1. Introduction

The objective of this effort was to develop a reference standard for analysis of nutrients in seawater that would ensure comparability of analytical data collected by different laboratories and facilitate onboard analysis of nutrients in seawater. Highly accurate nutrient data could thus become available. We have focused on developing a certified reference material for nutrients in seawater in a seawater matrix. The IOC–IAEA–UNEP Group of Experts on Standards and Reference Materials (UNESCO, 1991, 1992) have clearly stated the need to place a high priority on developing a reference material for nutrients in seawater (hereafter RMNS).

Currently, the only way to ensure comparability among nutrient analyses performed by different laboratories is to conduct interlaboratory comparison experiments that provide consensus values plus uncertainties for nutrient concentrations. The ICES Nutrient Intercomparison has been done 5 times since 1965 (UNESCO, 1965, 1967; ICES, 1967, 1977; Kirkwood et al., 1991; Aminot and Kirkwood, 1995), and efforts to ensure comparability among analyses in this field have been carried out for 30 years. In 2000 and 2002, NOAA/NRC intercomparisons between laboratories in the United States and Canada were carried out to certify a seawater certified reference material for nutrients as MOOS-1 provided by National Research Council Canada (Willie and Clancy, 2000; Clancy and Willie, 2003). The first certified reference material for nutrients in seawater in a seawater matrix was provided as MOOS-1 in 2003 by the National Research Council of Canada (Clancy and Willie, 2004). However, the nutrient concentrations of MOOS-1 were too low for analysis of nutrients in Pacific Ocean seawater and did not cover the concentrations of nutrients in other seawater samples.

Thus, in 2003 the present exercise was planned and conducted to make progress in this field. This intercomparison has two advantages over previous intercomparisons. First, nutrient concentrations of the distributed samples were set to cover the concentration range of nutrients in the Pacific Ocean, which has the highest nutrient concentrations among the open oceans of the world. Second, the distributed samples were prepared in a natural seawater matrix in a single bottle so that 4 determinands (nitrate, nitrite, phosphate, and silicic acid) could be simultaneously analyzed.

This report describes the exercise in detail and summarizes the results reported by the participants.

2. Samples

2.1 Sample preparation and timetable for intercomparison

Progress in preparing RMNS samples has been made over the past 10 years (Aoyama et al., 2006). For this study, seawater in a stainless steel container (volume 40 to 200 L) was autoclaved twice at 120 ºC for 2 h. A sample for analysis consisted of 90 mL of the autoclaved seawater in a polypropylene bottle. This procedure for preparing samples is based on a previously reported method for preparing a reference material for the determination of nutrients in seawater (Aminot and Kerouel, 1991, 1995). Sample homogeneity was confirmed by repeatability of measurement. Long-term storage of our RMNS samples for up to 4 years showed that the homogeneities and concentrations of nutrients were maintained for about 4 years (Aoyama et al., 2006).

The samples sent to the participants were prepared in 2001 and 2002. The nutrient concentrations in the samples were confirmed to be stable for at least several months before the samples were sent to the participants between March 2002 and December 2002. All participants had analyzed the samples and returned their results by April 2003.

2.2 Selection of determinands

The determinands of interest were Nitrate (or Nitrate + Nitrite), Nitrite, Phosphate, and Silicic acid.

2.3 Sample homogeneity

The homogeneities of the samples were measured separately. The homogeneities for 30 bottles of sample 3 are listed in table 1. Analytical precision was also estimated for 30 samples of natural seawater whose nutrient concentrations were similar to those of sample 3.

Table 1 Homogeneity of sample 3 and analytical precision

	Nitrate + nitrite	Phosphate	Silicic acid
Homogeneity of sample 3 (%)	0.44	0.80	0.15
Analytical precision (CV %)	0.34	0.32	0.16

Note: The concentrations of nutrients in natural seawater for the simultaneous analyses were 43 μmol kg^{-1} for nitrate + nitrite, 3.1 μmol kg^{-1} for phosphate, and 148 μmol kg^{-1} for silicic acid.

Figure 1 Samples sent to participants

For sample 3, the homogeneities for nitrate + nitrite, phosphate, and silicic acid were good considering the analytical precision for the analysis of natural seawater (table 1). Since the concentrations of nutrients in sample 3 were similar to those in the natural seawater used in this study, the homogeneities for nitrate + nitrite and silicic acid were of the same order of magnitude as, or better than, the analytical precision. The homogeneity for phosphate had a larger scattering, which was attributed to the nature of the RMNS sample itself, and not any analytical problem.

Samples 1, 2, 4, 5, and 6 were not analyzed due to a limited number of samples. It is, however, safe to assume that these samples were similar in nature to sample 3, since all samples were prepared by the same process.

3. Participants and response

By November 2002, 18 laboratories in 5 countries had replied to the call for participants. A total of 18 sets of samples were distributed. Appendix I lists the participants.

Results were submitted by 17 laboratories; a set of samples was returned by 1 laboratory because it was unable to meet the deadline for submission of results. One participant did not report results for nitrite. Four participants did not report results for nitrate, but did report results for nitrate + nitrite. In these cases, nitrate concentrations were calculated from the nitrate + nitrite and nitrite concentrations. Four participants did not report results for silicic acid.

The responses from the participants are summarized in table 2.

Table 2 Summary of responses from participants

Nutrient	Sample ID	Number of results	
		Received	Statistically treated
Nitrate + nitrite	1	15	16
	2	15	17
	3	15	17
	4	15	16
	5	15	17
	6	15	17
Nitrite	1	16	15
	2	16	16
	3	16	14
	4	16	14
	5	16	16
	6	16	16
Nitrate	1	13	15
	2	13	16
	3	13	15
	4	13	15
	5	13	16
	6	13	16
Phosphate	1	17	17
	2	17	17
	3	17	17
	4	17	17
	5	17	17
	6	17	17
Silicic acid	1	13	13
	2	13	13
	3	13	13
	4	13	13
	5	13	13
	6	13	13

4. Statistical treatment

4.1 Consensus mean, median, and standard deviation

Successive *t*-tests at the 95% confidence level were applied to the results to remove outliers before estimating the consensus mean, median, and standard deviation. Tests were applied until a stable mean was reached, and stable means were obtained at the second test for all sets of results.

4.2 Calculation of *Z*-scores

Z-scores were used to evaluate the performance of laboratories.

The *Z*-score for each analysis is defined as

$$Z_{par} = \mathrm{ABS}((C_{par} - C_{consensus})/P_{par}) \qquad (1)$$

where Z_{par} is the *Z*-score for an analysis; C_{par} is the concentration of an RMNS sample measured by a laboratory for the parameter of interest (nitrate, phosphate, or silicate); $C_{consensus}$ is the consensus sample concentration for the parameter of interest, described in section 4.1; and P_{par} is the standard deviation at the sample concentration for the parameter of interest.

Averages of the *Z*-scores for the 6 samples were calculated for nitrate (Z_{NO_3}), nitrite (Z_{NO_2}), phosphate (Z_p), and silicic acid (Z_s).

Averages of *Z*-scores for each laboratory were calculated for $Z_{NO_3} + Z_p$ and $Z_{NO_3} + Z_p + Z_s$.

5. Results

5.1 Raw results

Results reported by the participants are summarized in Appendix II.

5.2 Consensus medians, means, and standard deviations

The consensus medians, means, and standard deviations (table 3) were calculated using the data that passed the successive *t*-test applications described in section 4.1. The consensus means and medians are in excellent agreement for all parameters for all samples.

Table 3 Consensus medians, means, and standard deviations for the 6 samples

	Nitrite (µmol kg^{-1})			Nitrate (µmol kg^{-1})			Nitrate + nitrite (µmol kg^{-1})		
Sample	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**
1	0.02	0.02	0.01	0.05	0.04	0.03	0.07	0.06	0.04
2	0.14	0.13	0.06	17.5	17.4	0.7	17.7	17.6	0.6
3	0.01	0.01	0.01	35.4	35.3	0.3	35.4	35.4	0.3
4	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.04	0.04
5	0.90	0.91	0.02	13.0	13.1	0.2	14.0	14.0	0.2
6	0.24	0.23	0.09	38.4	38.2	1.1	38.5	38.4	1.0

	Phosphate (µmol kg^{-1})			Silicic acid (µmol kg^{-1})		
Sample	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**
1	0.09	0.09	0.02	2.03	2.06	0.23
2	1.25	1.25	0.04	66.3	66.4	2.0
3	2.14	2.14	0.07	136.0	135.7	2.3
4	0.09	0.09	0.03	2.09	2.09	0.31
5	1.10	1.10	0.04	73.4	73.8	2.4
6	2.74	2.74	0.10	134.0	133.8	2.5

5.3 Scatter plots and histograms of the results

Scatter plots for nitrate, phosphate, and silicic acid are shown in figures 2–4, respectively. For nitrate and phosphate, laboratories were sorted by order of reported concentration of nitrate and phosphate in sample 6, for which the nitrate and phosphate concentrations were the highest in the 6 samples sent to the participants. For silicic acid, laboratories were sorted by order of reported silicic acid concentration in sample 3, for which the silicic acid concentration was the highest in the 6 samples. In figures 2–4, error bars appear when they were reported.

Scatter plots and histograms for each parameter of each sample are shown in figures 5-1 to 9-6. The consensus value is shown at the top of each figure. In the scatter plots, error bars appear when they were reported. Each histogram interval is set to equal the corresponding standard deviation shown in table 4.

5.4 Comparison between consensus standard deviation of sample 3 and homogeneity of sample 3

For nitrate, the consensus standard deviations were only about double the homogeneities (table 4). For phosphate, the consensus standard deviations were 4.5 times greater than that of the homogeneities, and for silicic acid, the consensus standard deviation was more than 10 times greater than that of the homogeneities.

Table 4 Comparison between consensus standard deviation of sample 3 and homogeneity of sample 3

	Nitrate	**Phosphate**	**Silicic acid**
Homogeneity (%)	0.44	0.80	0.15
Standard deviation (CV %)	1.0	3.5	1.7

5.5 *Z*-scores

Z-scores, computed according to the method described in section 4.2, are summarized in table 5.
Laboratories 3, 5, 6, and 15 showed consistently good performance throughout the range of nutrients.

Table 5 Summary of *Z*-scores

Laboratory	Z_{NO_3}	Z_{NO_2}	Z_p	Z_s	$(Z_{NO_3}+Z_p)/2$	$(Z_{NO_3}+Z_p+Z_s)/3$
1	22.97	2.07	1.02	1.34	11.99	8.44
2	3.71	1.43	1.24	6.04	2.48	3.66
3	0.37	0.44	0.41	0.65	0.39	0.48
4	1.02	0.63	0.97	0.52	1.00	0.84
5	0.46	0.41	0.95	no data	0.71	not available
6	0.33	0.39	0.63	0.08	0.48	0.35
7	0.71	0.77	1.16	0.96	0.93	0.94
8	0.90	0.97	0.38	1.02	0.64	0.77
9	0.58	0.77	2.12	1.80	1.35	1.50
10	1.02	1.46	0.68	no data	0.85	not available
11	2.13	0.96	0.77	0.63	1.45	1.18
13	0.69	0.50	0.61	1.27	0.65	0.86
14	0.86	1.08	1.19	no data	1.02	not available
15	0.89	0.81	0.78	no data	0.83	not available
16	1.19	0.84	2.28	0.79	1.74	1.42
17	2.65	1.84	0.65	0.53	1.65	1.27
18	0.36*	no data	0.96	2.58	0.66	1.30

*Z_{NO_3} for this laboratory was not available; the listed value corresponds to $Z_{NO_3+NO_2}$.

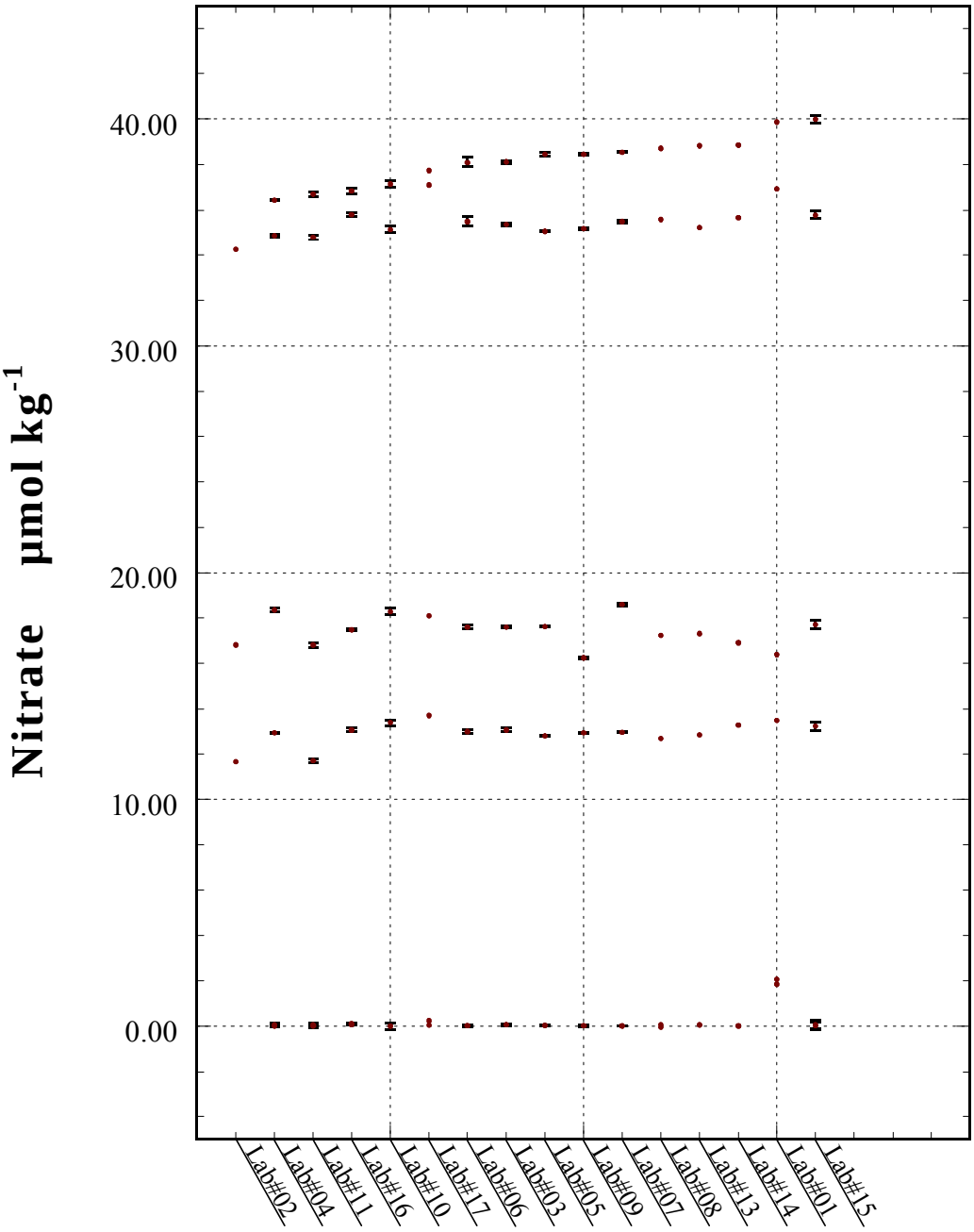

Figure 2 Nitrate results for sample 6: concentration *versus* laboratory number sorted by concentration.

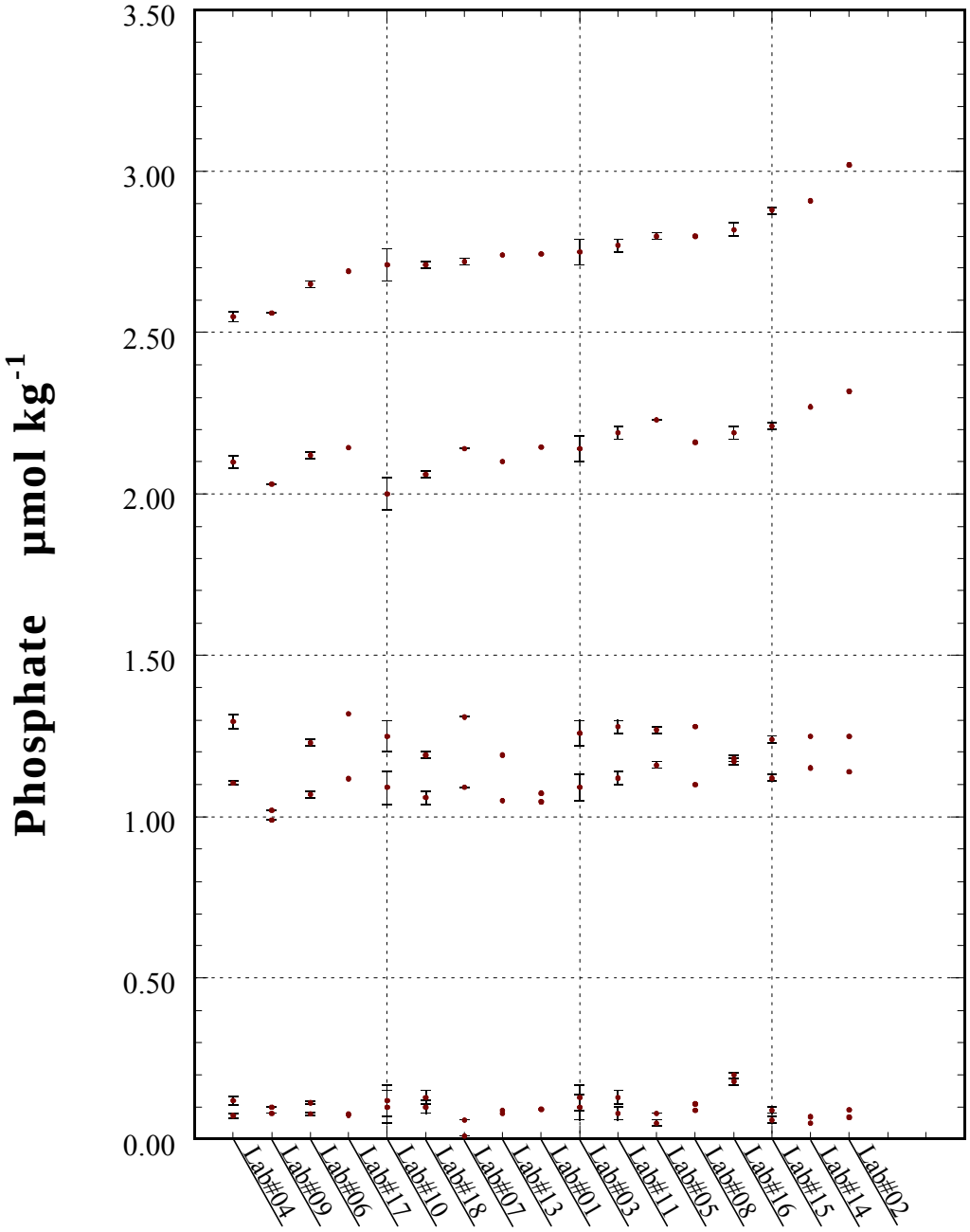

Figure 3 Phosphate results for sample 6: concentration *versus* laboratory number sorted by concentration.

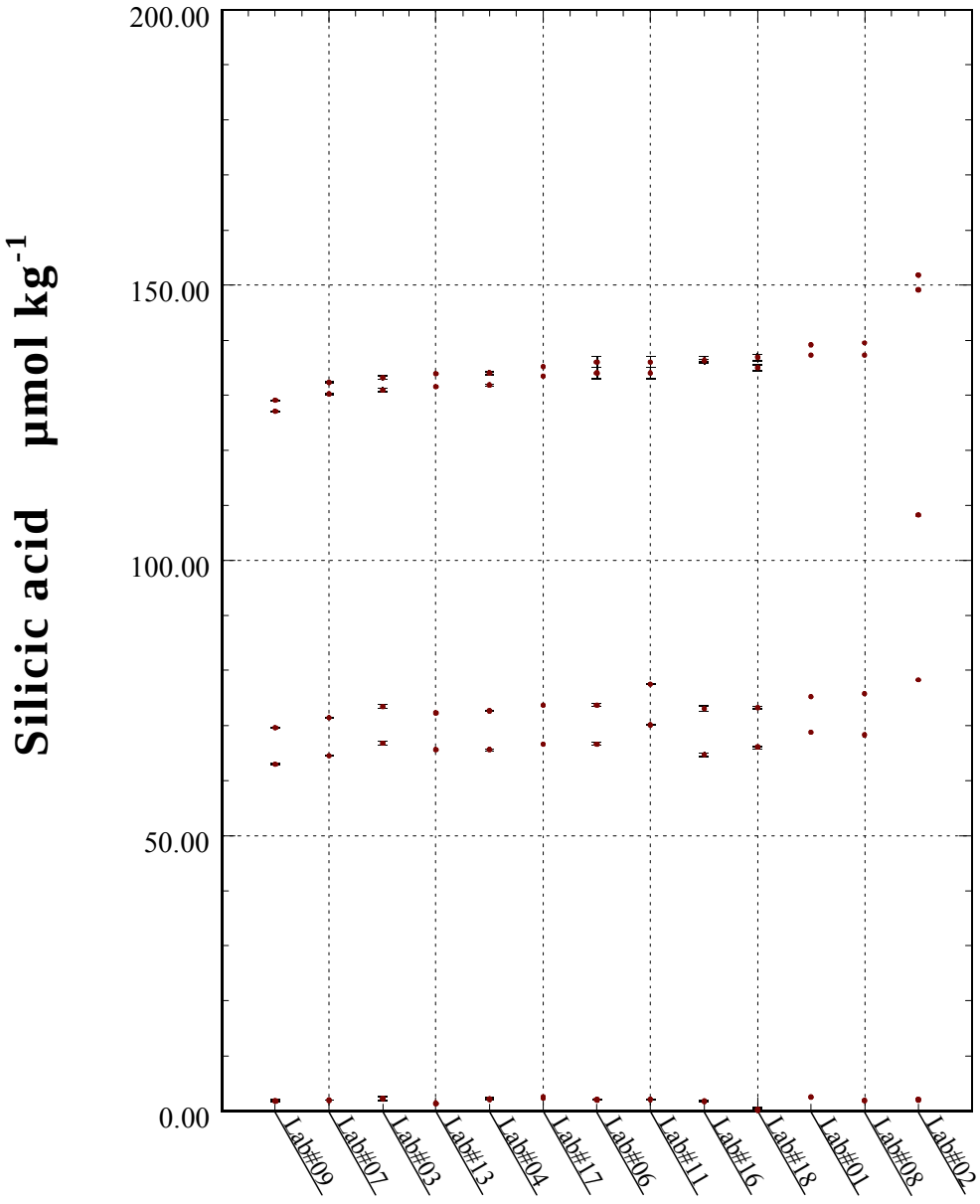

Figure 4 Silicic acid results for sample 3: concentration *versus* laboratory number sorted by concentration.

Sample 1 — Nitrite

Consensus Value: 0.02 ± 0.01 µmol kg^{-1}

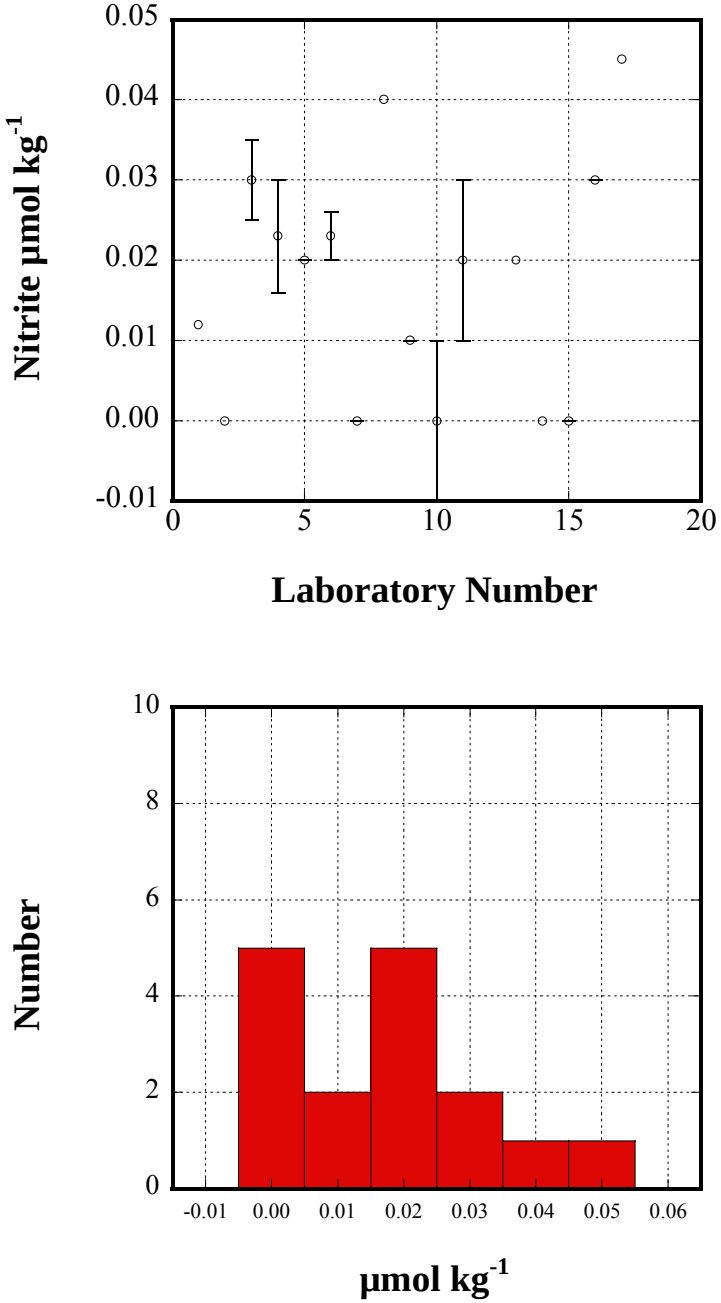

Figure 5-1 Nitrite results for sample 1: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 2 — Nitrite

Consensus Value: 0.13 ± 0.06 µmol kg^{-1}

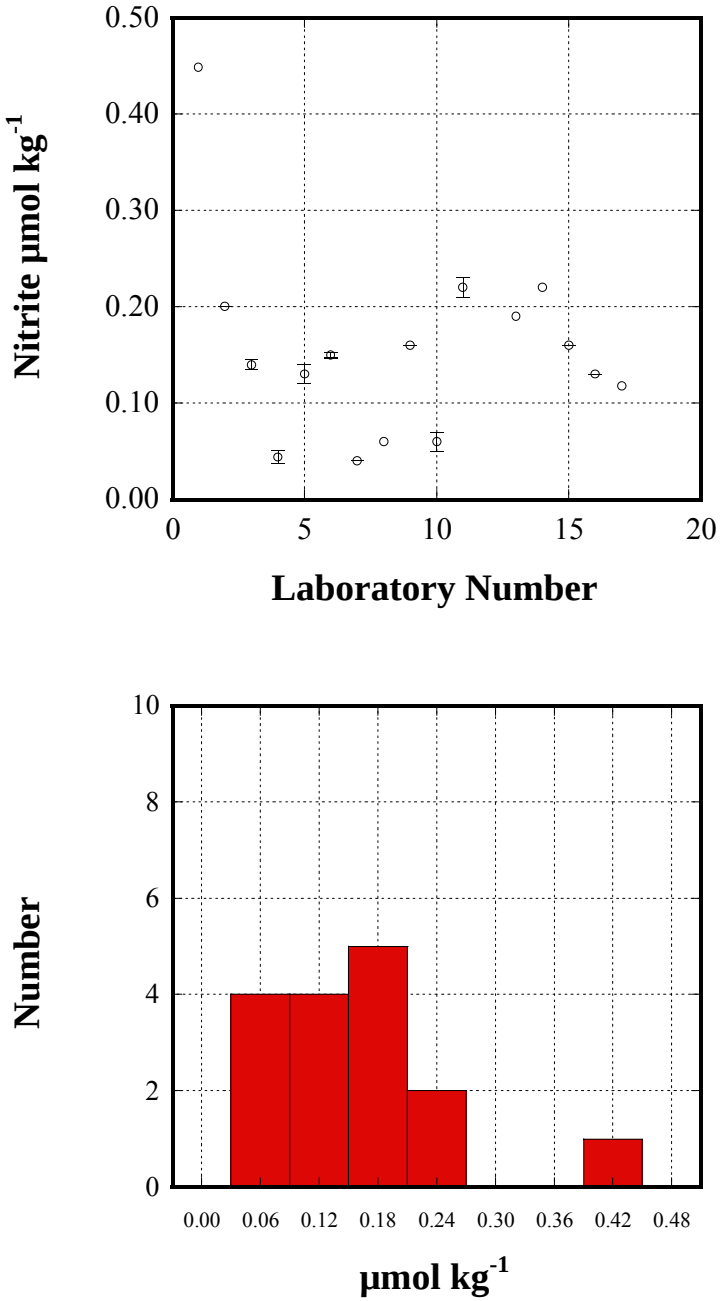

Figure 5-2 Nitrite results for sample 2: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 3 — Nitrite

Consensus Value: 0.01 ± 0.01 µmol kg^{-1}

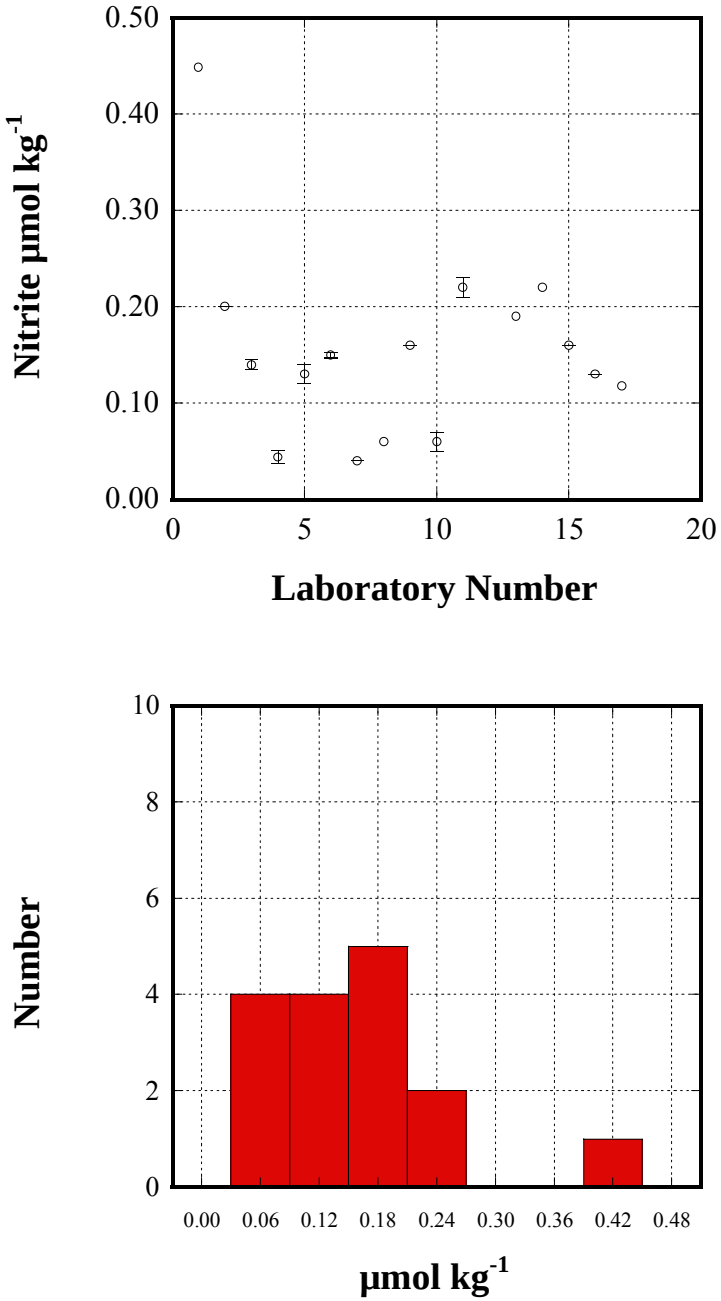

Figure 5-3 Nitrite results for sample 3: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 4 — Nitrite

Consensus Value: 0.02 ± 0.02 µmol kg^{-1}

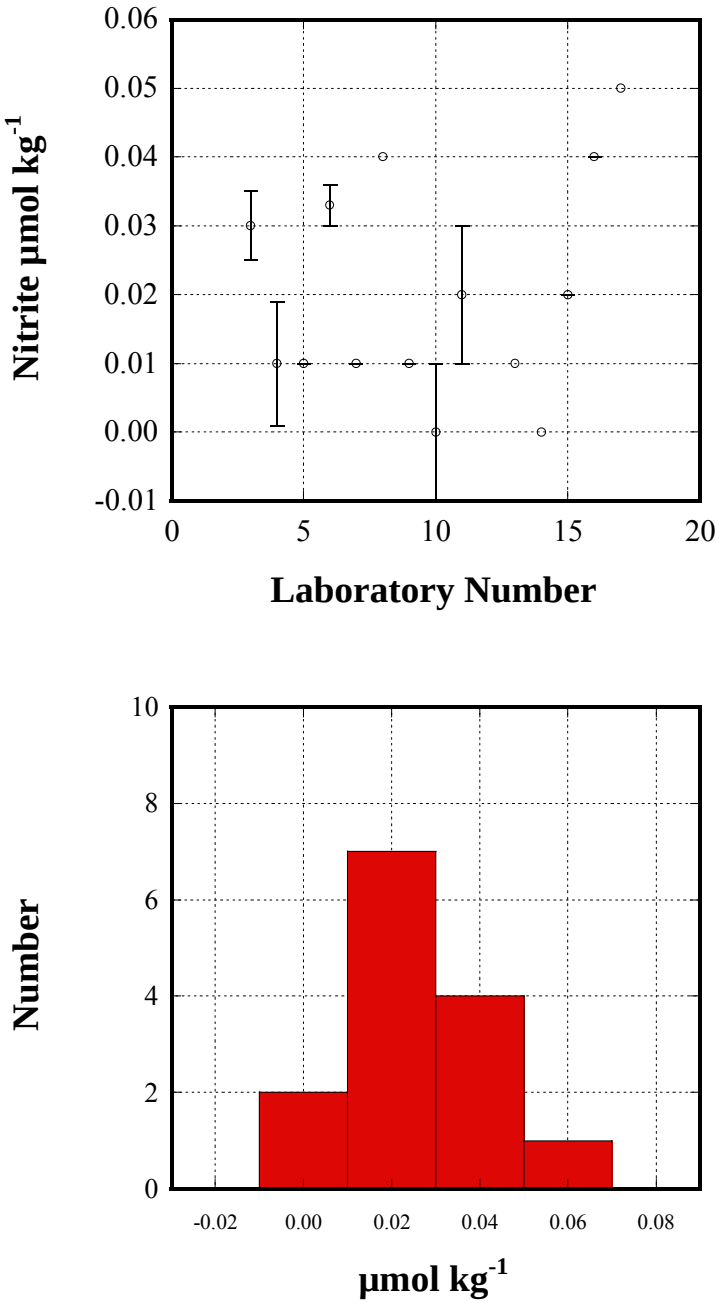

Figure 5-4 Nitrite results for sample 4: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 5 — Nitrite

Consensus Value: 0.91 ± 0.02 µmol kg^{-1}

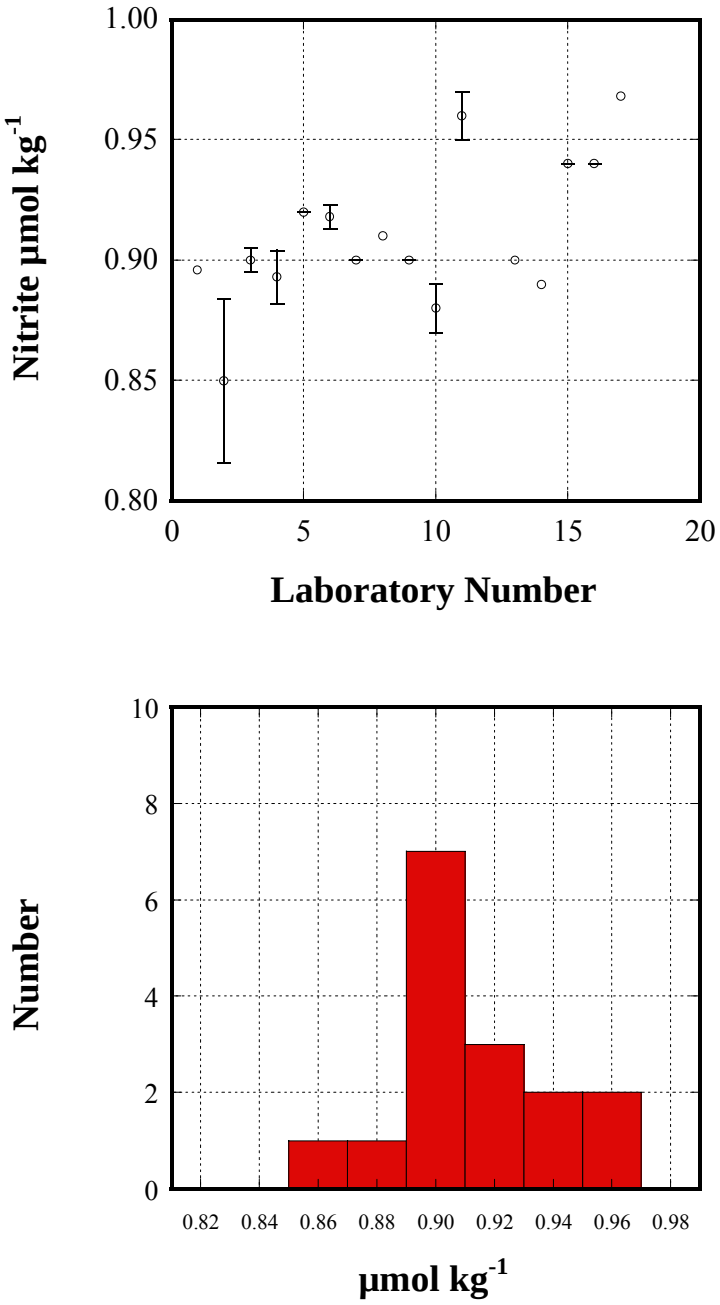

Figure 5-5 Nitrite results for sample 5: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 6 — Nitrite

Consensus Value: 0.23 ± 0.09 µmol kg^{-1}

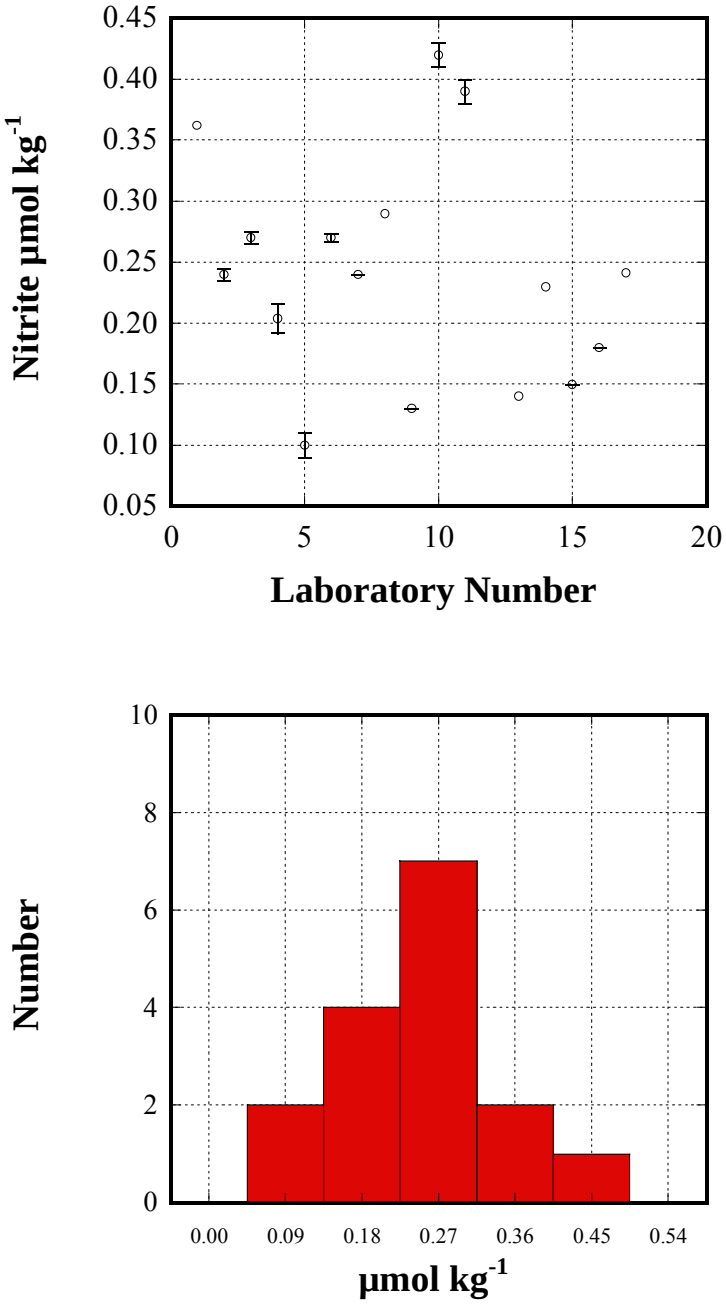

Figure 5-6 Nitrite results for sample 6: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 1 — Nitrate

Consensus Value: 0.04 ± 0.03 µmol kg^{-1}

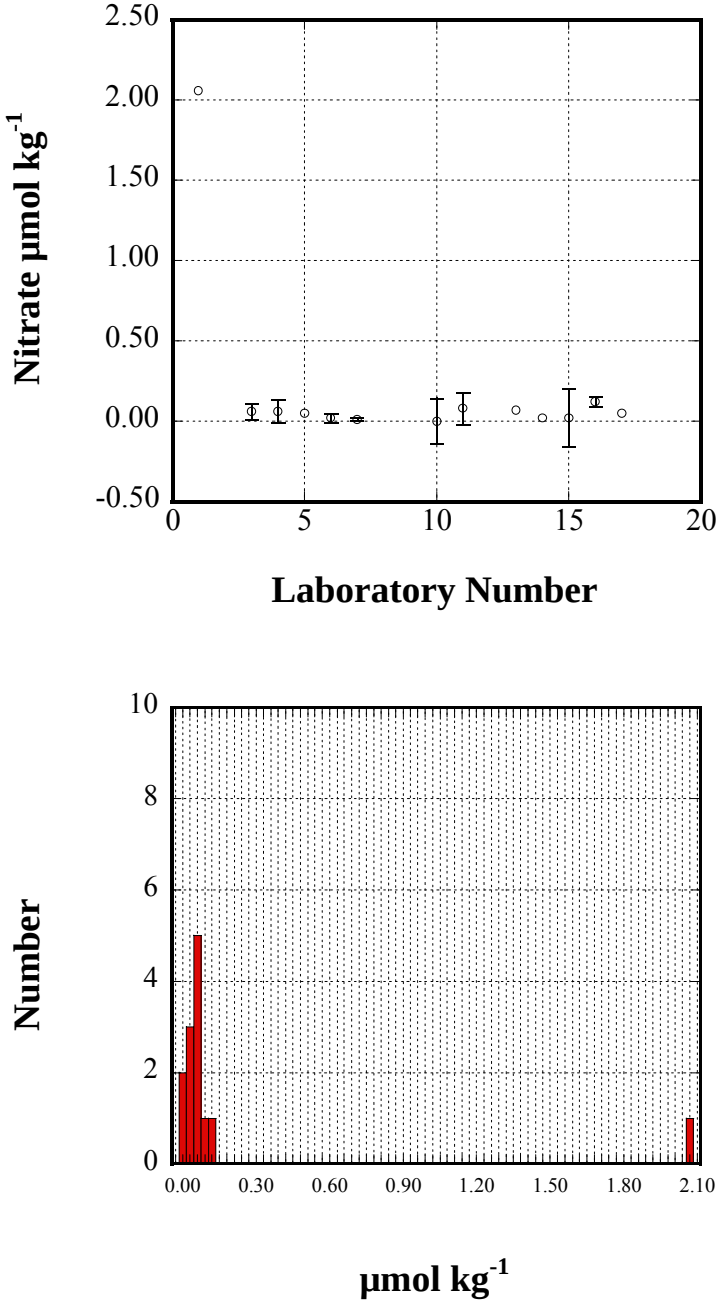

Figure 6-1 Nitrate results for sample 1: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 2 — Nitrate

Consensus Value: 17.4 ± 0.7 µmol kg^{-1}

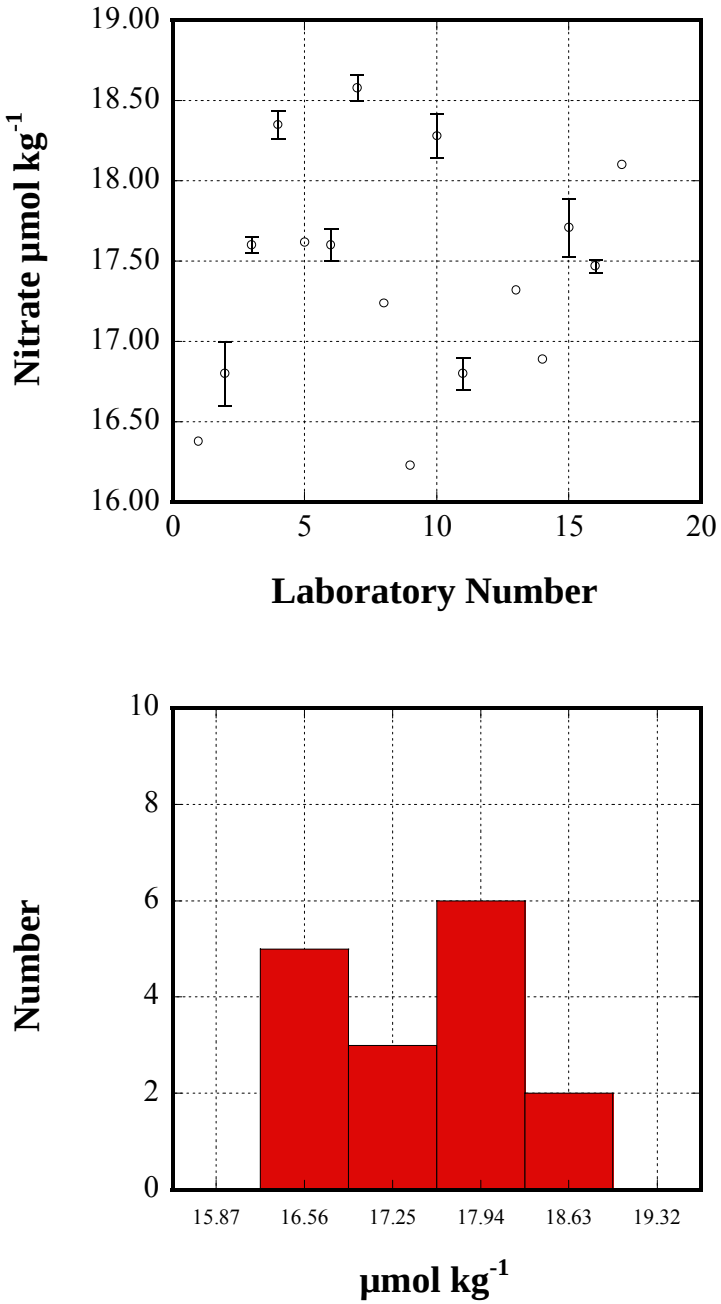

Figure 6-2 Nitrate results for sample 2: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 3 — Nitrate

Consensus Value: 35.3 ± 0.3 µmol kg^{-1}

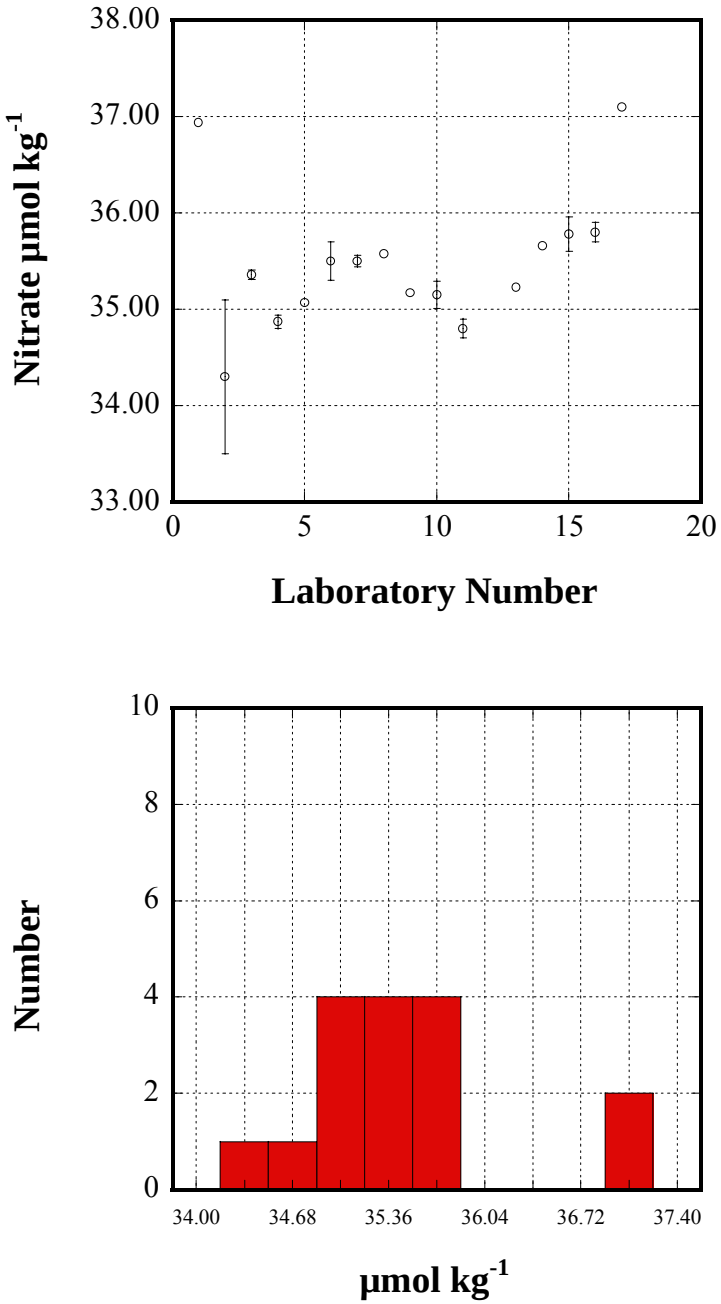

Figure 6-3 Nitrate results for sample 3: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 4 — Nitrate

Consensus Value: 0.02 ± 0.03 µmol kg^{-1}

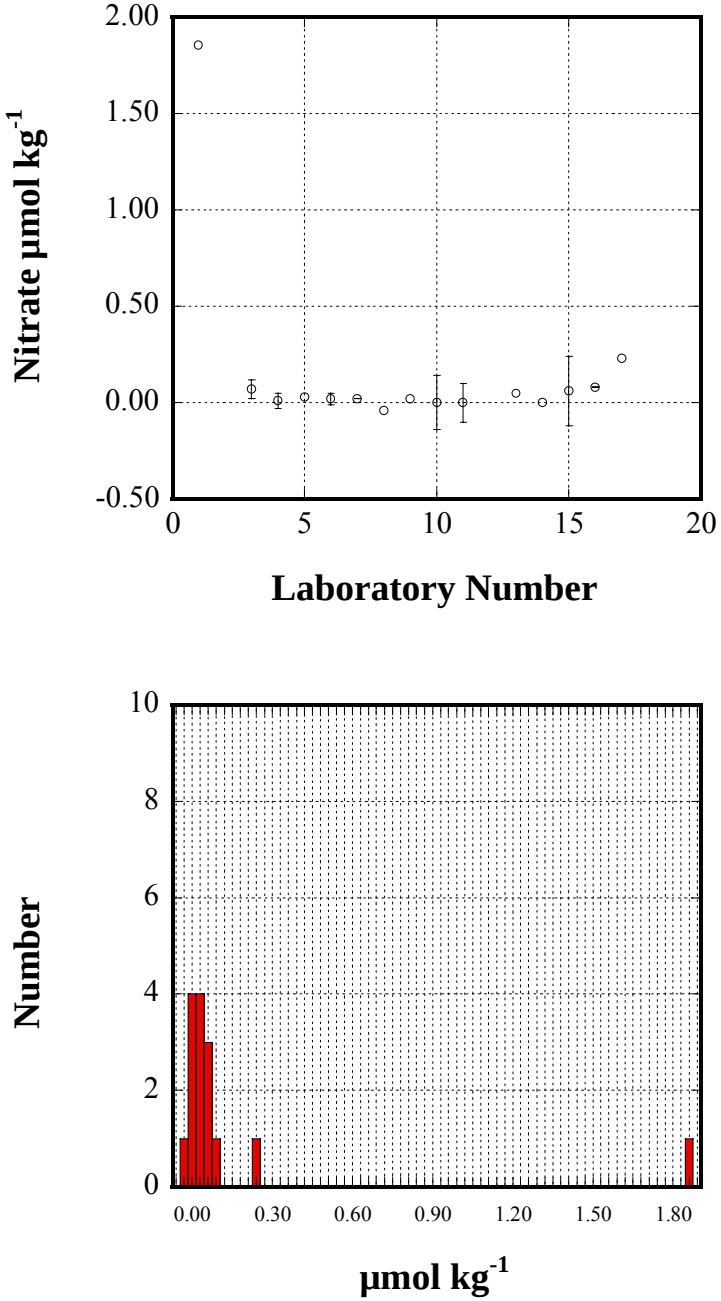

Figure 6-4 Nitrate results for sample 4: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 5 — Nitrate

Consensus Value: 13.1± 0.2 µmol kg^{-1}

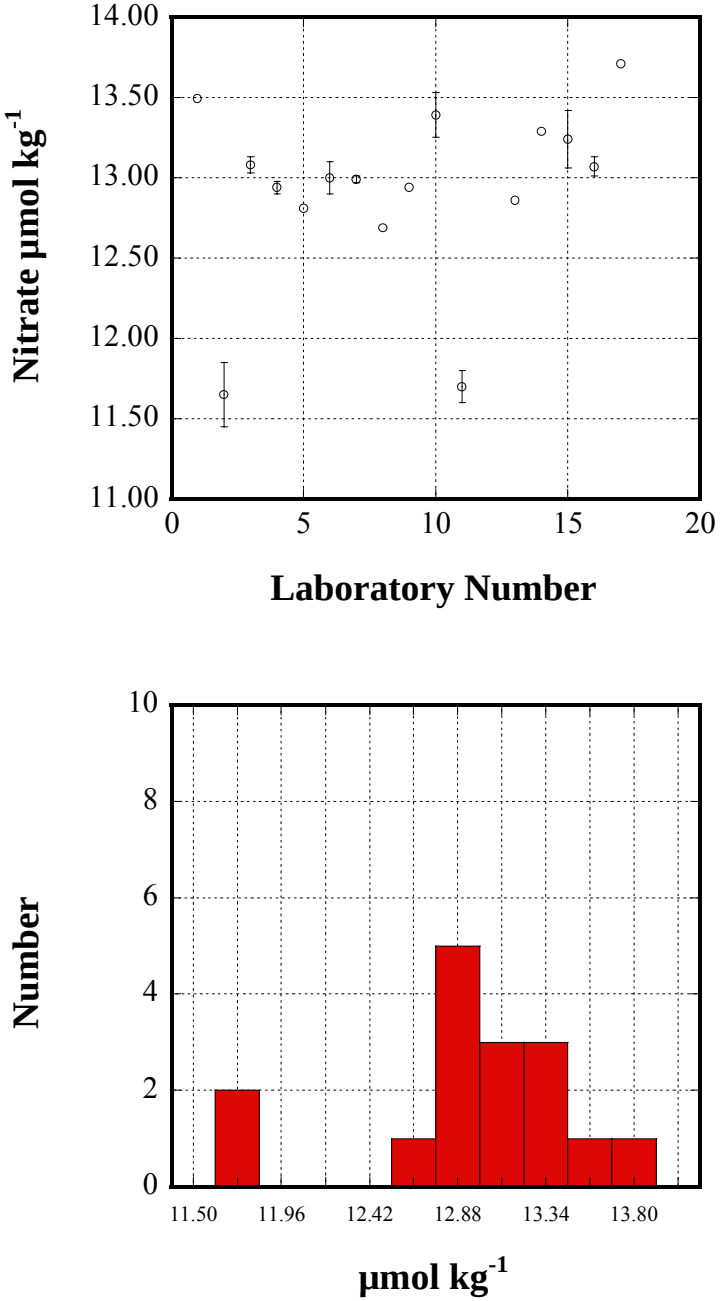

Figure 6-5 Nitrate results for sample 5: concentration *versus* laboratory number (upper panel)

Frequency distribution of concentration (lower panel).

Sample 6 — Nitrate

Consensus Value: 38.2 ± 1.1 µmol kg^{-1}

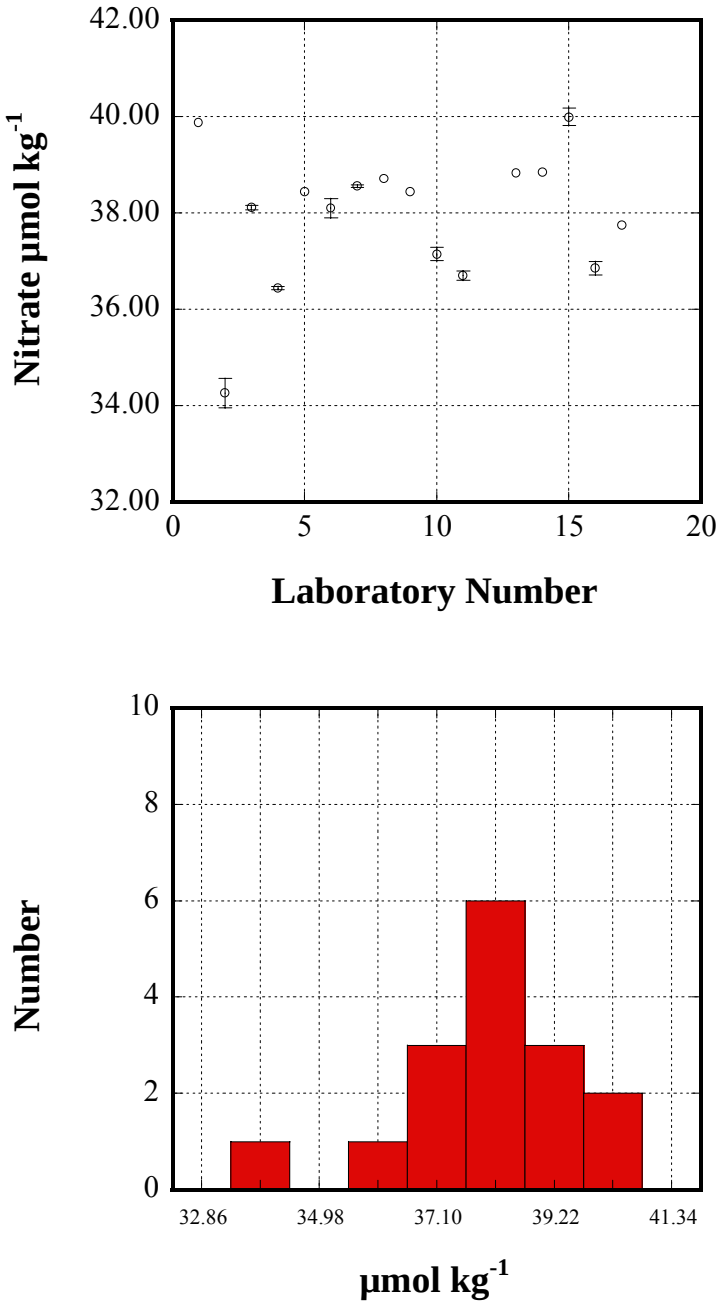

Figure 6-6 Nitrate results for sample 6: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 1 — Nitrate + Nitrite

Consensus Value: 0.06 ± 0.04 µmol kg^{-1}

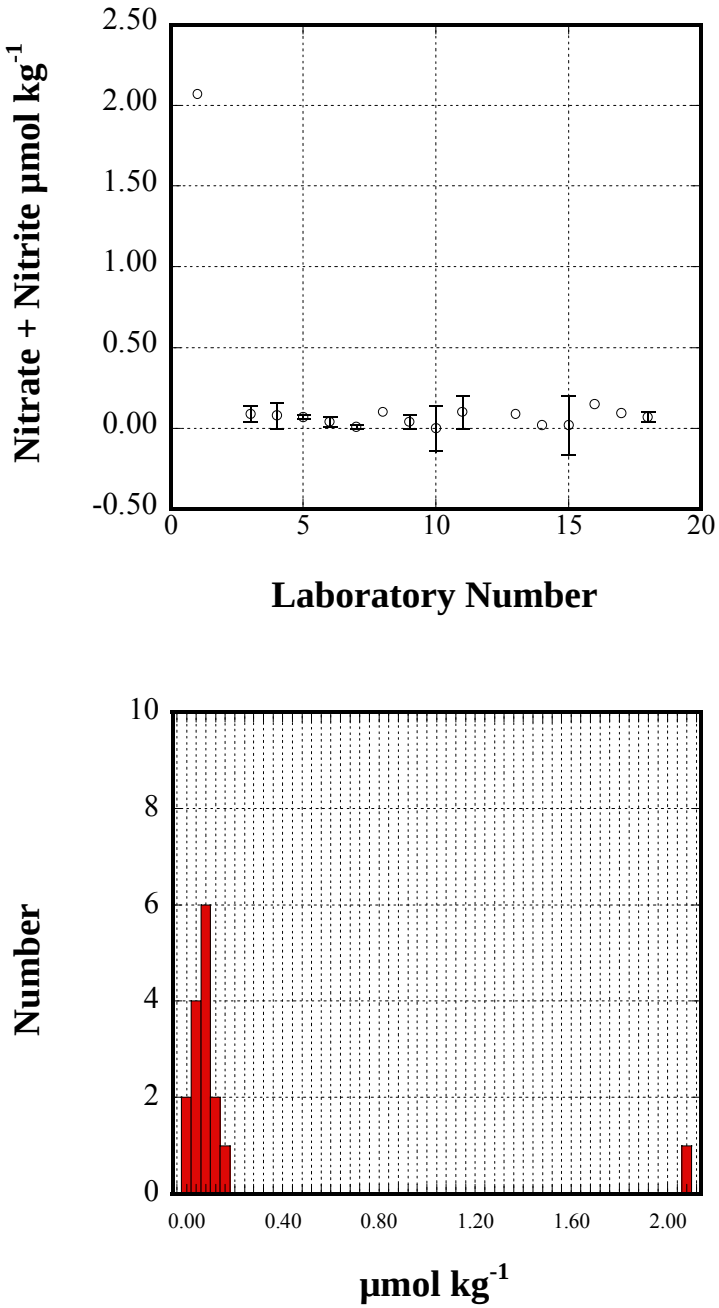

Figure 7-1 Nitrate + nitrite results for sample 1: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 2 — Nitrate + Nitrite

Consensus Value: 17.6 ± 0.6 ? mol kg^{-1}

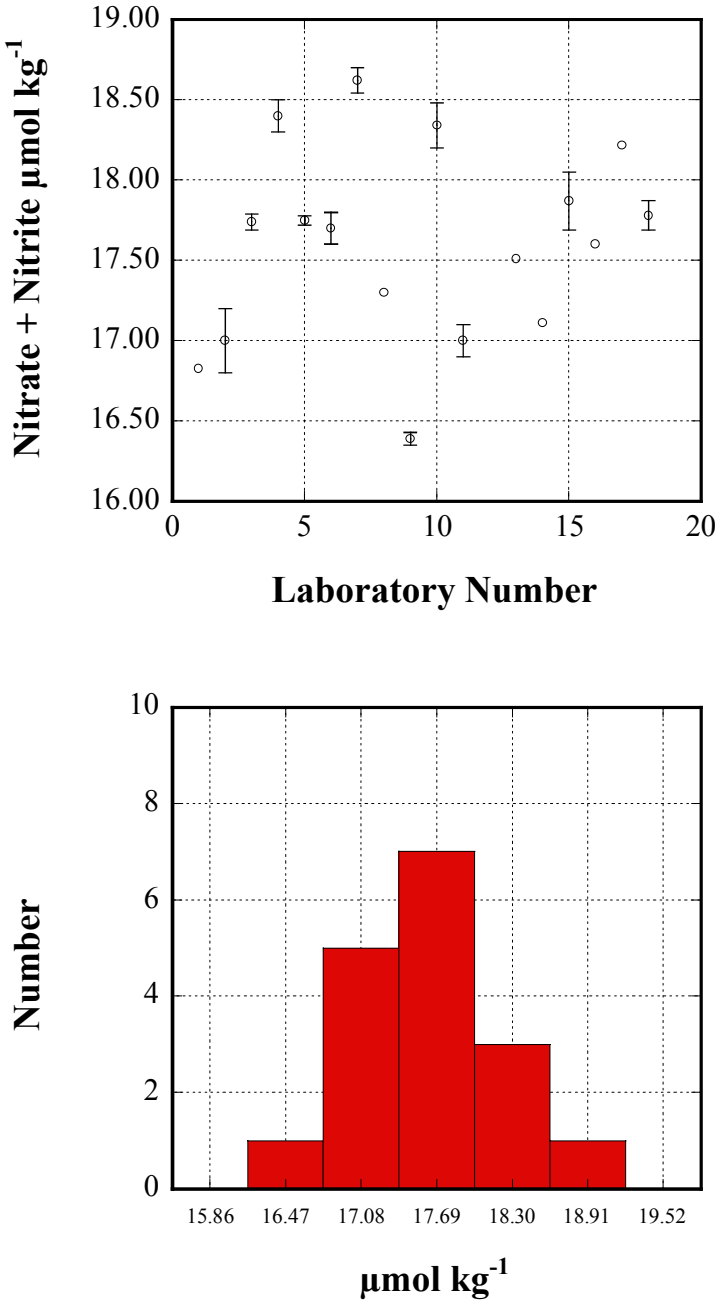

Figure 7-2 Nitrate + nitrite results for sample 2: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 3 — Nitrate + Nitrite

Consensus Value: 35.4 ± 0.3 µmol kg^{-1}

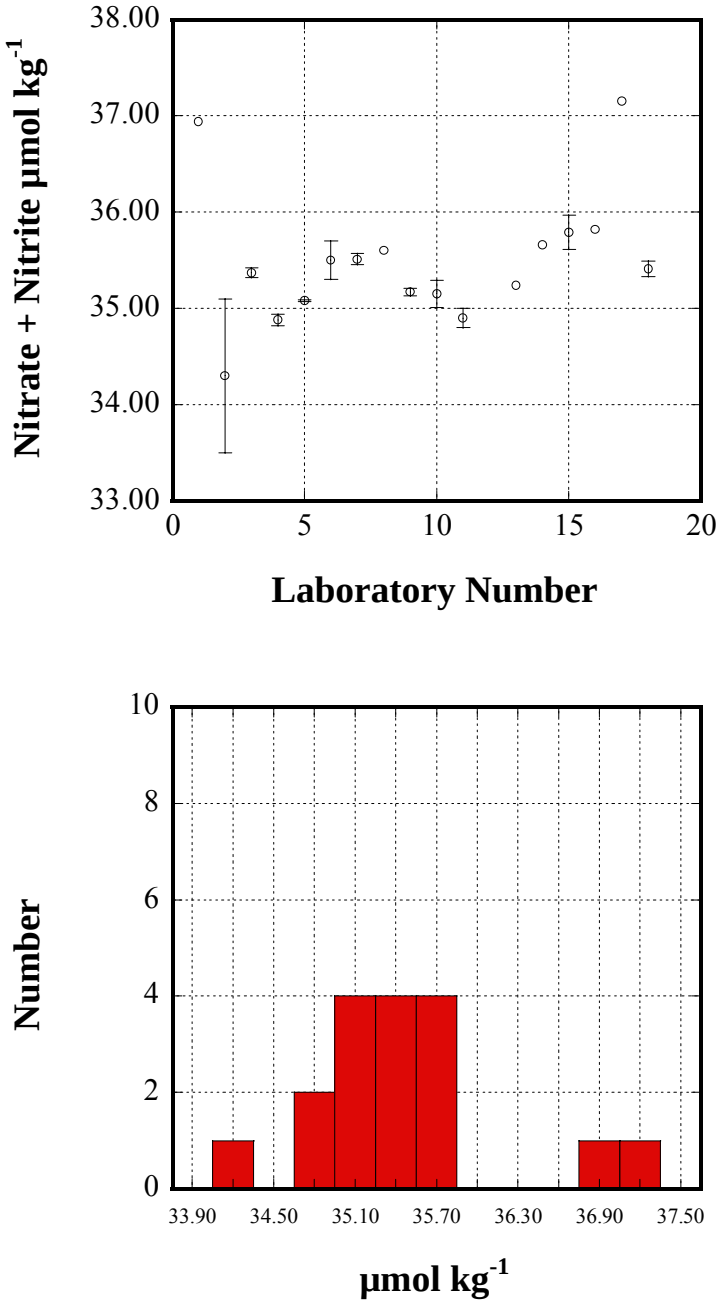

Figure 7-3 Nitrate + nitrite results for sample 3: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 4 — Nitrate + Nitrite

Consensus Value: 0.04 ± 0.04 µmol kg^{-1}

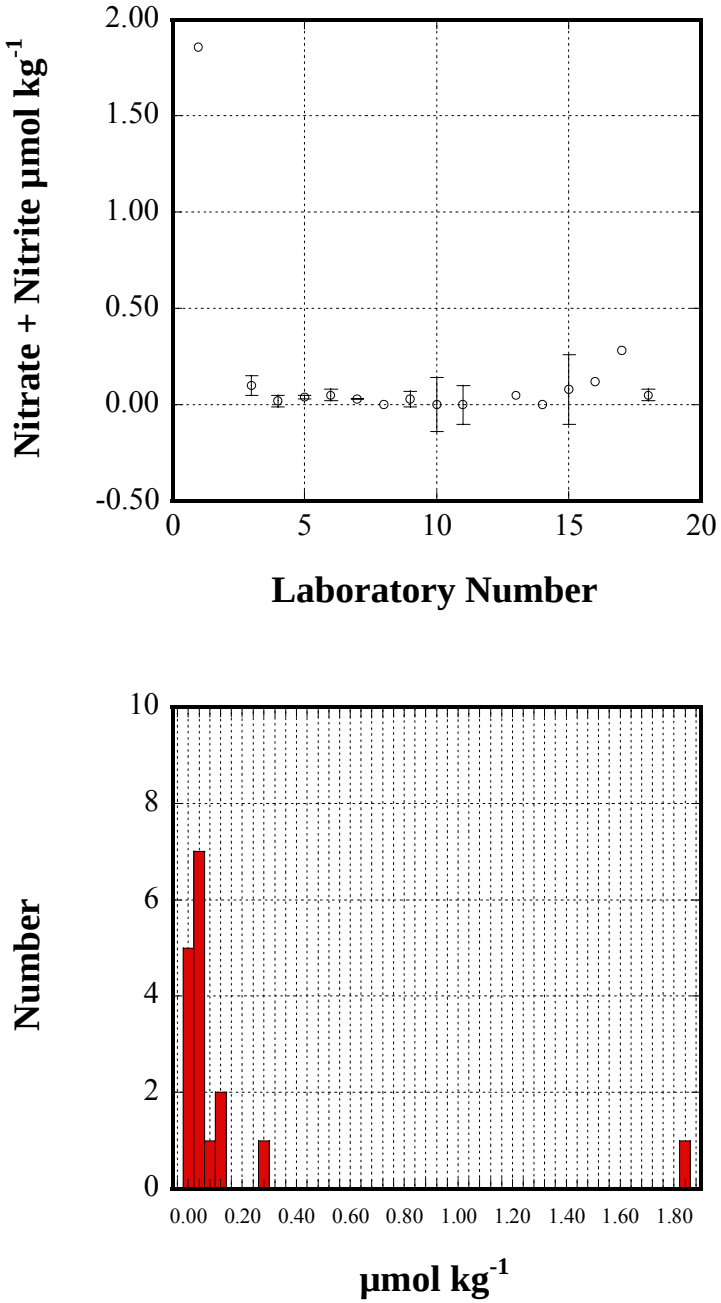

Figure 7-4 Nitrate + nitrite results for sample 4: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 5 — Nitrate + Nitrite

Consensus Value: 14.0 ± 0.2 µmol kg^{-1}

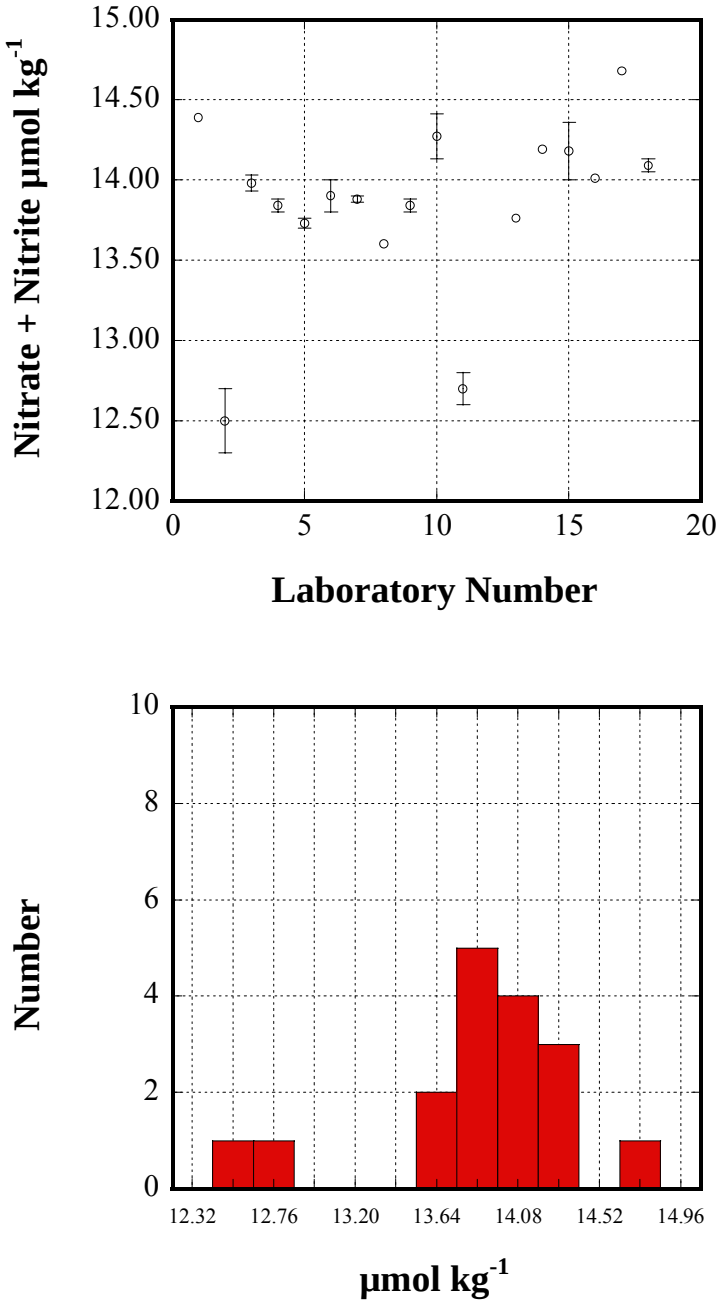

Figure 7-5 Nitrate + nitrite results for sample 5: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 6 — Nitrate + Nitrite

Consensus Value: 38.4 ± 1.0 µmol kg^{-1}

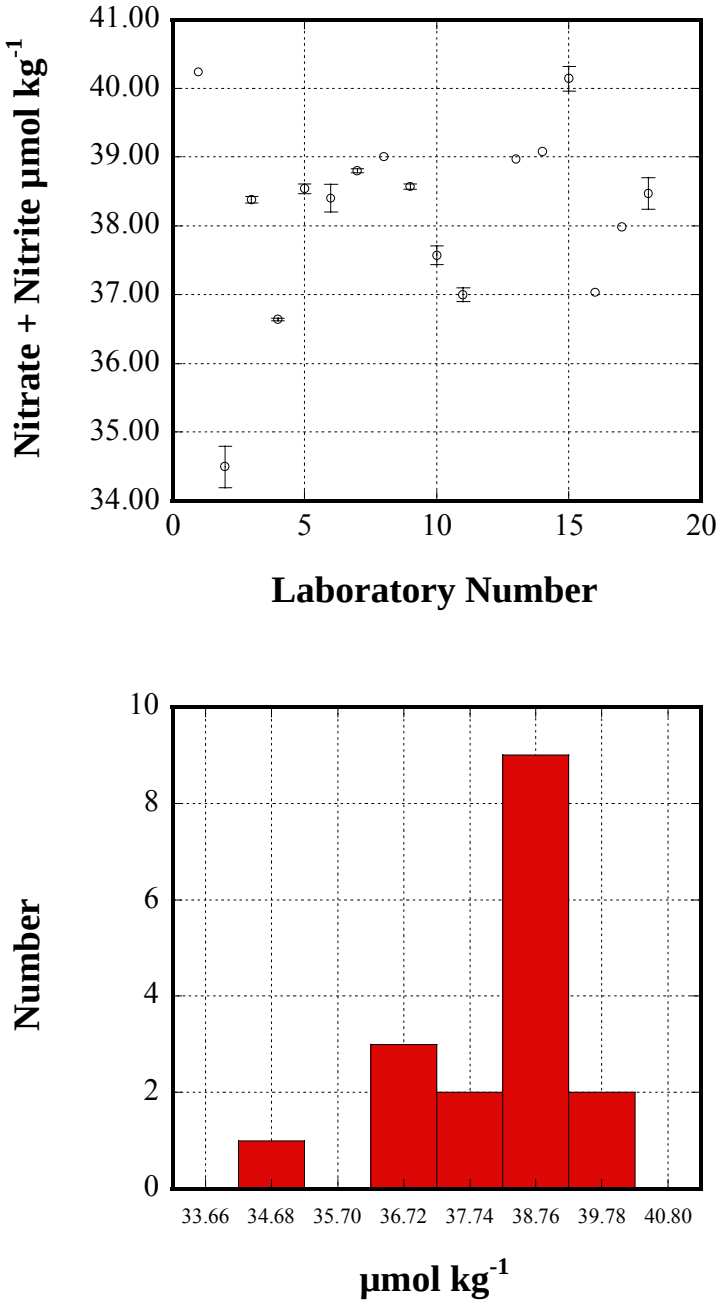

Figure 7-6 Nitrate + nitrite results for sample 6: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 1 — Phosphate

Consensus Value: 0.09 ± 0.02 µmol kg^{-1}

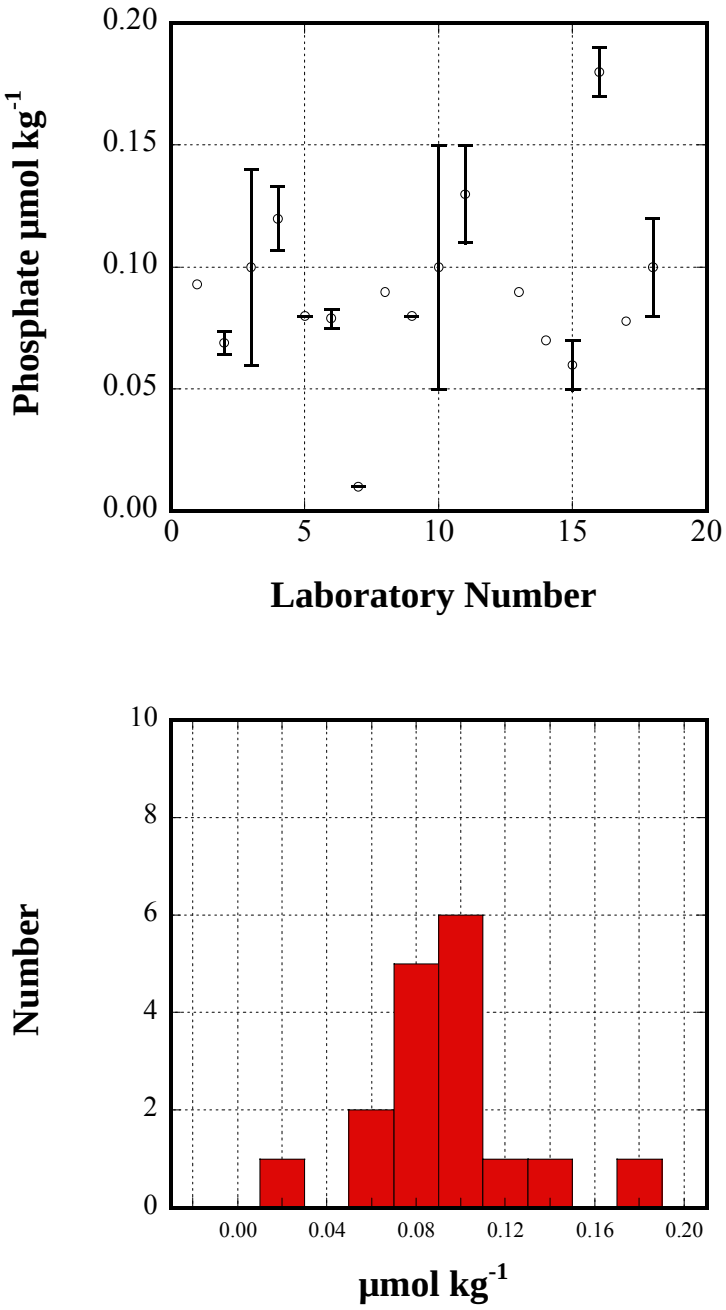

Figure 8-1 Phosphate results for sample 1: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 2 — Phosphate

Consensus Value: 1.25 ± 0.04 µmol kg^{-1}

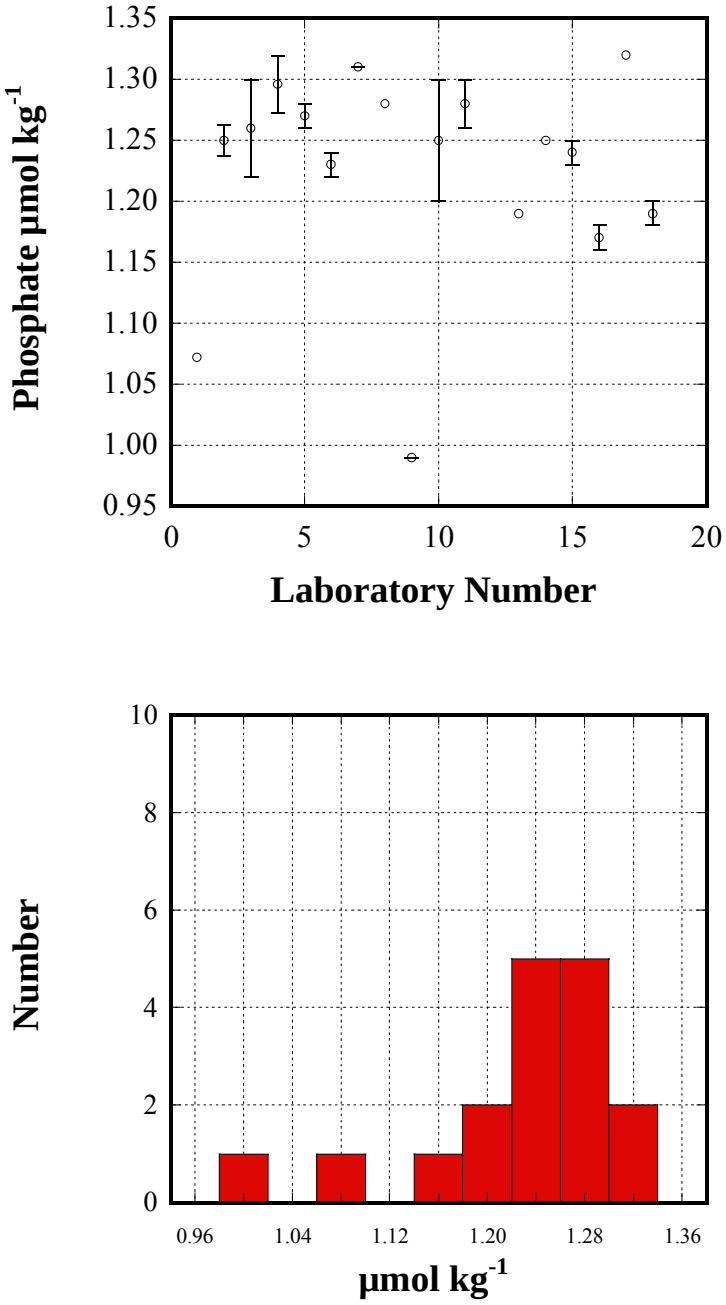

Figure 8-2 Phosphate results for sample 2: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 3 — Phosphate

Consensus Value: 2.14 ± 0.07 µmol kg^{-1}

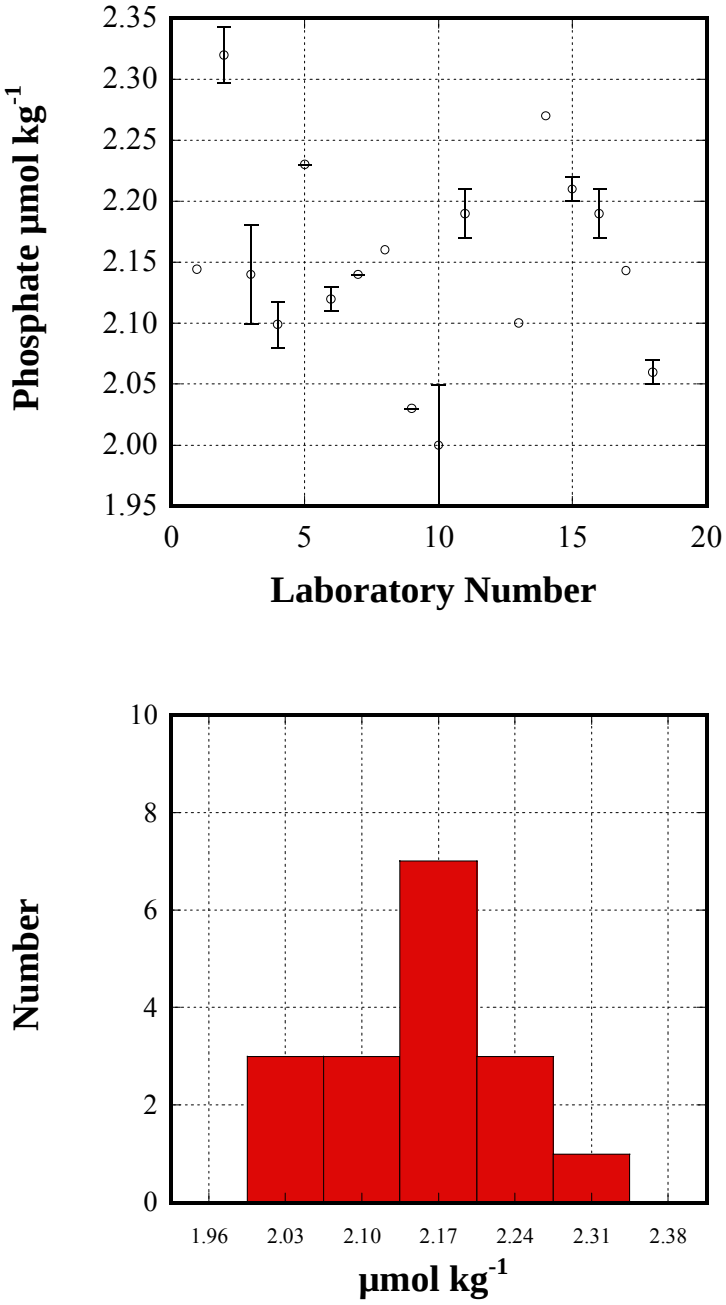

Figure 8-3 Phosphate results for sample 3: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 4 — Phosphate

Consensus Value: 0.09 ± 0.03 µmol kg^{-1}

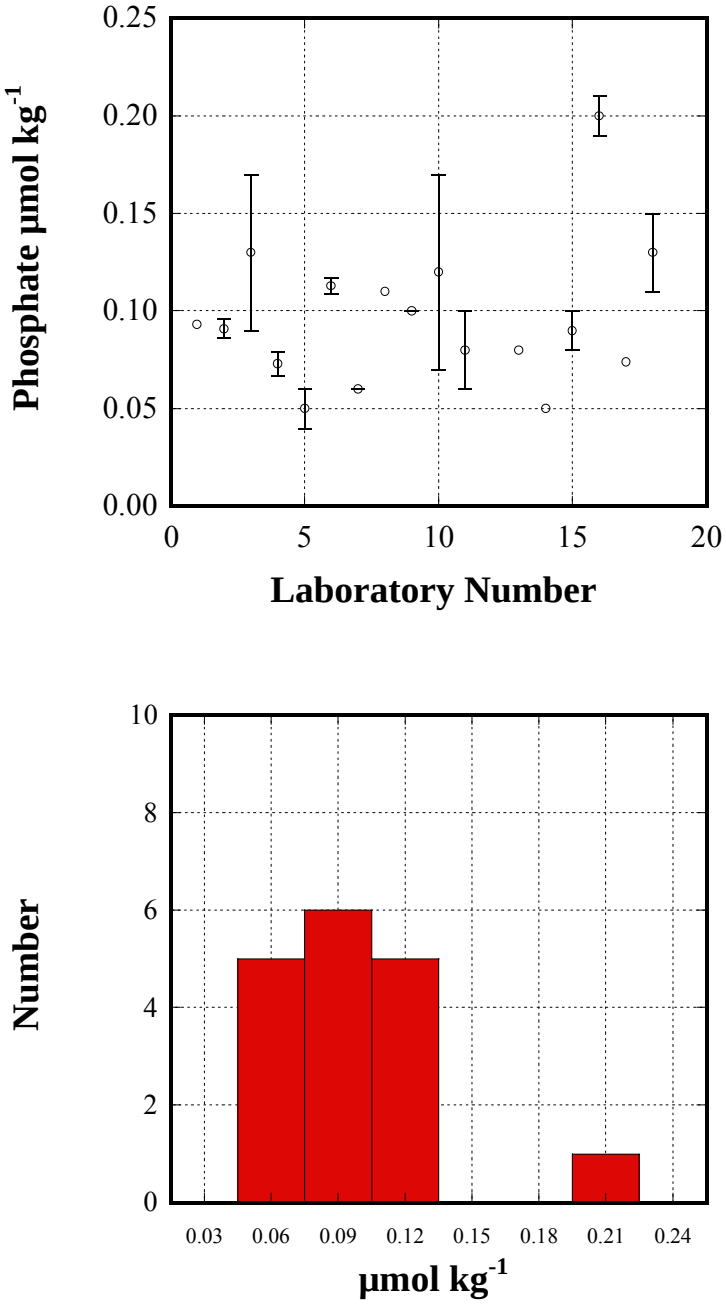

Figure 8-4 Phosphate results for sample 4: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 5 — Phosphate

Consensus Value: 1.10 ± 0.04 µmol kg^{-1}

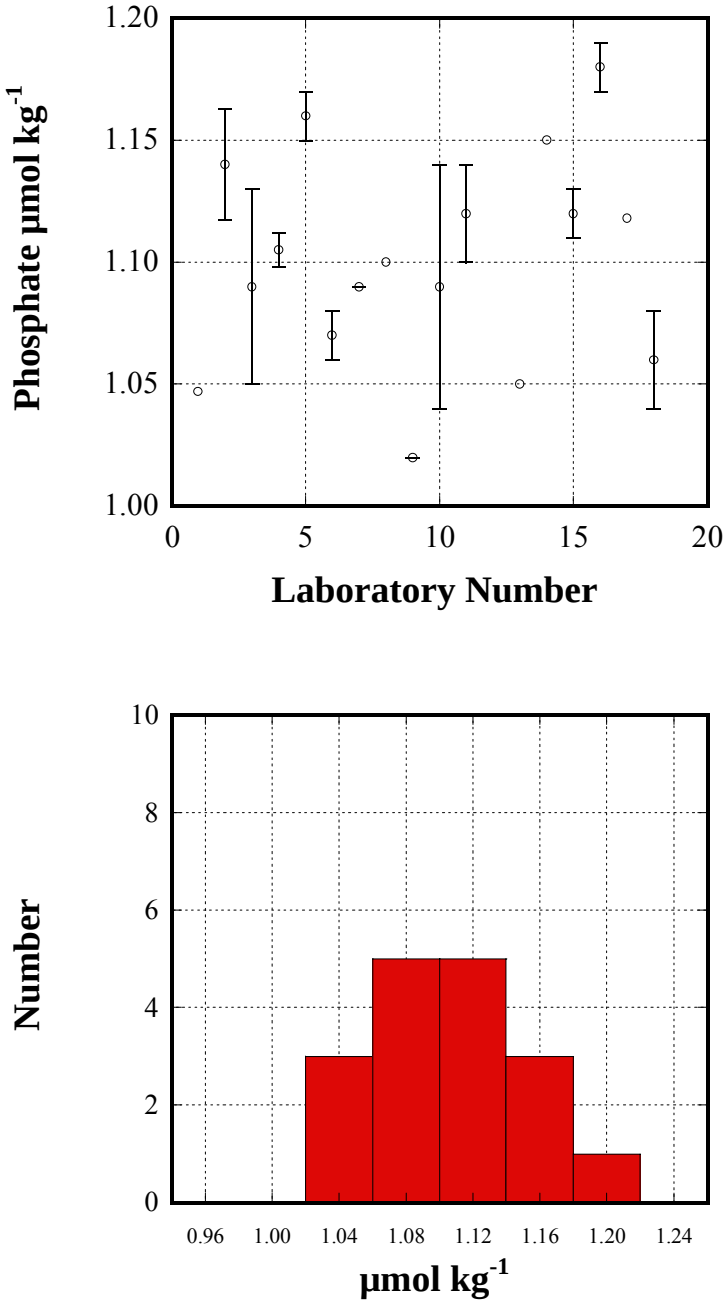

Figure 8-5 Phosphate results for sample 5: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 6 — Phosphate

Consensus Value: 2.74 ± 0.10 µmol kg^{-1}

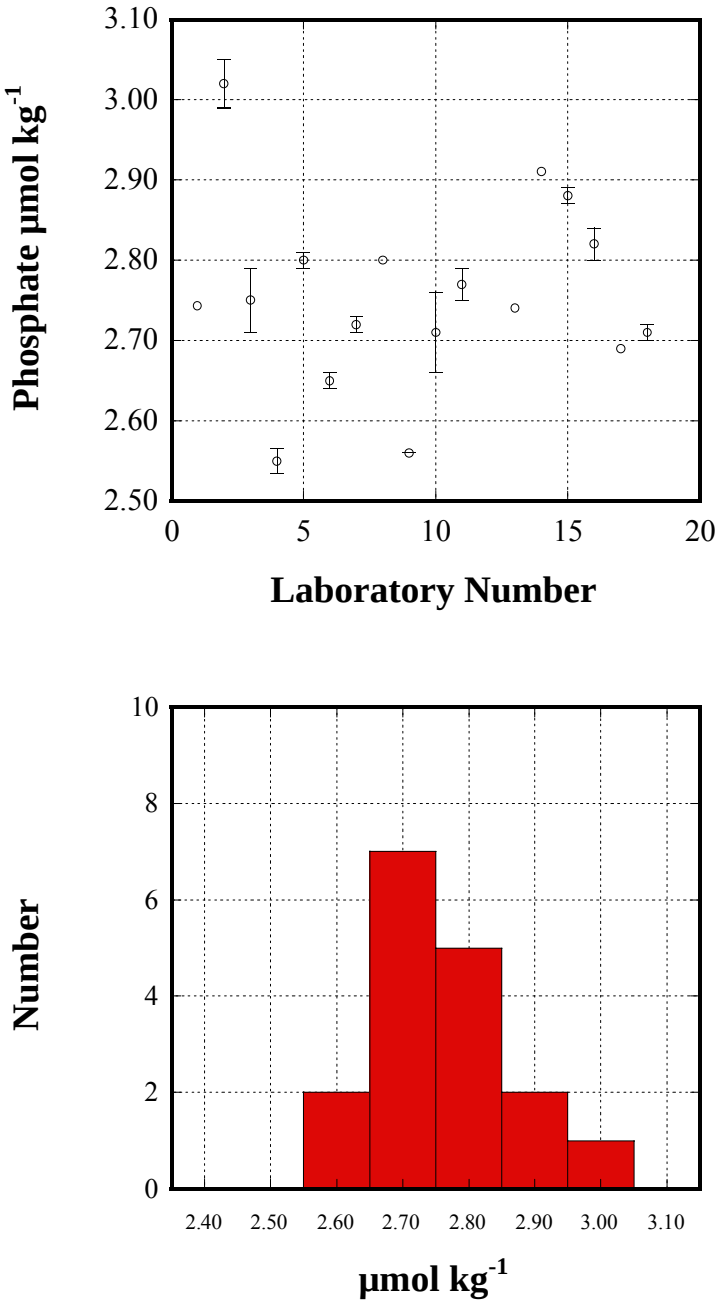

Figure 8-6 Phosphate results for sample 6: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 1 — Silicic acid

Consensus Value: 2.06 ± 0.23 µmol kg^{-1}

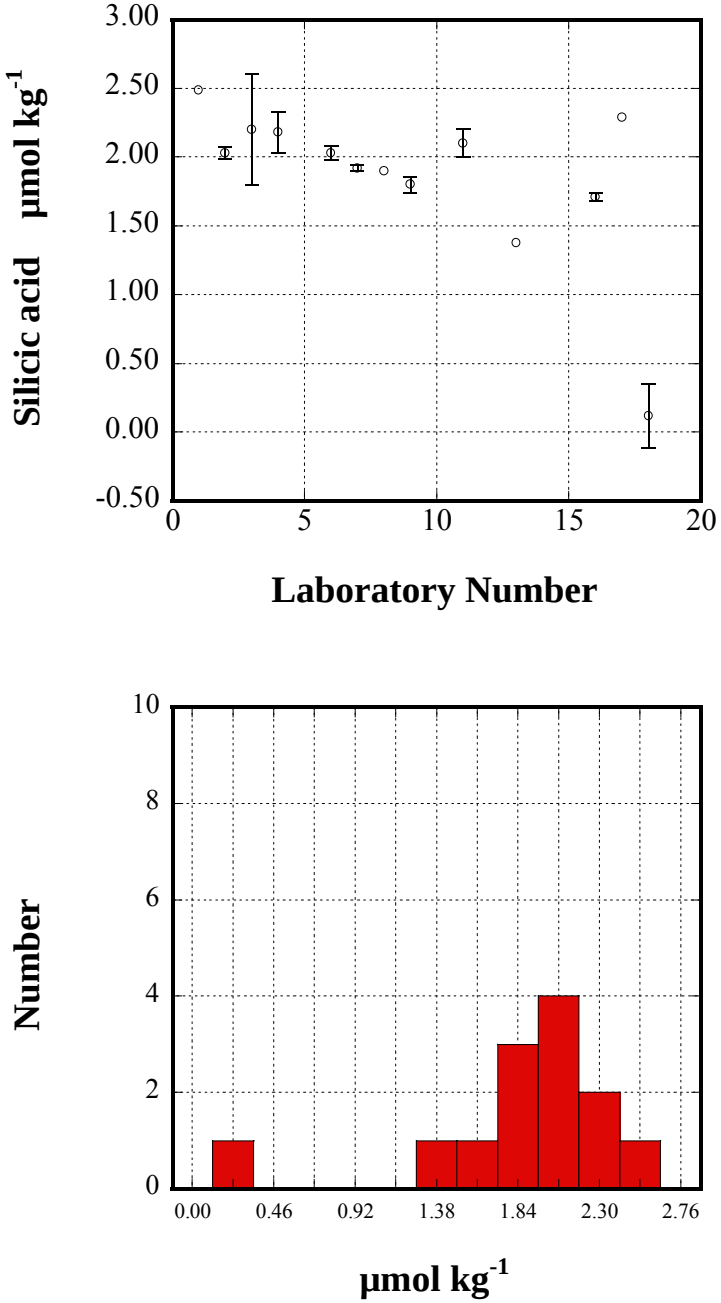

Figure 9-1 Silicic acid results for sample 1: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 2 — Silicic acid

Consensus Value: 66.4 ± 2.0 µmol kg^{-1}

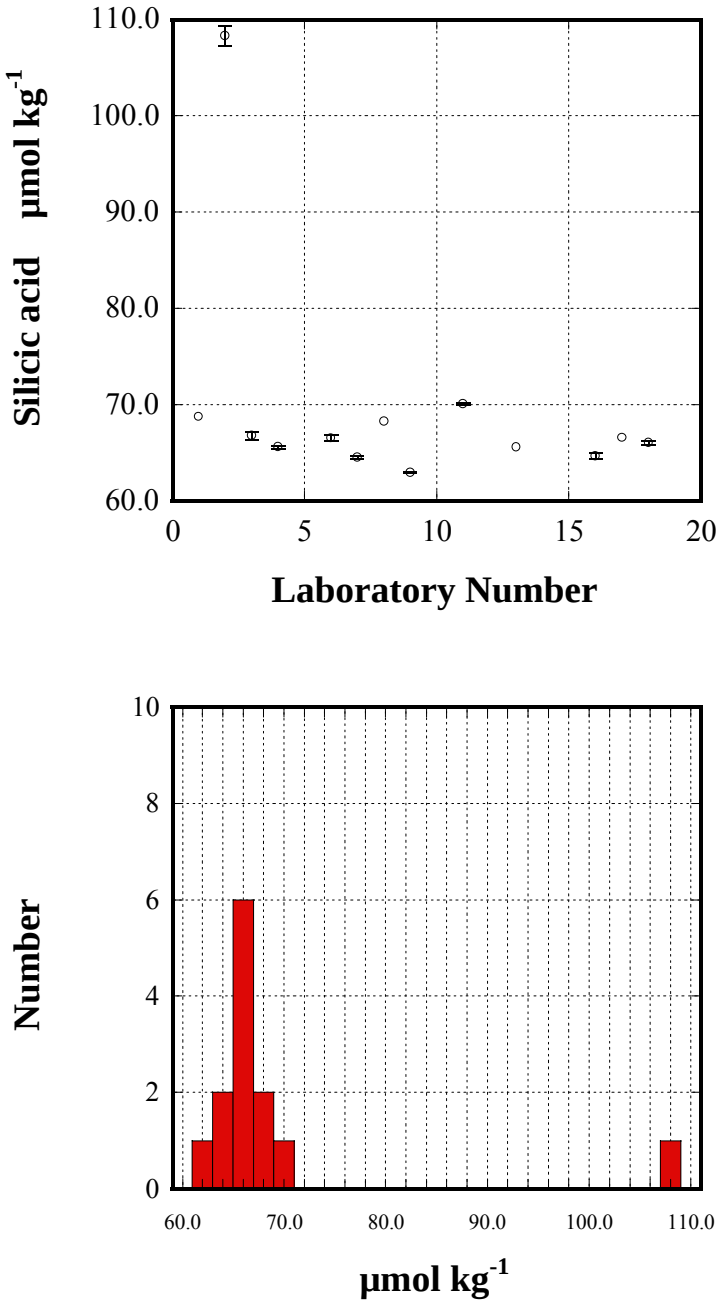

Figure 9-2 Silicic acid results for sample 2: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 3 — Silicic acid

Consensus Value: 135.7 ± 2.3 µmol kg^{-1}

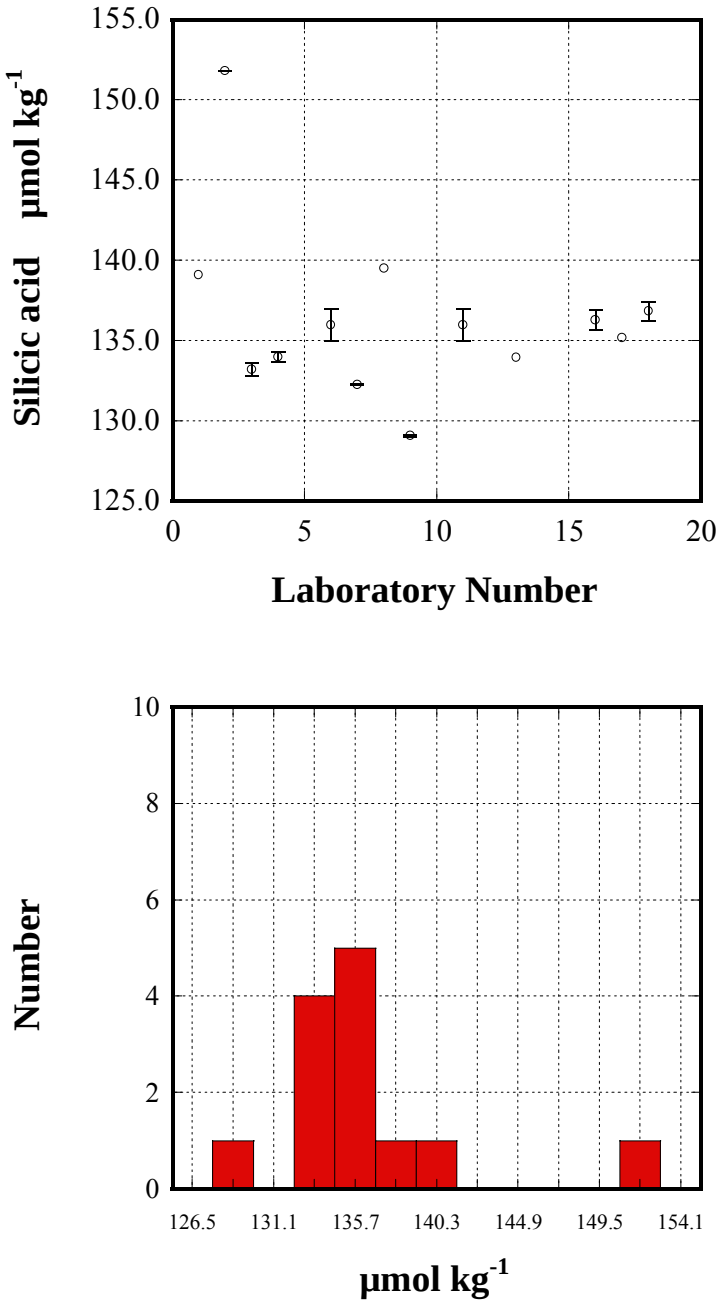

Figure 9-3 Silicic acid results for sample 3: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 4 — Silicic acid

Consensus Value: 2.09 ± 0.31 µmol kg^{-1}

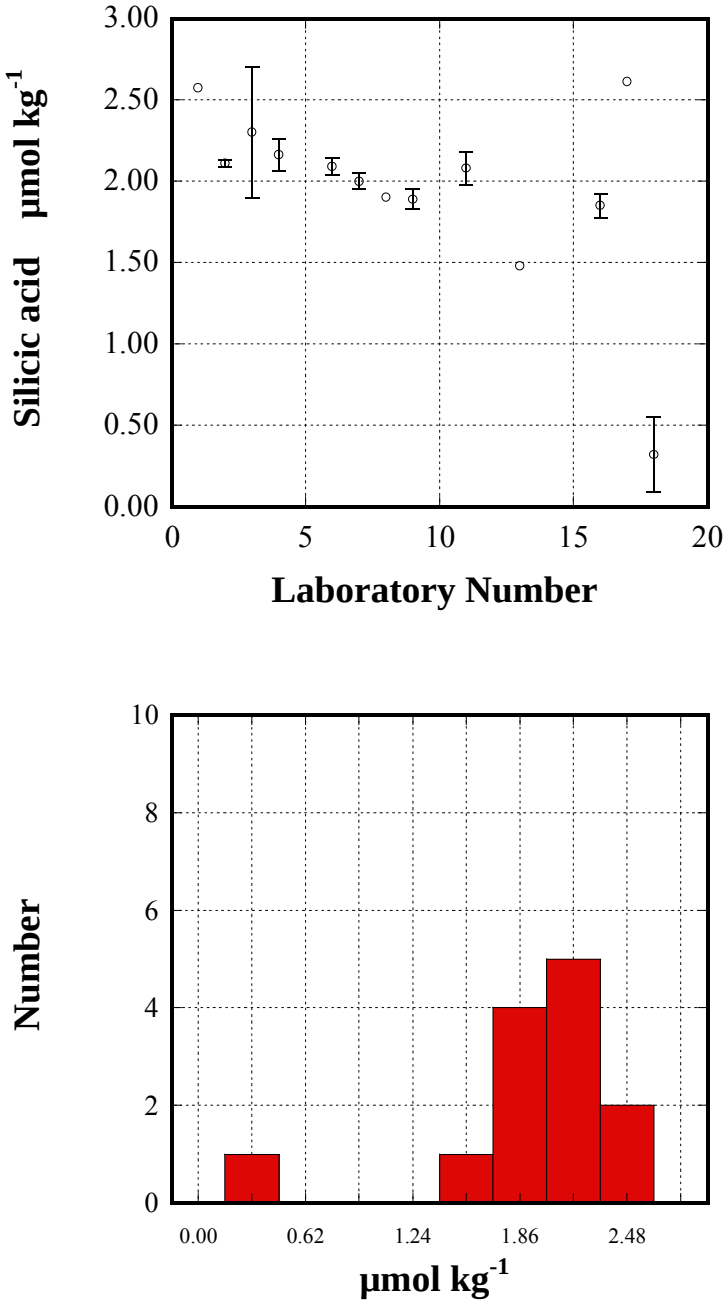

Figure 9-4 Silicic acid results for sample 4: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 5 — Silicic acid

Consensus Value: 73.8 ± 2.4 µmol kg^{-1}

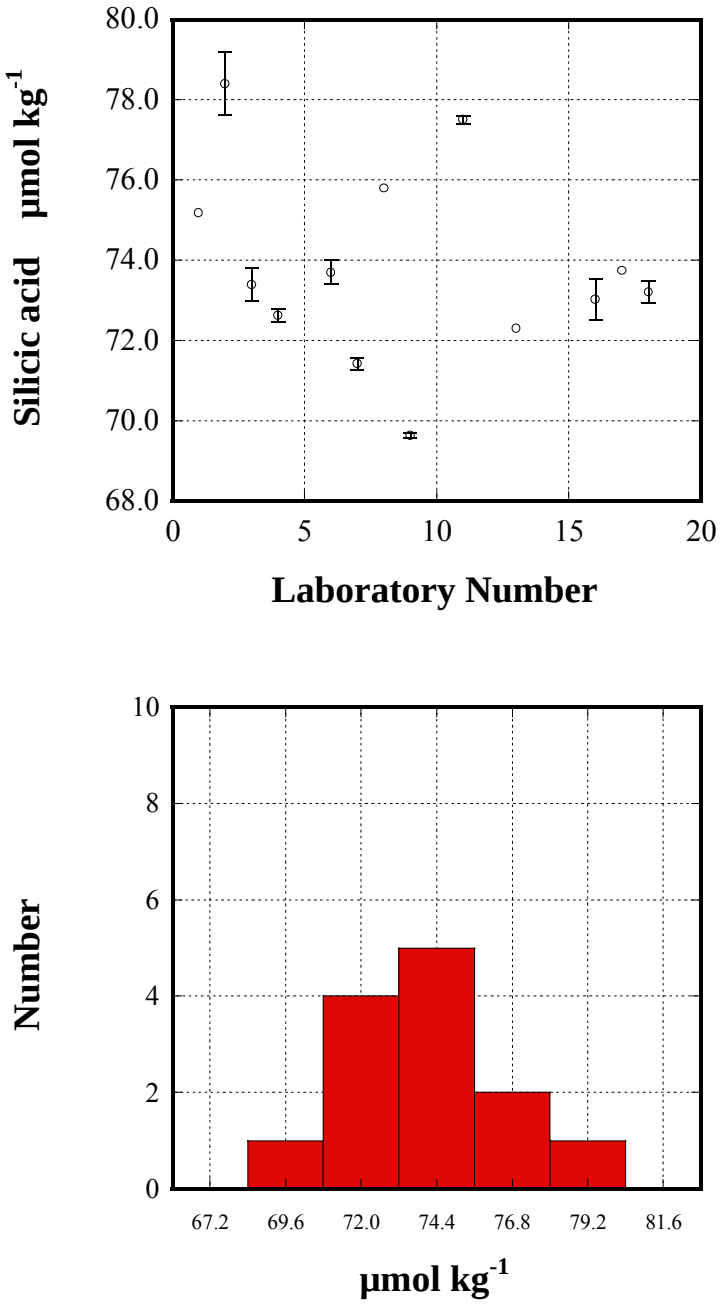

Figure 9-5 Silicic acid results for sample 5: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

Sample 6 — Silicic acid

Consensus Value: 133.8 ±2.5 µmol kg^{-1}

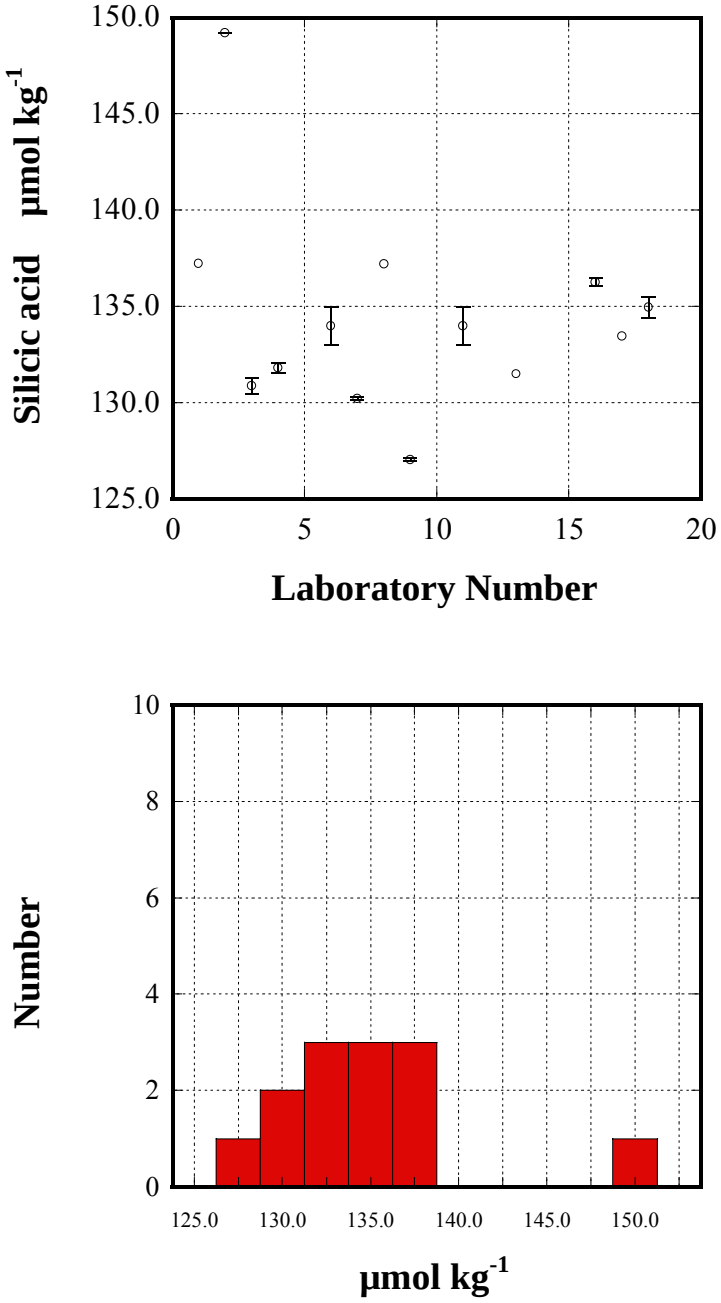

Figure 9-6 Silicic acid results for sample 6: concentration *versus* laboratory number (upper panel)
Frequency distribution of concentration (lower panel).

6. Conclusions

A total of 18 sets of 6 samples each were distributed in 5 countries. Results were returned by 17 laboratories in 5 countries. Although consensus concentrations were obtained for the 6 samples, the standard deviations were 4.5 times and more than 10 times larger than those of the homogeneities for phosphate and silicic acid, respectively. For nitrate, the standard deviations were only about double the homogeneities. These results indicate that variability in in-house standards of the participating laboratories — rather than analytical precision — is the primary source of interlaboratory discrepancy. Therefore use of a certified RMNS is essential for establishing nutrient data sets that can be compared across laboratories, especially for silicic acid and phosphate.

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