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

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INTRODUCTION

Previous nutrient intercomparisons/intercalibrations, conducted directly by
ICES or in which ICES has been involved, include the following exercises:

1965	Copenhagen	Report: UNESCO Technical Papers in Marine Sciences No. 3
1966	Copenhagen/ Leningrad	Report: UNESCO Technical Papers in Marine Sciences No. 9
1970	ICES/SCOR	Cooperative Research Report No. 67
1977	Kiel	Report of the Baltic Intercalibration Workshop in 1977
1982	Ronne	Baltic Marine Environment Protection Commission - Biological Workshop Report
1986	PEX	Baltic Patchiness Experiment Report 1989

In many cases, nutrient monitoring data form the most representative source
for environmental studies and modelling; however, in all the above
exercises, discrepancies have been found both in methods and in results.
This is especially true for field exercises and, while it is recognised that
the variability in environmental results includes the variability associated
with sampling and subsequent sample handling, differences in purely analyti-
cal methods are best detected by means of laboratory intercomparisons.
Field exercises are difficult to organise, and the effect of unknown factors
on the results can be considerable, as shown in the Joint International Multi-
ship Investigation of Patchiness in the Baltic Sea (PEX) exercise.

PARTICIPATION

Preliminary enquiries in 1987 indicated that a substantial number of labora-
tories would be interested in participating in a Nutrients Intercomparison
Exercise, and after the 1988 meeting of the ICES Marine Chemistry Working Group,
invitations were sent to ICES laboratories and various other potentially
interested bodies. Those indicating their willingness to participate were
asked to supply a detailed description of their phosphate method (in order to
assess susceptibility towards colorimetric interference from silicate).

On the basis of the plans for the exercise and the recommendation for its
conduct in the 1988 report of the Marine Chemistry Working Group, the issue
was considered at the 1988 ICES Statutory Meeting and the Council approved
its conduct in C.Res.1988/4:10. Invitations for participation were
subsequently issued by the ICES General Secretary to all ICES member countries
and to the Oslo and Paris Commissions and the Baltic Marine Environment
Protection Commission (Helsinki Commission).

In all, 85 laboratories (listed in Annex 1) agreed to take part.

SAMPLES

The preparation and origin of the four samples are fully described in Annex 4,
but, briefly, one sample, labelled C or D, was of natural oceanic water stored
in a glass bottle without added preservative or treatment of any kind.
Earlier samples from a nearby location had been shown to have a 'useful'
level of stability in respect of nitrate at about 16 $\mu mol/\ell$ and phosphate at
1.0-1.2 $\mu mol/\ell$. (All reference to 'nitrate' in this context should be
understood to mean nitrate+nitrite determined as nitrite.)

The T sample (T = temperature) was a natural shelf seas sample, filtered, bottled in glass, then sterilised by autoclaving, containing nitrate at around 7 µmol/l and phosphate at around 0.5 µmol/l.

Both V and P samples were essentially blanks for nitrate and phosphate. They were the same water, a low-nutrient water that had been allowed to become depleted in nitrate and phosphate by prolonged bulk storage in polyethylene before filtering and bottling, V in glass (verre) and P in polypropylene.
At the time of bottling, this water contained a small but measurable residual silicate concentration.

The leaching of silicate from glass storage bottles into samples obviously imposes limitations on the usefulness of samples C/D, T and V in a silicate context but it was hoped that some information could be gained on silicate interference in the determination of phosphate, particularly from the C/D samples which had been in glass for many months.

The history and preparation methods for the samples suggested that they should contain undetectable amounts of nitrite and ammonia, but participants were nevertheless encouraged to determine these if they wished (as it was anticipated that this might help to identify biases), although it was understood that nitrate and phosphate were of primary interest. Additionally, participants were invited to submit results for any other determinands that were routine in their laboratories.

DISTRIBUTION

Samples were sent mainly by air-mail in May 1989 from IFREMER, Brest, one set to those laboratories using continuous-flow analytical techniques (cfa), and two sets to those using manual methods.

Accompanying the samples were specific instructions (pp.33-34) for handling the samples, and a results report form (p.35). For each determination, two replicates were required. These replicates were to be significantly separated in time, preferably by at least 24 hours, each analysis requiring freshly prepared reagent solutions and freshly prepared calibration solutions though no particular method of calibration was stipulated.

This level of replication was necessarily a compromise between statistical and logistical considerations (some laboratories, for example, required 100 ml per replicate for the phosphate determination).

RESPONSE

In all, 68 laboratories responded. Those receiving samples and failing to respond are marked 'NR' (No Response) in Annex 1.

RESULTS

67 participants submitted results for nitrate+nitrite and 68 for phosphate. These were perceived from the beginning to be the nutrients of primary interest.

Respondents were sent a brief results summary in February 1990 (A1.6).

The results tabulated in Annex 2 include nitrate+nitrite, phosphate, silicate, nitrite, ammonia, total nitrogen and total phosphorus (two replicates each, in most cases). The implied precision of these is highly variable but they are listed exactly as submitted by the participants.

In cases where more than the required number of replicates was submitted, only result-1/day-1 and result-1/day-2 were retained, listed and processed.

The following pages 4-22 show scatter plots for the distribution and range of the results, and statistical summaries for each determinand.

STATISTICAL ANALYSIS

The aim of statistical analysis is to extract useful information from the data, and to describe the performance of laboratories, both individually, and as a community, summarised as follows:

a) Estimation of the consensus ('true') concentration of each determinand in each sample.

b) Evaluation of the performance of each individual laboratory (bias and repeatability).

c) Evaluation of the overall performance of laboratories (reproducibility), and identification of consistent laboratories.

For the determinands of primary interest (nitrate and phosphate) in samples C/D and T, the following results are presented.

1.   Consensus concentration estimation (and its standard error) obtained by a robust estimator (Annex 5a).

2.   Classical statistics on the full data-set, including mean, within laboratory s.d., between laboratory s.d. (repeatability), total s.d. (reproducibility) (Annex 5b).

3.   Classical statistics on a modal group of consistent laboratories, identified using cluster analysis (Annex 5b).

For other samples and determinands, only classical statistics have been used to aid in the interpretation of the data.

As the samples are, in effect, uncompromised reference materials, estimation of consensus ('true') concentrations is a particularly important step. This estimation necessarily comes from the full data set whatever its 'representativity' relative to the whole population of laboratories. In a statistical sense, representativity is guaranteed when using a random sampling design; therefore, in the present case, a bias cannot be excluded if participation, based on volunteers, does not correspond to a random sample (i.e. a statistically representative sample) from the whole population of laboratories. The use of robust estimators leads to consensus concentrations, admittedly biased, but insensitive to extreme values.

NITRATE+NITRITE

C/D results

Fig. 1. Scatter plot for C/D
        nitrate+nitrite results

The distribution of the
original full set of results
is shown.

The line in the figure
represents the 'consensus C/D
nitrate+nitrite concentration'

    16.19 µmol/l

Laboratory Number
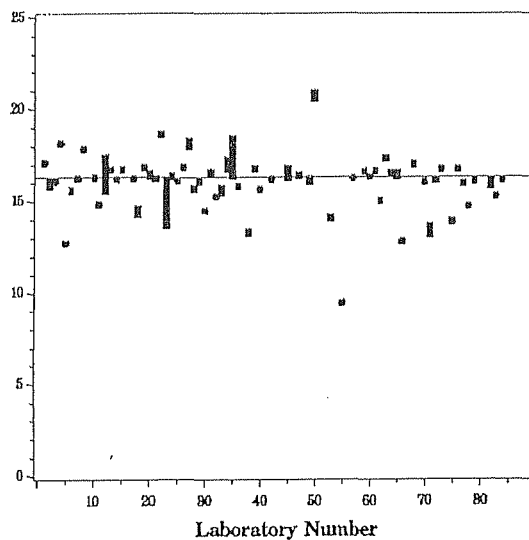
Table 1. Summary of the C/D nitrate+nitrite results (µmol/1).

	Full set	Consistent labs set
No. of labs	67	51
CONSENSUS (std error)	16.19(0.14)	–
mean	15.99	16.33
maximum (paired)	20.714	18.23
maximum (individual)	21.07	18.6
minimum (paired)	9.44	14.4
minimum (individual)	9.34	14.1
within s.d.	0.46	0.35
between s.d.	1.50	0.64
total s.d.	1.57	0.73
'' '' %	9.6	4.5

(See DISCUSSION Section, page 23)

NITRATE+NITRITE (continued)

T results

Fig. 2. Scatter plot for T
nitrate+nitrite results

The distribution of the
original full set of results
is shown.

The line in the figure
represents the 'consensus T
nitrate+nitrite concentration'

6.90 µmol/1

Laboratory Number
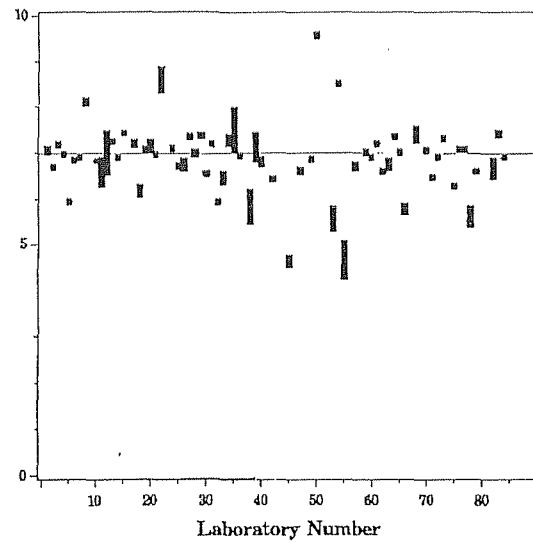
Table 2. Summary of the T nitrate+nitrite results (µmol/1).

	Full set	Consistent labs set
No. of labs	67	51
CONSENSUS (std error)	6.90 (0.06)	–
mean	6.84	7.0
maximum (paired)	9.572	7.5
maximum (individual)	9.643	8.0
minimum (paired)	4.635	6.43
minimum (individual)	4.22	6.25
within s.d.	0.22	0.18
between s.d.	0.76	0.24
total s.d.	.80	0.30
` ` ` ` %	11	4.3

(See DISCUSSION Section, page 23)

NITRATE+NITRITE (continued)

V and P results

The range of results from each laboratory is plotted for V and P samples.
(These are effectively 'blanks' for nitrate+nitrite.)

Fig. 3. Scatter plot for V
          nitrate+nitrite results
                (μmol/l).
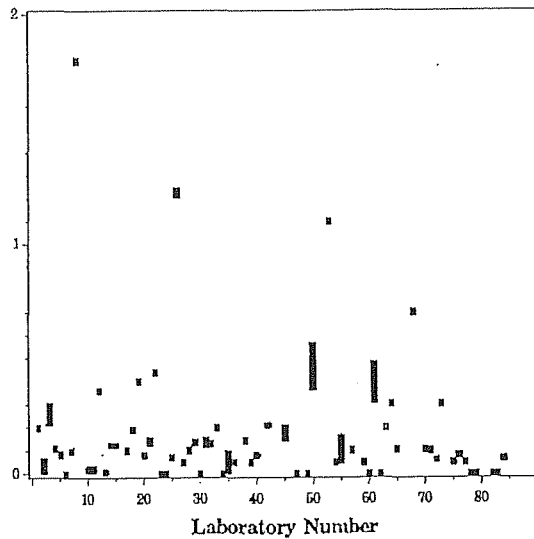
Fig. 4. Scatter plot for P
          nitrate+nitrite results
                (μmol/l).
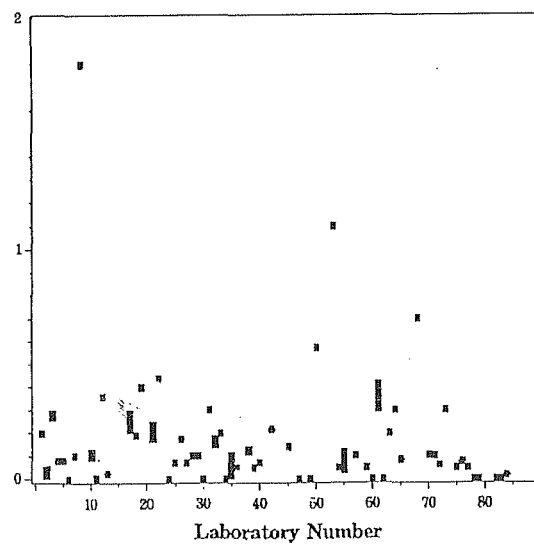
Note: For illustrative purposes, results reported as '< x' are shown as 'x'.

No statistical treatment was considered appropriate.

NITRATE+NITRITE (continued)

General tendency

The general tendency to produce systematically low or high values in both concentration ranges was also studied by plotting the high nitrate+nitrite (C/D) values against the medium ranges values (T). In general, those laboratories reporting low or high concentrations in the medium level samples, did so also in the high level samples.

Fig. 5.   Scatter plot of T vs
          C/D nitrate+nitrite

Solid lines represent
consensus concentrations.

Broken lines represent
± consistent labs total s.d.
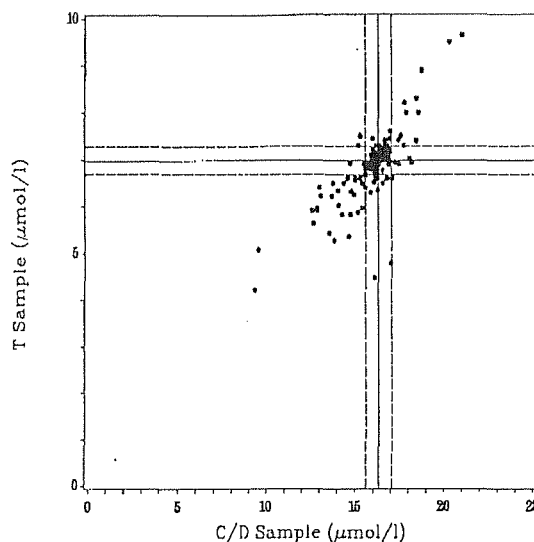
- **Manual vs continuous-flow performance**

The influence of the method of analysis (manual vs continuous-flow) was studied by comparing the distribution of means and standard deviations using the Student criteria.

Table 3.   Summary of manual and cfa results (nitrate+nitrite)

| | cfa | | manual | |
	high	medium	high	medium
No. of labs	44	43	17	17
mean	16.15	6.88	15.84	6.78
s.d.	1.29	0.77	1.38	0.49

In both concentration ranges, the results obtained from continuous flow methods tend to be higher than those from manual methods. However, on the basis of the Student t-test, the hypothesis of the two sets belonging to the same population cannot be rejected at the 95% confidence limit in either of the concentration ranges. Thus the difference between the means of the manual and continuous-flow data sets is not significant for nitrate+nitrite.

PHOSPHATE

C/D results

Fig. 6. Scatter plot for C/D
       phosphate results

The distribution of the
original full set of results
is shown.

The line in the figure
represents the 'consensus C/D
phosphate concentration'

   1.14 µmol/l

Laboratory Number
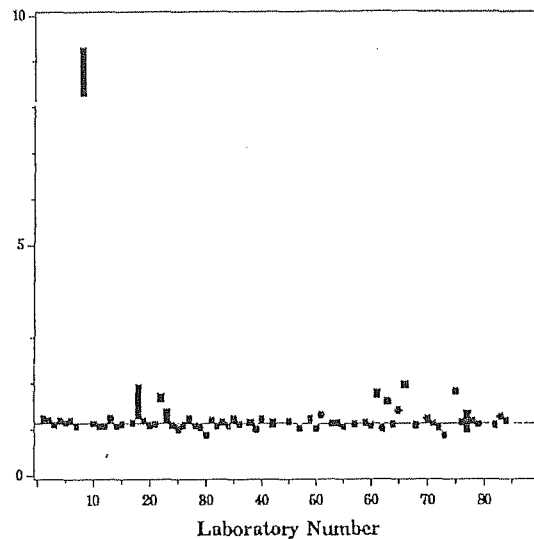
Table 4. Summary of the C/D phosphate results (µmol/l).

	Full set	Consistent labs set
No. of labs	68	58
CONSENSUS (std error)	**1.144**(0.012)	–
mean	1.31	**1.14**
maximum (paired)	8.775	1.32
maximum (individual)	9.32	1.42
minimum (paired)	0.895	0.895
minimum (individual)	0.85	0.85
within s.d.	0.14	0.08
between s.d.	0.94	0.09
total s.d.	0.94	0.12
＼＼ ＼＼ %	83	10

(See DISCUSSION Section, pages 23-25)

PHOSPHATE (continued)

T results

Fig. 7. Scatter plot for T
phosphate results

The distribution of the
original full set of results
is shown.

The line in the figure
represents the 'consensus T
phosphate concentration'

  0.55 µmol/1

Laboratory Number
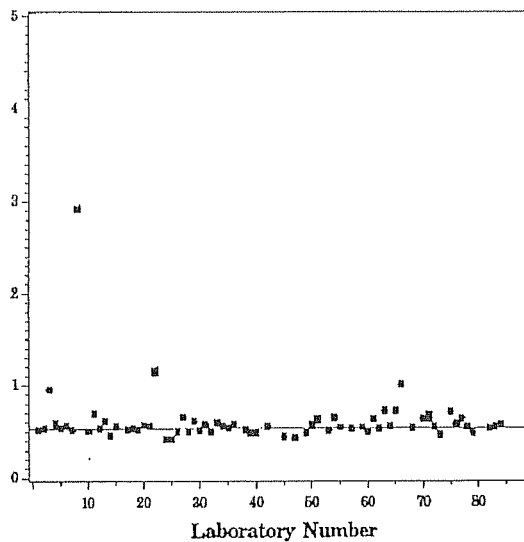
Table 5. Summary of the T phosphate results (µmol/1).

	Full set	Consistent labs set
No. of labs	67	58
CONSENSUS (std error)	0.548(0.009)	-
mean	0.61	0.54
maximum (paired)	2.925	0.69
maximum (individual)	2.96	0.72
minimum (paired)	0.42	0.42
minimum (individual)	0.41	0.41
within s.d.	0.03	0.03
between s.d.	0.31	0.05
total s.d.	0.31	0.06
'' '' %	58	11.6

(See DISCUSSION Section, pages 23-25)

PHOSPHATE (continued)

V and P results

The range of results from each laboratory is plotted for V and P samples. (These are effectively 'blanks' for phosphate.)

Fig. 8. Scatter plot for V
phosphate results
(μmol/1).
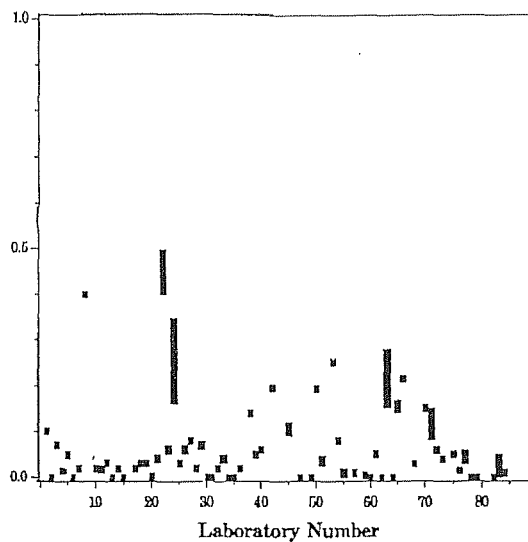
Fig. 9. Scatter plot for P
phosphate results
(μmol/1).
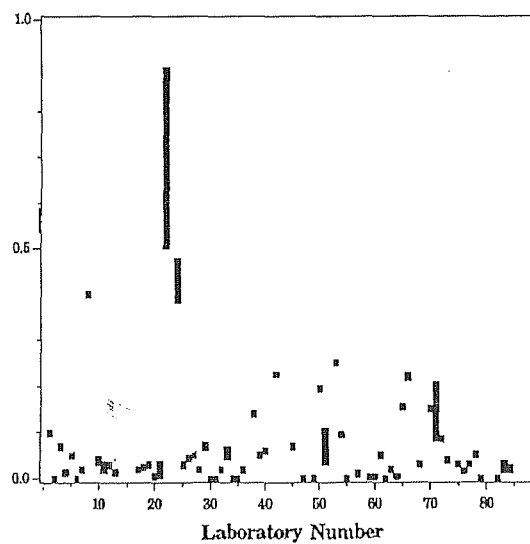
Note: For illustrative purposes, results reported as '< x' are shown as 'x'.

No statistical treatment was considered appropriate.

PHOSPHATE (continued)

General tendency

The general tendency to produce systematically low or high values in both
concentration ranges was also studied by plotting the high phosphate (C/D)
values against the medium ranges values (T). In general, those laboratories
reporting low or high concentrations in the medium level samples, did so also
in the high level samples.

Fig. 10. Scatter plot of T vs
C/D phosphate

Solid lines represent
consensus concentrations.

Broken lines represent
± consistent labs total s.d.
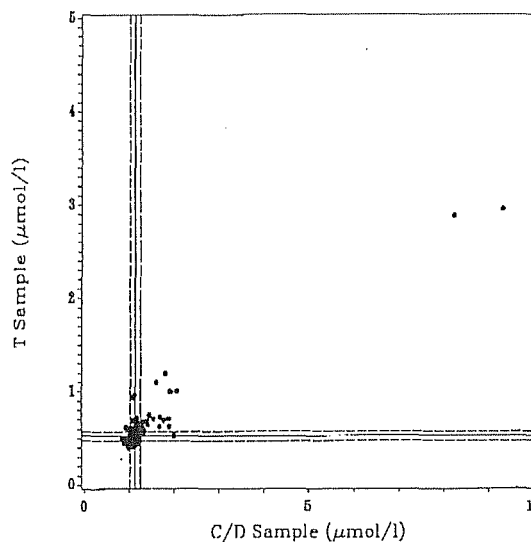
Several laboratories show a substantial positive bias in both C/D and T
samples, consistent with their bias in V and P samples (see figs 8 and 9).

Manual vs continuous-flow performance

The influence of the method of analysis (manual vs continuous-flow) was
studied by comparing the distribution of means and standard deviations using
the Student criteria. (The results of lab 8 were excluded)

Table 6. Summary of manual and cfa results (phosphate)

	cfa		manual	
	high	medium	high	medium
No. of labs	44	42	21	21
mean	1.19	0.57	1.22	0.58
s.d.	0.20	0.13	0.21	0.10

Again, in both concentration ranges, the difference between the means of the
manual and continuous-flow data sets is not significant.

SILICATE

Because of the composition of the sample bottles, sample P is of course the only sample where stable silicate concentrations can be expected.

P results

Fig. 11. Scatter plot for P
silicate results

The distribution of the
original full set of results
is shown.

The line in the figure
represents the 'consensus P
silicate concentration',
the mode of the 'full set'

0.60 µmol/l
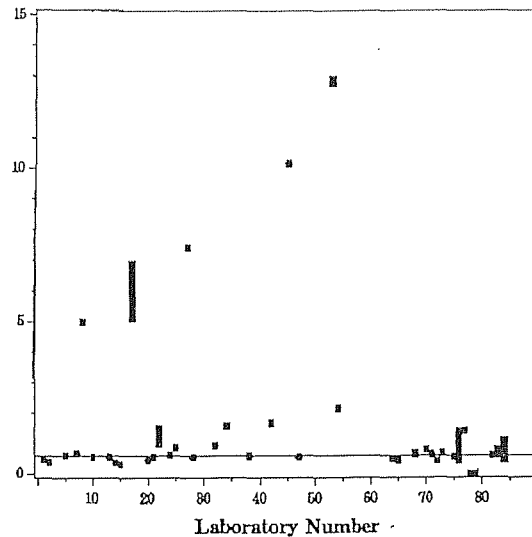
As the silicate concentration in this sample is close to the detection limit normally associated with this determination, the mode, rather than the mean, is considered the best estimate of its actual value.

Note: For illustrative purposes, results reported as '< x' are shown as 'x'.

Table 7. Summary of the P silicate results

Variable	Full set
No. of labs	40
mean	1.59
mode	0.60
s.d.	2.76
minimum	0
maximum	12.99

(See DISCUSSION Section page 25)

SILICATE (continued)

Although it is evident that the silicate concentrations in samples C/D, T and V cannot be considered stable, there is reason to believe that the T samples developed high silicate concentrations during the process of autoclaving, after which the silicate increased only slowly. Therefore the T silicate results can be used for qualitative information on the determination of moderately high silicate concentrations.

T results

Fig. 12. Scatter plot for T silicate results

The distribution of the original full set of results is shown.

The line in the figure represents the 'consensus T silicate concentration', for the samples in mid-1989.

approximately 100 μmol/l
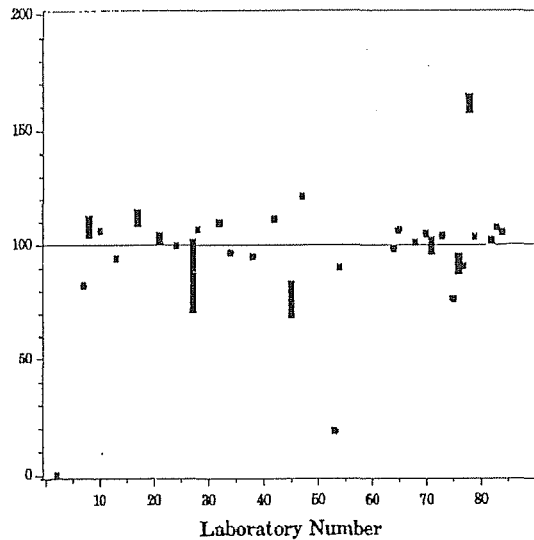
Note: For illustrative purposes, results reported as '> x' are shown as 'x'.

Table 8. Summary of the T silicate results

Variable	Full set
No. of labs	31
mean	95.1
mode	103.0
s.d.	29.8
minimum	0
maximum	166.0

NITRITE

Some laboratories reported '0' or '< x' concentrations.
These have been excluded from the statistics, but are shown in the scatter
plots as '0' and 'x' respectively.
The exceptionally high results of lab 83 for the C/D sample, and all of the
results from lab 8 have been excluded from the statistics.

Outliers were removed by means of the Student's t-distribution at 95%
confidence level.

Summary statistics are shown for 'full' and 'reduced' sets.

Fig. 13. Scatter plot for
C/D nitrite results

Table 9. Summary of
C/D nitrite results
(μmol/l).

Variable	full	reduced
No. of labs	15	14
mean	0.32	0.18
mode	0.30	0.30
s.d.	0.49	0.12
minimum	0.02	0.02
maximum	1.95	0.39

The line in the figure represents
the mean of the reduced set.
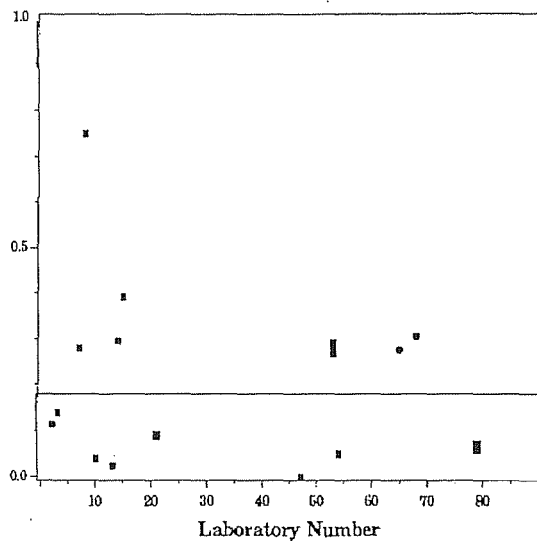
Fig. 14. Scatter plot for
T nitrite results

Table 10. Summary of the
T nitrite results
(μmol/l).

Variable	full set
No. of labs	10
mean	0.02
mode	0.02
s.d.	0.02
minimum	0.00
maximum	0.06

(no outliers)
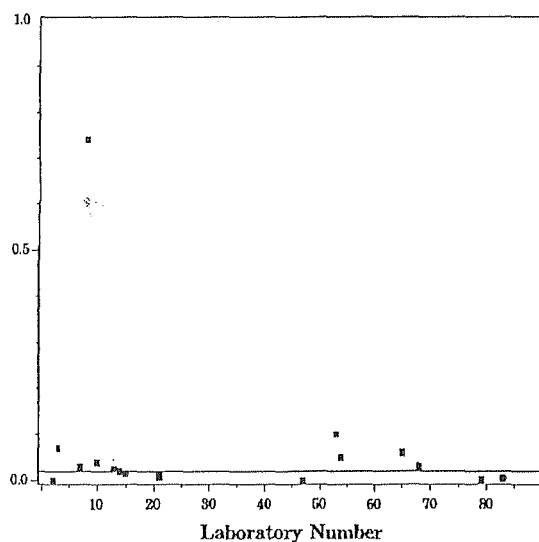
NITRITE (continued)

Fig. 15. Scatter plot for
         V nitrite results

Table 11. Summary of the
          V nitrite results
               (μmol/l).

Variable	full	reduced
No. of labs	9	8
mean	0.01	0.01
mode	0.01	0.01
s.d.	0.02	0.01
minimum	0.01	0.01
maximum	0.06	0.03

The line in the figure represents
the mean of the reduced set.
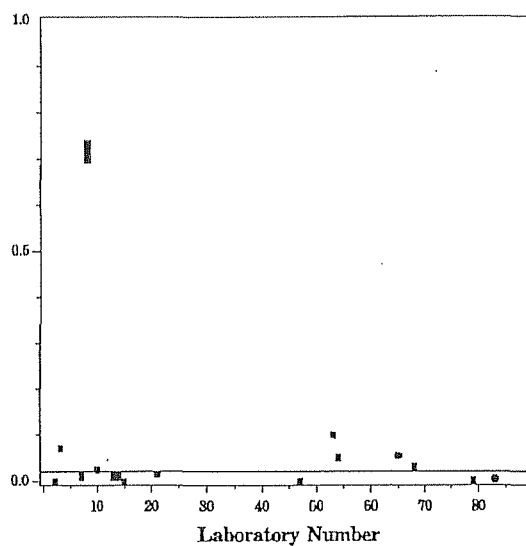
Fig. 16. Scatter plot for
         P nitrite results

Table 12. Summary of the
          P nitrite results
               (μmol/l).

Variable	full set
No. of labs	7
mean	0.02
mode	0.01
s.d.	0.02
minimum	0.01
maximum	0.05

(no outliers)
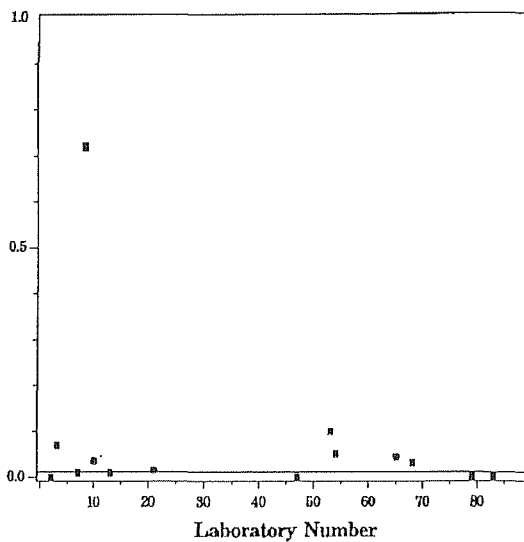
AMMONIA

Some laboratories reported '0' or '< x' concentrations.
These have been excluded from the statistics, but are shown in the scatter
plots as '0' and 'x' respectively.

Outliers were removed by means of the Student's t-distribution at 95%
confidence level.

Summary statistics are shown for 'full' and 'reduced' sets.

Fig. 17. Scatter plot for
C/D ammonia results

Table 13. Summary of the
C/D ammonia results
(μmol/1).

Variable	full	reduced
No. of labs	12	11
mean	1.33	0.55
mode	0.02	0.02
s.d.	2.69	0.47
minimum	0.02	0.02
maximum	10.60	1.73

The line in the figure represents
the mean of the reduced set.

Laboratory Number
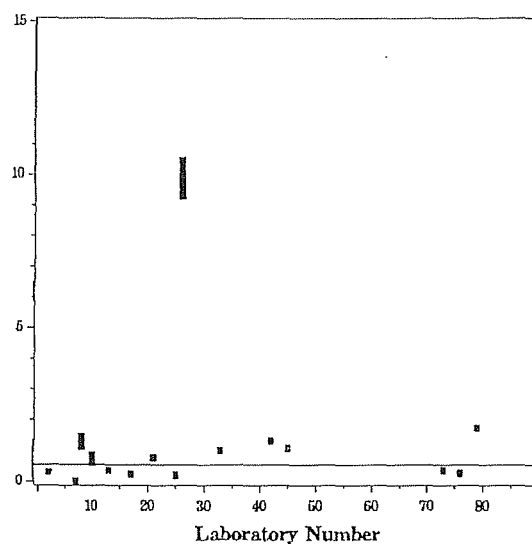
Fig. 18. Scatter plot for
T ammonia results

Table 14. Summary of the
T ammonia results
(μmol/1).

Variable	full	reduced
No. of labs	12	11
mean	0.75	0.59
mode	0.40	0.40
s.d.	0.73	0.50
minimum	0.05	0.05
maximum	2.60	1.54

The line in the figure represents
the mean of the reduced set.

Laboratory Number
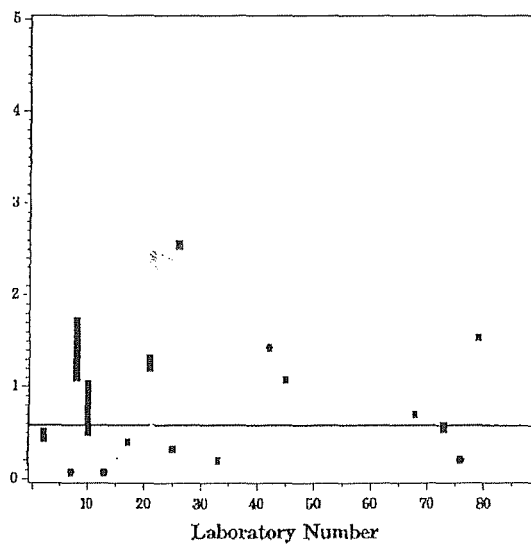
AMMONIA (continued)

Fig. 19. Scatter plot for
         V ammonia results

Table 15. Summary of the
          V ammonia results
              (μmol/1).

Variable	full	reduced
No. of labs	11	10
mean	0.67	0.60
mode	0.20	0.20
s.d.	0.60	0.52
minimum	0.02	0.02
maximum	2.10	1.60

The line in the figure represents
the mean of the reduced set.
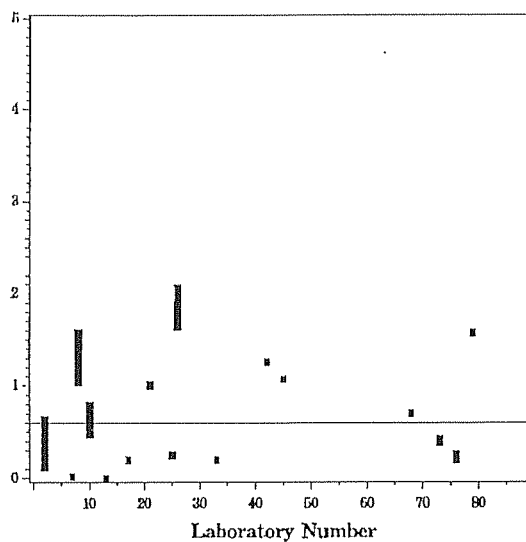
Fig. 20. Scatter plot for
         P ammonia results

Table 16. Summary of the
          P ammonia results
              (μmol/1).

Variable	full	reduced
No. of labs	13	12
mean	1.05	0.82
mode	0.02	0.02
s.d.	1.00	0.63
minimum	0.02	0.02
maximum	3.71	1.80

The line in the figure represents
the mean of the reduced set.
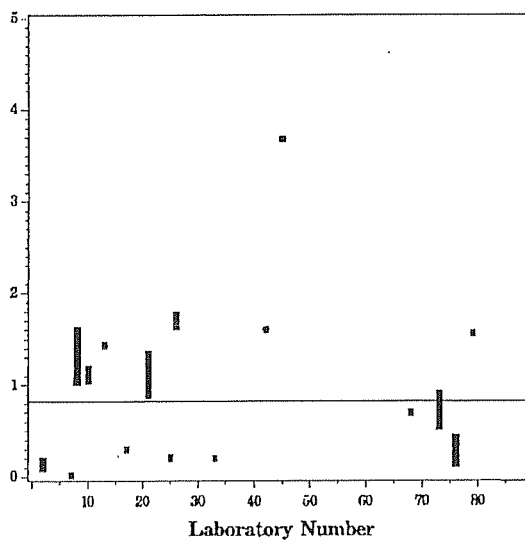
TOTAL NITROGEN

Five laboratories submitted results for total nitrogen.
Laboratory 84 reported results for DON (dissolved organic nitrogen); they have been included in the N_{tot} data set, assuming $N_{tot} = (NO_3+NO_2)+DON$, assuming negligible ammonia concentrations.

Since the total nitrogen concentration should remain constant in a sample bottle regardless of biological processes, all four sample types could be analysed.

On the basis of the Student's test, no values can be removed from any of the data sets.

Fig. 21. Scatter plot for C/D N_{tot} results

Table 17. Summary of the C/D N_{tot} results ($\mu mol/1$).

Variable	C/D
No. of labs	5
mean	22.04
s.d.	2.65
minimum	18.51
maximum	26.00

Laboratory Number
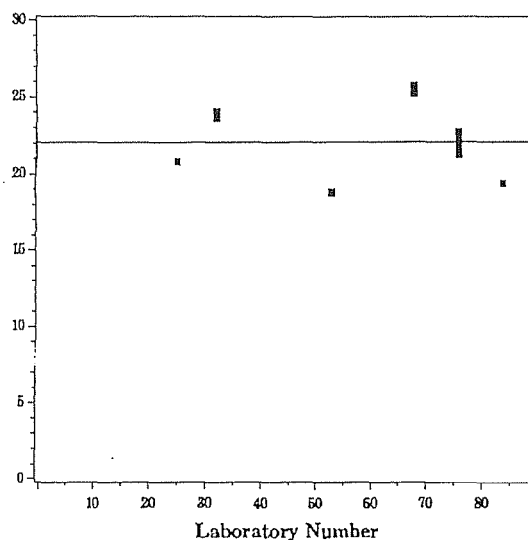
Fig. 22. Scatter plot for T N_{tot} results

Table 18. Summary of the T N_{tot} results ($\mu mol/1$).

Variable	T
No. of labs	5
mean	14.01
s.d.	2.14
minimum	11.59
maximum	18.00

Laboratory Number
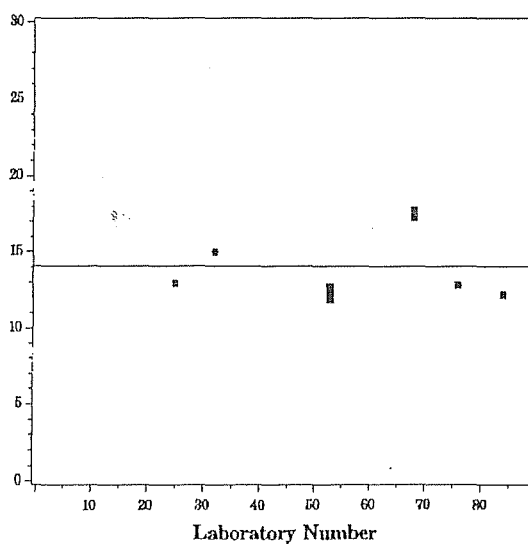
TOTAL NITROGEN (continued)

Fig. 23. Scatter plot for
V N_{tot} results

Table 19. Summary of the
V N_{tot} results
(μmol/1).

Variable   V

No. of labs   5
mean   8.79
s.d.   1.72
minimum   7.06
maximum   11.70

Laboratory Number
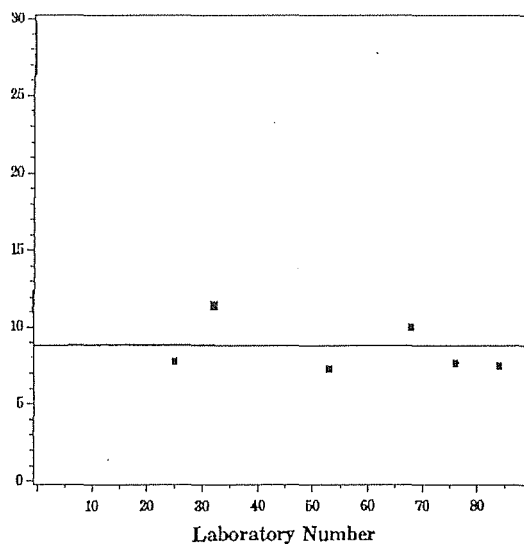
Fig. 24. Scatter plot for
P N_{tot} results

Table 20. Summary of the
P N_{tot} results
(μmol/1).

Variable   P

No. of labs   5
mean   8.43
s.d.   2.93
minimum   2.73
maximum   11.30

(see also page 22)

Laboratory Number
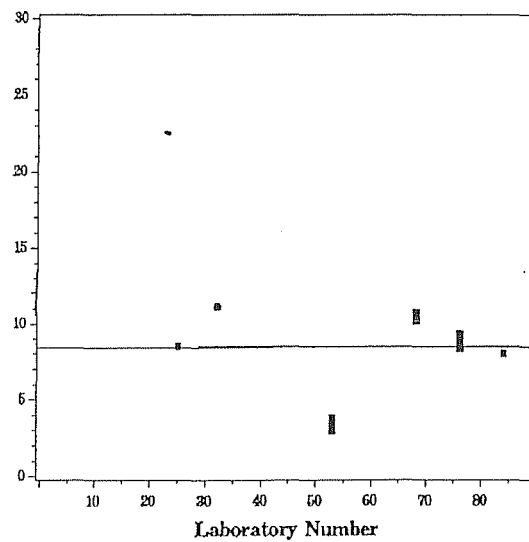
TOTAL PHOSPHORUS

Four laboratories submitted results for total phosphorus.

Laboratory 84 reported results for DOP (dissolved organic phosphorus); although not strictly equivalent, they have been included in the set of P_{tot} data, assuming $P_{tot} = PO_4 + DOP$.

Again, all four sample types can be considered.

Fig. 25. Scatter plot for C/D P_{tot} results

Table 21. Summary of the C/D P_{tot} results (μmol/1).

Variable	C/D
No. of labs	5
mean	1.25
s.d.	0.19
minimum	1.03
maximum	1.52
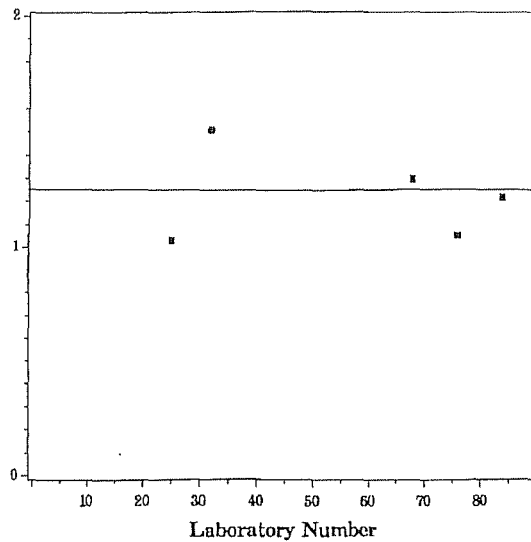
Fig. 26. Scatter plot for T P_{tot} results

Table 22. Summary of the T P_{tot} results (μmol/1).

Variable	T
No. of labs	5
mean	0.72
s.d.	0.20
minimum	0.48
maximum	0.97
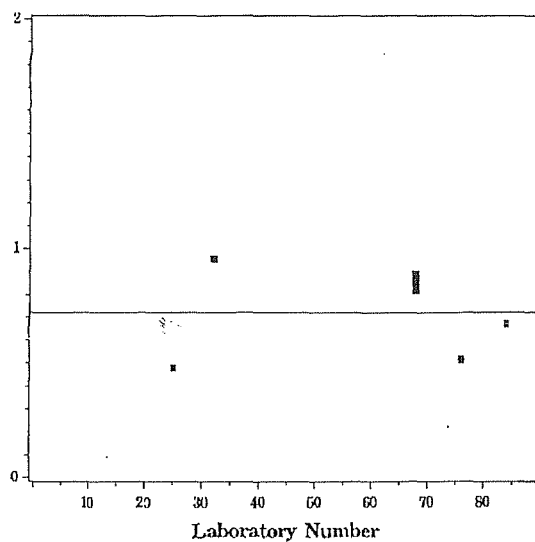
TOTAL PHOSPHORUS (continued)

Fig. 27. Scatter plot for
V P_{tot} results

Table 23. Summary of the
V P_{tot} results
(μmol/1).

Variable   V

No. of labs   5
mean   0.18
s.d.   0.13
minimum   0.00
maximum   0.30
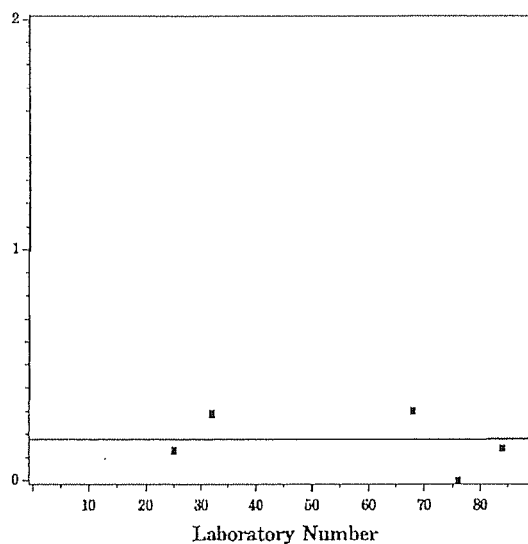
Fig. 28. Scatter plot for
P_{tot} results

Table 24. Summary of the
P P_{tot} results
(μmol/1).

Variable   P

No. of labs   5
mean   0.20
s.d.   0.15
minimum   0.00
maximum   0.40

TOTAL NITROGEN

Fig. 29. Scatter plot for
N_{tot} T,V,P vs C/D

Laboratories have a tendency
to produce systematically low
or high results in all four
samples
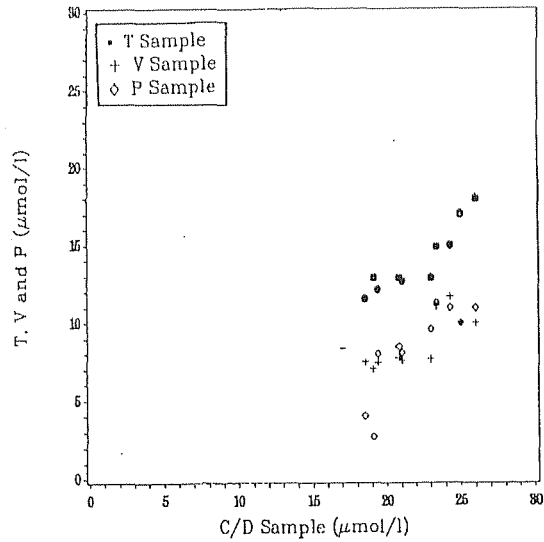
TOTAL PHOSPHORUS

Fig. 30. Scatter plot for
P_{tot} T,V,P vs C/D

As for N_{tot} laboratories
have a tendency to produce
systematically low or high
results in all four samples.
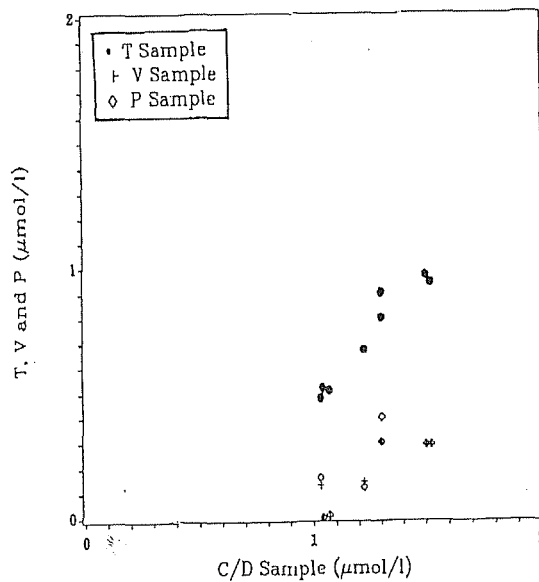
DISCUSSION

Nitrate+nitrite

Results for the C/D and T samples approximate to a Normal distribution, deviations from the mean being fairly equally spread above and below the mean. The general tendency illustrated by the Youden plot (fig 5), shows that laboratories exhibit relative systematic errors, which suggests that these deviations may be due mainly to errors in calibration.

Possible sample deterioration due to biological action was considered in cases where the interval between the first and second analyses extended to several weeks. Six laboratories reported two analyses separated by three weeks or more (labs 23, 24, 27, 42, 78 and 82) and, since there is no systematic difference between their two results (either in nitrate+nitrite or phosphate), samples can be considered unaltered during storage following the first opening of the bottles.

Phosphate

Results for the C/D and T samples show a marked skewness, the most serious deviations being on the high side of the means. Three possible reasons for this bias are proposed and discussed.

        a) Random contamination during analysis (any sample).

        b) Interference from silicate. (C/D samples, especially)
           (see Annex 3)

        c) Continuous-flow 'system-bias' (all samples).

a) Random contamination: It is well known that the determination of phosphate at natural sea water concentrations is generally more susceptible (than nitrate) to contamination due to handling, etc. This is a highly probable practical explanation for the C/D phosphate day-2 result of 1.99 from lab 18, whose phosphate results are otherwise acceptable.

b) Colorimetric interference from abnormally high silicate concentrations: There is, of course, no reference value for the phosphate concentration in the C/D samples at the time of bottling, but the mode (1.13) agrees well with the 1972 GEOSECS value for 1000 metres depth at Station 11 (the nearest to the origin of these samples. See Annex 4a).

This suggests that there is no serious general problem of silicate interference in participants' phosphate methods. This is in accord with Murphy and Riley's claim that their method is unaffected by the presence of 100 μmol/l silicate, and with Koroleff's similar claim for 200 μmol/l (see Grasshoff, K. et al. Methods of Seawater Analysis, 2nd Edition p127.)

C/D samples, at the time they were analysed by participants, had been in glass bottles for around 12 months and probably contained 400 ± 100 μmol/l depending on individual time, temperature and transit regimes after their dispatch from IFREMER, Brest.

However, individual phosphate methods substantially at variance with Koroleff's recommendations (see Annex 3) may well be more susceptible than 'normal' methods, and there is some evidence to suggest that lab 75's phosphate results include bias proportional to silicate content.

Lab 75's results	C/D	T	V	P
reported phosphate (mean)	1.83	0.70	0.05	<0.03
reported silicate (mean)	386	77	12.9	0.56
approximate bias	0.7	0.15	0.05	nil

The phosphate method used by Lab 75 differs markedly from those of other participants in that it uses hydrochloric rather than sulphuric acid, and at exceptionally high concentration.

One participant applied a correction for silicate to his phosphate results although his uncorrected results gave better agreement with the consensus concentrations than did the corrected versions.

The investigation of the effect of elevated levels of silicate on an individual phosphate method is good laboratory practice but the possibility of phosphate impurity in silicate salts, at concentrations capable of confusing the issue, must be considered.

To be certain the phosphate content of a silicate solution were acceptably low, would require a determination of that phosphate, and by a technique totally unrelated to the colorimetric method in question. (It is possible that some contradictory claims in the literature can be explained in these terms.)

It remains likely that within the range of colorimetric methods described in Annex 3, some will be more susceptible than others to interference from this source.

c) Continuous-flow 'system-bias': There are two possible contributing effects, both of which produce positive bias in saline samples. The extent of the bias is related to salinity, and the implications are most serious for high salinity samples that are low in phosphate; it follows that samples V and P are particularly susceptible.

    (i) Refraction   (ii) Precipitation

 i) Absorbance due to Refraction: This effect was described by Atlas et al. It is well known, widely appreciated, and need not be re-described here.

Details can be found in - A practical manual for use of the Technicon AutoAnalyser in sea water nutrient analysis: Revised Tech. Rep. 215 (1971) Oregon State Univ., 47 pp.

ii) Absorbance due to Precipitation: Precipitation can result from the use (or over-use) of certain wetting agents, particularly when the flow-cell effluent has a high sample concentration and that sample has a high salinity. The resultant light-scatter masquerades as an absorbance due to phosphate in the sample. Loder and Glibert drew attention to this in 1976 as did Grasshoff in 1983. Both specifically mentioned Levor IV but other wetting agents may be implicated, depending on the concentrations used.

The inclusion of samples V and P in this exercise, both effectively 'blanks' for phosphate (and nitrate), has proved useful in identifying labs that are possibly experiencing problems from this effect (particularly labs 22 and 66, see also the discussion section on silicate).

The details of phosphate methods supplied by participants (see Annex 3) contain evidence of the continued widespread use of levor IV despite these warnings.

There is a suspicion that some operators may use it at higher than the recommended concentrations 'to help things run more smoothly', unaware that the precise concentration may be critical.

Loder, T.C. and Glibert, P.M., 7th Technicon International Congress, (1976). Grasshoff, K., et al., Methods of Seawater Analysis 2nd Edition p369.

Silicate

Sample P, being 'almost-blank' for silicate, identified several labs with appreciable positive bias, 8, 17, 22, 27, 32, 34, 42, 45, 53.

The mode, 0.60 µmol/l is considered the best estimate of the silicate content of this sample.

Participants were not asked to supply details of their silicate procedure, but on the basis that those labs known to be using continuous-flow methods for phosphate are highly likely to use continuous-flow for silicate, it is also likely that the same wetting agent is preferred in both determinations. As an example it has been confirmed that lab 45 uses Levor IV in both, and it is one of the most seriously affected in the silicate determination.

Nitrite

Due to their method of preparation, P, V and T samples were assumed to be blank samples for nitrite. The reported values agree with this assumption: mean and standard deviation are close to the detection limit of current analytical procedures. Nevertheless, for these three samples, each laboratory reported similar values within the analytical precision of the method, which suggests a low but systematic blank bias for certain participants. These participants are invited to check their reagent blank or refractive index correction (in the case of cfa).

For C/D samples, there is some evidence for significant, but variable concentrations in some samples, the origin of which is unknown (contamination, biological activity). Due to this high variability, compared to T, V and P samples, statistics are inappropriate and the mean should not be considered a consensus concentration.

Ammonia

The large range of ammonia concentrations reported by the participants greatly exceeds that considered acceptable for unpolluted sea water. However, ammonia is recognised as a compound difficult to measure at low marine levels due to the risk of atmospheric contamination and analytical constraints mainly related to the determination of the blank.

The intercomparison samples, although not specially treated for ammonia stabilisation, should normally (bearing in mind their origin) contain little or no ammonia. If significant concentrations were present in the samples, either originally or as a consequence of any biochemical process, the results could be expected to be randomly distributed, but means and standard deviations are very similar for all four types of samples (C/D, T, V and P), as shown in Tables 13 to 16.

Consequently, revised statistics have been obtained for each series of four samples (C/D, T, V and P) for each laboratory except labs 8, 4 and 68 (too high detection limit), lab 26 (too high values) and lab 13 (too high standard deviation). In the remaining set of 20, 15 have a standard deviation $< \pm 0.20$ $\mu mol/l$ and 9 have a standard deviation $< \pm 0.10$ $\mu mol/l$, a reasonable figure in the range 0.03-1.58 $\mu mol/l$. For the whole set of 20 the standard deviation is ± 0.17.

The inter-sample variability for each laboratory thus appears significantly lower than the inter-laboratory variability for each type of sample.
The logical deduction is that the samples actually contain little or no ammonia and that the inter-laboratory variability originates from systematic analytical bias: consequently, most participants exhibit significant reagent blanks.

Laboratories are therefore invited to check their reagent blank according to the procedures described in original papers or in current handbooks of sea water analysis (with due attention to the refractive index correction, when using cfa).

Total nitrogen

Total N has been suspected of being greatly underestimated by persulphate methods (Suzuki and Sugimura, 1985), but, since this view was recently challenged (Walsh, 1989), the results are assumed to be comparable, whatever the method used. Contrary to inorganic forms, organic N (mainly composed of highly refractory material) is never depleted in sea water, consequently the concentration of N_{tot} is always considerably higher (by several micromoles per litre) than inorganic nitrogen ($NO_3 + NO_2 + NH_4$). All laboratories are consistent in this respect.

Nevertheless there is a tendency towards systematically high or low values, consistent with the nitrate+nitrite results submitted by these laboratories (again suggesting calibration problems).

SUZUKI, Y. and SUGIMURA, Y., Mar. Chem. 16, 83-97 (1985)
WALSH, T.W., Mar. Chem. 26, 295-311 (1989).

Total phosphorus

As in the case of N_{tot}, the organic forms of phosphorus are not decomposed and consequently in sea water, almost invariably, $P_{tot} > PO_4$.
Laboratory 76 reported results inconsistent with this assumption.

Laboratories tend to obtain systematically high or low concentrations in all sample types. Although the phosphate determination exhibits the same tendency, its range is insufficient to explain the observed P_{tot} differences. Since the inter-laboratory differences ($P_{tot}-PO_4$) vary by factors greater than 10, it can be assumed that the oxidation step alone is not responsible for these factors, and that the blank determination is again suspect.

STATISTICS

Following the February 1990 meeting of the Marine Chemistry Working Group, participants were sent a letter containing a brief summary of the progress of the Intercomparison Exercise and information on the nitrate+nitrite and phosphate concentrations in the four test samples. (see).

Their attention was drawn particularly to the phosphate concentrations claimed for C/D (1.199) and T (0.574) which were described as 'simple means', and that subsequent statistical treatment was expected to produce somewhat lower concentrations after the removal of results that were subject to predominantly positive bias.

It was not stated in the letter, but these simple means were not quite 'full-set' means since the results from lab 8 had been excluded by the coordinators on the basis that they were considered to be 'obvious outliers'.

(This explains the difference between the simple means circulated to participants in March 1990 and the full-set means shown on pages 8 and 9.)

The two statistical treatments described in Annex 5 are by no means the only methods that can be considered applicable to the data, but, despite their differing philosophies, they produce almost identical results; i.e. the robust estimator's consensus [Annex 5a] is very similar to the mean of the 'consistent laboratories',the modal group identified by cluster analysis in [Annex 5b].

For nitrate there are 51 consistent laboratories, for phosphate there are 58.

48 laboratories (70% of respondents) are consistent in both nitrate and phosphate, and these may be described as **Fully Consistent Laboratories**.

Laboratories **excluded** from this group are as follows –

a) on the basis of unacceptable nitrate+nitrite results –

    laboratories: 5 8 18 22 23 32 38 45 50 53 54 55 66 71 75 78

b) on the basis of unacceptable phosphate results –

    laboratories: 3 8 18 22 23 61 63 65 66 75

Approximately 10% of the laboratories were excluded by both a) and b).

A **distinguished group** of 12 laboratories (18% of the respondents) reported all eight individual replicates (for C/D and T, nitrate and phosphate) within one (consistent labs') standard deviation of the consensus concentrations.

    laboratories: 7 19 21 26 28 31 36 57 59 72 76 84

SUMMARY OF STATISTICS

The following table summarises:

a)   for nitrate+nitrite and phosphate

consensus concentrations for C/D and T samples, and anticipated results for
the V and P samples (based on the coordinators' knowledge of their method of
preparation and rigorous controls in witness samples).

b)   for silicate

consensus (mode) concentration for the P sample, and range for the C/D, T and
V samples, depending on date of analysis. (The lower and upper limits should
correspond approximately to April '89 and October '89 respectively, though
some higher concentrations than expected may be the result of storage at a
high ambient temperature.)

	nitrate+nitrite	phosphate	silicate(approx)
C/D	16.19	1.14	300-500
T	6.90	0.55	80-120
V	<0.1	<0.02	9-30
P	<0.1	<0.02	0.60

Consensus concentrations ±'total' standard deviations of the consistent
laboratories should be seen as realistically achievable, consequently
participants falling short of this level of analytical performance are urged
to examine their results closely and consider whether any of their techniques
may be in need of attention.

COORDINATORS' COMMENTS ON STATISTICS

Modern statistical techniques greatly assist in the interpretation of data
but they necessarily operate without the benefit of additional information
available only to the analytical chemist.
The following two examples of information of this kind are particularly rele-
vant to the pursuit of 'true' phosphate concentrations in samples C/D and T.

Example 1.   In the interests of simplicity, the statistical treatments
took no account of the relationship that samples V and P were effectively
'blanks' with respect to samples C/D and T, consequently where laboratories
show a consistent appreciable bias for V and P, their C/D and T results must
be assumed to be influenced by this known bias.

Example 2.   In the consensus estimation, both statistical techniques began
by using the mean of each lab's day-1 and day-2 pairs. In the particular
case of lab 18 (as described on page 23), use of the mean of 1.615 (derived
from 1.24 and 1.99) would be unacceptable to the analytical chemist.

Both of these examples suggest that the 'true' phosphate concentrations in samples C/D and T are slightly lower than the statistically derived consensus concentrations.

Future exercises, should they wish to avoid the estimation of consensus concentrations, will need to be Intercalibrations (rather than Intercomparisons), but this would require a supply of suitable Standard Reference Materials.

COORDINATORS' CONCLUDING REMARKS

1. The coordinators wish to express their concern over the lack of numerical appreciation shown by some participants in the reporting of their results.

a) In the context of this exercise, '0' can only be interpreted as <1, and, given that participants were informed that no sample contained phosphate at a concentration >2 µmol/l, a '0' result is clearly unacceptable.
0.0 and 0.00 we are inclined to accept, though <0.1 and <0.01 respectively would be preferred.

b) The precision and sensitivity implied by a nutrient concentration reported as, for example, 456.78 are totally unrealistic for colorimetric techniques applied to the analysis of seawater.
Reporting of this kind can only serve to detract from the general credibility of a laboratory, nevertheless it appears to be considered acceptable by more than 10% of the respondents.

2. The coordinators are satisfied that their decision to include more than one 'blank' was a worthwhile departure from established practice as this indicated the existence of serious biases that might otherwise have escaped attention.

3. The review of phosphate methods (Annex 3) demonstrated that earlier fears of a serious silicate interference problem in the phosphate determination were apparently unfounded; nevertheless, the information gathered gave strong clues to the origins of biases from other sources in both phosphate and silicate techniques.

4. Annex 3 also shows that 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.
Continuous-flow users are urged to examine their methods closely and assess how well they adhere to the conditions specified by the parent manual methods.

5. Finally, the coordinators, on behalf of the Marine Chemistry Working Group, wish to express their appreciation to all participants whose prompt responses made it possible for this work to be completed and reported within the originally proposed timescale.

Annex 1

List of Participants

1.	DIFMR	Charlottenlund	DK
2.	NERI	Charlottenlund	DK
3.	VBBK	Malmo	SWE
4.	IFM	Warnemünde	GDR
5.	SMHI	Goteborg	SWE
6.	UG	Goteborg	SWE
7.	IFM	Kiel	FRG
8.	LWKS-H	Kiel	FRG
9.NR	DHI	Hamburg	FRG
10.	UH	Hamburg	FRG
11.	RSAC	Fiskebackskil	SWE
12.	KML	Uddevalla	SWE
13.	IPMR	Bremerhaven	FRG
14.	IMWM	Gdynia	POL
15.	SFI	Gdynia	POL
16.NR	UG	Gdansk	POL
17.	NIWR	Oslo	NOR
18.	HAB	Bromma	SWE
19.	SNV	Solna	SWE
20.	ASKÖ	Stockholm	SWE
21.	NIOZ	Texel	NL
22.	NOASR	Den Helder	NL
23.	IMR	Bergen	NOR
24.	DIHR	Yerseke	NL
25.	RIJKS	Middelburg	NL
26.	ULB	Bruxelles	BEL
27.	MUMM	Oostende	BEL
28.	MAFF	Lowestoft	UK
29.	UEA	Norwich	UK
30.	ITE	Tallinn	USSR
31.	IAG	Tallinn	USSR
32.	FIMR	Helsinki	FIN
33.	DAFS	Aberdeen	UK
34.	BAS	Cambridge	UK
35.	FRBP	Edinburgh	UK
36.	NMRI	Hornefors	SWE
37.NR	IOS	Wormley	UK
38.	HRPB	Dingwall	UK
39.	LM	Rouen	F
40.	CRPB	Glasgow	UK
41.NR	NWWA	Warrington	UK
42.	SMBA	Oban	UK
43.NR	UCNW	Menai Bridge	UK
44.NR	WNRA	Caernarfon	UK
45.	WNRA	Llanelli	UK
46.NR	WWA	Bridgend	UK
47.	INTECHMER	Cherbourg	F
48.NR	DANI	Coleraine	UK
49.	UD	Dublin	IRL
50.	NIPPCR	Dublin	IRL
51.	DC	Dublin	IRL
52.NR	EOLAS	Shannon	IRL

53.	HS	Torshavn	Farö
54.	PML	Plymouth	UK
55.	UCG	Galway	IRL
56.NR	UCG	Galway	IRL
57.	IFREMER	Nantes	F
58.NR	SOBMR	Roscoff	F
59.	IFREMER	Brest	F
60.	UBO	Brest	F
61.	LM	Brest	F
62.	FORE	Six-Fours-Les-Plages	F
63.	LM	Bordeaux	F
64.	MRI	Reykjavik	ICE
65.	IOE	Palma	ESP
66.	IEO	La Coruna	ESP
67.NR	UA	Aviero	POR
68.	IH	Lisboa	POR
69.NR	INIP	Lisboa	POR
70.	METU	Icel	TUR
71.	IEO	Tenerif	ESP
72.	BIO	Dartmouth NS	CAN
73.	MLI	Mont-Joli Q	CAN
74.NR	UNH	Durham NH	USA
75.	NMFS	Highlands NJ	USA
76.	UM	Solomons MD	USA
77.	BBSR	Ferry Reach	Bermuda
78.	NOAA	Miami FL	USA
79.	SFRI	Cape Town	RSA
80.NR	DEMAST	Stellenbosch	RSA
81.NR	FI	Winnipeg MA	CAN
82.	IOS	Sidney BC	CAN
83.	OSU	Corvallis ON	USA
84.	UH	Honolulu HI	USA
85.NR	NLFW	Hildesheim	FRG

All 85 laboratories were sent samples.

Those that submitted no results are marked NR.

Dear Colleague,

ICES Marine Chemistry Working Group (MCWG)

INTERCOMPARISON EXERCISE - NUTRIENTS

You will find enclosed 4 samples sent to you from IFREMER, Centre de Brest, B.P. 70, 29263 Plouzane, France.

If the samples appear to be damaged in any way, please contact Alain Aminot at IFREMER without delay. (Telephone 98224361, FAX 98050473, TELEX 940627).

Prior to the analysis, these samples should be stored in a cool dark place, but not frozen.

Analyse the samples according to the instructions given on the attached sheet.

Record all relevant information and results on the reporting form provided and sent it to Dr M Perttila **before September 1989.**

The results of all analyses will be evaluated by Dr Pertilla before the end of 1989 and a preliminary report on this exercise will be submitted to the 1990 meeting of the MCWG.

A copy of the preliminary report will be sent to each participant.

Participants should be aware that when the results are published, their results will be readily identifiable by them **and by any other reader.**

On behalf of the MCWG we would like to thank you for participating in this exercise.

Yours sincerely,

D S Kirkwood & A Aminot

Enc

ICES MARINE CHEMISTRY WORKING GROUP : INTERCOMPARISON EXERCISE - NUTRIENTS

Instructions for Analysing Samples

1. Before opening sample bottles, on day-1 of the analysis --
    Allow the samples to attain laboratory temperature.
    Each sample has been pre-weighed, and, as a check against leakage or
    evaporative losses, record the gross bottle weights to the nearest
    10mg, and the date of the analysis.

2. Opening the sample bottles --
    Since two determinations, separated in time, must be performed, take
    great care when opening the samples and avoid unnecessary finger contact
    with the necks of the bottles and their closures.

3. After opening the sample bottles --
    Re-cap the sample bottles immediately after use, and, in the interval
    between the first and second analysis, store in a refrigerator (but do
    not freeze).

4. For each determination undertaken, we require two replicates. To keep
    the statistics of the exercise as simple and as meaningful as possible,
    we ask you to perform only TWO replicates, no more and no less, and to
    report both values.

5. It is recognised that participants with automated and miniaturised
    techniques may be able to perform an extended range of determinations.
    We invite these participants to submit results on any determination that
    is considered 'routine' in their laboratory, for example, 'total' - N and
    'total' - P, nitrite, silicate and ammonia. Participants using manual
    techniques requiring relatively large sample volumes may have to limit
    their range of determinations according to the amount of sample available
    to them, but these participants particularly are reminded that (NO_3+NO_2)
    and phosphate are the priority determinations in this exercise.

DEFINITION OF TERMS

phosphate : dissolved reactive orthophosphate

(NO_3+NO_2) = dissolved reactive (nitrate + nitrite)

silicate   : dissolved reactive silicate

replicates : determinations significantly separated in time, preferably by at
least 24 hours, each using freshly prepared reagent solutions and freshly pre-
pared calibration solutions.
(Successive Autoanalyser 'clone' peaks measured against a single calibration
peak are not considered to be replicates in the context of this exercise).

Note - The samples do not require filtration.
       No preservatives have been added.
       All samples can be assumed to have a nominal salinity of 35 PSS.
       Phosphate concentrations are believed to be < 2 micromoles per litre
       (NO_3+NO_2) concentrations are believed to be < 30 micromoles per litre
       The sample in the rectangular glass bottle can be assumed to have a
       silicate content in excess of 200 micromoles per litre, due to prolonged
       storage (10 months). This may have implications for its phosphate
       determination.

RESULTS OF THE ICES NUTRIENTS INTERCOMPARISON EXERCISE 1989

NAME AND ADDRESS OF LABORATORY :

SAMPLES RECEIVED ON :

SAMPLES ANALYSED ON :

All results must be expressed in micromoles per litre (μmol/l)

Sample reference				
Weight				
(NO_3+NO_2) : 1st 2nd				
Phosphate : 1st 2nd				
_____ : 1st 2nd				
_____ : 1st 2nd				
_____ : 1st 2nd				
_____ : 1st 2nd				
_____ : 1st 2nd				

This form must be sent to Dr Matti PERTTILA, Finnish Institute of Marine Research, P.O. Box 33, SF-00931 HELSINKI, FINLAND.

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
MARINE CHEMISTRY WORKING GROUP - INTERCOMPARISON EXERCISE - NUTRIENTS 1989

Following the MCWG's discussion of the draft Report on this exercise at its
recent meeting in Copenhagen, I am now in a position to publicise some of its
findings.

The full report (approximately 70 pages) is due to be considered by the ICES
Advisory Committee on Marine Pollution at its forthcoming meeting in June
1990, after which, publication as an ICES Cooperative Research Report is
anticipated, meanwhile, here is a brief summary.

A total of 85 laboratories were sent samples, and 68 returned results.

A copy of the results submitted by your laboratory is enclosed.

The four samples consisted of a natural oceanic water labelled C or D, a
natural shelf-seas sample T, and two further samples V and P, both essentially
"blanks" consisting of the same nutrients-depleted water, but in bottles of
different composition, V (verre) = glass, and P = polypropylene.

Mean concentrations (in µmol/1), and standard deviations, for
(nitrate+nitrite) and phosphate, for the C/D and T samples, reported by
participants are:

	(nitrate+nitrite)	s.d.	phosphate	s.d.
C/D	15.99	1.53	1.199	0.198
T	6.87	0.75	0.574	0.122

Note: These concentrations are simple means and should not be treated as
'final'. The phosphate data, in particular, show a marked skewness resulting
from positive analytical bias and it is anticipated that further statistical
treatment will produce consensus concentrations slightly but significantly
lower than those shown.

Concentrations in samples V and P should be below the detection limits of
normal seawater techniques (e.g. nitrate+nitrite <0.1 and phosphate <0.02).

The coordinators of the exercise are satisfied that their inclusion of two
'blanks', a departure from established practice, has produced much valuable
information on analytical bias and other interesting effects.

The probable sources of bias are discussed in some detail in the report, as
are the origin and preparation methods for the samples, a survey of the
phosphate methods used by participants, and possible interference from
silicate.

The report contains a full listing of results for these and other
determinands, statistical treatments, graphics, a list of participating
laboratories, and a means for the reader to identify the results of each
laboratory.

The MCWG wishes to thank all participants for supporting this exercise and
trusts that they will find the report a useful document when it becomes
available from ICES, Palaegade 2-4,
DK-1261 Copenhagen K, DENMARK.

Yours sincerely

Don Kirkwood
on behalf of D Kirkwood, MAFF Lowestoft UK
              A Aminot, IFREMER Brest, France
              M Perttila, FIMR Helsinki, Finland

Annex 2   PARTICIPANTS' RESULTS   (nitrate+nitrite)

lab no.	C/D	T	V	P
1	17.25	6.95	<0.2	<0.2
	17.00	7.15	—	—
2	16.29	6.73	0.00	0.00
	15.60	6.66	0.07	0.06
3	16.21	7.14	0.31	0.30
	16.00	7.21	<0.21	0.25
4	18.10	7.02	0.11	0.07
	18.23	6.94	0.11	0.09
5	12.59	5.91	0.09	0.07
	12.86	5.96	0.08	0.09
6	15.6	6.9	—	—
	15.6	6.8	0.0	0.0
7	16.11	6.90	<0.1	<0.1
	16.36	6.90	<0.1	<0.1
8	17.8	8.21	<1.8	<1.8
	17.9	8.00	<1.8	<1.8
10	16.30	6.86	0.01	0.08
	16.27	6.81	0.03	0.13
11	14.75	6.93	0.025	0.005
	14.95	6.25	0.014	0.005
12	17.6	7.5	<0.36	<0.36
	15.4	6.5	<0.36	<0.36
13	16.8	7.2	0	0.02
	16.7	7.3	0.02	0.03
14	16.20	6.88	0.12	—
	16.22	6.90	0.13	—
15	16.74	7.42	0.13	—
	16.74	7.43	0.12	—
17	16.1	7.1	<0.1	0.2
	16.4	7.3	<0.1	0.3
18	14.1	6.02	0.19	0.19
	14.8	6.32	0.19	0.19
19	16.79	7.07	<0.4	<0.4
	16.93	7.07	<0.4	<0.4
20	16.7	7.3	0.09	—
	16.2	7.0	0.07	—
21	16.43	6.98	0.16	0.16
	16.05	6.98	0.12	0.25
22	18.5	8.3	<0.44	0.44
	18.8	8.9	<0.44	<0.44
23	16.4	—	0.0	—
	13.5	—	0.0	—
24	16.4	7.1	0	0
	16.4	7.1	0	0
25	16.2	6.71	<0.07	<0.07
	16.1	6.71	<0.07	<0.07
26	16.9	6.9	1.2	0.18
	16.8	6.6	1.25	0.17
27	18.5	7.4	<0.05	<0.07
	17.8	7.3	<0.05	<0.07
28	15.5	6.9	<0.1	<0.1
	15.9	7.1	<0.1	<0.1
29	16.0	7.45	0.15	0.1
	16.2	7.30	0.12	0.1
30	14.4	6.5	0.0	0.0
	14.6	6.6	0.0	0.0
31	16.8	7.2	—	0.3
	16.4	7.2	—	0.3
32	15.16	5.88	0.13	0.13
	15.40	5.97	0.13	0.19
33	15.3	6.6	0.2	0.2
	15.9	6.3	<0.2	<0.2
34	16.58	7.12	0	0
	17.48	7.42	0	0
35	18.6	8.0	0.10	0.12
	16.2	7.0	0	0

36	15.82	6.939	<0.052	<0.052
	15.80	6.909	<0.052	<0.052
38	13.071	6.214	0.143	<0.1
	13.571	5.428	0.143	0.143
39	16.97	7.45	<0.05	<0.05
	16.59	6.78	<0.05	<0.05
40	15.7	6.93	<0.07	0.07
	15.6	6.69	0.09	0.07
42	16.30	6.35	0.20	0.21
	16.10	6.51	0.22	0.22
45	16.1	4.48	0.21	<0.14
	17.0	4.79	<0.14	<0.14
47	16.6	6.5	0	0
	16.2	6.7	0	0
49	16.4	6.9	0	0
	15.9	6.8	0	0
50	21.071	9.643	0.357	0.571
	20.357	9.500	0.571	0.571
53	14.30	5.83	<1.1	<1.1
	13.86	5.27	<1.1	<1.1
54	–	8.51	<0.05	<0.05
	–	8.49	<0.05	<0.05
55	9.54	5.08	0.17	0.13
	9.34	4.22	0.04	0.02
57	16.2	6.8	<0.1	<0.1
	16.3	6.6	<0.1	<0.1
59	16.6	7.0	<0.05	<0.05
	16.6	7.0	<0.05	<0.05
60	16.35	6.9	0.0	0.0
	16.3	6.9	0.0	0.0
61	16.62	7.22	0.49	0.43
	16.60	7.18	0.30	0.29
62	15.00	6.56	0.00	0.00
	15.00	6.62	0.00	0.00
63	17.1	6.6	<0.2	<0.2
	17.5	6.9	<0.2	<0.2
64	16.4	7.3	0.3	0.3
	16.7	7.4	0.3	0.3
65	16.71	7.00	0.11	0.09
	16.14	6.99	0.10	0.08
66	12.88	5.91	–	–
	12.67	5.64	–	–
68	17	7.2	<0.7	<0.7
	17	7.6	<0.7	<0.7
70	16.07	7.10	0.10	0.10
	15.95	6.98	0.11	0.11
71	13.0	6.4	0.1	0.1
	13.8	6.5	0.1	0.1
72	16.36	6.93	<0.06	<0.06
	15.93	6.85	<0.06	<0.06
73	16.7	7.3	<0.3	<0.3
	16.8	7.3	<0.3	<0.3
75	14.06	6.34	<0.05	<0.05
	13.72	6.22	<0.05	<0.05
76	16.6	6.99	0.07	0.07
	16.8	7.14	0.09	0.08
77	15.95	7.07	<0.05	<0.05
	–	–	–	–
78	14.75	5.83	0	0
	14.66	5.36	0	0
79	16.04	6.53	0	0
	16.14	6.64	0	0
82	16.3	6.9	0.0	0.0
	15.6	6.4	0.0	0.0
83	15.2	7.3	0.0	0.0
	15.3	7.5	0.0	0.0
84	16.13	6.90	0.08	0.01
	16.14	6.87	0.05	0.02

PARTICIPANTS' RESULTS   (phosphate)

lab no.	C/D	T	V	P
1	1.25	0.50	<0.1	<0.1
	1.25	0.54	—	—
2	1.16	0.53	0.00	0.00
	1.25	0.53	0.00	0.00
3	1.10	0.94	<0.07	<0.07
	1.13	0.97	<0.07	<0.07
4	1.19	0.58	0.009	0.009
	1.20	0.59	0.019	0.019
5	1.13	0.53	<0.05	<0.05
	1.19	0.55	<0.05	<0.05
6	1.19	0.57	0.0	0.0
	—	—	0.0	0.0
7	1.08	0.53	<0.02	0.02
	1.09	0.51	<0.02	<0.02
8	9.32	2.96	<0.4	<0.4
	8.23	2.89	<0.4	<0.4
10	1.07	0.47	0.02	0.03
	1.20	0.54	0.02	0.05
11	1.10	0.696	0.010	0.012
	1.06	0.693	0.025	0.04
12	1.06	0.52	<0.03	<0.03
	1.13	0.55	<0.03	<0.03
13	1.3	0.62	0	0.01
	1.2	0.61	0	0.02
14	1.08	0.45	<0.02	—
	1.10	0.46	<0.02	—
15	1.13	0.55	0.0	—
	1.13	0.56	0.0	—
17	1.13	0.52	<0.02	<0.02
	1.15	0.53	<0.02	<0.02
18	1.24	0.53	0.026	0.026
	1.99	0.53	0.034	0.026
19	1.19	0.52	<0.03	<0.03
	1.19	0.52	<0.03	<0.03
20	1.11	0.57	0.003	0.003
	1.09	0.57	0.003	0.01
21	1.12	0.55	0.05	0.00
	1.13	0.57	0.03	0.04
22	1.6	1.1	0.4	0.5
	1.8	1.2	0.5	0.9
23	1.46	—	0.07	—
	1.14	—	0.05	—
24	1.13	0.42	0.16	0.38
	1.10	0.42	0.35	0.48
25	1.03	0.42	<0.03	<0.03
	1.02	0.42	<0.03	<0.03
26	1.1	0.5	0.07	0.05
	1.1	0.5	0.05	0.04
27	1.3	0.68	<0.08	<0.05
	1.2	0.63	<0.08	<0.05
28	1.09	0.50	<0.02	<0.02
	1.11	0.50	<0.02	<0.02
29	1.08	0.6	0.06	0.06
	1.05	0.62	0.08	0.08
30	0.85	0.50	0.00	0.00
	0.95	0.51	0.00	0.00
31	1.22	0.59	—	0
	1.19	0.55	—	0
32	1.09	0.51	0.02	0.02
	1.07	0.49	0.02	0.02
33	1.20	0.56	0.05	0.04
	1.15	0.63	0.03	0.07
34	1.10	0.55	0	0
	1.10	0.57	0	0
35	1.2	0.52	0	0
	1.3	0.56	0	0
36	1.130	0.579	<0.019	<0.019
	1.119	0.573	<0.019	<0.019

(phosphate)

38	1.097	0.517	<0.14	<0.14
	1.259	0.516	<0.14	<0.14
39	1.09	0.53	<0.05	<0.05
	0.96	0.45	<0.05	<0.05
40	1.22	0.49	<0.06	<0.06
	1.25	0.49	<0.06	<0.06
42	1.04	0.57	0.19	0.22
	1.25	0.54	0.20	0.23
45	1.16	0.44	0.09	<0.07
	1.20	0.45	0.12	<0.07
47	0.98	0.41	0	0
	1.09	0.45	0	0
49	1.25	0.48	0	0
	1.25	0.50	0	0
50	1.097	0.516	0.194	0.194
	1.000	0.613	0.194	0.194
51	1.35	0.584	0.026	0.03
	1.29	0.674	0.048	0.11
53	1.20	0.52	<0.25	<0.25
	1.10	0.50	<0.25	<0.25
54	1.12	0.62	0.08	0.09
	1.18	0.68	0.08	0.10
55	1.05	0.54	0.00	0.00
	1.09	0.55	0.02	0.00
57	1.10	0.50	0.01	0.01
	1.15	0.55	<0.01	<0.01
59	1.15	0.55	<0.005	<0.005
	1.15	0.54	<0.005	<0.005
60	1.09	0.48	0.00	0.00
	1.11	0.52	0.00	0.01
61	1.89	0.63	<0.05	<0.05
	1.68	0.63	<0.05	<0.05
62	1.07	0.51	0.00	0.00
	1.00	0.54	0.00	0.00
63	1.69	0.73	0.28	<0.02
	1.54	0.71	0.15	<0.02
64	1.13	0.56	0.00	0.01
	1.12	0.55	0.00	0.00
65	1.39	0.69	0.14	0.15
	1.45	0.75	0.17	0.16
66	1.91	1.003	0.21	0.21
	2.06	1.008	0.22	0.23
68	1.13	0.53	<0.03	<0.03
	1.10	0.54	<0.03	<0.03
70	1.28	0.61	<0.15	<0.15
	1.22	0.65	<0.15	<0.15
71	1.11	0.59	0.08	0.08
	1.17	0.72	0.15	0.21
72	1.070	0.549	0.070	0.088
	1.062	0.546	0.051	0.083
73	0.90	0.48	<0.04	<0.04
	0.89	0.45	<0.04	<0.04
75	1.775	0.698	0.054	<0.03
	1.883	0.711	0.045	<0.03
76	1.18	0.60	0.02	0.02
	1.16	0.55	0.01	0.01
77	0.94	0.62	0.06	<0.03
	1.42	0.65	<0.03	<0.03
78	1.2	0.58	0	<0.05
	1.2	0.51	0	<0.05
79	1.18	0.48	0	0
	1.08	0.49	0	0
82	1.11	0.53	0.00	0.00
	1.13	0.53	0.00	0.00
83	1.26	0.55	0.05	0.04
	1.32	0.55	0.00	0.01
84	1.19	0.57	0.01	0.03
	1.19	0.57	0.01	0.01

PARTICIPANTS' RESULTS   (silicate)

lab no.	C/D	T	V	P
1	–	–	9.5	<0.5
	–	–	–	–
2	10.73	0.00	10.02	0.39
	–	0.18	10.14	0.39
5	–	–	–	0.63
	–	–	–	0.58
7	>180	83.2	11.7	0.62
	>180	81.9	11.5	0.74
8	358	103	<5	<5
	369	113	8.22	<5
10	394.97	106.38	9.61	0.56
	401.03	106.16	9.87	0.56
13	346.2	94.2	11.4	0.60
	–	–	11.7	0.52
14	–	–	–	0.36
	–	–	–	0.38
15	–	–	–	0.32
	–	–	–	0.34
17	349	116	15	5
	366	108	13	7
20	–	–	–	0.49
	–	–	–	0.42
21	324	105.8	9.42	0.59
	309	100.1	9.38	0.55
22	>>	>>	14.8	0.9
	>>	>>	15.5	1.6
24	>100	>100	13.90	0.64
	>100	>100	13.43	0.64
25	–	–	9.75	0.89
	–	–	9.90	0.85
27	505	103	15.2	7.4
	333.58	70.35	10.59	–
28	–	–	10.7	0.5
	395	107	11.0	0.6
32	333.8	108.2	12.2	0.94
	345.1	111.4	12.6	0:97
34	455.54	96.20	10.60	1.70
	466.66	97.59	11.38	1.47
38	499.9	94.22	13.50	0.616
	454.6	95.63	14.85	0.566
42	351.2	112.4	13.03	1.67
	365.7	110.4	12.29	1.69
45	444	84.8	19.3	10.2
	445	88.0	19.3	10.1
47	376.6	120.8	23.0	0.5
	362.4	121.5	22.4	0.6
53	–	17.97	21.28	12.99
	–	19.62	20.78	12.63
54	365	90.1	19.8	2.15
	–	–	19.6	2.17
64	502	97.7	17.0	0.6
	502	99.1	17.2	0.4
65	356.25	105.78	9.75	0.44
	370.13	107.14	10.24	0.52
68	>>25	>>25	16	0.8
	435	101	16	0.8
70	402.21	104.65	12.54	0.80
	404.02	105.20	13.05	0.80
71	–	95.5	37.2	0.6
	572.2	103.8	37.9	0.7
72	–	–	–	0.429
	–	–	–	0.420
73	397	103	9.5	0.69
	395	105	9.6	0.69
75	383.8	75.6	12.76	0.557
	389.7	77.7	13.08	0.563
76	372	96.4	10.0	1.5
	418	86.8	8.4	0.3
77	>140	90.64	27.67	1.41
	–	–	–	–
78	418.24	166	11.90	0
	410.93	157.2	11.34	0
79	>270	103.3	10.89	0
	–	–	11.16	0
82	off scale	104	12.7	0.5
	off scale	100	12.4	0.7
83	>500	106.9	34.4	0.9
	717.6	108.2	34.4	0.5
84	391.05	105.12	16.20	1.18
	397.60	106.25	16.09	0.35

PARTICIPANTS' RESULTS   (nitrite)

lab no.	C/D	T	V	P
2	0.11	0.00	0.00	0.00
	0.12	0.00	0.00	0.00
3	0.14	<0.07	<0.07	<0.07
	0.14	<0.07	<0.07	<0.07
7	0.28	0.03	<0.01	<0.01
	0.28	0.03	<0.01	<0.01
8	0.75	0.74	0.69	0.71
	0.75	0.74	0.74	0.73
10	0.04	0.04	0.02	0.03
	0.04	0.04	0.03	0.04
13	0.03	0.02	0.01	0.01
	0.02	0.03	0.01	0.01
14	0.29	0.02	<0.01	—
	0.30	0.02	<0.01	—
15	0.39	0.02	0.0	—
	—	0.01	0.0	—
21	0.08	0.00	0.02	0.02
	0.10	0.02	0.01	0.01
47	0	0	0	0
	0	0	0	0
53	0.30	<0.1	<0.1	<0.1
	0.26	<0.1	<0.1	<0.1
54	0.05	0.05	0.05	0.05
	0.05	0.05	0.05	0.05
65	0.27	0.06	0.05	0.04
	0.28	0.06	0.06	0.05
68	0.30	<0.03	<0.03	<0.03
	0.31	<0.03	<0.03	<0.03
79	0.05	0	0	0
	0.08	0	0	0
83	1.88	0.01	0.01	0.00
	1.95	0.00	0.00	0.00

(ammonia)

lab no.	C/D	T	V	P
2	0.41	0.55	0.68	0.22
	0.23	0.39	0.08	0.06
7	<0.02	0.06	<0.02	<0.02
	<0.02	0.09	<0.02	<0.02
8	1.00	1.06	<1	<1
	1.59	1.76	1.62	1.64
10	0.98	1.07	0.83	1.22
	0.51	0.47	0.44	1.01
13	0.37	0.05	0	1.43
	0.38	0.10	0	1.44
17	0.3	0.4	0.2	0.3
	0.2	0.4	0.2	0.3
21	0.89	1.16	1.05	0.85
	0.67	1.35	0.96	1.38
25	0.21	0.29	0.29	0.21
	0.21	0.36	0.21	0.21
26	10.6	2.5	2.1	1.8
	9.2	2.6	1.6	1.6
33	1.0	0.2	<0.2	<0.2
	—	—	—	—
42	1.28	1.42	1.25	1.62
	1.39	1.44	1.27	1.59
45	<1.07	<1.07	<1.07	3.65
	<1.07	<1.07	<1.07	3.71
68	—	<0.7	<0.7	<0.7
	—	<0.7	<0.7	<0.7
73	0.34	0.50	0.35	0 51
	0.39	0.62	0.46	0.94
76	0.20	0.23	0.30	0.10
	0.33	0.19	0.16	0.46
79	1.73	1.54	1.57	1.56
	—	—	—	—

lab no.	C/D	T	V	P
25	20.8	12.9	7.78	8.49
	—	—	—	—
32	24.3	15.0	11.7	11.0
	23.4	14.9	11.1	11.3
53	19.07	12.91	7.06	2.73
	18.51	11.59	7.50	4.06
68	25	17	10	10
	26	18	10	11
76	23.0	12.9	7.7	9.6
	21.0	12.7	7.6	8.1
84	19.36	12.16	7.48	8.03
	—	—	—	—

(Total P)

lab no.	C/D	T	V	P
25	1.03	0.48	0.13	0.16
	—	—	—	—
32	1.50	0.97	0.29	0.29
	1.52	0.94	0.29	0.29
68	1.3	0.8	0.3	0.3
	1.3	0.9	0.3	0.4
76	1.07	0.51	0.00	0.01
	1.04	0.52	0.00	0.00
84	1.22	0.67	0.14	0.12
	—	—	—	—

Annex 3

REVIEW OF PARTICIPANTS' METHODS FOR THE DETERMINATION OF PHOSPHATE

D. S. Kirkwood

INTRODUCTION

At an early stage in the planning of this Intercomparison Exercise, it was recognised that the storage of natural sea water in such a way as to maintain stable nutrient concentrations, is a problem area which continues to occupy marine chemists.

Of particular concern was the dissolution of silicate which occurs during storage in glass bottles; this not only rules out a meaningful silicate determination, but also may have implications for the phosphate determination.

The most widely used methods for phosphate and silicate have much in common in their colour-chemistry, both being based on complexes with molybdenum at low pH, and various authors have investigated and reported on the susceptibility of their phosphate methods to interference from elevated levels of silicate.

Anticipating the possibility of problems of this nature, a comprehensive survey of participants' methods for the determination of phosphate was considered prudent in order to attempt to assess the susceptibility of individual methods while the analysis of the samples by the participants proceeded.

The ICES-MCWG decided that submission of full details of phosphate technique should be a condition of participation, and the excellent response showed that laboratories found no difficulty in complying.

The majority of participants' methods cited Murphy and Riley (1962) (ref 1), and most of the remainder were obviously based directly or indirectly on that work; however, no one appears to use the procedure of Murphy and Riley precisely as published.

Participants' methods are listed anonymously, but individuals should have no difficulty identifying their own method in the appropriate table, if prepared to do a few small calculations.

I.   MANUAL METHODS

Classification of Manual Methods: (Table 1)

The 28 manual methods are readily classified into THREE distinct
groups according to their immediate origin.

(1)   Murphy and Riley (M&R)

9 methods with a final acidity (sulphuric) of around 200 mM, sample
volume around 80% of final volume, and generally using a single
reagent solution.

(2)   Strickland and Parsons (S&P) (refs 2a, b), adaptation of M&R.

9 methods with a final acidity of around 115 mM, sample volume around
90% of final volume, and invariably using a single reagent solution.

(3)   Koroleff (K), adaptations of M&R.

10 methods with a final acidity of around 100 mM, sample volume
generally >95% of final volume, and using a split system requiring
the addition of two reagents separately, but simultaneously.

Description of Manual Methods

(1)   The precise details of Murphy and Riley's procedure are as follows:

To 40 ml of sample in a 50 ml volumetric flask, 8.0 ml of 'single
mixed reagent' is added; mixing is followed by dilution to volume, further
mixing, standing at room temperature for a minimum of 10 minutes, then
Absorbance is measured at 880 nm.

The mixed reagent contains sulphuric acid (1250 mM), and ammonium
molybdate (4.855 mM) (MWt 1235.9), hence an acid/molybdate ratio of 257,
and cell-concentrations 200/0.777.

Also in the mixed reagent are ascorbic acid and antimony potassium
tartrate, but as these are not considered central to the debate, they are
not discussed further.

(2)   Strickland and Parsons

Such was Strickland and Parsons' praise for Murphy and Riley's
procedure, (they described it as '... so superior to other methods in
terms of the rapidity and ease of analysis that it probably represents the
ultimate in sea-going techniques'), it is surprising that they chose to
alter it.

Their procedure uses 100 ml of sample, and 10 ml of mixed reagent
identical to M&R's, but this disproportionately high sample volume results
in substantially reduced cell (cuvette) concentrations of 114/0.441 (cf
M&R 200/0.777).   While it is evident from simple spectrophotometric
considerations, that progression from M&R to S&P should give a slight

improvement in sensitivity, the reason behind S&P's significantly lower acidity than M&R's is not clear.

S&P nevertheless described their procedure as 'after that of Murphy and Riley' and there is ample evidence that numerous authors have followed and/or modified the S&P procedure, then ascribed their own variation to M&R, possibly without consulting the original work (this appears to be the case for five of the nine methods listed under S&P in Table 1).

(3) Koroleff

Koroleff addressed the problem of the instability of the single mixed reagent, which, according to M&R, 'does not keep for more than 24 hours'. Several authors and participants have offered estimates of the working life of the mixture (ranging from 6 hours upwards), but, given general agreement that the problem is one of gradual loss of sensitivity towards phosphate, Koroleff's approach would seem to have much to commend it, that is, to split the components of the mixture into two relatively stable solutions, then add them separately but simultaneously to the sample. In Table 1, methods of this kind are described as 'split', but to facilitate comparison with other manual methods, these two separate reagents are treated as a single mixed reagent for the purpose of calculating the reagent-concentrations (R concs) and cell-concentrations (cell-concs).

Koroleff's (1965) method (K.1.0) (ref 3), after modification to accommodate a larger sample volume (K.2.0) was incorporated into the 'New Baltic Manual' (1972) (ref 4), p 44-48.

Koroleff's method in Grasshoff, Edition 1 (ref 5) p 117-121 (K.3.0) has cell reagent concentrations 97/0.374, close to those of S&P although it is described as 'a modification of the Murphy and Riley procedure'.

A fourth variation (K.4.0) (ref 6) was published in 1979.

In Grasshoff, Edition 2 (ref 7) p 126-131, in the description of yet another method, (K.5.0), having cell concentrations 104/0.389, is the following additional sentence, 'This lower final acidity is also used by Strickland and Parsons (1968)' (ref 2b). K.5.0 apportions the sulphuric acid between the two reagents, but is otherwise quite similar to K.3.0.

The reasons for his choice of cell concentrations are not stated, but, according to Grasshoff, Koroleff investigated the role of the acid/molybdate ratio in the prevention of interference from silicate and concluded that a range of 247-309 was optimal at a final reaction pH <1.0.

This range was later extended to 247-371 (Grasshoff, Edition 2).

In view of these recommendations, it is perhaps surprising that Koroleff did not consistently adopt mid-range ratios for his later methods; K.2.0 has 278, the mid-point of the earlier range, but K.3.0, K.4.0 and K.5.0 have ratios of 260, 274 and 267 for no apparent reason.

The Swedish Standard Method SS028126 (1984) refers specifically to Koroleff's chapter in Grasshoff, Edition 1, (ref 5), and although it contains features that show Koroleff's influence (e.g. the split reagent), its authors have reverted to cell concentrations similar to those of Murphy and Riley, hence its classification as M&R.8. There are similar features in M&R.7, Fonselius and Carlberg's method in the New Baltic Manual (ref 4), p 37-43.

Methods M&R.0, K.1.0, K.2.0 and K.5.0, although not used by any laboratory in their original unmodified forms, are included in the Table 1 to illustrate the changes that have been made to them, or other points of discussion.

All of the manual methods examined conform to Koroleff's 247-371 range.

II. CONTINUOUS-FLOW METHODS

Classification of Continuous-Flow Methods: (Table 2)

A chemical basis of classification for continuous-flow methods was not so obvious as for manual methods, but the table begins with ten unpublished methods (group 0.), which participants have based directly on published manual methods.

Methods terminating with .0 are precisely as published, unmodified by participants, and although five methods 1.3.0, 3.1.0, 10.0, 11.0 and 12.0 are not used by any participants, these have been included in Table 2 to illustrate the subsequent changes made to them, or other points of discussion.

Variations of similar methods are ranked according to decreasing cell concentrations, and acid/molybdate ratios in brackets () indicate values outside Koroleff's later recommended range.

'S-dilution' is the dilution factor for the sample, obtained by dividing the total liquid flow (tlf) arriving at the cell-debubbler, by the net sample flow (after subtracting the effect of any debubbler fitted to the sampling line prior to the point of first reagent addition).

'R-dilution' is the dilution factor for the reagent containing the sulphuric acid and molybdate. In this case (tlf) is divided by the flow

value for this reagent (or that of the single mixed reagent if mixing takes place anywhere before the point of addition to the sample line).

Description of Continuous-Flow Methods:

(1)   Technicon

Technicon equipment is used by the majority of participants, many of whom rely on methods supplied by Technicon, or variations of these, but a few participants have shown that they are prepared to make changes where they consider it appropriate.

Techicon's method 155-71W, with little or no modification, is favoured by a number of users, but its acidity of 290 mM appears to have no basis in the literature.

Several users have split the components of M&R's single mixed reagent into a two reagent system in order to overcome the instablity problem already discussed in the section on manual methods. These are described as 'split' in Table 2.

(2)   Treguer et al. (refs 8,9)

The next largest group shows a preference for the methods of Treguer and co-workers who incorporated a simple 'post-pump, pre-sample' mixing system to produce M&R's mixed reagent on demand, an elegant way to avoid the reagent instability problem while remaining as true to the original manual method as possible; single reagent methods using this device are shown in Table 2 distinguished by '*'.

Treguer also describes alternative low-sensitivity and high-sensivity versions, one being in effect a dilution of the other, but both having the same final acidity and acid/molybdate ratio.

(3)   Grasshoff

In his 1965 method (3.1.0) (refs 10,11), Grasshoff appears to have been the first worker, in a continuous-flow context, to split Murphy and Riley's single solution into two components in the interests of reagent stability.

By 1976 Grasshoff had made a general reduction in flow-rates, presumably to accommodate simultaneous operation with other determinands (3.2.0) (ref 5) p 281-283. The acidity was changed from 159 to 211 mM though the acid/molybdate ratio of 258 was retained.

Grasshoff's 1983 method (3.3.0) (ref 7) p 368-370, is substantially different from both earlier methods, with little or no explanation for the changes. The acidity is 68 mM and the acid/molybdate ratio of 149 is actually outside the range recommended by Koroleff elsewhere in the same book (p 126).

(4)   Eberlein and Kattner (ref 12)

Eberlein and Kattner are among the few who clearly have consulted the original work and have attempted to reproduce M&R's conditions and concentrations.

Their method can claim to be 'based on' M&R's in a very precise sense.

(5 & 6)   Chemlab and Skalar

These manufacturers are less frequently encountered than Technicon. Methods supplied by their users contained no literature references to their sources and they appear better suited to higher concentrations than are generally found in unpolluted sea water.

Both Skalar methods have an acid/molybdate ratio outside Koroleff's recommended range.

Other Continuous-Flow Methods

Two unpublished methods, (A) and (B), both based on Koroleff's manual procedures, are worth further comments.

(A)   Method 0.2.1 uses acid/molybdate ratio 278 (the mid-point of Koroleff's 247-309 range in Grasshoff, Edition 1) as the starting point for recalculating the reagent formulation, and can be said to be firmly based on Koroleff's earlier manual procedures.

(B)   Method 0.2.2, although it claims to be based on Koroleff's manual procedure in Grasshoff, Edition 2, has Murphy and Riley's acid concentration.

(C)   By the late 60's, continuous-flow methods were in common use in oceanographic work and Strickland and Parsons included a section on 'Automated Nutrient Analysis' in their updated manual of 1968 (ref 2b).

In their general discussion of continuous-flow (p 119) they describe how 'reagents are added to the sample in the correct order and relative amounts'.

The method they describe (p 135-6) shows that they followed these directions precisely, but, although not evident in the text, they clearly chose Murphy and Riley's cell-concentrations as their new starting point for the conversion to continuous-flow, rather than the concentrations they had used in their own well-established manual method.

A split reagent system was also included, and later workers would have been well advised to follow this method (11.0), but there is little evidence of its widespread acceptance.

(D) Chan and Riley (ref 14) were only a little less meticulous in their (1966) continuous flow version of the M&R manual method. They retained the original reagent formulation and chose a combination of pump-tubes that gave a close approach to the required cell concentrations (12.0).

(E) One recently published method (8.0) (ref 13) follows the examples of (C) and (D) and attempts to reproduce M&R's conditions even more closely. In addition to the required attention to flow-cell concentrations, the method includes Treguer's device to produce M&R's single mixed reagent on demand.

Only three laboratories use methods that are considered materially different from that of M&R (F) (G) and (H).

(F) Method 9.0 (ref 15) uses sodium molybdate $Na_2MoO_4.2H_2O$ rather than the customary ammonium salt $(NH_4)_6Mo_7O_{24}.4H_2O$. (This is taken into account in the acid/moybdate ratio calculation for Table 2).

(G) Oregon State University's (unpublished) variation of Method 10.0 (ref 16) omits KSbtartrate, uses hydrazine sulphate (at 70°C) as reductant, and claims a 15% sensitivity enhancement compared to M&R's chemistry, with the added advantage that in the absence of antimony, coating of the cell and its troublesome consequences can be avoided.

(H) One participant uses Technicon's method 812-86T, designed for their 'TRAACS 800' system. As this method uses hydrochloric rather than sulphuric acid it was considered inappropriate to include it in the present classification scheme.

NOTES ON TABLES AND FIGURES

(a) In some cases, acidity was difficult to calculate accurately; for example, where a method requires the addition of x ml of concentrated sulphuric acid (of unstated Specific Gravity or assay) to y ml of molybdate solution, and the resultant mixture is not diluted to a specified volume.

One particular 'recipe' of this kind was very common, (140 ml acid + 900 ml water, as in Strickland and Parsons). In practice, due to the non-additve volumetric nature of these components, this mixture approximates closely to 1000 ml at room temperature, and several methods refer to this particular solution as 5.0N or 2.5M. Consequently, 'concentrated' is taken to mean 17.86M and where participants' methods do not specify a unit for acid concentration, this figure was substituted in order to derive the data in Tables 1 and 2.

(b) Table 3 shows the origin of each group of methods, and is also a key to the full literature references of methods that are listed but not discussed in detail.

(c) Figures 1 and 2 show –

> (i) Koroleff's revised limits for the acid/molybdate ratio (247-371) (full lines).
>
> (ii) Murphy and Riley's concentrations (200/0.777) (broken lines).
>
> (iii) The range of participants 'concentrations.

DISCUSSION

Several participants appear to have made quite arbitrary changes to the methods on which they claimed to be based, the resulting chemical divergence produces a factor of 7.4 between the acidities of extreme methods (450/61).

Example 1: A participant describes his method (2.1.1) as 'following the method described by Treguer and Le Corre ... modification in the bath temperature'.

Close examination reveals (in addition to the temperature variation) a substantial change consisting of a 27% increase in final acidity as a result of the use of the same flow-tubes as T&LeC, but put together in a very slightly different manner. (In this method, T&LeC's reagent mixture, 0.8 + 0.32 ml/min is added in its entirety to the sample line, rather than as in T&LeC's method, 0.8 ml/min (71%) of this mixture, using a re-entry line).

Example 2: Method M&R.5 is a miniaturised discrete analyser method and is therefore not strictly speaking a manual method. Nevertheless, it is more appropriate to classify it as manual rather than continuous-flow. This method has the highest cell acidity (450 mM), a unique reagent formulation, is unpublished and cites no literature.reference.

Example 3: Although not strictly pertinent to this study, it is interesting to note that the method recommended by the Environmental Protection Agency (USA) in 1971 (13.0) (ref 17), was not only substantially different from its Technicon contemporary (1.1.0), but it appears to be the only continuous-flow method to have used the Strickland and Parsons manual procedure as its starting point, in spite of S&P's preference for M&R's method as their starting point, three years earlier (see (C) on p 6).

While it can be said of these methods that they will all produce results, such disparities are unlikely to be in the best interests of the users of a reaction which produces a blue-coloured complex of uncertain composition.

Because of excessive dilution in their hydraulic systems, the majority of continuous-flow methods are, in practice, less sensitive than the basic manual methods, but this need not be so.

Table 2 contains no fewer than 15 methods with sample dilution factors equal to, or less than that of Murphy and Riley's method (1.25). However, there are several methods with S-dilution factors >4 which were probably designed primarily for waste-waters etc., rather than unpolluted sea-water, and their applicability to the Intercomparison samples must be considered questionable.

The author suggests that, particularly in the context of continuous-flow techniques, the term 'based on' should be used only in a restricted sense to describe methods satisfying certain well-specified criteria. Flow-rated tubing is generally understood to produce flows within ±10% of the nominal value, consequently, the reagent formulation for a continuous-flow method should be such that the theoretical concentrations in the flow-cell do not differ from those in the spectrophotometer cell of the manual method by any more than the ±10% associated with the tubing. Nine of the 53 participants' continuous-flow methods satisfy those conditions, (those within the 10% circle in Figure 2).

ACKNOWLEDGEMENTS

Figure 1 — Manual Methods

Figure 1 — Manual Methods

Figure 2 — Automated Methods
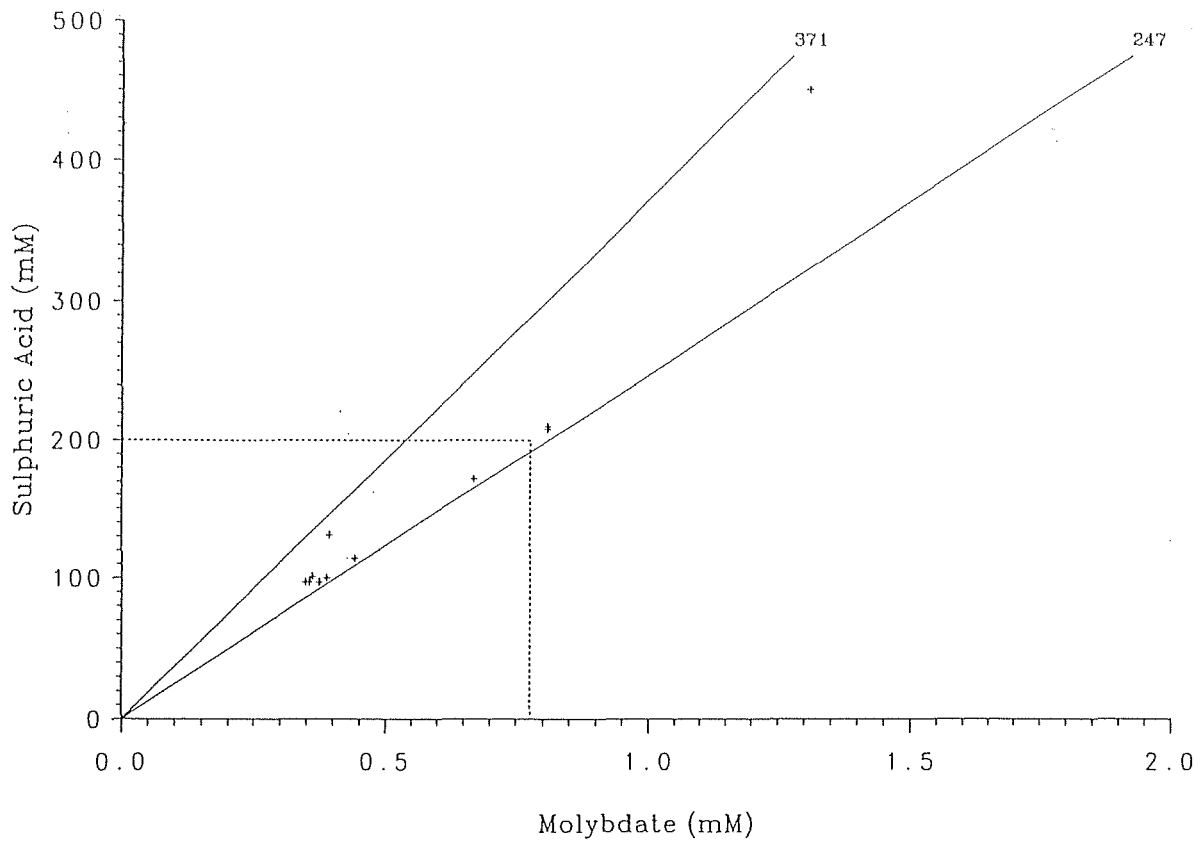
Figure 2 — Automated Methods
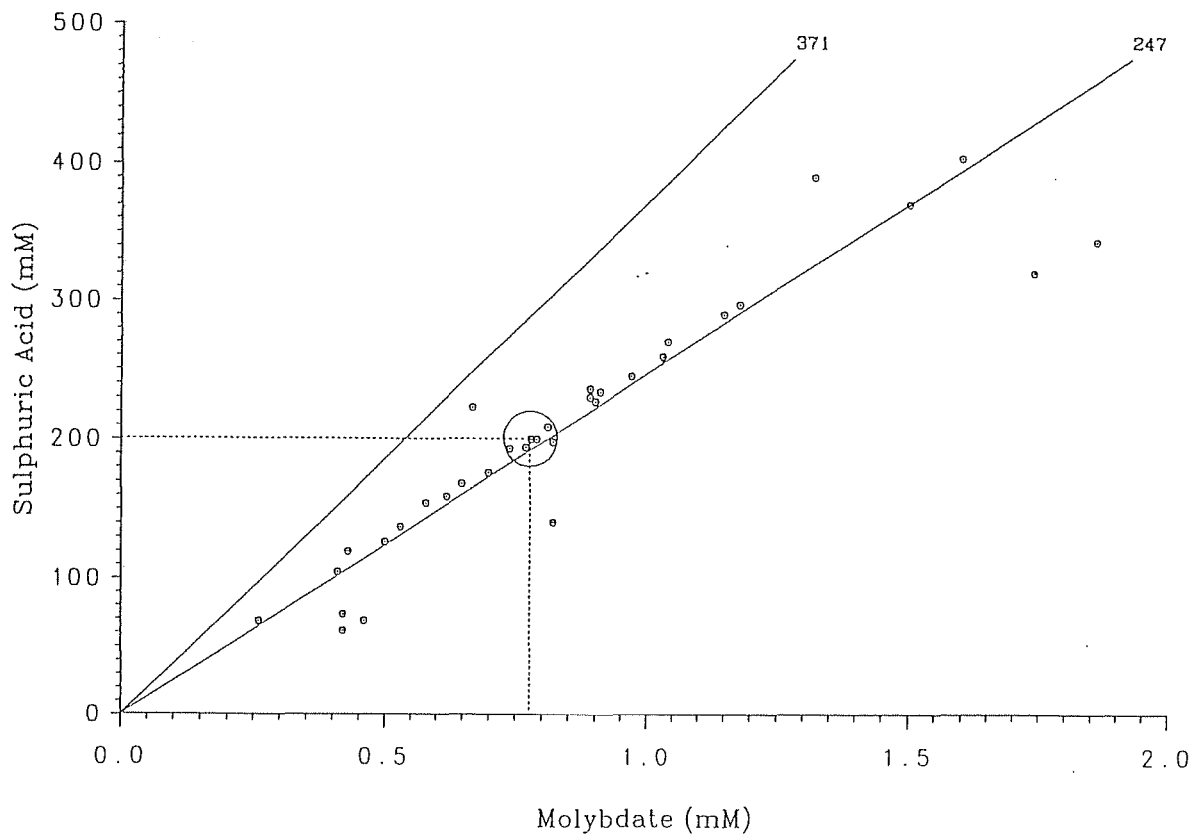
Table 1   MANUAL METHODS

method	reagent	S-vol	R-vol	F-vol	R-dil	R-concs	ratio	cell-concs	
#M&R.0	single	40	8	50	6.25	1250/4.855	257	200/0.777	
M&R.1	"	50	10	60	6.0	"	"	208/0.809	
M&R.2	"	50	8	58	7.25	"	"	172/0.670	
M&R.3	"	25	5	30	6.0	"	"	208/0.809	
M&R.4	"	20	4	24	"	"	"	"	2
M&R.5	"	10	2	12	"	"	"	"	
M&R.6	"	0.4	0.225	0.625	2.78	1250/3.641	343	450/1.311	
M&R.7	split	25	3.5+1.5	30	6.0	1250/4.855	257	208/0.809	
M&R.8	"	"	1.0+1.0	27	13.5	2650/10.52	252	196/0.779	
S&P.0	single	100	10	110	11.0	1250/4.855	257	114/0.441	5
S&P.1	"	50	5	55	"	"	"	"	2
S&P.2	"	40	4	50	12.5	"	"	100/0.388	2
#K.1.0	split	25	1.0+0.25	26.25	21.0	2080/7.586	274	99/0.361	
#K.2.0	"	35	1.0+0.25	36.25	29.0	2912/10.46	278	100/0.361	
K.2.1	"	50	1.43+0.36	51.79	28.9	2908/10.45	278	101/0.361	
K.2.2	"	50	1.0+0.33	51.33	38.6	3910/14.04	278	101/0.364	
K.2.3	"	25	0.7+0.7	26.4	18.9	1827/6.555	279	97/0.348	
K.2.4	"	25	1.0+1.0	27.0	13.5	1268/4.572	277	94/0.339	
K.3.0	"	35	1.0+1.0	37.0	18.5	1800/6.92	260	97/0.374	3
K.4.0	"	"	"	"	"	1800/6.57	274	97/0.355	
#K.5.0	"	50	1.0+1.0	52.0	26.0	2700/10.11	267	104/0.389	
K.5.1	"	50	1.0+0.5	51.5	34.3	4500/13.5	334	131/0.393	
K.5.2	"	25	0.5+0.5	26.0	26.0	2700/10.11	267	104/0.389	

M&R Murphy and Riley
S&P Strickland and Parsons
K   Koroleff
Methods not used by participants
vol (volumes), S- sample   R- reagent(s)   F- final
Where multiple laboratories use **precisely the same method**, the
number of laboratories is shown in the column on the far right.

(A total of 28 laboratories, using 19 different manual methods)

Table 2   CONTINUOUS-FLOW METHODS

method	reagent	S-dilution	R-dilution	R-concs	ratio	cell-concs		
0.1.1	single	1.23	5.35	1250/4.855	257	234/0.908		
0.1.2	split	1.30	8.75	1750/6.937	252	200/0.793 %		
0.1.3	"	1.26	7.88	1250/4.855	257	159/0.617		
0.1.4	"	1.10	22.8	3200/18.67	(171)	140/0.819		
0.1.5	"	1.61	5.31	725/2.816	257	137/0.531		
0.2.1	"	1.05	30.5	3640/13.08	278	119/0.429		3
0.2.2	"	1.17	13.5	1260/4.599	274	93/0.341		
0.2.3	"	1.40	7.0	1386/5.764	(240)	198/0.823 %		
1.1.0	single	2.31	4.22	1225/4.855	252	290/1.151		4
1.1.1	split	"	"	"	"	"		
1.1.2	"	"	3.03	"	"	404/1.602		2
1.1.3	"	1.28	6.65	1725/6.838	"	259/1.028		
1.1.4	single	1.92	5.0	1225/4.855	"	245/0.971		
1.1.5	single*	1.50	3.02	714/2.697	265	236/0.893		
1.1.6	single	3.45	6.30	1225/4.855	252	194/0.770 %		
1.1.7	"	1.12	9.70	"	"	126/0.501		
1.1.8	"	1.69	5.87	613/2.428	"	104/0.414		
1.2.0	split	1.46	6.35	1441/5.712	"	227/0.900		
1.3.0	"	1.26	9.8	600/4.151	(146)	61/0.424		
1.3.1	"	"	"	720/4.151	(173)	73/0.424		
#1.4.0	single	6.53	4.98	1429/4.855	294	287/0.976		
1.4.1	"	4.81	3.67	"	"	390/1.324		
2.1.0	single*	2.46	4.21	1250/4.954	252	297/1.176		4
2.1.1	"	2.69	3.3	"	"	370/1.503		
2.2.0	"	1.90	4.21	"	"	297/1.176		2
2.2.1	"	1.69	7.12	"	"	176/0.696		
3.1.0	split	1.55	5.63	893/3.464	258	159/0.616		
3.1.1	single*	1.25	10.0	1925/7.444	259	193/0.744 %		
3.2.0	split	"	"	2090/8.091	258	209/0.809 %		
3.2.1	"	1.23	10.75	"	"	"		
3.3.0	"	1.13	17.6	1197/8.091	(148)	68/0.459		
3.3.1	"	1.40	7.0	1072/4.046	265	153/0.578		
4.0	single	1.26	6.31	1225/4.855	252	194/0.769 %		3
5.1.0	"	2.98	5.44	1250/4.855	257	230/0.893		
5.1.1	split	"	"	"	"	"		
5.2.0	single	4.22	5.87	1307/3.947	331	223/0.472		
6.1.0	split	5.44	2.08	714/3.884	(184)	343/1.864		
6.2.0	"	4.19	2.23	"	(")	320/1.739		
7.0	"	1.33	10.6	1786/6.878	260	168/0.649		
8.0	single*	1.23	5.35	1070/4.155	257	200/0.777 %		
9.0	single	2.38	6.25	423/1.599	264	68/0.256		
#10.0	split	1.24	10.33	2975/24.27	(123)	288/2.349		
10.1	"	1.13	18.0	4864/18.77	259	270/1.043		
#11.0	"	1.19	9.31	1867/7.190	260	200/0.772 %		
#12.0	"	1.21	5.76	1250/4.855	257	217/0.843 %		
#13.0	"	1.56	10.76	"	"	116/0.451		

\#   Methods not used by participants.
*   'post-pump' mixing. See item (2) on page 6 of text.
() shows acid/molybdate ratios outside Koroleff's range.
% shows methods within 10% of Murphy & Riley's concentrations.
Where multiple laboratories use **precisely the same method**, the
number of laboratories is shown in the column on the far right.

(A total of 53 laboratories, using 41 different continuous-flow methods)

...

Table 3   ORIGIN OF METHODS

(manual)

M&R	Murphy and Riley, (1962)	1
S&P	Strickland and Parsons, (1965) p47-51	2a
"	" " " , (1968) p49-52	2b
K.1	Koroleff, UNESCO, Copenhagen, (1965)	3
K.2	" , New Baltic Manual, p44-48	4
K.3	" , in Grasshoff, Edn. 1 p117-121	5
K.4	" , Meri., (1979)	6
K.5	" , in Grasshoff, et al., Edn. 2 p125-130	7

(continuous-flow)

0.1	Unpublished, based directly on M&R	1
0.2.1	" , " . " " K.2	4
0.2.2	" , " " " K.4	6
0.2.3	" , " " " K.5	7
1.1	Technicon 155-71W (manufacturer's literature)	—
1.2	" (NL) 113-73W (" ")	—
1.3	" (Scand.) 78-4 (" ")	—
1.4	" 253-80E (" ")	—
2.1	Treguer and Le Corre, (1975) (low sensitivity version)	8
2.2	" " " , " (high " ")	8
3.1	Grasshoff, (1965)	10, 11
3.2	" , Edn. 1, (1976) p281-283	5
3.3	" , et al., Edn, 2, (1983) p368-370	7
4	Eberlein and Kattner, (1987)	12
5	Chemlab (manufacturer's literature)	--
6	Skalar (" ")	--
7	Mostert, (1983)	18
8	Kirkwood, (1989)	13
9	Eisenreich, Bannerman and Armstrong, (1975)	15
10	Bernhardt and Wilhelms, (1967)	16
11	Strickland and Parsons, (1968) p135-6	2b
12	Chan and Riley, (1966)	14
13	Environmental Protection Agency (USA), (1971)	17

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<u>ADDENDUM to Annex 3</u>

Recent work by Pai, Yang and Riley describes a comprehensive study of the effects of variation of acidity and molybdate concentration on the determination of phosphate by the original Murphy and Riley method.

The acid/molybdenum ratio was shown to be crucial, influencing not only the form of the final reduced complex but also playing a vital role in the control of the reaction kinetics.

Normal colour formation is observed only at ratios between 210-280.

[The paper specifies a ratio range of 60-80, but due to differences in the expression of reagent concentrations, a conversion factor of 7/2 applies. 210-280 is the equivalent range in terms consistent with those used throughout ANNEX 3.]

Silicate interference was again demonstrated to be minimal so long as Murphy and Riley's original concentrations and conditions pertain.

Reference:

Effects of acidity and molybdate concentration on the kinetics of the formation of the phosphoantimonylmolybdenum blue complex. Su-Chen Pai, Chung-Cheng Yang, and Riley, J. P., Analytica Chimica Acta, 229 (1990) 115-120.

During an oceanographic cruise in 1987, RV CIROLANA occupied a
hydrographic station off SE Greenland on 27/06/87 at 63°33.19N
36°24.34W.

Water samples were obtained from various depths and analysed on
board.

In the course of experiments that were not central to the
primary objectives of this cruise, sub-samples of the water
from 1000 m depth were shown to maintain their nutrients
concentrations over a period of many weeks at room temperature,
and without added preservatives.

MCWG Chemical Oceanography sub-Group members referred to this
water as 'Greenland Water', and it is now referred to as GW87.

Several laboratories sequentially analysed the same sample
bottles over a period of months and achieved a level of
agreement sufficient to suggest that this water (GW87) could be
considered potentially useful for collaborative work on
nutrients. (Unfortunately, stocks were now exhausted.)

On a subsequent cruise in 1988, RV CIROLANA occupied a position
within a few kilometers of that of GW87 and obtained a bulk
sample GW88.
A rosette of EIGHT 30litre Niskin bottles was deployed in the
following manner to check the vertical homogeneity of the water
column in the vicinity of the sampling depth.

Bottle 1 1100 m
Bottles A B C D E F (SIX Niskins) all at 1000 m
Bottle 8 900 m

On board determination of nutrients immediately after sampling,
demonstrated that all EIGHT samples were indistinguishable.

Sub-samples were taken in 200 ml clear glass bottles, closed by
a polyethylene neck insert secured by a hard plastic screw-cap.
These bottles were used as received from the manufacturer,
(Besser, Hamburg) i.e. no washing or previous use.
They were rinsed once with 20-30 ml of GW88, directly from the
drain-tap of the Niskin bottle.

Directly from each of the two Niskins C and D, without
filtration, 90 sub-samples were obtained and subsequently
stored in three wooden crates each containing 30 bottles. (A
total of 180 bottles in 6 crates.)
The sequence of labelling of the bottles allows the filling and
storage history of each to be traced; for example, C223 which
was sent to O. Vagn Olsen, Charlottenlund, was the 23rd bottle
in crate No 2 (sub-sample 53) from Niskin C.

It should be noted that while a 'useful stability' is claimed
for these samples they must not be considered sterile.
Experiments have shown that several days of exposure to bright
sunlight can induce biological action which causes nutrient
depletion.

Annex 4 b - PREPARATION OF SAMPLES.

Preparation of samples T, V and P and control of homogeneity and stability.

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1. INTRODUCTION

This paper deals with the preparation and control of samples for the ICES nutrient intercomparison exercise 1989 with particular reference to nitrate and phosphate.

According Berman's definition (in Vijverberg and Cofino, 1987), samples of this kind are considered to be uncompromised reference materials (URM). This implies an homogeneous material, similar in type to the samples to be analysed (ACMP, 1988) and which must be stable.

Another type of reference material, a standard reference material (SRM), already exists for nutrients, the standard solutions of Sagami Chemical Research Center. However, these standards are not similar to the samples to be analysed, since they are prepared either in fresh water, or in 30.5 g/l NaCl solution. In addition, each bottle contains only 50 ml of water, which is insufficient for most participants using manual methods.

From our previous experiments on the preparation of natural stable intercomparison samples for nitrate and phosphate (Aminot and Kerouel, 1989 a and b), we concluded that two kinds of samples can be prepared :

- blank samples, obtained by light depletion of unfiltered water ;
- samples with normal concentrations, stabilized by autoclaving.

We think that reference materials prepared in this way should satisfy conditions close to that proposed by Ambe (1975) for the Sagami standards, i.e. having "the highest possible accuracy attainable at the technical advancement today". In particular, we think that the inter-sample homogeneity and the stability must be as high as possible, if possible within the normal precision of the method, during the course of the exercise.

Consequently, we undertook the following control measures.

2. EXPERIMENTAL

2.1. Analytical methods

Nitrate and phosphate were analysed with Technicon Autoanalyzers, according to the methods of Treguer and Le Corre (1975). However, for phosphate, the mixed reagent flow was reduced to half its original value in order to keep the chemistry as close as possible to that of Murphy and Riley's method (1962). Note that nitrate must be understood as nitrate + nitrite.

2.2. Standards cross-control

Our normal laboratory standard solutions have been checked against standards from other origins. Thus, nitrate and phosphate primary standards were exchanged with P. Morin and P. Le Corre (Laboratoire d'Océanographie Chimique, Université de Bretagne Occidentale, Brest). We also used Merck Titrisol HNO_3 standards (old and fresh) for nitrate checks. In no case was a deviation greater than $\pm$ 1 % observed between our standard and any other.

2.3. Standardization range

Multiple points standardization was always used in order to bracket the sample concentration and to minimize random errors from glassware.

2.4. Blanks

Milli-Q water was used as baseline and 'zero' concentration water. For seawater the refractive index correction was performed according to Treguer and Le Corre (1975).

3. PREPARATION OF SAMPLES

3.1. Bottles

Two types of bottles were used :

– Polypropylene (PP) bottles, 250 ml, narrow neck ; most of them from Kartell and a few from Nalgene.

– Plain glass bottles, 200 ml and 250 ml, narrow neck, with liner-free PP screw cap.

Before use, bottles were washed in a washing-machine with phosphate-free detergent and distilled water. The bottles were pre-numbered in the sampling order.

3.2. Preparation of seawater

3.2.1. Depleted seawater

Depleted seawater for blank samples was sampled in April 1988 in the bay of Brest and stored in a closed polyethylene carboy (100 litres), in light and at room temperature. Successive analyses indicated that nitrate and phosphate had reached undetectable levels after 70 days. Part of the water was then subsampled and subsequent controls confirmed that nitrate and phosphate remained undetectable in both the carboy and the samples whether in PP or glass bottles.

Intercomparison blank samples were prepared with the seawater remaining in the carboy after the preliminary checks. This water was submitted to no particular preservation treatment. Its salinity is assumed to be close to 34.

3.2.2. Seawater to be autoclaved

Water with normal concentrations of nitrate (# 7 µmol/l) and phosphate (# 0.5 µmol/l) was prepared by mixing coastal seawater and North Atlantic Surface Water (provided by Don Kirkwood), both of them aged in polyethylene carboys. Consequent to the results of our preliminary experiments, the pH of this water was lowered to 7.2, by addition of hydrochloric acid, just before sampling, in order to avoid phosphate modification during and after autoclaving. The salinity of this water is assumed to be close to 35.

3.3. Sampling

The bottles were filled in numerical order from the bulk of seawater contained in a polyethylene carboy. Control bottles were sampled in the series at pre-selected intervals in order to detect any drift during sampling. During the sampling operation latex gloves were worn.

The bottles were rinsed twice with about 10 ml of the water to be sampled, then filled, tighly capped and placed in darkness.

Two series of depleted seawater samples were prepared : the series named V(i) in glass bottles and the series named P(i) in PP bottles. Both series of bottles were filled on 25 April 1989. The series of samples with normal nutrients concentrations, named T(i), was prepared and autoclaved on 26 April 1989.

3.4. Autoclaving

The samples were autoclaved all together about two hours after the beginning of sampling. A 200 l chamber Lequeux KL autoclave was used. Autoclaving was performed under pressure in an atmosphere saturated with water vapour. The sterilization cycle included a heating stage controlled by a Pt probe placed in a witness bottle, a 120 $^\circ$C steady temperature stage for 20 min, then an accelerated cooling stage under compressed air.

After autoclaving, the samples were stored in darkness at room temperature.

4. SAMPLE CONTROL

Four types of controls were performed :

1 - Control of the homogeneity and stability of the intercomparison series.

2 - Control of light effect.

3 - Control of effect of sample contact with the cap.

4 - Control of stability after the bottles have been opened.

4.1. Control of homogeneity and stability of the intercomparison series

The intercomparison series was checked on three occasions during the course of the exercise :

1st control : within one day of sample preparation (25-27 April 1989),

2nd control : 56 days after sample preparation (21 June 1989),

3rd control : 155 days after sample preparation (28 September 1989).

All samples were stored in darkness at room temperature.

4.1.1. Depleted water

The three controls were performed on the same set of bottles. An aliquot of the sample was taken for each control and the bottle was then immediately stoppered and replaced in darkness.

The results on blank samples are summarized in table 1 and shown in figure 1. For nitrate, the overall statistical results are 0.02 ± 0.01 µmol/l either in plastic bottles or in glass bottles. For phosphate, the results are 0.002 ± 0.002 µmol/l and 0.001 ± 0.001 µmol/l respectively in plastic and glass bottles. Whatever the material of the bottles and the storage time, the maximum concentration encountered was 0.05 µmol/l for nitrate and 0.006 µmol/l for phosphate. These results demonstrate that these samples can really be considered as blank samples according to the detection limits usually quoted for these nutrients.

4.1.2. Autoclaved water

In order to ensure sample sterility, a new set of bottles was used for each control.

The results of analyses of autoclaved samples are summarized in table 1 and shown in figure 2. The overall results, whatever the storage time, indicate concentrations of 6.95 ± 0.03 µmol/l for nitrate and 0.542 ± 0.003 µmol/l for phosphate. The results show the high stability of the samples. Indeed, their concentrations remain identical (within the standard deviation of the analyses) throughout the intercomparison exercise. The relative standard deviation of the control series never exceed 0.4 % for nitrate and 0.6 % for phosphate. Throughout the entire control experiment, the maximum deviation from the mean was 0.06 µmol/l for nitrate and 0.006 µmol/l for phosphate.

Table 1 :

Statistical results of control of intercomparison samples series.

	Nitrate			Phosphate		
	control 1 day 1	control 2 day 56	control 3 day 155	control 1 day 1	control 2 day 56	control 3 day 155
	DEPLETED WATER IN PP BOTTLES					
Number of samples	9	9	4	9	9	4
Average	0.01	0.02	0.02	0.001	0.004	0.002
Standard deviation	< 0.01	0.01	0.02	< 0.001	0.002	0.001
	DEPLETED WATER IN GLASS BOTTLES					
Number of samples	9	9	9	9	9	9
Average	0.02	0.03	0.02	0.001	0.001	0.001
Standard deviation	0.01	0.02	0.01	0.001	0.001	< 0.001
	AUTOCLAVED WATER					
Number of samples	16	10	4	16	10	4
Average	6.93	6.98	6.95	0.540	0.543	0.544
Standard deviation	0.03	0.01	0.01	0.002	0.003	0.003
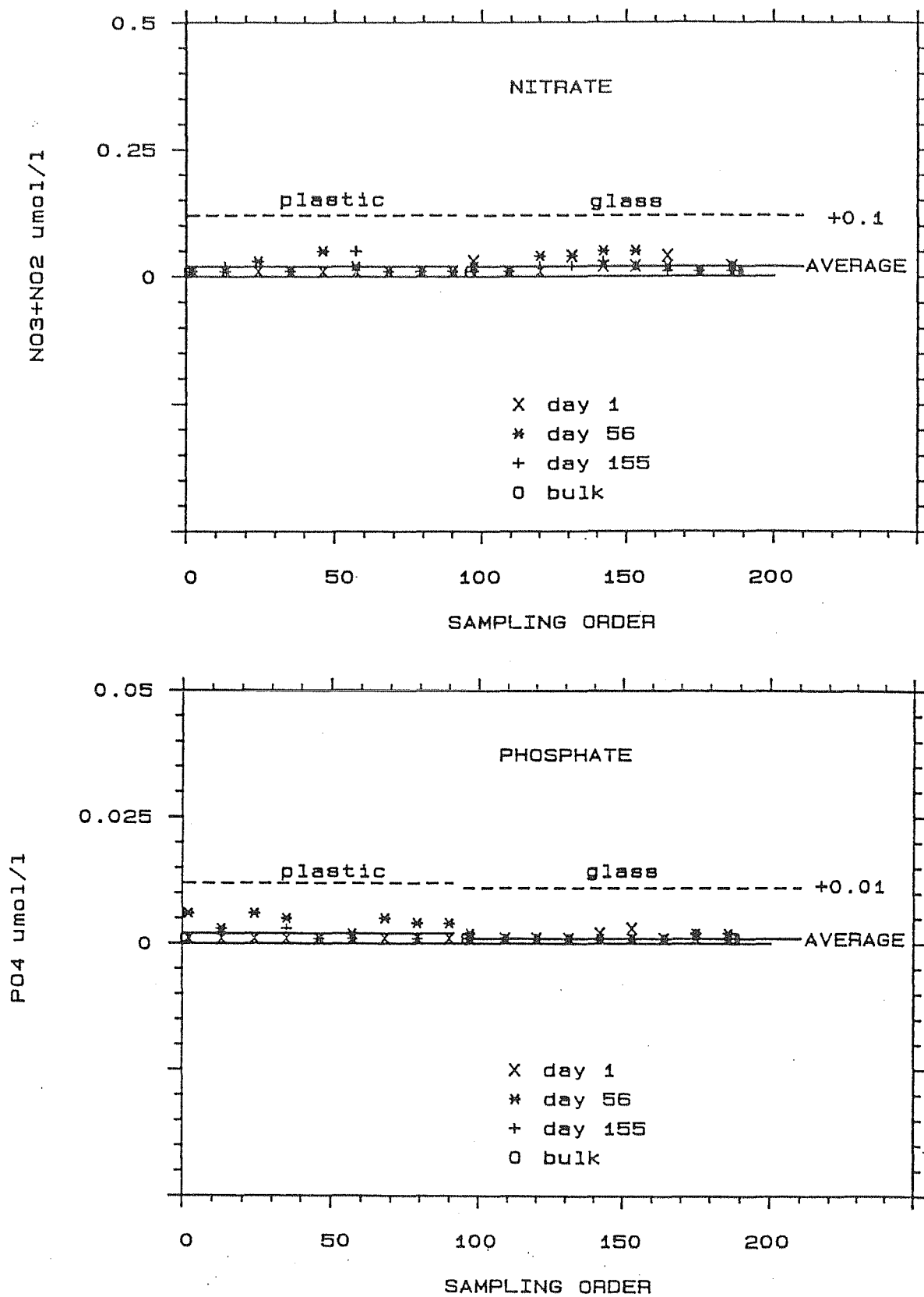
Figure 1 : Results of homogeneity and stability control for nitrate and phosphate in samples of depleted water, bottled either in plastic or in glass.

CONTROL OF AUTOCLAVED SAMPLES
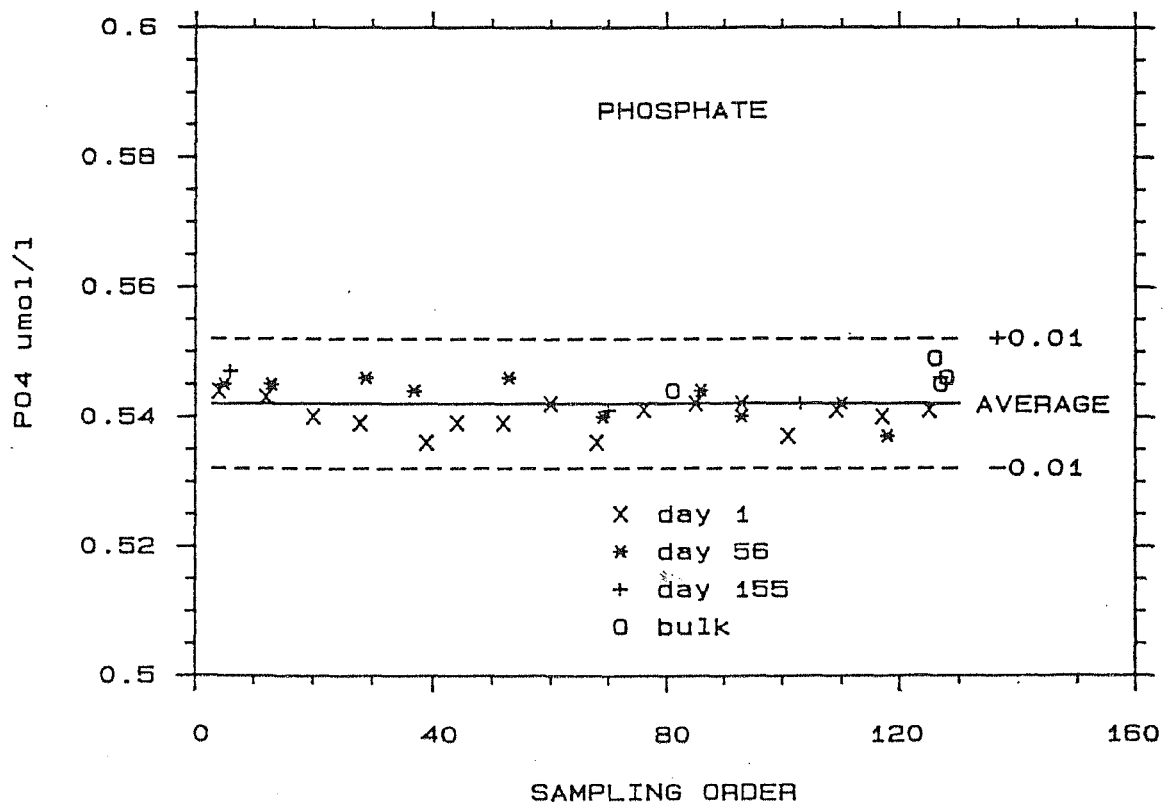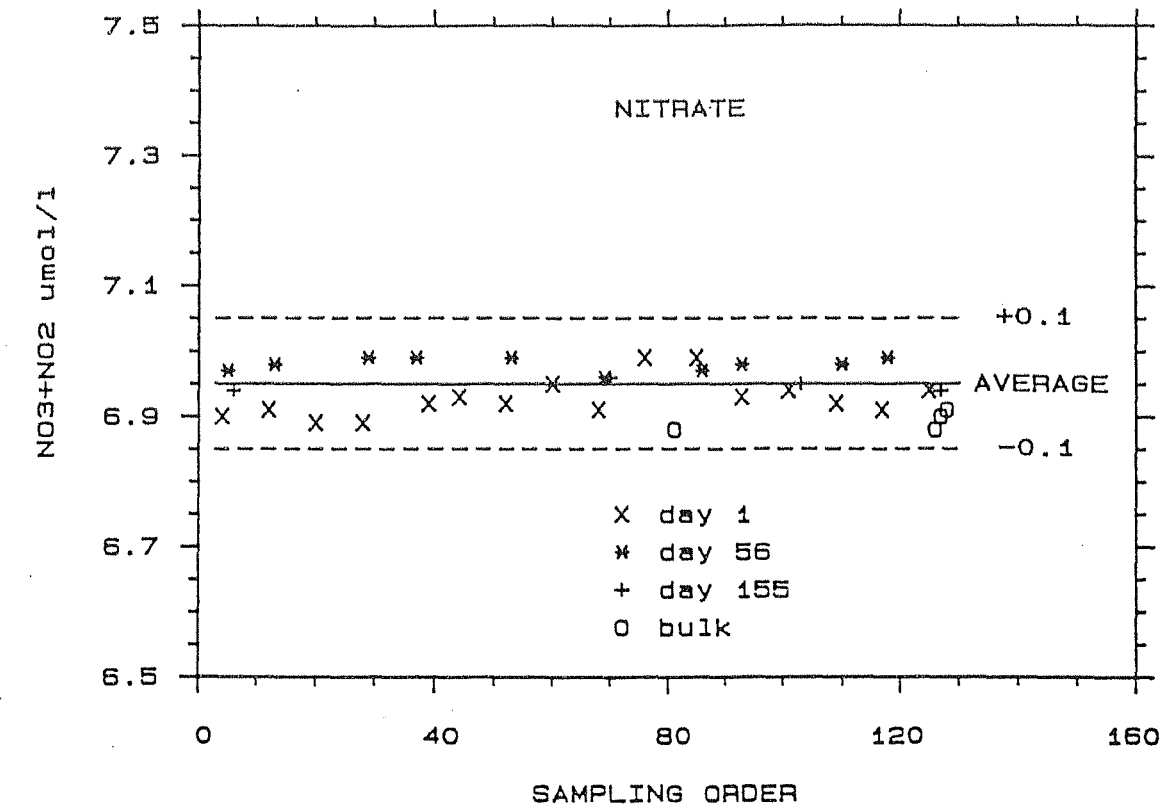
Figure 2 : Results of homogeneity and stability control for nitrate and phosphate in autoclaved samples.

We conclude that the range of concentration variations from one sample to another and versus time is significantly lower than the typical stated precision for the determination of nitrate and phosphate (i.e. 0.1 μmol/l for nitrate and 0.01 μmol/l for phosphate). Autoclaved samples thus appear to be a reliable reference material for intercomparison exercises on nitrate and phosphate.

4.2. Control of light and cap contact effect

The effect of light on depleted water was not checked since the samples came from a bulk of water stored in light for many months and in which the concentrations of nitrate and phosphate have been shown to remain stable at an undetectable level.

However, as the effect of light on autoclaved samples was unknown, they needed to be checked. Due to an insufficient number of samples in the intercomparison series to allow extra controls, another series was prepared a few days later, from the same bulk of water in order to check light and cap contact effects (the concentrations of this new series are very close to those of the intercomparison samples).

The bottles were placed just behind a north-west window, thus receiving, in addition to laboratory light, direct sun irradiation for several hours each day during late spring and summer.

Since we aimed only to know the effect of light, the series was not analysed immediately after preparation because high inter-bottle homogeneity had been previously established.

The results are summarized in table 2 and shown in figure 3. Like the previous series, they confirm the stability and homogeneity of the samples. But in addition they demonstrate that neither light nor cap contact have any effect within the precision of our determinations (rsd < 0.6 % (0.04 μmol/l) for nitrate and < 1.2 % (0.006 μmol/l) for phosphate).

Table 2 :

Comparison of nitrate and phosphate concentrations (μmol/l) in different storage conditions.

Days of storage	Number of samples	NITRATE mean s			PHOSPHATE mean s		
		upright darkness	upright light	lying down darkness	upright darkness	upright light	lying down darkness
54	5	6.75 0.02	6.76 0.04	6.74 0.03	0.500 0.005	0.500 0.006	0.501 0.004
153	4	6.71 0.01	6.71 0.02	6.71 0.03	0.497 0.004	0.499 0.006	0.503 0.005
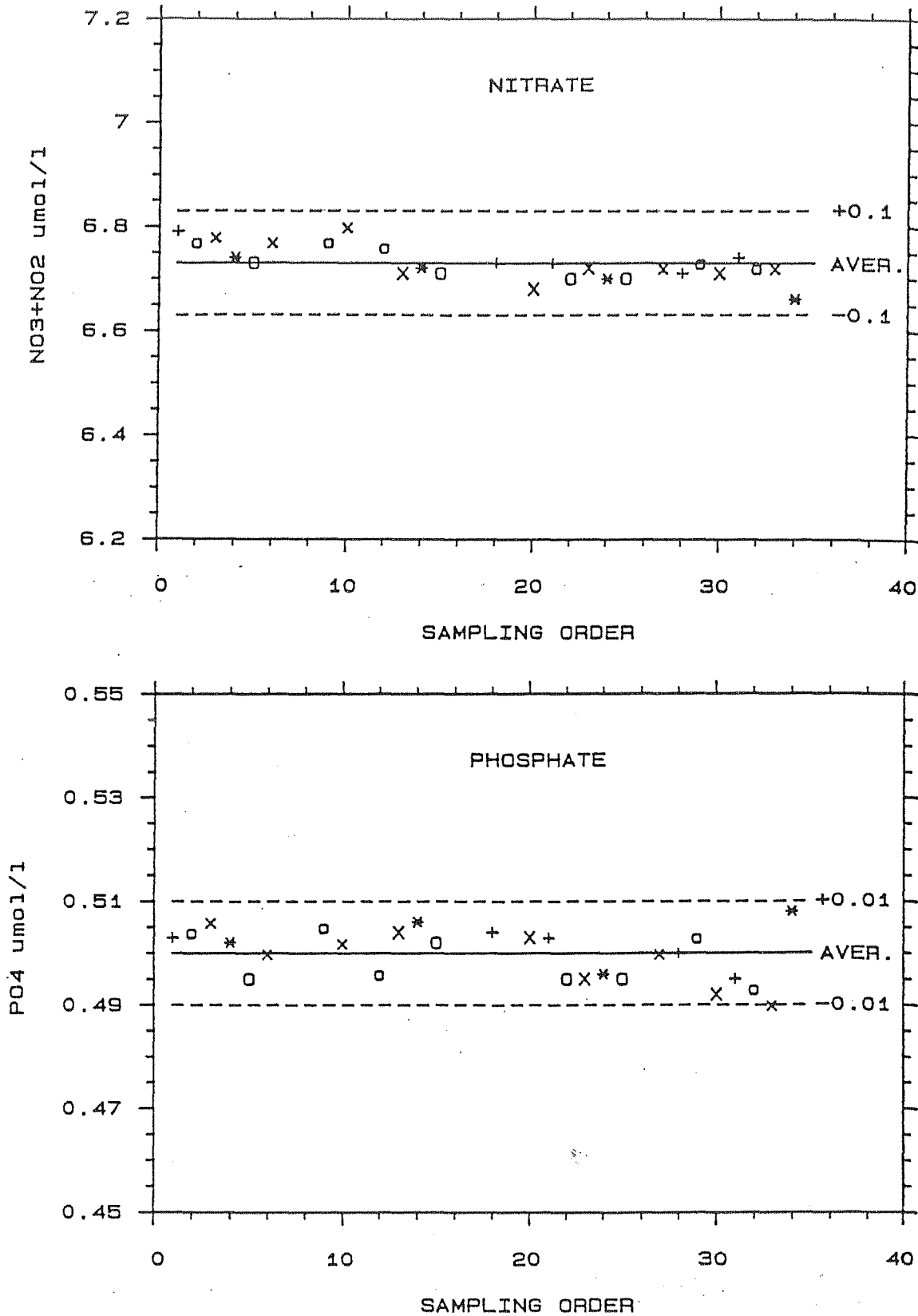
Figure 3 : Comparison of nitrate and phosphate concentrations in autoclaved
samples stored in different conditions and time of storage.
Mean is for normal storage condition, upright in darkness.
Upright, darkness   : o 54 days, O 153 days
Upright, light   : x   "   , X   "
Laying down, darkness : +   "   , *   "

4.3. Stability of samples in bottles which have been opened

The intercomparison procedure included two determinations of nutrients, in each bottle, separated in time by at least one day. For the unsterilized depleted water the other controls (see § 4.1) showed the stability of the samples analysed three times.

For autoclaved undepleted samples, concentration may change after bottles have been opened, due to the high probability of introduction of microorganisms.

The stability of these samples in bottles that have been opened was checked on the first control series. After the first determination, seven bottles were tighly capped again and stored in darkness together with the other samples. Then, 55 days later, a second determination was performed in the same bottles.

As shown by the results (table 3), no change occured in the samples within the precision of the determination. Since no special precaution has been taken in handling and pouring the samples, it seems that there is an extremely low probability of observing any change in bottles which have been opened and kept in darkness for only a few days.

Table 3 :

Stability of nitrate and phosphate concentrations (μmol/l ; mean $+$ s) in bottles which have been opened (storage upright in darkness).

Days after first opening	0	55
Nitrate	6.93 $\pm$ 0.03 ; n = 16	6.94 $\pm$ 0.01 ; n = 7
Phosphate	0.540 $\pm$ 0.002 ; n = 16	0.545 $\pm$ 0.002 ; n = 7

5. CONCLUSION

The controls performed on the intercomparison samples prepared for the ICES nutrient intercomparison exercise 1989 lead to the following conclusions :

- the inter-sample homogeneity and the stability throughout the exercise have remained within limits significantly narrower than the typical stated precision of nitrate ($\pm$ 0.1 μmol/l) and phosphate ($\pm$ 0.01 μmol/l) determinations ;

- no light or cap contact effect could be detected ;

- stability of the samples is maintained even after an aliquot has been properly taken from the bottle.

These conclusions show that the depleted and autoclaved samples meet all the requirements for an uncompromised reference material and are totally suitable for intercomparison exercises.

6. BIBLIOGRAPHY

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Annex 5a

ROBUST ESTIMATION OF 'CONSENSUS VALUES' FOR INTERCOMPARISON MATERIAL

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INTRODUCTION

The four data sets (C/D No_3, T NO_3, C/D PO_4 & T PO_4) under consideration relates to the classical problem of estimating a location parameter in the presence of outliers. It is well recognised that the arithmetic mean is inappropriate in this case, because of its high sensitivity to extreme values. Two strategies are then conceivable:

 i) Use a rejection procedure, which implies more or less some kind of parametric assumptions in order to decide whether a given observation belongs (or not) to the bulk of the data. Furthermore, this leaves unanswered the question : what do the statistics computed on the 'reduced sample' really estimate?

ii) Use a robust estimate of location, which explicitly reweights each observed value of the original sample ('the full sample'). This is the method retained here, and briefly presented in the following chapter.

1. Material and methods

The aim of the analysis is the estimation of unknown, but fixed, values (ie, nutrient concentrations); thus, a frequentist point of view will be adopted, rather than a bayesian one. It is also desirable to quantify the precision of the estimates, which is done by computing the sampling variance of the estimators. Inferences can also be done by means of confidence intervals.

These requirements highlight the necessity of building a rigourous conceptual framework. Without entering technical details, the basic ideas are:

- the sampling unit is the laboratory, each sampling unit yielding four characteristics, ie, numerical values of measured nutrient concentrations,

- the laboratories were selected according to a simple random sampling scheme: this is the key assumption, in the sense that it determines the linkage between the sample and a 'parent population' of laboratories, thus allowing any kind of statistical inference, not only those based on the procedure applied here*.

*However, it should be pointed out that the necessity of this assumption vanishes if the objective of the study is restricted to a pure description of the data sets, without seeking inferences about the parent population.

According to the above context, the observed concentrations of a given nutrient are considered as realisations of iid (independent and identically distributed), positive valued and continuous random variables. The examination (even brief) of the sample histograms suggests them to be generated by stochastic processes whose density function are one-side (PO_4) or two-side (NO_3) heavy-tailed. Whatever the underlying processes, they appear quite 'distant' from the gaussian one, and it must be expected that the arithmetic mean will lose its optimality properties; an estimator more resistant to suspect 'leverage points' is thus preferred.

It is beyond the scope of this paper to give an accurate definition of the various concepts and tools of statistical robustness theory. For a comprehensive, mathematical exposure, the reader is referred to the book of HAMPEL *et al.* (1986). The estimator retained in this study is the HAMPEL's 2,4,8 three-part redescending M-estimator, whose influence function slides to zero in the tails, thus rejecting smoothly distant outliers. From a purely heuristic point of view, the influence function provides some guidance to assess the (approximate and standardised) effect of any additional observation on the estimator.

What is the meaning of the label '2,4,8'? Let's first define e, which estimates the scale parameter 'median absolute deviation':

   e = median of the absolute residuals from the sample median.

Denoting by T the location estimate, and by x a current observation, then:

   $|x-T|>8e$: the observation x is discarded;

$4e<|x-T|<8e$: 'zone of increasing doubt', the influence of x decreases
                continuously between the 'not too bad' and the 'clearly
                bad' values;

$2a<|x-T|<4e$: the influence of x is constant and bounded; to give an
                informal picture, it is a zone of 'median-like behaviour';

   $|x-T|<2a$: zone of 'arithmetic mean-like behaviour'.

The 2,4,8 estimator is thus a weighted least-squares estimator; the data-dependent weights equal one for data with small residuals (like for ordinary least squares), zero for outliers, and vary continuously between 1 and 0 for data with intermediate residuals.

The results given below provide graphical sketches of two finite sample versions of the influence function : a discrete one obtained with the jackknife, and a continuous one (the TUKEY's sensitivity curve).

Finally, under the assumption of an iid sample (*vide supra*), information about the sampling distribution of the robust estimator can be obtained using a resampling procedure. This is done by applying EFRON's bootstrap; nonparametric confidence intervals are obtained with the percentile method (see EFRON, 1982).

2. Results

The robust estimates of 'consensus concentrations' are presented in table 1. As it is usually done with the arithmetic mean, the standard deviation of the sampling distribution of the estimator is called here 'standard error'; 80%

bootstrap confidence intervals are also given. The last two rows contain the values obtained for the arithmetic mean.

DATA SET	C/D NO_3	T NO_3	C/D PO_4	T PO_4
Number of labs.	66	66	68	67
Consensus concentration (standard error)	16.19 (0.14)	6.903 (0.063)	1.144 (0.012)	0.548 (0.009)
80% C.I.	(16.00,16.36)	(6.815, 6.973)	(1.131, 1.161)	(0.535,0.560)
Arithmetic mean (standard error)	15.99 (0.19)	6.869 (0.093)	1.311 (0.114)	0.609 (0.038)

Table 1. Point and interval estimates of consensus values.

Figure 1 shows the empirical influence functions of the Hampel's M-estimator for the four data sets. On the abscissa scale (real line), the positions of the observed concentrations are indicated by vertical bars : this reveals an obvious outlier in data sets C/D PO_4 and T PO_4 (high responses of laboratory No. 8, which cannot be explained only by the admissible 'analytical noise'). These extreme values are interesting *per se*, regarding their information content about the heterogeneity of the parent population of laboratories (and the probability of occurrence of gross errors). However, every careful statistician would reject these data points before estimating a location parameter : this is what the redescending estimator exactly does, as illustrated on figure 1, and clearly shown if estimates are recomputed after rejection of laboratory No. 8 :

DATA SET	C/D PO_4(-lab. 8)	T PO_4(-lab. 8)
Consensus concentration	unmodified	unmodified
80% C.I.	(1.131, 1.160)	(0.535, 0.559)
Mean (Std. error)	1.199 (0.024)	0.574 (0.015)

Table 2. Same as table 1, after deletion of response of lab. No. 8.

Figure 2 shows the histograms of the bootstrap replicates, which give, under the assumption of simple random sampling, a representation of the sampling distribution of the estimator.

Discussion and conclusion

It is worth noting that robust statistics are parametric in essence, but the fact that parametric models constitute at most approximations (usually of empirical nature) to reality is explicitly taken into account. Procedures are implemented in such a way they maintain a good behaviour not only under the model, but also in its neighbourhood. As a consequence, when compared to the strict classical parametric framework, inferences are based upon weaker assumptions.

What about the choice of the estimator? The robust estimator used here possesses some desirable properties : the influence of any fixed fraction (<50%) of wrong observations is restricted, distant outliers are thrown out (and identified for a separate treatment), the rejection procedure increasing smoothly with the distance (no 'hard jump' in the influence function). Furthermore, it is little affected by local inaccuracies (eg, grouping, rounding), and as good as possible under the 'ideal parametric model', ie the normal distribution. However, one must keep in mind the great variety of robust estimators : there is no unique answer to the question of 'the best procedure of general use', the diversity of situations encountered in applied statistics requiring several complementary tools.

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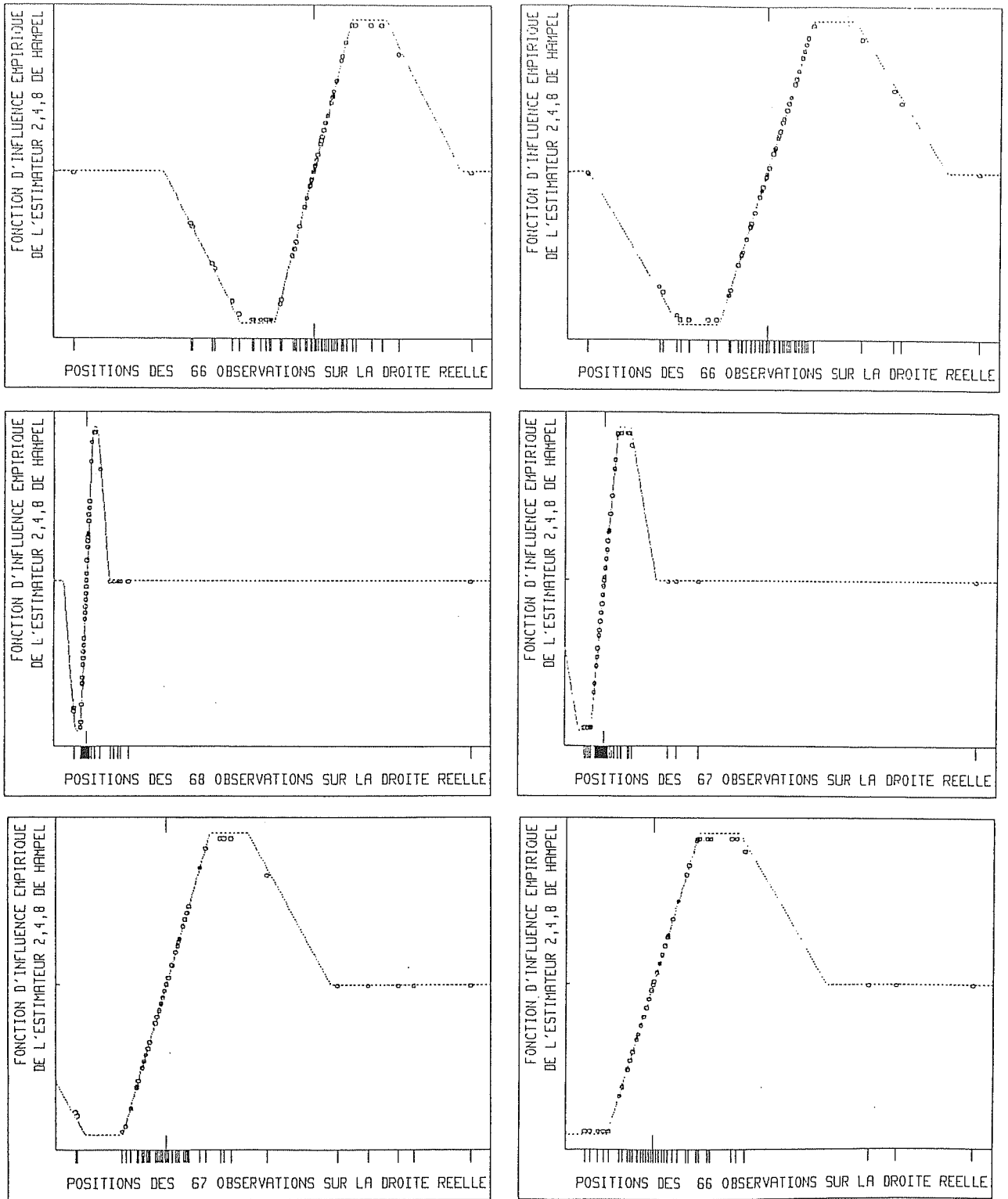
Fig. 1 : Plot of finite-sample influence function *vs*. domain of observed concentrations (vertical bars on the abscissa scale). Dotted line : TUKEY's sensitivity curve ; open symbols : jackkknife computed values (one for each data point). Left : C/D data sets ; right : T data sets ; top : NO_3 ; middle :PO4, full set ; bottom : PO_4 data sets, without responses of lab. 8.

76
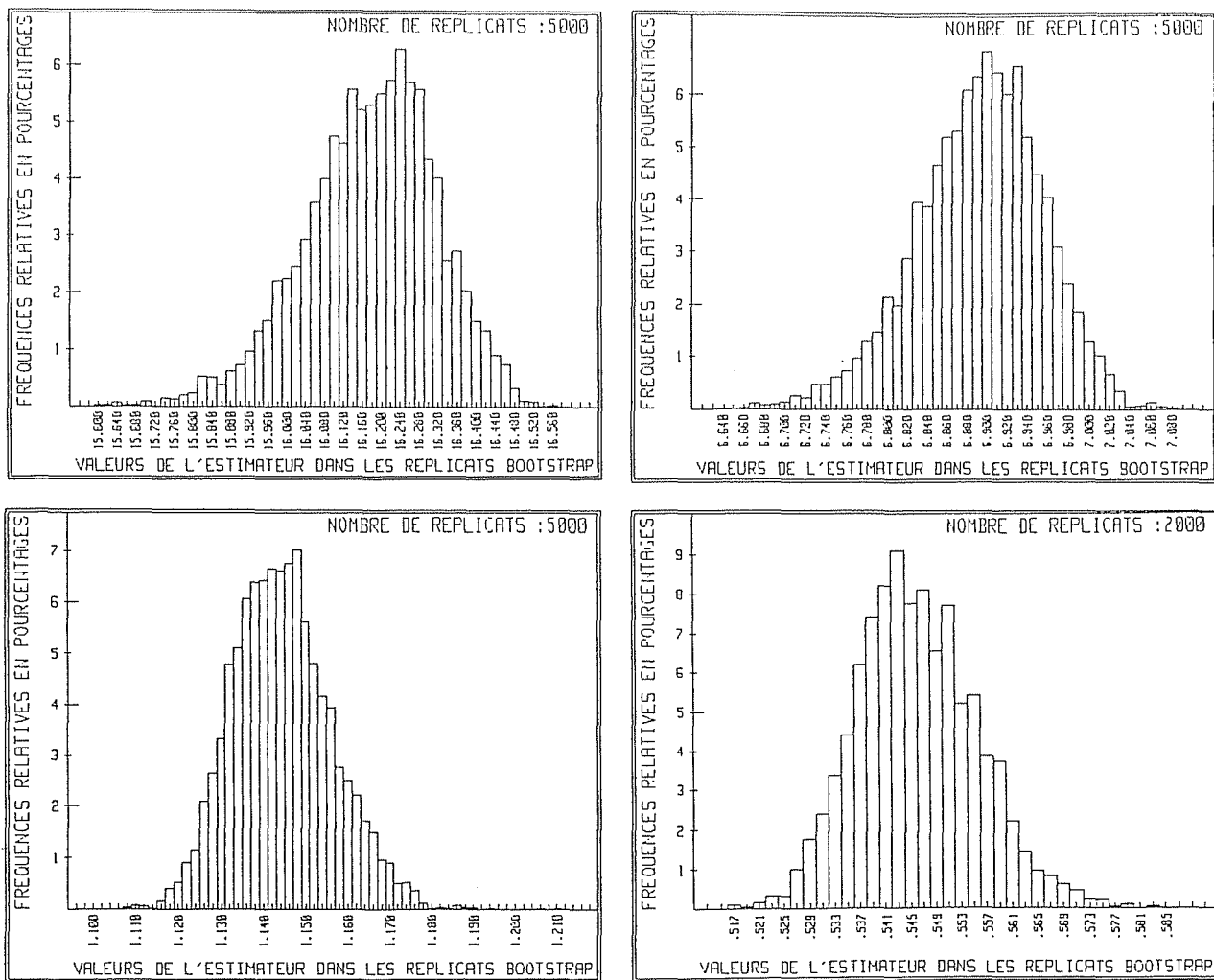
Fig. 2 : Histograms, 2000 to 5000 bootstrap replicates of Hampel's estimates, give a bootstrap estimate of the estimator sampling distribution (under the assumption of iid samples of laboratories).

Annex 5b.

ICES 1990 Nutrients Intercomparison

M.D. Nicholson   (MAFF - Lowestoft)

1. Introduction.

This report examines data collected during the ICES (Fourth) Intercomparison Exercise for nutrients in sea-water. Four samples were analysed in duplicate (once on each of two separate days) for a variety of determinands including nitrate and phosphate. The samples consisted of one high level sample (C/D), one medium sample (T), and two low level samples not statistically analysed here.

This report summarises analyses of the high and medium samples of nitrate and phosphate. The objectives of the analyses were to quantify the characteristics of the analytical accuracy with which nutrients are measured and to estimate the nominal concentrations of phosphate and nitrate in each sample.

The characteristics of analytical accuracy are

bias :- the average value obtained by a laboratory for a particular nutrient concentration *minus* the true concentration.

within lab. s.d. :- the standard deviation of repeated analyses within a laboratory. Usually called precision. Sometimes called repeatability.

between lab. s.d. :- the standard deviation between laboratories.

total s.d. :- The square root of the sum of the squares of the within and between s.d.'s. Sometimes called reproducibility. This is the standard deviation that would apply to a measurement made by a laboratory chosen at random.

The statistical analyses used to measure these characteristics are described in Annex A (Exploration of data) and Annex B (Measuring the notional mean concentration).

2. Results.

From the exploration of the data, it was clear that, for a particular nutrient, results for a majority of laboratories were similar, and those for a smaller group of laboratories were different, and different from each other. This might be expected for this type of experiment.

The results from the majority were extracted and are assumed to provide a baseline of achievable analytical performance. The results are given in Table 1. The percentage of laboratories in this group was approximately 75% for nitrate, 85% for phosphate. Reproducibility was about 5% for nitrate, 10% for phosphate.

These results measure the analytical performance for the subset of laboratories which are in agreement. However, the complete data set provides a snapshot of the analytical performance of all laboratories at the time of the exercise. However, most laboratories would perform better than average; a small proportion would do much worse.

The second part of Table 1 gives the performance characteristics from all of the data. Phosphate is measured with an apparent average bias of the order of 10%, but reproducibility of 50% or more. The results for nitrate show almost no bias, and an overall reproducibility of 10%.

Table 1. Summary of nitrate and phosphate results.

	Nitrate		Phosphate	
	C/D	T	C/D	T
a. Consistent Laboratories.				
No. Labs.	51	51	58	58
Mean	16.3	7.0	1.14	0.54
Within s.d.	0.35	0.18	0.08	0.03
%	2.1	2.6	7.0	5.7
Between s.d.	0.64	0.24	0.06	0.05
%	3.9	3.4	5.2	10.2
Total s.d.	0.73	0.30	0.10	0.06
%	4.5	4.3	8.7	11.6
b. All Results.				
No. Labs.	66	66	68	67
Mean	16.0	6.9	1.31	0.61
Within s.d.	0.46	0.22	0.14	0.03
%	2.8	3.1	12	5.7
Between s.d.	1.50	0.76	0.94	0.31
%	9.2	11	82	58
Total s.d.	1.57	0.80	0.94	0.31
%	9.6	11	83	58
Bias	-0.31	-0.12	0.17	0.07
%	-2	-2	15	13

Appendix A Exploration of data.

   The data were summarised graphically in several ways, shown in Figures 1 and 2 for nitrate and phosphate respectively. Each figure consists of six graphs as follows.

Top/left: plot of the laboratory mean against the laboratory standard deviation for the C/D sample. This reveals laboratories which have exceptional means (relatively biased) or exceptionally large standard deviations (poor precision). If both are exceptional, there could be bias and poor precision, or a single, curious observation.

Top/right: as top/left for T sample.

Middle/left: plot of the within-laboratory standard deviations against their expected chi-square order statistics for the C/D sample. If the duplicate results are at least approximately Normally distributed, and precision is the same for all laboratories, this should be a straight line. The slope of the straight line provides an estimate of the average precision across laboratories.

Middle/right: as middle/left for T sample.

Bottom/left: plot of the C/D mean for each laboratory against the corresponding T mean. This is usually called a Youden plot. If there is bias and it is sustained throughout the period of the exercise, it should be present in both samples, and points will fall along a straight line with positive slope. Poor precision and mistakes will appear as points distant from the main group, but not on the line of bias. Bias is present for both nitrate and phosphate.

Bottom/right: Plot of the differences between the C/D sample results from each day plotted against the corresponding differences for the T sample. This will demonstrate any day to day bias within laboratories, which will again appear as points along a straight line with positive slope. There is some evidence of short term bias for nitrate, but less for phosphate.

Fig 1 : Nitrate
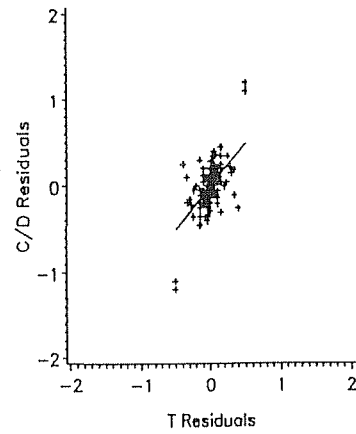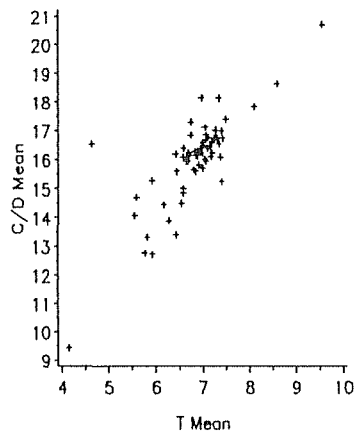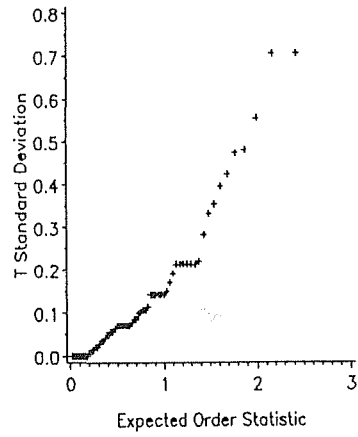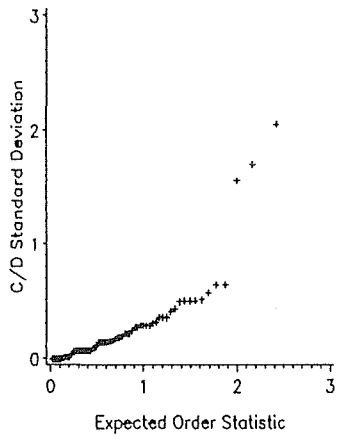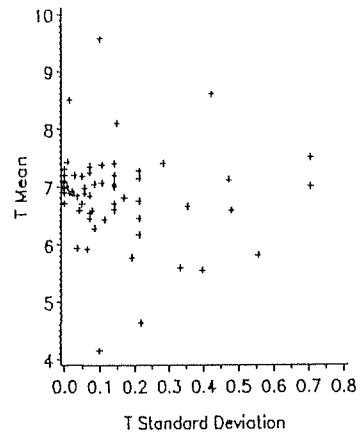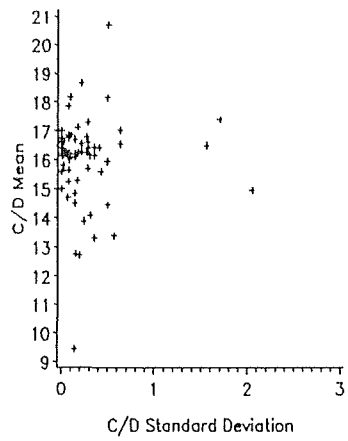
Fig 2 : Phosphate
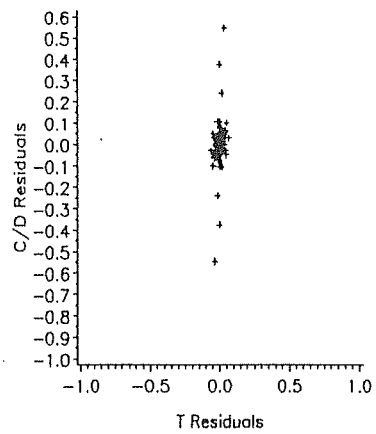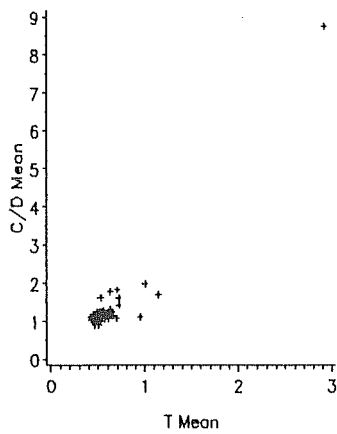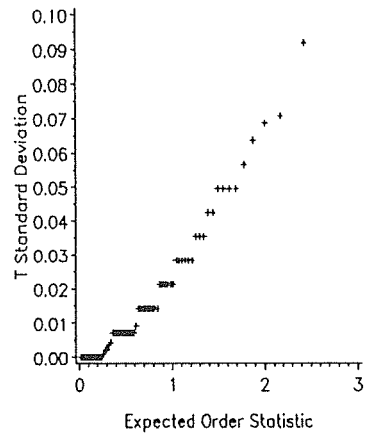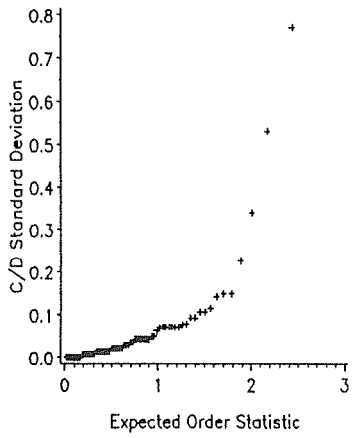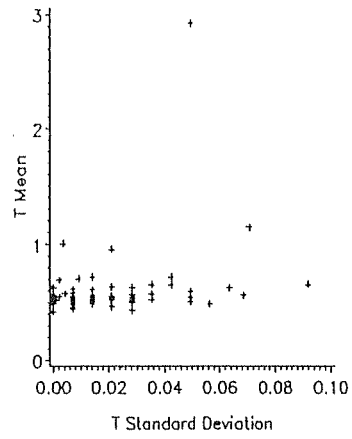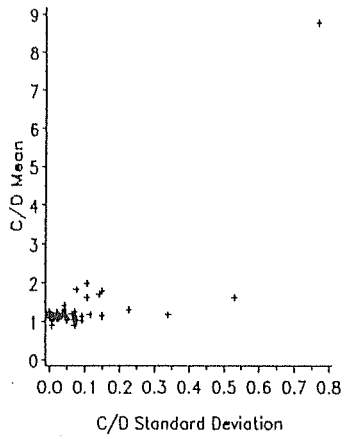
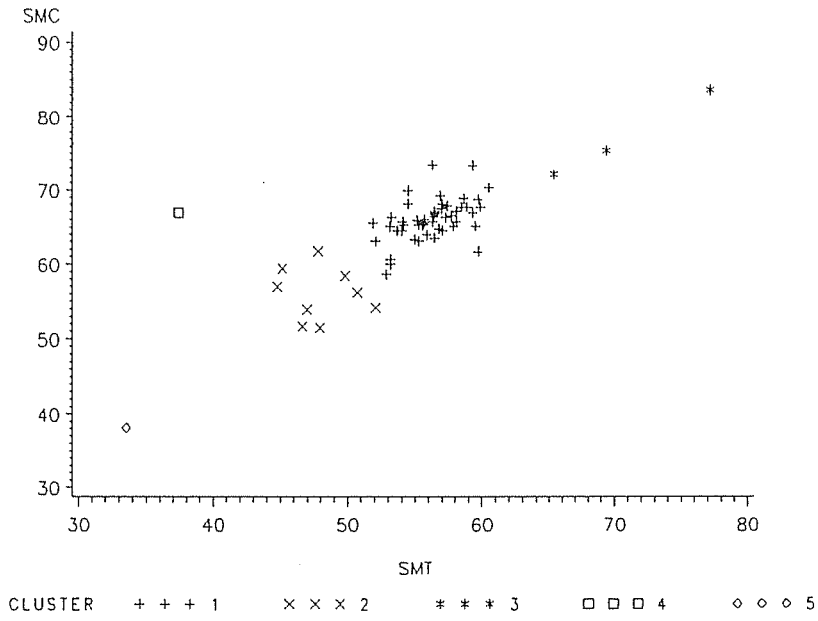## Appendix B   Estimating the Notional Mean Concentration.

The notional concentrations of the C/D and T samples may be estimated by the average concentrations from the largest group of laboratories which, in some sense, are consistent with each other.

The technique chosen here exploits Youden plots of the laboratory C/D sample means against the T sample means. If the duplicates are approximately Normally distributed, roughly 95% of the plotted means, standardized by their standard errors, should lie within a circle of radius 2.6. Complete-linkage cluster analysis and a distance criterion based on the average within-laboratory standard deviations were used to identify groups of laboratories for which this criterion was met. The performance characteristics and notional concentrarions were estimated by the C/D and T means from the largest group.

The resulting clusters are shown in Figures 3a and b for nitrate and phosphate respectively.

Figure 3

3a. Nitrate

3b. Phosphate