A Comparison of Preservation Techniques for Dissolved Nutrient Analyses

by
Betty A. Salley

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Environmental Protection Agency,
Chesapeake Bay Monitoring Program

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INTRODUCTION

The ideal procedure for handling water quality samples is to process and analyze immediately after collection. For the present discussion "processing" includes filtering, chilling, freezing, and the addition of acid or other chemicals to reduce or stop bacterial transformation of the constituent to be measured in the sample. Since immediate processing and analysis of samples is rarely possible, scientists, water quality managers, and analysts must determine an appropriate alternative to immediate analysis. The purpose of this study is to compare alternative processing and preservation techniques.

The US Environmental Protection Agency (USEPA) has published guidelines that include a table of "Required Containers, Preservation Techniques, and Holding Times" (Federal Register, 1991). The procedures established by EPA allow persons to apply for variances from the prescribed preservation techniques and indicated that "(S)ufficient data should be provided to assure such variance does not adversely affect the integrity of the sample" (Federal Register, 1991). But even with this guideline scientists and other Federal Agencies continue to differ in the preferred method of preservation (Venrick and Hayward, 1985) depending on factors such as the use of the resulting data, the data quality necessary to meet the intended uses, and the characteristics of the water. The choice of the preservation method has practical implications, too. For example, a number of dissolved nutrient measurements (eg NO_2NO_2+$N0_3$, PO_4F, and NH_4) could be determined from a single sample as long as acid has not been used as a preservative. This study particularly looks at preservation of a sample used for simultaneous analyses.

The Chesapeake Bay water quality monitoring program involves a number of institutions and laboratories. Through the collective efforts of the managers and the analysts, much has been accomplished to assure comparability of the laboratory analyses. In many instances, differences in procedures have been reduced or eliminated, but some differences remain. Sampling in the mainstem of Chesapeake Bay occurs from large vessels of sufficient size to allow water samples to be filtered and chilled or frozen on-board. The vessels for sampling in the Virginia tributaries are much smaller and do not allow immediate processing on-board. One aspect of the present study is to compare immediate to delayed processing to determine what effects that may have on the resultant data. A second aspect concerns the EPA requirement that certain parameters require the addition of sulfuric acid to samples to lower the pH below 2. This currently is not the standard practice among the Chesapeake Bay mainstem monitoring program participants. However, the standard practice among laboratories is to freeze the filters, which are used for chlorophyll and other particulate analyses, and to freeze filtered samples (with the exception of silica), especially when the analysis will be delayed beyond the maximum holding times established by EPA. The purpose of the study was to determine how these treatments, or combinations of treatments, affect the dissolved nutrient concentrations.

STUDY DESIGN

The law of conservation of mass dictates that the total amount of an element should remain constant, unless some portion is made volatile or otherwise allowed to escape. The relative amounts of the various species of that element, however, can be altered through chemical and biological transformations (eg, ammonia can be converted to nitrate). In the present study the dissolved fractions of the nutrients nitrogen and phosphorus are examined. Both freshwater and saline samples were examined, since both matrices are included in the monitoring program.

For the mainstem of the Chesapeake Bay, processing of samples occurs shortly (< 1 hour) after collection. Samples collected in the Virginia tributaries are chilled immediately, but typically they are not processed until the following day. The study, therefore, included samples that were filtered immediately and samples filtered 24 hours after the onset of the study.

When processing and/or analysis must be delayed, two preservation techniques are widely used: lowering the temperature and/or lowering the pH. The Chesapeake Bay monitoring program uses both cooling and freezing as preservation techniques, but to date acidification has not been used. Some members of Region III EPA staff support current Chesapeake Bay Program preservation procedures while other EPA staff suggest that monitoring samples should be acidified (personal communication, Cook). The present study included cooling to 4°C (with 1 to 7 day holding times), acidification (with 0 to 7 day holding times) and freezing to -15°C (with holding times up to 28 days).

Many prior studies appear to have been designed and conducted with the objective of establishing one method as superior to another. The purpose of the present study, however, was to identify the advantages and disadvantages of each preservation technique used.

SAMPLE COLLECTION AND HANDLING

A freshwater sample was taken from the James River at Jordon Point (Hopewell, VA), a location well above the limit of saltwater intrusion (salinity was less than 0.5 ppt). The saline sample was taken from the York River at Gloucester Point, VA (salinity of 17 ppt). The samples were stored in carboys which were refrigerated that evening and processed the next day.

Freshwater and saline samples were handled in an identical fashion. The sample was kept homogeneous by continual mixing while subsampling. The refrigeration temperature was 4°C and freezing temperature was -15°C. Subsamples which were acidified were checked to ensure a pH of 2. Each sample was analyzed to determine the concentration of each of the following dissolved constituents:

Nitrite	NO_2
Nitrate plus Nitrite	$NO_3 + NO_2$
Ammonia	NH_3
Orthophosphate	PO_4

The dissolved nutrients were analyzed according to *Methods for determination of Chemical Substances in Marine and Estuarine Environmental Samples* (EPA, 1992) with the exception of Ammonia. Ammonia was analyzed according to *Methods for Chemical Analysis of Water and Wastes* (EPA, 1979). A minimum of seven replicates was analyzed per preservation group for each of the above nutrients. The saline samples were corrected for refractive index for nitrite, phosphate and nitrate. The fresh water samples did not require refractive index correction. The instruments used were *Technicon* Autoanlyzer II and *Orion* continuous flow analyzers.

EXPERIMENTAL TREATMENTS

The four preservation treatments included in the study were: (1)filtering, (2)chilling, (3)freezing, and (4)addition of acid. Processing and analysis occurred at varying times after the start of the study and sometimes combinations of treatments were examined. In order to identify each sample, the following scheme was used:

S or F	indicates a Saline or a Freshwater sample;
N or A	indicates No acid added or Acidified;
c or f	indicates whether the sample was chilled or frozen; and
x/y	indicates the day on which the sample was filtered (x) and the day on which the sample was analyzed (y).

The sequence of sample collection, processing, and analysis is given below. Table 1 shows the treatments or combination of treatments employed. Regardless of treatment all samples were filtered before analysis.

Day 1: Sample collection; storage in carboys in refrigerator.

Day 0: Sample Processing and/or analysis.

A. Subsamples filtered and either
   1. analyzed (SNc-0/0, FNc-0/0); or
   2. refrigerated for later analysis (SNc-0/1 and SNc-0/7; FNc-0/1 and FNc-0/7); or
   3. frozen for later analysis (SNf-0/7 and SNf-0/28, and FNf-0/7 and FNf-0/28).

B. Sample filtered and acidified and either
   1. analyzed (SAc-0/0 and FAc-0/0); or
   2. refrigerated for later analysis (SAc-0/1 and SAc-0/7, and FAc-0/1 and FAc-0/7).

C. Samples taken from carboy and refrigerated with
   1. acid added on Day 0, filtered and analyzed on Day 1 (SAc-1/1 and FAc-1/1); or
   2. filtered and analyzed on Day 1 (SNc-1/1 and FNc-1/1).

Table 1. Summary of Preservation Treatments and Holding Times

	DAY 0	DAY 1	DAY 7	DAY 28
No acid, immediate filtration, chilled	SNc-0/0 FNc-0/0	SNc-0/1 FNc-0/1	SNc-0/7 FNc-0/7	
No acid, immediate filtration, frozen			SNf-0/7 FNf-0/7	SNf-0/28 FNf-0/28
No acid, chilled, filtration next day		SNc-1/1 FNc-1/1		
Acid, immediate filtration, chilled	SAc-0/0 FAc-0/0	SAc-0/1 FAc-0/1	SAc-0/7 FAc-0/7	
Acid, chilled, filtration next day		SAc-1/1 FAc-1/1		

DATA PROCESSING AND PRESENTATION

Mean, minimum, and maximum concentrations plus standard deviation and coefficient of variation for the replicate analyses were calculated for each sample (Tables 2 through 5). The results also are summarized graphically in Figures 1 through 8.

Tukey's Studentized range test (SAS Institute Inc., 1985) was used to check for significant differences among the treatments. The calculations were performed for each nutrient; saline and freshwater samples data sets were kept separate. This test lists the means of each treatment in descending concentration and then groups the means using the mean square error of the treatments to find the subgroups with no significant difference (with alpha at 0.05). The results are presented in Table 6.

We note that the concentration of ammonia and nitrate plus nitrite was much higher for the freshwater samples than for the saline samples. These subsamples were diluted 1:20 prior to ammonia analysis. This extra step in the processing could cause a larger variance between treatments.

RESULTS AND DISCUSSION

The study did not incorporate filtration immediately after sample collection. For convenience, the samples were collected from the two rivers one day (Day -1) and the laboratory component of the study began the following day (Day 0). At that time, aliquots of the samples were filtered and analyzed (SNc-0/0 and FNc-0/0); in the remainder of the report we refer to these as the "initial values". These initial values are assumed to be the "truest" estimate of nutrient concentrations at the time the laboratory component of the study began; therefore concentrations for other treatments will be contrasted with the initial values.

ORTHOPHOSPHATE (Table 2; Figures 1 and 5)

Saline

The initial value for York River sample (SNc-0/0) was 0.0010 mg/L. All the saline treatments agreed except for samples which were not filtered until the next day (SNc-1/1 and SAc-1/1). Hence, we concluded that filtration, or the lack thereof, was the only significant factor in the preservation of the saline orthophosphate samples.

Freshwater

The initial value of the James River sample (FNc-0/0) was 0.0243 mg/L, 24 times greater than the initial value of the saline sample. In general, the treatments agreed with the initial value except for the acidified samples. The sample acidified and then filtered the next day (FAc-1/1), showed an almost 300 % increase in concentration. FAc-0/0 showed a slight increase, but FAc-0/7 was only 67 % of the initial value.

Other studies have shown that freezing an orthophosphate freshwater sample adversely affects the results due to precipitation of some phosphate (Johnson et al., 1975). This study showed no such effect. In fact, close agreement between the initial value and the filtered frozen samples that were not acidified (FNf-0/7 and FNf-0/28) was observed.

NITRATE PLUS NITRITE DATA (Table 4; Figures 2 and 6)

Saline

The initial value for the York River sample (SNc-0/0) was 0.0727 mg/L. SNc (chilled) samples analyzed on day 1 and day 7 showed a slight drop in concentration on day 1, but no difference on day 7. The SNf (frozen) samples were significantly lower on day 7 and day 28 than the initial value. In addition, the samples which were filtered on Day 1 (both acidified and not acidified), although similar to each other, were significantly lower than the initial value. Lastly, the sample which was filtered and acidified on Day 0, but not analyzed until Day 7 (SAc-0/7) had a significantly lower concentration.

The initial value was the highest concentration in this set of data. However, the lowest value (SNf-0/28) was within 90% of the initial value.

Freshwater

The initial value for the James River sample (FNc-0/0) was 0.4948 mg/L. These samples, which were analyzed along with the saline samples, required a dilution by a factor of 20, thus introducing an additional source of error. Interestingly, only two samples were significantly different from the initial value. FNf-0/7 and FNc-0/7 were significantly higher than the initial value. For the freshwater samples, the initial value was the lowest measured; directly opposite the results of the saline samples. There was no obvious explanation for this result. The difference between the highest concentration and the initial value was less than 10%.

AMMONIA (Table 3; Figures 3 and 7)

Saline

The initial value for the York River sample (SNc-0/0) was 0.0123 mg/L. SNf-0/7 and SAc-1/1 were the only treatments not significantly different. The 95 % confidence limits for all treatments except SNc-1/1 overlap the variance of the initial value. This sample, which was unacidified and not filtered until day 1, showed a definite loss of ammonia.

Freshwater

The initial value for the James River sample (FNc-0/0) was 2.0262 mg/L. This sample required a dilution factor of 20 which could have increased the variance between treatments. In general the treatment values agreed with the initial value. Contrary to its saline counterpart, FNc-1/1, which was not acidified or processed until day 1, showed no loss of ammonia. FNf-0/28 was significantly higher.

NITRITE (Table 5; Figures 4 and 8)

Nitrite is considered an unstable species of nitrogen and the concentrations were very low compared to other nitrogen species from the same sample.

Saline

The initial value for the York River sample (SNc-0/0) was 0.0035 mg/L. SNf-0/7, SAc-0/0 and SNc-0/1 agreed with the initial value. SNc-1/1, SNf-0/28 and SNc-0/7 were significantly different, but the greatest concentration difference was only 0.0007 mg/L. The SAc-1/1 and SAc-0/7 concentrations were only 0.00091 and 0.00029 mg/L, respectively, clearly indicating that acidification and delayed filtration resulted in lower nitrite values.

Freshwater

The initial value for the James River sample (FNc-0/0) was 0.0251 mg/L, almost an order of magnitude higher than the saline sample. In general, all treatments agreed with the initial value except for the acidified samples. FAc-0/0 was 20% higher and FAc-0/7 4% less than the initial value. FAc-1/1 was even lower than FAc-0/7.

The reason for the higher concentration of FAc-0/0 is not clear. The nitrate plus nitrite samples did not show a corresponding decrease, but the ammonia value was slightly lower than the initial ammonia value. Since the nitrite concentration in both the saline and freshwater samples was small compared to the other nitrogen species, any gain or loss is difficult to attribute to an increase or decrease in the concentration of another nitrogen species or as loss from the sample as gas.

SUMMARY

Examination of the four preservation treatments (filtering, chilling, acidification, freezing) and combinations thereof, indicated that some treatments did not maintain sample integrity. This conclusion varied depending on the nutrient species and sample salinity.

The results indicated that filtration should be performed first, to avoid altering the particulate portion of the sample and, consequently, altering the dissolved portion as well. The need to filter first was most essential when filtering was used in conjunction with other methods of preservation such as acidification or freezing.

Chilling was found to maintain the integrity of a filtered sample, but chilling without filtering a saline sample resulted in an orthophosphate concentration increase by the next day.

Regardless of salinity, the treatment which most affected sample integrity for orthophosphate and nitrite was acidification (filtered or unfiltered) with analysis the next day or later. Acidification did not alter ammonia and nitrate plus nitrite concentrations.

Filtered frozen samples were found, in most cases, to agree with the initial value. Orthophosphate and nitrite results were not statistically different after 7 days. Results for ammonia and nitrate plus nitrite were less satisfactory. Differences in ammonia concentrations that were statistically significant were observed for some holding times. Statistically significant differences for both holding times were observed for nitrate plus nitrite saline samples.

Freshwater sample concentrations ranged from 7 to 165 times higher than the saline sample concentrations, depending on the nutrient. Given the concentration differences, it was difficult to compare the two matrixes. However, one instance where a clear difference in results between the matrices was observed. The unacidified, unfiltered saline sample showed a significant loss over 24 hours of ammonia, but the freshwater sample did not.

RECOMMENDATIONS

This study indicates that filtering is the most important part of the preservation process and should be carried out as soon as possible after sample collection. Filtered samples should be chilled if analyses are to be performed within the week, preferably within 24 hours. If the analysis time may be delayed, the filtered samples should be frozen.

The addition of acid before filtering is contraindicated as a preservation technique for orthophosphate. Also, it has been well established by prior studies that acid preservation, whether before or after filtration, will cause nitrite loss. This study showed similar nitrite losses, indicating that acidification is not appropriate for nitrite samples.

Table 2. Orthophosphate Data (concentration in mg/L)

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	C.V.
York River						
SAc-0/0	7	0.00000	0.00000	0.00000	0.00000	
SAc-0/7	7	0.00012	0.00017	0.00000	0.00050	132.559
SNf-0/7	7	0.00111	0.00033	0.00080	0.00180	29.957
SNc-0/0	7	0.00099	0.00066	0.00050	0.00240	66.635
SNc-0/1	7	0.00096	0.00011	0.00070	0.00100	11.847
SNc-0/7	7	0.00043	0.00049	0.00000	0.00110	114.875
SNf-0/28	7	0.00154	0.00080	0.00060	0.00260	51.562
SAc-1/1	7	0.01173	0.00262	0.00910	0.01680	22.324
SNc-1/1	7	0.00434	0.00106	0.00230	0.00530	24.436
James River						
FAc-0/0	7	0.02933	0.00042	0.02890	0.03010	1.429
FAc-0/7	7	0.01634	0.00067	0.01580	0.01780	4.072
FNf-0/7	7	0.02564	0.00062	0.02430	0.02600	2.423
FNc-0/0	7	0.02426	0.00058	0.02320	0.02480	2.402
FNc-0/1	7	0.02823	0.00057	0.02700	0.02870	2.032
FNc-0/7	9	0.02477	0.00063	0.02360	0.02560	2.546
FNf-0/28	7	0.02417	0.00081	0.02340	0.02580	3.350
FNc-1/1	7	0.06973	0.00024	0.06930	0.06990	0.339
FNc-1/1	7	0.02496	0.00038	0.02410	0.02510	1.514

Table 3. Ammonia Data (concentration in mg/L)

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	C.V.
York River						
SAc0/0	7	0.01076	0.00076	0.01010	0.01220	7.097
SAc-0/7	9	0.01566	0.00221	0.01280	0.01920	14.143
SNf-0/7	10	0.01366	0.00162	0.01070	0.01590	11.825
SNc-0/0	7	0.01229	0.00098	0.01130	0.01350	7.953
SNc-0/1	7	0.01056	0.00114	0.00970	0.01210	10.812
SNc-0/7	9	0.01401	0.00210	0.01150	0.01660	14.986
SNf-0/28	7	0.01043	0.00080	0.00900	0.01140	7.625
SAc-1/1	7	0.01164	0.00027	0.01140	0.01210	2.318
SNc-1/1	7	0.00737	0.00056	0.00660	0.00850	7.622
James River						
FAc-0/0	7	1.94240	0.02546	1.91420	1.97250	1.311
FAc-0/7	9	1.98873	0.07678	1.92370	2.09310	3.861
FNf-0/7	10	2.12287	0.04018	2.07770	2.19060	1.893
FNc-0/0	7	2.02624	0.04050	1.98140	2.10690	1.999
FNc-0/1	7	2.03371	0.02260	2.00600	2.06300	1.111
FNc-0/7	7	2.12023	0.02821	2.08800	2.17010	1.331
FNf-0/28	7	2.16487	0.03163	2.10970	2.20870	1.461
FAc-1/1	7	2.07457	0.02204	2.03500	2.11100	1.062
FNc-1/1	7	2.02771	0.00785	2.01500	2.03900	0.387

Table 4. Nitrate plus Nitrite Data (concentration in mg/L)

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	C.V.
York River						
SAc0/0	7	0.07130	0.00069	0.07010	0.07190	0.972
SAc-0/7	7	0.06851	0.00023	0.06830	0.06900	0.342
SNf-0/7	7	0.06651	0.00027	0.06630	0.06680	0.402
SNc-0/0	7	0.07270	0.00049	0.07210	0.07330	0.674
SNc-0/1	7	0.06954	0.00089	0.06780	0.07040	1.283
SNc-0/7	9	0.07210	0.00205	0.06780	0.07410	2.838
SNf-0/28	7	0.06489	0.00050	0.06440	0.06570	0.773
SAc-1/1	7	0.06843	0.00077	0.06710	0.06910	1.118
SNc-1/1	7	0.06944	0.00084	0.06840	0.07040	1.213
James River						
FAc-0/0	7	0.50166	0.01144	0.48100	0.51710	2.281
FAc-0/7	7	0.50994	0.01323	0.48320	0.52480	2.595
FNf-0/7	7	0.52151	0.00598	0.51440	0.53100	1.146
FNc-0/0	7	0.49481	0.00454	0.49310	0.50510	0.917
FNc-0/1	7	0.51011	0.00975	0.50090	0.52670	1.912
FNc-0/7	7	0.53249	0.00718	0.52060	0.54140	1.348
FNf-0/28	7	0.49740	0.01197	0.48220	0.50880	2.406
FAc-1/1	7	0.50643	0.00690	0.50090	0.51380	1.362
FNc-1/1	7	0.50274	0.00488	0.50090	0.51380	0.970

Table 5. Nitrite Data (concentration in mg/L)

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	C.V.
York River						
SAc-0/0	7	0.00347	0.00005	0.00340	0.00350	1.406
SAc-0/7	7	0.00029	0.00004	0.00020	0.00030	13.229
SNf-0/7	8	0.00354	0.00014	0.00330	0.00380	3.980
SNc-0/0	7	0.00351	0.00007	0.00340	0.00360	1.964
SNc-0/1	7	0.00346	0.00014	0.00330	0.00360	4.042
SNc-0/7	7	0.00396	0.00011	0.00380	0.00410	2.865
SNf-0/28	7	0.00406	0.00022	0.00370	0.00430	5.485
SAc-1/1	7	0.00091	0.00009	0.00080	0.00110	9.841
SNc-1/1	7	0.00424	0.00014	0.00410	0.00450	3.293
James River						
FAc-0/0	7	0.02994	0.00011	0.02980	0.03010	0.379
FAc-0/7	7	0.00947	0.00013	0.00940	0.00970	1.324
FNf-0/7	7	0.02533	0.00045	0.02480	0.02590	1.776
FNc-0/0	7	0.02509	0.00026	0.02480	0.02540	0.988
FNc-0/1	7	0.02513	0.00031	0.02450	0.02540	1.252
FNc-0/7	7	0.02459	0.00023	0.02430	0.02490	0.922
FNf-0/28	7	0.02309	0.00119	0.02160	0.02530	5.158
FAc-1/1	7	0.00270	0.00008	0.00260	0.00280	3.024
FNc-1/1	7	0.02331	0.00027	0.02270	0.02350	1.173

Table 6. **Tukey's Studentized Range (HSD) Test**
Means with the same letter are not significantly different.
Underlined means show agreement

A. Orthophosphate

York River MINIMUM SIGNIFICANT DIFFERENCE=.00177

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.0117	7	SAc-1/1
	B	0.0043	7	SNc-1/1
	C	0.0015	7	SNf-0/28
	C	0.0011	7	SNf-0/7
	C	0.0010	7	SNc-0/0
	C	0.0010	7	SNc-0/1
	C	0.0004	7	SNc-0/7
	C	0.0001	7	SAc-0/7
	C	0.0000	7	SAc-0/0

James River MINIMUM SIGNIFICANT DIFFERENCE=975E-6

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.0697	7	FAc-1/1
	B	0.0293	7	FAc-0/0
	C	0.0282	7	FNc-0/1
	D	0.0256	7	FNf-0/7
E	D	0.0250	7	FNc-1/1
E	D	0.0248	9	FNc-0/7
E		0.0243	7	FNc-0/0
E		0.0242	7	FNf-0/28
	F	0.0163	7	FAc-0/7

B. Nitrate plus Nitrite

York River MINIMUM SIGNIFICANT DIFFERENCE=.00165

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.0727	7	SNc-0/0
	A	0.0721	9	SNc-0/7
	A	0.0713	7	SAc-0/0
	B	0.0695	7	SNc-0/1
	B	0.0694	7	SNc-1/1
	B	0.0685	7	SAc-0/7
	B	0.0684	7	SAc-1/1
	C	0.0665	7	SNf-0/7
	C	0.0649	7	SNf-0/28

B. (continued) Nitrate plus Nitrite

James River MINIMUM SIGNIFICANT DIFFERENCE=.01548

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.5325	7	FNc-0/7
B	A	0.5215	7	FNf-0/7
B	C	0.5101	7	FNc-0/1
B	C	0.5099	7	FAc-0/7
B	C	0.5064	7	FAc-1/1
	C	0.5027	7	FNc-1/1
	C	0.5017	7	FAc-0/0
	C	0.4974	7	FNf-0/28
	C	0.4948	7	FNc-0/0

C. Ammonia

York River MINIMUM SIGNIFICANT DIFFERENCE=.00232

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.0157	9	SAc-0/7
B	A	0.0140	9	SNc-0/7
B	A C	0.0137	10	SNf-0/7
B	D C	0.0123	7	SNc-0/0
	D C	0.0116	7	SAc-1/1
	D	0.0108	7	SAc0/0
	D	0.0106	7	SNc-0/1
	D	0.0104	7	SNf-0/28
	E	0.0074	7	SNc-1/1

James River MINIMUM SIGNIFICANT DIFFERENCE=.06615

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	2.1649	7	FNf-0/28
B	A	2.1229	10	FNf-0/7
B	A	2.1202	7	FNc-0/7
B	C	2.0746	7	FAc-1/1
	C	2.0337	7	FNc-0/1
D	C	2.0277	7	FNc-1/1
D	C	2.0262	7	FNc-0/0
D	E	1.9887	9	FAc-0/7
	E	1.9424	7	FAc-0/0

D. Nitrite

York River MINIMUM SIGNIFICANT DIFFERENCE=213E-6

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.0042	7	SNc-1/1
B	A	0.0041	7	SNf-0/28
B		0.0040	7	SNc-0/7
	C	0.0035	8	SNf-0/7
	C	0.0035	7	SNc-0/0
	C	0.0035	7	SAc0/0
	C	0.0035	7	SNc-0/1
	D	0.0009	7	SAc-1/1
	E	0.0003	7	SAc-0/7

James River MINIMUM SIGNIFICANT DIFFERENCE=802E-6

TUKEY	GROUPING	MEAN	N	SAMPLE
	A	0.0299	7	FAc-0/0
	B	0.0253	7	FNf-0/7
	B	0.0251	7	FNc-0/1
	B	0.0251	7	FNc-0/0
	B	0.0246	7	FNc-0/7
	C	0.0233	7	FNc-1/1
	C	0.0230	7	FNf-0/28
	D	0.0095	7	FAc-0/7
	E	0.0027	7	FAc-1/1

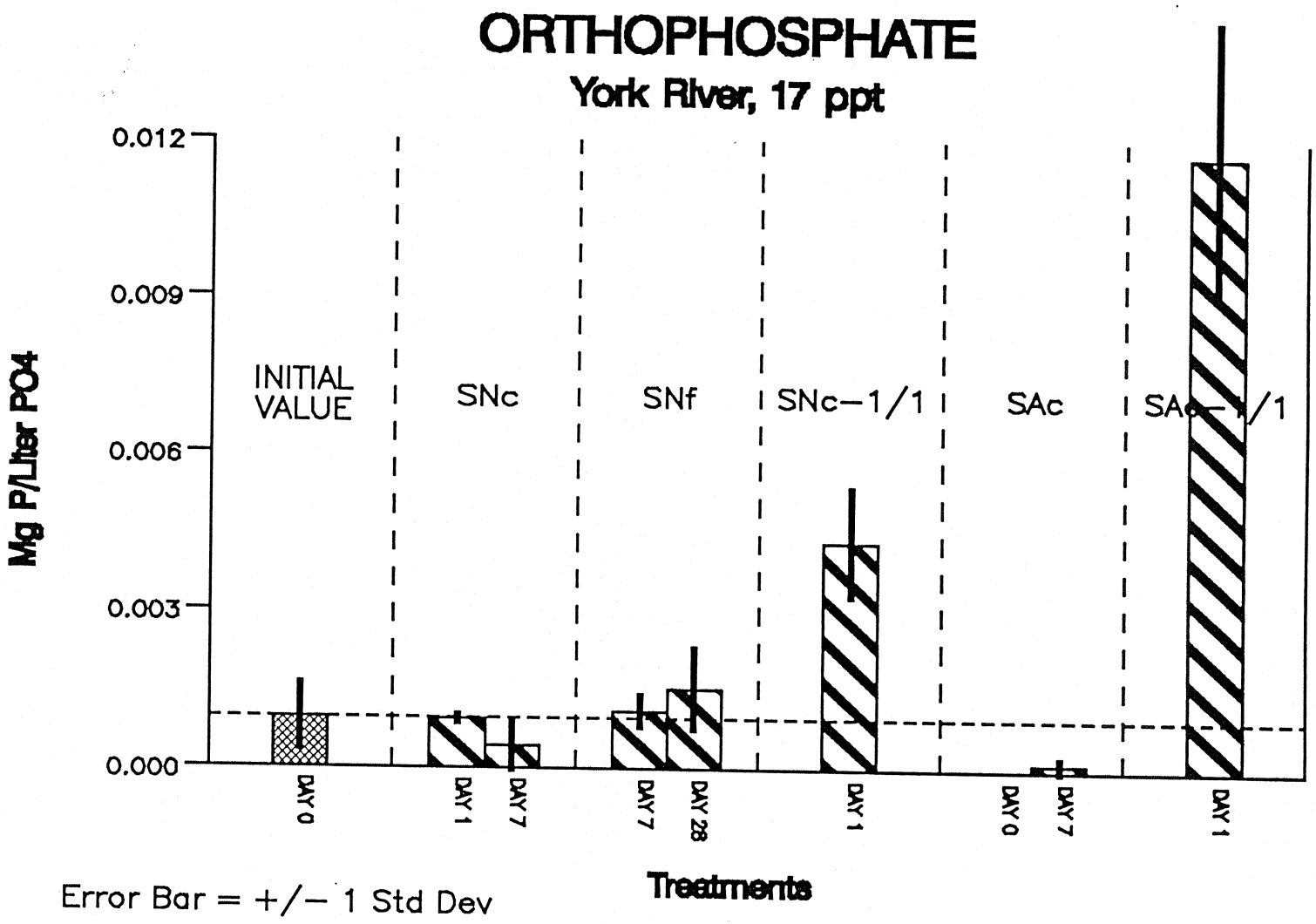
ORTHOPHOSPHATE
York River, 17 ppt
Mg P/Liter PO4
0.012
0.009
0.006
0.003
0.000
INITIAL VALUE
SNc
SNf
SNc—1/1
SAc
SAc—1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/— 1 Std Dev

Figure 1.

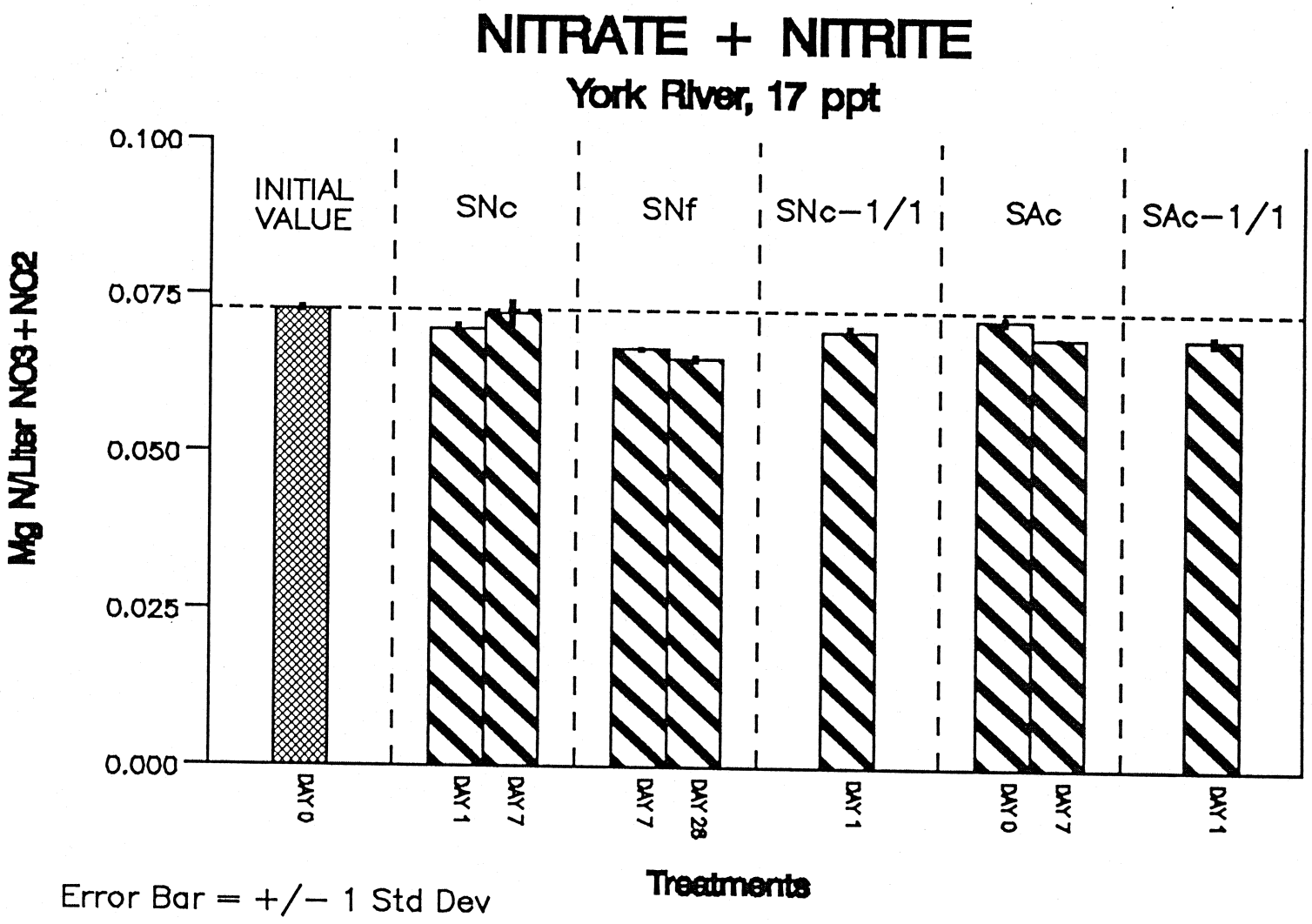
NITRATE + NITRITE
York River, 17 ppt
Mg N/Liter NO3+NO2
0.100
0.075
0.050
0.025
0.000
INITIAL VALUE
SNc
SNf
SNc−1/1
SAc
SAc−1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/− 1 Std Dev

Figure 2.

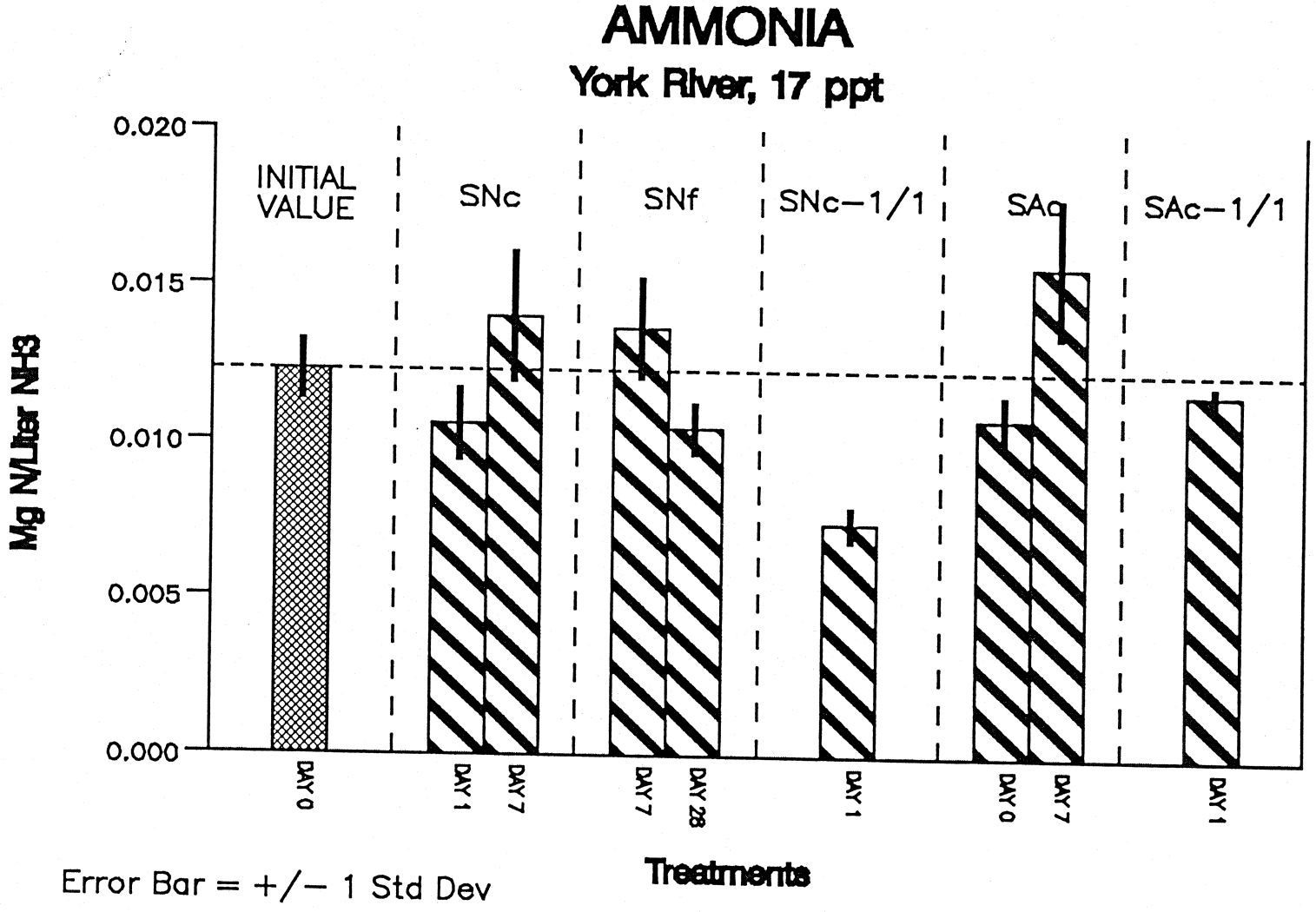
AMMONIA
York River, 17 ppt
0.020
0.015
0.010
0.005
0.000
Mg N/Liter NH3
INITIAL VALUE
SNc
SNf
SNc−1/1
SAc
SAc−1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/− 1 Std Dev

Figure 3.

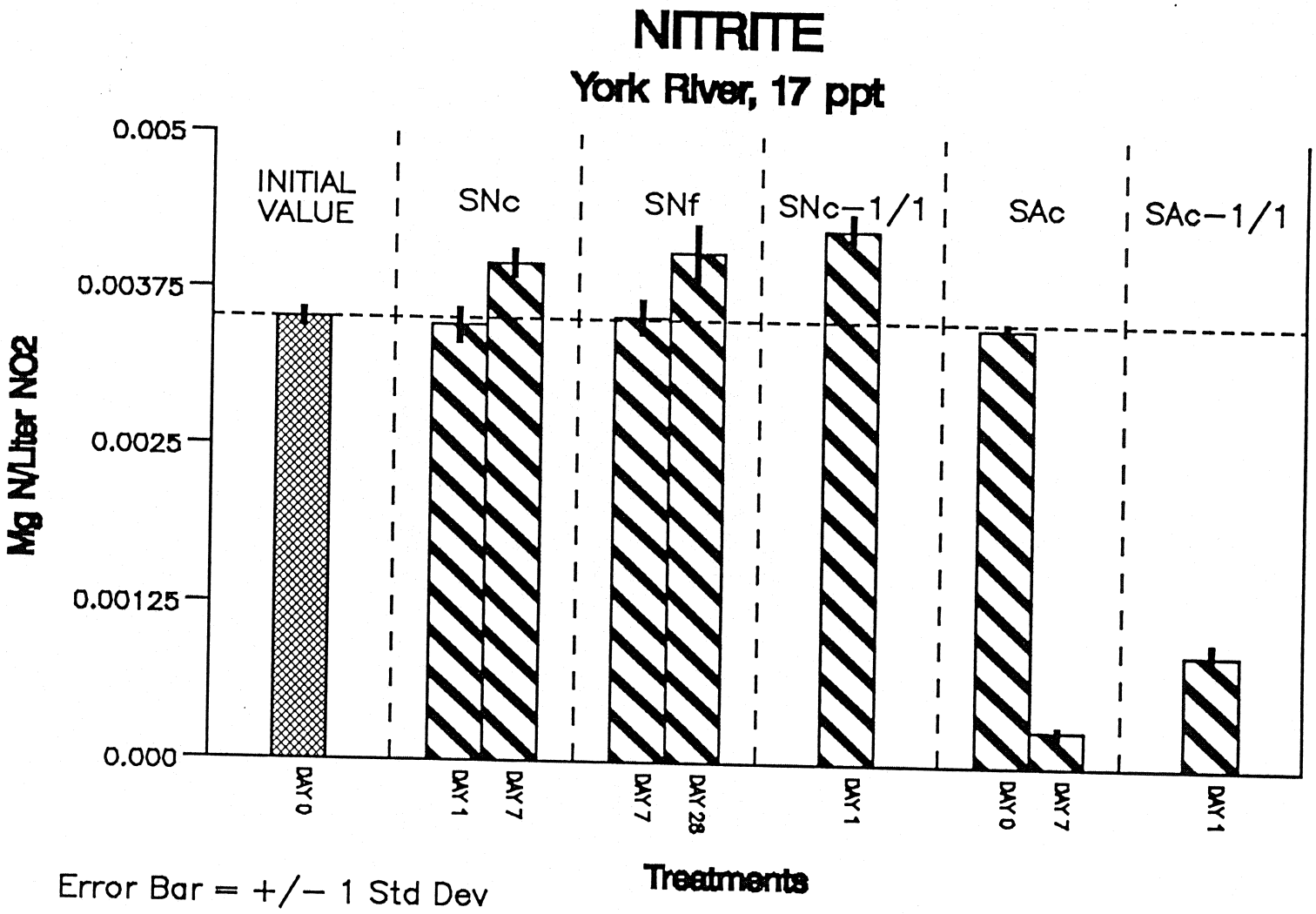
NITRITE
York River, 17 ppt
0.005
0.00375
0.0025
0.00125
0.000
Mg N/Liter NO2
INITIAL VALUE
SNc
SNf
SNc—1/1
SAc
SAc—1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/— 1 Std Dev

Figure 4.

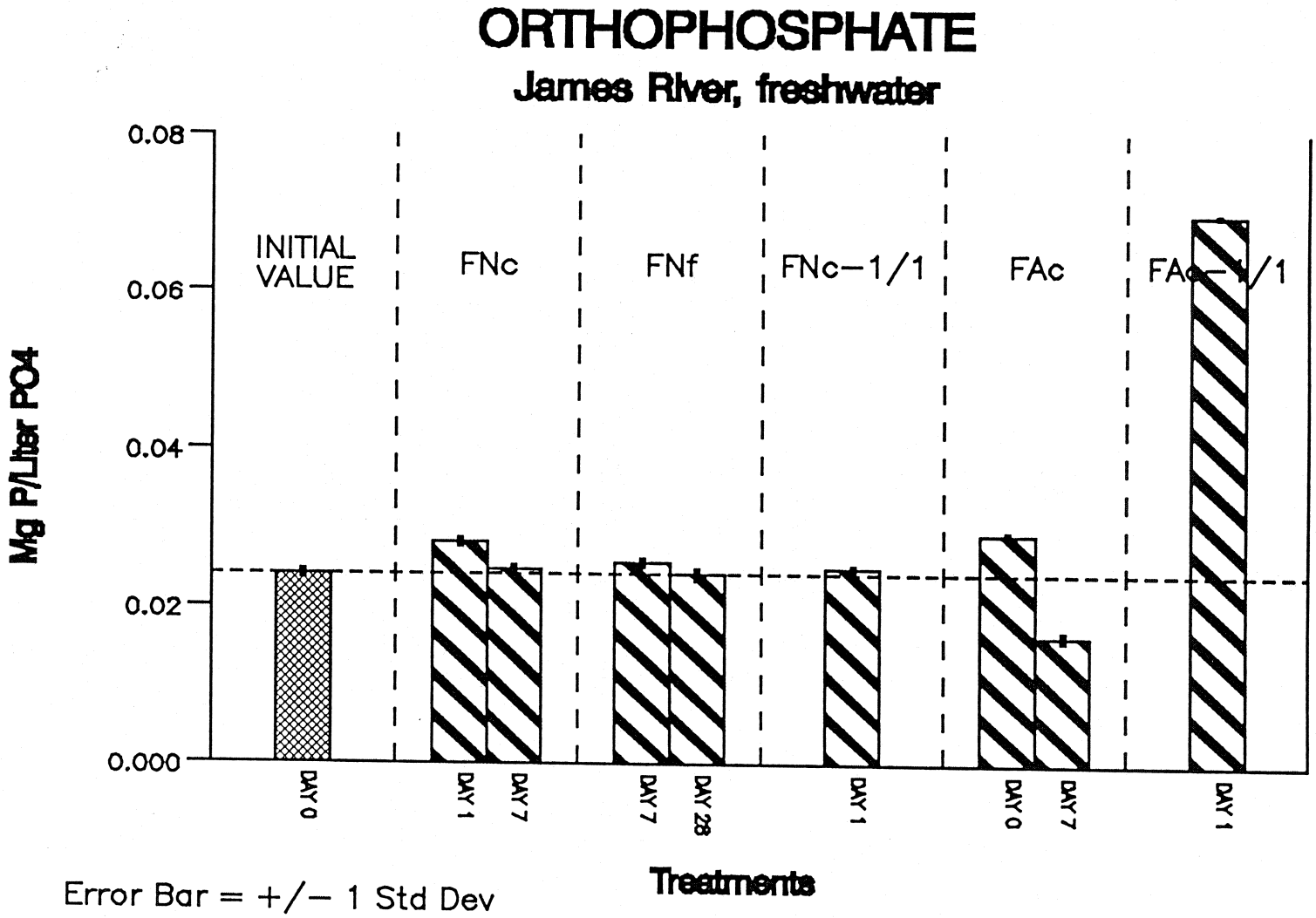
ORTHOPHOSPHATE
James River, freshwater
0.08
0.06
0.04
0.02
0.000
Mg P/Liter PO4
INITIAL VALUE
FNc
FNf
FNc−1/1
FAc
FAc−1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/− 1 Std Dev

Figure 5.

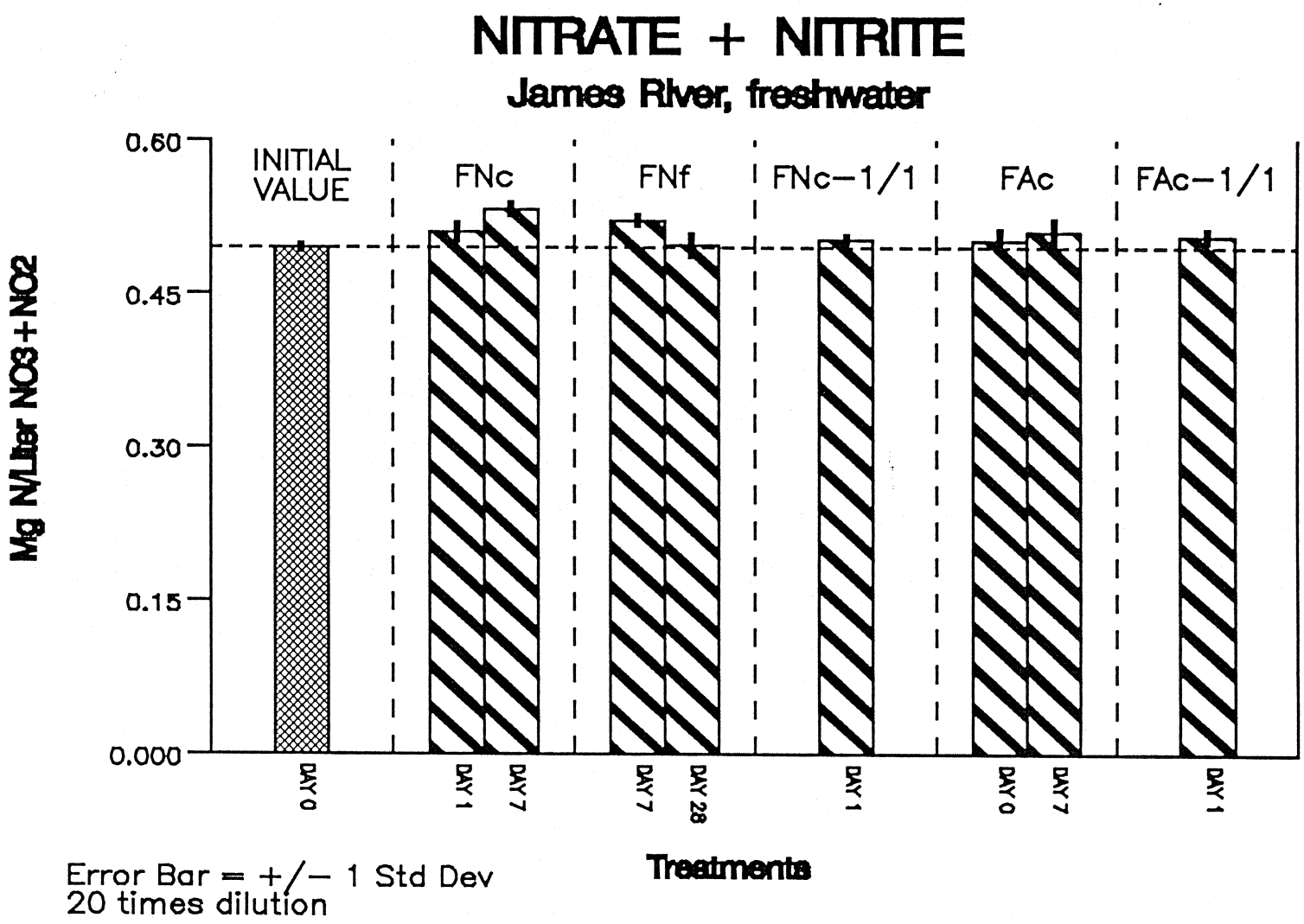
NITRATE + NITRITE
James River, freshwater
Mg N/Liter NO3+NO2
0.60
0.45
0.30
0.15
0.000
INITIAL VALUE
FNc
FNf
FNc−1/1
FAc
FAc−1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/− 1 Std Dev
20 times dilution

Figure 6.

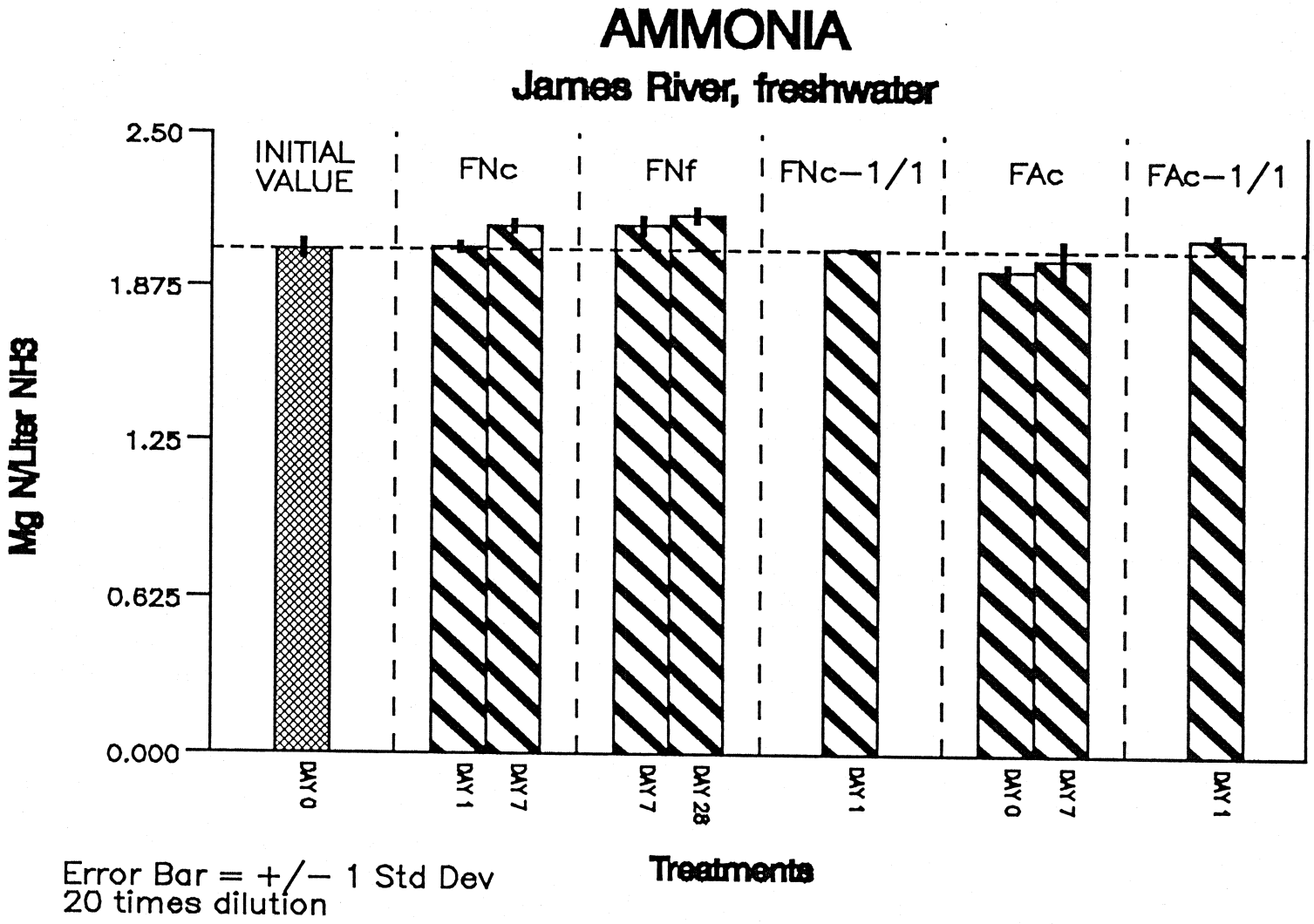
AMMONIA
James River, freshwater
2.50
1.875
1.25
0.625
0.000
Mg N/Liter NH3
INITIAL VALUE
FNc
FNf
FNc−1/1
FAc
FAc−1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/− 1 Std Dev
20 times dilution

Figure 7.

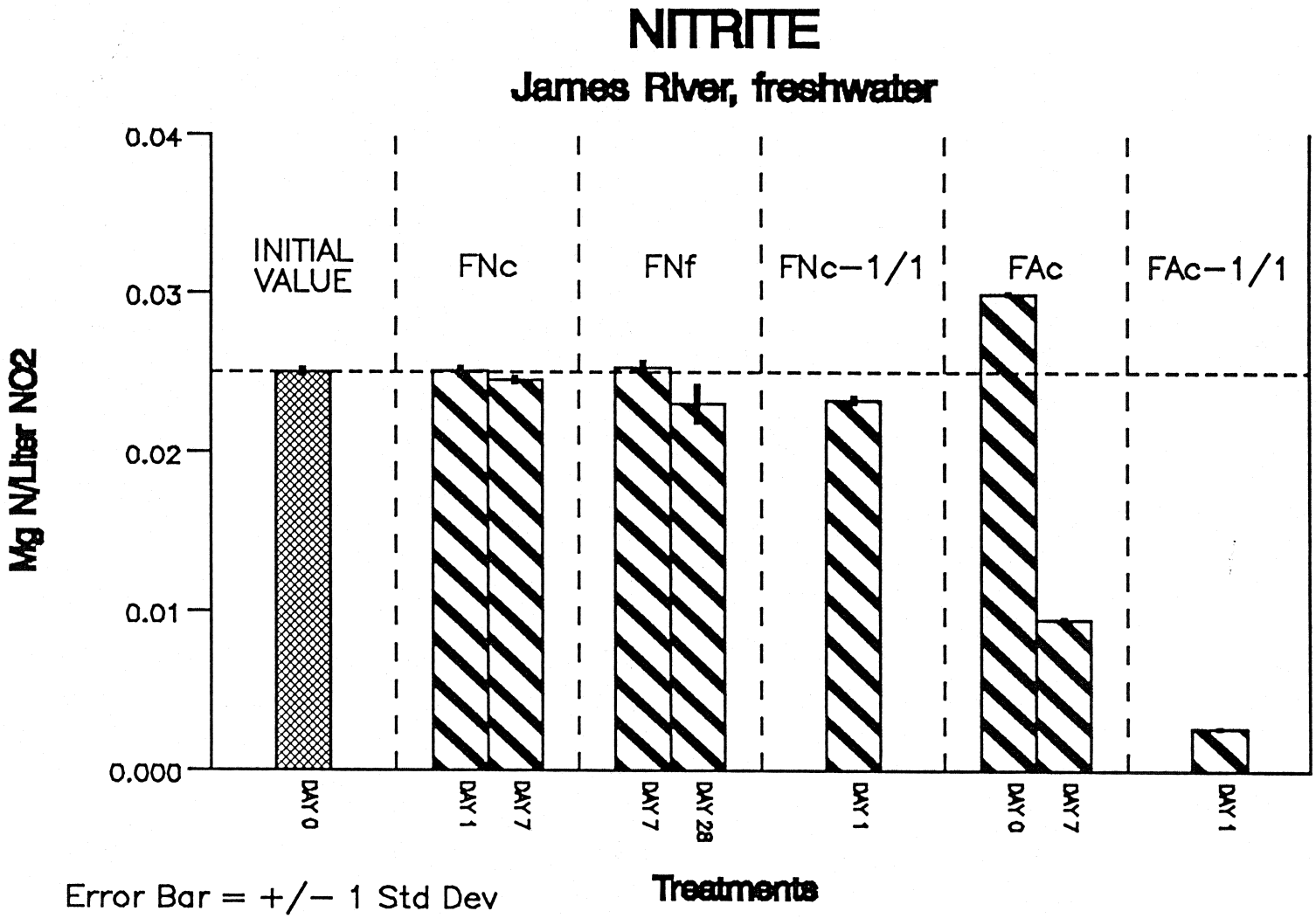
NITRITE
James River, freshwater
0.04
0.03
0.02
0.01
0.000
Mg N/Liter NO2
INITIAL VALUE
FNc
FNf
FNc−1/1
FAc
FAc−1/1
DAY 0
DAY 1
DAY 7
DAY 7
DAY 28
DAY 1
DAY 0
DAY 7
DAY 1
Treatments
Error Bar = +/− 1 Std Dev

Figure 8.

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