EVALUATION OF SOME TECHNIQUES FOR PRESERVING NUTRIENTS IN STORED SEAWATER SAMPLES

ELIZABETH L. VENRICK AND THOMAS L. HAYWARD
Marine Life Research Group
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California 92093

ABSTRACT

We examined the effects of freezing, acidification, and acidification plus freezing on the concentrations of nitrate, phosphate, and silicate in seawater samples. We compared polyethylene and polystyrene containers, and determined preservation effects after storage periods of 1, 3, 6, and 12 months.

Because of the magnitudes of the concentration changes and their variability, none of the tested procedures was judged satisfactory for our purposes. Interpretation of results was complicated by strong interactions among treatments and between treatments and water sources. If nutrient preservation is necessary, the effects of preservation should be determined for the specific conditions of the study.

RESUMEN

Examinamos los efectos de la congelación, la acidificación y acidificación más congelación sobre las concentraciones de nitratos, fosfatos y silicatos en muestras de agua marina. Comparamos recipientes de polietileno y poliestireno, y determinamos los efectos de la preservación al cabo de 1, 3, 6, y 12 meses de almacenamiento.

Debido a las magnitudes y variabilidad de los cambios en la concentración ninguno de los procedimientos examinados se consideró satisfactorio para nuestros propósitos. La interpretación de los resultados se vió complicada por las intricadas interacciones entre tratamientos y entre lugares de origen de las muestras. Si la preservación de nutrientes es necesaria, sus efectos deben ser determinados para las condiciones específicas de cada estudio.

INTRODUCTION

In 1982, when planning for the 1984 CalCOFI surveys began, the planning committee agreed about the importance of adding measurements of chlorophyll and primary production to the standard data set. We recognized, however, that these additions would not be financially feasible without some cutbacks in other areas. As one possible cost reduction, we considered eliminating the shipboard analyses of nutrients by postponing analyses until the samples were returned to the facilities at Scripps Institution of Oceanography (SIO), thereby eliminating sea pay and overtime for the chemists. The feasibility of this move depended upon our ability to preserve the nutrient samples in a simple and effective manner for at least one month.

In January 1983 we began a series of nutrient preservation experiements within the central North Pacific and the California Current. We examined the effects of three different preservation procedures (freezing, acidification, and freezing plus acidification) on the concentration of three nutrients (nitrate, phosphate, and silicate) in two container types (polyethylene bottles and polystyrene tubes). We determined the preservation effects after storage for periods of 1, 3, 6, and 12 months. The effects of storage on nitrite were also determined. Because initial nitrite concentrations were always low, concentration changes were examined only in relation to concomitant changes in nitrate.

We ran five separate experiments, using water samples from different depths and different locations (Table 1), providing a variety of nutrient concentrations and, presumably, a variety of chemical and biological histories. All samples were oceanic; none were from upwelled or turbid waters.

The effectiveness of various preservation strategies has been examined previously; reviews are given by Riley (1975) and Grasshoff (1976). The definition of "effective procedure" undoubtedly varies from study to study depending on the degree of accuracy and/or precision required by the investigator. Freezing at $-10°$ to $-20°C$ has been demonstrated to successfully preserve phosphate, nitrate, and silicate (Collier and Marvin 1953; Strickland and Parsons 1968; Thayer 1970; Truesdale 1971; MacDonald and McLaughlin 1982); however, recommendations as to the necessity for prefiltration or quick-freezing vary. Numerous other workers have reported inferior results with frozen samples unless they are chemically stabilized (Murphy and Riley 1956; Gilmartin 1967; Jenkins 1968; Charpiot 1969). Depression of silicate levels in frozen samples caused by the polymerization of reactive silicate has been reported (Kobayashi 1967). This may be reversed by extended periods of thawing. Polymerization is not expected in samples of high

[Manuscript received December 9, 1984.]

TABLE 1
Origin of Samples Used in Study

Experiment #	Date (Jan. 1983)	Location		Depth of sample (m)	Initial concentration µmol/l		
					NO_3	PO_4	SiO_3
1.	13	24°52.4'N	156°13.4'W	15	0.02	0.060	0.62
2.	14	27°16.9'N	155°16.3'W	995	44.32	3.020	131.04
3.	18	27°57.7'N	141°38.6'W	140	1.50	0.155	3.90
4.	22	28°49.4'N	127°14.4'W	180	1.86	0.320	4.60
5.	26	33°11.3'N	118°22.8'W*	90	9.58	1.006	11.16

*CalCOFI 90.37

(oceanic) salinities (Burton et al. 1970), at least not for storage periods less than 5 months (MacDonald and McLaughlin 1982), and, at present, there is no accepted protocol for the length and means of thawing samples.

An alternative preservation strategy is to reduce the pH of the sample sufficiently to preclude biological activity. Acidification of samples with hydrochloric acid has been reported to preserve silicate (Mullin and Riley 1955), and sulfuric acid has been used to preserve silicate (Grasshoff 1976) and phosphate (Charpiot 1969). Other workers have warned against the acidification of phosphate samples because of the hydrolysis of labile organic phosphate (Jenkins 1968; Grasshoff 1976). At SIO hydrochloric acid has been used to stabilize samples in several studies, particularly when nitrate is to be analyzed (J. Gieskes, pers. comm.). The combination of acidification and freezing was recommended by Riley (1975), but no experimental justification was presented.

Although plastic containers are essential for preserving silicate (Mullin and Riley 1956), a number of workers have reported loss of phosphate from samples stored in polyethylene, especially soft polyethylene (Murphy and Riley 1956; Heron 1962; Hassenteufel et al. 1963). Other workers have stored samples for phosphate analysis in polyethylene containers without noticeable loss of phosphate (Charpiot 1969; Thayer 1970), and samples are briefly held in polyethylene tubes during autoanalyzer use. Acidification of samples should prevent the absorption of phosphate by polyethylene (A. Mantyla, pers. comm).

The disparity of the conclusions in previous studies suggests that the results of specific studies may have limited general applicability (e.g., Gilmartin 1967; Thayer 1970), both because of differences in the biological and chemical systems being studied and because of differences in the needs of various workers. The present investigation was specific to the requirements of the CalCOFI program. Since we could not expect to analyze nutrients less than one month after collection, this was the shortest storage period considered. Also, preservation is cost-effective only if it does not greatly increase the cost of analysis ashore and if the shipboard treatment is rapid and does not require skilled chemists or special materials. This latter requirement precluded many preservation procedures, such as those requiring filtration before preservation or quick-freezing in dry ice.

METHODS

Analytical procedures were those routinely used on CalCOFI surveys by the Physical and Chemical Oceanographic Data Facility (PACODF; Atlas et al. 1971). Control samples consisted of four or five replicate subsamples drawn into standard autoanalyzer tubes that had been rinsed with 1N HCl and triple-rinsed with distilled water. Nutrient concentrations were determined with an Hitachi autoanalyzer within 24 hours of collection. The same autoanalyzer was used for all subsequent analyses.

Preservation Methods

Frozen samples. We drew four replicate subsamples into each container type and placed them in a blast freezer at −10°C within one hour of collection. Samples remained frozen until the night before anlysis, when they were defrosted at room temperature.

Acidified samples. We drew four replicate subsamples into each container type. To each subsample we added 0.1 ml of concentrated HCl, using an Eppendorf pipette. Samples were capped and stored at room temperature. Prior to determining nutrients, we checked the pH of the samples with Hydrion paper to ascertain that acid had been added, and then neutralized them with the addition of 60 mg of sodium carbonate, returning the pH to 7-8. Controlled neutralization is essential because the subsequent nitrate analysis is sensitive to pH values outside this range.

Acidified and frozen samples. We drew four replicate subsamples into each container type and acidified them before freezing. Prior to analysis, we thawed and neutralized samples as described above.

Container Types

Polystyrene tubes. These were 16-ml, disposable, screw-capped test tubes. Because they are sterilized by the manufacturer, preliminary rinsing was not necessary.

Polyethylene bottles. These were 50-ml bottles, which were rinsed with 1N HCl and triple-rinsed with distilled water before use. In experiment 5, we substituted 60-ml autoanalyzer tubes like those routinely used to collect and hold nutrient samples at sea. Because we were depending upon materials at hand, we did not standardize the type of polyethylene used. In experiments 1 and 3, bottles were soft polyethylene; in the others they were linear polyethylene. In retrospect, this lack of control was a mistake.

Because we added a constant volume of acid to sample containers of varying sizes, the resultant pH of the preserved sample varied. In all cases it was below 2.

Simulated Catastrophes

In addition to the treatments described above, we simulated two types of catastrophe and determined their effects after one month. For these, we stored samples in tubes, except in experiment 4, when we used bottles.

No preservation. We stored samples at room temperature without acidification.

Accidental thaw. We simulated the effects of a power failure by removing samples from the freezer and allowing them to thaw at room temperature overnight before refreezing. We were particularly interested to see whether acidification of frozen samples would buffer them against the effects of an accidental thaw.

Performance Criteria

We used three criteria to evaluate the performance of the various preservation procedures.

Quality of the autoanalyzer output. One unexpected consequence of sample preservation was an increase in the noise of the autoanalyzer output signal, which made it difficult to accurately determine the peak height. In some cases, particularly phosphate and nitrite stored for 12 months, the noise was severe enough to completely prevent estimation of peak height, resulting in loss of data. In order to quantify this effect, we ranked the output traces for each sample from one to three, with one signifying a normal trace, two a noisy trace that could still be read with some degree of certainty, and three a trace that could not be interpreted with any degree of assurance.

Accuracy. We measured the accuracy of the preservation procedure as the absolute value of the deviation of the mean concentration of the four preserved replicates from the mean of the four or five replicates analyzed on board ship.

Precision. We measured the precision of the four preserved samples by their variance. Change in precision was evaluated by comparing the variance of the preserved replicates with the variance of the four or five replicates analyzed on board ship.

Statistical Analysis

The experimental design was originally intended for a single, multidimensional analysis of variance. Unfortunately, the data showed significant heteroskedasticity, which could not be removed by simple data transformation. In addition, there were significant interaction terms, which complicated interpretation of the results. Ultimately, we reorganized the data into a series of two-way designs and analyzed with the Friedman nonparametric two-way analysis of variance (Tate and Clelland 1959; Conover 1971). Under the assumption of no significant differences between primary effects (preservation method, container type, or preservation time) the test statistic, W, can be approximated by

$$W = \text{observed SS} \left(\sum_{i=1}^{m} R_i\right) / \text{maximum SS} \left(\sum_{i=1}^{m} R_i\right)$$

where

$$\text{observed SS} \left(\sum_{i=1}^{m} R_i\right) = \sum_{j=1}^{n} \left[\sum_{i=1}^{m} R_{ij}\right]^2 - \left\{\frac{\left[\sum_{j=1}^{n}\sum_{i=1}^{m} R_{ij}\right]^2}{n}\right\}$$

and

$$\text{maximum SS} \left(\sum_{i=1}^{m} R_i\right) = (m^2)(n^3 - n)/12$$

where items are ranked 1 through n across the factor being tested, and m = the number of sets of ranks.

For large values of m or n, the chi-square approximation was used:

$$x^2_{n-1} = m(n\text{-}1)(W)$$

We retained outlying values in the analyses and, unless otherwise noted, used only complete experiments.

For example, in the case where the effect of storage time on nitrate concentration is being tested ($n = 5$, $m = 17$), the appropriate sum of squares has components caused by storage time within samples frozen in tubes, within samples frozen in bottles, within samples acidified in tubes, etc. Thus the total sum of squares may be decomposed to examine the effect of storage time on samples stored in bottles, regardless of preservation method, or the effect of time on frozen samples regardless of container type. Or the effect of storage time on samples frozen in tubes may be compared with the effect on samples acidified in tubes. In this way, the components of the primary analysis may be examined for interaction effects, which cannot be tested directly. The calculated "probability" values associated with ANOVAs performed on subsidiary units have uncertain statistical meaning, but they indicate the relative strength of the primary effect within the various subunits. In addition, since accuracy and precision are independent measures of performance, they can be combined into a single measure for each nutrient, and the statistics for all nutrients may be combined to give an overall estimate of the relative success of the three preservation methods.

We are concerned with three factors: preservation procedure (freezing, acidification, and acidification plus freezing), container type (polystyrene tubes and polyethylene bottles), and storage time (1, 3, 6, and 12 months). To reduce the effects of multiple testing, effects of the preservation procedure, which we consider our major concern, were tested for significance at $P = .05$. The effects of container type were considered to be significant at $P = .025$, and the effects of storage time at $P = .01$.

When we obtained a significant ANOVA between three or more factors, we tested differences between specific levels of the primary factor by means of an a posteriori test (Nemenyi 1963), carried out at $P = .05$. Results are indicated by the pattern of underlining in Tables 2-4. For instance, in Table 2, the accuracies of the nitrate determinations changed significantly with time of storage according to the Friedman ANOVA ($P < .01$). The four storage times are listed from left to right in order of decreasing accuracy. The results of the Nemenyi analysis indicate that samples stored for 1 month are significantly more accurate than samples stored for 6 and 12 months, but are not significantly different from samples stored for 3 months; samples stored for 3 months are significantly more accurate than samples stored for 12 months, but are not different from samples stored for 1 or 6 months; samples stored for 6 months are not significantly more accurate than samples stored for 12 months. These relationships are indicated by a series of three overlapping underlines.

We examined the effects of the simulated catastrophes with a Friedman two-way ANOVA in which the six levels of the primary factor were the three preservation procedures, together with the three catastrophes: samples stored without preservation; samples frozen, thawed, and refrozen; and samples acidified and frozen, thawed, and refrozen. Secondary factors were the initial conditions of the five experiments.

RESULTS

The experimental results are summarized in Tables 2 to 4. The complete data set is available on request from the senior author.

Nitrate and Nitrite (Table 2)

There was no detectable increase in the noise of the nitrate signal caused by preservation. However, increased signal noise did result in loss of nitrite data, and consequently in nitrate data, especially in acidified samples stored for 12 months.

For nitrate there was a significant difference between preservation procedures only among the samples stored in autoanalyzer tubes in experiment 5, where there was a strong tendency ($P < .01$, accuracy and precision combined) for acidified samples to be best and frozen samples worst. When this subset was removed from the analysis, there was a tendency for frozen samples to be superior ($P < .10$, accuracy and precision combined).

For all procedures and all containers, there was a significant increase in the concentration of nitrate with time, accompanied by a loss of accuracy and decrease in precision.

There were also significant changes of nitrite over time ($P < .05$). However, no general trend of increase or decrease with time could be detected. There was no evidence of correlation between concentrations of nitrite and nitrate, either within or between experiments. Thus, changes in nitrate concentration could not be explained solely as reduction to or oxidation of nitrite.

Phosphate (Table 3)

For all samples, there was a significant increase in the noise of the phosphate autoanalyzer signal over time. The signal quality of samples run on board ship or stored for only one month was significantly better than that of samples stored for longer periods of time. The increase in noise with time was strongest in samples acidified or acidified and frozen and stored in tubes. Regardless of storage time, signals from frozen

TABLE 2
Summary of Nitrate Changes in Stored Seawater Samples

Factor	Criterion	ANOVA probability	Performance Best → Worst	
Preservation method	q	>.20	F A A+F	
F: frozen	a	<.20	F A+F A	
A: acidified	p	>.20	F A A+F	
	a+p	>.20	F A+F A	
Container type	q	>.20	B T	
T: tube	a	>.20	T B	
B: bottle	p	>.20	B T	
	a+p	>.20	T B	
Storage time in months	q	=.10	12 1 3 C 6	
C: control	c	<.01*	C 1 3 6 12	(increase)
	a	<.01*	1 3 6 12	
	p	<.05	C 1 3 12 6	

q: quality of the autoanalyzer signal

a: accuracy of the preserved samples

p: precision of the preserved samples

c: nitrate concentration in the preserved samples

Treatments are listed according to performance, left to right from best to worst. Underlining indicates the results of a posteriori tests, performed only when ANOVA results are significant (*).

samples had significantly less noise than did samples that were acidified and frozen, whereas signals from acidified samples were intermediate. There was no effect of container type.

The chemists who did the analyses believe that much of the problem can be attributed to the release of carbon dioxide in the sample when it is neutralized prior to analysis. Such bubbles are known to interfere with the proper operation of the autoanalyzer. The problem is more severe with phosphate because of the small diameter of the phosphate tube and because, unlike the other nutrients, the sample is not diluted with distilled water during analysis. However, if signal deterioration results solely from the production of bubbles, we would expect it to be independent of storage time, but dependent upon the volume of the sample (since the absolute amounts of acid and base added were the same for all volumes). Neither was the case in our samples, suggesting that other factors may be involved.

The precision of the phosphate determination is affected by the accuracy with which the autoanalyzer traces can be read. However, in most cases, changes in precision were greater than could be explained by uncertainty in the signal alone.

The preservation procedure affected neither the accuracy nor the precision of phosphate determinations, except for samples stored in tubes, where the procedure did influence precision ($P < .05$). Frozen samples were more precise than samples acidified and

TABLE 3
Summary of Phosphate Changes in Stored Seawater Samples

Factor	Criterion	ANOVA probability	Performance Best Worst
Preservation method	q	<.02*	F A A+F
F: frozen	a	>.20	F A+F A
A: acidified	p	>.20	F A A+F
	a+p	>.20	F A A+F
Container type	q	>.20	B T
T: tube	a	=.05	T B
B: bottle	p	<.10	T B
	a+p	<.01*	T B
Storage time in months	q	<.01*	C 1 3 12 6
C: control	c	<.025	C 3 1 6 (increase)
	a	<.01*	1 6 3
	p	<.01*	C 1 6 3

q: quality of the autoanalyzer signal

a: accuracy of the preserved samples

p: precision of the preserved samples

c: phosphate concentration in the preserved samples

Treatments are listed according to performance, left to right from best to worst. Underlining indicates the results of a posteriori tests, performed only when ANOVA results are significant (*).

frozen; precision of acidified samples was intermediate. This effect was not evident among samples stored in bottles and may in part reflect the deterioration of the autoanalyzer signal resulting from neutralization of the acid in the smaller-volume tubes.

When both accuracy and precision were considered together, pooling all storage procedures, the performance of tubes was significantly better than that of bottles. However, this effect was greatest in samples that were either frozen or acidified; samples that had been acidified and frozen showed no difference between container types.

The statistical analysis of length of storage time on accuracy and precision did not include the samples stored for 12 months because of the small number of these samples available. In all experiments, there was a tendency for the phosphate concentration to increase with time of storage, accompanied by a significant loss of accuracy and precision. Since this occurred both in frozen and in acidified samples, it did not appear to be solely related to the acidification of the sample and the resultant breakdown of organic material. Moreover, since the concentration increased in both container types, there was no evidence of differential adsorption of phosphate by the plastics, as has been suggested by previous workers (Murphy and Riley 1956; Heron 1962; Hassenteufel et al. 1963).

In general, these data indicate that the best phosphate results may be obtained from samples frozen in polystyrene tubes and analyzed within one month.

TABLE 4
Summary of Silicate Changes in Stored Seawater Samples

Factor	Criterion	ANOVA probability	Performance Best	Worst
Preservation method	q	>.20	A+F	A--F
F: frozen	a	>.20	A+F	A F
A: acidified	p	>.20	A+F	F A
	a+p	>.20	A+F	F A
Container type	q	>.20	T	B
T: tube	a	=.01*	T	B
B: bottle	p	<.05	T	B
	a+p	<.01*	T	B
Storage time in months	q	>.20	1 3 12 6	C
C: control	c	>.20	C 1 3 12 6	(decrease)
	a	<.01*	C 1 3 12 6	
	p	<.01*	C 1 3 6 12	

q: quality of the autoanalyzer signal

a: accuracy of the preserved samples

p: precision of the preserved samples

c: silicate concentration in the preserved samples

Treatments are listed according to performance, left to right from best to worst. Underlining indicates the results of a posteriori tests, performed only when ANOVA results are significant (*). Dash indicates ties.

Silicate (Table 4)

Both accuracy and precision of samples stored in polystyrene tubes were greater than those of samples stored in polyethylene bottles.

In all experiments, there was a significant loss of accuracy with increased storage time, related to a tendency for the concentration of silicate to decrease. This tendency was strongest for samples acidified and frozen in tubes ($P < .05$). A similar decrease with time was not evident in samples stored in bottles. Within tubes and bottles there was a significant decrease in precision over time, with the major period of decrease occurring during the first month of storage.

Interaction Effects

Numerous interaction effects were apparent in the results of this experiment. The more easily interpretable have been pointed out. In addition, there frequently appeared to be interactions between the storage procedure or the container type and the initial concentration of the nutrient. For instance, in the case of phosphate frozen in tubes (Figure 1), after one month there was a significant decrease in concentration in experiment 1, where the initial phosphate concentration was low (Mann Whitney U test, $P < .01$). However, in experiment 2, where initial concentration was high, there was a significant increase in concentration ($P < .01$).

The effect of time on the nutrient concentration of stored samples was confounded with any differences in the day-to-day standardization and operation of the autoanalyzer. This latter source of variability is not

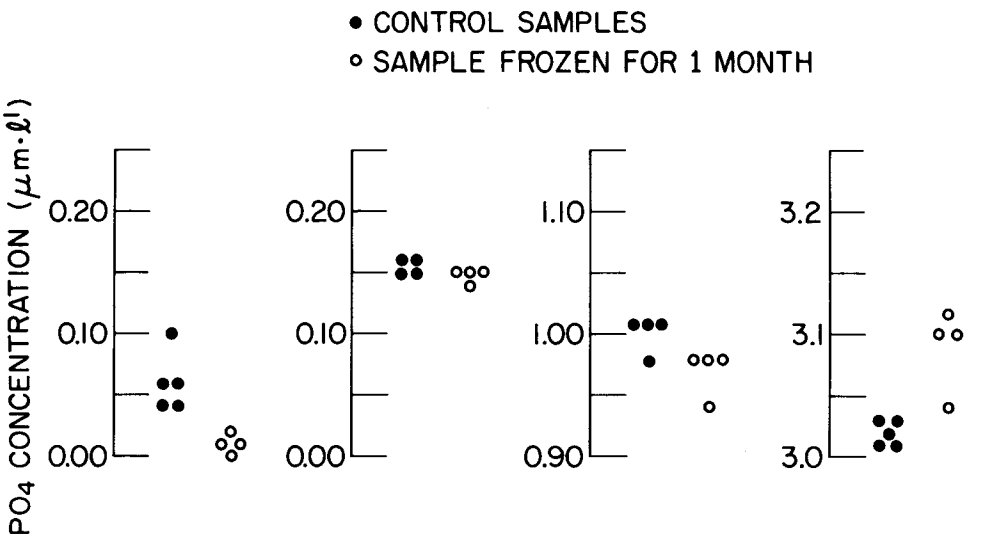

Figure 1. Interaction between initial concentration of phosphate and the change in concentration after freezing for one month in polystyrene tubes. Experiments 1, 3, 5, and 2, from left to right.

expected to have any temporal trend. However, it is not appropriate to blame operator error for any variability that does not exhibit a linear trend with time. The frozen samples generally showed highest concentrations of all nutrients after 6 months of storage. This pattern was not apparent in samples that had been acidified or acidified and frozen. Although all 6-month samples were analyzed by the same chemists, frozen samples were run the day before the others. We initially suspected that the high concentrations in the frozen samples were due to operator variability rather than true temporal changes. However, we are unable to postulate a source of operator error of such magnitude. In fact, the graphs for nitrate and phosphate from experiment 2, after 6 months, were off scale for the only time during the entire experiment.

The numerous interaction effects, especially those involving initial nutrient concentration or time of storage, make precise predictions about preservation of future samples risky.

Simulated Catastrophes

When the three simulated catastrophes were considered and compared with the three preservation procedures for both nitrate and phosphate, there were significant differences between treatments ($P < .05$). The primary outliers were related to the absence of treatment, which caused phosphate concentrations to decrease in all samples, and nitrate concentrations to increase in samples with very high or low initial concentrations but to decrease in samples with intermediate concentrations. There were no significant differences in precision. There was no evidence of treatment effect on either accuracy or precision of silicate determinations; silicate preserved as well in untreated samples as in preserved samples.

We could detect no decrease in accuracy or precision resulting from an accidental overnight thawing. Thus there is no evidence that adding acid before samples are frozen protects them from deterioration during a thaw.

CONCLUSIONS

When the data from all nutrients are weighted equally and combined, the most accurate and precise storage procedure is freezing; acidification, without freezing is the least satisfactory. The differences, however, are not significant ($P < .25$). Storage of samples in polystyrene tubes gave significantly better results than storage in polyethylene bottles ($P<.01$).

With respect to this latter result, we cannot distinguish between the effect of the smaller volume and the polystyrene material. From a technical standpoint, 16 ml represents an absolute minimum needed for the autoanalyzer. A second analysis, should the need arise, is not possible, and any loss of sample may preclude adequate rinsing. While the smaller volume may have advantages in terms of rapid freezing and thawing, the tubes were also superior to the bottles when samples were acidified. Thus, circumstantial evidence suggests that the difference between the performances of tubes and bottles was due primarily to the container material. A better container may be a 50-ml polystyrene bottle. More research into this is warranted.

The purpose of this study was not only to identify the best preservation procedure, but to determine whether any would be an adequate substitute for shipboard analyses. The error introduced by preservation was small relative to the range of values found within the California Current, and data from preserved sam-

ples would be adequate for mapping major horizontal and vertical distributions. However, general distributions within the California Current have been described in the past, and it is now the details and anomalies that are receiving most attention. Unfortunately, the relatively large error at the lowest values (e.g., after one month $> \pm 100\%$ for nitrate, $> \pm 33\%$ for phosphate, and $> +200\%$ for silicate) would complicate the use of these data for investigations into the relationships between the depth or slope of the nutricline and the biomass or productivity of the euphotic layer (e.g., Eppley et al. 1978, 1979; King and Devol 1979; Hayward and McGowan 1985). Moreover, the error introduced at higher concentrations, although relatively small, is equivalent to the variance observed at 300 m at a single station over a 28-year period, and this error would hinder calculations such as long-term anomalies of mass transport (e.g., Haury and Shulenberger 1982). Thus, because the magnitude of the error introduced by any of the storage procedures tested was sufficient to preclude many of the uses which we anticipated for the data, we concluded that nutrient storage during the 1984 CalCOFI surveys could not be justified.

When our results are considered in the context of previous studies of this sort, the only conclusion is the impossibility of valid generalization. The results of different nutrient storage procedures appear to depend strongly upon the type of water being sampled and upon specific details of the analytical techniques employed. Preservation of nutrient samples should be considered a strategy of last resort. However, any program that depends upon preservation of water samples for future nutrient analysis must independently determine the error expected to be introduced by the specific procedures selected. The question of whether the additional analytical error is acceptable can then be addressed.

ACKNOWLEDGMENTS

Much of the chemical information in this paper was contributed by others. We especially thank George Anderson, David Bos, and Arnold Mantyla. In addition, chemists from the Physical and Chemical Oceanographic Data Facility analyzed the endless replicate samples, an exceedingly uninteresting task. In gratitude for this invaluable help, we will happily supply each of our assistants with a bottle of wine, on request. This offer will not be repeated elsewhere. The work was supported by the Marine Life Research Group of Scripps Institution of Oceanography.

LITERATURE CITED

Atlas, E.L., J.C. Callaway, R.D. Tomlinson, L.I. Gordon, L. Barstow, and P.K. Park. 1971. A practical manual for use of the Technicon autoanalyzer in sea water nutrient analysis; revised. Oregon State University Tech. Rep. 215, Ref. No. 71-22.

Burton, J.D., T.M. Leatherland, and P.S. Liss. 1970. The reactivity of dissolved silicon in some natural waters. Limnol. Oceanog. 15:472-476.

Charpiot, R. 1969. Technique de conservation des echantillons d'eau de mer pour le dousage de phosphates, nitrites, nitrates, silice et bore. Cahiers Oceanog. 21:773-793.

Collier, A.W., and K.T. Marvin. 1953. Stabilization of the phosphate ratio of sea water by freezing. Fish. Bull. 79:71-76.

Conover, W.J. 1971. Practical nonparametric statistics. John Wiley & Sons, New York, 462 p.

Eppley, R.W., E.H. Renger, and W.G. Harrison. 1979. Nitrate and phytoplankton production in southern California coastal water. Limnol. Oceanog. 24(3):483-494.

Eppley, R.W., C. Sapienza, and E.H. Renger. 1978. Gradients in phytoplankton standing stocks and nutrients off southern California in 1974-76. Estuarine and Coastal Mar. Sci. 7:291-301.

Gilmartin, M. 1967. Changes in inorganic phosphate concentration occurring during seawater sample storage. Limnol. Oceanog. 12:325-328.

Grasshoff, K. 1976. Methods of seawater analysis. Verlag Chemie, New York, 317 p.

Hassenteufel, W., R. Jagitsch, and F.F. Koczy. 1963. Impregnation of glass surface against sorption of phosphate traces. Limnol. Oceanog. 8:152-156.

Haury, L., and E. Shulenberger. 1982. Horizontal transport of phosphorus in the California Current. CalCOFI Rep. 23:149-159.

Hayward, T. L., and J.A. McGowan. 1985. Spatial patterns of chlorophyll, primary production, macrozooplankton biomass, and physical structure in the central North Pacific Ocean. J. Plankt. Res. 7:147-167.

Heron, J. 1962. Determination of phosphate in water after storage in polyethylene. Limnol. Oceanog. 7:316-321.

Jenkins, D. 1968. The differentiation, analysis, and preservation of nitrogen and phosphorus forms in natural waters. Adv. Chem. Ser. 73:265-280.

King, F.D. and A.H. Devol. 1979. Estimates of vertical eddy diffusion through the thermocline from phytoplankton nitrate uptake in the mixed layer of the eastern tropical Pacific. Limnol. Oceanog. 24(4):645-651.

Kobayashi, J. 1967. Silica in fresh water estuaries. *In* H.L. Golterman and R.S. Clymo (eds.), Chemical environments in the aquatic habitat. North-Holland, Amsterdam, p. 41-55.

MacDonald, R.W., and F.A. McLaughlin. 1982. The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate from coastal and estuarine waters. Water Res. 16:95-104.

Mullin, J.B., and J.P. Riley. 1955. The spectrophotometric determination of nitrate in natural waters, with particular reference to sea-water. Analyt. 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. Analyt. Chim. Acta 14:318-319.

Nemenyi, P. 1963. Distribution-free multiple comparisons. Ph.D. dissertation, Princeton, 127 p.

Riley, J.P. 1975. Analytical chemistry of seawater. *In* J.P. Riley and G. Skirrow (eds.) Chemical oceanography, vol. 3. Academic Press, London (2nd ed.), p.193-514.

Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Brd. Can., Bull. 125, 195 p.

Tate, M.W. and R.C. Clelland. 1959. Nonparametric and shortcut statistics. Interstate Printers, Danville, Illinois, 171 p.

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.

Truesdale, V.W. 1971. A modified spectrophotometric method for the determination of ammonia (and amino-acids) in natural waters, with particular reference to sea water. Analyst 96:584-590.