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STABILIZATION OF THE CARBOHYDRATE CONTENT OF SEA WATER SAMPLES

It is the practice during many oceanographic investigations to analyze sea water samples immediately after collection. This has been necessary due to difficulties involved in stabilizing a sample during storage. Collier and Marvin (1953) have demonstrated that the inorganic to organic phosphorus ratio of a sea water sample can be effectively stabilized for long periods of time by freezing. The purpose of this experiment was to determine if carbohydrate materials (see Collier, *et al.* 1953) could be similarly stabilized.

A sea water medium that contained a bacterized culture of the organism *Prorocentrum* sp. was chosen because such a medium is relatively rich in dissolved organic material. A portion of the medium was first centrifuged through a Sharples centrifuge

to remove as many organisms as possible and was then thoroughly mixed. Fifty-ml portions were then dispensed into clean 25 × 200 mm culture vials and capped with solid polyethylene screw caps. All cap-glass junctions were sealed with plastic insulating tape. Of the 130 vials filled, half were frozen and stored at minus 18°C and half were stored at room temperature. Groups of 8 vials were taken from both the frozen and unfrozen material at the intervals indicated in Figure 1. Employing the method of Zein-Eldin and May (1958), four measurements of the carbohydrate content of each sample were made, making a total of 32 determinations per time interval.

Immediately prior to storage, the carbohydrate concentration in the centrifuged sea water medium was estimated at $3.8 \pm$

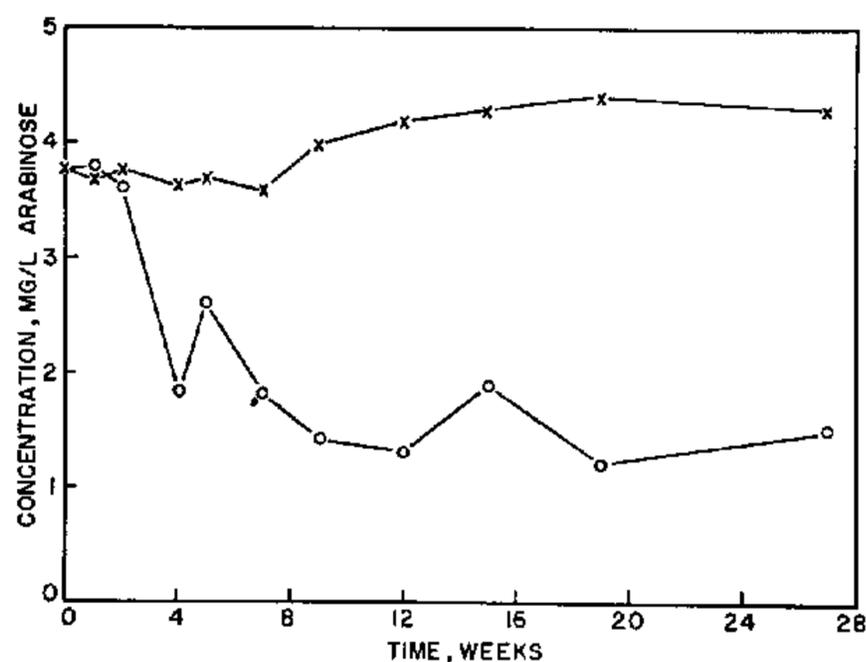


FIG. 1. Carbohydrate content of sample plotted against storage time of sample. The two plots $x-x-x$ and $o-o-o$ indicate mean values of 8 frozen and unfrozen samples respectively at each time interval.

0.1 mg/L arabinose equivalents. Subsequent estimates from the frozen and unfrozen material are plotted against time in Figure 1. Estimates from the frozen material did not vary more than ± 0.15 mg/L for a period of 7 weeks. In contrast, carbohydrate concentration in the material not frozen generally diminished as length of storage time increased. The somewhat erratic values obtained in the medium not frozen might be expected since biotic activity within different vials undoubtedly proceeds at different rates.

Comparison of the two curves shows that freezing stabilizes the carbohydrate content of sea water for periods up to 7 weeks. Since the average deviation within groups of frozen samples was less than 0.1 mg/L at any one period (Table 1), a reasonable assumption is that the increase in mean values after the 7th week was not due to sampling variation, but rather to unknown factors. For example, bacteria or fungi not

TABLE 1. Standard error of means of each frozen group

Group	Elapsed time Weeks	Mean (mg/L)	Standard error (mg/L)
1	1	3.70	0.096
2	2	3.80	0.082
3	4	3.65	0.047
4	5	3.70	0.043
5	7	3.65	0.057
6	9	4.0	0.032
7	12	4.2	0.038
8	15	4.3	0.030
9	19	4.4	0.078
10	27	4.3	0.083

eliminated during the process of centrifugation could have survived the freezing temperatures. The growth of these organisms might have caused the increase of arabinose equivalents.

This supposition suggests that further investigation should be conducted on the biological activities of micro-organisms at freezing temperatures and during the time lapse between thawing and analysis of samples.

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REFERENCES

- COLLIER, A. W. AND K. T. MARVIN. 1953. Stabilization of the phosphate ratio of sea water by freezing. U.S. Fish and Wildl. Serv., Fish. Bull., **79**: 71-76.
- COLLIER, A. W., S. M. RAY, A. W. MAGNITSKY AND J. O. BELL. 1953. Effect of dissolved organic substances on oysters. U.S. Fish and Wildl. Serv., Fish. Bull., **84**: 166-185.
- ZEIN-ELDIN, Z. P. AND B. Z. MAY. 1958. Improved n-Ethylcarbazole determination of carbohydrates with emphasis on sea water samples. *Analyt. Chem.*, **30**: 1935-1941.