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Abstract

Water samples were collected from Lake Ontario during April and November, filtered (0.45 μm) and immediately analyzed onboard ship for the nutrients soluble reactive phosphorus, nitrate plus nitrite, and ammonia. Replicates were stored in glass bottles at 4°C and reanalyzed within eight days. Statistical analysis showed that soluble reactive phosphorus decreased by 11% and 13% and nitrate plus nitrite by 7% and 6%, whereas ammonia increased by 75% on one cruise and decreased by 37% on the other.

Résumé

Des échantillons d'eau ont été recueillis dans le lac Ontario en avril et en novembre, filtrés (0.45 μm) et analysés immédiatement à bord du bateau pour en déterminer les éléments nutritifs suivants : phosphore réactif soluble, nitrates et nitrites et ammoniac. Des doubles ont été conservés dans des bouteilles de verre à 4°C et analysés dans l'espace de huit jours. L'analyse statistique montre que la teneur en phosphore réactif soluble a diminué de 11 % et de 13 % et celle des nitrates et des nitrites de 7 % et de 6 % alors que celle de l'ammoniac a augmenté de 75 % dans le cas d'une expédition et diminué de 37 % dans l'autre.

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INTRODUCTION

Considerable research (Eichholz *et al.*, 1965; Thayer, 1970; Bowditch *et al.*, 1976; Macdonald *et al.*, 1980) has been conducted to study the effect of preservation and storage on the chemical characteristics of water samples. Soluble reactive phosphorus (SRP), nitrate plus nitrite ($NO_3 + NO_2$) and ammonia (NH_3) have received particular attention (Murphy and Riley, 1956; Gilmartin, 1967; Jenkins, 1968; Howe and Holley, 1969; Thayer, 1970; Armstrong, 1972; Degobbis, 1973; Klingaman and Nelson, 1976). Stored without treatment, the concentration of these nutrients can undergo significant change. These changes are rapid, occurring within 30 min of sampling (Collier and Marvin, 1953).

There has been little agreement concerning the optimal preservation technique for these nutrients. To stabilize the SRP content of water samples, Collier and Marvin recommended quick-freezing and storage at sub-zero temperatures. Strickland and Parsons (1968) advised analysis within 2 h of sampling but agreed that freezing would stabilize the sample for several months. Gilmartin (1967) and Philbert (1973), in studying the effect of freezing on nutrient concentrations, found decreased SRP levels in the thawed samples. Various sources suggested mercuric chloride as an effective preservative in conjunction with either refrigeration (Environmental Protection Agency, 1971; Klingaman and Nelson, 1976) or freezing (Jenkins, 1968; American Public Health Association, 1971). Henriksen (1969), however, found that in the presence of HgCl, SRP levels increased. Bowditch *et al.* (1976) determined the most acceptable preservation technique to be storage in glass bottles at 4°C. Yet Murphy and Riley (1956) and Klingaman and Nelson (1976) reported large changes in SRP concentrations using this technique. Murphy and Riley (1956) considered storing samples at 20°C, in the dark, with the addition of various preservatives (sodium fluoride, chloroform, aluminum hydroxide and thorium carbonate). Their conclusion, only to be refuted by Jenkins (1968), was that chloroform (0.7% v/v) worked best.

To preserve nitrogen samples, Bowditch *et al.* (1976) recommended storage at 4°C. Klingaman and Nelson (1976) and Thayer (1970), however, found that large concentration changes resulted. Jenkins (1968) advised the addition of mercuric chloride, prior to refrigeration, for short-term storage of ammonia, nitrate plus nitrite and, except for nitrite samples, sulphuric acid for long-term storage. Although this met with acceptance from Howe and Holley (1969) and the American Public Health Association (1971), it had previously been found to be inadequate for stabilizing ammonia (Kreps, 1934; Cooper, 1937; Redfield and Keys, 1938). Freezing was suggested as an alternative (Marvin and Proctor, 1965; Klingaman and Nelson, 1976), but Degobbis (1973) and Newell (1967) reported concentration changes with increased variability attributed to the rupturing of plant and animal cells during freezing and thawing. Phenol was found to stabilize NH_3 concentrations for up to two weeks (Degobbis, 1973).

The Water Quality Branch, Ontario Region, conducts a wide variety of studies requiring measurement of the phosphorus and nitrogen levels in water. In light of the inconclusiveness of these studies, the analyses of soluble reactive phosphorus, nitrate plus nitrite, and ammonia are performed, whenever possible, in the field at the time of sampling. As this is not always convenient, however, samples are sometimes collected and stored at 4°C. A comparative study was initiated in 1982 to determine whether changes occur in the concentration of these nutrients as a result of storage. In 1982, during the April 26-30 and November 19-24 Lake Ontario surveillance cruises, samples were collected for the analysis of these nutrients. The resulting data were then subjected to statistical analysis to determine (i) whether there were real changes in the concentration of SRP, ($NO_3 + NO_2$) and NH_3 as a result of storage, and if so, the magnitude of the changes and (ii) a confidence interval for the magnitude of change.

METHODS

Sampling was conducted at 94 stations on Lake Ontario at depths of 1 m, 10 m, 25 m, as well as 10 m and 2 m from the bottom during isothermal conditions and at 1 m, 1 m above and 1 m below the thermocline, and 10 m and 2 m from the bottom during stratified conditions.

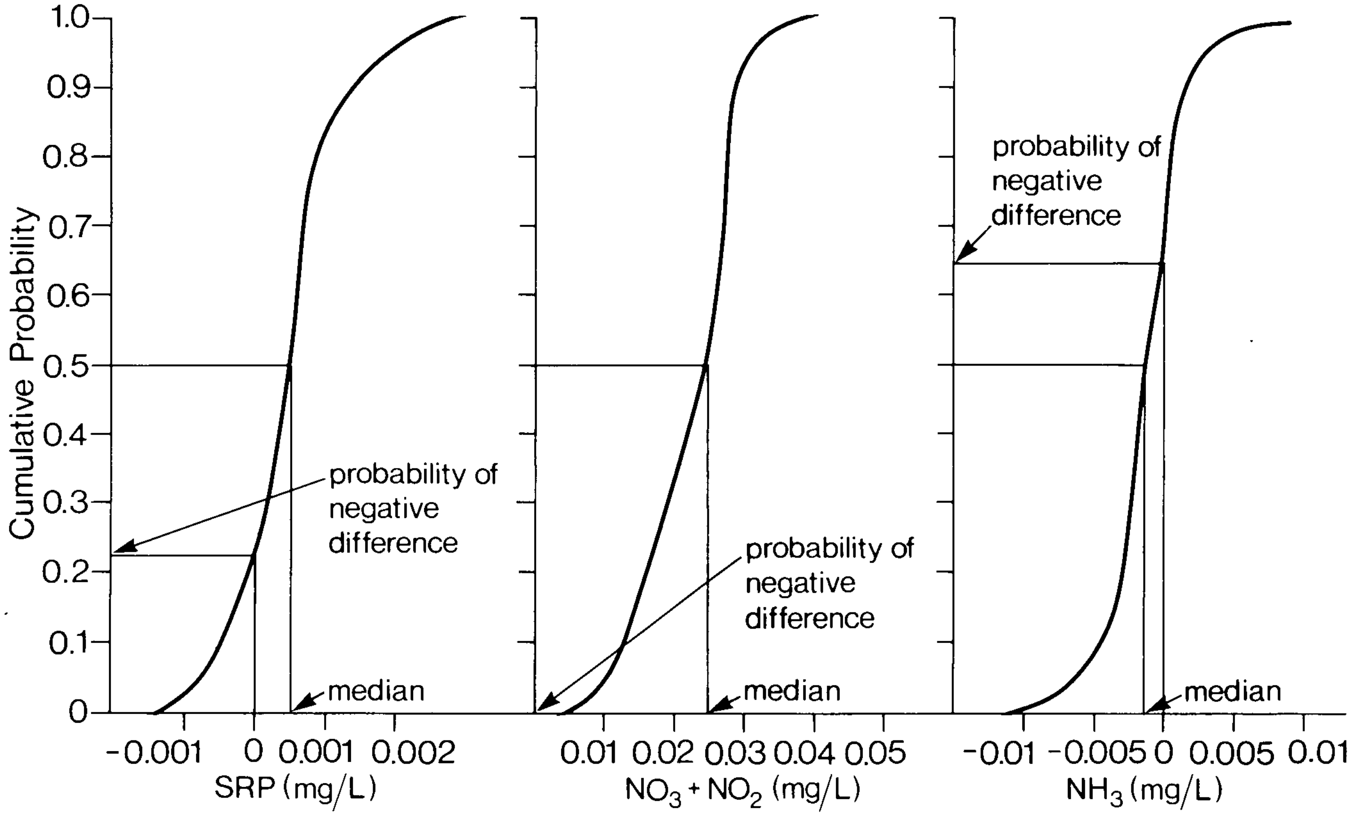

Figure 1. The empirical cumulative distribution functions for the differences between the shipboard and stored sample analyses (April 26-30).

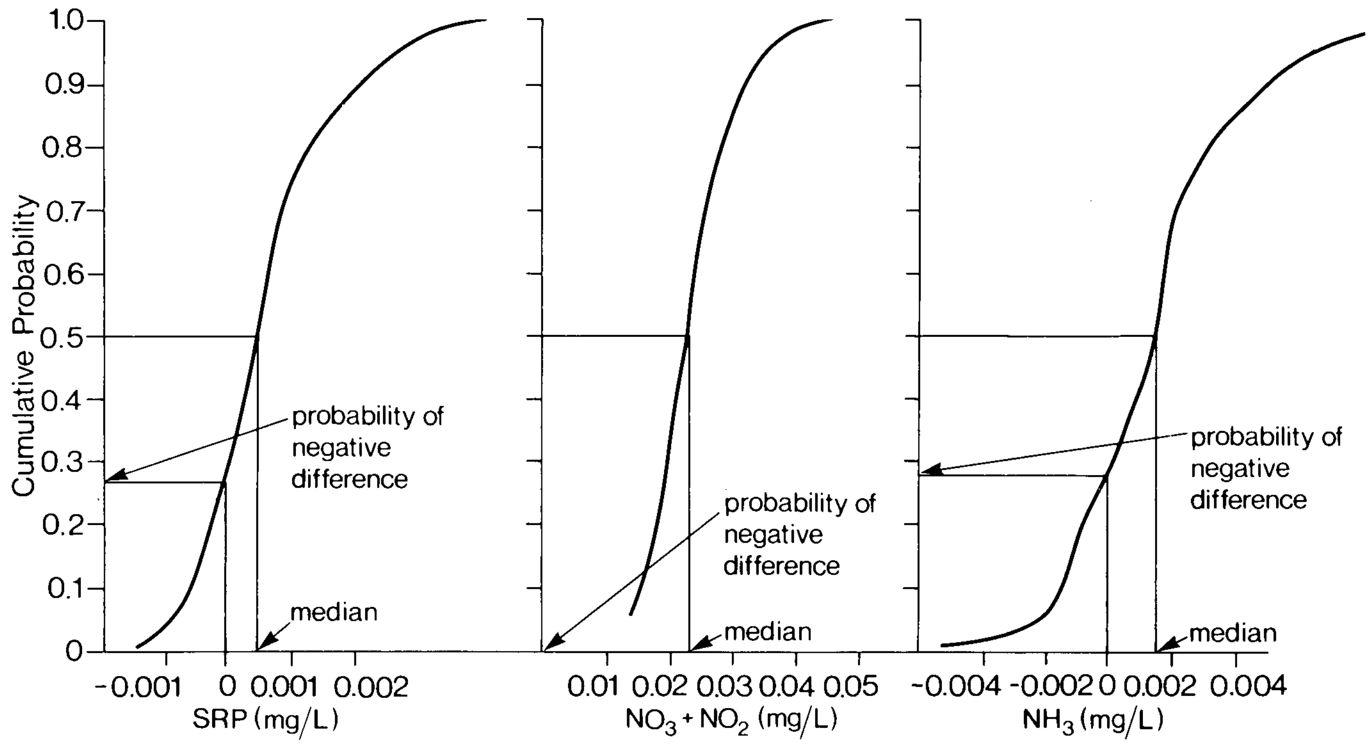

Figure 2. The empirical cumulative distribution functions for the differences between the shipboard and stored sample analyses (November 19-24).

Samples from each depth were filtered through 0.45-µm membrane filters, then split into two sets of replicates and stored in 125-mL glass bottles with plastic snap-on caps. One set was refrigerated (4°C) for future analysis; the other underwent immediate analysis in the shipboard laboratory for soluble reactive phosphorus, nitrate plus nitrite and ammonia. During the April 26-30 and November 19-24 cruises, 374 and 370 samples, respectively, were collected. Replicate analyses were conducted May 4-5 and November 24-25 following each cruise.

Soluble reactive phosphorus was measured using an automated colorimetric stannous chloride method. Nitrate plus nitrite was determined by an automated cadmium reduction, and ammonia, by an automated colorimetric phenate procedure (Environment Canada, 1979).

The statistical analyses were performed in the following manner. Let (x_i, y_i), $i = 1,2...n$ be the results available for each of the nutrients, where n is the number of split samples, x_i is the concentration of the nutrient if the chemical analysis was performed immediately (shipboard), and y_i is the corresponding concentration for the stored water sample. The common approach for testing the difference between x and y is to perform a matched paired t test. On inspection of the results, however, it was noted that some values of x and y were below the detection limit. To accommodate the censored data, modification of the paired t test would have been necessary. This posed a complicated computational problem, leading us to consider another approach which, although slightly less efficient than the t test (provided the difference (x_i-y_i) is normally distributed), is well suited for use in case of censoring and results in robust inferences, i.e., inferences independent of the form of the distribution of (x_i-y_i). This approach is the non-parametric sign test (Lehmann, 1975).

The Sign Test

The sign test is based on replacing the difference between the random variables x_i and y_i by a new random variable Z_i, which is defined as follows:

$$Z_i = \begin{cases} +1 & \text{if } x_i > y_i \\ -1 & \text{if } x_i < y_i \end{cases}$$

If $x_i = y_i$ the pair (x_i, y_i) is disregarded, since it supplies no information on the difference between x_i and y_i. Also the pair is disregarded when both x_i and y_i are below the detection level. Under the null hypothesis (H_0) of no difference between x_i and y_i, the random variable Z_i is binomial with probability $= 0.5$. The binomial distribution can be used to evaluate the exact significance level, α, for testing H_0 when M (the number of Z_i values) is small. For large M, an approximate value for α can be obtained using the normal approximation to the binomial distribution.

Estimation of the Size of the Difference

Lehmann (1975) showed that when the sign test is used, the most natural estimate for the difference between (x_i-y_i) is the median of the differences.

Confidence Interval for the Difference

Let d_i be the difference between x_i and y_i ($i = 1,2 ...M$) and $d_{(i)}$ be the ith largest value of d_i, i.e., $d_{(1)} \leq d_{(2)} \leq ... \leq d_{(M)}$. The $1-2\gamma$ confidence interval for the median u can be expressed as $d_{(i)} \leq u \leq d_{(M+1-i)}$ and the value of i is determined from the equation

$$\text{Prob}(u \leq d_{(i)}) \simeq \Phi\left[\frac{2i-(N+1)}{\sqrt{N}}\right]$$

where Φ is the normal cumulative distribution function.

RESULTS AND DISCUSSION

Table 1 lists the detection limits for the three nutrients and the number and percentage of samples reported below these levels. Also given are the median concentrations of the shipboard and stored sample analyses (x_i and y_i, respectively).

Figure 1 presents the plots of the empirical cumulative distribution functions (CDF) of the difference between the shipboard and stored sample analyses for the April cruise. The CDF for obtaining negative differences (i.e., storage results are higher than shipboard results) are 0.22, 0 and 0.64 for SRP, $(NO_3 + NO_2)$ and NH_3, respectively (Fig. 1). These values strongly indicate that the distribution is not symmetric about zero and that storage results in (i) the reduction of the concentrations of SRP and $(NO_3 + NO_2)$ and (ii) the increase of the concentration of NH_3. Also shown on each graph is the estimated median of the distribution which is larger than zero for SRP and $(NO_3 + NO_2)$ and smaller than zero for NH_3. The results for the November cruise are shown in Figure 2. The CDF for obtaining negative differences are 0.27, 0 and 0.28 for SRP, $(NO_3 + NO_2)$ and NH_3, respectively. Also, the medians of the three nutrients are larger than zero (Fig. 2). From this it appears that the results for SRP and $(NO_3 + NO_2)$ are consistent for the two cruises, whereas the NH_3 results indicate an opposite storage effect.

Table 2 presents the results of application of the statistical analysis to the nutrient data. The values of the

Table 1. Detection Limits, Number (and Percentage) of Samples Reported below These Levels and Medians of Shipboard and Stored (Laboratory) Sample Analyses

Nutrient	Detection limit (dl)	No. of samples < dl (%)				Median			
		April 26-30		November 19-24		April 26-30		November 19-24	
		Ship	Lab	Ship	Lab	Ship	Lab	Ship	Lab
SRP	0.0002	0(0)	5(1)	0(0)	0(0)	0.0047	0.0041	0.0046	0.0040
$NO_3 + NO_2$	0.005	0(0)	0(0)	0(0)	0(0)	0.365	0.342	0.358	0.336
NH_3	0.001	50(13)	12(3)	23(6)	107(29)	0.002	0.003	0.004	0.003

Note: All concentrations are reported in milligrams per litre.

Table 2. Summary of the Statistical Analyses of the Three Nutrients

Cruise	Nutrient	No. of differences (−)	(+)	Total	Sign test	Median of differences	95% Confidence interval Lower limit	Upper limit	% Change
April 26-30	SRP	70	288	358	11.47*	0.0005	0.0004	0.0006	11
	$NO_3 + NO_2$	1	373	374	19.18*	0.025	0.024	0.027	7
	NH_3	192	108	300	− 4.91*	− 0.0015	− 0.0020	− 0.0009	− 75
November 19-24	SRP	94	257	351	8.65*	0.0005	0.0004	0.0006	11
	$NO_3 + NO_2$	0	370	370	38.42*	0.023	0.022	0.024	6
	NH_3	64	238	302	9.96*	0.0015	0.001	0.002	37

* Value is significant at the 1% level.

sign test are highly significant ($p < 0.01$) indicating (i) for the April cruise, storage resulted in an increase in the concentration of NH_3 and a reduction in the concentration of the other two nutrients and (ii) for the November cruise, storage resulted in a reduction in the concentration of the three nutrients. The estimates of the median of the difference between the ship and laboratory analyses and the limits of the 95% confidence interval for the median are also given in the table. The final column of the table lists the percent change in concentration resulting from storage, calculated as

$$\%\ \text{change} = \frac{\text{Median of the difference } (x_i - y_i)}{\text{Median of shipboard analysis } (x_i)} \times 100$$

It appears that the percent change was constant for SRP and ($NO_3 + NO_2$) for the two cruises, but substantially different for NH_3. This might be due to a change in the concentration of the nutrients in the samples, i.e., the November concentration of NH_3 was double that of the April cruise, whereas SRP and ($NO_3 + NO_2$) concentrations remained relatively constant.

The loss of ($NO_3 + NO_2$) and subsequent increase in NH_3 concentrations on the first cruise may be indicative of the dominant bacterial species present in the water at that time, i.e., nitrifying bacteria were more sensitive to low temperatures than were those responsible for ammonification (Klingaman and Nelson, 1976). Since the levels of both nutrients decreased on the November cruise, there must be another explanation, such as algal uptake or adsorption. Likewise, decreased SRP concentrations may be a result of phosphate utilization by a developing bacterial population (Gilmartin, 1967) or adsorption onto detritus.

In conclusion, storage of unpreserved water samples in glass bottles at 4°C has been found to be insufficient for the stabilization of SRP, ($NO_3 + NO_2$) and NH_3 concentrations for periods extending up to eight days. Until better preservation techniques have been established, continued immediate (shipboard) analysis of these nutrients is recommended.

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