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DOES FREEZING OF NUTRIENT SAMPLES CAUSE ANALYTICAL ERRORS?

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As it is not always possible to analyse nutrient samples on board ship during research cruises, it is necessary to preserve them for analysis ashore, and freezing is by far the simplest and seemingly the most accurate means of preservation. To verify this statement, a series of experiments on samples analysed both at sea and later ashore (after freezing) was conducted. Freezing is apparently a viable method for preserving samples destined for analysis of nitrate and low silicate (<30–40 mmol·m^{-3}), but phosphate samples are seemingly not well preserved when frozen. In the latter case, the deficits between analysis of fresh samples aboard ship and frozen duplicates ashore vary randomly. To determine normal trends in nutrient distribution it seems feasible to store samples in the frozen state, but for highly accurate work, as would be necessary for WOCE studies, it is advisable to analyse samples at sea immediately after collection.

Aangesien dit nie altyd moontlik is om voedingsoutmonsters aan boord gedurende 'n navorsingsvaart te ontleed nie, is dit noodsaaklik om hulle te preserveer vir analise later op land. Bevriesing van die monsters is verreweg die maklikste en skynbaar ook die mees akkurate metode van bewaring. Om dit te bevestig, is 'n reeks eksperimente uitgevoer deur gebruik te maak van monsters wat ter see ontleed is en waarvan duplikate bevries is vir latere ontleding op land. Dit wil voorkom asof bevriesing 'n gangbare bewaringsmetode van monsters is vir nitraat- en lae (<30–40 mmol·m^{-3}) silikaatontleding. Fosfaatmonsters word blykbaar nie goed bewaar deur bevriesing nie aangesien die verskille tussen vars monsters wat aan boord ontleed is, en hulle bevrore duplikate wat op land ontleed is, onreëlmatig wissel. Vir die vasstelling van normale neigings van voedingsoutverspreiding blyk bevriesing 'n gangbare metode vir die berging van monsters te wees, maar vir hoogs akkurate werk, soos benodig vir WOCE-studies, word aanbeveel dat monsters ontleed word onmiddellik nadat hul ter see versamel is.

The analysis of nutrients (ammonia, nitrate, nitrite, phosphate and silicate) in seawater is necessary for purposes such as characterization of water masses and studies on phytoplankton production and pollution. As these nutrients are utilized by phytoplankton and bacteria for growth, and nutrient uptake is rapid (Dugdale and Goering 1967, Dugdale *et al.* 1981), it is obviously important, in surface waters at least, to analyse the samples as soon as possible after collection. Ideally, analysis should start within an hour of sampling (Strickland and Parsons 1972, Riley 1975), but there will undoubtedly be occasions when this is not possible for reasons of time and equipment constraint. For example, sampling may take place far from a laboratory, or there may be insufficient space on the research vessel for either the necessary analytical equipment or trained personnel. In such cases, some form of preservation of samples is necessary.

Preservation techniques for seawater have been studied by many researchers (see, for example, Strickland and Parsons 1972, Riley 1975, Grasshoff 1976, Aminot and Chaussepied 1983 for reviews of some of this work). Unfortunately the studies have given rise to considerable confusion over the years, with inconsistent and conflicting results being the norm. Much of the inconsistency is the result of the many variables in the storage techniques. Examples are whether the samples were filtered, the materials in which they were stored, whether additives were used to poison biological reactions, and storage temperature. Possibly the most important, however, is the origin of the sample itself, because this determines the original concentration of species of interest and thus the relative error acceptable in the analysis (Macdonald and McLaughlin 1982, Venrick and Hayward 1985).

Even standard reference works are not immune to inconsistency; for example, *Standard Methods for the Examination of Water and Waste Water* (APHA, AWWA, WPCF 1985) states in Table 105.1 that samples for nitrate, nitrite or phosphate should be frozen or refrigerated. However, in the individual methodologies of the same work (pp. 392, 441), it is stated that $HgCl_2$ can be added as a preservative.

Most of the nutrient work within the Sea Fisheries Research Institute in Cape Town is carried out in areas known for large variations in the nutrient concentrations in the water column. Such variations apply to both time and space because of upwelling (e.g. nitrate

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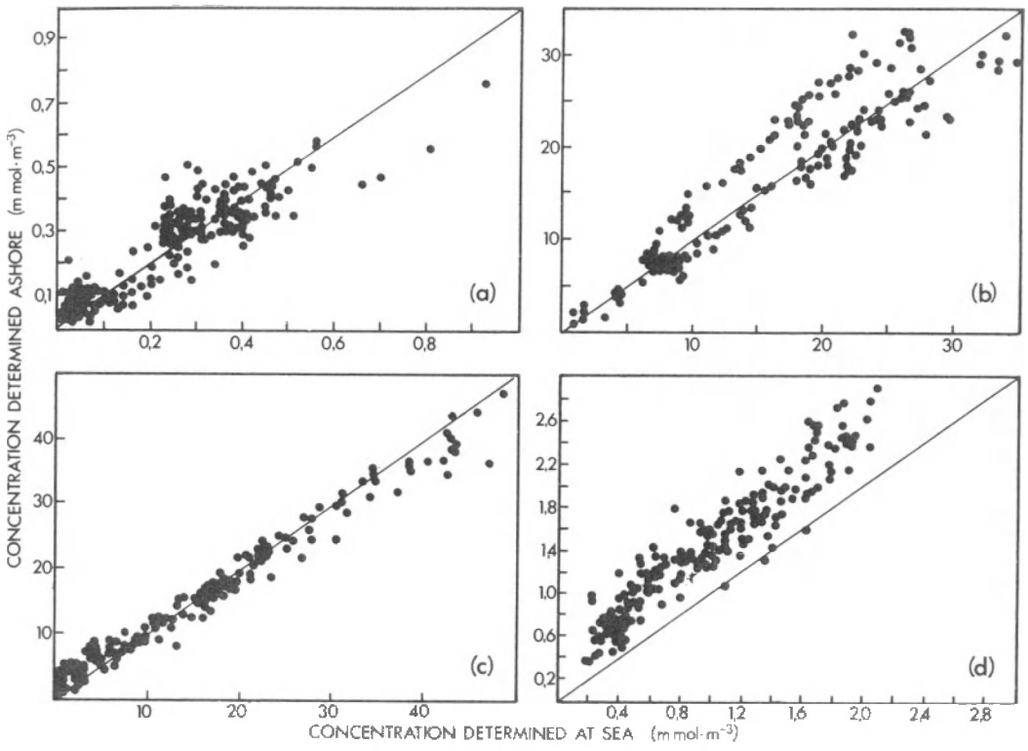

Fig. 1: Analyses of samples from Cruise 067, comparing analysis at sea with analysis on land after freezing, the lines showing a 1:1 correlation — (a) nitrite, (b) nitrate, (c) silicate, (d) phosphate

concentration in St Helena Bay on South Africa's west coast varies between 0 and 30, phosphate between 0,1 and 3 and silicate between 2 and 45 mmol·m^{-3} respectively — Bailey and Chapman 1985, in prep.), or because the samples are taken from deep stations in the South Atlantic, South Indian or Antarctic oceans with large nutrient-concentration gradients. Because of forthcoming commitments to the World ocean Circulation Experiment (WOCE), and because of changes in emphasis in studies of upwelling areas, it is critical that the methodology currently in use for dealing with nutrient samples is realistic and able to give accurate results. The tests included herein were made with that in mind.

CURRENT METHODOLOGY

Little work in the Sea Fisheries Research Institute in Cape Town is carried out on turbid coastal or estuarine waters, and therefore filtration, one of the major causes of problems in analysis (Riley 1975, Grasshoff 1976, Aminot and Chaussepied 1983), is very rarely necessary as a routine measure. The exception is when manual analyses of nitrite are being run with 10-cm path-length cells; then turbidity can be a problem. In such cases, nitrite samples of about 150 ml are filtered through Whatman GF/C or GF/F papers. Otherwise, samples are taken in aged high-density polyethylene tubes, with screwcaps of the same material. Each of these holds about 30 ml of sample, which is sufficient for two analyses of each sample for four parameters, allowing for rinsing of the sample vials of the auto-analyser. Analysis for nitrate, phosphate and silicate, and also nitrite if it is not analysed manually aboard ship, is by TECHNICON Auto-Analyzer (Mostert 1983, 1988). A separate sample for manual analysis of nitrite is taken in a 50 ml pyrex measuring cylinder (Strickland and Parsons 1972). Samples for ammonia and nitrite analysis are generally analysed manually aboard ship, because their values are usually below 3 and 1 mmol·m^{-3} respectively, and delaying analysis would therefore have a greater effect on measurement

Table I: Comparison of analyses of samples from Cruise 067

Nutrient	Samples	*n*	Slope	*SE*	Intercept	*SE*	*r*
Nitrite	1–80	79	0,723	0,055	0,099	0,078	0,8310
	81–160	78	0,756	0,025	0,030	0,046	0,9594
	161–205	45	0,928	0,070	0,021	0,058	0,8951
	All	202	0,766	0,027	0,063	0,070	0,8933
Nitrate	1–80	67	1,241	0,024	0,706	1,256	0,9882
	81–160	73	0,872	0,019	0,817	1,621	0,9839
	161–205	44	1,050	0,021	−1,646	0,985	0,9914
	All	184	0,955	0,028	1,522	3,112	0,9305
Silicate	1–80	80	0,872	0,011	1,787	1,170	0,9941
	81–160	78	0,881	0,016	1,395	2,001	0,9873
	161–205	45	0,901	0,016	2,345	1,287	0,9933
	All	203	0,879	0,009	1,789	1,618	0,9900
Phosphate	1–80	79	1,291	0,036	0,281	0,148	0,9708
	81–160	79	1,077	0,029	0,315	0,158	0,9727
	161–205	45	0,886	0,068	0,511	0,192	0,8929
	All	203	1,110	0,026	0,355	0,192	0,9485

accuracy than it would with the other nutrients. If samples cannot be analysed *in situ*, they are immediately frozen at −20°C and stored vertically for periods of up to two months before analysis ashore, as recommended by Aminot and Kerouel (1979). Ammonia analysis is not considered in this paper.

Various workers (e.g. Mullin and Riley 1955, Murphy and Riley 1956, Jones 1963, Gilmartin 1967, Jenkins 1967, Charpiot 1969) have suggested that nutrient samples be preserved with mercuric chloride, chloroform or acid. Others (e.g. Fitzgerald and Faust 1967, Jenkins 1968, Thayer 1970, Strickland and Parsons 1972, Morse *et al.* 1982, Venrick and Hayward 1985) suggest that the use of preservatives leads to unreliable results. Because surface seawater samples contain very low concentrations of nutrients, addition of preservatives is likely to cause more problems by way of contamination than it solves.

MATERIAL AND METHODS

During Cruises 067 and 071 of the R.S. *Africana* (in September 1988 and April 1989 respectively), samples were taken for nutrient analysis. The samples were obtained with a multi-bottle rosette sampler containing either 12 × 5ℓ bottles (Cruise 067) or 24 × 8ℓ bottles (Cruise 071), which were triggered at the depth required. Nitrite was analysed manually (Strickland and Parsons 1972) during Cruise 067 only, reagents being added within 30 minutes of the samples being taken. Samples for nitrate, phosphate and silicate analysis were kept at 4°C in the dark until analysed by autoanalyser (Mostert 1983, 1988), generally within 3–4 hours. The residuum after subsamples had been poured into autoanalyser vials (about 20 mℓ) was frozen in the same tubes into which they had been drawn and stored at −20°C until the ship docked. Samples were analysed a second time by means of the same methodology, but also including nitrite, within two weeks. All frozen samples were defrosted by standing in hot water (approx. 70°C) in batches of 40 directly before analysis ashore. Thus, the samples had been kept frozen for varying periods of up to about five weeks. Totals of 205 samples were obtained from Cruise 067, and 310 from Cruise 071.

During *Africana* Cruise 076 in September 1989, two 5ℓ bulk samples of deep water were obtained from 2 500 m (Station A9023 at 26°59,4′S, 12°19,1′E) and 2 990 m (Station A9204 at 25°01,2′S, 12°25,1′E) in the Cape Basin. Bottom depths were 3 693 and 3 002 m respectively. The samples were kept in plastic carboys at ambient temperature until the ship returned to Cape Town, when aliquots were subsampled into the normal nutrient tubes. Ten replicates of each were analysed on the day of subsampling and a further five in duplicate after freezing at −20°C for 16, 28 and 56 days. All analyses of this water were for phosphate and silicate only. Samples are rarely stored for longer than six weeks after collection in the Institute, and it was for that reason that an eight-week freezing period was selected.

RESULTS

Cruise 067

Samples during Cruise 067 were taken during an in-

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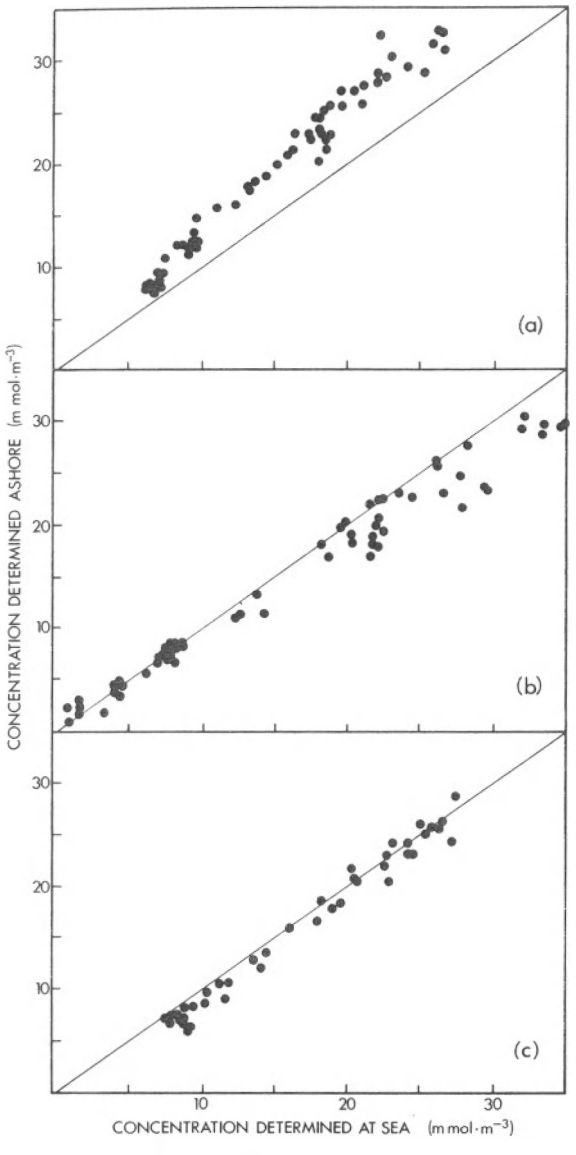

Fig. 2: Nitrate samples from Cruise 067 separated into sections corresponding to each day's analysis ashore — (a) Samples 1–80, (b) Samples 81–160, (c) Samples 161–205

shore leg from Walvis Bay to Lüderitz and are therefore indicative of the normal range of samples found during experimental work in upwelling areas. Data for the four nutrients are shown in Figure 1 and the relevant statistical information in Table I. The apparent existence of two separate sets of data for nitrate is immediately clear, and the data were therefore split into three groups (Samples 1–80, 81–160 and 161–205) corresponding to the three sets in which the samples were analysed ashore. Samples 1–80 provided all the aberrant data (Fig. 2). The number of data points for nitrate was lower than the remainder because several went off-scale when analysed ashore. These data were removed from the data set before statistical analysis.

While all four data sets in Figure 1 show some scatter, it is particularly pronounced for phosphate and nitrite, as shown by the lower correlation coefficients for these parameters. There is also a suggestion that concentrations of silicate analysed ashore may be lower than those analysed at sea, particularly at concentrations higher than 30 mmol·m^{-3}. These observations were investigated further by examining the deficits (D) between the two sets of data, defined as the difference between the samples measured at sea and ashore:

$$D = [X_{sea}] - [X_{shore}] \ .$$

The calculated deficits were plotted against the values for each parameter measured at sea, and the result is shown in Figure 3. There is clearly a change from a negative to a positive deficit in the case of nitrite and silicate, but for phosphate and nitrate there was no apparent trend. When the nitrate deficits were separated into those from Samples 1–80 and 81–205, however, results were conflicting. For the first group of samples, the deficit increased negatively as the nitrate concentration increased, but for the latter group it increased positively. These results are probably dependent on the calibration of the autoanalyser during each of the runs — during the analysis of Samples 1–80, the shore analyses produced figures a factor of 1,2 higher than those analysed at sea, whereas Samples 81–205 produced figures rather lower (Table I).

The distributions of the magnitude of the deficits (Fig. 4) reveal that the data probably do not come from normal populations. Considerable tailing is apparent for all parameters, although this is partly compensated for when the nitrate data are split into Samples 1–80 and 81–205, and when silicate values (measured at sea) of over 30 mmol·m^{-3} are ignored. Random error is clearly, therefore, not the only variable at work.

Cruise 071

Samples taken during Cruise 071 were taken from the first 17 stations between Cape Town and Tristan da Cunha. All the stations were deep, bottom depth being between 2 520 m and 5 200 m. Samples were taken throughout the water column, with 73 (23,9%) being taken from below 3 000 m. Paired samples were taken at seven stations, and three sets of 7, 7 and 8 duplicates were obtained at other sites. All these duplicates were

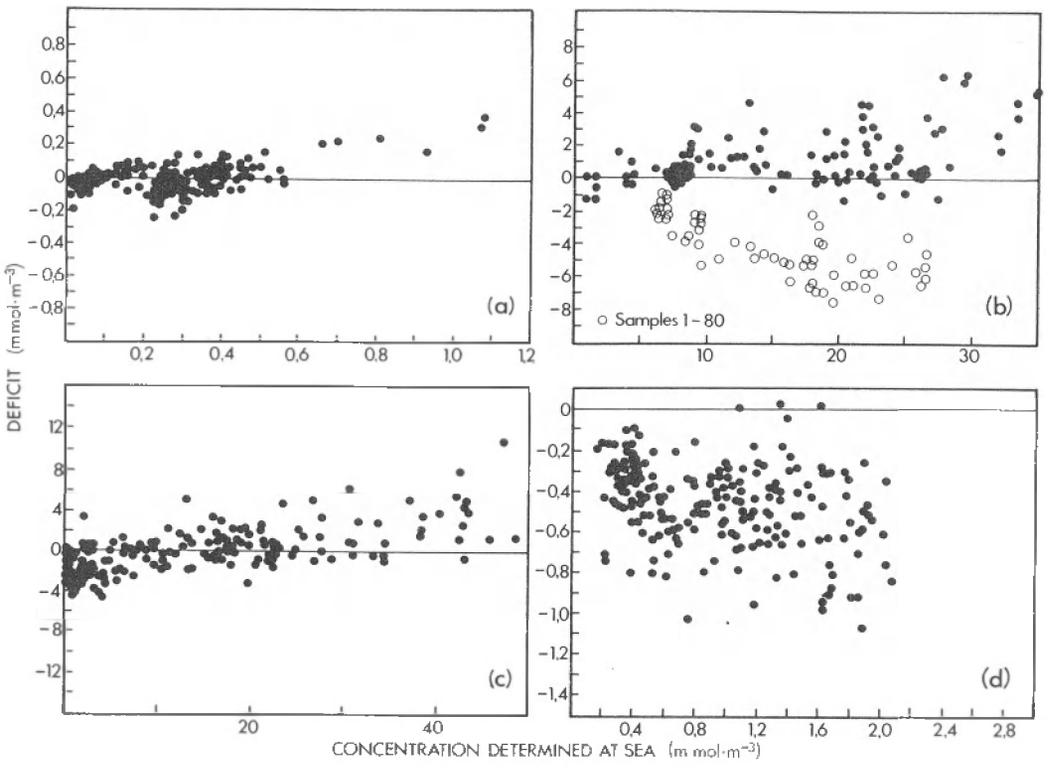

Fig. 3: Deficits (concentration measured at sea minus concentration measured ashore) for samples from Cruise 067 — (a) nitrite, (b) nitrate, with Samples 1–80 distinguished, (c) silicate, (d) phosphate

taken from separate Niskin bottles (i.e. in the last case eight bottles were activated at the same depth and one sample was taken from each). A further 34 sets of duplicates were obtained during the cruise and analysed at sea as a check on inter-bottle and sampling variability; details are available from the authors. The variability introduced by the sampling and analytical methodology was very low for the set of duplicates analysed at sea, variability between duplicates being 0,55 per cent (σ = 0,54, n = 43) for nitrate, 1,38 per cent (σ = 1,58, n = 43) for phosphate and 0,33 per cent (σ = 0,89, n = 44) for silicate. In all instances, the variability was calculated as 100 $(n_1-n_2)/(n_1+n_2)$ for paired data and 100 σ/M where more than two data points were available.

Freezing introduced considerably more variation into the analyses. The data for Station A8447a are compared in Table II. While the means for nitrate and phosphate changed only slightly, the difference probably being attributable to day-to-day variability with the autoanalyser, mean values of silicate were considerably lower after freezing, and the differences were highly significant (Student's t-test, $p < 0,01$). From Table II it is also clear that freezing resulted in considerable increases in the scatter of the data for all three parameters, with the variability, calculated as above, being 3,04 per cent for nitrate, 3,74 per cent for

Table II: Comparison of data from Station A8447a

Nutrient	Pressure (db)	Concentration determined at sea (mmol · m⁻³)		Concentration determined ashore (mmol · m⁻³)	
		Mean	*SD*	Mean	*SD*
Nitrate	785	28,17	0,04	28,21	0,91
	815	29,04	0,05	29,56	0,69
	886	30,14	0,21	30,70	0,71
Silicate	754	19,02	0,12	17,91	0,54
	815	23,10	0,12	21,53	0,38
	886	28,19	0,12	25,32	0,46
Phosphate	754	1,930	0,022	2,008	0,102
	815	2,032	0,033	2,116	0,070
	886	2,123	0,035	2,241	0,042

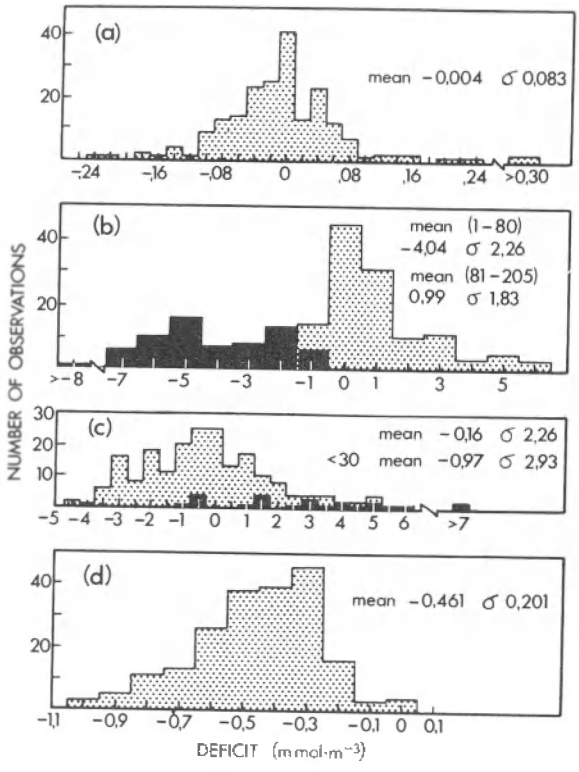

Fig. 4: Distribution of deficits from Cruise 067, shaded portions denoting nitrate samples 1–80 and silicate concentrations (measured at sea) greater than 30 mmol·m^{-3} respectively — (a) nitrite, (b) nitrate, (c) silicate, (d) phosphate

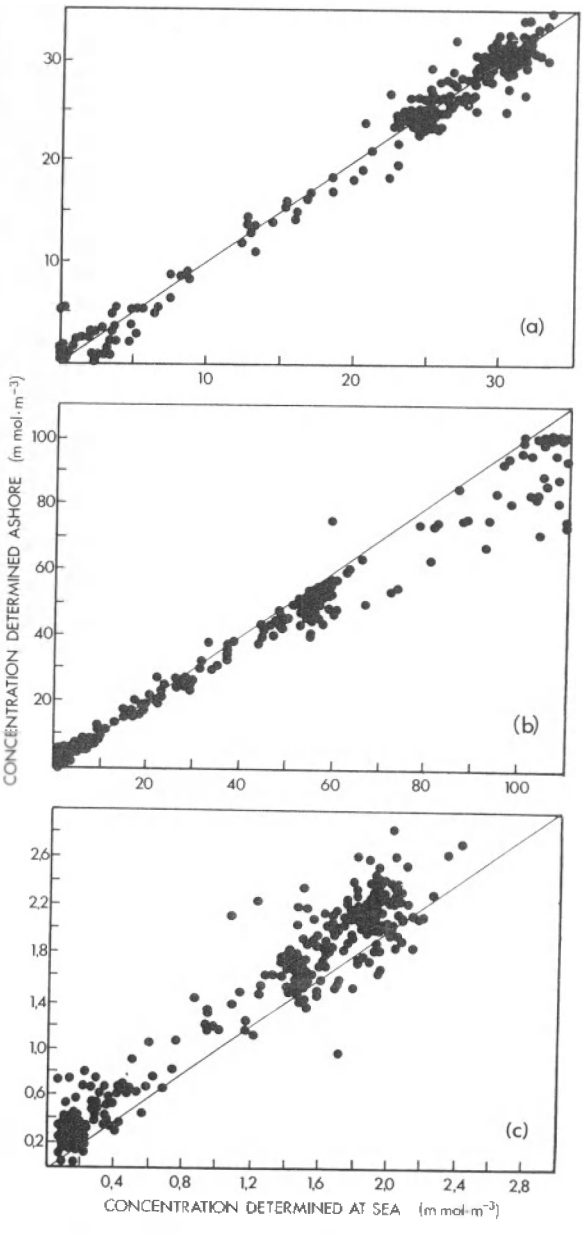

Fig. 5: Analyses of samples from Cruise 071, comparing analysis at sea with analysis on land after freezing, the lines showing a 1:1 correlation — (a) nitrate, (b) silicate, (c) phosphate

phosphate and 2,66 per cent for silicate.

The data for Cruise 071 are shown in Figure 5. In this case there were no apparent differences between the daily runs and all data have been grouped. Phosphate values were again higher and silicates lower after freezing, with the discrepancy for silicate increasing with increasing concentration (Fig. 6). The magnitude of the deviations (Fig. 7) approximated to normality for all three nutrients when silicate concentrations greater than 30 mmol·m^{-3} were ignored.

Cruise 076 freezing experiments

On each occasion that samples were analysed, five aliquots of the two samples were allowed to thaw (slowly) at room temperature overnight. For the 28- and 56-day analyses, additional aliquots were thawed quickly in hot water immediately before analysis. The mean and range for each set of samples are given in Table III.

It is clear that the apparent concentration of both

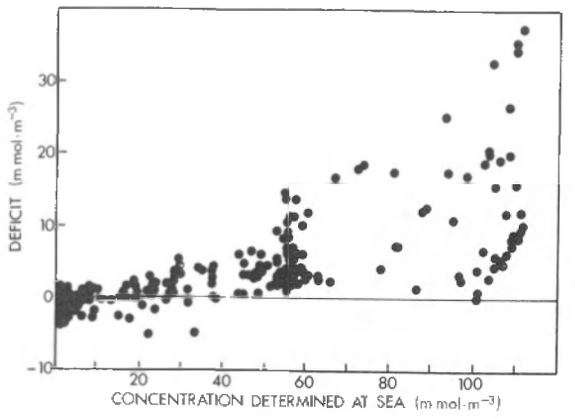

Fig. 6: Deficits (concentration measured at sea minus concentration measured ashore) for silicate samples from Cruise 071

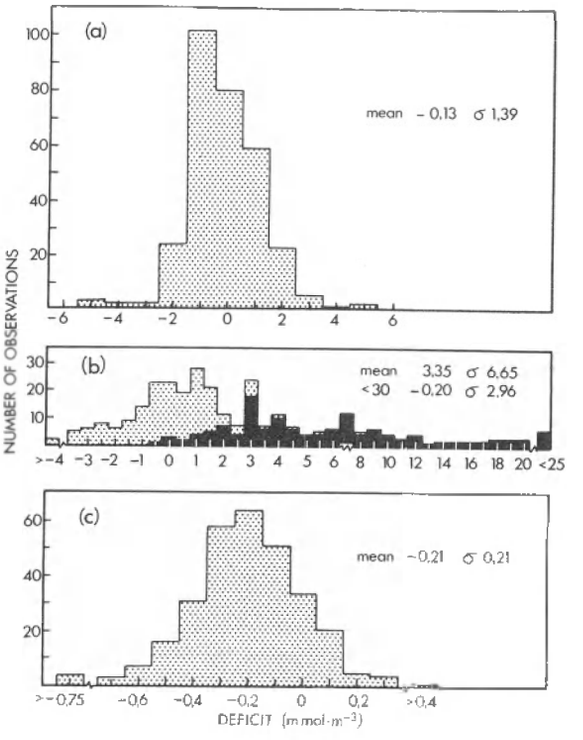

Fig. 7: Distribution of deficits from Cruise 071, shaded portions denoting silicate concentrations (measured at sea) greater than 30 mmol·m^{-3} — (a) nitrate, (b) silicate, (c) phosphate

phosphate and silicate decreased after storage for two months. The decline was 6,6 and 8,3 per cent for the two silicate samples, and 10,7 and 16,4 per cent for phosphate, when they were allowed to thaw slowly, the method suggested by Macdonald and McLoughlin (1982). When hot water was used for thawing and the samples were analysed immediately afterwards, then the decrease was the same for silicate but considerably less for phosphate (1,5 and 8,8% respectively). Similarly, the scatter for the five aliquots all increased with time, except for the quickly thawed samples of phosphate which had less scatter than those thawed slowly at the same time.

DISCUSSION

The results show that the measured concentrations of all nutrients varied to some extent after being subjected to freezing. The largest discrepancies occurred with silicate, particularly at high levels, and phosphate (Figs 3 and 7). Whereas variability of silicate after storage has been long known and is thought to be

Table III: Results of long-term freezing experiment. Samples defrosted at (a) room temperature or (b) in hot water. Standard deviation in parentheses

State of sample	Silicate (mmol · m^{-3})		Phosphate (mmol · m^{-3})	
	Sample 1	Sample 2	Sample 1	Sample 2
Unfrozen (original)	85,88 (0,17)	85,35 (0,27)	1,32 (0,003)	1,59 (0,005)
Frozen for:				
16 days	84,18 (1,27)	85,11 (1,20)	1,22 (0,039)	1,44 (0,075)
28 days	(a) 83,79 (2,44)	82,14 (1,07)	1,27 (0,076)	1,38 (0,038)
	(b) 82,74 (3,28)	83,76 (1,32)	1,31 (0,030)	1,45 (0,022)
56 days	(a) 80,22 (2,36)	78,29 (3,13)	1,18 (0,134)	1,33 (0,065)
	(b) 80,33 (1,97)	79,28 (1,74)	1,30 (0,062)	1,45 (0,016)

caused by slow rates of depolymerization (see Macdonald and McLoughlin [1982] and references therein), it was previously thought (Burton *et al.* 1970) that storage by freezing for less than two months was acceptable for saline ($S > 27 \times 10^{-3}$) samples. Macdonald *et al.* (1986) suggested that, if the silicate concentration is less than 70 mmol.m^{-3}, storage for a month should have no effect on the apparent concentration even when samples are analysed immediately after thawing, but that up to 24 hours of thawing would be necessary at higher concentrations or where salinity is low. Even with longer thaw-times, they found that precision deteriorated. Similar conclusions about decreasing accuracy were reached by Morse *et al.* (1982) and Venrick and Hayward (1985), although the former authors found that their silicate concentrations apparently increased with time of storage.

The present results suggest that the deviations begin at lower concentrations than found by Macdonald *et al.* (1986), with the threshold being near 30–40 mmol·m^{-3} (Figs 1 and 5), but that less than 10-percent error generally results even at high concentrations of silicate (Table III), although precision is less. For silicate concentration < 30 mmol·m^{-3} (Figs 4 and 7), there was no apparent bias in the results. The bias at higher concentrations was not completely corrected even by allowing a longer period for thawing (Table III).

The results of the phosphate analyses were not as clearcut as for silicate. While the comparison of the samples taken on Cruises 067 and 071 showed an increase in concentration if samples were frozen and analysed ashore, the long-term storage experiment showed a decrease. Precision again decreased on freezing. Macdonald and McLaughlin (1982) showed minimal (<3%) decreases in concentration of phosphate after freezing. Murphy and Riley (1956) suggested that adsorption of phosphate to polythene during long-term storage could result in apparently decreased concentrations of phosphate, and they proposed acidification as a solution. However, Morse *et al.* (1982) found large changes in their acidified samples, possibly caused by phosphate contamination of the acid itself, and they and Venrick and Hayward (1985) found that the phosphate concentration in stored samples tended to increase with time. Gilmartin (1967), who admittedly was working in an inshore, highly eutrophic, area with great turbidity, suggested that a balance was set up in stored samples between enzymatic decomposition of organic material, tending to increase nutrient concentrations, and phosphate utilization by bacteria, tending to decrease them. Cell lysis during the freezing process might also result in increased concentrations of nutrients. It is difficult, however, to conceive of such large increases in samples taken from the open ocean, particularly at depths where little or no particulate matter is present, although the difference in thawing time may have allowed some adsorption during the long experiment (Table III).

Phosphate is probably the most difficult to measure of the nutrients considered here. Reproducibility is around the 3 per cent range at 1 mmol·m^{-3} (Grasshoff 1976, Mostert 1983), although it deteriorates at lower concentrations. The variation in slope from unity in the results of Cruise 071 (Fig. 5) are probably therefore within the expected variability of the method. The negative bias found in both cruises, however, is much higher than expected (Figs 4 and 7). The data analysed at sea agreed to within 2–3 per cent with accepted literature values from the GEOSECS and AJAX expeditions, and the negative bias was therefore either caused by storage or by a systematic error in the analysis. Changes in standard concentration are unlikely, because the same bulk concentrated standard was used in the laboratory and at sea for each cruise.

Nitrate samples were generally successfully preserved by freezing, although the precision decreased slightly. No increase was detected on storage, in agreement with Macdonald and McLaughlin (1982). Morse *et al.* (1982) and Venrick and Hayward (1985) reported large increases in nitrate in frozen samples, with the maximum variability in surface samples, which have very low values anyway. Whereas some low-nitrate samples exhibited high variability, the deviations were generally less than 10 per cent, and no particular bias was found (Figs 4 and 7). As nitrite values were all low (<1 mmol·m^{-3}), it is perhaps not surprising that there was considerable variability and that the slopes of the regressions have departed from unity.

CONCLUSIONS

It would seem that freezing is a viable method for the preservation of samples for nitrate analysis, and also for silicate in surface samples where concentrations are less than 30–40 mmol·m^{-3}. At higher concentrations, although the deviations from the original concentrations were reduced by allowing the samples to thaw for longer periods, they were not eliminated entirely (Table III). However, phosphate samples are apparently not well preserved when frozen, and the deterioration is more marked when samples are allowed to thaw slowly which, as stated, is necessary for improved determination of silicate concentration.

Whether the magnitude of the deviations after freezing is acceptable depends on the use to which the data are to be put. For pollution studies, for which one is generally interested in knowing only whether concentrations exceed a set standard, or where the levels are

so high anyway that relative concentrations are sufficient, errors of the order of 10 per cent may be acceptable and freezing is then a reasonable method of sample preservation. Nevertheless, for deep-sea work, where high accuracy is required, and for process studies in euphotic zones, where nutrient concentrations are low, samples should preferably be analysed at sea. As an example, WOCE studies require nitrate, phosphate and silicate concentrations to be determined to 1, 1–2 and 3 per cent respectively for accuracy and full-scale precision (U.S. WOCE 1989). This standard may not even be attainable for phosphate with the present methodology, and certainly it does not appear possible for any nutrient unless samples are analysed at sea.

LITERATURE CITED

AMINOT, A. and M. CHAUSSEPIED 1983 — *Manuel des Analyses Chimiques en Milieu Marin*. Brest; CNEXO: 395 pp.

AMINOT, A. and R. KEROUEL 1979 — Exercise d'intercalibration RNO 1978: sels nutritifs. (NO_3^-, NO_2^-, PO_4^{3-}). Préparation des échantillons et résultats. *Bull. Trimestriel MECV – CNEXO Réseau National d'Observation de la Qualité du Milieu Marin* **11**: 179–213.

APHA, AWWA, WPCF 1985 — *Standard Methods for the Examination of Water and Wastewater, 16th Ed.* Washington; American Public Health Association: 1268 pp.

BAILEY, G. W. and P. CHAPMAN 1985 — The nutrient status of the St Helena Bay region in February 1979. In *South African Ocean Colour and Upwelling Experiment*. Shannon, L. V. (Ed.). Cape Town; Sea Fisheries Research Institute: 125–145.

BAILEY, G. W. and P. CHAPMAN (in preparation) — Short term variability during an Anchor Station study in the southern Benguela upwelling system in Autumn 1987; chemical and physical oceanography.

BURTON, J. D., LEATHERLAND, T. M. and P. S. LISS 1970 — The reactivity of dissolved silicon in some natural waters. *Limnol. Oceanogr.* **15**: 472–476.

CHARPIOT, R. 1969 — Technique de conservation des échantillons d'eau de mer pour le dosage de phosphates, nitrites, nitrates, silice et bore. Méthodes d'analyses et résultats à partir des prélèvements à des stations profondes au large de Monaco et en Atlantique Nord. *Cah. Oceanogr.* **21**: 773–793.

DUGDALE, R. C. and J. J. GOERING 1967 — Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* **12**(2): 196–206.

DUGDALE, R. C., JONES, B. H., MacISAAC, J. J. and J. J. GOERING 1981 — Adaptation of nutrient assimilation. *Can. Bull. Fish. Aquat. Sci.* **210**: 234–250.

FITZGERALD, G. P. and S. L. FAUST 1967 — Effect of water sample preservation methods on the release of phosphorus from algae. *Limnol. Oceanogr.* **12**(2): 332–335.

GILMARTIN, M. 1967 — Changes in inorganic phosphate concentration occurring during seawater sample storage. *Limnol. Oceanogr.* **12**(2): 325–328.

GRASSHOFF, K. 1976 — *Methods of Seawater Analysis*. New York; Verlag Chemie: 317 pp.

JENKINS, D. 1967 — Analysis of estuarine waters. *J. Wat. Pollut. Control Fed.* **39**: 159–180.

JENKINS, D. 1968 — The differentiation, analysis, and preservation of nitrogen and phosphorus forms in natural waters. In *Trace Inorganics in Water*. Gould, R. G. (Ed.). Washington, D.C.; American Chemical Society: 265–280 (Advances in Chemistry Series **73**).

JONES, P. G. W. 1963 — The effect of chloroform on the soluble inorganic phosphate content of unfiltered sea water. *J. Cons. perm. int. Explor. Mer* **28**: 3–7.

MacDONALD, R. W. and F. A. McLAUGHLIN 1982 — The effect of storage by freezing on dissolved inorganic phosphate nitrate and reactive silicate for samples from coastal and estuarine waters. *Wat. Res.* **16**(1): 95–104.

MacDONALD, R. W., McLAUGHLIN, F. A. and C. S. WONG 1986 — The storage of reactive silicate samples by freezing. *Limnol. Oceanogr.* **31**(5): 1139–1142.

MORSE, J. W., HUNT, M., ZULLIG, J., MUCCI, A. and T. MENDEZ 1982 — A comparison of techniques for preserving dissolved nutrients in open ocean seawater samples. *Ocean Sci. Engng* 7(1): 75–106.

MOSTERT, S. A. 1983 — Procedures used in South Africa for the automatic photometric determination of micronutrients in seawater. *S. Afr. J. mar. Sci.* **1**: 189–198.

MOSTERT, S. A. 1988 — Notes on improvements and modifications to the automatic methods for determining dissolved micronutrients in seawater. *S. Afr. J. mar. Sci.* **7**: 295–298.

MULLIN, J. B. and J. P. RILEY 1955 — The spectrophotometric determination of nitrate in natural waters, with particular reference to sea-water. *Analytica chim. Acta* **12**: 464–480.

MURPHY, J. and J. P. RILEY 1956 — The storage of sea-water samples for the determination of dissolved inorganic phosphate. *Analytica chim. Acta* **14**: 318–319.

RILEY, J. P. 1975 — Analytical chemistry of sea water. In *Chemical Oceanography, 2nd Ed.* **3**. Riley, J. P. and G. Skirrow (Eds). London; Academic Press: 193–514.

STRICKLAND, J. D. H. and T. R. PARSONS 1972 — A practical handbook of seawater analysis, 2nd Ed. *Bull. Fish. Res. Bd Can.* **167**: 310 pp.

THAYER, G. W. 1970 — Comparison of two storage methods for the analysis of nitrogen and phosphorus fractions in estuarine water. *Chesapeake Sci.* **11**(3): 155–158.

U.S. WOCE 1989 — U.S. WOCE Implementation Plan. *U.S. WOCE Implementation Rep.* **1**: 176 pp.

VENRICK, E. L. and T. L. HAYWARD 1985 - Evaluation of some techniques for preserving nutrients in stored seawater samples. *Rep. Calif. coop. oceanic Fish. Invest.* **26**: 160–168.