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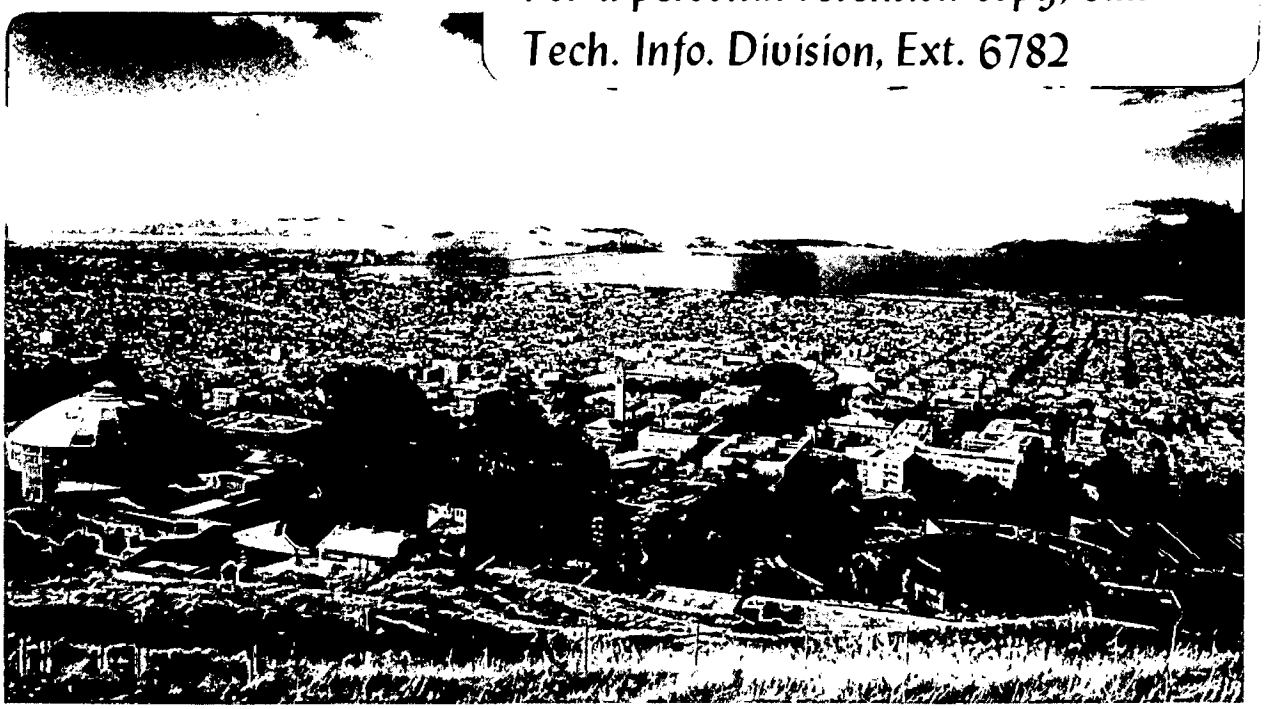

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DISCLAIMER

A COMPARISON OF TECHNIQUES FOR PRESERVING DISSOLVED NUTRIENTS IN OPEN OCEAN SEAWATER SAMPLES

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SUMMARY

The accurate determination of dissolved nutrient concentrations in natural seawater is essential to the characterization of oceanographic conditions at potential Ocean Thermal Energy Conversion (OTEC) plant sites, and for monitoring the influence of operating plants on the marine environment. Although spectrophotometric analytical techniques have been developed and carefully tested for determining the concentration of most nutrients of interest in seawater, major problems have been found in preserving original nutrient concentrations in seawater samples which cannot be analyzed immediately upon collection. Because this is frequently not practical it has been necessary to devise methodologies for storing samples in a manner which will prevent "significant" changes in concentrations of nutrients between the time of collection and analysis.

A survey of recent literature on methods for preserving nutrients indicates that the major factors which have been considered are: filtration and type of filter, material and history of storage containers, the influence of light, storage temperature and how it is achieved, the effectiveness of various acids, poisons and preservatives, and the source of the sample. The results of these previous investigations are frequently conflicting and appear to be strongly dependent on the source (e.g. coastal, estuarine, etc.) of the sample. Only rarely was more than one factor investigated and no comprehensive studies of open ocean seawater, similar to that which is expected in the vicinity of OTEC plants, were found to have been conducted.

In order to determine the best methods for nutrient concentration preservation, in samples to be collected as part of the OTEC program, and if any methodology produces results which are satisfactory, a comprehensive study of nutrient preservation techniques was conducted on surface and deep seawater samples collected in the Gulf Stream east of Miami, Florida. Two sample collection cruises were undertaken. Samples from the first cruise were preserved by a large number of different techniques encompassing most of the various reported preservation techniques in different combinations. Based on the results obtained from this first study, samples from the second cruise were preserved by a much smaller number of methods, and factors such as the rate of freezing, manual versus automated analysis and bottle to bottle variations were studied.

The results of this study indicate that none of the preservation techniques is satisfactory for near-surface open ocean seawater. Nutrient concentrations in such samples are generally very low and are, consequently, extremely sensitive to such processes as the breakdown of organic matter and adsorption-desorption reactions with storage containers as well as the usual problems associated with analyzing near the detection limit. The results for deep water samples, where the nutrient concentrations are frequently up to 100 times higher than in surface waters, are substantially better. However, even in these samples changes in nutrient concentrations on the order of 30% are common. One interesting finding was that the degree of preservation was not substantially improved by going to complex techniques involving freezing and chemical additives. Consequently, storage of filtered (polycarbonate 0.45 μm) samples in aged polyethylene bottles, at 2°C in the dark is recommended for samples which must be stored.

INTRODUCTION

The preservation of nutrients in natural water samples between the time of collection and analysis has been a persistent problem. Previous investigations have indicated that significant changes start occurring almost immediately upon sample collection. Rapid analysis following sample collection is generally impossible because of the rate of sample collection and number of different nutrients to be determined and/or the logistics of getting samples to a laboratory where analyses are to be performed. A great deal of effort has consequently been spent on developing methods for preserving nutrient concentrations in natural water samples during sample handling and storage.

The nutrient preservation problem can be divided into two major areas depending on the time involved between sample collection and analysis. The first area involves sample preservation for a few minutes to a few hours. The second area involves sample preservation over extended periods of time of up to several months.

A survey of the existing literature (De Gobbis, 1973; Fitzgerald and Faust, 1967; Gilmartin, 1967; Howe and Holley, 1969; Jenkins, 1968; Maynard and Hopkins, 1973; Proctor, 1962; Thayer, 1970) on nutrient preservation techniques has failed to find any comprehensive studies on open ocean surface and deep seawater. Most studies have been carried out in lakes, estuaries, and near-shore marine environments or on sunthetically prepared solutions. Many of the findings are contradictory, and there is a general consensus that the effectiveness of different preservation techniques is strongly dependent on the source of the sample. Since in open ocean samples the

level of biological activity can generally be expected to be significantly lower and anthropogenic nutrient sources of less concern, relative to the near-shore areas in which most nutrient preservation studies have been carried out, it is advisable to carry out a detailed nutrient preservation study on open ocean water samples.

The general factors and methods considered in nutrient preservation are limited. The first consideration is whether or not the sample is to be filtered and, if so, by what means and with what type of filter. The second consideration is what type of container is to be used for sample storage. The third factor is the temperature at which the sample is to be stored and how that temperature is arrived at. The last factors to be considered are: should the sample be poisoned, by which poison, when, and at what concentration.

PREVIOUS STUDIES

Filtration

Filtration of water samples for nutrient analysis has been used as a technique for removing (at least partially) organisms, which can take up and release nutrients through biological processes and after death during decomposition, and particulate matter which produces increased turbidity blanks and on which surface exchange reactions can occur. In surveying the literature on nutrient preservation and storage techniques it was found that for many of the studies the sample was not filtered. However, most evidence indicates that filtration prior to subsequent manipulations is desirable and increases the effectiveness and reproducibility of preservation and storage techniques (e.g. Proctor, 1962; Rigler, 1964; Fitzgerald and Faust, 1967; Gilmartin, 1967; De Gobbis, 1973; American Public Health Association, 1981).

Although it is anticipated that filtration should not generally be as important in open ocean samples, which generally have far lower particulate concentrations than the nearshore and estuarine environment in which the filtration studies were carried out, it may still be advisable to filter open ocean water samples.

Another important consideration is the source and type of material from which the filter is made. Studies have indicated that even after extensive washing cellulose acetate (e.g. Millipore) filters "bleed" both phosphatic (Jenkins, 1968) and nitrogenous (Maynard and Hopkins, 1973) nutrients. Glass fiber filters can contribute silicate contamination (Fanning, 1981, personal communication).

Contamination problems from polycarbonate filters (Nuclepore®) have not been reported and it, therefore, appears to be advisable to use polycarbonate instead of cellulose acetate filters or glass fiber filters. However, clogging can be a problem with polycarbonate filters.

Storage Container

A major problem in the handling and storage of water samples in which nutrient concentrations are to be determined is the interactions that occur between the various nutrients and the surfaces which they encounter during filtering, storage and analytic manipulation. A survey of the literature on this topic indicates that little reported work has been done on this problem. Generally, only glass and polyethylene containers have been considered, although a few researchers have considered Teflon® and polypropylene.

Most studies on the effects of container composition have been carried out with regard to reactive phosphate. Hassenteufel, et al. (1963) found that the uptake of orthophosphate was about three times greater on

polyethylene and polyvinyl chloride than on glass and that Teflon was the best container material. Their results indicated that treatment of glass containers with 0.5 to 1.0% hydrofluoric acid greatly retarded orthophosphate uptake. The American Public Health Association (1981) also recommends glass in preference to polyethylene for storage of samples in which phosphate is to be determined. They recommend rinsing the glass several times with hot hydrochloric acid followed by several rinses with distilled water. Degobbis (1973) found that for ammonia untreated glass released ammonia while polyethylene bottles took it up. Glassware cleaned with chromic acid caused little change in ammonia concentration. Both polyethylene and glass bottles were found to work well for samples which were quick frozen. Strickland and Parsons (1972) recommend use of polyethylene bottles for all sample storage. Iverson (1979, personal communication) has found that polypropylene bottles generally are better for sample storage than are polyethylene bottles. For samples in which silica is to be determined, it has been assumed that it is advisable to keep contact with glass to a minimum. The relative merits of the different glass and plastic containers for nitrate and nitrite are unreported. In conclusion, the type of storage bottle preferred depends on the nutrient and storage conditions.

Temperature

It has generally been found that immediate cooling or freezing of samples along with storage in the dark is one of the most effective methods for preserving nutrient concentrations in natural samples. For samples which are to be analyzed within a few hours of collection, storage in the

dark a 4°C is recommended (e.g. Jenkins, 1968; Strickland and Parsons, 1972). There is also general agreement that for long term storage, quick freezing in a dry ice-alcohol bath and storage at minus 10 to minus 20°C is best (e.g. Proctor, 1962; Jenkins, 1968; Thayer, 1970; Strickland and Parsons, 1972; De Gobbis, 1973; Maynard and Hopkins, 1973; American Public Health Association, 1981). However, there does seem to be controversy about how erratic the results are when samples are frozen for long periods of time. De Gobbis (1973) recommends thawing frozen samples in a warm water bath at 30-40°C followed by immediate analysis for ammonia. All storage facilities should not be used for storing volatile chemical compounds or decomposing organics (food, vegetation, sediments, etc).

Poisoning

The basic concept in poisoning a sample is to introduce a substance which will kill organisms so that they will not alter the nutrient concentrations in the sample. The use of a wide variety of poisons, including organics such as phenol and chloroform, mercuric chloride and sulphuric acid has been reported in the literature. The results of these studies are highly variable and appear to be strongly dependent on the source of the samples and what other manipulation such as filtering and freezing are carried out on the sample.

A number of studies have been carried out on the use of poison to preserve phosphorus nutrient concentrations. Jenkins (1968) found that on untreated water samples both chloroform and mercuric chloride gave reasonable results while acid preservation was unsatisfactory. This he attributed to the lability of condensed phosphates. It was recommended that 40 mg/L mercury was the best for both long term and short term storage. He found that 5 ml/L chloroform caused a slight drop in soluble

reactive phosphate and a rise in insoluble phosphate for unfiltered samples. The American Public Health Association (1981) recommends storage with mercury, and is against use of acid or chloroform as a preservative for samples in which phosphate is to be determined. Gilmartin (1967) found that chloroform stabilized frozen samples over short periods of time, such as during sample thawing and analysis for phosphate. Thayer (1970) found that on frozen samples, chloroform had no effect on the reactive phosphate concentration. Strickland and Parsons (1972) state that mercury poisoning should not be used in samples in which phosphate is to be determined since it can interfere with standard analytic techniques of phosphate. These reports are clearly contradictory. Several researchers recommend storage with mercury but Strickland and Parsons warn against. There were no strong recommendations for chloroform.

Use of acid as a poison for nitrogen nutrients is not acceptable since it destroys nitrite (e.g. Jenkins, 1968; Howe and Holley, 1969). Thayer (1970) has found for frozen samples that chloroform causes variations in nitrate and nitrite concentrations, and Degobbis (1973) has found that chloroform significantly increases the amount of variability of ammonia in frozen samples. Howe and Holley (1969) found that 42 mg/L mercuric chloride was the best poison for preserving nitrate and nitrite. Maynard and Hopkins (1973) found that 40 mg/L mercuric chloride preserved nitrate and nitrite up to at least 8 hours for filtered samples, but that it slightly increased variability in frozen samples. Degobbis (1973) did not investigate the effect of mercury poisoning on ammonia preservations, but found chloroform poisoning unsatisfactory

and that 0.4 grams per 100 milliliters of phenol is an effective poison.

In conclusion, the literature does not point to any one poison for all nutrients but suggests that mercuric chloride (about 40 mg/L) should be examined as a potentially useful poison. However, a careful determination must be made of what interferences may result with phosphate determinations.

Conclusions - Literature Survey

A survey of the existing literature on nutrient preservation and storage techniques for natural water samples indicates the following:

1) No comprehensive study has been made using open ocean surface and deep seawater.

2) No study has been made on which preservation techniques were examined for orthophosphate, total phosphate, nitrate, nitrite, ammonia and silica on the same water samples.

3) Most nutrient preservation studies have concentrated on only one or two aspects of the problem with little attention being paid to the interactions between all of the preservation techniques.

4) Studies of inshore temperature waters indicate that immediate filtration through a filter with pore size of 0.4 microns or less is important. Cellulose acetate and glass fiber filters are generally unacceptable as they can contribute nutrients. Polycarbonate filters (e.g. Nuclepore®) appear to have the best overall performance.

5) With the exception of samples to be analyzed for silica, the literature suggests that acid cleaned glass bottles are the best storage containers.

6) If samples are to be analyzed shortly after collection, storage in a cool (4°C) dark place is advisable. For long term storage

quick freezing in a dry ice-acetone bath and storage at -20°C is best. Quick thawing in a water bath at 30-40°C is recommended.

7) No general purpose poison is recommended but mercuric chloride at concentrations of 40 mg Hg/L should be investigated as a poison, although it may interfere with phosphate analyses at very low phosphate concentrations.

This summary of available data on nutrient preservation indicates a lack of information regarding the preservation of nutrient concentrations in seawater typical of the open ocean. Also, there have been few attempts to look at more than one aspect of preservation techniques at a time. Based on these findings, it was decided to carry out an experimental investigation of the behavior of nutrient concentrations in preserved seawater samples from open ocean surface and deep waters. In order to make the study as complete as possible, it was also deemed wise to look at a wide matrix of preservation technique variables including storage container material, storage temperature, different preservatives and length of storage time.

METHODS AND PROCEDURES

Sample Collection: In order to collect seawater samples typical of open ocean waters, a sampling site in the central Gulf Stream east of Miami, Florida was chosen. Two sample collection trips were made, the first in June, 1979, (25° 47' N, 79° 56' W, water depth 516 m), and the second in November, 1980, (25° 25' N, 79° 56' W, water depth 700 m). Deep water samples were collected from a depth of 500 m using GoFlow® bottles. Surface waters were collected using a teflon lined submersible pumping system.

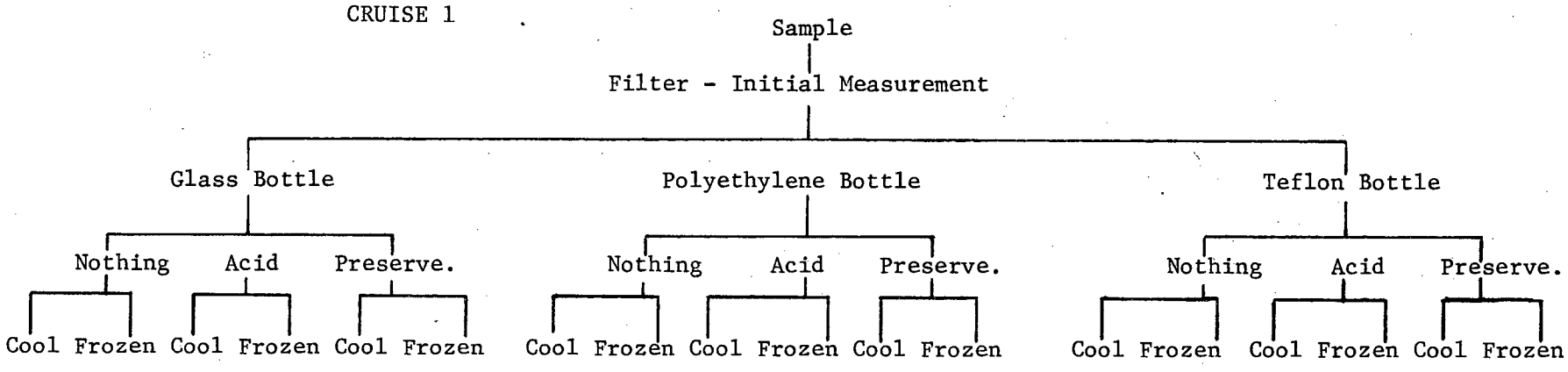
Preservation Methods: After collection, all water samples were filtered through a seawater washed 0.4 micrometer Nuclepore® filter. Subsamples of the surface and deep water samples were taken for analysis at the same time preservations were being made. Three types of bottles were used in the first study; glass, polyethylene, and Teflon. In the second study only glass and polyethylene bottles were used. All bottles were of approximately 125 ml volume. The glass and polyethylene bottles had all been used for seawater nutrient measurements for several years. The Teflon bottles were new.

In the first study, six types of preservation were used for each bottle type. Samples were stored both frozen and cool (2°C) in the dark with either no additives, acidified or with a preservative (Figure 1). Acidification involved addition of 0.1 ml of concentrated HCl to each bottle to bring the pH to approximately 2.5. Alcoholic phenol (5 ml of solution, 100 g phenol/L EtOH)was to preserve samples for ammonia analysis. For all other nutrients, 0.3 ml of 5% (w/w) $HgCl_2$ solution was added to each bottle.

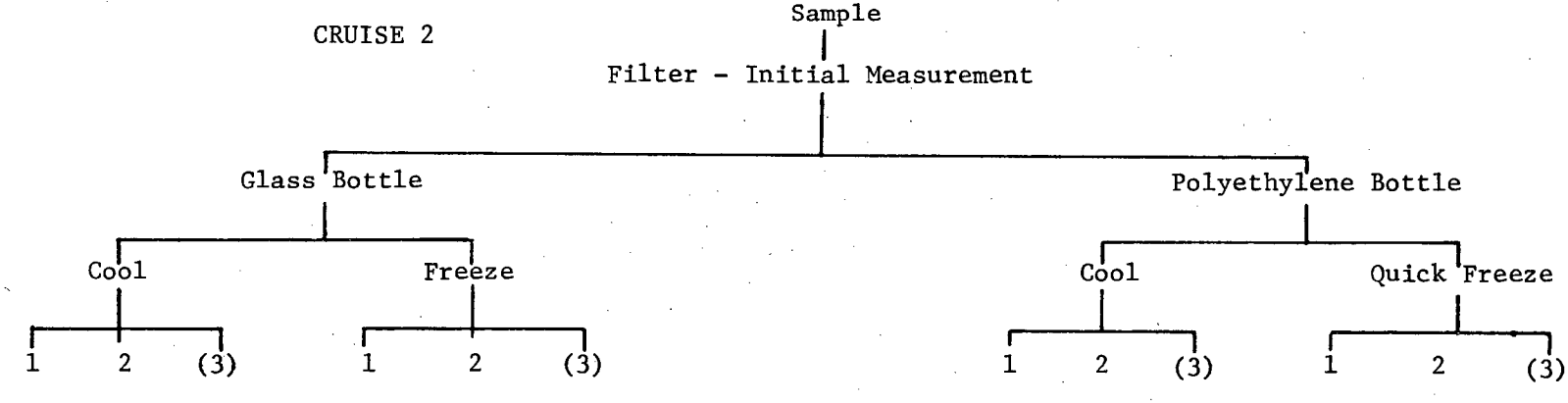
Because dry ice and/or liquid nitrogen are not usually available on long open ocean cruises in sufficient quantities throughout the cruise to permit quick freezing of nutrient samples, samples were frozen in normal freezers in the first study. The time required for complete freezing of the sample under these conditions was usually several hours. Samples were stored for 1, 9, 30 and 60 days.

In the second study, no acid or preservatives were added to the samples. Samples were preserved both frozen and cool in the dark. In this study the samples in polyethylene bottles were rapidly frozen in a standard

Figure 1 Preservation "Flow" Chart



Time (days): 1, 7, 30, 60.



Time (days): 1, 2, 7.

Bottle numbers in parenthesis for 7 day manual analysis.

dry ice-acetone bath. Glass sample bottles broke when subjected to quick freezing. Samples were stored for 1, 2, and 7 days and duplicate bottles were used.

Analytical Methods: Two basic types of analytical methods were used: manual with a GCA McPherson digital spectrophotometer, and automated with a Technicon Autoanalyzer model CSM-6. In general, the manual methods presented by Strickland and Parsons (1972), and automated manifold setups of Grasshoff (1976) were closely followed. Ammonia was determined only on samples from the first cruise. This was done manually using a Beckman DU-2 spectrophotometer.

METHODS OF ANALYSIS - MANUAL

Nitrite: Reactive nitrite is analyzed according to the procedure of Strickland and Parsons (1972), which is based on the classical Griess reaction as applied to seawater by Bendschneider and Robinson (1952). Nitrite is reacted with acid sulphanilamide and the resulting diazo complex reacted with N-(1-napthyl)-ethylenediamine to form an azo dye with an absorption at 545 nm. The detection limit is 0.05 µM.

Nitrate: Reactive nitrate is analyzed by the method presented by Strickland and Parsons (1972), which was derived from the procedure developed by Wood, Armstrong and Richards (1967). The nitrate in the sample is reduced by passage through a cadmium-copper column to nitrite. The resulting solution is analyzed for nitrite as previously described. Nitrate is obtained by taking the difference between the nitrate-nitrite (nitrate plus nitrite) analysis and the nitrite analysis. The detection limit is 0.05 µM.

Ammonia: The method of analyzing ammonia is taken from Strickland and Parsons (1972) and is based on the procedure for ammonia analysis of Solorzano (1969). The sample is reacted with an alkaline citrate medium containing sodium hypochlorite and phenol. The reaction is catalyzed with sodium nitroprusside. A blue indophenol color is measured at an absorption at 640 nm. The detection limit is 0.1 μM.

Orthophosphate: The procedure used for determining orthophosphate was that presented by Strickland and Parsons (1972), it is based on the method of Murphy and Riley (1962). Molybdic acid is allowed to react with the sample and the resulting heteropoly acid is reduced with ascorbic acid. The reaction rate is enhanced by trivalent antimony. The resulting blue solution is analyzed at an absorption at 885 nm. Detection limit is 0.03 μM.

Total Phosphorus: Total phosphorus is determined by the method presented by Strickland and Parsons (1972), which is based on the procedure of Hansen and Robinson (1953). The sample is evaporated with perchloric acid and the residue heated to oxidize all the organic phosphorus to orthophosphate. The resultant sample is analyzed by the previously described method for orthophosphate. This method was also used for preparation of samples for total phosphate analysis on the autoanalyzer by the method for orthophosphate described in the automated analysis section of this report. Detection limits are close to those reported for orthophosphate.

Silica: Reactive silica is analyzed by the method of Strickland and Parsons (1972), which is a modification of the technique of Mullin and Riley (1955). Molybdic acid reacts with dissolved silica to form a

silcomolybdate complex. The complex is reduced to form a blue color which is analyzed at an absorption at 810 nm. The only modification to the procedure of Strickland and Parsons (1972) is to allow the sample and solution to react for 20(±0.5) minutes as suggested by Fanning and Pilson (1973). The detection limit is 0.1 μM.

Automated Methods

Nitrite, nitrate, orthophosphate, and silica were all determined by autoanalyzer as described by Grasshoff (1976). The chemistry for determination of these nutrients outlined in the manual methods was followed, with the only exception being that silica was determined at 660 nm instead of the 810 nm used in the manual method. Detection limits are: orthophosphate 0.02 μM; nitrite and nitrate 0.4 μM; silica 0.5 μM.

RESULTS

General Considerations

Analytical results from the first and second cruises are presented in Tables 1 and 2, respectively. The concentrations presented are averages from quadruplicate analyses of samples from the first cruise and triplicate analyses of samples from the second cruise. The reason for the reduction in the number of replicate analyses was the relatively small standard deviations found (see Table 3a). The reduction in the number of replicates did not significantly change the values of the standard deviations.

TABLE 1a. NITRATES (+ NITRITES ~ 0) AVERAGE CONCENTRATIONS CRUISE 1.

CONTAINER	TEMPERATURE	PRESERVATIVE	DAY 1		DAY 7		DAY 30		DAY 60	
			SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP
Polyethylene	Cool	None	0	42	0	31	0	29	0	26
		Hg	0	43	0	31	0	30	0	26
		Acid	0.6	41	0	28	0	26	0	23
	Frozen	None	0	44	0	32	0	29	0	26
		Hg	0	44	0	31	0	29	0	26
		Acid	0.7	41	0	29	0	26	0	24
Glass	Cool	None	0	31	0	31	0	29	0	27
		Hg	-	32	0	31	0	28	0	26
		Acid	1.0	29	0	28	0	26	0	24
	Frozen	None	0	22	0	31	2.4	25	0	23
		Hg	0	27	0	31	0	27	0	26
		Acid	0	24	0	26	0	25	0	24
Teflon	Cool	None	-	-	-	-	-	-	1.5	26
		Hg	-	-	-	-	-	-	0	27
		Acid	-	-	-	-	-	-	0	24
	Frozen	None	-	-	-	-	-	-	0	-
		Hg	-	-	-	-	-	-	0	27
		Acid	-	-	-	-	-	-	0	24

All concentrations in μmoles per liter. Initial concentrations: surface 0.04; deep 20

TABLE 1b. AMMONIA AVERAGE CONCENTRATIONS CRUISE 1.

CONTAINER	TEMPERATURE	POISON	DAY 1		DAY 7		DAY 30		DAY 60	
			SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP
Polyethylene	Cool	None	0.5	3.4	0.4	3.9	1.0	1.4	0	0.7
		A.P.	0.6	2.6	0.1	2.9	0.3	1.8	0	1.9
		Acid	1.2	3.0	0.1	4.4	0.5	1.8	0.2	2.7
	Frozen	None	0.5	3.4	-	4.7	0.3	1.8	0.2	5.9
		A.P.	0.4	3.4	0.1	2.4	0.2	1.8	0.2	2.4
		Acid	1.1	3.2	0.3	2.3	0.3	2.8	0.2	4.2
Glass	Cool	None	1.5	6.0	0.4	0.5	2.2	0.5	6.7	0.3
		A.P.	1.6	3.6	0.1	2.2	0.5	0.3	5.8	0.2
		Acid	1.2	6.1	0.2	1.5	2.1	1.4	2.8	4.5
	Frozen	None	0.8	4.1	0.4	0.1	1.0	1.9	6.0	1.3
		A.P.	0.8	9.5	0.4	1.3	3.2	0.4	4.1	0.7
		Acid	3.3	1.6	3.2	0.5	4.9	1.5	8.5	3.6
Teflon	Cool	None	-	-	-	-	-	-	0.1	1.2
		A.P.	-	-	-	-	-	-	-	-
		Acid	-	-	-	-	-	-	-	-
	Frozen	None	-	-	-	-	-	-	0.4	2.8
		A.P.	-	-	-	-	-	-	-	-
		Acid	-	-	-	-	-	-	-	-

All concentrations in μmoles per liter. Initial concentrations: surface 0.6; deep 3.2.

TABLE 1c. ORTHOPHOSPHATE AVERAGE CONCENTRATIONS CRUISE 1.

CONTAINER	TEMPERATURE	POISON	DAY 1 SURFACE	DAY 1 DEEP	DAY 7 SURFACE	DAY 7 DEEP	DAY 30 SURFACE	DAY 30 DEEP	DAY 60 SURFACE	DAY 60 DEEP
Polyethylene	Cool	None	0.05	2.1	0.05	1.18	0.05	1.6	0	1.6
		Hg	0.04	2.0	0.05	1.8	0.05	1.7	0.08	1.7
		Acid	0.10	2.9	0.10	1.6	0.08	1.7	0.10	1.3
	Frozen	None	0.04	3.0	0.09	1.8	0.04	1.7	0.05	1.4
		Hg	0.02	2.8	0.05	1.8	0.08	1.7	0.07	1.6
		Acid	0.07	2.6	0.09	1.7	0.05	1.6	0.05	1.5
Glass	Cool	None	0.02	1.8	0.05	1.8	0.05	1.6	0.09	1.7
		Hg	-	2.2	0.05	1.9	0.10	1.8	0.09	1.7
		Acid	0.12	1.9	0.18	1.6	0.10	1.6	0.10	1.6
	Frozen	None	0.02	1.2	0.06	1.8	0.08	1.6	0.06	1.7
		Hg	0.02	2.2	0.10	1.8	0.08	1.7	0.05	1.7
		Acid	0.09	2.1	0.21	1.6	0.10	1.5	0.10	1.5
Teflon	Cool	None	-	-	-	-	-	-	0.05	1.7
		Hg	-	-	-	-	-	-	0.10	1.7
		Acid	-	-	-	-	-	-	0.10	1.6
	Frozen	None	-	-	-	-	-	-	0.08	1.7
		Hg	-	-	-	-	-	-	0.07	1.7
		Acid	-	-	-	-	-	-	0.10	1.5

All concentrations in μmoles per liter. Initial concentrations; surface 0.03; deep 1.0.

TABLE 1d. TOTAL PHOSPHATE AVERAGE CONCENTRATIONS CRUISE 1.

CONTAINER	TEMPERATURE	POISON	DAY 1 SURFACE	DAY 1 DEEP	DAY 7 SURFACE	DAY 7 DEEP	DAY 30 SURFACE	DAY 30 DEEP	DAY 60 SURFACE	DAY 60 DEEP
Polyethylene	Cool	None	2.8	2.7	1.3	2.4	1.0	2.5	1.0	2.7
		Acid	0.9	2.2	1.1	2.4	1.0	3.0	1.1	2.8
	Frozen	None	1.0	2.2	1.1	2.8	1.3	2.7	1.0	2.8
		Acid	1.0	1.8	1.0	2.3	1.3	2.6	1.1	2.8
Glass	Cool	None	4.3	4.4	1.1	4.2	1.5	2.9	1.3	2.8
		Acid	3.7	4.3	1.0	4.0	1.7	2.5	1.3	2.8
	Frozen	None	3.8	3.9	3.9	4.3	1.3	2.6	1.1	2.7
		Acid	3.6	3.6	2.7	2.4	1.4	3.0	1.1	2.8
Teflon	Cool	None	-	-	-	-	-	-	1.1	2.7
		Acid	-	-	-	-	-	-	-	-
	Frozen	None	-	-	-	-	-	-	1.0	2.7
		Acid	-	-	-	-	-	-	-	-

All concentrations in μmoles per liter. Initial concentrations: surface 0.9; deep 2.7.

TABLE 1e. SILICA AVERAGE CONCENTRATIONS CRUISE 1.

CONTAINER	TEMPERATURE	POISON	DAY 1 SURFACE	DAY 1 DEEP	DAY 7 SURFACE	DAY 7 DEEP	DAY 30 SURFACE	DAY 30 DEEP	DAY 60 SURFACE	DAY 60 DEEP
Polyethylene	Cool	None	4	13	3	10	2	12	2	14
		Hg	2	13	3	10	2	12	2	14
		Acid	3	16	5	11	7	17	12	23
	Frozen	None	2	15	3	10	2	12	2	15
		Hg	2	14	3	10	2	12	2	15
		Acid	3	13	5	11	6	16	12	25
Glass	Cool	None	3	28	3	85	3	14	5	44
		Hg	4	20	3	10	3	11	4	61
		Acid	5	23	6	25	11	22	18	100
	Frozen	None	2	17	3	12	2	-	3	30
		Hg	-	15	4	13	3	20	3	26
		Acid	-	28	6	37	14	34	19	34
Teflon	Cool	None	-	-	-	-	-	-	14	15
		Hg	-	-	-	-	-	-	2	15
		Acid	-	-	-	-	-	-	12	24
	Frozen	None	-	-	-	-	-	-	2	15
		Hg	-	-	-	-	-	-	2	15
		Acid	-	-	-	-	-	-	12	24

All concentrations in μmoles per liter. Initial concentrations: surface 6; deep 9.

TABLE 2a. NITRATE +NITRITE AVERAGE CONCENTRATIONS FROM CRUISE 2

CONTAINER	TEMPERATURE	BOTTLE	DAY 1 SURFACE	DAY 1 DEEP	DAY 2 SURFACE	DAY 2 DEEP	DAY 3 SURFACE	DAY 3 DEEP
Glass	Cool	1	0.7	33	0.6	33	0.5	32
		2	0.8	32	0.6	32	0.5	32
		Manual	-	-	-	-	1.5	28
Polyethylene	Cool	1	1.2	31	0	33	0	31
		2	0.8	31	0.4	32	0	31
		Manual	-	-	-	-	0.5	37
Glass	Frozen	1	0.7	32	0.6	31	0.4	32
		2	0.9	39	0.5	30	0.4	32
		Manual	-	-	-	-	0	28
Polyethylene	Frozen	1	0.5	38	0.6	38	0	32
		2	0.6	27	0.6	31	0	34
		Manual	-	-	-	-	0.7	28

All concentrations in µmoles per liter. Initial concentrations by autoanalyzer : Surface 0.0, deep 24. Initial concentrations by manual method: Surface 0.3, deep 26.

TABLE 2b. ORTHOPHOSPHATE AVERAGE CONCENTRATIONS FROM CRUISE 2

CONTAINER	TEMPERATURE	BOTTLE	DAY 1 SURFACE	DAY 1 DEEP	DAY 2 SURFACE	DAY 2 DEEP	DAY 7 SURFACE	DAY 7 DEEP
Glass	Cool	1	0	1.8	0	1.8	0.32	2.0
		2	0	-	0	1.8	0	1.9
		Manual	-	-	-	-	0	2.0
Polyethylene	Cool	1	0.08	1.9	0.17	2.0	0.06	2.0
		2	0.08	1.9	0.09	2.5	0.08	2.1
		Manual	-	-	-	-	0.01	2.0
Glass	Frozen	1	0.03	2.6	0.11	1.7	0.05	2.1
		2	0	2.2	0	1.6	0	2.0
		Manual	-	-	-	-	0.03	2.0
Polyethylene	Frozen	1	0.09	1.8	0.07	1.7	0.29	2.4
		2	0.06	1.6	0.04	1.6	0.06	2.3
		Manual	-	-	-	-	0	1.8

All concentrations in μmoles per liter. Initial concentrations by autoanalyzer: Surface 0.06, deep 1.9.

TABLE 2c. SILICA AVERAGE CONCENTRATIONS FROM CRUISE 2

CONTAINER	TEMPERATURE	BOTTLE	DAY 1		DAY 2		DAY 7	
			SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP
Glass	Cool	1	9	35	3	19	4	22
		2	0	16	9	19	12	22
		Manual	-	-	-	-	8	17
Polyethylene	Cool	1	0	19	0	17	4	18
		2	0	18	0	17	4	18
		Manual	-	-	-	-	0	17
Glass	Frozen	1	10	22	5	18	23	22
		2	9	21	16	18	33	-
		Manual	-	-	-	-	12	17
Polyethylene	Frozen	1	0	19	0	20	2	19
		2	0	19	0	20	3	21
		Manual	-	-	-	-	0	15

All concentrations in μmoles per liter. Initial concentrations by autoanalyzer: surface 2, deep 16. Initial concentrations by manual method: surface 0, deep 16.

TABLE 3a. STANDARD DEVIATION OF QUADRUPLICATE ANALYSES

NUTRIENT	SURFACE	DEEP
Nitrate	0.03	0.2
Ammonia	0.2	0.1
Orthophosphate	0.008	0.02
Total Phosphate	0.02	0.03
Silica	0.02	0.1

TABLE 3b. BOTTLE TO BOTTLE VARIABILITY

NUTRIENT	CONTAINER	$\overline{\Delta}$ SURFACE	$\overline{\Delta}$ DEEP
Nitrate	Glass	0.1	2
	Polyethylene	0.1	4
Orthophosphate	Glass	0.09	0.1
	Polyethylene	0.07	0.1
Silica	Glass	7	5
	Polyethylene	0	1

$\overline{\Delta}$ = Average bottle to bottle concentration variation

TABLE 3c. APPROXIMATE TOTAL PERCENT UNCERTAINTY

		PERCENT UNCERTAINTY	
NUTRIENT	CONTAINER	SURFACE	DEEP
Nitrate	Glass	30	6
	Polyethylene	30	10
Ammonia*	-	40	4
Phosphate	Glass	150	5
	Polyethylene	130	5
Total Phosphorus	Glass	150	5
	Polyethylene	130	5
Silica	Glass	150	25
	Polyethylene	4	5

*Assumed that bottle to bottle variability same as for orthophosphate.

To determine bottle to bottle variability, samples from the second cruise were stored in duplicate bottles. (Results in Table 3b). In general, the bottle to bottle variations were significantly larger than the variability in analytical results found from within a given bottle.

In Table 3c the estimated total uncertainty, in percent of concentration, of surface and deep water samples for the different nutrients studied are summarized. The large percent uncertainties in the surface concentrations are to be expected as the concentrations detected are so close to and frequently below detection limits for the method. Furthermore, their absolute values are so low that very minor levels of contamination or absorption could significantly change the concentrations. The estimated uncertainties for deep water nutrient concentrations are 10% or less for all nutrients except silica stored in glass bottles. In these bottles absorption and/or dissolution of silica from the glass surfaces of the bottles is probably the major factor contributing to the larger uncertainty.

An examination of the nutrient concentration data for samples stored for different lengths of time indicates that there are no significant trends in concentrations with time of storage, with the obvious exception of silicate samples preserved in glass and acid. This leads to the conclusion that for storage periods of up to two months, the length of time which a sample is stored under constant conditions does not significantly influence nutrient concentrations. However, in many cases nutrient concentrations do vary substantially from initial concentrations indicating that preservation methods and storage result in changes in concentrations.

In order to more readily recognize changes in nutrient concentrations, results for different periods of storage have been averaged and this average concentration compared as a ratio to the initial concentrations. These ratios are presented in Tables 4 and 5 for the results of cruises 1 and 2, respectively. Samples from the first cruise were stored with various poisons or preservatives. Acid was found to be a particularly unsatisfactory preservative (details follow in individual nutrient discussion). It was, therefore, excluded in calculating average concentration ratios from both time and preservation method. The concentration ratios are presented in Table 6. The concentration ratios presented in Tables 4 through 6 and the percent uncertainty in concentration values for stored samples presented in Table 3c will form the foundation for the following discussion of the preservation behavior of individual nutrients. Surface water nitrate plus nitrite data from the first cruise have not been used because the initial concentration was at the limit of detection and over 90% of the stored samples had concentrations below the detection limit. There was not clear trend for samples with concentrations above the detection limit.

INDIVIDUAL NUTRIENTS

Nitrate

Nitrate was determined as nitrite after previous determinations found nitrite to be either below the limit of detection or insignificant relative to the nitrate concentration. These nitrate concentrations are actually nitrate plus nitrite, with the nitrite contributing a negligible amount to the nitrate concentration.

TABLE 4. TIME AVERAGED NUTRIENT CONCENTRATION RATIOS FROM CRUISE 1.

RATIO OF AVERAGE PRESERVED NUTRIENT CONCENTRATIONS TO INITIAL VALUES

CONTAINER	TEMPERATURE	POISON	O-PHOSPHATE		Σ-PHOSPHORUS		NITRATE		SILICA		AMMONIA	
			SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP
Polyethylene	Cool	None	1.3	1.8	0.5	1.0	-	1.6	1.0	1.4	0.8	0.7
		Hg*	1.8	1.8	-	-	-	1.6	0.8	1.4	0.4	0.7
		Acid	3.1	1.9	0.4	1.0	-	1.5	2.3	1.9	1.1	0.9
	Frozen	None	1.8	2.0	0.5	1.0	-	1.6	0.8	1.4	0.6	1.3
		Hg*	1.8	2.0	-	-	-	1.6	0.8	1.4	0.6	0.7
		Acid	2.2	1.8	1.2	0.9	-	1.5	2.0	1.8	0.8	0.9
Glass	Cool	None	1.8	1.7	2.3	1.3	-	1.5	1.2	4.7	4.7	0.5
		Hg*	2.7	1.9	-	-	-	1.5	1.6	2.8	3.5	0.5
		Acid	4.2	1.7	2.1	1.2	-	1.3	3.3	4.7	2.8	1.0
	Frozen	None	1.8	1.6	2.8	1.2	-	1.3	0.8	2.2	3.6	0.6
		Hg*	2.1	1.8	-	-	-	1.4	0.8	2.0	3.7	0.9
		Acid	4.2	1.7	2.4	1.1	-	1.2	3.2	3.7	8.7	0.6
Teflon	Cool	None	1.7	1.7	1.2	1.0	-	1.3	4.7	1.6	0.2	0.4
		Hg*	0.3	1.7	-	-	-	1.3	0.7	1.7	-	-
		Acid	0.3	1.6	-	-	-	1.2	4.0	2.7	-	-
	Frozen	None	2.7	1.7	1.1	1.0	-	-	0.7	1.7	0.7	0.9
		Hg*	2.3	1.7	-	-	-	1.3	0.7	1.7	-	-
		Acid	0.3	1.5	-	-	-	1.2	4.0	2.7	-	-

* For ammonia Hg was not used as a poison, but a phenol alcohol solution was used as a preservative.

TABLE 5. TIME AVERAGED NUTRIENT CONCENTRATION RATIOS FROM CRUISE 2

			*RATIO OF AVERAGED PRESERVED NUTRIENT CONCENTRATIONS					
			NITRATE		O-PHOSPHATE		SILICA	
CONTAINER	TEMPERATURE	METHOD	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP
Glass	Cool	Auto	4	1.3	0.9	1.0	6	1.4
		Manual**	10	1.1	0.0	1.1	8	1.1
	Frozen	Auto	4	1.3	0.5	1.1	16	1.3
		Manual	2	1.1	0.5	1.1	12	1.1
Polyethylene	Cool	Auto	4	1.2	1.6	1.1	1.3	1.1
		Manual	3	1.5	0.2	1.1	0.0***	1.1
	Frozen	Auto	3	1.3	1.7	1.0	0.8	1.2
		Manual	5	1.1	0.0	0.9	0.0***	0.9

* Ratio relative to initial average manual and automated concentrations

** Determined for only one storage time (7 days).

*** Determined for only polyethylene. In all cases no silicon was determined, so these ratios could be considered to be 1. When glass containers were used there was a substantial increase in silica concentration.

TABLE 6. TIME AND PRESERVATIVE AVERAGED CONCENTRATION RATIO FROM CRUISE 1.

CONTAINER	TEMPERATURE	O-PHOSPHATE		Σ-PHOSPHORUS		NITRATE		SILICA		AMMONIA	
		SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP	SURFACE	DEEP
Polyethylene	Cool	1.6	1.8	0.5	1.0	-	1.6	0.9	1.4	0.6	0.7
	Frozen	1.8	2.0	0.5	1.0	-	1.6	0.8	1.4	0.6	1.0
	Average	1.7	1.9	0.5	1.0	-	1.6	0.8	1.4	0.6	0.9
Glass	Cool	2.3	1.8	2.3	1.3	-	1.5	1.4	3.8	4.1	0.5
	Frozen	2.1	1.7	2.8	1.2	-	1.4	0.8	2.1	3.7	0.8
	Average	2.2	1.8	2.6	1.3	-	1.5	1.1	3.0	3.9	0.7
Teflon	Cool	1.0	1.7	1.2	1.0	-	1.3	2.7	1.7	0.2	0.4
	Frozen	2.5	1.7	1.1	1.0	-	1.3	0.7	1.7	0.7	0.9
	Average	1.8	1.7	1.2	1.0	-	1.3	1.7	1.7	0.5	0.7

Data for samples in which acid was used as the preservative was excluded because of its general disagreement with all other data.

All nitrate concentrations for surface water samples for the second cruise determined after preservation, show major increases relative to the initial concentrations, with the ratio of preserved to initial values ranging from 2 to 10. (Nitrate concentrations from surface waters obtained during the first cruise were generally close to or below detection limits.) This indicates that it is not possible using any of the techniques studied to quantitatively preserve near-surface open ocean seawater nitrate concentrations. Because concentration of nitrate in open ocean surface water was close to our lower limit of detection, small changes in absolute concentration, lead to major changes in relative concentrations.

The concentration of nitrate in deep waters is approximately 100 times that of the near-surface waters. At this higher concentration the uncertainty as a percent of the analytical values decreases by a factor in excess of 4 compared to those found for near surface concentrations. Also, the ratio of the preserved to initial nitrate concentrations in the deep water samples is much closer to 1.

In examining the results from Cruise 1, it was not possible to determine a statistically significant difference between samples preserved with different additives and no additives. The time and preservative averaged ratios for Cruise 2 presented in Table 6 also indicate that there is no significant difference between samples stored in a frozen state or cool.

On Cruise 1, there was a slight advantage to storage in Teflon, where the concentration ratio averaged 1.3. When samples were stored in glass, the concentration ratio averaged 1.5; in polyethylene, the concentration ratio

averaged 1.6. Results from the second cruise were similar to those obtained from the first cruise, with the concentration ratio averaging 1.3 and no significant difference occurring between samples stored frozen or cool. The results from the second cruise did not indicate a significant difference between samples stored in glass and polyethylene bottles. The average concentration ratio obtained by manual analysis was 1.2. The average was strongly influenced by the cool polyethylene sample which had a ratio of 1.5. All other concentration ratios obtained by the manual method of analysis were 1.1. These results indicate that nitrate concentrations in preserved samples of deep seawater tend to increase, with the increase generally being on the order of 30 percent and not strongly dependent on the type of preservation added (or not), temperature of storage or type of bottle in which the sample is stored.

Ammonia

Ammonia was determined only on samples from the first cruise, by the manual method previously described. No bottle to bottle replication was carried out on these samples and the approximate uncertainty in the concentrations presented in Table 3c do not, therefore, include this factor which was found to be significant for other nutrients. Ammonia exhibited no significant storage time dependent concentrations trends. In general, the concentration of ammonia in stored samples was highly erratic exhibiting both major increases (up to 8 times initial concentrations) and decreases (to only 20% initial values), in both surface and deep water samples. Results well within the uncertainty of the initial values were obtained for both surface and deep water samples preserved cool in polyethylene with acid added. Whether these good

results indicate that this is the method for stabilizing ammonia concentrations or merely fortuitous can only be ascertained by further testing. However, considering the generally poor results obtained by this preservation method for other nutrients, it is probable that these results for ammonia are largely a coincidence.

Orthophosphate

Initial orthophosphate concentrations from near-surface water samples were close to the detection limit. The bottle to bottle variation in preserved samples were extremely high, averaging 140%. This high level of uncertainty probably due to analytical problems inherent near the limit of detection, makes interpretation of the preservation results from near-surface samples highly tenuous. There was no distinct trend in concentration ratios with length of storage period and the results from the various methods of storage are highly erratic ranging from ratios of 4.2 to 0. These results are similar to those for nitrate from near-surface seawater and reflect the fact that at very low concentrations minor influences can cause major changes in relative concentrations.

The results of storing deep water samples from the first cruise all exhibited an increase in orthophosphate concentration in stored samples. This increase was from 50 to 100 percent and did not show any significant correlation with preservation method or bottle type. Samples from the second cruise exhibited no significant change in phosphate concentration with preservation, all averaging within 10% of the initial concentration, independent of bottle type or method of storage. Also, both manual and autoanalyzer results for preserved samples were in good agreement. The

difference in these results from the two cruises is not easily explained and, perhaps, simply reflects that preservation results can, even for a large number of analyses on single sample, be variable. One difference in preserving the samples from the first and second cruises was the time between analysis and preservation. Although these two operations took place at the same time, the time required in the first study, due to the much larger number of samples and more complex methods of storage, was considerably longer than in the second study.

Total Phosphorus

Total phosphorus was determined only on samples from the first cruise. Again, no preservation time dependent trends were observed. Also, the preservation method did not cause major changes in the relative concentrations. Near-surface water samples gave consistent results for a given bottle type with concentration ratios relative to initial values averaging 0.5 for polyethylene bottles, 2.6 for glass bottles and 1.2 for Teflon bottles. Storage temperature did not make a significant difference in concentrations. Generally excellent results were found for all bottles and preservation methods on deep water samples. Polyethylene and Teflon bottles had average concentration ratios of 1.0, while the concentration ratios in the glass bottles average 1.3.

Silica

Dissolved silica concentrations showed no time dependent trends of significance other than when acid was used as a preservative. Both near-surface and deep water samples to which acid was added exhibited major increases in dissolved silica concentrations. The

results from the near-surface samples were erratic, with larger variations found in the samples from the second cruise where the initial concentration was lower by an average factor of 6.

All methods of preservation resulted in major concentration increases in deep water samples from the first cruise, with the largest increases consistently coming from samples stored in glass bottles. Deep water silica concentrations determined for samples from the second cruise were lower when determined by the manual method and when stored in polyethylene bottles. For samples stored in polyethylene bottles and analyzed by the manual method an ideal preservation concentration ratio of one was found. The highest ratio of stored to initial concentrations was found for samples stored in glass bottles at a cool temperature. Samples stored cool in polyethylene bottles and analyzed by autoanalyzer averaged within 10% of initial concentrations for deep water samples from the second cruise, but exhibited a 40% increase in deep water samples from the first cruise.

CONCLUSIONS

The results of this study of a variety of nutrient preservation techniques for open ocean seawater from near the ocean surface and deep sea indicate that none of the techniques produce reliable preservation of nutrient concentrations in near-surface seawater where concentrations are frequently near or below detection limits. The results for deep water, while substantially better than those found for shallow water, indicate that for most nutrients changes in concentrations occur during storage which are unacceptably large for many purposes.

The length of time which samples were stored; storing the samples at cool temperatures in the dark, frozen and quick frozen; adding acid, poisons and preservatives; and using bottles made of different materials - all had very little or no influence on the preservation of most nutrients. a major exception was that silica behaved erratically in glass bottles. Consequently, if nutrients must be stored, the simplest method of storage (at $2^{\circ}C$ in the dark with no additives) in aged polyethylene bottles is recommended for open ocean seawater samples.

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