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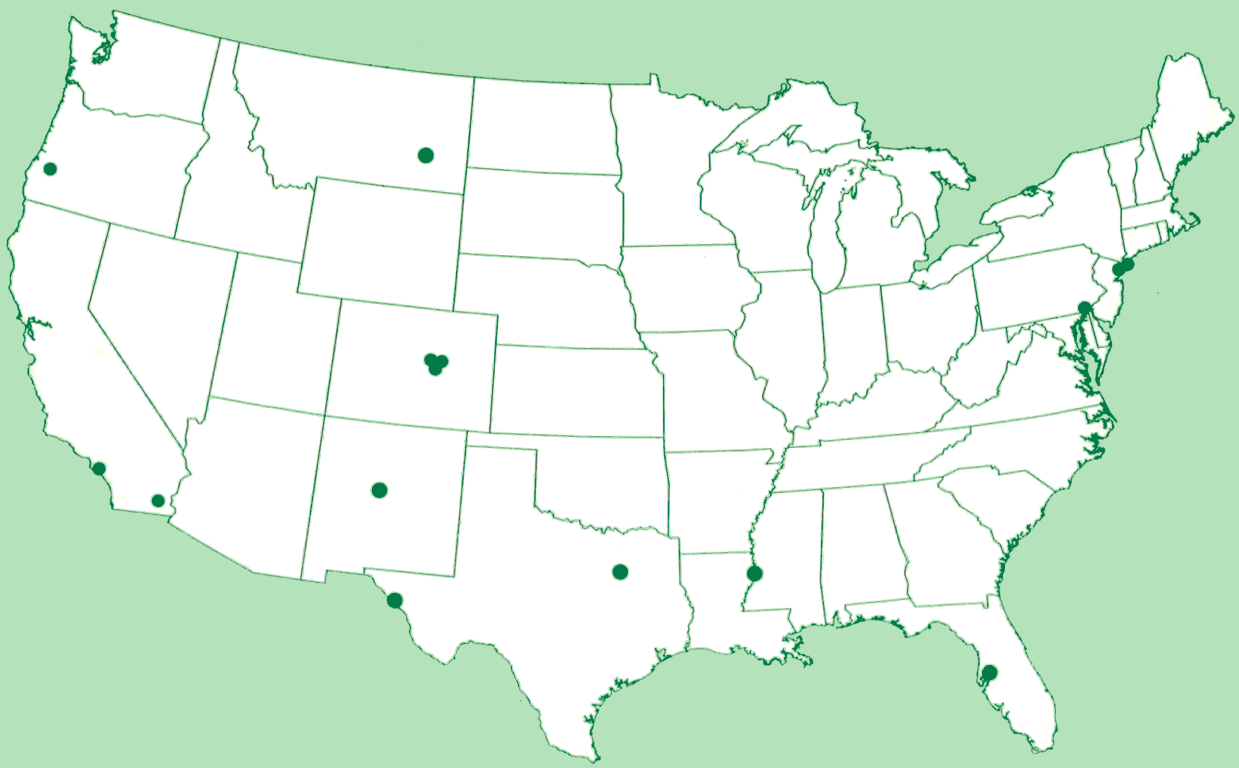
U.S. GEOLOGICAL SURVEY NUTRIENT PRESERVATION EXPERIMENT— EXPERIMENTAL DESIGN, STATISTICAL ANALYSIS, AND INTERPRETATION OF ANALYTICAL RESULTS

Water-Resources Investigations Report 98-4118



U.S. Department of the Interior
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Cover illustration—Map showing stations in the nutrient preservation experiment. (From Patton and Truitt, 1995, p. 3) Explanation: • Chemical Assessment Site

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By Charles J. Patton and Edward J. Gilroy

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U.S. DEPARTMENT OF THE INTERIOR

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CONVERSION FACTORS AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
centimeter (cm)	3.94×10^{-1}	inch
liter (L)	2.64×10^{-1}	gallon
microliter (µL)	2.64×10^{-7}	gallon
micrometer (µm)	3.94×10^{-5}	inch
milligram (mg)	3.53×10^{-5}	ounce
milliliter (mL)	2.64×10^{-4}	gallon
millimeter (mm)	3.94×10^{-2}	inch

Degree Celsius (°C) may be converted to degree Fahrenheit (°F) by using the following equation:

$$°F = 9/5\ (°C) + 32.$$

Abbreviated water-quality units used in this report are as follows:

M	molarity (moles per liter)
mg/L	milligrams per liter
mg-N/L	milligrams-nitrogen per liter
mg-P/L	milligrams-phosphorus per liter
µS/cm	microsiemens per centimeter at 25°C

Other abbreviations also used in this report:

≈	approximately
>	greater than
<	less than
±	plus or minus

ANCOVA	analysis of covariance
ANOVA	analysis of variance
ASTM	American Society for Testing and Materials
DKN	dissolved Kjeldahl nitrogen
DP	dissolved phosphorus
MDL	method detection limit
MPV	most probable value
NPDES	National Pollution Discharge Elimination System
NWQL	National Water Quality Laboratory
NASQAN	National Stream Quality Accounting Network
OWQ	Office of Water Quality
RSD	relative standard deviation
TKN	total Kjeldahl nitrogen
TP	total phosphorus
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
v/v	volume per volume

U.S. Geological Survey Nutrient Preservation Experiment—Experimental Design, Statistical Analysis, and Interpretation of Analytical Results

By Charles J. Patton and Edward J. Gilroy, U.S. Geological Survey

ABSTRACT

This report describes the experimental details and interprets results from a study conducted by the U.S. Geological Survey (USGS) in 1992 to assess the effect of different sample-processing treatments on the stability of eight nutrient species in samples of surface-, ground-, and municipal-supply water during storage at 4 degrees Celsius (°C) for about 30 days. Over a 7-week period, splits of filtered- and whole-water samples from 15 stations in the continental United States were amended at collection sites with sulfuric acid (U.S. Environmental Protection Agency protocol), mercuric chloride (former U.S. Geological Survey protocol), and ASTM (American society for Testing and Materials) Type I deionized water (control) and then shipped by overnight express to the USGS National Water Quality Laboratory (NWQL). At the NWQL, the eight nutrient species were determined in splits from each of the 15 stations, typically, within 24 hours of collection and at intervals of 3, 7, 14, 22, and 35 days thereafter. Ammonium, nitrate plus nitrite, nitrite, and orthophosphate were determined only in filtered-water splits. Kjeldahl nitrogen and phosphorus were determined in both filtered-water and whole-water splits.

Data on which this report is based, including nutrient concentrations in synthetic reference samples determined concurrently with those in real samples, are extensive (greater than 20,000 determinations) and have been published separately. In addition to confirming the well-documented instability of nitrite in acidified samples, this study also demonstrates that when biota are removed from samples at collection sites by

0.45-micrometer membrane filtration, subsequent amendment with sulfuric acid or mercury (II) provides no statistically significant improvement in nutrient concentration stability during storage at 4°C for 30 days. Biocide amendment had no statistically significant effect on the 30-day stability of phosphorus concentrations in whole-water splits from any of the 15 stations, but did stabilize Kjeldahl nitrogen concentrations in whole-water splits from three data-collection stations where ammonium accounted for at least half of the measured Kjeldahl nitrogen.

INTRODUCTION

Ambient concentrations of dissolved and suspended plant nutrients in samples of natural water are notorious for changing in unpredictable ways during storage. Two recent review articles (Sharp and others, 1993; Robards and others, 1994) note critical needs to characterize changes in nutrient concentrations that may occur between sample collection and analysis and to establish effective treatments to minimize the changes. Microorganisms, either present in the water or introduced into it inadvertently during collection and processing operations, are commonly presumed agents of such changes. For this reason, treatments intended to stabilize ambient nutrient concentrations in samples prior to analysis typically involve one or more of the following techniques:

- adding biocides to samples,
- chilling or freezing samples to inhibit microbiological activity, or
- filtering samples to remove microorganisms.

U.S. Environmental Protection Agency (USEPA) and U.S. Geological Survey (USGS) protocols for preserving nutrient samples entail several combinations of these three techniques. With the exception of samples collected for nitrite and orthophosphate determinations, the USEPA requires that nutrient samples collected for compliance-monitoring purposes must be adjusted to pH < 2 with sulfuric acid and stored at 4°C (U.S. Environmental Protection Agency, 1990). Holding times up to 28 days are allowed.

Because nitrite is unstable in acidic solution (Brezonic and Lee, 1966; Howe and Holley, 1969; Williams, 1979; Delfino, 1979; Roman and others, 1991) and condensed phosphates (if present) can hydrolyze and contribute to apparent orthophosphate concentrations (Robards and others, 1994), samples collected for determination of these analytes[1] are not acidified. Instead, they are chilled—and field filtered in the case of orthophosphate—immediately, and must be analyzed within 48 hours.

Until January 1994, USGS recommended adding mercury (II) to nutrient samples at the collection site and storing them at 4°C. This practice avoided the potential of acid hydrolysis of condensed phosphates and oxidation of nitrite during storage, and thus simplified sample collection and processing operations relative to the USEPA protocol (U.S. Environmental Protection Agency, 1990). Increased concerns about worker safety and the possibility of contaminating samples collected for low-level mercury determinations, however, prompted the USGS to consider alternative field treatments to stabilize nutrient concentrations in samples during shipment and storage prior to analysis at the USGS National Water Quality Laboratory (NWQL). In 1992, the USGS collected samples and produced analytical data necessary to compare statistically the effectiveness of then current USGS and USEPA nutrient sample preservation protocols with each other and with a third protocol that involved field filtration and chilling, or in the case of whole-water samples, chilling only. Complete data used for statistical analysis in this work were published by Patton and Truitt (1995). Statistical evaluation of these data—the subject of this report—resulted in a technical memorandum, which announced the discontinuation of amending samples collected for nutrient analysis with mercury (II) (U.S. Geological Survey Office of Water Quality Technical Memorandum No. 94.16, 1994).

[1] **Analyte** as used in this report is the substance being identified and measured in an analytical determination.

Note that for many years pharmaceutical and food-processing industries have recognized the effectiveness of membrane filtration as a means to sterilize, and thereby to extend the shelf life of, medicines, foodstuffs, and beverages (American Society for Testing and Materials, 1990; Dickenson, 1992). In contrast, the community of water-quality specialists typically views filtration as a means to define an operational boundary between dissolved and particulate constituents—particularly in the case of trace metal analyses (Horowitz and others, 1996a, p. 954–963; Horowitz and others, 1996b, p. 3398–3400; Shiller and Taylor, 1996). In the case of samples collected for analysis of dissolved nutrients—ammonium, nitrate, nitrite, and orthophosphate—however, the rationale for filtration prior to analysis is different. In general, such samples are filtered either to remove biota capable of metabolizing nutrients or to remove any light-scattering particles that otherwise would interfere with photometric determinations, or both. Whereas zooplankton, phytoplankton, and most bacteria are retained by 0.45-µm filters, it is well established that 0.2-µm filtration is required to remove the smallest species of bacteria that are found in nature (American Society for Testing and Materials, 1990; Dickenson, 1992; Robards and others, 1994, p.149, fig. 1). The 0.45-µm pore size membrane filters used in this study were chosen because they conform to current USGS field practice and USEPA filtration guidelines, but it should be clear from the following discussion that filters with a nominal pore size of 0.2 µm would have been more appropriate. Furthermore, sample-processing equipment and sample containers used in this study were not sterile. An extensive introduction to modern container sterilization processes can be found at a Web site maintained by SteriGenics International in Fremont, California (http://www.sterigenics.com/medical/medical.htm). Gamma ray and electron-beam sterilization methods are effective and inexpensive. Thus, protocols for processing nutrient samples described in U.S. Geological Survey Office of Water Quality Technical Memorandum No. 94-16 likely would benefit from modifications discussed in the "Conclusions" section of this report.

Finally, readers may wonder why results and conclusions of a prior study (Fishman and others, 1986), which supported a policy—in place at the USGS from 1980 through 1994—of amending nutrient samples with mercury (II) at collection sites, differ from results and conclusions reported here. The Fishman study reported that nutrients in water samples amended with mercury (II) and chilled were more stable over the 16-day duration of the experiment than nutrients in samples that were chilled only or amended with sulfuric acid or chloroform and chilled. It is important to note, however, that whereas bulk samples collected for the Fishman study were filtered prior to splitting and biocide amendment, the 10-µm nominal pore size of the filters used was too large to retain bacteria and phytoplankton (Salonen, 1979; Dickenson, 1992; Robards and others, 1994). For this reason, natural water used in the Fishman study probably would have retained much of its prefiltration biological activity. Hence the necessity of adding a biocide for 16-day storage stability is reasonable and does not contradict results and conclusions of the present study, which led to the USGS to announce an end to the former USGS policy of collection-site amendment of nutrient samples with mercury (II) (U.S. Geological Survey Office of Water Quality Technical Memorandum No. 94.16).

Purpose and Scope

This report describes a study to assess the effect of different sample-processing treatments on the stability of eight nutrient species in samples of surface-, ground-, and municipal-supply water during storage at 4°C for about 30 days. The three primary objectives of this study follow:

1. To determine whether biocide amendments enhance storage stability (about 30 days at 4ºC) of dissolved nutrients in natural-water samples from which biota have been removed or substantially reduced by 0.45-µm membrane filtration at collection sites.

2. To determine whether biocide amendments enhance storage stability of total Kjeldahl nitrogen and total phosphorus in natural, whole-water samples.

3. To choose samples of different water types collected from widely separated geographical locations and to incorporate sufficient collection-site and laboratory control samples and replicates into the experimental design, so that results from this study are statistically supportable and broadly applicable to future USGS water-quality assessment work.

In addition to the preceding primary objectives, the experimental design of this study also provides the means to gain insight into a number of ancillary questions, including:

- Does water type affect nutrient concentration storage stability?
- Are particular nutrients unstable following any of the three amendment treatments?
- Are samples with high nutrient concentrations more or less stable than those with low nutrient concentrations during approximately 30-day storage at 4ºC?
- How does the 30-day stability of nutrient concentrations in real samples compare with that of nutrient concentrations in pristine-matrix, synthetic check standards?
- Does repeated opening and closing of sample containers affect the 30-day storage stability of nutrients in the water samples?
- Is there a clear-cut relation between the temporal stability of nutrient concentrations in a particular sample and the biological activity in that sample for any of the three treatments?

The scope of the work included collection and processing—splitting, filtering, adding amendments—of surface-, ground-, and municipal-supply water samples at 15 widely distributed sites within the continental United States and shipping them to the NWQL for first analyses within 24 hours of collection. The sample collection phase of this study began on April 13, 1992, and ended on May 26, 1992. Upon receipt at the NWQL, processed samples from each collection site were analyzed in quadruplicate for ammonium, nitrate,

nitrite, dissolved Kjeldahl nitrogen, orthophosphate, dissolved phosphorus, total Kjeldahl nitrogen, and total phosphorus. Quadruplicate analyses of splits from each collection site were performed five more times, typically at intervals of 3, 7, 14, 22, and 35 days after collection. Analytical work for this study began on April 13, 1992, and ended on June 29, 1992. The data on which this study is based have been published separately (Patton and Truitt, 1995).

Acknowledgments

This report benefited substantially from thorough and thoughtful reviews by Robert Runyon (Region 2, U.S. Environmental Protection Agency). Giles Miller (Kentucky Department of Water), as a member of the Interagency Task Force for Methods (ITFM), also provided helpful comments and an informal review. Other ITFM members, particularly Herb Brass (Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency) provided opportunities to present and discuss preliminary results of this study. Laurie A. Shea (Microcal Software, Northampton, Mass.) deserves special thanks for outstanding technical support for Origin 4.1™ graphics software, which was used to prepare all figures in this report.

Helpful collague reviews also were provided by USGS staff, including David Mueller, National Synthesis Team for the National Water-Quality Assessment Program (NAWQA); and Jeffrey Pritt and Juan Vasquez (National Water Quality Laboratory).

A number of individuals at the NWQL merit special thanks for assistance with various aspects of work reported here. Peter Rogerson endorsed the project proposal and was instrumental in negotiating an experimental design that was sound scientifically and acceptable to a broad range of interested parties within the USGS. Will Lanier, Peggy

Omara-Lopez, and Mark Vigil kindly shared equipment and supplies. Juan Vasquez loaned the pair of block digesters used for Kjeldahl nitrogen and phosphorus determinations. Jon Raese contributed consistently helpful editorial assistance. Barbara Kemp provided expert manuscript preparation of this report and its companion data report (Patton and Truitt, 1995). Mark Sandstrom supplied financial resources, valuable discussions, and encouragement throughout the project. Merle Shockey was especially helpful in providing opportunities to present research results at ITFM meetings.

This project is an outgrowth of discussions within the Nutrient Component of the Environmental Task Group Committee of the USGS chartered by David A. Rickert and chaired by Franceska Wilde at the USGS in Reston, Virginia. Special thanks are due to Rickert and Timothy L. Miller, who recognized the need for this study and supplemented it with funds from their budgets. Kathleen K. Fitzgerald used her extensive knowledge of the USGS National Stream Quality Accounting Network (NASQAN) Program and her skill in searching its historical data base to find potential sites from which the surface-water stations included in this study were selected. She also helped locate suitable ground-water collection sites, arranged for the loan of cone splitters used in the study, and served as a liaison between USGS sample collectors and the NWQL.

We are saddened by the untimely death of Earl Paul Truitt (1948–93), whose enthusiasm for the analytical work upon which the report is based never waned, even as his health did. Earl's colleagues at the National Water Quality Laboratory still miss his camaraderie and able assistance.

NUTRIENT PRESERVATION EXPERIMENTAL DESIGN

Even 10 years ago, the field and laboratory logistics required for an experiment of this scope and magnitude would have been daunting. By 1992, however, advances in at-site sample collection methods and equipment, analytical methods including instrumentation and ancillary, computer-based data acquisition and processing hardware and software, and the availability of overnight shipping services made the work described here seem feasible. Nonetheless, this study was labor intensive and required a great deal of planning and coordination among field and laboratory personnel. Surface-water samples, with the exception of station 12, were collected at NASQAN stations, which on the basis of historical data were expected to differ significantly in nutrient concentrations and suspended-sediment load. (See tables 13 through 21 in Appendix B.) Ground-water samples were collected from long-established stations in New Jersey, New Mexico, and Colorado. A map showing sample-collection stations can be found in a previous report (Patton and Truitt, 1995, p. 3) and on the cover of this report. See figure 31 in Appendix A for box plots of concentration ranges for dissolved and total nutrient in samples collected at the 15 stations included in this study.

Between April 13, 1992, and May 25, 1992, composite, 14-L natural-water samples were collected from 15 widely separated sites within the continental United States. At collection sites, 6 L each of whole water and filtered water from the composite sample were split into 1-L bottles and amended with either ASTM Type I deionized water (control), mercury (II), or sulfuric acid. The result was one pair of filtered water samples and one pair of whole-water samples for each of the three amendment treatments. The 12, field-processed samples from each collection site are referred to as *large-bottle splits* throughout this report.

As soon as processing operations were completed, large-bottle splits were packed in ice and shipped to the NWQL by overnight express. Immediately upon arrival at the NWQL, one filtered and one whole-water large-bottle split from each treatment group—six bottles in

all—were selected according to a preassigned random design and partitioned with 10-port cone splitters (Ward and Harr, 1990, p. 36 and 37) into 125-mL, brown polyethylene bottles. Throughout this report, these samples are referred to as *small-bottle splits*. Refer to figure 2 in Patton and Truitt (1995, p. 6) for a diagram of field and laboratory splitting operations.

Unlike large-bottle splits, which were opened and reclosed on each analysis date, small-bottle splits were opened only on the date of analysis and then discarded. Comparison of nutrient concentrations in large- and small-bottle splits from the same station, therefore, provides an indicator of the propensity of samples to become contaminated by multiple exposures to the laboratory environment. The decision to partition half of the large-bottle splits into small bottles at the NWQL was made to minimize the complexity of sample-processing operations at collection sites. Typically, large- and small-bottle splits for each treatment group from each collection site were analyzed for eight nutrient species within 24 hours of collection. Thereafter, determinations were repeated on each sample set at approximate intervals of 3, 7, 14, 22, and 35 days. When the experiment ended on June 29, 1992, well over 20,000 analytical determinations had been performed on water samples, synthetic check standards, and calibrants.

How This Experiment Differed from Some Previous Ones

The experimental design of this study differed substantially from several previous studies (Fishman and others, 1986; Salley and others, 1986; Prentice and Bender, 1987; Avanzino and Kennedy, 1993) in one or more aspects that readers should remember as they consider data and interpretations presented later in this report. Processing operations, storage conditions, and time scales in this and above-cited studies are listed in table 1.

Table 1. Processing operations, storage conditions, and time scales in selected studies

[µm, micrometer; temp., temperature; °C, degrees Celsius; >, greater than; ≈, approximately]

Reference	Processing location	Amendments	Filtration (µm)	Storage temp. (°C)	Duration (days)
Fishman and others (1986)	Laboratory	Hg (II), H_2SO_4, $HCCl_3$, none	10	4	16
Salley and others (1986)	Laboratory	H_2SO_4, none	.45	4, -20	28
Prentice and Bender (1987)	Laboratory	H_2SO_4, none	none	4	>30
Avanzino and Kennedy (1993)	Collection sites	none	.45	-16	>365
Patton and Truitt (1995)	Collection sites	Hg (II), H_2SO_4, H_2O	.45	4	≈ 35

Analytes, water types, and the number of sample-collection sites for the same five studies are listed in table 2. In discussions that follow, *previous studies* refer specifically and collectively to those cited in tables 1 and 2.

Table 2 — Near Here

Table 2. Analytes, water types, and number of sample collection sites in selected studies

[S, surface water; G, ground water; D, drinking water; E, estuarine water; N, National Pollution Discharge Elimination System (NPDES) sites; DKN, dissolved Kjeldahl nitrogen; DP, dissolved phosphorus; TKN, total Kjeldahl nitrogen; TP, total phosphorus; —, no data]

Reference	Analytes		Water types					Sites
	Dissolved	Whole-water	S	G	D	E	N	
Fishman and others (1986)	—	NH_4^+, NO_2^-, NO_3^-+NO_2^- PO_4^{3-}, TKN, TP	x		x			5
Salley and others (1986)	NH_4^+, NO_2^-, NO_3^-+NO_2^- PO_4^{3-}, DP	TKN, TP				x		4
Prentice and Bender (1987)	—	NH_4^+, NO_3^-+NO_2^-, TKN, TP			x		x	[1] 7
Avanzino and Kennedy (1993)	NH_4^+, NO_3^-+NO_2^-, PO_4^{3-}	—	x	x				3
Patton and Truitt (1995)	NH_4^+, NO_2^-, NO_3^-+NO_2^- PO_4^{3-}, DKN, DP	TKN, TP	x	x	x			15

[1]Each analyte was determined in water samples from only three sites, but not the same three sites.

Note particularly that in this study, all sample-processing operations—splitting into 1-L bottles, 0.45-µm membrane filtration, adding amendment solutions, chilling—were performed at collection sites. Thus, any nutrient concentration stability imparted to samples by any combination of these treatments typically was in effect within an hour of sample collection. Samples in the Avanzino and Kennedy (1993) study also were processed—0.45-µm filtration and freezing—at collection sites. In the other three previous studies, however, bulk, whole-water samples were transported from collection sites to laboratories where they were stored in refrigerators. A few hours to several days elapsed before bulk samples were split, filtered, and amended with biocides. During this time, nutrients in biologically active samples could have changed, distorting postprocessing "initial" concentration estimates.

In this study, initial nutrient concentrations usually were determined within one day of sample collection. In the case of surface-, ground-, and municipal supply-water samples collected in Colorado—stations 1, 9, and 6, respectively—initial nutrient concentrations were

determined within a few hours of collection. The interval between sample collection and initial nutrient concentration determinations was similar in the study by Salley and others (1986). In the studies by Fishman and others (1986) and Prentice and Bender (1987), two days typically elapsed between sample collection and determination of "initial" nutrient concentrations, which generally followed immediately after laboratory processing operations. Six months to 3 years elapsed between sample collection and first analyses in the study by Avanzino and Kennedy (1993).

The present study included ambient surface- and ground-water samples that varied widely in concentration range and geographical location within the continental United States. Furthermore, with the exception of station 12, surface-water samples were collected at NASQAN stations, which have been monitored for nutrient concentrations and suspended-sediment loads for many years (Alexander and others, 1996; also see tables 13 through 21 in Appendix B). Previous studies generally were more regional in scope. Samples in the study by Fishman and others (1986), for example, all were collected in metropolitan Denver. Samples were restricted to estuarine water from the Chesapeake Bay in the study by Salley and others (1986), whereas all those in the study by Avanzino and Kennedy (1993) were collected from Little Lost Man Creek in Humbolt County, California. Samples in the study by Prentice and Bender (1987) came either from drinking-water supplies or from industrial effluent discharge sites.

Most samples in the Prentice and Bender (1987) study had nutrient concentrations in excess of ambient levels, whereas samples in the Salley and Avanzino studies had low-level nutrient concentrations. Furthermore, the Prentice and Fishman studies added nutrients of interest (*spiking*) to samples as part of processing operations. In the case of the Prentice study, nutrient concentrations in unspiked samples were not reported. No samples in the present study were spiked with analyte species. This study also was unique in that USEPA check standards (Nutrient Concentration 1 and Nutrient Concentration 2) were determined

concurrently with each batch of environmental samples. These check standards were chosen because they are supplied as concentrates and could be prepared in deionized water matrices containing the same amendment solutions that were added to natural-water samples. Daily nutrient concentrations in these pristine-matrix check standards were compared with those in natural-water samples to assess the contribution of analytical method variability to the overall nutrient concentration variability measured in real samples. Other quality-control data that were collected throughout the course of this study, such as long-term blank concentrations and cadmium column reduction efficiency, can be found in the "Analytical Methods and Procedures" section that follows. Finally, the present study is unique in that at its conclusion, microbiological activity in small-bottle splits from each station was assessed by tritiated adenine uptake experiments (Karl, 1993) and by fluorescent dye-staining techniques.

Sampling Sites and Schedules

Sampling site locations and collection dates are listed in table 3 (Patton and Truitt, 1995, p. 4). The laboratory analysis schedule for samples from each collection site are listed in table 22 of Appendix B in the present report. Typically, nutrient concentrations in samples were determined within 1 day of the collection date and on days 3, 7, 14, 22, and 35 thereafter.

Table 3 — Near Here

Table 3. Station and laboratory identifiers of samples used in nutrient preservation study

[NWQL, National Water Quality Laboratory]

Laboratory identification	Station number	Description	Collection date	First analysis date (Julian)
[1]Station 1	06720500	South Platte at Henderson, Colo.	4/13/92	4/13/92 (104)
[2]Station 2	10254670	Alamo River, drop 3 near Calipatria, Calif.	4/16/92	4/18/92 (109)
Station 3	01389500	Passaic River at Little Falls, N.J.	4/20/92	4/21/92 (112)
Station 4	06326500	Powder River at Locate, Mont.	4/23/92	4/24/92 (115)
Station 5	11103000	Los Angeles River at Long Beach, Calif.	4/27/92	4/28/92 (119)
[1]Station 6	(3)	Municipal supply (NWQL tap water)	4/27/92	4/27/92 (118)
Station 7	08370500	Rio Grande at Fort Quitman, Tex.	4/30/92	5/1/92 (122)
Station 8	14312260	South Umpqua River near Roseburg, Oreg.	5/4/92	5/5/92 (126)
[1]Station 9	394037104391601	Colorado local well number = SC0406533CCC!	5/4/92	5/4/92 (125)
Station 10	393134074335201	New Jersey well (UID 010938)	5/7/92	5/8/92 (129)
Station 11	345833106185101	New Mexico well (station name = 09N.06E.29.244 MOSIER)	5/11/92	5/12/92 (133)
Station 12	01576540	Mill Creek near Eshelman Mill Road at Lyndon, Pa.	5/14/92	5/15/92 (136)
Station 13	07288000	Yazoo River at Redwood, Miss.	5/18/92	5/19/92 (140)
Station 14	08062800	Trinity River at Trinidad, Tex.	5/21/92	5/22/92 (143)
[2]Station 15	02248000	Spruce Creek at Samsula, Fla.	5/26/92	5/28/92 (148)

[1]Time zero station, that is, first samples were analyzed on the day that they were collected.

[2]Samples were shipped with the wrong priority. First samples were analyzed 2 days after samples were collected.

[3]Station number does not exist.

Experimental Design Considerations in Sample Processing and Sequence of Analyses

To lessen the possibility either of masking amendment effects or of interpreting withdrawal order effects as amendment effects, the order for withdrawing composite samples from 14-L churn splitters into 1-L bottles and the amendment for each bottle were assigned randomly according to one of the following three patterns:

	Amendment solution					
Filling order	1	2	3	4	5	6
Withdrawal pattern 1:	H_2O	Hg(II)	H_2SO_4	H_2O	Hg(II)	H_2SO_4
Withdrawal pattern 2:	Hg(II)	H_2SO_4	H_2O	Hg(II)	H_2SO_4	H_2O
Withdrawal pattern 3:	H_2SO_4	H_2O	Hg(II)	H_2SO_4	H_2O	Hg(II)

Additional details about the labels attached to each bottle set to achieve these random assignments can be found in the following "Analytical Methods and Procedures" section. Withdrawal patterns assigned to 1-L bottles used at each of the 15 sample collection sites in this study are listed as follows.

Station number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Withdrawal pattern	2	2	3	2	1	3	1	1	1	2	3	3	1	3	2

Similarly, the association of cone splitter port number (0–9) and dates of analysis for small-bottle splits were randomized.

Analytical Methods and Procedures

As described previously (Patton and Truitt, 1995, p. 2–7), all operations—small-bottle split preparation, analytical determinations, and data reduction and preliminary analysis—were performed by Patton and Truitt using laboratories and equipment of the Methods Research and Development Program at the NWQL. RFA-300, air-segmented continuous

flow analyzers (Alpkem Corp., Clackamas, Ore.), operated with pecked sampling and bubble-gated detectors (Patton and Wade, 1997), were used for all colorimetric determinations. Two independent analyzers—a four-channel system configured for simultaneous determination of ammonium, nitrate plus nitrite, nitrite, and orthophosphate and a two-channel system configured for simultaneous determination of Kjeldahl nitrogen and phosphorus—were used in this study. Modules used to assemble these analyzers were model 301 samplers, model 302 peristaltic pumps with air-bars, model 313 analytical cartridge bases, model 314 power modules, model 305A photometers, and model 311, dual-pen, 25.4-cm strip chart recorders. Inside diameters of analytical cartridge components were 1 mm or less. Rates of analyses for the four-channel and two-channel analyzers were 120 per hour and 90 per hour, respectively.

Prior to Kjeldahl nitrogen and phosphorus analysis, samples were digested, batchwise, using a pair of Tecator Digestion System 40, model 1016 block digesters (Perstorp Analytical, Inc., Silver Springs, Md.), as described previously (Patton and Truitt, 1992, p. 5 and 6). Except as noted, USGS analytical methods were used (Patton and Truitt, 1992; Fishman, 1993), which are listed in table 4 along with numbers of approximately equivalent

Table 4 — Near Here

USEPA methods (U.S. Environmental Protection Agency, 1993). Minimum concentrations in the *Analytical Range* column of table 4 are method detection limits, calculated as specified by the U.S. Environmental Protection Agency (1990).

Table 4. U.S. Geological Survey methods and approximately equivalent U.S. Environmental Protection Agency methods used for determination of nutrients in this study

[USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; mg/L, milligram per liter]

Analyte	USGS method	USEPA method	Analytical range (mg/L)
Ammonium	I-2522-90	350.1	0.01 – 2.00
Nitrate plus nitrite	I-2545-90	353.2	.02 – 5.00
Nitrite	I-2540-90	353.2	.003 – 1.00
Dissolved Kjeldahl nitrogen	I-2515-91	351.2	.05 – 10.00
Total Kjeldahl nitrogen	I-4515-91	351.2	.05 – 10.00
Orthophosphate	I-2601-90	365.1	.004 – 1.00
Dissolved phosphorus	I-2610-91	365.4	.01 – 2.00
Total phosphorus	I-4610-91	365.4	.01 – 2.00

Long-term reagent stability was improved by using separate molybdate and ascorbic acid reagents in orthophosphate method and separate sulfanilamide and N-(1-Naphthy)ethylenediamine reagents in nitrate-nitrite methods as described previously (Antweiler and others, 1993).

A Soft-Pack ™ (Alpkem Corp., Clackamas, Ore.), personal-computer-based data acquisition and processing system was used to control samplers and to acquire and process photometric detector data from both analyzers. This system, which was equipped with a 12-bit analog-to-digital converter, located peak maxima in photometric detector signals, and after applying appropriate baseline drift and digestion blank corrections, converted them into concentration units using second-order, least squares polynomial calibration functions estimated from calibrants included with each batch of environmental samples. Strip-chart recordings of detector outputs were used solely to monitor analyzer performance and to document analyzer performance in archived data, which are stored at the NWQL. A wedge-

type bar-code reader (Welch Allyn, Skaneateles Falls, N.Y.) interfaced through the computer keyboard provided direct transfer of bar-coded identifiers from labels on large- and small-bottle splits, calibrants, check standards, and blanks into SoftPack™ sample identification files. Bar-code entry of sample and reference solution identifiers greatly speeded laboratory data entry and processing operations. Concentration data acquired from individual runs[2] with the SoftPack™ system were exported to 1-2-3™ (Lotus Development Corp., Cambridge, Mass.) and EXCEL™ (Microsoft Corp., Redmond, Wash.) spreadsheets for sorting, consolidation, and preliminary statistical analysis. Flat files (ASCII format) of consolidated data were exported to the USGS Data General minicomputer system where group statistical analyses were prepared using STATIT (Statware, Inc., Corvallis, Ore.) software. All data graphs in this report were produced with ORIGIN™ version 3.78 or 4.1 software (Microcal Software, Inc., Northampton, Mass.).

Hand-held EDP-Plus™ motorized microliter pipets (Rainin Instrument Company, Inc., Woburn, Mass.) were used to prepare calibrants and check standards, to dispense samples into Kjeldahl digestion tubes, and, when necessary, to dilute samples prior to analyses. Three interchangeable liquid ends permitted dispensing volumes in the range from 2 to 10,000 µL. Gravimetric calibration of these pipets using all three liquid ends indicated that digitally set and dispensed volumes agreed to within 1 percent or less and that repeatability of dispensed volumes was 0.5 percent or less in accordance with the manufacturer's specifications.

Three sets of mixed standards and sampler wash solutions, whose matrices matched those of acid-, mercury (II)-, and water-amended (control) samples, were prepared for use with the four-channel analyzer. Matching the matrix of calibrants and wash solutions with

[2]***Run*** as used in this report is a group of samples (60 – 90, typically) analyzed together by the same analytical instrument in association with a common set of calibrants and check standards.

those of samples is a common practice in continuous flow analysis (Patton and Wade, 1997), which minimizes errors caused by refractive index differences and chemical reactivity that would otherwise occur. Nominal concentrations of ammonium, nitrate plus nitrite, nitrite, and orthophosphate in these mixed calibrants are listed in table 5.

Table 5. Nominal concentrations of ammonium, nitrate plus nitrite, nitrite, and orthophosphate in mixed calibrants

Calibrant	Nominal concentration, in milligrams per liter			
	NH_4^+-N	NO_3^- + NO_2^--N	NO_2^--N	PO_4^{3-}-P
1	2.00	5.00 (4.00 + 1.00)	1.00	1.00
2	1.50	3.75 (3.00 + 0.75)	.75	.75
3	1.00	2.50 (2.00 + 0.50)	.50	.50
4	.50	1.25 (1.00 + 0.25)	.25	.25
5	.25	.62[5] (0.50 + 0.12[5])	.12[5]	.10
6	.10	.25 (0.20 + 0.05)	.05	.05

Calibrants for the four-channel analyzer were prepared in 100-mL volumes as needed at intervals of about 2 weeks. Acid-matrix calibrants were prepared daily within minutes of use because nitrite is unstable in acidic solutions. Even so, the slope of the nitrite calibration function for acid-matrix calibrants was about 15 percent less than those for mercury (II)- and water-matrix calibrants because of rapid decomposition of nitrite. Similar effects were observed in unmixed, acid-matrix nitrite check standards, where concentrations decreased steadily from the beginning to the end of each run.

A single set of mixed standards for Kjeldahl nitrogen and phosphorus determinations, which contained organic nitrogen, as glycine, and organic phosphorus, as sodium glycerophosphate, was prepared for use with the two-channel analyzer. These calibrants were not matrix-matched because the digestion reagent contained sulfuric acid, potassium

sulfate, and mercury (II) in concentrations much greater than those contributed by amendment solutions. The wash solution, however, was formulated to match the matrix of resolvated Kjeldahl digests as previously described (Patton and Truitt, 1992). Nominal concentrations of glycine and sodium glycerophosphate in these mixed calibrants are listed in table 6. Calibrants for the two-channel analyzer were prepared in 250-mL volumes as needed at intervals of about 2 weeks.

Table 6. Nominal concentrations of glycine and sodium glycerophosphate in mixed calibrants

	Nominal concentration, in milligrams per liter	
Calibrant	Nitrogen	Phosphorus
1	10.0	2.00
2	7.50	1.50
3	5.00	1.00
4	1.75	.35
5	.50	.10

Lot numbers and preparation dates for check standards (USEPA Nutrient Concentration 1 and Nutrient Concentration 2), which were obtained at no charge from the USEPA and a commercial source (SPEX Industries, Inc., Edison, N.J.), are listed in table 7.

Table 7 — Near Here

Table 7. U.S. Environmental Protection Agency (USEPA) nutrient concentration data

[—, no data; WP, label identifier; SPEX, vendor name]

Preparation date (Julian day)	WP	Lot number	Batch number	Source
	USEPA nutrient concentration 1			
4/13/92 (104)	486	—	—	USEPA
4/21/92 (112)	861	1188	4797	SPEX
4/30/92 (121)	861	1188	4784	SPEX
5/11/92 (132)	861	1188	4877	SPEX
5/20/92 (141)	861	1188	4793	SPEX
6/01/92 (153)	861	1188	4786	SPEX
6/15/92 (167)	861	1188	4796	SPEX
	USEPA nutrient concentration 2			
4/13/92 (104)	486	—	—	USEPA
4/21/92 (112)	862	1188	6586	SPEX
4/28/92 (119)	862	1188	6585	SPEX
5/04/92 (125)	862	1188	3141	SPEX
5/20/92 (141)	862	1188	6639	SPEX
6/15/92 (167)	862	1188	6658	SPEX

As was the case for calibrants, 100-mL volumes of Nutrient Concentration 1, containing ammonium, nitrate, and orthophosphate, were prepared in matrices that corresponded to the three amendments, whereas 250-mL volumes of Nutrient Concentration 2, containing organic nitrogen and organic phosphorus, were prepared in a deionized water matrix only. Four replicates of USEPA Nutrient Concentration 1 with the appropriate matrix were included in each batch of samples determined on the four-channel analyzer. A single aliquot of USEPA Nutrient Concentration 2 was digested in each batch of 40 calibrants,

blanks, and samples. Most probable values (MPV) and standard deviations for USEPA check standards Nutrient Concentration 1 and Nutrient Concentration 2 follow. Note that Nutrient Concentration 1 does not contain nitrite.

Check standard	Analyte	Most probable value (mg/L)	Standard deviation (mg/L)
Nutrient 1 (lot 1188)	Ammonium nitrogen	1.98	0.16
Nutrient 1 (lot 1188)	Nitrate nitrogen	1.99	.14
Nutrient 1 (lot 1188)	Orthophosphate phosphorus	.39	.04
Nutrient 2 (lot 1188)	Kjeldahl nitrogen	4.95	.44
Nutrient 2 (lot 1188)	Total phosphorus	1.53	.11

Average concentrations and standard deviations of blank solutions determined concurrently with each batch of samples are listed in table 8. Blank concentrations for dissolved nutrients were negligible. Blank concentrations about 0.05 mg-N/L for Kjeldahl nitrogen and 0.01 mg-P/L for phosphorus were significantly greater than zero, so their contribution to recorded peak heights was removed by subtraction prior to estimation of calibration functions and calculation of concentrations. Blank concentrations for these analytes in table 8 are the result of this blank correction procedure.

Table 8 — Near Here

Table 8. Blank concentrations determined during the 77-day course nutrient preservation experiment

[avg., average; s.d., standard deviation; n, number of points. All concentrations in milligrams nitrogen or phosphorus per liter.]

	Amendment					
	Acid		Mercury (II)		Water control	
Analyte	avg. ± s.d.	*n*	avg. ± s.d.	*n*	avg. ± s.d.	*n*
Ammonium	0.01 ± .02	86	0.01 ± 0.01	86	0.011 ± 0.009	90
Nitrate plus nitrite	.04 ± .02	77	.03 ± .02	82	.03 ± .02	82
Nitrite	.009 ± .009	86	.005 ± .004	90	.005 ± .003	87
Orthophosphate	.00 ± .01	86	.002 ± .005	90	.004 ± .005	88
Phosphorus	.000 ± .007	106	.000 ± .006	112	-.001 ± .006	112
Kjeldahl nitrogen	.00 ± .03	110	.00 ± .03	116	.00 ± .04	110

Average concentrations for percent reduction efficiency—defined as the concentration ratio of unmixed nitrate and nitrite calibrants with nominal concentrations of 1 mg-N/L multiplied by 100—of packed bed cadmium reactors used for nitrate plus nitrite determinations are listed in table 9. As explained in the Nitrate Plus Nitrite section, which appears later in this report, the greater than 100-percent reduction efficiency calculated in the acid matrix is an artifact caused by decomposition of nitrite.

Table 9. Percent reduction of nitrate to nitrite by packed bed cadmium reactors calculated during the 77-day course of this study

	Matrix		
	Water	Mercury	Acid
Average reduction efficiency (percent)	102	100	123
Standard deviation (percent)	2	4	13
Number of points	131	133	128

Table 10 lists concentrations estimated when mixed calibrants containing ammonium, nitrate, nitrite, and orthophosphate were subjected to Kjeldahl digestion and then determined as nitrogen and phosphorus on the two-channel analyzer. Concentrations in the "found" columns demonstrate consistency between nominal concentrations of organic and inorganic calibrants. Note that neither nitrate nor nitrite is determined by the Kjeldahl nitrogen method.

Table 10. Inorganic (four-channel analyzer) calibrants determined on the two-channel analyzer after Kjeldahl digestion on Julian date 105, 1992

[mg-P/L, milligrams-phosphorus per liter; mg-N/L, milligrams-nitrogen per liter; ID, identifier]

Calibrant ID	Phosphorus concentration (mg-P/L)		Nitrogen concentration (mg-N/L)	
	Nominal	Found	Nominal	Found
4CH BLANK	0	-0.01	0	0
4CH CAL1	1.00	1.02	2.00	2.00
4CH CAL2	.75	.74	1.50	1.46
4CH CAL3	.50	.49	1.00	1.02
4CH CAL4	.25	.24	.50	.48
4CH CAL5	.10	.11	.25	.27
4CH CAL6	.05	.04	.10	.13

Sample Containers and Sample Container Preparation

Brown polyethylene bottles (1-L capacity, 28-mm screw-cap closures) were used exclusively to ship large-bottle splits to the NWQL for nutrient analyses. They were cleaned, labeled, and packaged as kits by Patton and Truitt for field-sampling personnel as follows. Bottles were cleaned by rinsing them first with ASTM Type I deionized water, then with three, 15-mL volumes of 5 percent v/v HCl solution, and again with ASTM Type I deionized

water. After the final rinse, vigorous, manual shaking removed excess water from bottles before they were capped tightly and labeled. Labels were color coded—red for acid-amended, green for mercury (II)-amended, and blue for ASTM Type I deionized water-amended (control) samples—to minimize the potential for bottle mix-ups during field and laboratory operations. A set of four labels was affixed to each bottle. The first three indicated 5-digit (large-bottle splits) or 6-digit (small-bottle splits) laboratory identification numbers, sample types (field-filtered, designated "FC" for filter chilled or whole-water, designated "RC" for raw chilled), and amendment ("ACID," "MERCURY," or "WATER CONTROL"). These were both alphanumeric and bar-code readable to streamline laboratory operations. A fourth label, consisting of a single digit from 1 to 6, was affixed to the bottle shoulders, which indicated the filling order for filtered and whole-water large-bottle splits. These were assigned according to a random design to ensure that no amendment treatment would be associated with a particular filling order at the collection site or with a particular analysis order at the NWQL (see “Experimental Design Considerations in Sample Processing and Sequence of Analyses”). More details about labeling protocols are provided elsewhere (Patton and Truitt, 1995, p. 7). Kits composed of 12 clean, labeled 1-L bottles (two to contain filtered samples, two to contain whole-water samples for each of the three amendments), ampoules containing 4-mL volumes of amendment solutions (sulfuric acid, mercuric chloride, ASTM Type I deionized water), and a two-page set of instructions for collecting, processing, and shipping samples were sent to sample collectors several weeks in advance of scheduled collection dates.

In addition to the 1-L bottles that were prepared for use at sample-collection sites, 125-mL capacity, brown polyethylene bottles with 28-mm screw-cap closures were similarly cleaned and labeled to receive small-bottle splits filled at the NWQL as previously described (Patton and Truitt, 1995). Six sets of 10, 125-mL bottles—three sets to contain sulfuric acid-, mercury (II)-, and ASTM Type I deionized water-amended filtered water, and three

sets to contain sulfuric acid-, mercury (II)-, and ASTM Type I deionized water-amended whole water—were prepared for each of the 15 stations. Bottles in each set were assigned numbers between 0 and 9, corresponding to the cone splitter port from which they were filled. These numbers allowed random selection of small-bottle splits so temporal analysis orders would not be associated with particular cone splitter ports as described earlier under the "Experimental Design Considerations in Sample Processing and Sequence of Analyses" section.

Amendment Solutions

Amendment solutions used at all sample-collection sites were obtained commercially (Eagle Pitcher, Miami, Okla.) on a custom basis. Four-mL volumes of each solution were packaged by the vendor in 5-mL glass ampoules in lots of 150 ampoules. The designation and composition of amendment solutions are listed as follows:

Amendment	Color code	Composition
Acid	Red	4.5 ***M*** sulfuric acid (1 part ultrapure concentrated H_2SO_4 plus 3 parts ASTM Type I deionized water)
Mercury (II)	Green	13 milligrams $HgCl_2$ plus 100 milligrams NaCl per milliliter of ASTM Type I deionized water
Water (control)	Blue	ASTM Type I deionized water

Upon receipt at the NWQL, the contents of about 5 percent of these ampoules were dissolved individually in 1 L of ASTM Type I deionized water. In all cases, measured dissolved and total nutrient concentrations in these solutions were comparable to blank concentrations. Color-coded labels corresponding to those of sample bottles were affixed to remaining ampoules.

Sample Collection Protocols

Surface-water samples were collected using USGS standard isokinetic samplers equipped with glass or plastic bottles of 1- or 3-L capacity (Ward and Harr, 1990, p. 9–11). Ground-water samples were collected using peristaltic or positive displacement pumps by standard USGS protocols (Koterba and others, 1995). Approximately 13 L of each surface- and ground-water sample were composited in 14-L churn splitters (Ward and Harr, 1990, p. 34–36). The municipal supply-water sample (station 6) was not composited. Instead a cold water tap at the NWQL was turned on and allowed to flow for 10 minutes, and then six, 1-L bottles were rinsed and filled in rapid succession. These samples were not filtered prior to addition of preservatives; hence, no filtered-water sample data exist for this station.

Sample Splitting

Composite samples of surface or ground water contained in 14-L churn splitters were processed at collection sites as follows. With continuous churning, six whole-water sample bottles ("RC" labels) were successively rinsed (three, 10- to 15-mL sample volumes), filled with homogenized sample, and capped. Sufficient head space was left in each bottle to accommodate 4-mL additions of amendment solutions. Bottles were filled successively (1–6) as indicated by their filling order labels (see preceding discussion under “Experimental Design Considerations in Sample Processing and Sequence of Analyses”; also see “Analytical Methods and Procedures”). When bottling of the whole-water samples was completed, churning was stopped and water remaining in the churn was pumped by use of a peristaltic pump through a 142-mm diameter, 0.45- m pore size membrane filter housed in a plastic assembly. The six filtered-water sample bottles ("FC" labels) were rinsed three times with 10- to 15-mL volumes of filtered sample, filled successively (1–6) as indicated by their filling order labels, and capped. Sufficient head space was left in each bottle to accommodate 4-mL additions of amendment solutions.

Sample Amendment

The 12 large-bottle splits then were sorted according to their amendment solution labels and dosed with appropriate amendment solutions contained in glass ampoules as described under “Analytical Methods and Procedures.” Four mL of mercuric chloride solution was added to each of the four bottles with "MERCURY" labels. Four mL of sulfuric acid solution was added to each of the four bottles with "ACID" labels. Four mL of ASTM Type I deionized water was added to each of the four bottles with "WATER CONTROL" labels. Bottles were recapped, inverted several times to effect mixing between samples and amendment solutions, packed in ice, and shipped the same day by overnight express to the NWQL.

STATISTICAL ANALYSIS OF EXPERIMENTAL RESULTS

Summary of Statistical Analyses

This study was designed to test the null hypothesis that collection-site amendments had no effect on the stability of eight nutrient species during storage at 4°C for about 30 days. Results of statistical analyses are listed in table 11. The first assumption tested was that any concentration changes over time for a given sample and nutrients could be reasonably described by a linear function. The temporal stability of a particular constituent was measured using a General Linear Model, analysis of covariance (ANCOVA). In this model, time was the covariant whereas amendment treatment, split size (large or small), and water source (surface-, ground-, or municipal-supply) were the explanatory categorical variables. Stability over time was assumed to hold if the regression coefficient associated

Table 11 — Near Here

Table 11. Results of statistical analyses of factors that influence stability of nutrient concentrations during storage at 4°C for about one month

[DKN, dissolved Kjeldahl nitrogen; TKN, total Kjeldahl nitrogen; DP, dissolved phosphorus; TP, total phosphorus]

Analytes	Stability			Bottle effects	Source effects	Amendment effects	Real compared to synthetic	Biological activity
	Acid	Mercury	Water					
Ammonium	X	X	X	none	none	none	none	possible
Nitrate plus nitrite	X	X	X	none	none	none	none	none
Nitrite	no	X	X	none	none	unstable in acid	no data	none
DKN	X	X	X	none	none	none	none	none
TKN	X	X	X	none	none	none	none	possible
Orthophosphate	X	X	X	none	none	none	none	none
DP	X	X	X	none	none	none	none	none
TP	X	X	X	none	none	none	none	none

with time was not statistically significant at the 95 percent significance level ($\alpha = 0.05$). Differences caused by categorical variables were evaluated by testing the associated regression coefficients at $\alpha = 0.05$. The assumption of linearity over time was rejected with a high level of confidence ($\alpha < 0.01$) in almost all cases, using a lack of fit test for replicate data in linear regression. The assumption of linearity over time was also rejected for logarithmic transforms of these data, indicating that some mechanism other than a linear or exponential change over time is occurring. Of the eight nutrients in this study, only nitrite showed a statistically significant functional relation with time for acid-amended splits. This result was expected because the instability of nitrite in acidified water samples is well documented (Brezonic and Lee, 1966; Howe and Holley, 1969; Delfino, 1979; Williams, 1979; Roman and others, 1991) and is explained for the most part by chemical oxidation of nitrite to nitrate.

At this point, it is worth noting that a similar lack of statistically significant linearity over time was observed in a previous holding-time study (Prentice and Bender, 1987, p. 8.). Paradoxically, Prentice and Bender used the linear model anyway to project maximum nutrient holding times presented in their study. Careful review of nutrient concentration as a function of time data from several of the previous studies suggests that minor, random excursions about the initial concentration rather than a monotonic increase or decrease are the norm in nutrient holding-time studies.

Because of the aforementioned lack-of-fit test results, the assumption of linearity over time, and hence the ANCOVA model, was discarded for the other seven nutrient species included in this study. In its place, the analysis of variance (ANOVA) model was used. The ANOVA factors were (a) time order of analysis and (b) amendment treatment. The response variable in each ANOVA was the concentration measured at any time *t*, divided by the average initial (time zero) concentration. Here, analyte concentration changes over time were scaled by dividing them by initial ("true") concentrations for large- and small-bottle splits from each of the 15 collection sites. The ANOVA results on the main factors of time order of analysis and amendment did yield some statistically significant results for each analyte at $\alpha = 0.05$. Interaction plots of time order of analysis and amendments for each analyte and station, however, revealed no "best" amendment for all analytes and stations. "Best" was taken to mean least variation over time from initial concentration. Again there is no monotonic pattern to the variations in the mean levels over time (see figs. 1 and 2).

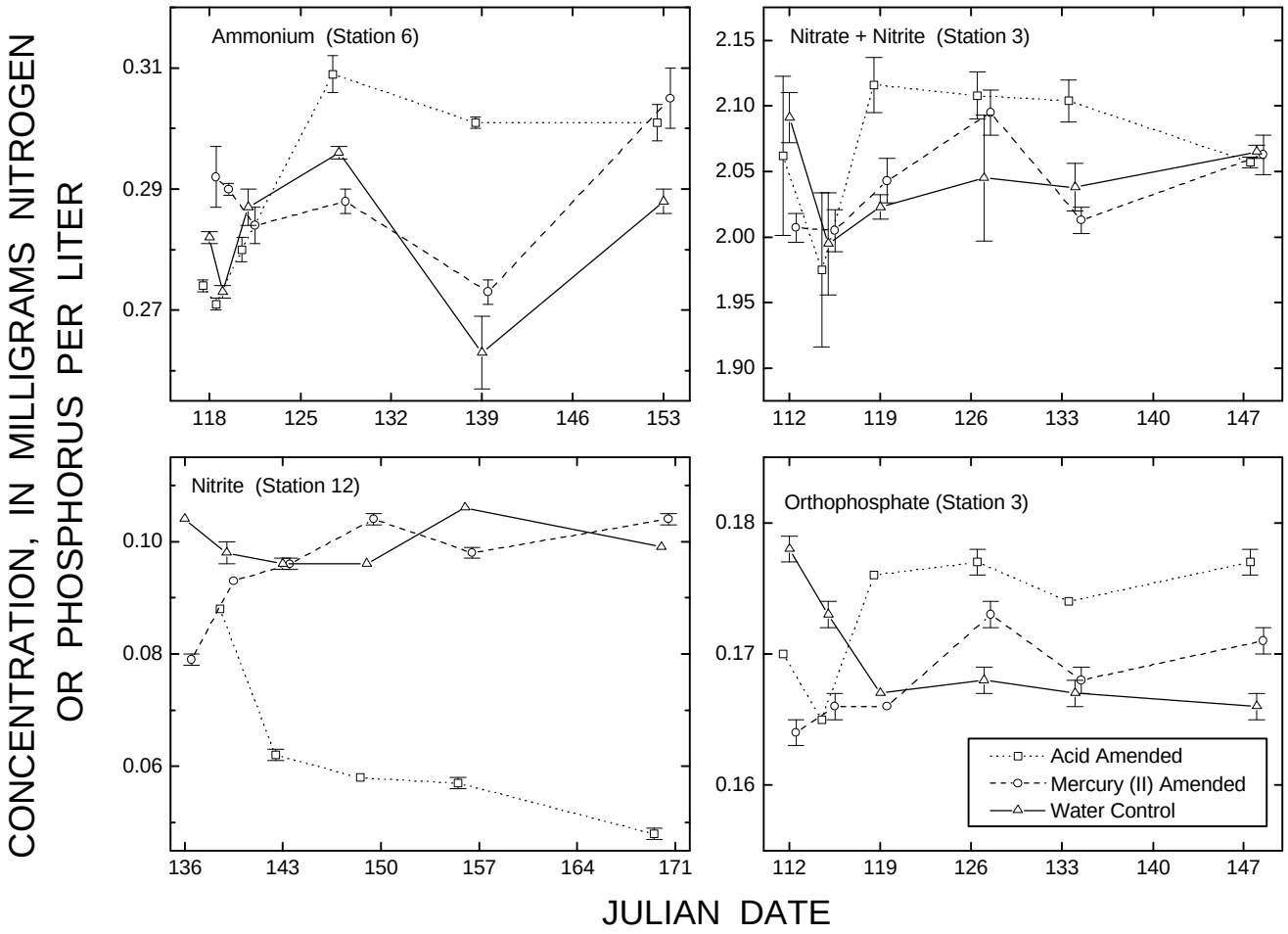

Figure 1. Typical plots of dissolved inorganic nutrient concentrations in relation to determination date in small-bottle splits for each amendment group. Symbols for acid- and mercury (II)-amended splits are offset by ± 0.5 Julian day for clarity. Error bars indicate ± 1 standard deviation.

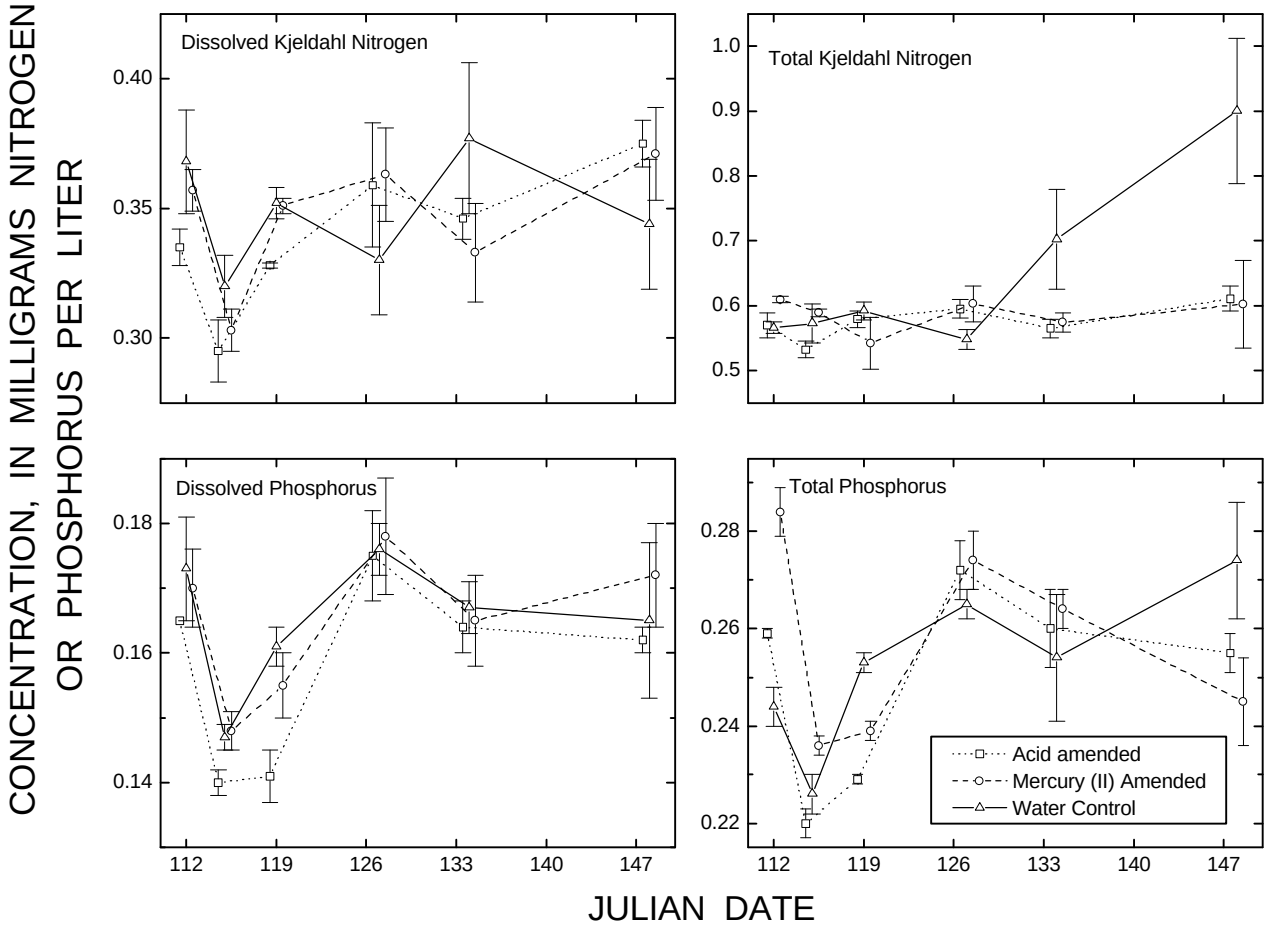

Figure 2. Typical plots of Kjeldahl nitrogen and phosphorus concentrations in relation to determination date in small-bottle splits from station 3 for each amendment group. Symbols for acid- and mercury (II)-amended splits are offset by ± 0.5 Julian day for clarity. Error bars indicate ± 1 standard deviation.

Temporal Concentration Variation of Analytes in Check Standards from the U.S. Environmental Protection Agency

Because temporal changes in nutrient concentrations were not monotonic, the possibilities of either random contamination introduced during sample-handling operations at the NWQL or the lack of control in analytical methods, or both, were broached. USEPA check standards—Nutrient Concentration 1 (containing ammonium, nitrate, and orthophosphate) and Nutrient Concentration 2 (containing organic nitrogen and organic phosphorus), which were analyzed concurrently with environmental samples—provided a means to investigate these possibilities. Note that new batches of check standards were prepared at intervals of about 2 weeks as described in the preceding "Analytical Methods and Procedures" section. The measured concentrations of these check standards were standardized by subtracting the USEPA-reported most probable value (MPV) for each analyte and dividing by either (1) the USEPA-reported standard deviation for each analyte, or (2) standard deviations estimated from regression equations maintained by USGS Standard Reference Water Sample, whichever was least. With the exception of eight concentrations for orthophosphate in the initial batch of USEPA Nutrient Concentration 1—determined on Julian dates 104, 109, and 112—that were censored (see Patton and Truitt, 1995, table 121, p. 130), none of the control charts indicate any laboratory contamination during this time period.

Analyte concentrations in USEPA check standards also were subjected to General Linear Model analyses. As was the case for environmental samples, the assumption that analyte concentrations changed linearly over time was rejected with a high level of confidence. Hence there is no evidence of either random or deterministic contamination of samples during storage and analysis at the NWQL. Control charts of all USEPA check standards analyzed during the 77-day course of this study are presented in figures 25

through 29 (see Appendix A). As shown in table 12, the pooled relative standard deviation (RSD)—that is, standard deviation divided by average concentration, also known as *coefficient of variation* or CV—of all amendments for ammonium and nitrate was about 2 percent, that for orthophosphate and phosphorus was about 3 percent, and that for Kjeldahl nitrogen was about 5 percent. In table 12, *n* indicates the number of repeat determinations performed on the check standards between Julian dates 104 and 181 that were averaged to obtain percent RSD estimates. In the case of RSD estimates for Nutrient Concentration 1 (ammonium, nitrate, and orthophosphate), each reported concentration included in the average was itself the average of four replicate, within-run determinations. In the case of RSD estimates for Nutrient Concentration 2 (Kjeldahl nitrogen and phosphorus), averages are based on individual determinations of check standards that were digested and determined concurrently with each batch of environmental samples.

Table 12. Precision of analytical methods based on repeat determinations of U.S. Environmental Protection Agency check standards

[RSD, relative standard deviation, in percent; n, number of points; —, no data]

Analyte	Amendment							
	All		Acid		Mercury (II)		Water	
	RSD (percent)	*n*	RSD (percent)	*n*	RSD (percent)	*n*	RSD (percent)	*n*
Ammonium	2.4	267	2.2	88	2.7	89	2.1	90
Nitrate	2.1	269	2.0	89	1.9	90	1.3	90
Nitrite[1]	—	—	6.0	125	4.4	123	6.0	128
Orthophosphate	2.8	256	2.2	86	2.6	85	3.2	85
Kjeldahl nitrogen	4.7	412	6.9	135	3.0	138	3.0	139
Phosphorus	2.7	413	2.6	137	1.9	138	3.3	138

[1]NWQL check standard.

Temporal Concentration Variation of Analytes in Environmental Samples

In the sections that follow, relative standard deviation (*RSD*) is one criterion used to evaluate stability of individual analytes during storage in acid-, mercury (II)-, and water-amended large- and small-bottle splits from each of the 15 stations. Note that for analytical methods in general, *RSD* is approximately constant for analyte concentrations greater than or equal to about 10 times the method detection limit (MDL); below this concentration threshold, *RSD* increases asymptotically as analyte concentrations approach the MDL (Green, 1996—see fig. 2, p. 308A). This result is in contrast to standard deviation, which increases monotonically as analyte concentration increases. These trends are shown in figure 3 for ammonium concentrations at each of the 15 stations in relation to standard deviation (a) and relative standard deviation (b). The other seven analytes included in this study showed similar characteristics.

RSD estimates for analytes in environmental samples typically resulted from averaging 24 concentrations—four within-run replicates determined on six different days. When

Fig. 3 — Near Here

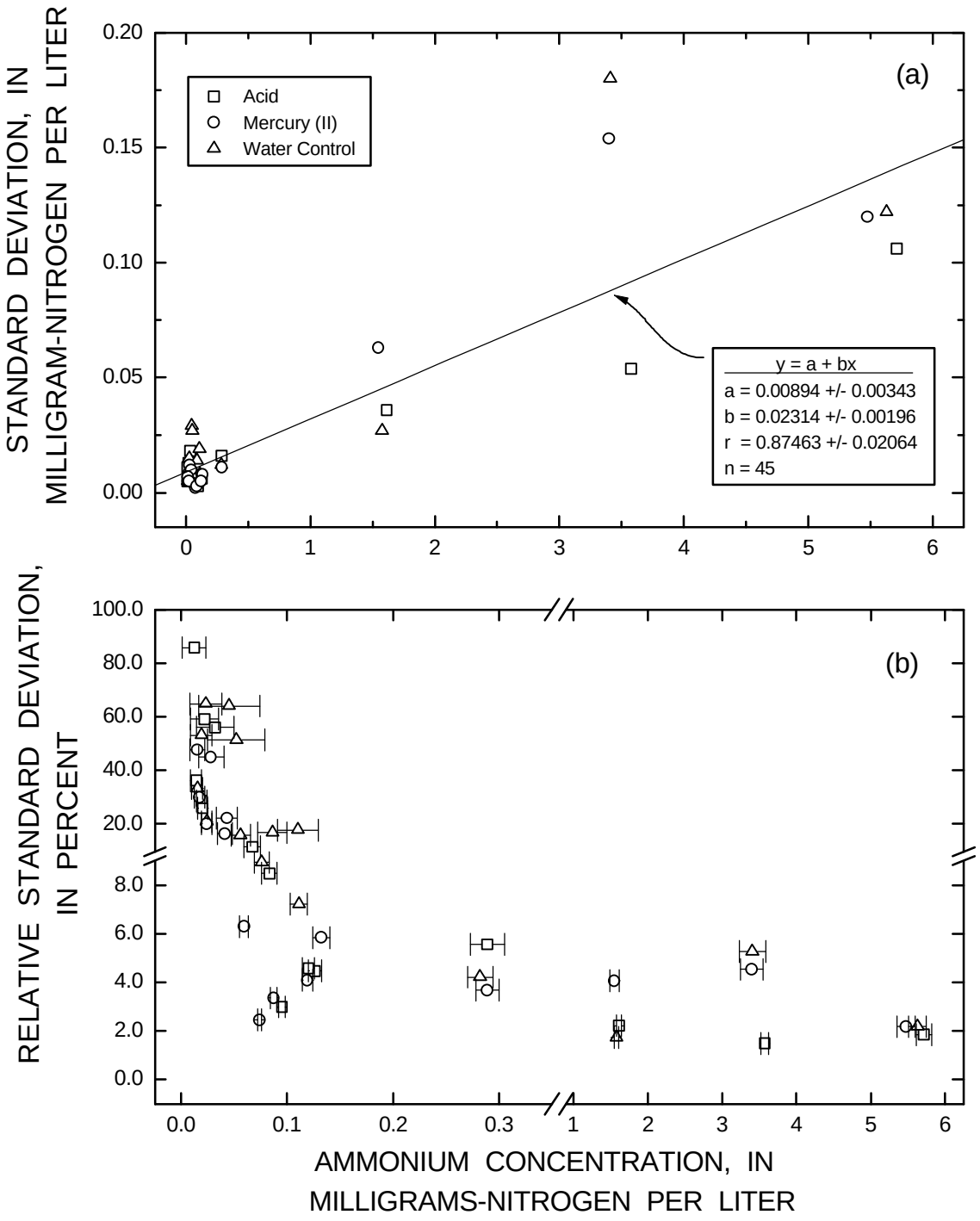

Figure 3. Six-determination-date-average concentrations of ammonium in small-bottle splits for the three amendments from each collection site plotted as a function of (a) standard deviation and (b) relative standard deviation. Error bars in figure 3b indicate ±1 standard deviation.

six-determination-date average concentrations for individual stations, treatments and analytes exceeded 10 MDLs, their relative standard deviations were pooled using the equation that follows (Anderson, 1987):

$$\%\ RSD_{\text{pooled}} = \sqrt{\frac{(RSD_1)^2 \times df_1 + (RSD_2)^2 \times df_2 + \ldots + (RSD_n)^2 \times df_n}{df_1 + df_2 + \ldots + df_n}} \times 100 \qquad (1)$$

In equation 1, *RSD* is the six-determination-date relative standard deviation of concentration for a particular station, analyte, and amendment, whereas *df*—the degrees of freedom—is one less than the number of concentrations used to calculate *RSD*. See table 13 for pooled *RSD* estimates of each of the eight analytes. The number of stations included in pooled *RSD* estimates are within parentheses under the ***Station numbers*** heading of this table.

Data in table 13 are expected to be independent of analyte concentration because *RSD*s for environmental samples were pooled only when the six-determination-date average concentrations for a given station, amendment, and analyte exceeded 10 MDLs. This is also the case for pooled RSD estimates in table 12, since analyte concentrations in USEPA check standards all exceeded 10 MDLs. Thus, pooled RSDs for individual amendments and analytes in table 12 (pristine matrix check standards) provide estimates of analytical method variability, whereas those in table 13 (variable matrix environmental samples) provide estimates of the sum of analytical method variability and variability caused by matrix type and treatment effects.

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Table 13 – Near Here

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Table 13. Pooled relative standard deviation (RSD) of analyte concentrations in small-bottle splits of environmental samples from stations at which six-determination-date average concentrations were greater than or equal to 10 method detection limits

Analyte	Station numbers	Pooled percent relative standard deviation (number of points)		
	(number of stations)	Acid	Mercury	Water
Ammonium	1-3, 5, 6, 15 (6)	3.7 (144)	4.4 (144)	[1] 8.0 (144)
Nitrate plus nitrite	1-3, 5, 7, 9-14 (11)	3.6 (256)	2.0 (263)	2.7 (264)
Nitrite	1-3, 5, 7, 12, 14 (7)	45.3 (164)	6.8 (168)	4.5 (167)
Dissolved Kjeldahl nitrogen	1, 2, 5, 7, 9, 14, 15 (7)	6.8 (165)	10.1 (169)	9.2 (172)
Total Kjeldahl nitrogen	1-5, 7, 9, 12-15 (11)	8.3 (258)	4.8 (265)	16.9 (264)
Orthophosphate	1-3, 5, 7, 12, 14, 15 (8)	3.5 (186)	1.8 (188)	3.2 (188)
Dissolved phosphorus	1-3, 5, 7, 12, 14 (7)	5.1 (168)	5.4 (172)	5.5 (172)
Total phosphorus	1-5, 7, 9, 12-15 (11)	6.0 (256)	7.2 (257)	5.8 (263)

[1]Exclusion of ammonium concentrations for water-amended, small-bottle splits from station 15 on the final determination date (Julian day 181) results in a pooled RSD of 4.5 percent; n = 140.

INTERPRETATION OF ANALYTICAL RESULTS

Ammonium Concentrations

The 30-day storage stability of ammonium in field-filtered samples from each of the 15 collection sites was statistically equivalent for each of the three amendments. This was the case for both small- and large-bottle splits (see figs. 4 a–c). The striking correspondence between minor, ammonium concentration fluctuations in large- and small-bottle splits shown in these figures supports the inference that repeated opening of the large-bottle splits to remove small volumes of sample for reanalysis during the holding-time study had little effect on ammonium storage stability. Storage stability of ammonium was statistically equivalent in surface- and ground-water samples, although ammonium concentrations in ground-water splits from all three collection sites were near the method detection limit. Furthermore, analyses of variance and covariance revealed no relation between initial ammonium concentrations and storage stability.

Figures 4 a–c also show the close correspondence of between-day, ammonium concentration fluctuations in environmental samples and in concurrently determined USEPA check standards. Comparison of data listed in tables 12 and 13 indicates that analytical method variability accounts for about half of the about 5 percent between-day ammonium concentration variability observed for environmental samples.

Figure 4 a–c — Near Here

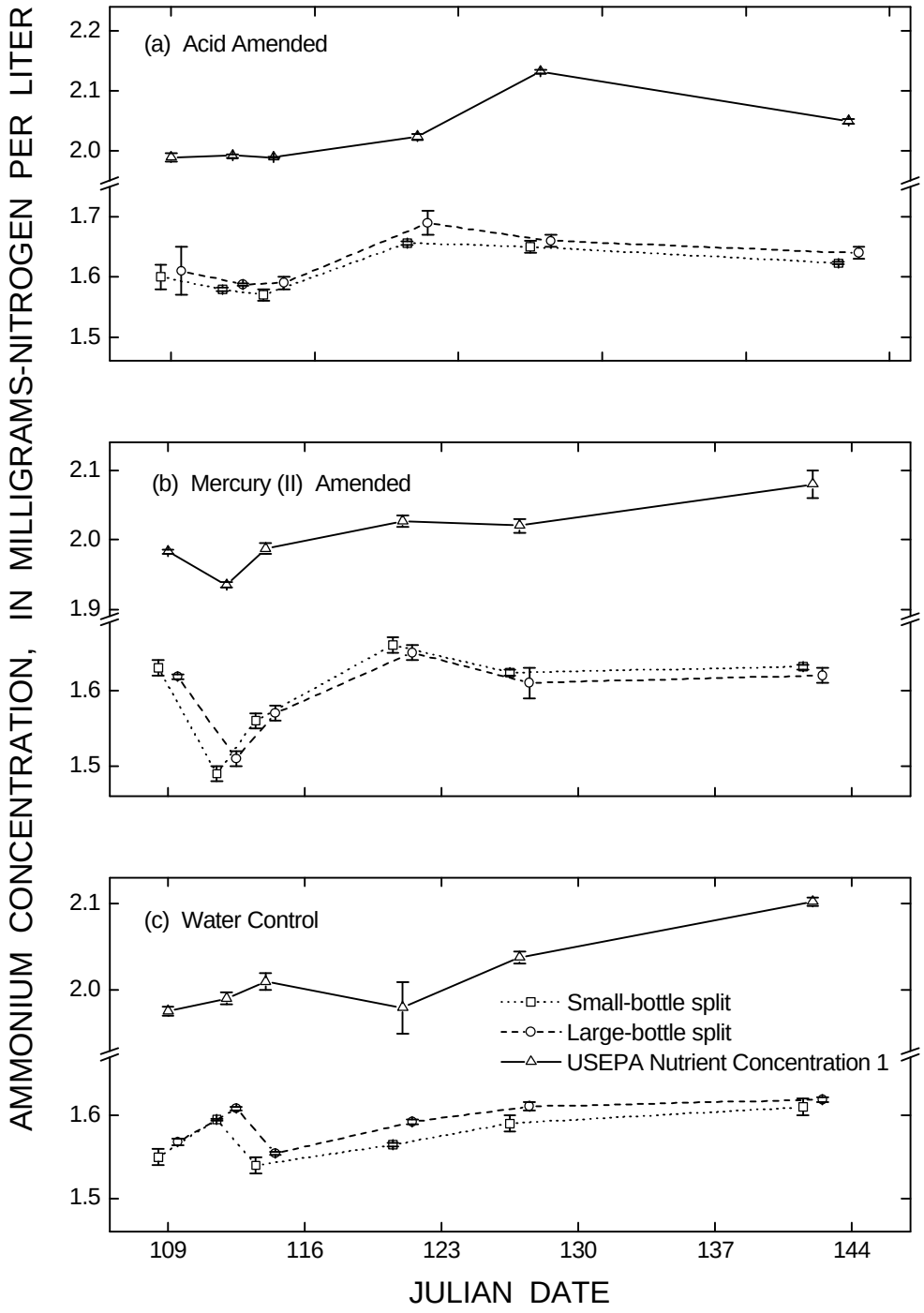

Figure 4. Plots of ammonium concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 2 along with concurrently determined U.S. Environmental Protection Agency (USEPA) check standards. In these plots, symbols for large- and small-bottle splits are offset by ±0.5 Julian day for clarity.

Six-determination-date averages and standard deviations of ammonium concentrations in small-bottle splits from each collection site are shown in figure 5. In this figure, error bars indicate one standard deviation. Note that the larger error bars on water-amended, small-bottle splits from stations 14 and 15 are caused by about 30 percent decreases (≈ 0.04 mg NH_4^+–N/L) in ammonium concentrations that had occurred on the last determination date relative to the previous five. The same effect was observed in large-bottle, water-amended splits from these two stations, which suggests sample processing equipment or sample containers, or both, may have introduced biota into these samples after they were filtered. This hypothesis is supported by tritiated adenine uptake values (an indicator of biological activity) for water-amended, small-bottle splits from these two stations, which were higher than those from other stations (see fig. 30 in Appendix A). Greater than average between-day variability in ammonium concentrations for samples from stations 7 and 9 is also shown in figure 5. The high salt content of samples from station 7 (specific conductance = 4,250 µS/cm) and station 9 (specific conductance = 3,000 µS/cm) relative to that of sampler wash solutions caused periodic refractive index changes in the analytical stream, which in turn increased the background absorbance (see "Analytical Methods and Procedures"). These perturbations should be greatest for water-amended samples, which were analyzed using a deionized water wash solution. Such refractive index effects are most apparent in samples with concentrations near the MDL, as was the case for ammonium concentrations in samples from stations 7 and 9.

Figure 5 — Near Here

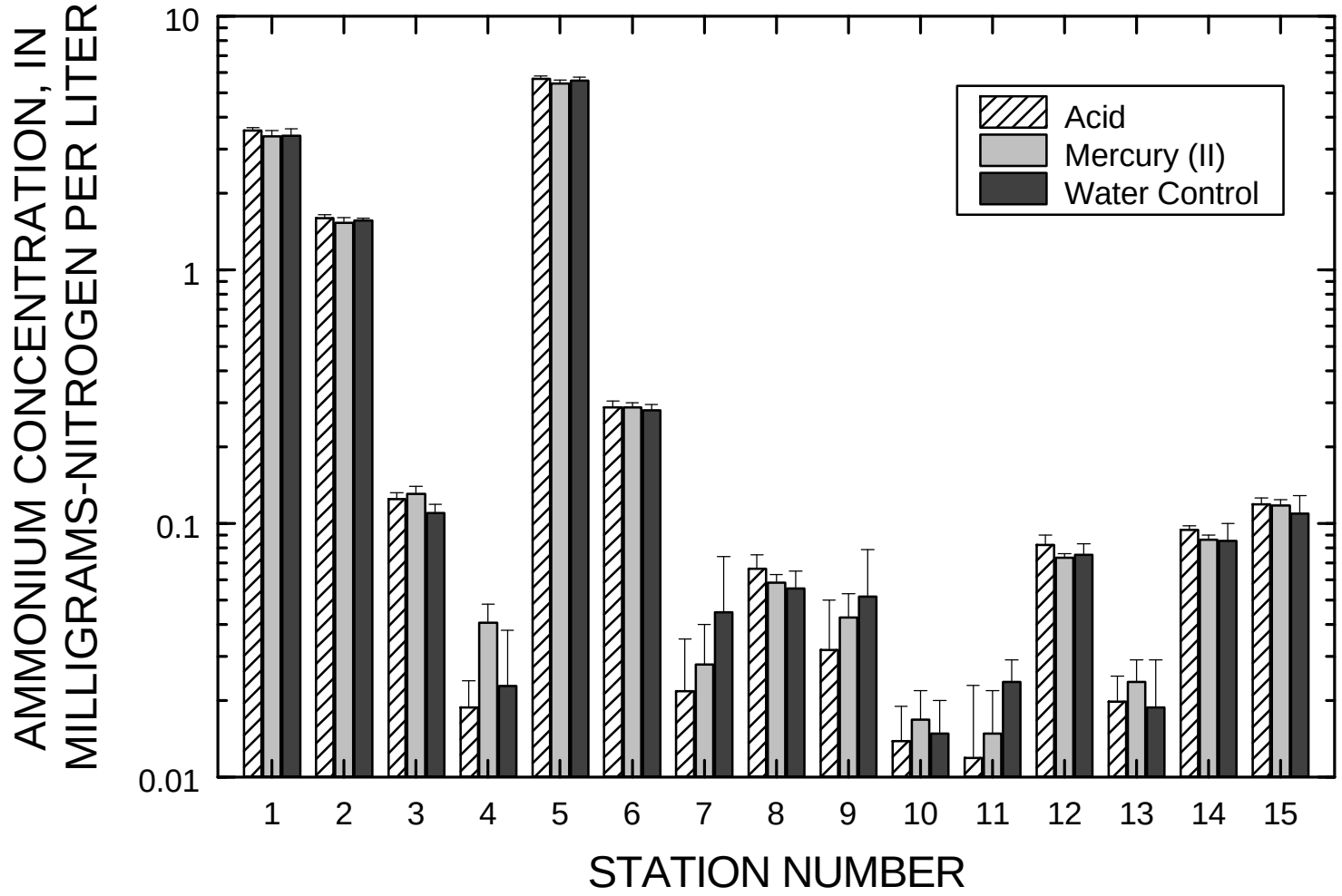

Figure 5. Bar chart of six-determination-date-average concentrations of ammonium determined in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation. See supporting text for additional details.

Nitrate Plus Nitrite

The 30-day storage stability of nitrate plus nitrite in field-filtered samples from each of the 15 collection sites was statistically equivalent for each of the three amendments. This was the case for both small- and large-bottle splits (see figures 6 a–c). The striking correspondence between minor, nitrate plus nitrite concentration fluctuations in large- and small-bottle splits shown in these figures supports the inference that repeated opening of the large-bottle splits to remove small volumes of sample for reanalysis during the holding-time study had little effect on nitrate plus nitrite storage stability. Storage stability of nitrate plus nitrite was statistically equivalent in surface- and ground-water samples, where initial concentrations ranged from 0.04 to 8.7 mg-N/L in the former and from 2.4 to 56 mg-N/L in the latter. As was the case for other analytes, analyses of variance and covariance revealed no relation between initial nitrate plus nitrite concentrations and storage stability.

Six-determination-date averages of nitrate plus nitrite concentrations in small-bottle splits from each collection site are shown in figure 7. In this figure, error bars indicate one standard deviation. The 11 stations with nitrate plus nitrite concentrations in excess of 10 MDLs have a pooled RSD of about 3 percent for all three amendments (see table 12)—roughly one-third greater than that observed for concurrently determined USEPA check standards (see table 12).

The extent of reduction of nitrate to nitrite was assessed several times during each run because nitrate was determined as nitrite after reduction to that species with cadmium metal ($NO_3^- + Cd^0 + 2\ H^+ \rightarrow NO_2^- + Cd^{2+} + H_2O$). Assessment involved sequential determination of a nitrite and a nitrate standard, each having a nominal concentration of 1 mg-N/L. Percent

Figure 6 & 7 — Near Here

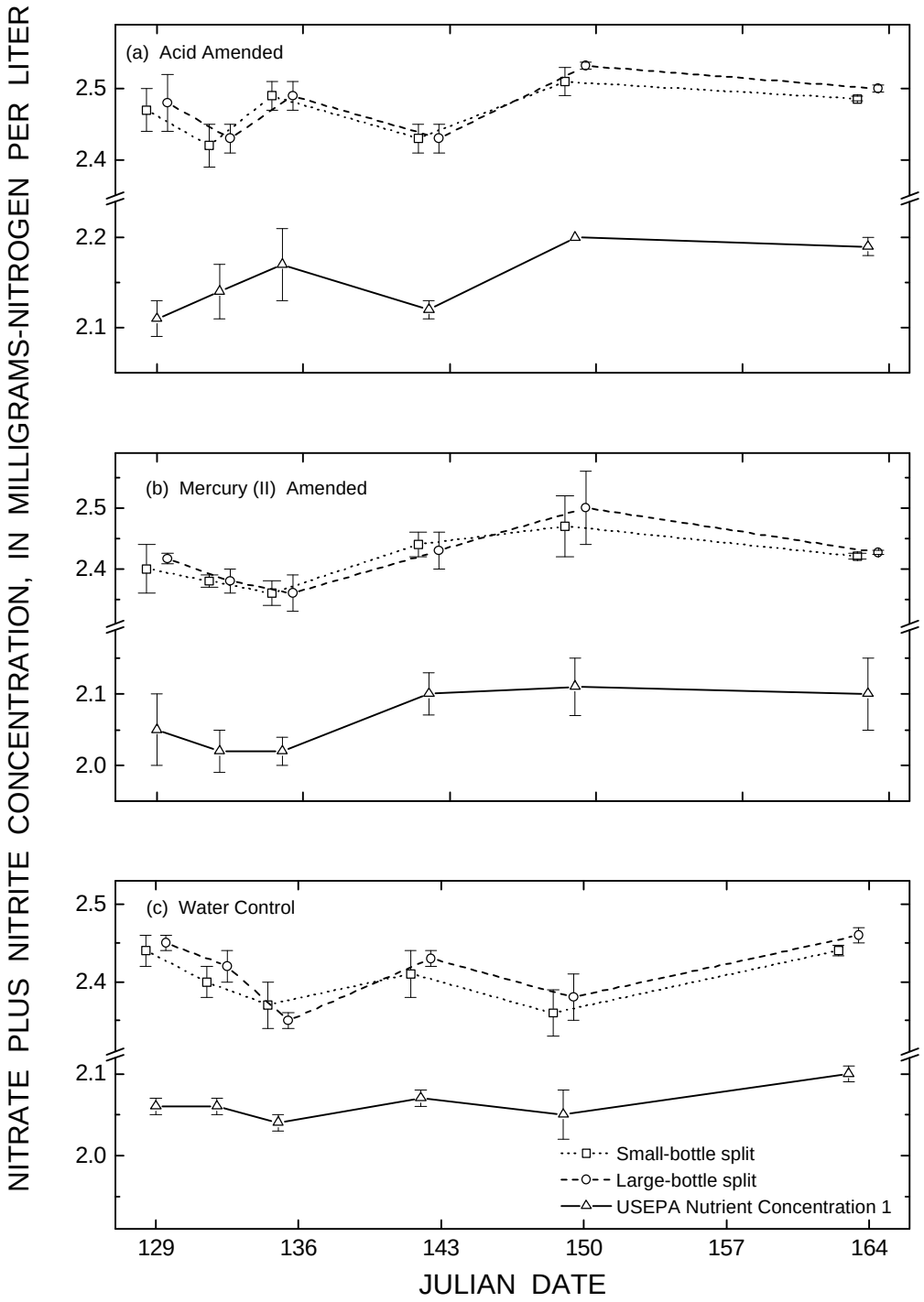

Figure 6. Plots of nitrate plus nitrite concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 10 along with concurrently determined U.S. Environmental Protection Agency (USEPA) check standards. In these plots, symbols for large- and small-bottle splits are offset by ±0.5 Julian date for clarity.

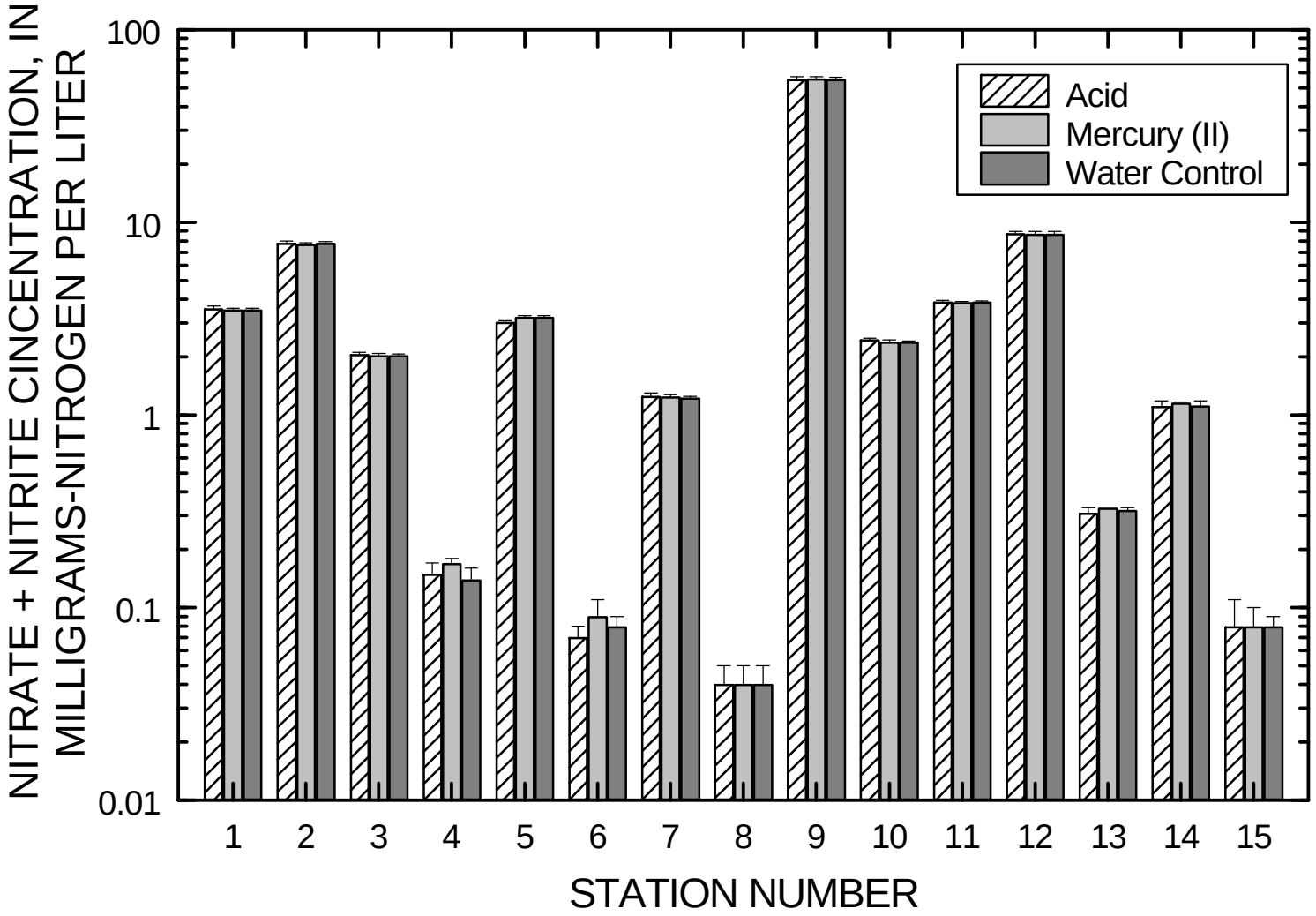

Figure 7. Bar chart of six-determination-date-average concentrations of nitrate plus nitrite determined in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation.

reduction was defined as the concentration ratio of nitrate to nitrite multiplied by 100. Three pairs of these check standards were prepared in matrices that corresponded to the three amendments for environmental samples. As presented in the "Analytical Methods and Procedures" section, long-term (between April 13, and June 29, 1992) percent reduction was 102 ± 2 in the water-control matrix, 100 ± 4 in the mercury (II) matrix, and 123 ± 13 in the sulfuric acid matrix. High percent reduction in the acid matrix is an artifact caused by the instability of nitrite in the acid matrix, as discussed in the "Analytical Methods and Procedures" section.

Between-day nitrate plus nitrite concentration fluctuations in acid-, mercury (II)-, and water-amended splits from four stations at which the initial concentrations of these species differed substantially are shown in figure 8. Note that although the nitrite concentrations in acid-amended splits (also shown in fig. 8) decrease monotonically in relation to time, nitrate plus nitrite concentrations in acid-amended splits remain constant. Inspection of data in this figure, however, indicates that relative to water or mercury (II) amendments, acid amendment may, for some samples, destabilize nitrate plus nitrite concentrations during storage—see plots (b) and (d)—or result in low estimates of nitrate plus nitrite concentrations—see plot (c).

Nitrite

No statistically significant differences in 30-day storage stability of nitrite in field-filtered, large- or small-bottle splits from the 15 collection sites were detected between mercury and water amendments. Nitrite was unstable at all concentrations in acid-amended splits, however, as shown in figure 9. Here nitrite concentrations are plotted as a function of determination date for small-bottle splits from stations 1, 2, 5, and 7, which had initial concentrations in the range of 0.04 to 1.8 mg-N/L. Note that initial nitrite concentrations were always lower in acid-amended splits than in mercury (II)-amended or

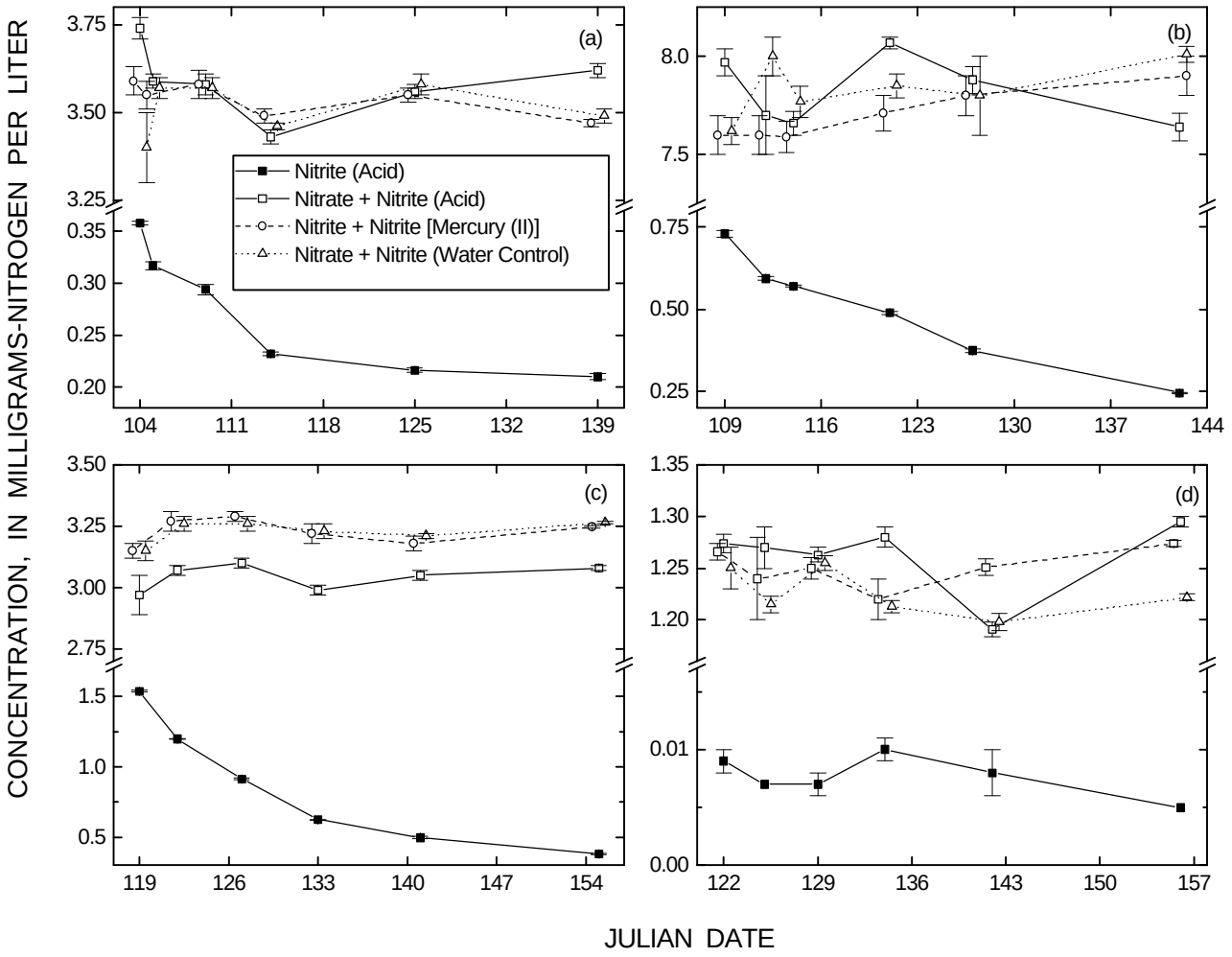

Figure 8. Between-day fluctuations in nitrate plus nitrite concentrations in small-bottle splits as a function of analysis date for stations 1 (a), 2 (b), 5 (c), and 7 (d). Error bars indicate ± 1 standard deviation. Symbols for mercury (II)- and water-amended splits are offset by ± 0.5 Julian day for clarity.

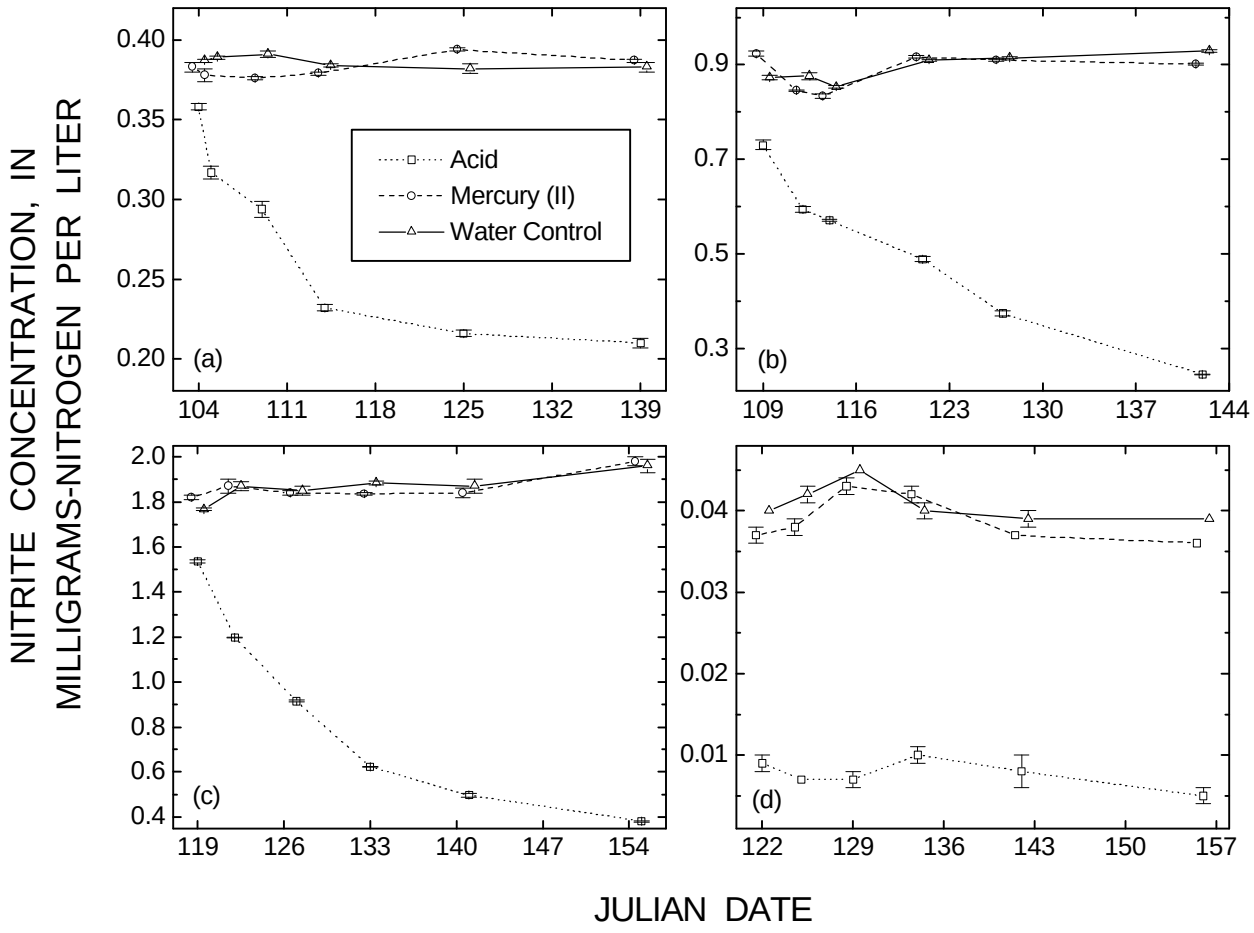

Figure 9. Plots of nitrite concentrations in acid-, mercury (II)-, and water-amended small-bottle splits as a function of analysis date for stations 1 (a), 2 (b), 5 (c), and 7 (d). Error bars indicate ±1 standard deviation. Symbols for mercury (II)- and water-amended splits are offset by ±0.5 Julian day for clarity.

water-amended ones and that when initial nitrite concentrations were low—as at station 7, for example—most of the nitrite in acid-amended splits had decomposed prior to initial determinations. As mentioned previously, the instability of nitrite in acidified water samples is well documented (Brezonic and Lee, 1966; Howe and Holley, 1969; Delfino, 1979; Williams, 1979; Roman and others, 1991), and is explained for the most part by chemical oxidation of nitrite to nitrate. Six-determination-date averages and standard deviations of nitrite concentrations in small-bottle splits for each of the three amendments as a function of station number are shown in figure 10. The instability of nitrite in acid-amended splits (lower average concentrations and higher standard deviations) is evident in this figure.

As was the case for other analytes included in this study, between-day fluctuations of nitrite concentrations in large- and small-bottle splits typically corresponded closely (see fig. 11). The single exception was the water-amended, large-bottle split from station 1, in which the nitrite concentration dropped precipitously—from ≈0.35 to ≈0.01 mg-N/L—between the fifth and the final measurement dates; nitrite concentrations in the corresponding small-bottle splits remained constant over this interval. As discussed in the "Analytical Methods and Procedures" section, the small-bottle splits were selected at random on each analysis date, used once, and discarded, whereas large-bottle splits were subject to repeated openings and closings. Interestingly, concentrations of other analytes in this large-bottle split remained constant throughout the experiment. It is possible that the large-bottle split became contaminated with microorganisms, which, in the absence of biocides, could have metabolized nitrite. It is also possible that nitrite in this split was oxidized or reduced to an unanalyzed species by some purely chemical pathway. Regardless of the mechanism involved, this result underscores what seasoned analysts know from experience—that in some cases, obtaining accurate estimates of nutrient concentrations depend critically on analyzing samples as quickly as possible after they are collected and on minimizing exposure of samples to the external environment.

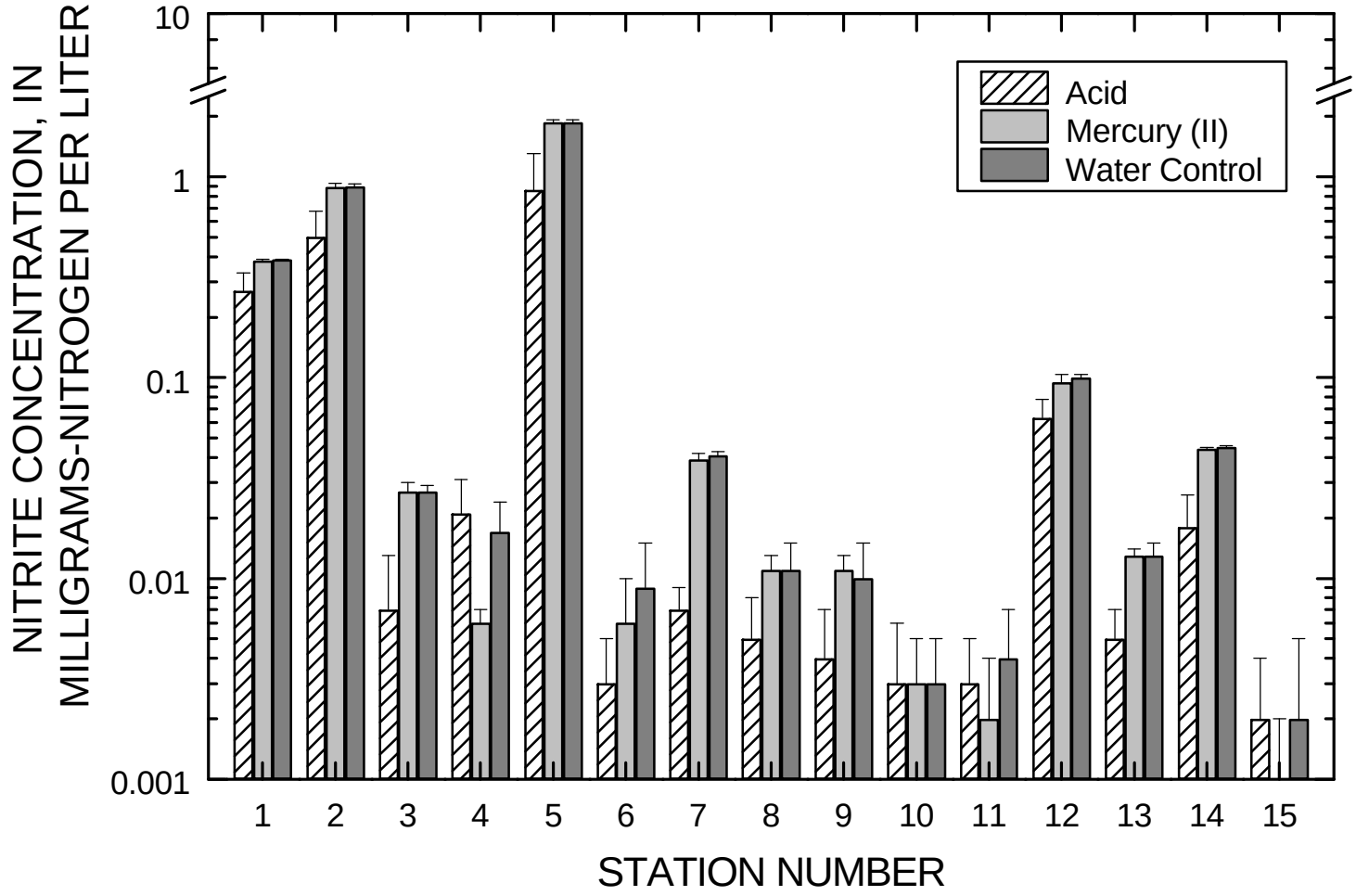

Figure 10. Bar chart of six-determination-date-average concentrations of nitrite determined in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation.

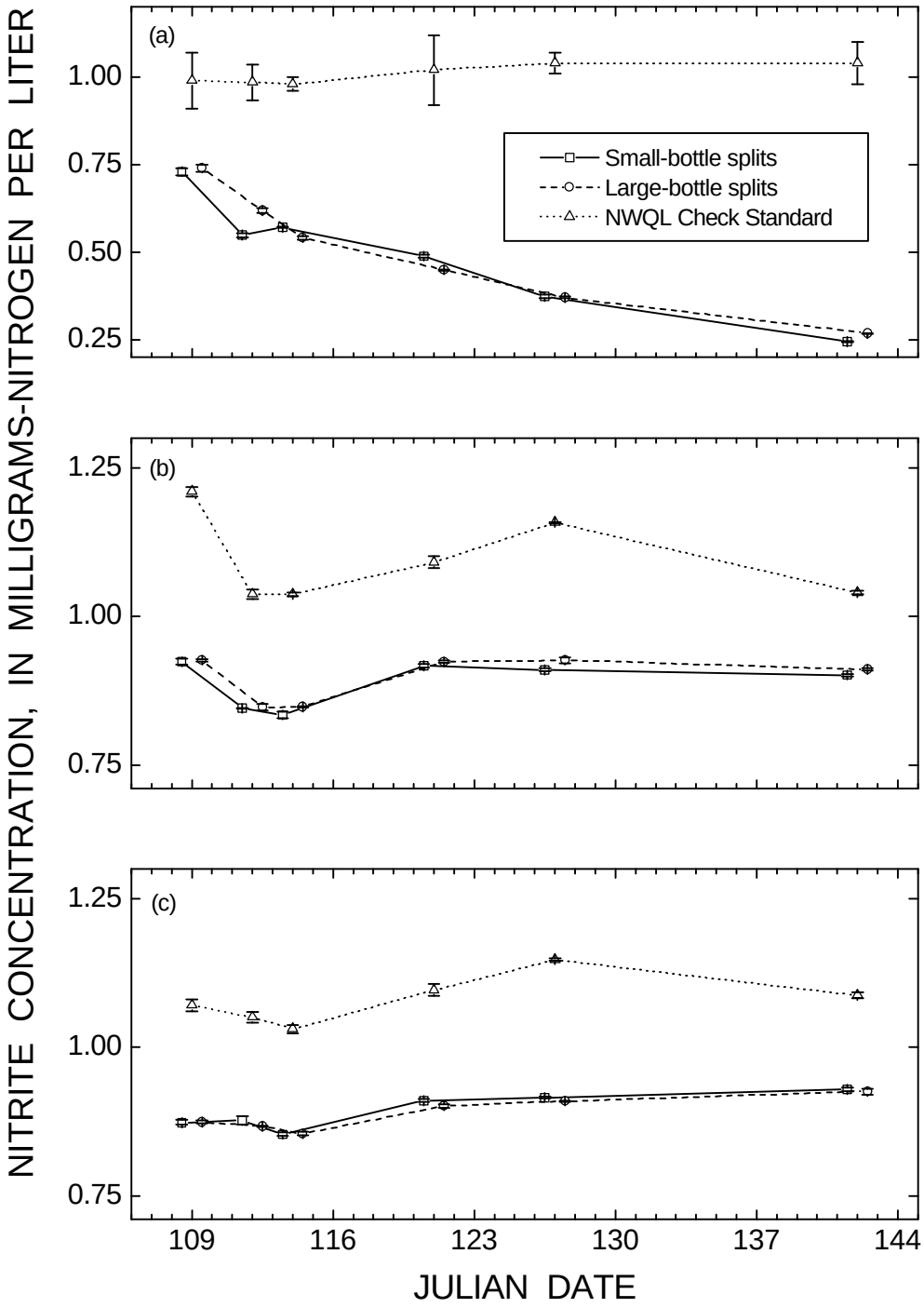

Figure 11. Plots of nitrite concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 2 along with concurrently determined National Water Quality Laboratory (NWQL) prepared nitrite check standards. In these plots, symbols for large- and small-bottle splits are offset by ±0.5 Julian day for clarity.

Although no certified check standard was available for nitrite, the relative standard deviation of the 1 mg NO_2^--N/L standards prepared in each amendment matrix, which were used to check the cadmium reactor reduction efficiency, was about 6 percent (see table 12). The acid-amended check standards were prepared just prior to use for each run, and therefore exhibited less variation than natural samples. The seven stations with nitrite concentrations in excess of 10 MDLs had a pooled RSD of about 7 percent for mercury (II)-amended splits and 5 percent for water-amended splits (see table 13)—about the same as that observed for NWQL check standards (see table 12). Storage stability of nitrite was statistically equivalent in surface- and ground-water splits, although nitrite concentrations in ground-water splits from all three collection sites were near the method detection limit.

Orthophosphate

The 30-day storage stability of orthophosphate in field-filtered samples from each of the 15 collection sites was statistically equivalent for each of the three amendments. This was the case for both small- and large-bottle splits (see figs. 12 a–c). The correspondence between minor, orthophosphate concentration fluctuations in large- and small-bottle splits shown in these figures strongly supports the inference that repeated opening of the large-bottle splits to remove small volumes of sample for reanalysis during the holding-time study had little effect on orthophosphate storage stability. Note that the greater between-day variation observed for acid-amended splits from station 7 (see fig. 12) is atypical and careful review of unprocessed data provided no clues to its cause. As discussed below, however, the nonmonotonic nature of between-day variations in acid-amended splits is inconsistent with a mechanism involving hydrolysis of polyphosphates.

For the eight stations at which the six-determination-date average orthophosphate concentration exceeded 10 MDLs, pooled RSD estimates were about 3 percent (see table 13), similar to that observed for concurrently determined USEPA check standards

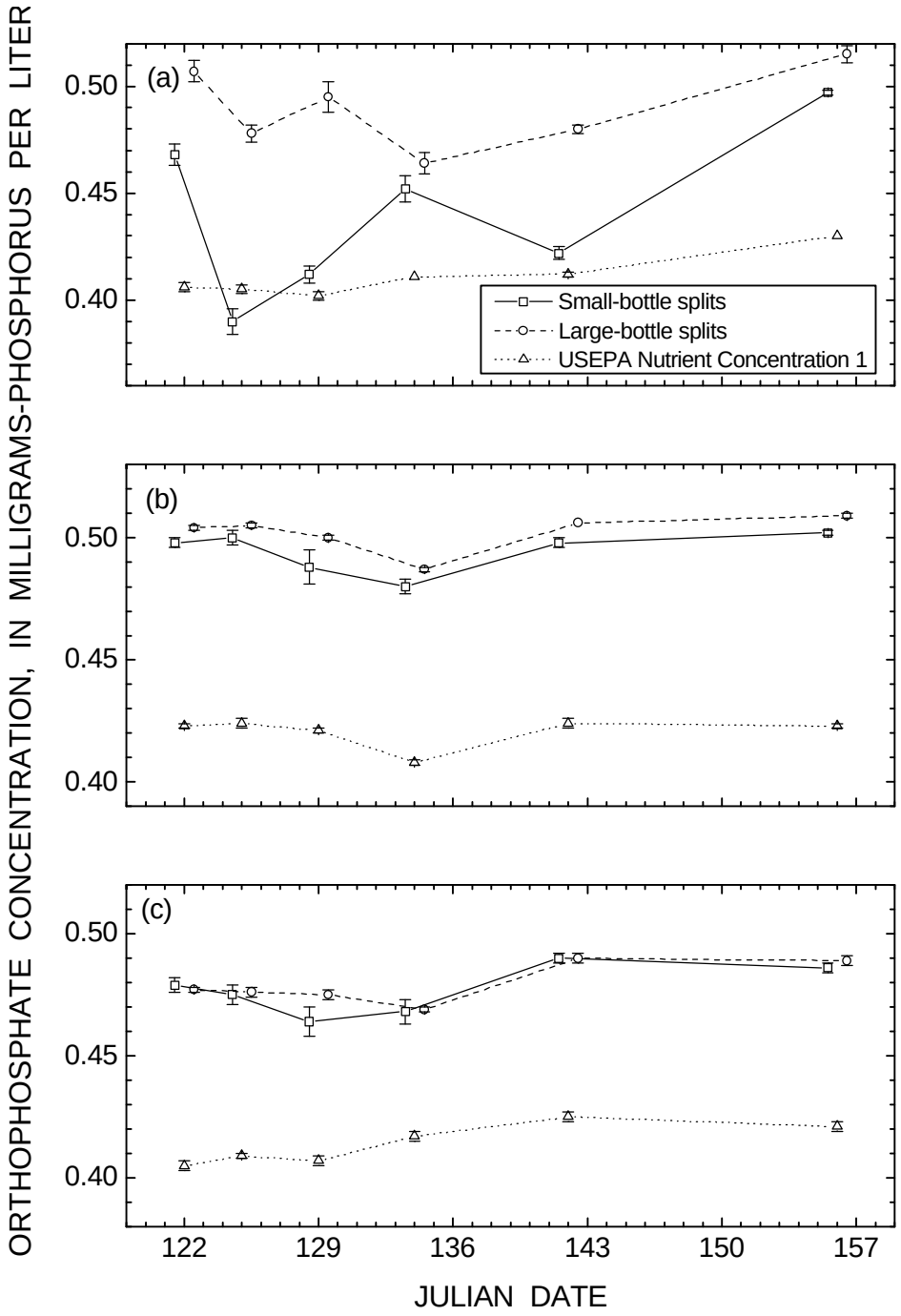

Figure 12. Plots of orthophosphate concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 7 along with concurrently determined U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 1 check standards. In these plots, symbols for large- and small-bottle splits are offset by ±0.5 Julian day for clarity.

(see table 12). Thus analytical method variability accounts for most of the between-day orthophosphate concentration variability observed in environmental samples. Storage stability of orthophosphate was statistically equivalent in surface and ground water, although orthophosphate concentrations in ground-water splits from all three collection sites were near the method detection limit. Furthermore, analyses of variance and covariance revealed no relation between initial orthophosphate concentrations and storage stability.

Six-determination-date averages and standard deviations of orthophosphate concentrations in small-bottle splits from each collection site are shown in figure 13. The poor precision of orthophosphate concentrations determined in all amendment treatments for splits from station 9 has two causes. The first is a refractive index mismatch between reagent blanks (deionized water matrix) and the samples caused by the high salt content of the latter—Cl^- ≈ 160 mg/L; SO_4^{-2} ≈ 1,200 mg/L; specific conductance ≈3,000 µS/cm—(see data for well 21 in table 6 of Gaggiani, 1991, p. 123 and 124). The second is color reaction interference (Duff and Stuart, 1971) caused by the high nitrate concentration in these samples. Small (≈0.02 mg-P/L), but analytically significant, changes in orthophosphate concentration that occurred during the last half of the measurement periods in water-amended splits from stations 13, 14, and 15 account for the larger error bars for this treatment evident in figure 13. These changes may have been biologically mediated, since small-bottle splits from these stations exhibited higher tritiated adenine uptake (an indicator of biological activity) than those from other stations (see fig. 30 in Appendix A).

We anticipated that the apparent orthophosphate concentration in acid-amended samples from at least some of the stations would increase significantly during storage because of acid hydrolysis of polyphosphate compounds. As shown in figure 14, the close correspondence of orthophosphate concentrations in splits for all three amendments (panels *a–c*) and the lack of any monotonic increase of orthophosphate concentrations in

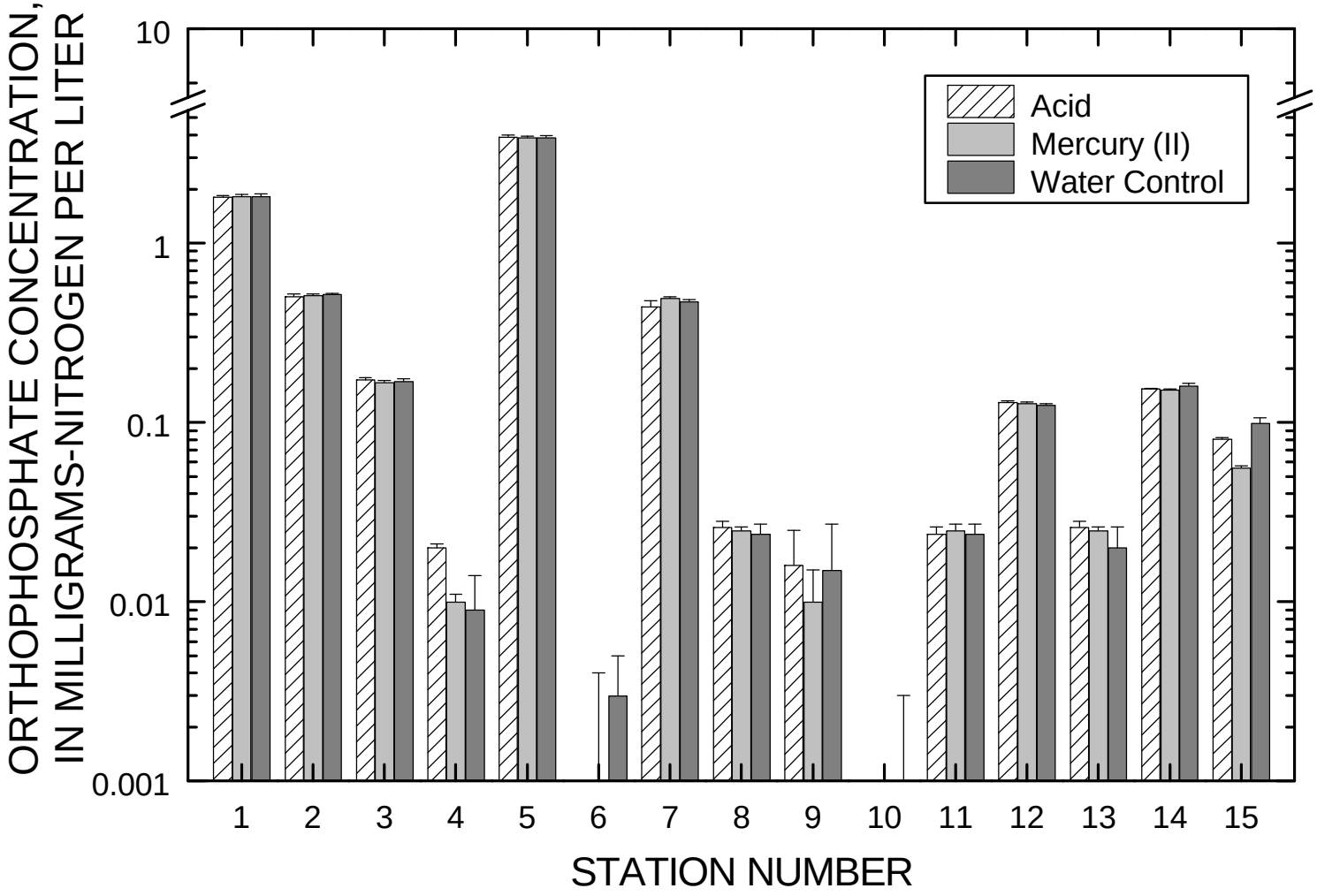

Figure 13. Bar chart of six-determination-date-average concentrations of orthophosphate determined in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation. Break in the y-axis occurs between 5.5 and 8.7 mg-P/L.

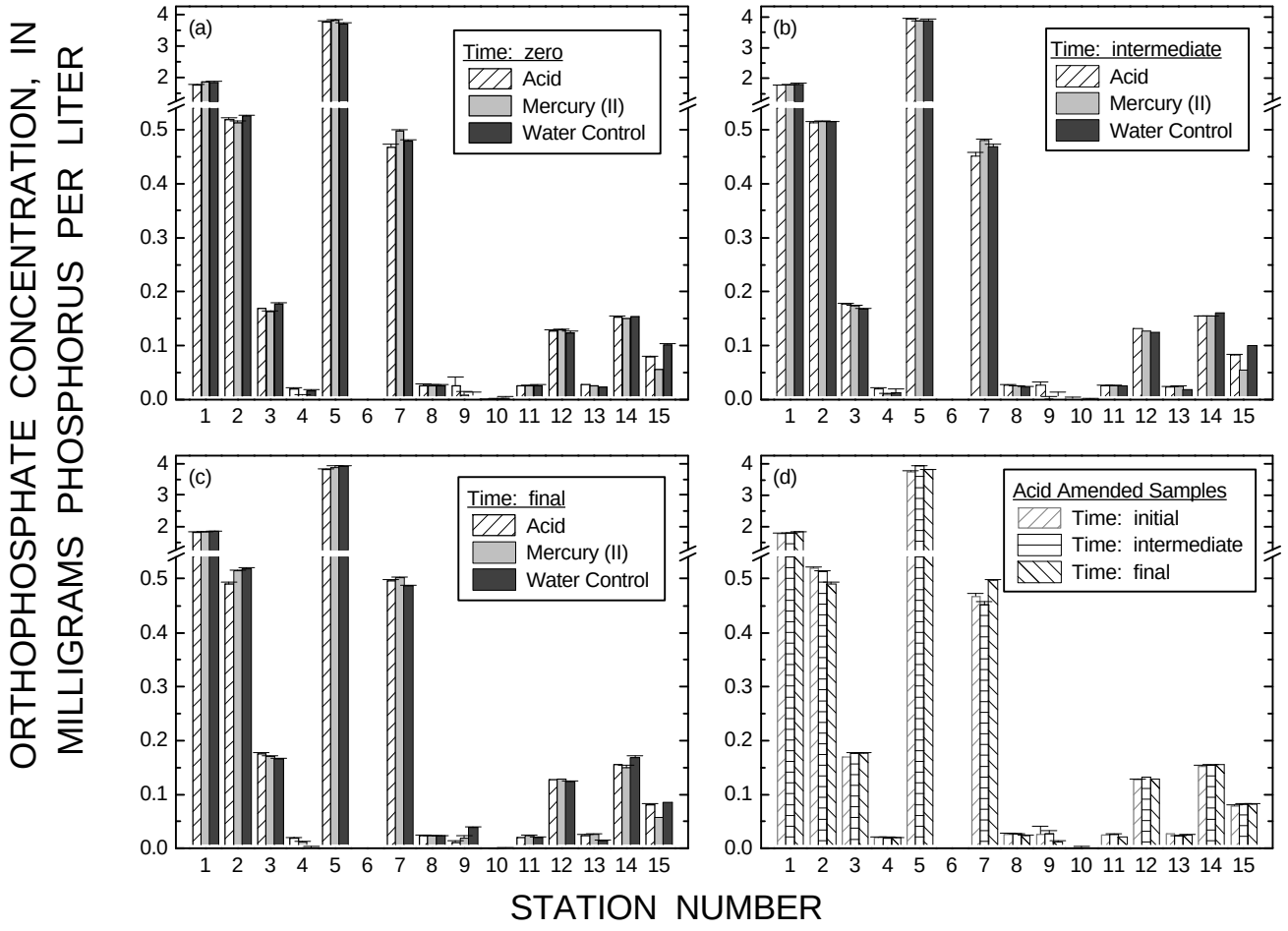

Figure 14. Bar charts of initial (a), intermediate (b), and final (c) orthophosphate concentrations in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. In panel (d), the three columns centered over each station number represent one of the three measurement times for acid-amended splits only, as identified on the figure. Error bars indicate 1 standard deviation.

acid-amended splits at initial, intermediate (day 21, typically), and final measurement times indicates that this did not occur (panel *d*). It seems possible that previously reported increases of orthophosphate concentration in acid-amended samples during storage may have been caused by release of orthophosphate from lysed cells in whole-water samples, rather than hydrolysis of polyphosphates. It may also be possible that the absence of polyphosphates in modern household detergents has caused ambient concentrations of polyphosphates in natural water to decrease substantially over the past 20 years.

Kjeldahl Nitrogen

Dissolved samples

The 30-day storage stability of dissolved Kjeldahl nitrogen in field-filtered samples from each of the 15 collection sites was statistically equivalent for each of the three amendments. This was the case for both small- and large-bottle splits (see figs. 15 a–c). The correspondence between minor, dissolved Kjeldahl nitrogen concentration fluctuations in large- and small-bottle splits shown in these figures strongly supports the inference that repeated opening of the large-bottle splits to remove small volumes of sample for reanalysis during the holding-time study had little effect on dissolved Kjeldahl nitrogen storage stability. Storage stability of dissolved Kjeldahl nitrogen was statistically equivalent in surface- and ground-water samples, where initial concentrations ranged from about 0.2 to about 7 mg-N/L in the former and from <0.05 to about 1.5 mg-N/L in the latter. Analyses of variance and covariance revealed no relation between initial dissolved Kjeldahl nitrogen concentrations and storage stability.

Analytical method variability accounted for only about 50 percent of the between-day concentration variations in mercury (II)- and water-amended splits and 25 percent of the between-day concentration variations in acid-amended splits (see tables 12 and 13) on the basis of pooled RSD estimates for the seven stations where dissolved Kjeldahl nitrogen concentrations exceeded 10 MDLs and those of concurrently determined USEPA check standards.

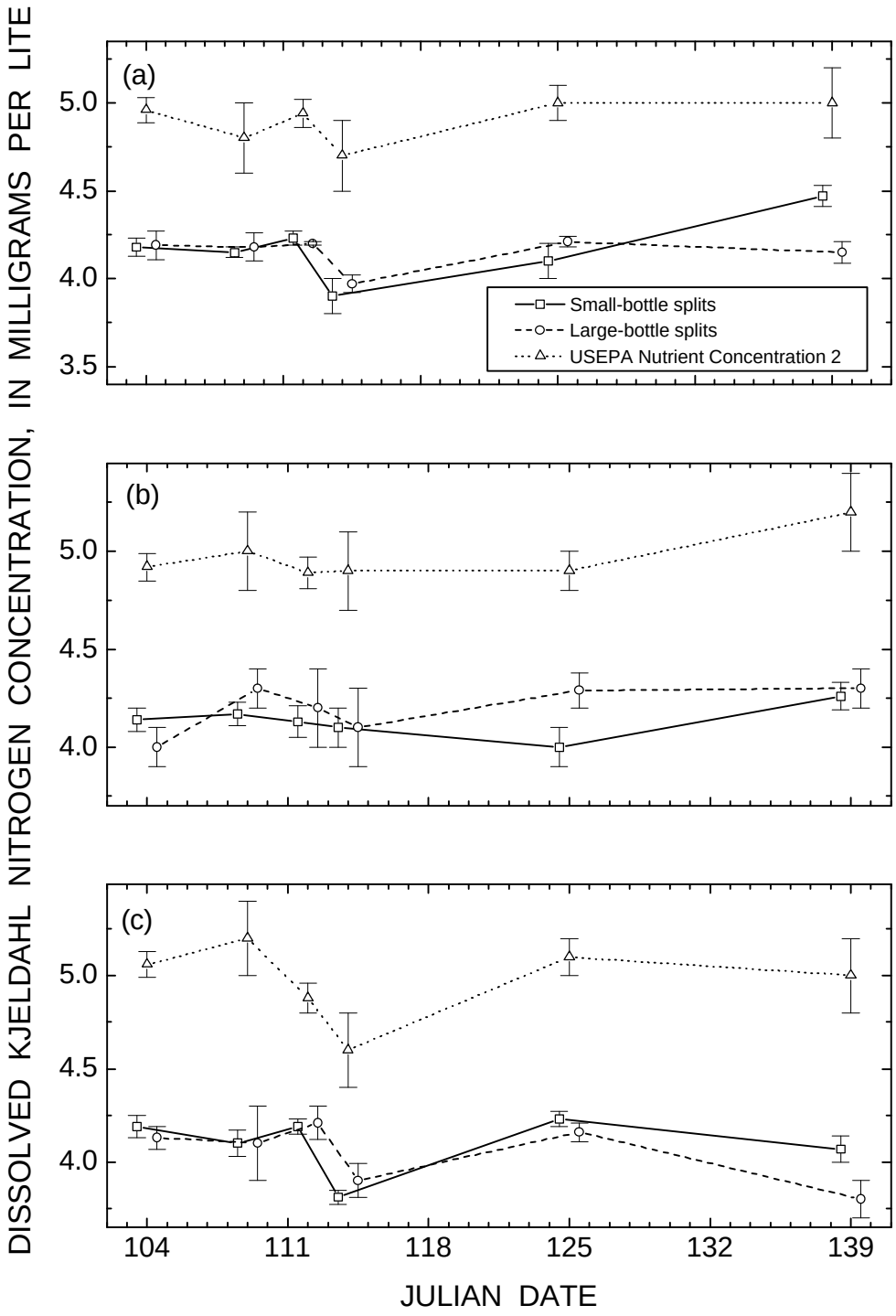

Figure 15. Plots of dissolved Kjeldahl nitrogen concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 1 along with concurrently determined U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 2 check standards. Error bars indicate ±1 standard deviation. Symbols for large- and small-bottle splits are offset by ±0.5 Julian day for clarity.

Six-determination-date dissolved Kjeldahl nitrogen concentration averages for acid-, mercury (II)-, and water-amended small-bottle splits from each of the 15 collection sites are shown in figure 16.

Whole-water samples

Storage stability of Kjeldahl nitrogen concentrations in whole-water splits was equivalent to that observed for filtered-water splits with the exception of water-amended splits from stations 1, 2, and 5. As shown in figure 17, Kjeldahl nitrogen concentrations in water-amended, whole-water splits from these stations began to decrease monotonically after the first week of storage. Kjeldahl nitrogen concentrations in corresponding acid- and mercury (II)-amended, whole-water splits from these stations remained relatively constant over their entire measurement periods (fig. 17). Lack of storage stability in water-amended splits from these stations is understandable with reference to figure 18. Average ammonium, dissolved Kjeldahl nitrogen, and total Kjeldahl nitrogen concentrations determined in acid-amended, small-bottle splits from each of the 15 stations, which are plotted in figure 18, are representative of concentrations determined in the other amendments and split sizes. Again, with reference to figure 18, note particularly that ammonium concentrations at stations 1, 2, and 5 all exceed 1 mg-N/L and constitute about 75, 50, and 60 percent, respectively, of the Kjeldahl nitrogen concentration. Furthermore, net decreases in Kjeldahl nitrogen concentrations in water-amended splits from each of these stations are roughly equivalent to initial ammonium concentrations in corresponding filtered-water splits. Hence, concentrations that changed during storage likely were caused by losses in the ammonium fraction of Kjeldahl nitrogen, rather than in organic nitrogen or particulate nitrogen fractions. Loss of ammonium from whole-water splits not amended with biocides during extended storage is consistent with a mechanism involving either nitrification ($NH_4^+ \rightarrow NO_3^-$) or denitrification ($NH_4^+ \rightarrow N_2\uparrow$) by bacteria, because in either case, end products

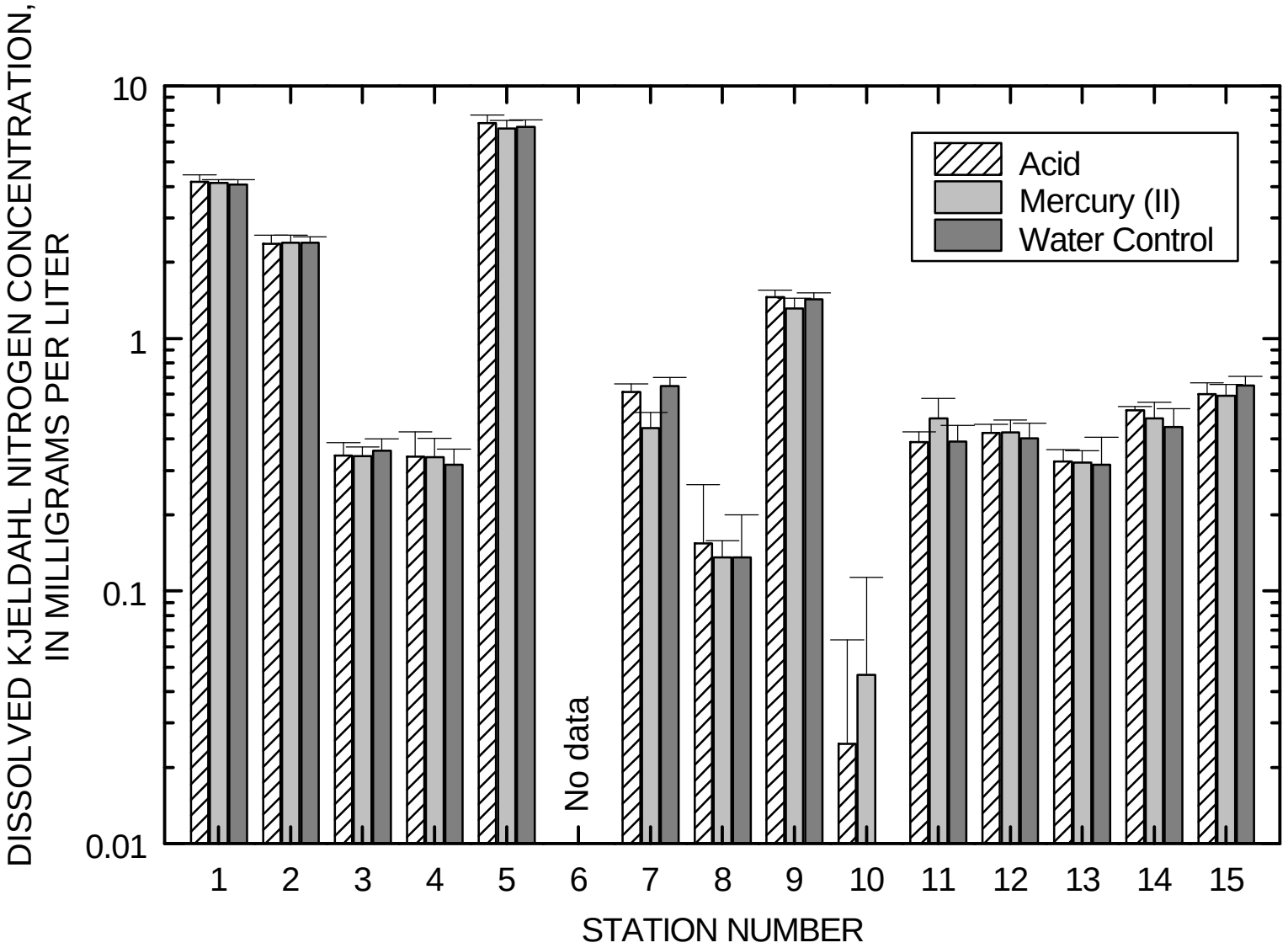

Figure 16. Bar chart of six-determination-date-average concentrations of dissolved Kjeldahl nitrogen determined in filtered, small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation.

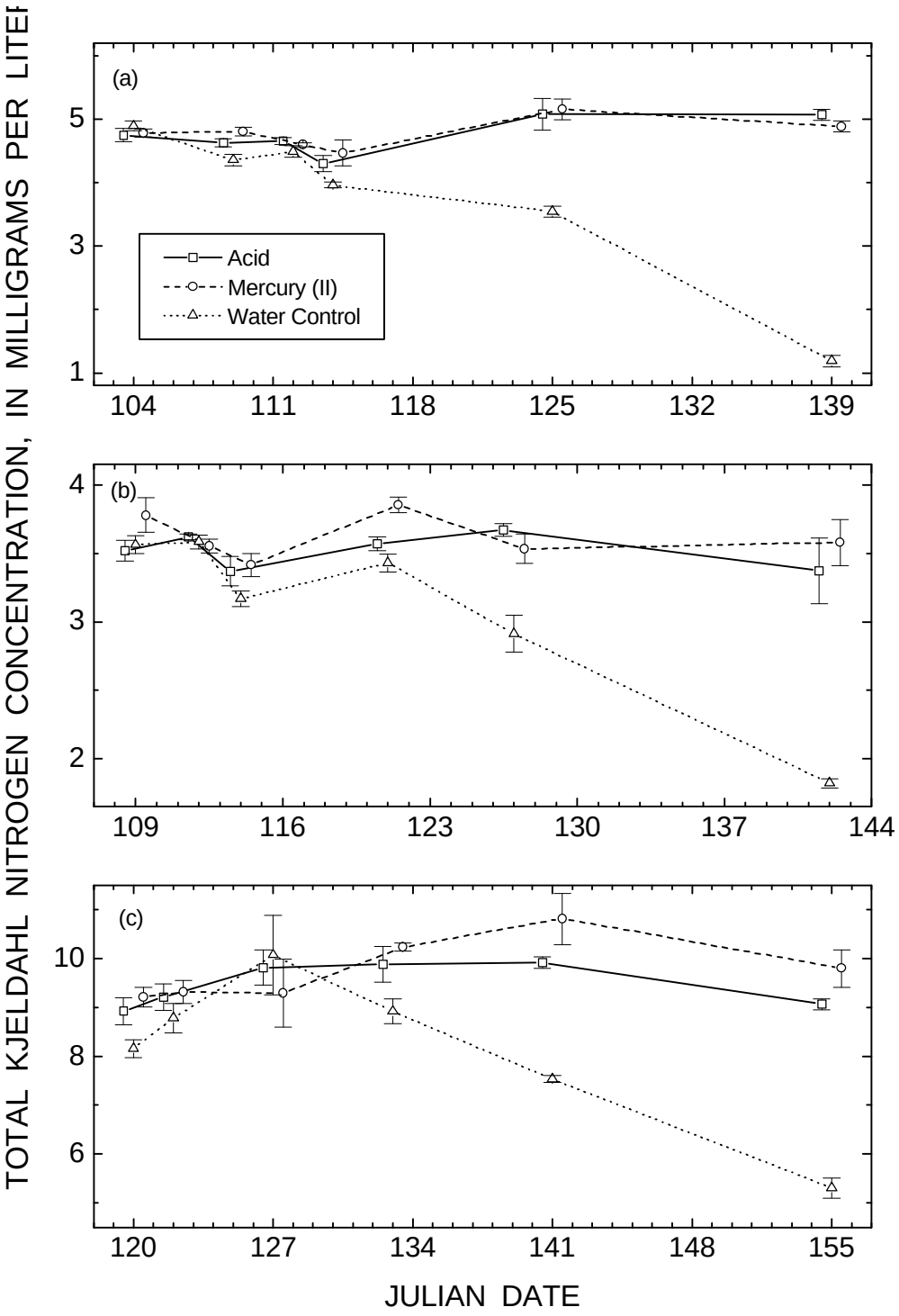

Figure 17. Plots of Kjeldahl nitrogen concentrations in whole-water, small-bottle splits as a function of analysis date for stations 1 (a), 2 (b), and 5 (c). Error bars indicate ±1 standard deviation. Symbols for acid- and mercury (II)-amended splits are offset by ±0.5 Julian day for clarity.

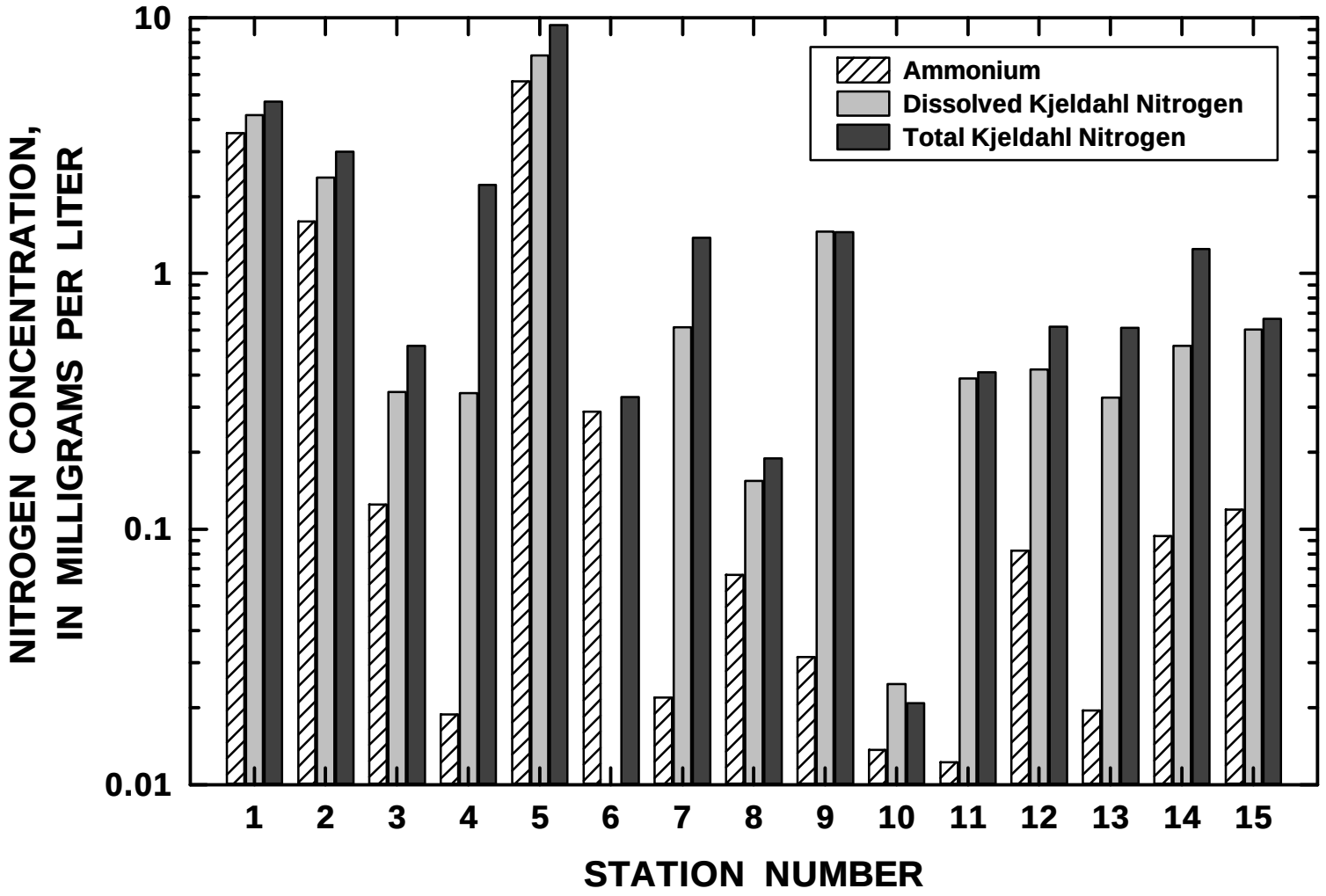

Figure 18. Bar chart of six-determination-date-average concentrations of ammonium, dissolved Kjeldahl nitrogen, and total Kjeldahl nitrogen determined in acid-amended, small-bottle splits from each of the 15 stations.

would not be included in the Kjeldahl nitrogen fraction. The storage stability of Kjeldahl nitrogen in water-amended, whole-water splits from station 6 may at first seem anomalous, since ammonium constitutes 100 percent of the Kjeldahl nitrogen concentration. Note, however, that station 6 is unfiltered tap water (municipal-supply drinking water) from the NWQL in which the biological activity is negligible. Six-determination-date total Kjeldahl nitrogen concentration averages for acid-, mercury (II)-, and water-amended small-bottle splits from each of the 15 collection sites are shown in figure 19.

Phosphorus

Dissolved samples

No statistically significant differences in 30-day storage stability of dissolved phosphorus (the sum of orthophosphate, hydrolyzable phosphorus, and dissolved organic phosphorus) in field-filtered, large- or small-bottle splits from the 15 collections sites were detected among the three amendments. The close correspondence in between-day fluctuations of dissolved phosphorus concentrations in large- and small-bottle splits is shown in figure 20 a–c. This correspondence between large- and small-bottle splits was typical for all stations. The pooled relative standard deviation of all amendments for the 7 stations with dissolved phosphorus concentrations in excess of 10 MDLs was about 5 percent (see table 13), whereas that of concurrently determined USEPA check standards was about 3 percent (see table 12). Thus, analytical method variability accounts for only 60 percent of the between-day concentration variations observed in natural-water samples. Dissolved phosphorus storage stability was statistically equivalent in surface- and ground-water samples, although concentrations in the latter from all three collection sites were near the method detection limit. Analyses of variance and covariance revealed no relation between initial dissolved phosphorus concentrations and storage stability.

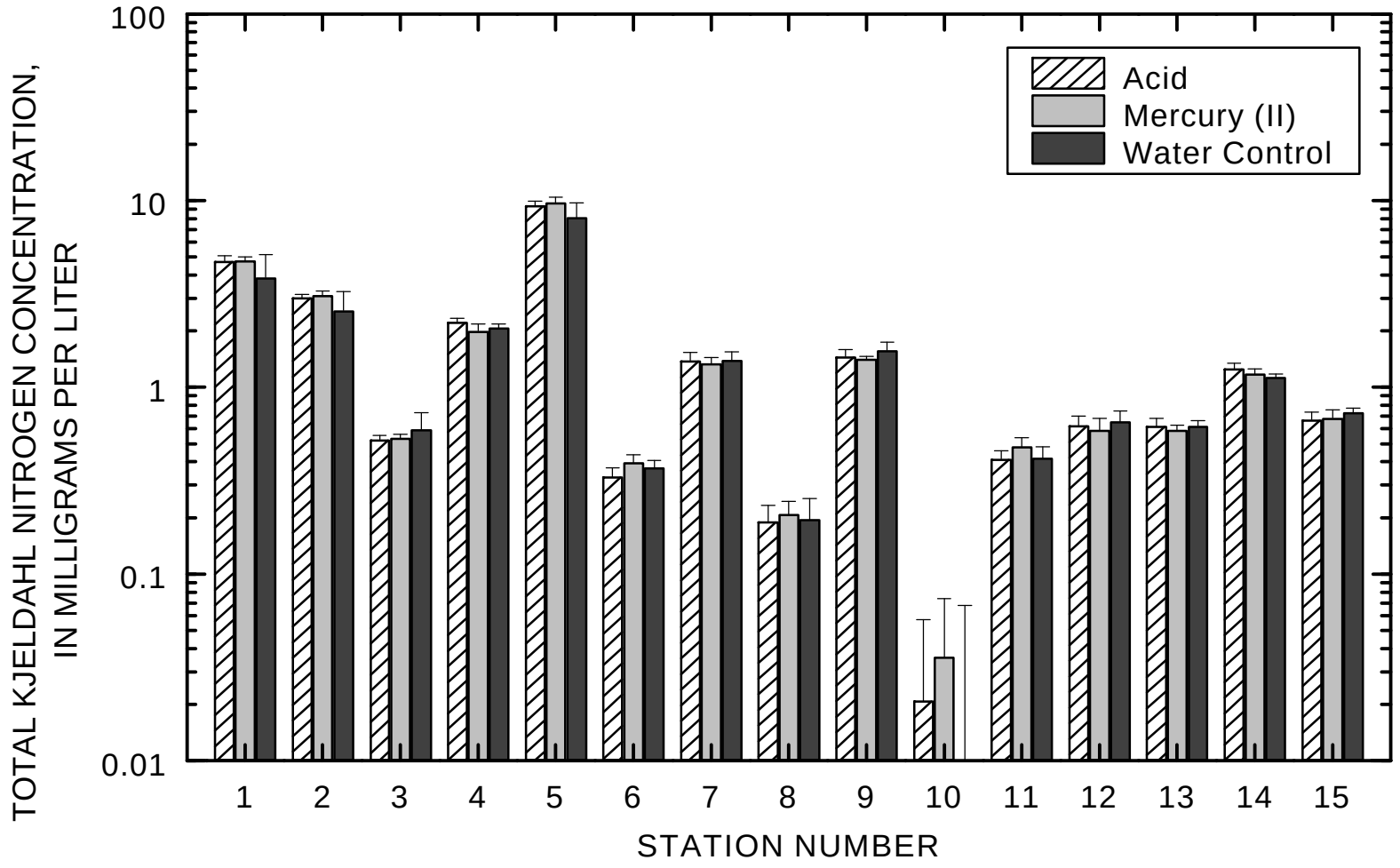

Figure 19. Bar chart of six-determination-date-average concentrations of total Kjeldahl nitrogen determined in whole-water, small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation.

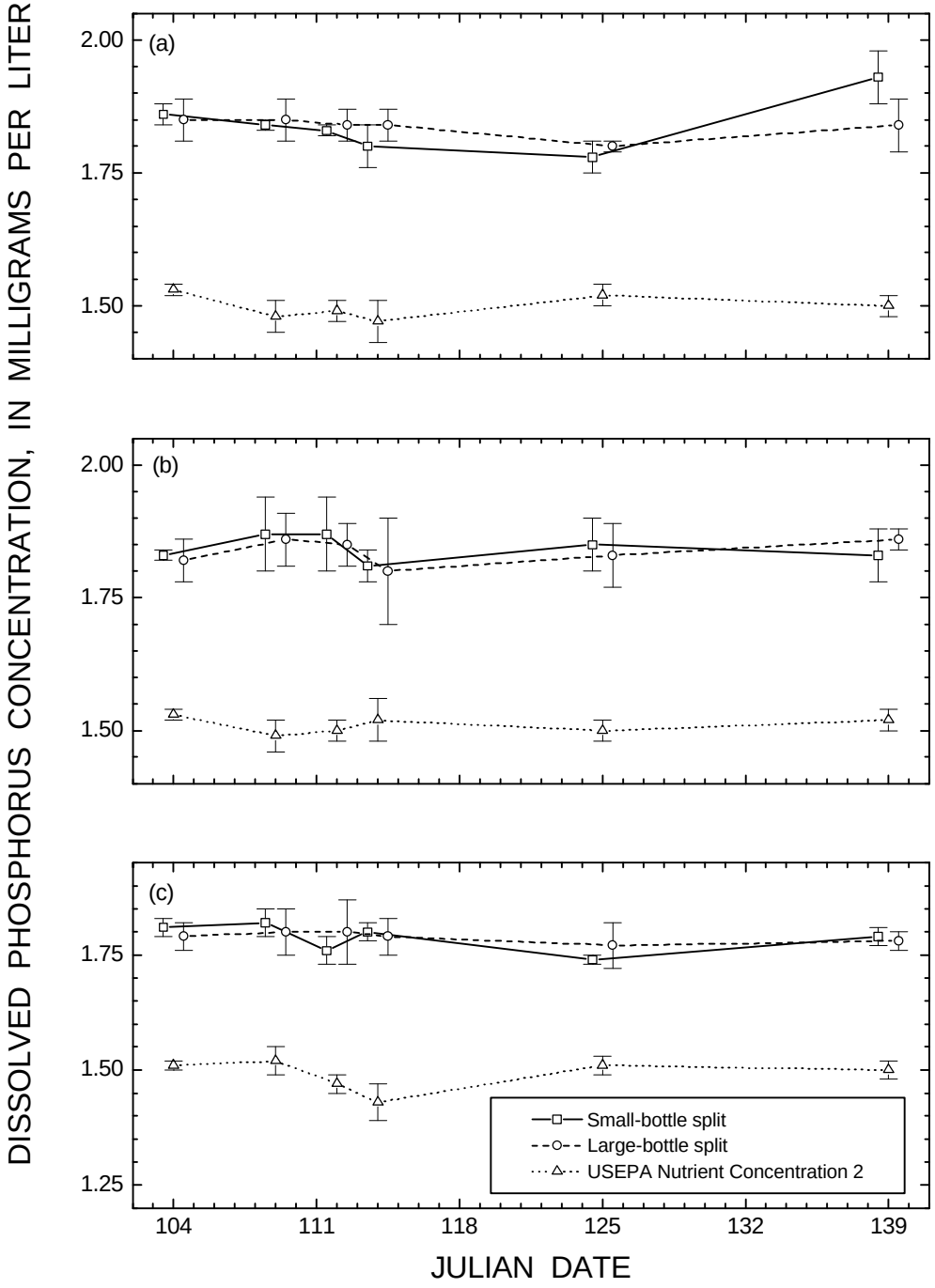

Figure 20. Plots of dissolved phosphorus concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 1 along with concurrently determined U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 2 check standards. Error bars indicate ±1 standard deviation. Symbols for large- and small-bottle splits are offset by ±0.5 Julian day for clarity.

Six-determination-date dissolved phosphorus concentration averages for acid-, mercury (II)-, and water-amended small-bottle splits from each of the 15 collection sites are shown in figure 21. The lack of precision evident for mercury (II)-amended, small-bottle splits from station 9 may be the result of some type of unresolved bottle mix-up—accidental substitution of whole-water splits for filtered-water splits, possibly—that occurred on the first analysis date.

Whole-water samples

Storage stability of total phosphorus (the sum of dissolved phosphorus and particulate phosphorus) concentrations in whole-water splits was equivalent to that observed for filtered-water splits. Typically between-day, total phosphorus concentration fluctuations in large- and small-bottle splits were closely correlated as shown in figure 22 a–c. The pooled relative standard deviation of all amendments for the 11 stations with total phosphorus concentrations in excess of 10 MDLs was about 6 percent (see table 13), whereas that of concurrently determined USEPA check standards was about 3 percent (see table 12). Total phosphorus storage stability was statistically equivalent in surface- and ground-water samples, where initial concentrations ranged from <0.01 to about 5 mg-P/L in the former and from <0.01 to about 0.2 mg-P/L in the latter. Analyses of variance and covariance revealed no relation between initial total phosphorus concentrations and storage stability. Six-determination-date total phosphorus concentration averages for acid-, mercury (II)-, and water-amended small-bottle splits from each of the 15 collection sites are shown in figure 23.

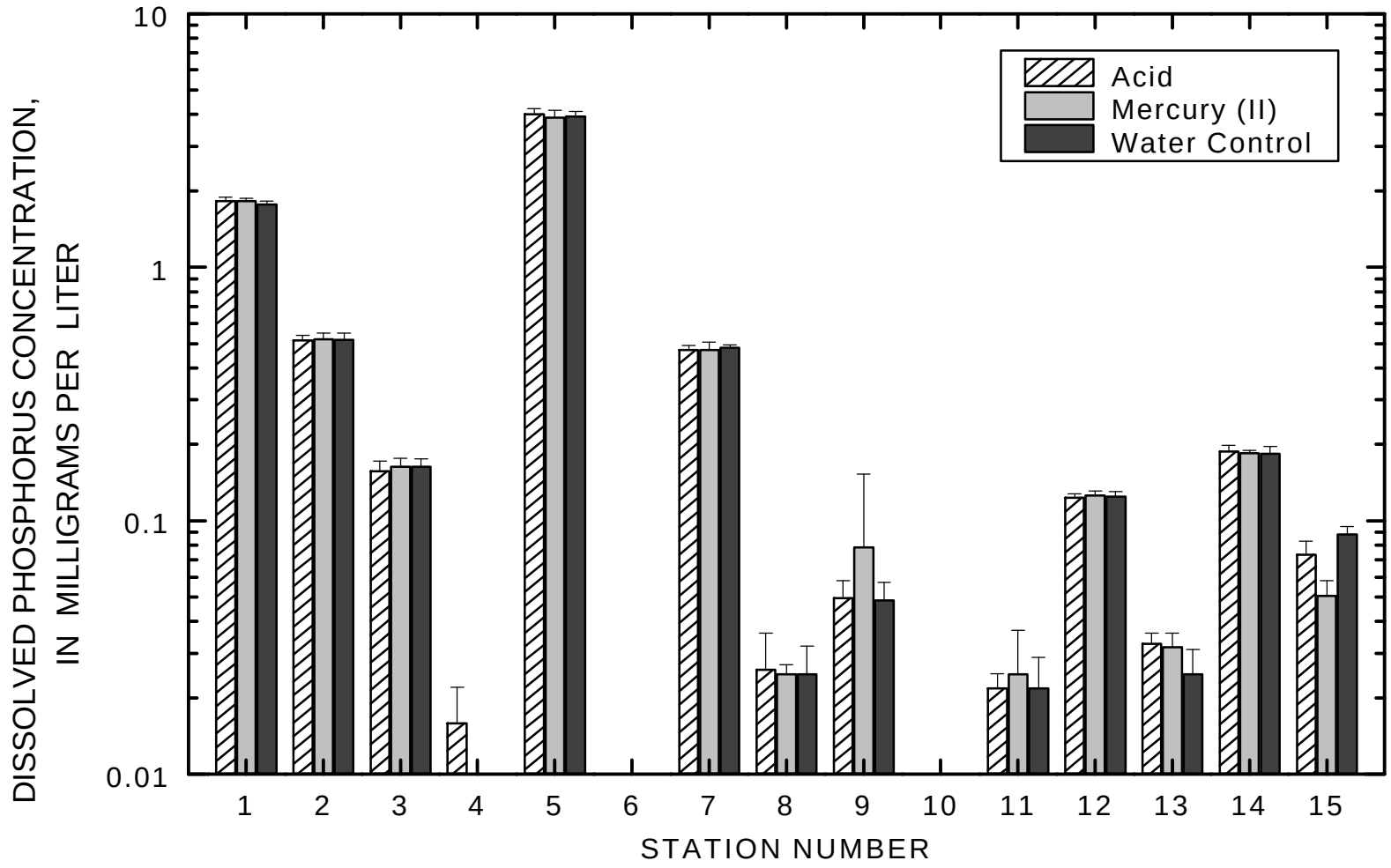

Figure 21. Bar chart of six-determination-date-average concentrations of dissolved phosphorus determined in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation. There are no data for station 6. Concentrations for mercury- and water-amended samples from station 4 and all samples from station 10 were less than the method detection limit.

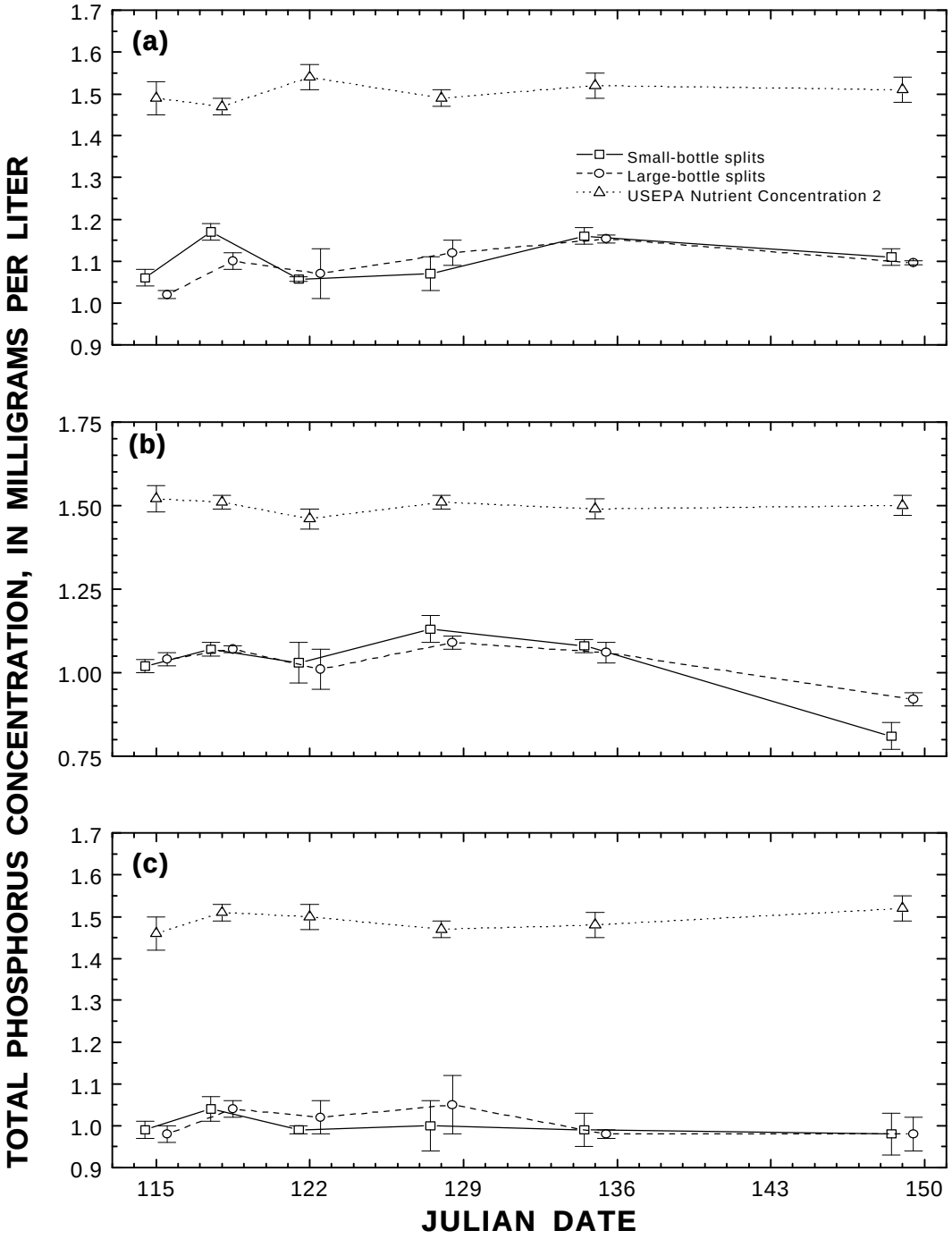

Figure 22. Plots of total phosphorus concentrations in (a) acid-amended, (b) mercury (II)-amended, and (c) water-amended large- and small-bottle splits for station 4 along with concurrently determined U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 2 check standards. Error bars indicate ±1 standard deviation. Symbols for large- and small-bottle splits are offset by ±0.5 Julian day for clarity.

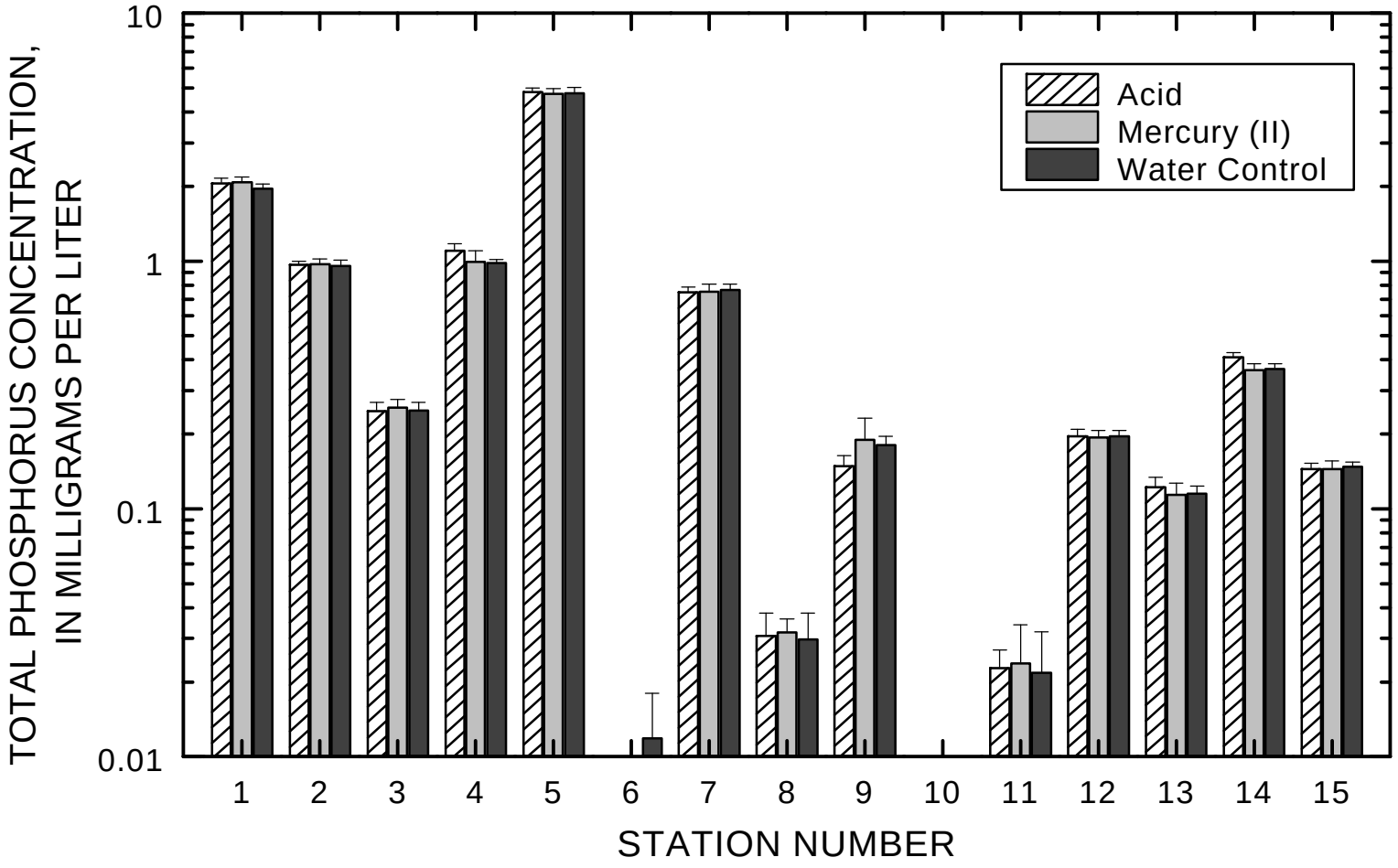

Figure 23. Bar chart of six-determination-date-average concentrations of total phosphorus determined in small-bottle splits from each of the 15 stations. The three columns centered over each station number represent one of the three amendments identified on the figure. Error bars indicate 1 standard deviation. See supporting text for additional details.

The relative concentrations of phosphorus species in water-control, small-bottle splits from each of the 15 stations are shown in figure 24. These concentrations are representative of speciation observed for the other amendments and split sizes. It is apparent that orthophosphate is the predominant species in filtered-water splits (fig. 24). Interestingly, no statistically significant decreases in phosphorus concentrations were observed in water-amended (control), whole-water splits (see preceding Kjeldahl Nitrogen section), presumably because any microbially assimilated phosphorus was released in measurable form (PO_4^{3-}) during the digestion step.

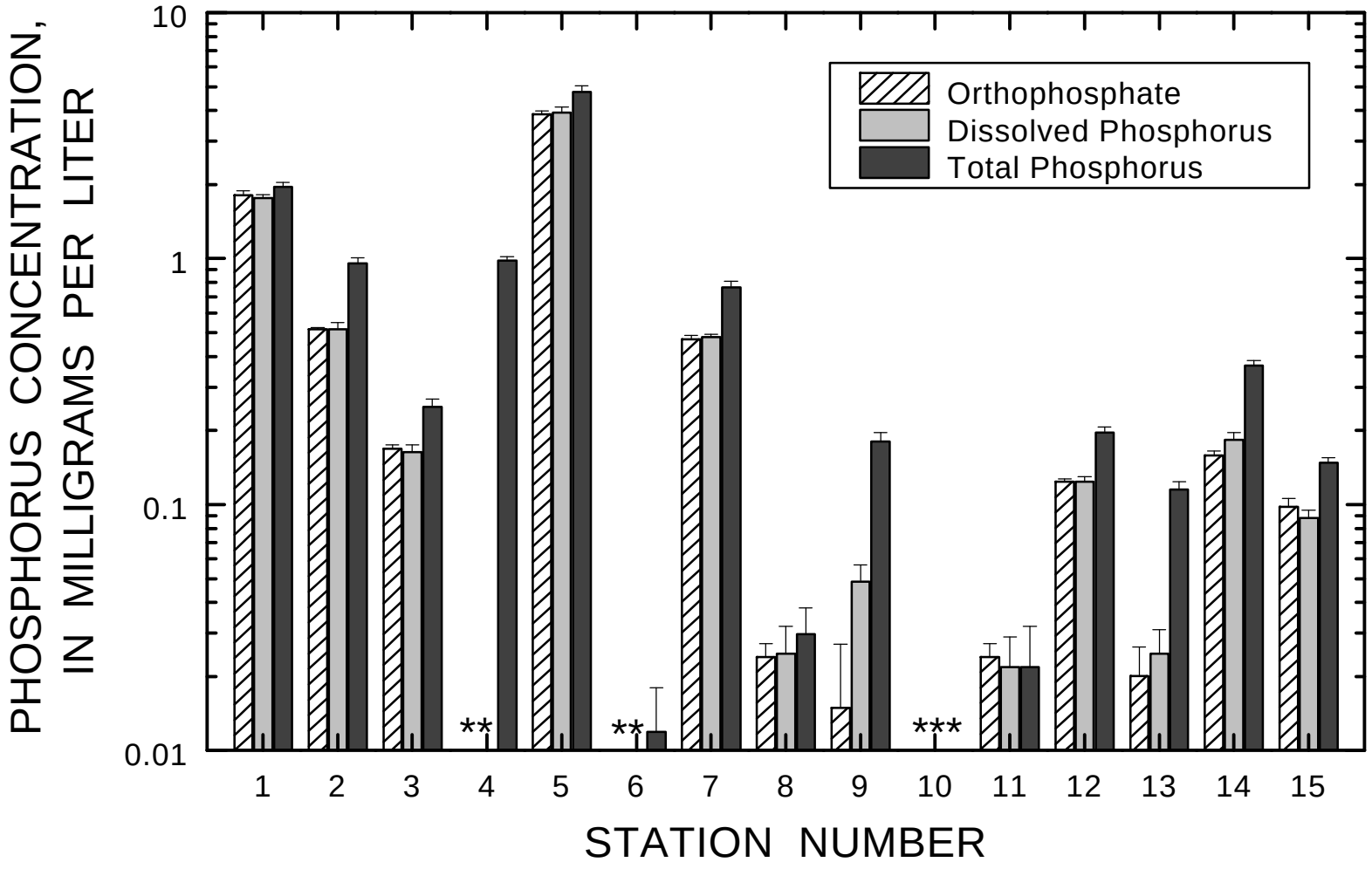

Figure 24. Bar chart of six-determination-date-average concentrations of orthophosphate, dissolved phosphorus, and total phosphorus determined in water-control, small-bottle splits from each of the 15 stations. Asterisks indicate concentrations less than method detection limits.

CONCLUSIONS

Major conclusions of this study, which correspond by number with objectives listed in the "Introduction" section, follow:

1. When collection-site sample processing includes 0.45-µm membrane filtration, subsequent amendment of samples with sulfuric acid or mercury (II) does not result in statistically significant changes in the stability of dissolved nutrient species during 30-day storage at 4°C. The close correspondence of minor concentration fluctuations in large- and small-bottle splits documented in this study provides reasonable assurance that repeated opening of bottles that may be required for repeat analyses will have little effect on storage stability.
2. Amendment of whole-water samples with sulfuric acid or mercury (II) does not result in statistically significant changes in the stability of total phosphorus concentrations during 30-day storage at 4°C. For some whole-water samples that contain high initial concentrations of ammonium—and presumably high biological activity—sulfuric acid or mercury (II) amendment may be necessary to ensure the stability of Kjeldahl nitrogen concentrations during 30-day storage at 4°C.
3. Sample-collection sites, water types, and initial nutrient concentrations did not significantly affect the 30-day storage stability of the eight nutrient species determined in this study. Considering the broad scope of this study—samples were collected throughout the United States; samples contained a wide range of nutrient and suspended-sediment concentrations; and a large data set (more than 20,000 analyses) was evaluated—results should be applicable to future USGS water-quality assessment work.

In addition, the authors offer the following suggestions for changes in collection-site sample processing that may further stabilize ambient nutrient concentrations in samples during storage:

1. Amend at collection sites the whole-water samples—collected for Kjeldahl nitrogen and phosphorus determinations—with a biocide to ensure month-long stability of ammonium during storage at 4°C. Sulfuric acid and mercury (II) are equally effective in this regard, but the former (0.5 mL of 4.5 ***M*** sulfuric acid per 125-mL sample) is preferable because it conforms to present USEPA protocols and it is less toxic than mercury (II).

2. If the first suggestion is adopted, then replace the opaque, brown polyethylene bottles currently used to store whole-water nutrient samples with translucent bottles because obtaining a representative subsample during digest preparation may depend critically on analysts' ability to estimate the amount of suspended solids and its settling rate by visual inspection. Such inspections are possible only if sample bottles are translucent. Filtered nutrient samples still can be collected, shipped, and stored in brown bottles.

3. Change the nominal pore size of filters used to process samples collected for determination of dissolved nutrients from 0.45 to 0.2 μm for reasons already discussed in the Introduction.

4. Use sterile bottles for nutrient sample collection and storage.

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APPENDIX A — FIGURES 25 THROUGH 31

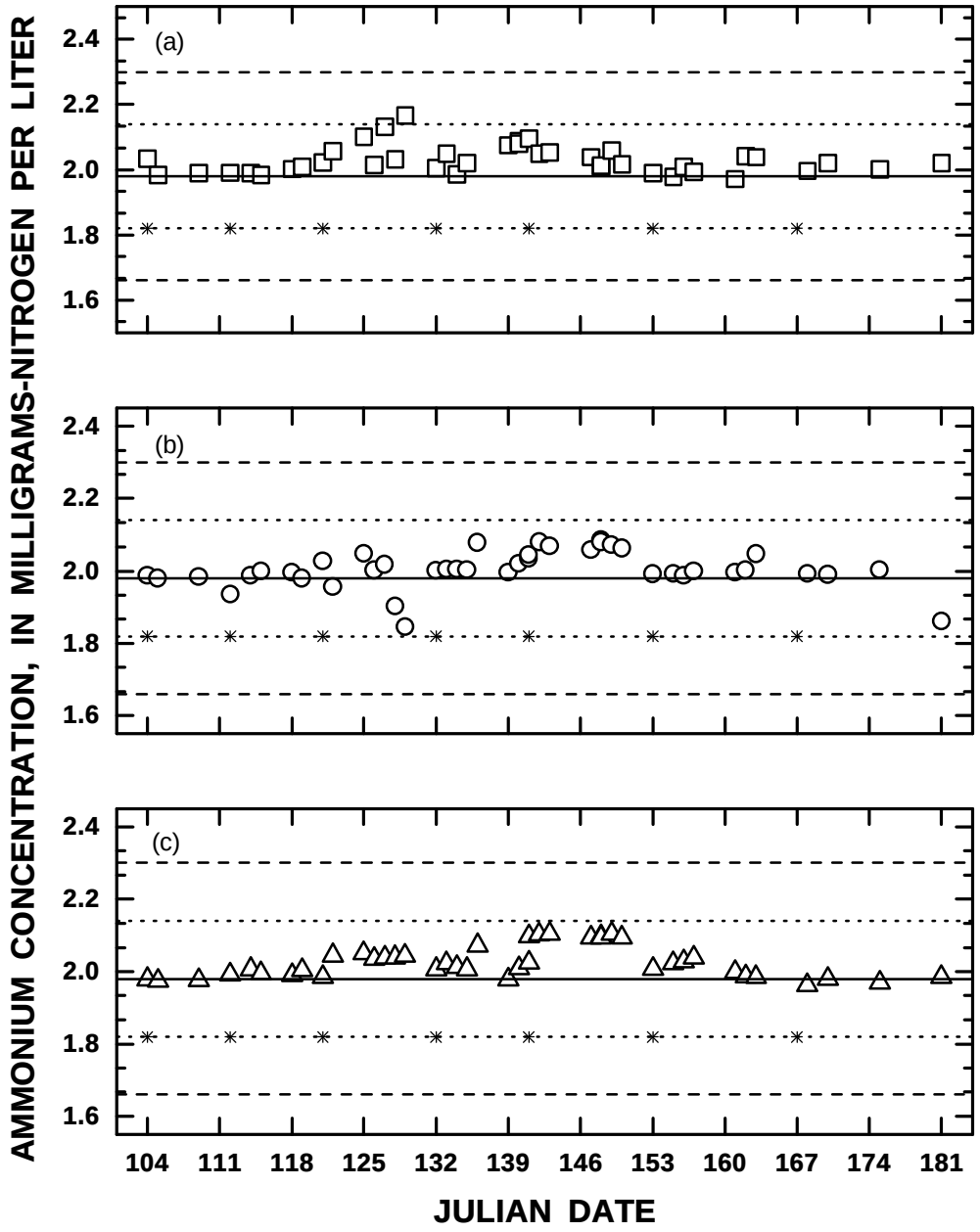

Figure 25. Plots of dissolved ammonium concentration in relation to Julian date for U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 1 check standards prepared in ASTM Type I deionized water (a) amended with sulfuric acid, (b) amended with mercury (II), and (c) control. Plotted points indicate the average of four replicate concentrations, which were determined concurrently with each batch of environmental samples. The associated ±1 standard deviation (s.d.) error bars, which are omitted to improve clarity, typically fall within the boundaries of plotted symbols. In each plot, central solid lines, inner dotted line pairs, and outer dashed line pairs indicate USEPA-supplied most probable concentrations, ±1 s.d., and ±2 s.d. (95% confidence intervals), respectively. Star-shaped symbols along bottom, dotted lines indicate preparation dates for new batches of check standards.

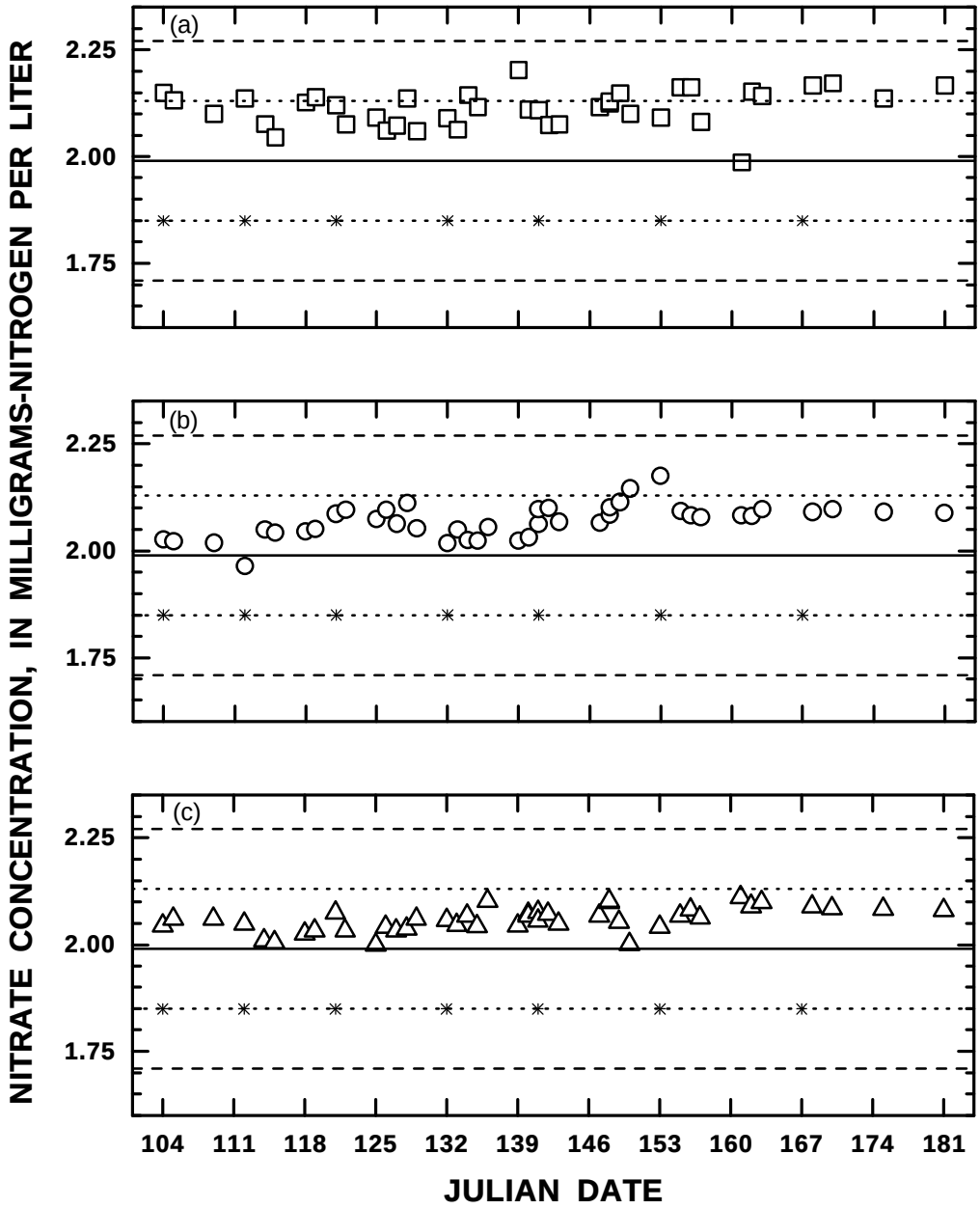

Figure 26. Plots of dissolved nitrate concentration in relation to Julian date for U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 1 check standards prepared in ASTM Type I deionized water (a) amended with sulfuric acid, (b) amended with mercury (II), and (c) control. Plotted points indicate the average of four replicate concentrations, which were determined concurrently with each batch of environmental samples. The associated ±1 standard deviation (s.d.) error bars, which are omitted to improve clarity, typically fall within the boundaries of plotted symbols. In each plot, central solid lines, inner dotted line pairs, and outer dashed line pairs indicate USEPA-supplied most probable concentrations, ±1 s.d., and ±2 s.d. (95% confidence intervals), respectively. Star-shaped symbols along bottom, dotted lines indicate preparation dates for new batches of check standards. Note that this check standard does not contain nitrite.

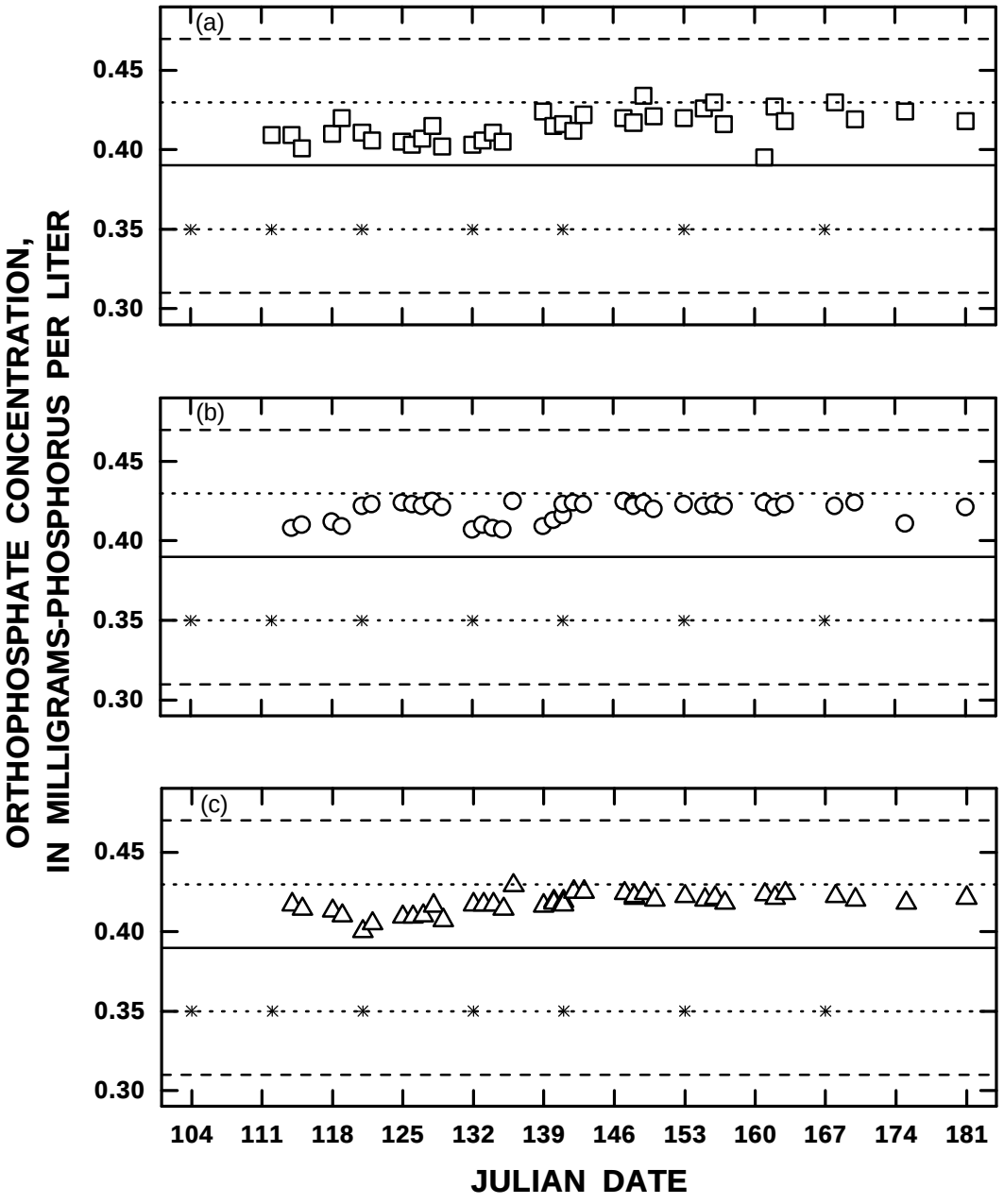

Figure 27. Plots of dissolved orthophosphate concentration in relation to Julian date for U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 1 check standards prepared in ASTM Type I deionized water (a) amended with sulfuric acid, (b) amended with mercury (II), and (c) control. Plotted points indicate the average of four replicate concentrations, which were determined concurrently with each batch of environmental samples. The associated ±1 standard deviation (s.d.) error bars, which are omitted to improve clarity, typically fall within the boundaries of plotted symbols. In each plot, central solid lines, inner dotted line pairs, and outer dashed line pairs indicate USEPA-supplied most probable concentrations, ±1 s.d., and ±2 s.d. (95% confidence intervals), respectively. Star-shaped symbols along bottom, dotted lines indicate preparation dates for new batches of check standards.

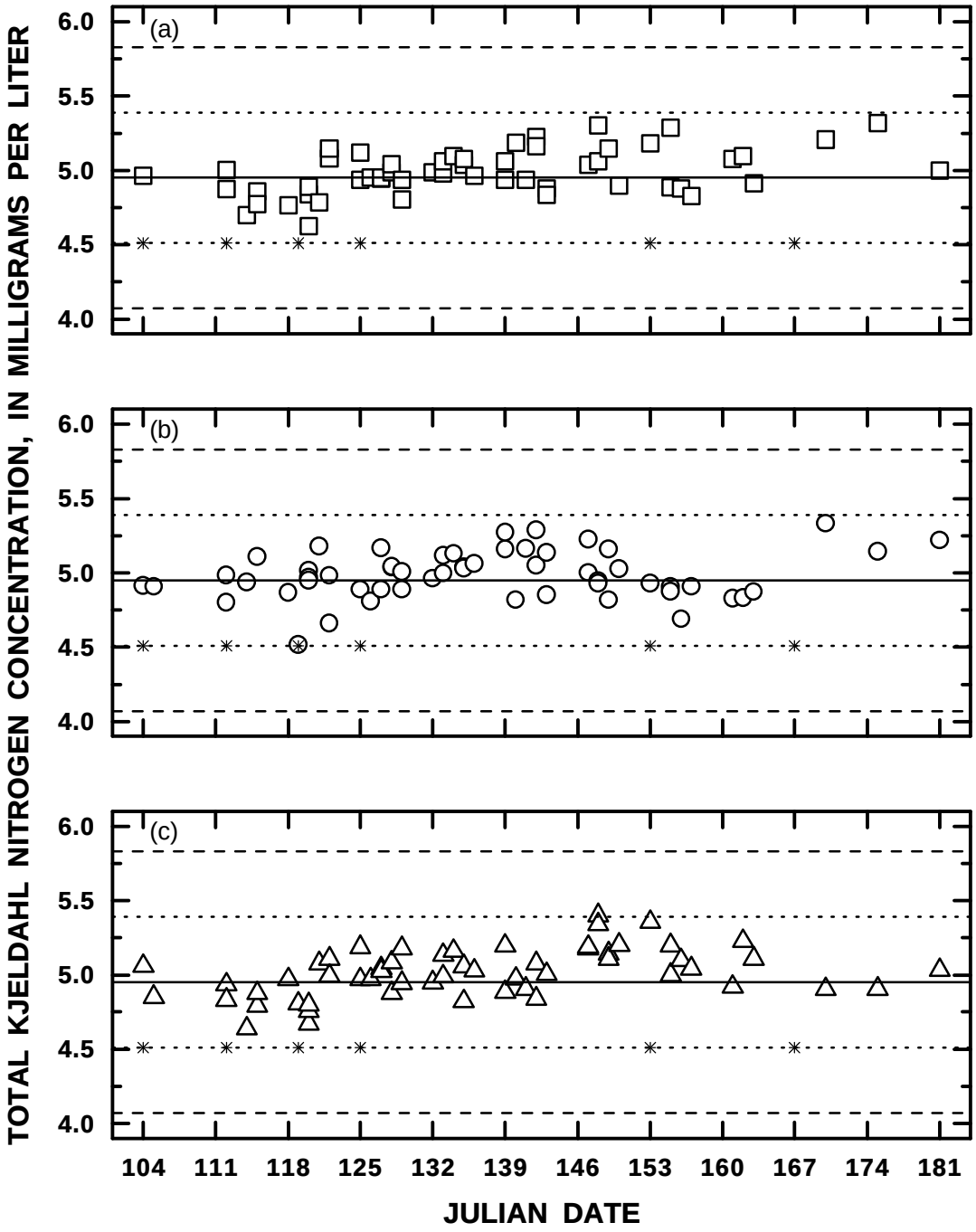

Figure 28. Plots of Kjeldahl nitrogen concentration in relation to Julian date for U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 2 check standards prepared in ASTM Type I deionized water (a) amended with sulfuric acid, (b) amended with mercury (II), and (c) control. Plotted points indicate individual concentrations of check standards, which were digested and determined concurrently with each batch of environmental samples. In each plot, central solid lines, inner dotted line pairs, and outer dashed line pairs indicate USEPA-supplied most probable concentrations, ±1 s.d., and ±2 s.d. (95% confidence intervals), respectively. Star-shaped symbols along bottom, dotted lines indicate preparation dates for new batches of check standards.

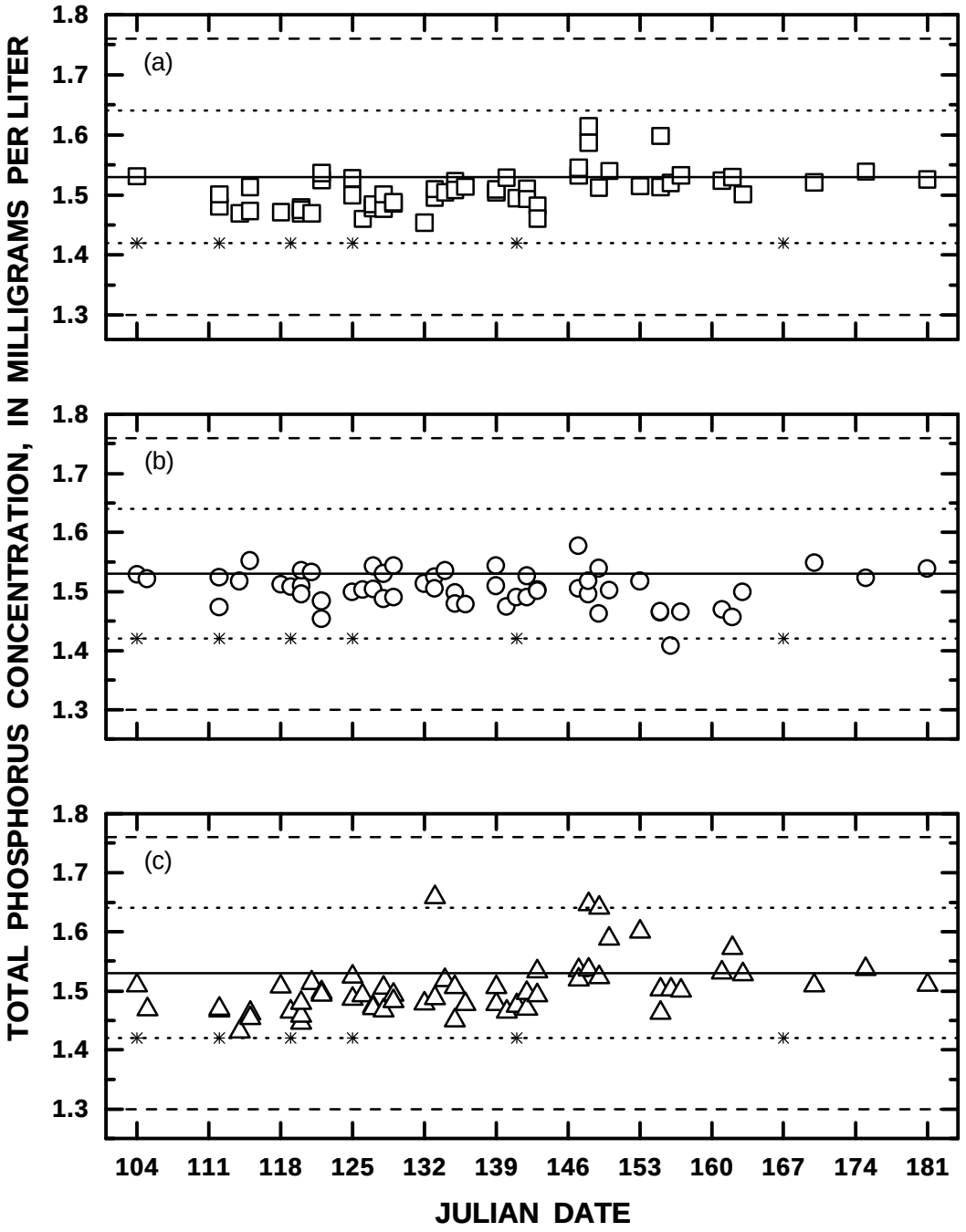

Figure 29. Plots of total phosphorus concentration in relation to Julian date for U.S. Environmental Protection Agency (USEPA) Nutrient Concentration 2 check standards prepared in ASTM Type I deionized water (a) amended with sulfuric acid, (b) amended with mercury (II), and (c) control. Plotted points indicate individual concentrations of check standards, which were digested and determined concurrently with each batch of environmental samples. In each plot, central solid lines, inner dotted line pairs, and outer dashed line pairs indicate USEPA-supplied most probable concentrations, ±1 s.d., and ±2 s.d. (95% confidence intervals), respectively. Star-shaped symbols along bottom, dotted lines indicate preparation dates for new batches of check standards.

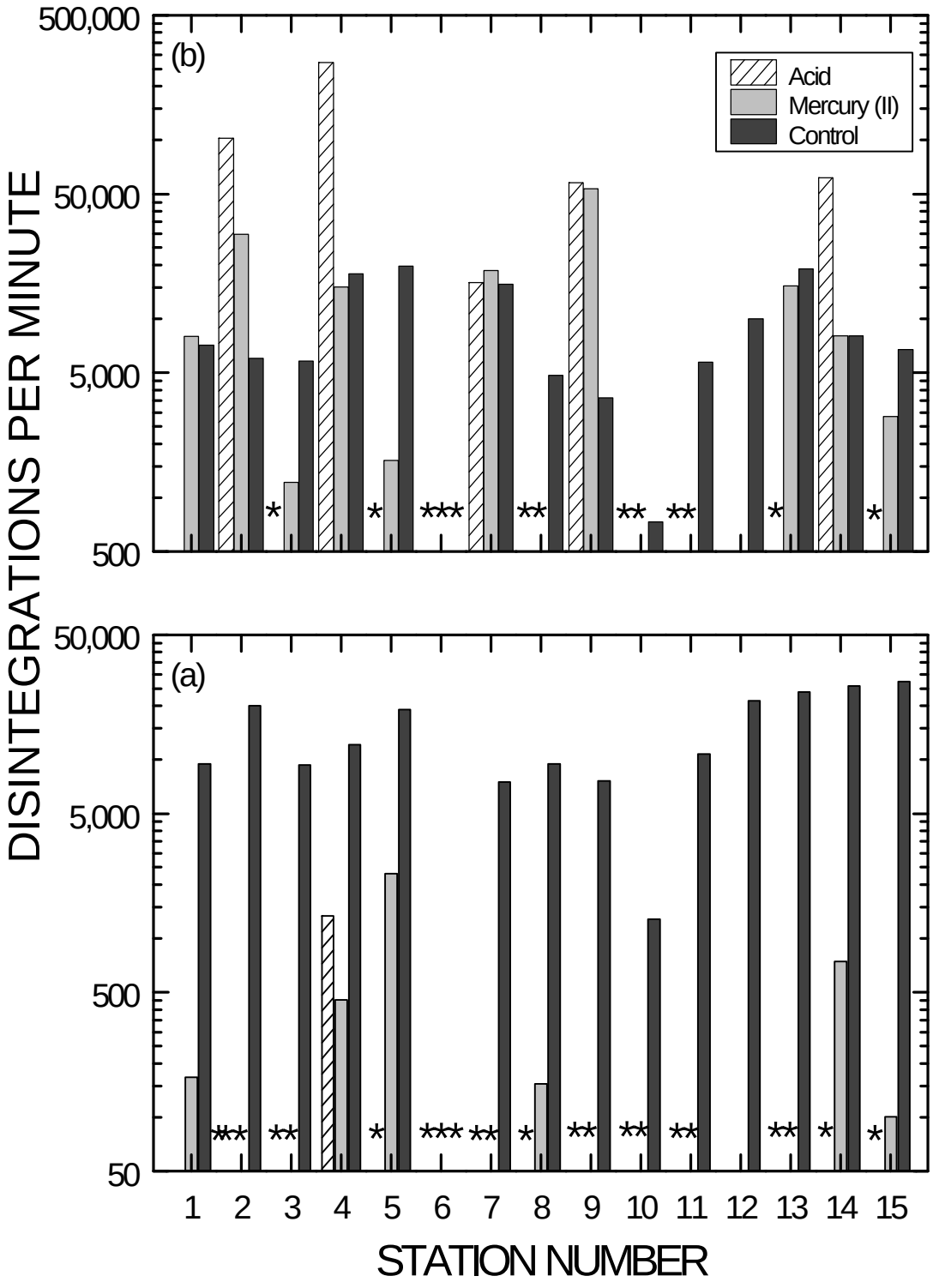

Figure 30. Biological activity in filtered (a) and whole-water (b), small-bottle splits at the end of the experiment as estimated by the tritiated adenine uptake method. Asterisks indicate values less than 50 disintegrations per minute.

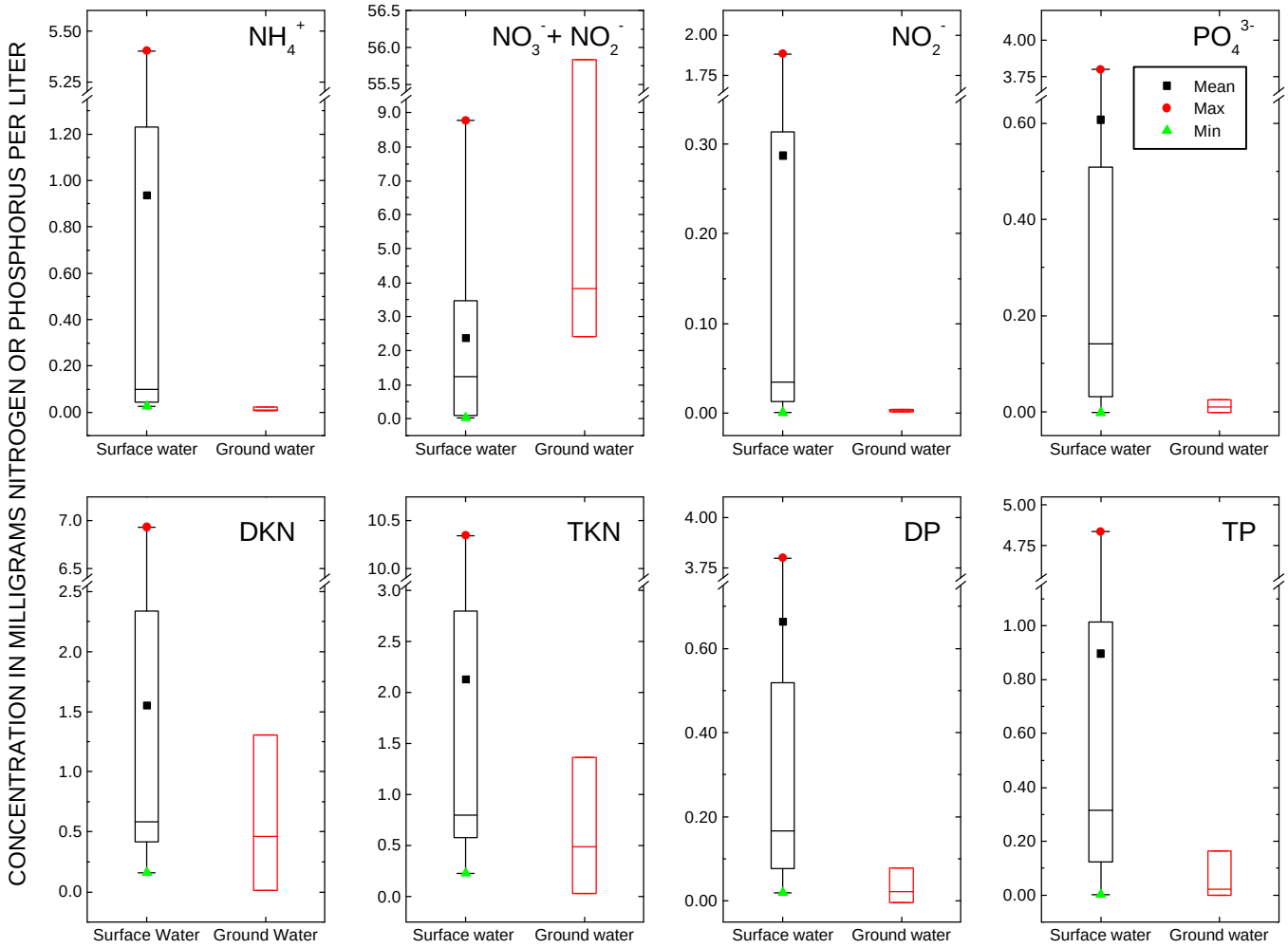

Figure 31. Box plots of concentration ranges for dissolved and total nutrient samples collected at the 15 stations included in this study. In these plots, hinges indicate median concentrations, gates indicate 25th and 75th percentiles, and whiskers indicate 5th and 95th percentiles.

APPENDIX B — TABLES 14 THROUGH 23

Table 14. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of dissolved phosphorus (lab code 666) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than]

Laboratory identification	Station number	Dissolved phosphorus concentration (mg-phosphorus/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	1.84 ± 0.05	2.66 (n=11)	4.50	1.00
Station 2	10254670	0.52 ± 0.03	.40 (n=14)	.70	.18
Station 3	01389500	0.17 ± 0.01	.23 (n=55)	.88	.07
Station 4	06326500	0.008 ± 0.007	.02 (n=24)	.05	< .01
Station 5	11103000	3.9 ± 0.2	.74 (n=15)	2.30	.09
Station 7	08370500	0.48 ± 0.03	.58 (n=21)	1.40	.08
Station 8	14312260	0.025 ± 0.005	.08 (n=23)	.26	.02
[1] Station 12	01576540	0.13 ± 0.01	[2].15 (n=71)	[2].66	[2].03
Station 13	07288000	.032 ± 0.004	.04 (n=17)	.09	< .01
Station 14	08062700	0.185 ± 0.007	1.62 (n=24)	4.9	.09
[2] Station 15	02248000	0.051 ± 0.007	.09 (n=14)	.42	.04

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 15. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of dissolved Kjeldahl nitrogen (lab code 623) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; —, no data]

Laboratory identification	Station number	Dissolved Kjeldahl nitrogen concentration (mg-nitrogen/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	4.1 ± 0.1	—	—	—
Station 2	10254670	2.4 ± 0.1	—	—	—
Station 3	01389500	0.35 ± 0.03	—	—	—
Station 4	06326500	0.34 ± 0.06	—	—	—
Station 5	11103000	6.9 ± 0.6	—	—	—
Station 7	08370500	0.45 ± 0.06	—	—	—
Station 8	14312260	0.14 ± 0.03	—	—	—
[1]Station 12	01576540	0.42 ± 0.03	—	—	—
Station 13	07288000	0.33 ± 0.03	—	—	—
Station 14	08062700	0.49 ± 0.07	—	—	—
Station 15	02248000	0.60 ± 0.06	—	—	—

[1]Not a NASQAN site.

Table 16. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of dissolved ammonium nitrogen (lab code 608) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than; —, no data;—, no data]

Laboratory Identification	Station number	Dissolved ammonium nitrogen concentration (mg-nitrogen/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	3.4 ± 0.2	10.0 (n=11)	19.0	4.0
Station 2	10254670	1.55 ± 0.06	1.78 (n=14)	5.4	.32
Station 3	01389500	0.132 ±0 .008	.40 (n=55)	1.80	.01
Station 4	06326500	0.040 ± 0.007	.06 (n=23)	.16	< .01
Station 5	11103000	5.5 ± 0.1	2.08 (n=15)	8.5	.03
Station 7	08370500	0.03 ± 0.01	1.01 (n=21)	4.9	.01
Station 8	14312260	0.059 ±0 .004	.08 (n=24)	.22	.01
[1]Station 12	01576540	0.074 ± 0.002	[2].092 (n=70)	[2] 1.66	—
Station 13	07288000	0.024 ± 0.005	.06 (n=17)	.14	< .01
Station 14	08062700	0.087 ± 0.003	.20 (n=24)	1.10	.02
Station 15	02248000	0.118 ± 0.006	.12 (n=14)	.72	.04

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 17. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of dissolved orthophosphate (lab code 671) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than; —, no data]

Laboratory Identification	Station number	Dissolved orthophosphate concentration (mg-phosphorus/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station	06720500	1.82 ± 0.05	2.42 (n=11)	3.7	1.10
Station 2	10254670	0.511 ± 0.008	.36 (n=14)	.63	.13
Station 3	01389500	0.168 ± 0.003	.20 (n=55)	.79	.01
Station 4	06326500	0.010 ± 0.002	.01 (n=24)	.03	< .01
Station 5	11103000	3.89 ± 0.06	.63 (n=15)	2.2	.04
Station 7	08370500	0.49 ± 0.01	.55 (n=21)	1.3	.05
Station 8	14312260	0.025 ± 0.001	.06 (n=23)	.27	.01
[1]Station 12	01576540	0.128 ± 0.002	[2].11 (n=71)	[2].66	—
Station 13	07288000	0.025 ± 0.001	.03 (n=17)	.07	< .01
Station 14	08062700	0.152 ± 0.002	1.52 (n=24)	4.3	.07
Station 15	02248000	0.056 ± 0.001	.08 (n=14)	.36	.03

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 18. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of dissolved nitrate plus nitrite (lab code 631) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than; —, no data]

Laboratory identification	Station number	Dissolved nitrate plus nitrite concentration (mg-nitrogen/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	3.54 ± 0.05	2.66 (n=11)	4.50	1.00
Station 2	10254670	7.7 ± 0.2	.40 (n=14)	.70	.18
Station 3	01389500	2.04 ± 0.04	.23 (n=55)	.88	.07
Station 4	06326500	0.166 ± 0.007	.02 (n=24)	.05	< .01
Station 5	11103000	3.23 ± 0.06	.74 (n=15)	2.30	.09
Station 7	08370500	1.25 ± 0.03	.58 (n=21)	1.40	1.25
Station 8	14312260	0.04 ± 0.01	.08 (n=14)	.26	.02
[1]Station 12	01576540	8.7 ± 0.3	[2] 9.5 (n=71)	[2] 13	[2] 2.24
Station 13	07288000	0.329 ± 0.006	.04 (n=17)	.09	< .01
Station 14	08062700	1.16 ± 0.01	1.62 (n=24)	4.90	.09
Station 15[C]	02248000	0.08 ± 0.02	.09 (n=14)	.42	—

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 19. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of dissolved nitrite (lab code 613) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than]

Laboratory identification	Station number	Dissolved nitrite concentration (mg-nitrogen/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	0.383 ± 0.007	0.32 (n=11)	0.58	0.13
Station 2	10254670	0.89 ± 0.04	.61 (n=14)	1.10	.31
Station 3	01389500	0.027 ± 0.003	.06 (n=55)	.19	.01
Station 4	06326500	0.006 ± 0.001	.01 (n=23)	.04	< .01
Station 5	11103000	1.87 ± 0.06	.84 (n=15)	1.40	.12
Station 7	08370500	0.039 ± 0.003	.11 (n=21)	.27	.01
Station 8	14312260	0.011 ± 0.002	.02 (n=24)	.15	< .01
[1]Station 12	01576540	0.10 ± 0.01	[2].05 (n=43)	[2].11	[2].01
Station 13	07288000	0.013 ± 0.001	.01 (n=17)	.03	< .01
Station 14	08062700	0.044 ± 0.001	.09 (n=24)	.47	.01
Station 15	02248000	0.001 ± 0.001	.02 (n=14)	.15	< .01

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 20. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of whole-water phosphorus (lab code 665) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than]

Laboratory identification	Station number	Whole-water phosphorus concentration (mg-phosphorus/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	2.09 ± 0.09	4.8 (n=11)	22	1.1
Station 2	10254670	0.98 ± 0.04	.54 (n=14)	.90	.23
Station 3	01389500	0.26 ± 0.02	.33 (n=54)	.93	.09
Station 4	06326500	1.0 ± 0.1	.62 (n=28)	5.7	< .01
Station 5	11103000	4.8 ± 0.2	1.43 (n=15)	2.8	.64
Station 7	08370500	0.76 ± 0.05	.83 (n=21)	1.8	.23
Station 8	14312260	0.032 ± 0.006	.09 (n=42)	.31	.03
[1]Station 12	01576540	0.20 ± 0.01	[2]1.09 (n=162)	[2] 7.2	[2].03
Station 13	07288000	0.12 ± 0.01	.21 (n=17)	.83	< .01
Station 14	08062700	0.37 ± 0.02	1.73 (n=24)	5.0	.11
Station 15	02248000	0.15 ± 0.01	.14 (n=14)	.50	.05

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 21. Measured (small-bottle, mercury (II)-amended splits) and historical concentrations of whole-water Kjeldahl nitrogen (lab code 625) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg, milligrams; L, liter; *n*, number of points; ±, plus or minus; <, less than]

Laboratory identification	NASQAN station number	Whole-water Kjeldahl nitrogen concentration (mg-nitrogen/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
Station 1	06720500	4.8 ± 0.2	12 (n=11)	28	3.0
Station 2	10254670	3.1 ± 0.2	3.0 (n=14)	5.4	1.5
Station 3	01389500	0.53 ± 0.03	1.18 (n=54)	2.5	.4
Station 4	06326500	2.0 ± 0.2	1.23 (n=24)	6.4	.2
Station 5	11103000	9.8 ± 0.7	4.4 (n=15)	10	1.7
Station 7	08370500	1.4 ± 0.1	2.4 (n=21)	6.9	.7
Station 8	14312260	0.21 ± 0.05	.38 (n=42)	.9	< .2
[1]Station 12	01576540	0.59 ± 0.09	[2] 2.5 (n=163)	[2] 21	[2].2
Station 13	07288000	0.59 ± 0.04	.76 (n=17)	1.0	.3
Station 14	08062700	1.18 ± 0.08	1.2 (n=24)	2.5	.5
Station 15	02248000	0.69 ± 0.08	1.2 (n=14)	1.8	.6

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995.

Table 22. Historical concentrations of suspended sediment (lab code 80154) at National Stream Quality Accounting Network (NASQAN) stations included in this study

[mg/L, milligrams per liter; *n*, number of points; ±, plus or minus, <, less than; —, no data]

Laboratory identification	NASQAN station number	Suspended sediment concentration (mg/L)			
		This study (*n*=24)	Historical data (December 1987 to June 1991)		
		Average ± standard deviation	Average (sample size)	Maximum	Minimum
[2]Station 1	06720500	—	28 (n=6)	65	8
[2]Station 2	10254670	—	—	—	—
Station 3	01389500	—	20 (n=18)	44	2
Station 4	06326500	—	1,654 (n=20)	8,910	25
Station 5	11103000	—	13 (n=11)	23	8
Station 7	08370500	—	576 (n=17)	2,180	192
Station 8	14312260	—	8 (n=20)	28	< 1
[1]Station 12	01576540	—	[2] 690 (n=147)	[2] 11,000	[2] 2
Station 13	07288000	—	200 (n=16)	395	66
Station 14	08062700	—	173 (n=24)	752	28
Station 15	02248000	—	16 (n=9)	63	4

[1]Not a NASQAN site.
[2]Data collected from February 1992 to July 1995

Table 23. Schedule of analyses for nutrient preservation experiment

[STA No., station number]

	Week 1 (4/13 - 4/17) Julian date/day of week					**Week 2** (4/20 - 4/24) Julian date/day of week					**Week 3** (4/27 - 5/01) Julian date/day of week					**Week 4** (5/04 - 5/08) Julian date/day of week				
STA	104	105	106	107	109	111	112	113	114	115	118	119	120	121	122	125	126	127	128	129
No.	M	T	W	Th	Sa	M	T	W	Th	F	M	T	W	Th	F	M	T	W	Th	F
1	1	2			[1]3, 2		[1]3		4							5				
2					1		2		3					4				5		
3							1			2		3						4		
4										1	2				3				4	
5												1	[1]1		2			3		
6											1	2		3					4	
7															1	2				3
8																	1			2
9																1	2		3	
10																				1
11																				
12																				
13																				
14																				
15																				

[1]Numbers pertain to analysis schedules for Kjeldahl nitrogen and phosphorus determinations that do not correspond with analysis schedules for ammonia, nitrate + nitrite, nitrite, and orthophosphate determinations.

Table 23 Schedule of analyses for nutrient preservation experiment—Continued

	Week 5 (5/11 - 5/15) Julian date/day of week					**Week 6** (5/18 - 5/22) Julian date/day of week					**Week 7** (5/25 - 5/29) Julian date/day of week					**Week 8** (6/01 - 6/05) Julian date/day of week				
STA	132	133	134	135	136	139	140	141	142	143	146	147	148	149	150	153	154	155	156	157
No.	M	T	W	Th	F	M	T	W	Th	F	M	T	W	Th	F	M	T	W	Th	F
1						6														
2									6											
3			5										6							
4				5										6						
5		4						5										6		
6						5										6				
7			4						5										6	
8		3						4					5							
9				4								5								
10	2			3					4					5						
11		1			2		3						4					5		
12					1	2				3				4					5	
13							1			2		3						4		
14										1		2			3					4
15													1		2	3				4

Table 23 Schedule of analyses for nutrient preservation experiment—Continued

	Week 9 (6/08 - 6/12) Julian date/day of week					**Week 10** (6/15 - 6/19) Julian date/day of week					**Week 11** (6/22 - 6/26) Julian date/day of week					**Week 12** (6/29 - 7/03) Julian date/day of week				
STA	160	161	162	163	164	167	168	169	170	171	174	175	176	177	178	181	182	183	184	185
No.	M	T	W	Th	F	M	T	W	Th	F	M	T	W	Th	F	M	T	W	Th	F
1																				
2																				
3																				
4																				
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9		6																		
10				6																
11							6													
12									6											
13		5										6								
14				5								6								
15							5									6				