participants now stands at 110 laboratories.

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INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

Dear Colleague

Fifth Intercomparison Exercise for Nutrients in Sea Water "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 Sea Water "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 Sea Water "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

Alain Aminot and Don Kirkwood

ICES FIFTH INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEA WATER "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).

1. 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).

2. 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 minimize 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.

3. 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.

4. 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.

ICES FIFTH INTERCOMPARISON EXERCISE FOR NUTRIENTS IN SEA WATER "NUTS IC/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
$NO_3 + NO_2$	_____	_____	_____
NO_2	_____	_____	_____
NO_3 (by subtraction)	_____	_____	_____

	Sample 4	Sample 5	Sample 5
PO_4	_____	_____	_____
NH_4	_____	_____	_____

THESE RESULTS SHOULD BE SENT TO:

Alain Aminot
IFREMER, Centre de Brest,
BP 70
29280 Plouzane
France

Plouzane, 1993

O/Ref.:DEL/93

Object: **ICES Fifth Intercomparison Exercise for Nutrients in Sea Water "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 there 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	$NO_3 + NO_2$	NO_2	NO_3	sample	PO_4	NH_4
	1				4		
	2				5		
	3				6		

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

Thank you.

Yours sincerely.

ALAIN AMINOT

22 April 1993

Dear Colleague

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

We have not yet received results from your laboratory for the fifth ICES Intercomparison Exercise for Nutrients in Sea Water.

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 not 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

Alain Aminot and Don 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 Sea Water (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 1996/1997).

Yours sincerely

Alain Aminot and Don Kirkwood

ANNEX 2

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

LIST OF PARTICIPANTS

QUASIMEME participants (56) are indicated by "Q" in column 2.
NUTS I/C 4 participants (61) are indicated by their I/C 4 laboratory number in column 3.

Lab. No. NUTS I/C 5	QUASIMEME Participant	Lab. No. NUTS I/C 4	Institute Name and City	Country
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			University of British Columbia, Vancouver, BC	CANADA
10		82	IOS Sidney, BC (A)	"
11			" " (B)	"
12		72	Bedford Institute of Oceanography, Dartmouth, NS	"
13			SIO-SOA, Hanzhou	CHINA
14	Q		Water Quality Institute, Hørsholm	DENMARK
15	Q	1	Danish Institute for Fisheries and Marine Research, Charlottenlund	"
16	Q	2	National Environmental Research Institute, Roskilde	"
17		31	IEE-TT University, Tallinn	ESTONIA
18		30	EMI, Tallinn	"
19		53	HS, Tórshavn	FAROE ISLANDS
20	Q	32	Finnish Institute of Marine Research, 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	"

Lab. No. NUTS I/C 5	QUASIMEME Participant	Lab. No. NUTS I/C 4	Institute Name and City	Country
33	Q	8	LWKS-H, Kiel	GERMANY (18)
34		7	Institut für Meereskunde, Univ. Kiel	"
35	Q		SAUN, Stralsund	"
36	Q	4	Institut für Ostseeforschung, Warnemünde	"
37			Universität Rostock	"
38			BAH-MH, Helgoland	"
39			NLfO-FK, Norderney	"
40		10	Universität Hamburg	"
41			Bran & Luebbe, Hamburg	"
42			UHAU, Hamburg	"
43	Q		BSH, Hamburg	"
44		13	Alfred Wegner Institute for Polar & Marine Research, Bremerhaven	"
45			ZFKM, Wilhelmshaven	"
46			GKSS-FG, Geesthacht	"
47			Universität 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	Institute of Marine Research, Reykjavik	ICELAND
55	Q		Fisheries Research Centre, 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	Netherlands Institute for Sea Research, Texel	NETHERLANDS
66		22	TNO-AMRL, Den Helder	"
67	Q	25	Rijkswaterstaat–Tidal Waters Division, Middelburg	"
68	Q	24	NIOO-CEMO, Yerseke	"
69			AA&A, Auckland	NEW ZEALAND
70	Q	23	Institute of Marine Research, Bergen	NORWAY
71			University of Bergen	"
72		17	Norwegian Institute for Water Research, Oslo (A)	"
73	Q		Norwegian Institute for Water Research, Oslo (B)	"

Lab. No. NUTS I/C 5	QUASIMEME Participant	Lab. No. NUTS I/C 4	Institute Name and City	Country
74			SBSF, Hisøy	NORWAY (cont.)
75		15	Sea Fisheries Institute, Gdynia	POLAND
76		14	Institute of Meteorology and Water Management, Gdynia	"
77	Q		University of Aveiro	PORTUGAL
78	Q		DGQA-CIA, Lisboa	"
79	Q	68	Institute Hydrografico, Lisboa	"
80			University of Qatar, Doha	QATAR
81			CSIR, Congella, Natal	SOUTH AFRICA
82	Q		AZTI-SIO, Pedernales	SPAIN (8)
83	Q	66	IEO, La Coruña	"
84			IIM-CSIC, Vigo	"
85			CEAB-CSIC, Blanes	"
86	Q		LCT-CONTOX, Madrid	"
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		Swedish Meteorological & Hydrological Institute, Norrkoping	"
96		12	KML, Uddevalla	"
97	Q	11	RSAS, Fiskebäckskil	"
98	Q	5	Swedish Meteorological & Hydrological Institute, 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	Scottish Office Agriculture & Fisheries Department, Aberdeen	"
106		42	Scottish Marine Biological Association, Oban	"
107	Q	35	Forth River Purification Board, Edinburgh	"
108	Q	40	Clyde River Purification Board, Glasgow	"
109			North West Region, National Rivers Authority, Carlisle	"
110			DANI, Belfast	"
111	Q		DED-ISC Lisburn	"
112			University of Liverpool, Port Erin, IOM	"
113			University of Liverpool	"
114			MBCC, Bangor	"
115		29	University of East Anglia, Norwich	"

Lab. No. NUTS I/C 5	QUASIMEME Participant	Lab. No. NUTS I/C 4	Institute Name and City	Country
116			Anglian-National Rivers Authority, Peterborough	UK (cont.)
117	Q	28	Ministry of Agriculture, Fisheries & Food, Lowestoft	"
118	Q	45	Welsh-National Rivers Authority, Llanelli	"
119			Wallace-Evans, Bridgend	"
120			Institute of Oceanographic Sciences, Wormley	"
121			University of Southampton	"
122			South Western Region, National Rivers Authority, Exeter	"
123		54	Plymouth Marine Laboratory, Plymouth (A)	"
124	Q		Plymouth Marine Laboratory, Plymouth (B)	"
125			University of Plymouth	"
126		83	OS University, Corvallis, OR	USA (7)
127			University of New Hampshire, Durham, NH	"
128			University of Rhode Island, Narragansett, RI	"
129		76	University of Maryland, Solomons, MD	"
130	Q		Texas A & M University, College Station, TX	"
131		78	National Oceanic and Atmospheric Administration, Miami, FL	"
132		84	University of Hawii, Manoa, Honolulu, HI	"

ANNEX 3

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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1 INTRODUCTION

Intercomparisons are important tools for quality assurance of chemical analysis. They rely on the distribution of a ***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). As the aim of the fourth exercise was to check only nitrate and phosphate determination performances, natural untreated waters were convenient. Indeed, these determinands are the final products of the mineralization oxidation steps in which nitrogen and phosphorus are involved and, therefore, they 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 a 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 results reported by the participants.

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 contolled 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, problems should not normally be encountered. Grouping nitrate and nitrite together seemed the best compromise, since they are determined using the same reagents. Ammonia and phosphate remained for the second bottle. Two bottles containing approximately 140 ml each appeared convenient even for laboratories using manual methods.

2.1.3 Stabilization 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 any remaining organisms are killed or inhibited.

We filtered the water through glass fibre filters (Whatman GF/C) of approximately 1 µm pore size. After filtering, the water was heat sterilized (120 °C for 20 min) without the addition of preservatives, some of which 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 (four months) before the bottles were sent to the participants. Thereafter, all samples were stored at around 20 °C. The participants were advised on how to 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 Fourth Intercomparison Exercise for Nutrients in Sea Water (NUTS I/C 4). They have proved to be satisfactory, particularly with respect to the cover. 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 replaced with insufficient care (i.e., 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 the step-by-step 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 the expected value.

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 a reference material, since such material is assumed to be stable over the intended time period. In this case, 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 the reference material, however, none are available for ammonia. Additionally, these standards are not prepared in sea water and hence are not reference materials 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 bracketed with working standards which are run frequently 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 as close as possible to the samples in composition but which does not contain the determinand at detectable concentrations. Distilled or demineralized water usually satisfies this condition depending on the nutrient concerned. However, as always is the case for trace analysis, the purity of these waters is 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" or RIB, according to the procedures described by Tréguer and Le Corre (1975).

3 OPERATIONAL PROCEDURES

3.1 Preparation and Testing Scheme

Flow charts for the preparation and testing of the reference material are shown in Figures A3.1 and A3.2.

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 those for phosphate and ammonia. This allowed more attention to be focused on the preparation of standards or spiking solutions 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 nutrient 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 a second 100 l carboy (B) and then subsampled into a third 60 l polypropylene carboy (C) for spiking and bottling. All equipment receives great attention in order to limit 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 demineralized water acidified with H_2SO_4 (0.5 mol/l). The inner walls of the container 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 demineralized 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 of Milli-Q water (0.5 % of the carboy capacity), then determining the nutrients in that water. The nitrate and nitrite concentrations found ($\leq$ 0.02 µmol/l) would have been insufficient to contaminate the depleted sea water.

Taking into account the satisfactory results obtained with nitrate and nitrite, the same cleaning procedure was applied in the preparation of the samples for phosphate and ammonia. However, no check was undertaken since the concentrations of these two determinands before spiking were 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 (cap). The glass bottles and the polypropylene caps were cleaned separately before use.

Before commencing the preparation of the reference material, a set of bottles was washed in a washing machine piped with demineralized water (SADON Cartridge System) and using a phosphate-free detergent (Neodisher UW). Following this cleaning procedure, the possibility of filling the bottles with the 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 demineralized water was found to be sufficient for cleaning new bottles and these should be capped immediately for storage before use. In addition, a rinse with a small volume of sample water immediately before filling was found to be necessary.

3.2.3 Acid contribution

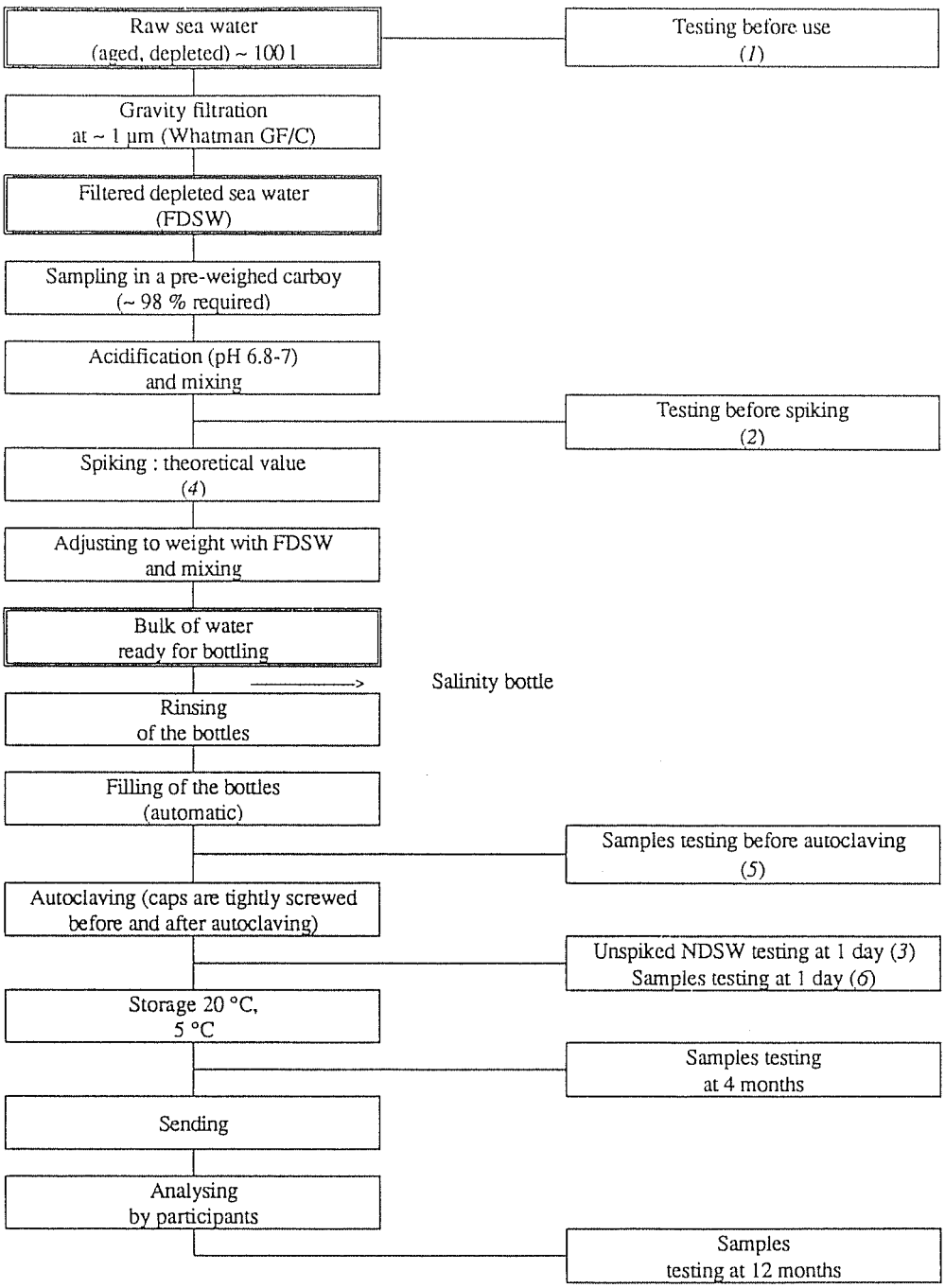
Hydrochloric acid is used to prevent precipitation of phosphate observed during autoclaving. Two lots of acid were checked after dilution with Milli-Q water at concentrations three times that required for the preparation of the reference material. At normal added quantities, nitrate, nitrite, ammonia,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

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 concentrated nutrient solutions. These concentrates are prepared using the following crystallized salts from J.T. Baker, the purity of which is guaranteed by certificates of analysis (see Table A3.1).

Figure A3.1. Flow chart for the preparation of the reference material for nutrients. The italicized numbers in parentheses refer to the code numbers in Tables A3.9, A to E.



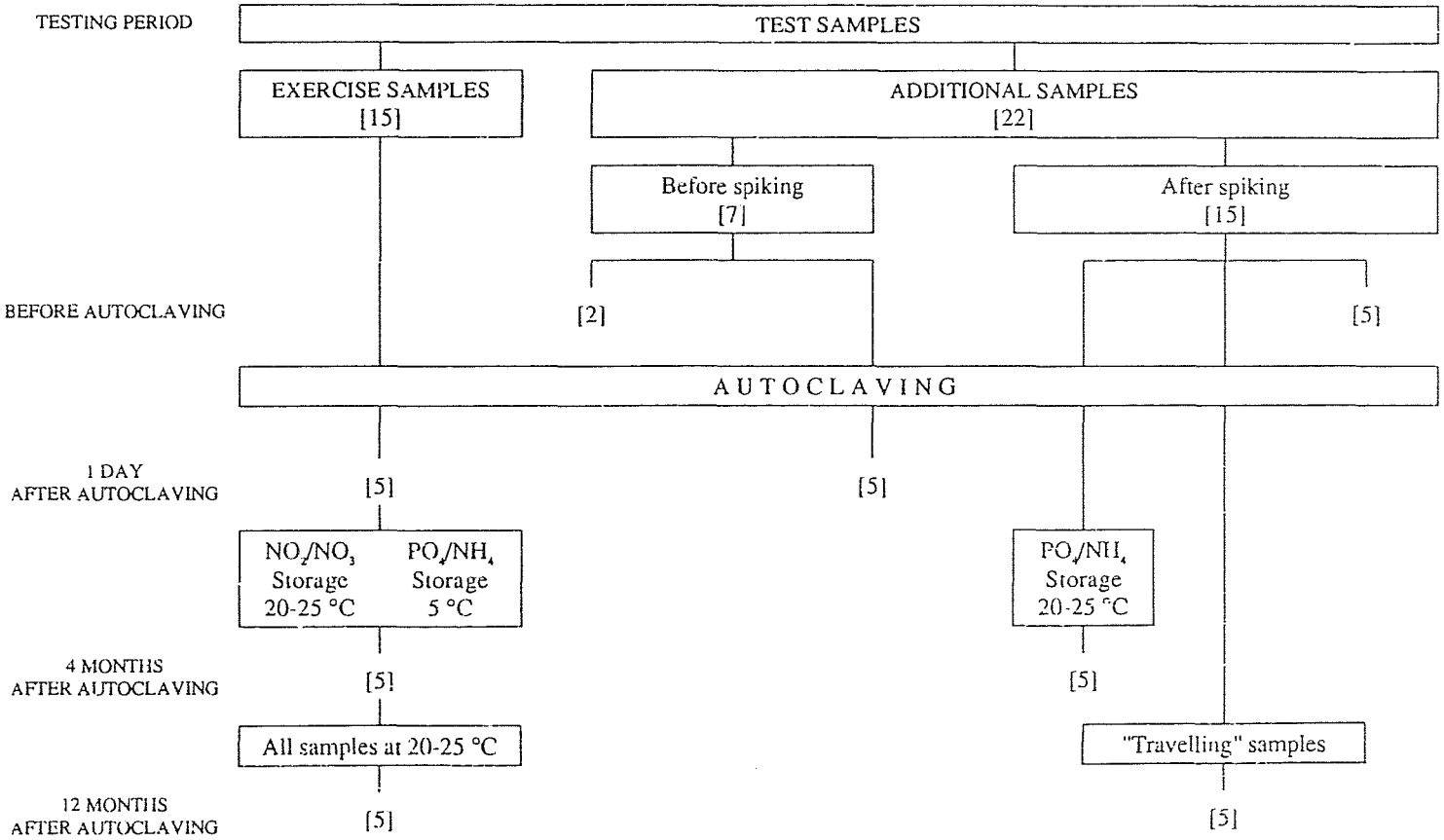


Figure A3.2. Flow chart for testing of reference materials for nutrients. The minimum numbers of samples are shown in brackets.

Table A3.1. Purity of salts used for the preparation of the spiking solutions.

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 in small glass vessels for approximately 2 hours at 105 °C. 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 that 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 into account 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 (g):*

$$m_S = 0.50059 \text{ g}$$

- *Mass of water to be added (g):*

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

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 = 1013 ± 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 percent (and is therefore negligible).

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 transferred with caution into the pre-weighed bottle (4000 g Sartorius 1364MP electronic balance, 0.01 g resolution) using a wash-bottle; the rest of the water is then added up to the required quantity (the balance is zeroed with the empty bottle on, hence the total weight equals water plus salt weights). The bottle is tightly capped and the salts are 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 A3.2.

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) that were carefully rinsed, dried, and stored in a desiccator before use. A 400 g Sartorius 1265MP electronic balance with a resolution of 0.001 g was used for these concentrates.

Table A3.2. Preparation of the spiking solutions.

Nutrient	Salt	Molar mass (g)	Required concentration (mmol/l)	Expected volume of solution	Required salt mass m_t (g)	Weighed salt mass m_s (g)	Theoretical water 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 first used 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 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 as that of pure water, and corrected for air boyancy (see Section 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 (practical salinity scale) 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 Section 3.3.1, above).

The density of sea water at 20 °C and 35 PSS, according to Cox *et al.* (1970), is 1024.8 g/l, hence the density factor $\left(d_{sw}^{20} - d_a^{t,P}\right)$ is (1024.8 – 1.2) = 1023.6. As the salinity was expected to be in the range 34.5–35 PSS, the rounded value 1023 was used. Further determinations gave 34.9 to 35.4 PSS, which leads to a slight under-estimation of the density by 0.04–0.10 %. Hence, the actual concentrations 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 sea water is then added up to the required mass. Table A3.3 summarizes the solutions, masses, and volumes involved in the preparation of the samples.

3.4 Determination of Nutrient Concentrations

3.4.1 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 (1962) reagents 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 has been made accordingly.

The performance of the methods from replicates of standards is summarized in Table A3.4.

Table A3.3. Preparation of the sea water samples for bottling.

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

Table A3.4. 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 values 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, therefore leading to variable signal intensity for similar concentrations.

Testing Period	Data	Nitrate		Nitrite		Ammonia		Phosphate	
1 day	Level (µmol/l)	1.1	27	1.1	27	1.2	2.2	0.6	1.9
	n	4	8	4	8	6	5	4	5
	mean (mV)	273	2560	273	2560	920	1608	996	3272
	SD (mV)	3.3	5.8	3.3	5.8	3.3	8.5	1.7	1.3
	RSD (%)	1.2	0.2	1.2	0.2	0.4	0.5	0.2	0.04
4 months	Level (µmol/l)	10	27	10	27	1.7	4.7	0.4	1.9
	n	3	5	3	5	5	4	5	7
	mean (mV)	3738	3169	3738	3169	1251	3422	575	3219
	SD (mV)	1	2.6	1	2.6	1.1	5.4	1.1	2.7
	RSD (%)	0.03	0.08	0.03	0.08	0.09	0.2	0.2	0.08
12 months	Level (µmol/l)	9.5	10.5	10	27	1.5	4.5	0.25	1.9
	n	3	3	3	5	6	4	4	5
	mean (mV)	3333	3710	3738	3169	1249	2786	549	2641
	SD (mV)	8.4	6.6	8.4	6.6	7.2	9.6	2.2	3.0
	RSD (%)	0.3	0.2	0.3	0.2	0.6	0.3	0.4	0.1

3.4.2 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.

3.4.2.1 Concentrated solutions

Concentrated solutions are prepared in the same way as the spiking solutions and using the same salts (see Section 3.3.1). However, for the calibration, they were prepared by another analyst and their concentrations are different from those of the spiking solutions (Table A3.5).

3.4.2.2 Working solutions

Working solutions are obtained by dilution of the concentrated solutions with nutrient-depleted sea water using volumetric glassware and pipettes. 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 necessarily 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 too large a 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.

3.4.2.3 Volumetric tools checkings

Automatic pipettes are now currently used in laboratories. Previous work has shown that there is no risk of adsorption of phosphate onto 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. The results are summarized in Table A3.6. The pipette was always set in direct mode using a new tip each time (only one pipetting with each 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 entire range. A separate examination of results at 500 µl and 1000 µl settings 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 1000 µl, it can be considered that the pipette does not introduce biases greater than around 0.1 %.

The dilutions of the concentrated solutions were done using class A volumetric flasks of various capacities (100 to 1000 ml). Their accuracy, which is normally within ± 0.1 % (100 ml) to ± 0.04 % (1000 ml) of nominal volume, was also checked in previous work and found to be in agreement with the stated accuracy (see Table A3.7).

Table A3.5. 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 concentration (mmol/l)	Expected volume of solution	Required salt mass m_t (g)	Weighed salt mass m_s (g)	Theoretical water 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

Table A3.6. Checking of the electronic pipette.

Date of checking	Pipette setting	Water temperature (°C)	Correction factor	Weight (mg)	Volume (µl)	Difference from setting (µl)
23/06/1992	500	25.5	1.0041	498.5	500.5	+ 0.5
	1000	25.5	1.0041	997.8	1001.9	+ 1.9
15/09/1992	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
	1000	23.2	1.0036	999.7	1003.2	+ 3.2
	1000	23.2	1.0036	996.3	999.9	− 0.1
	1000	23.2	1.0036	995.1	998.7	− 1.3
14/10/1992	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
	1000					− 0.9 ± 2

Table A3.7. Checking of volumetric flasks.

Nominal value (mL)	Water temperature (°C)	Correction factor	Weight (g)	Volume (m)	Relative difference (%)
100	21.5	1.0032	98.812	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

3.4.2.4 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 A3.8. They show that the differences between standards are all within about ± 1 % of the reference.

3.4.3 Blanks

The importance of blanks has been indicated in Section 2.6.2, above. The most important problem 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 a series of system blanks for nitrate, nitrite, ammonia and phosphate. These results are summarized in Table A3.9.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 besystematically 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 cannot 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 a 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 (see Table A3.9) 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 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 1 to 9 June 1993 and led to 0.207 ± 0.005 µmol/l. Given this satisfying stability (± 0.01 µmol/l) over a one-year working period, the blank was considered unbiased.

Table A3.8. Comparison of concentrated solutions of nutrients used for testing the reference material samples. All values are given relative to the new concentrate prepared at each step. Extra concentrates are also tested.

Step (date)	Concentrate	Nitrate		Nitrite		Ammonia		Phosphate	
		means diff. %	RSD % (n)	means diff. %	RSD % (n)	means diff. %	RSD % (n)	means diff. %	RSD % (n)
1	Standard 1	ref.	0.2 (3)	ref.	0.5 (4)	ref.	0.3 (2)	ref.	0.1 (2)
May/June	Spiking solution	− 0.2	0.1 (3)	+ 0.7	0.1 (4)	− 0.5	0.4 (5)	− 1.0	0.1 (5)
1992	Dilut-it BAKER	–	– –	–	– –	− 0.5	0.1 (2)	+ 1.1 (2)	0.1
2	Standard 2	ref.	0.1 (4)	ref.	0.1 (4)	ref.	0.4 (3)	ref.	0.1 (4)
Sept./Oct	Standard 1	+ 0.5	0.1 (4)	0.0	0.2 (4)	− 0.4	0.1 (2)	0.0	0.2 (3)
1992	Standard 3	ref.	0.4 (5)	ref.	0.2 (5)	ref.	0.1 (5)	ref.	0.1 (5)
3	Standard 2	0.0	0.9 (4)	− 1.0	0.1 (4)	− 0.6	0.1 (5)	0.1	0.1 (5)
May 1993	Standard 1	− 0.2	0.6 (2)	− 0.3	< 0.1 (3)	− 0.5	0.1 (2)	0.0	< 0.1 (2)

Table A3.9. System blanks (RIB) as determined by the authors using their Autoanalyzer II Technicon. The blanks are converted into their equivalent in micromoles per litre of the corresponding nutrients. Salinities of the reference material (RM) waters are as follows: RM1 = 35.44 (PSS); RM2 = 35.34; RM3 = 35.45; RM4 = 35.34; RM5 = 34.85; RM6 = 35.25.

Nutrient	RM	Date in 1992	Blanks (µmol/l)			Limit of detection (= 3 x SD) (nmol/l)
			n	mean	SD	
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	10.043	< 0.0001	< 1
	2	20 May	11	0.043	0.0008	2.5
	3	21 May	13	30.040	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

3.4.4 Signal recording and treatment

The output signal from the colorimeters is recorded on a paper chart recorder and simultaneously on a computer, via a 10 V/12 bit electronic device. The signal is sampled at 20 Hertz and averaged 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.

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 concentrations. All these figures are summarized in Table A3.10, 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 Section 5, below).

The results show good agreement between expected and measured added concentrations (Table A3.10, A to E). For nitrate, nitrite and phosphate, the differences remain lower than or close to one percent for the intermediate and high levels or at the limit of the analytical possibilities for the low level (i.e., ≤ 0.002 µmol/l for nitrite and ≤ 0.03 µmol/l for nitrate; no spike at low level for phosphate). For ammonia, differences between measured and expected concentrations 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 A3.11.

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

Table A3.10.A. Concentrations of nutrients in the samples: measured and expected values in micromoles per litre. Code numbers refer to the italicized numbers in Figure A3.1.

NITRATE + NITRITE				
Code	**Reference**	**Low Level**	**Intermediate Level**	**High Level**
MEASURED CONCENTRATIONS				
		mean ± sd (n)	mean ± sd (n)	mean ± sd (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	– 0.02
(6 – 5)	Sample	+ 0.03	– 0.02	*
ADDED CONCENTRATIONS				
4	*Theoretical*	1.44	10.50	27.40
	Amount Measured			
(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 was not determined, but the autoclaving effect is assumed to be 0.00 µmol/l, the mean of the five other effects.

Table A3.10.B. Concentrations of nutrients in the samples: measured and expected values in micromoles per litre. Code numbers refer to the italicized numbers in Figure A3.1.

NITRITE				
Code	**Reference**	**Low Level**	**Intermediate Level**	**High Level**
MEASURED CONCENTRATIONS				
		mean ± sd (n)	mean ± sd (n)	mean ± sd (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	– 0.02
(6 – 5)	Sample	+ 0.03	– 0.02	*
ADDED CONCENTRATIONS				
4	***Theoretical***	1.44	10.50	27.40
	Amount Measured			
(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 was not determined, but the autoclaving effect is assumed to be + 0.003 µmol/l, the mean of the five other effects.

Table A3.10.C. Concentrations of nutrients in the samples: measured and expected values in micromoles per litre. Code numbers refer to the italicized numbers in Figure A3.1.

NITRATE (by subtraction)				
Code	**Reference**	**Low Level**	**Intermediate Level**	**High Level**
	MEASURED CONCENTRATIONS			
		mean ± sd (n)	mean ± sd (n)	mean ± sd (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)	9.55 ± 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
	Amount Measured			
(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 A3.10.D. Concentrations of nutrients in the samples: measured and expected values in micromoles per litre. Code numbers refer to the italicized numbers in Figure A3.1.

AMMONIA				
Code	**Reference**	**Low Level**	**Intermediate Level**	**High Level**
	MEASURED CONCENTRATIONS			
		mean ± sd (n)	mean ± sd (n)	mean ± sd (n)
1	Raw NDSW	–	–	–
	Not autoclaved SW			
2	Prepared NDSW		0.07 ± 0.006 (3)	0.08 ± (0) (3)
5	Sample	0.13 ± 0.006 (3)	1.52 ± 0.03 (5)	4.57 0.008 (5)
	Autoclaved SW			
3	Prepared NDSW		0.28 ± (0) (3)	0.25 ± 0.006 (3)
6	Sample	0.34 ± 0.01 (7)	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
	Amount Measured			
(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 A3.10.E. Concentrations of nutrients in the samples: measured and expected values in micromoles per litre. Code numbers refer to the italicized numbers in Figure A3.1.

PHOSPHATE				
Code	**Reference**	**Low Level**	**Intermediate Level**	**High Level**
	MEASURED CONCENTRATIONS			
		mean ± sd (n)	mean ± sd (n)	mean ± sd (n)
1	Raw NDSW	–	–	–
	Not autoclaved SW			
2	Prepared NDSW		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		0.030 ± 0.003 (3)	0.040 ± 0.004 (3)
6	Sample	0.072 ± 0.003 (7)	0.473 ± 0.007 (6)	1.830 ± 0.011 (10)
	Autoclaving effect			
(3 – 2)	Prepared NDSW	+ 0.02	0.00	– 0.02
(6 – 5)	Sample	+ 0.03	– 0.02	*
	ADDED CONCENTRATIONS			
4	***Theoretical***	1.44	10.50	27.40
	Amount Measured			
(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

$s \leq 0.015$ µmol/l 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.

4.2.2 Stability

The stability can be evaluated by the variation of the average concentrations of the test samples (see Table A3.11) measured under strictly identical conditions, i.e., new concentrated standards prepared 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 levels, respectively. No obvious trend can be observed, as the differences remain 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 levels, 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 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 this has been previously demonstrated (Aminot *et al.*, 1992). Hence, the samples were stored at 5 °C before their distribution 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: a 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 of storage at 5 °C seems to have efficiently stabilized 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 eight 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 condition modifications with a possible increase or decrease in the sample temperature (including possible freezing), and shaking of the samples. To obtain information about such modifications, special intercomparison packages were sent to 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 also sent 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 A3.12) 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 detected 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-month checking time have been pooled to obtained the values in Table A3.11.

Table A.3.11. Results of homogeneity and stability testing.

Level	Time after autoclaving	n	Mean (µmol/l)	Range (µmol/l)	SD (µmol/l)	RSD (%)
			NITRATE + NITRITE			
	1day	5	**1.44**	1.43 - 1.44	0.002	0.1
LOW	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
	1 day	5	**10.42**	10.38 - 10.45	0.03	0.3
INTERMEDIATE	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
	1 day	12	**27.27**	27.23 - 27.32	0.02	0.07
HIGH	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			
	1day	5	**0.142**	0.142 - 0.143	0.0004	0.3
LOW	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
	1 day	5	**0.498**	0.496 - 0.499	0.001	0.2
INTERMEDIATE	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
	1 day	12	**1.389**	1.379 - 1.395	0.005	0.4
HIGH	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)			
	1day	5	**1.29**	1.29 - 1.30	0.003	0.2
LOW	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
	1 day	5	**9.92**	9.88 - 9.95	0.03	0.3
INTERMEDIATE	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
			AMMONIA			
	1day	7	**0.34**	0.32 - 0.36	0.015	4
LOW	4 months	5	**0.31**	0.29 - 0.32	0.014	5
	12 months	5	**0.33**	0.31 - 0.34	0.011	3
	1 day	7	**1.78**	1.76 - 1.80	0.012	0.7
INTERMEDIATE	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
	1 day	10	**4.85**	4.83 - 4.88	0.02	0.4
HIGH	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			
	1day	7	**0.072**	0.070 - 0.076	0.003	4
LOW	4 months	5	**0.074**	0.071 - 0.078	0.003	4
	12 months	5	**0.088**	0.085 - 0.091	0.002	3
	1 day	6	**0.473**	0.461 - 0.479	0.007	1.5
INTERMEDIATE	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
	1 day	10	**1.830**	1.808 - 1.844	0.011	0.6
HIGH	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

For phosphate, a slight positive shipment effect is shown by the test. This effect is an increase in concentrations and standard deviations. It is attributed to an increase in the dissolution rate of the glass into the sea water samples induced mainly by the shaking of samples during transport (the mean silicate concentration increased by about 40 µmol/l compared with sedentary samples). Surprisingly, the magnitude of the increase was 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.

For ammonia, the results are more complex. It can be seen (Table A3.12) that some discrepancy does exist between travelling and sedentary samples. A few outlying results are found and this needs 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. All data were then examined in detail (Table A3.13).

The places from where samples had been returned were divided into two groups. In group 1 were 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 were the confirmed participants who sent back their samples because they were unable to analyse them. These packages had been opened by the participants and stored for several months in their laboratories before being returned.

It is obvious from this test that the samples in group 1 exhibit no increase in concentration (apart from one bottle with a broken cap); all samples with concentrations higher than expected belong 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 a 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 are one of the major factors for obtaining reliable data for ammonia. The present exercise cannot therefore discriminate between storage conditions and analytical capabilities.

In addition to the increase in ammonia, one outlying value was 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) led 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 such a sample being 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 nutrient concentrations to the samples requires some comments. We have pointed 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 thus 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 and, thus, on our detection limit. Table A3.9 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 standardization can only have an insignificant effect on the low concentrations in these waters. Only errors in system blank evaluations could be incriminated (see Section 3.4.3, above).

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 have been identified at this stage: the purity of the original dried salts and potential losses either during the addition step or via physical and biochemical processes in the carboy before bottling.

Table A3.12. Comparison of nutrient concentrations (µmol/l) in samples kept in the laboratory and samples having travelled to various countries. The storage time is 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
		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 A3.13).

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 leaching 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

A3.13. 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	-
Spain	*0.64*	*1.99*	
Ireland	0.32	1.74	
France (Bordeaux)	0.32		4.73
			4.80
		-	4.75
			4.78
Mean**	0.34	1.75	4.76

* Excluded (cap broken).

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

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 presented in Table A3.10, 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 Section 4, above).

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 (see Table A3.10, A, B and C).

Considering phosphate, the problem is complicated by the dissolution from glass into the sample. Summing storage and shipment effects led to rigorous expressions of 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 yields:

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 and high concentration 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 imply, 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 %.

Table A3.14. Assigned concentrations for nitrate, nitrite, ammonia, and phosphate (confidence level = 95 %).

	Low	Intermediate	High
Nitrate + nitrite	1.47 ± 0.02	10.48 ± 0.10	27.43 ± 0.10
Nitrite	0.143 ± 0.002	0.505 ± 0.020	1.406 ± 0.020
Ammonia	0.34 ± 0.03	1.83 ± 0.05	4.86 ± 0.05
Phosphate	0.08 ± 0.02	0.495 ± 0.04	1.85 ± 0.04

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 contributions can be found in Section 4, above. Note that

$$s^2_{autoclaving} = s^2_{beforeautoclaving} + s^2_{afterautoclaving}$$

We have pointed out that storage and shipment have no detectable effect on the variability except for phosphate.

From these considerations, the concentrations shown in Table A3.14, with 95 % confidence intervals, were attributed to the samples. Note that for phosphate the variability originating from the storage drift was pooled with the other sources of variability.

6 COMPARISON OF ESTIMATIONS FROM VARIOUS ORIGINS

Three sources of estimation of the nutrient concentrations in the reference material are available: the assigned concentrations (see Section 5); the concentrations measured in the test samples in our laboratory throughout the duration of the exercise (see Section 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 for 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 approximately Normal distribution is obtained (stable mean). The results are summarized in Table A3.15, with ranges at the 95 % confidence level.

The agreement among 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 additional confirmation of the stability of the samples.

7 SUMMARY AND CONCLUSIONS

The aim of this work was to produce and test reference materials 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. In this exercise nitrite and ammonia have been included in order to more widely cover 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 leaching 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, assignment of concentration values 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, these 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 exercise.

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,

Table A3.15. Comparison of the sample concentrations obtained from three separate sources.

	Concentrations and intervals at the 95 % confidence level (± 2 s.d.)								
	Assigned			Measured			From participants' 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 is shown in brackets.

showing that there is no systematic bias due to sample preservation problems.

In conclusion, the reference materials for nutrients in sea water prepared for the present exercise met the expected requirements.

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ANNEX 4

RESULTS SUBMITTED BY PARTICIPATING LABORATORIES

The following comments refer to the results reported in Table A4.1.

Remarks on participants' response

- Laboratories 92 and 112 sent results in obviously incorrect 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 4.1. 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 4.1. 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
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 4.1. 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
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 4.1. 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
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 4.1. 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
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

Table 4.2. 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 4.2. 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*
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 4.2. 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*
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 4.2. Regression analysis data for individual laboratories.

s.d. = standard deviation (µmol/l); prop. = proportional error (%); const. = constant error (µmol/l); *se* = standard error

	NITRATE + NITRITE			NITRITE			NITRATE			AMMONIA			PHOSPHATE		
Lab No.	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 4.2. 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*
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 4.2. 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*
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 4.2. 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*
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 5

CONSENSUS DATA DETERMINATION

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