

U.S. Department of the Interior  
U.S. Geological Survey

# ***Relations of Biological Indicators to Nutrient Data for Lakes and Streams in Pennsylvania and West Virginia, 1990-98***

*by Robin A. Brightbill and Edward H. Koerke*

**Water-Resources Investigations Report 03-4033**

*In cooperation with the*

**PENNSYLVANIA DEPARTMENT OF ENVIRONMENTAL PROTECTION and the  
U.S. ENVIRONMENTAL PROTECTION AGENCY**

New Cumberland, Pennsylvania  
2003

**U.S. DEPARTMENT OF THE INTERIOR**

GALE A. NORTON, Secretary

**U.S. GEOLOGICAL SURVEY**

Charles G. Groat, Director

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

---

For additional information  
write to:

District Chief  
U.S. Geological Survey  
215 Limekiln Road  
New Cumberland, Pennsylvania 17070-2424  
Email: [dc\\_pa@usgs.gov](mailto:dc_pa@usgs.gov)  
Internet Address: <http://pa.water.usgs.gov>

Copies of this report may be  
purchased from:

U.S. Geological Survey  
Branch of Information Services  
Box 25286  
Denver, Colorado 80225-0286  
Telephone: 1-888-ASK-USGS

# CONTENTS

	Page
Abstract . . . . .	1
Introduction . . . . .	2
Purpose and scope . . . . .	2
Study area . . . . .	3
Acknowledgments . . . . .	4
Approach . . . . .	5
Data sources . . . . .	5
Data analysis . . . . .	5
Algae . . . . .	5
Macroinvertebrates . . . . .	8
Relations among chlorophyll <i>a</i> , nutrients, other water-quality characteristics, and habitat and macroinvertebrate indices . . . . .	9
Comparison among nutrient ecoregions . . . . .	9
Predictive relations between chlorophyll <i>a</i> and nutrients . . . . .	13
Predictive relations between chlorophyll <i>a</i> and nutrients for nutrient ecoregions VII, VIII, IX, and XI . . . . .	16
Nutrient ecoregion VII . . . . .	16
Nutrient ecoregion VIII . . . . .	20
Nutrient ecoregion IX . . . . .	22
Nutrient ecoregion XI . . . . .	25
Ecological indicators of nutrient enrichment . . . . .	36
Considerations for future research . . . . .	39
Summary . . . . .	40
References cited . . . . .	42
Appendix—Laboratory methods used by different governmental agencies . . . . .	45

## ILLUSTRATIONS

	Page
Figures 1-2. Maps showing:	
1. Lake sites for which chlorophyll <i>a</i> and nutrient data were available within the nutrient ecoregions of Pennsylvania and West Virginia, 1990-98 . . . . .	3
2. Stream sites for which chlorophyll <i>a</i> and nutrient data were available within the nutrient ecoregions of Pennsylvania and West Virginia, 1990-98 . . . . .	4
3-28. Graphs showing the:	
3. Distribution of pH, specific conductance, alkalinity/acid neutralizing capacity, total nitrogen concentrations, total phosphorus concentrations, phytoplankton chlorophyll <i>a</i> , and Secchi depth in lakes by nutrient ecoregions of Pennsylvania and West Virginia, 1990-98 . . . . .	10
4. Distribution of pH, specific conductance, alkalinity/acid neutralizing capacity, total nitrogen concentrations, total phosphorus concentrations, periphyton chlorophyll <i>a</i> , and turbidity in streams by nutrient ecoregions of Pennsylvania and West Virginia, 1990-98 . . . . .	14
5. Relation between log of phytoplankton chlorophyll <i>a</i> concentrations and the log of concentrations of total nitrogen (A) and total phosphorus (B) in lakes within nutrient ecoregion VII, Pennsylvania, 1990-98 . . . . .	16
6. Relation between observed and predicted logs of phytoplankton chlorophyll <i>a</i> concentrations in lakes within nutrient ecoregion VII, Pennsylvania, 1990-98 . . . . .	17
7. Relation between log of periphyton chlorophyll <i>a</i> concentrations and the log of concentrations of total nitrogen and total phosphorus in streams within nutrient ecoregion VII, Pennsylvania, 1990-98 . . . . .	18
8. Relation between observed and predicted logs of periphyton chlorophyll <i>a</i> concentrations in streams within nutrient ecoregion VII, Pennsylvania, 1990-98 . . . . .	19
9. Relation between logs of phytoplankton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in lakes within nutrient ecoregion VIII, Pennsylvania, 1990-98. . . . .	21
10. Relation between log of periphyton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in streams within nutrient ecoregion VIII, Pennsylvania, 1990-98. . . . .	21
11. Relation between log of phytoplankton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in lakes within nutrient ecoregion IX, Pennsylvania, 1990-98 . . . . .	22
12. Relation between observed and predicted logs of phytoplankton chlorophyll <i>a</i> concentrations in lakes within nutrient ecoregion IX, Pennsylvania, 1990-98 . . . . .	23
13. Relation between logs of periphyton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in streams within nutrient ecoregion IX, Pennsylvania, 1990-98 . . . . .	24

## ILLUSTRATIONS—Continued

		Page
Figure	3-28. Graphs showing the:	
	14. Relation between observed and predicted logs of periphyton chlorophyll <i>a</i> concentrations in streams within nutrient ecoregion IX, Pennsylvania, 1990-98 .....	24
	15. Relation between log of phytoplankton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in lakes during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98 .....	25
	16. Relation between log of phytoplankton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in lakes during winter months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98 .....	25
	17. Relation between observed and predicted logs of phytoplankton chlorophyll <i>a</i> concentrations in lakes during summer months within ecoregion XI, Pennsylvania and West Virginia, 1990-98 .....	26
	18. Relation between observed and predicted phytoplankton chlorophyll <i>a</i> concentrations in lakes during winter months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98 .....	27
	19. Relation between log of periphyton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in lakes during summer months within nutrient ecoregion XI, West Virginia, 1990-98 .....	28
	20. Relation between log of periphyton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in lakes during winter months within nutrient ecoregion XI, West Virginia, 1990-98 .....	28
	21. Relation between observed and predicted logs of periphyton chlorophyll <i>a</i> concentrations in lakes during summer months within nutrient ecoregion XI, West Virginia, 1990-98 .....	29
	22. Relation between observed and predicted logs of periphyton chlorophyll <i>a</i> concentrations in lakes during winter months within nutrient ecoregion XI, West Virginia, 1990-98 .....	30
	23. Relation between log of periphyton chlorophyll <i>a</i> concentrations and the logs of concentrations of total nitrogen and total phosphorus in streams during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98 .....	31
	24. Relation between observed and predicted logs of periphyton chlorophyll <i>a</i> concentrations in streams during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98 .....	32
	25. Relation between log of phytoplankton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in streams during summer months within nutrient ecoregion XI, West Virginia, 1990-98 .....	33

## ILLUSTRATIONS—Continued

	Page
Figure 3-28. Graphs showing the:	
26. Relation between log of phytoplankton chlorophyll <i>a</i> concentrations and logs of concentrations of total nitrogen and total phosphorus in streams during winter months within nutrient ecoregion XI, West Virginia, 1990-98 .....	33
27. Relation between observed and predicted logs of phytoplankton chlorophyll <i>a</i> concentrations in streams during summer months within nutrient ecoregion XI, West Virginia, 1990-98 .....	34
28. Relation between observed and predicted logs of phytoplankton chlorophyll <i>a</i> concentrations in streams during winter months within nutrient ecoregion XI, West Virginia, 1990-98 .....	35

## TABLES

	Page
Table 1. Explanatory variables examined for significance in regressions of the log of chlorophyll <i>a</i> concentrations for nutrient ecoregions VII, VIII, IX, and XI lakes, Pennsylvania and West Virginia, 1990-98 . . . . .	7
2. Explanatory variables examined for significance in regressions of the log of chlorophyll <i>a</i> concentrations for nutrient ecoregions VII, VIII, IX, and XI streams, Pennsylvania and West Virginia, 1990-98 . . . . .	7
3. Results of multiple-stage Kruskal-Wallis test for significant differences in median values of water-quality constituents data from lakes in nutrient ecoregions VII, VIII, IX, and XI, Pennsylvania and West Virginia, 1990-98 . . . . .	9
4. Results of multiple-stage Kruskal-Wallis test for significant differences in median values of water-quality constituent data from streams in nutrient ecoregions VII, VIII, IX, and XI, Pennsylvania and West Virginia, 1990-98 . . . . .	13
5. Regression equation statistics for phytoplankton chlorophyll <i>a</i> concentrations in nutrient ecoregion VII lakes, Pennsylvania, 1990-98 . . . . .	17
6. Regression equation statistics for periphyton chlorophyll <i>a</i> concentrations in nutrient ecoregion VII streams, Pennsylvania, 1990-98. . . . .	19
7. Regression equation statistics for phytoplankton chlorophyll <i>a</i> concentrations in nutrient ecoregion IX lakes, Pennsylvania, 1990-98. . . . .	22
8. Regression equation statistics for summer-season periphyton chlorophyll <i>a</i> concentrations in nutrient ecoregion IX streams, Pennsylvania, 1990-98. . . . .	24
9. Regression equation statistics for summer-season phytoplankton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI lakes, Pennsylvania and West Virginia, 1990-98 . . . . .	26
10. Regression equation statistics for winter-season phytoplankton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI lakes, Pennsylvania and West Virginia, 1990-98 . . . . .	27
11. Regression equation statistics for summer-season periphyton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI lakes, West Virginia, 1990-98 . . . . .	29
12. Regression equation statistics for winter-season periphyton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI lakes, West Virginia, 1990-98 . . . . .	30
13. Regression equation statistics for summer-season periphyton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI streams, Pennsylvania and West Virginia, 1990-98 . . . . .	31
14. Regression equation statistics for summer-season phytoplankton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI streams, West Virginia, 1990-98. . . . .	34
15. Regression equation statistics for winter-season phytoplankton chlorophyll <i>a</i> concentrations in nutrient ecoregion XI streams, West Virginia, 1990-98. . . . .	35
16. Summary of influential lake explanatory variables related to lake chlorophyll <i>a</i> through regression analysis for each nutrient ecoregion and season . . . . .	37
17. Summary of influential stream explanatory variables related to stream chlorophyll <i>a</i> through regression analysis for each nutrient ecoregion and season . . . . .	38

## CONVERSION FACTORS AND ABBREVIATIONS

<u>Multiply</u>	<u>By</u>	<u>To obtain</u>
	<u>Length</u>	
foot (ft)	0.3048	meter
mile (mi)	1.609	kilometer
	<u>Area</u>	
square mile (mi <sup>2</sup> )	2.590	square kilometer
	<u>Temperature</u>	
degree Fahrenheit (°F)	°C=5/9 (°F-32)	degree Celsius

Abbreviated water-quality units used in report:

ft/mi, feet per mile

µg/L, micrograms per liter

µg/m<sup>2</sup>, micrograms per square meter

µS/cm, microsiemens per centimeter at 25 degrees Celsius

mg/L, milligrams per liter

mg/m<sup>2</sup>, milligrams per square meter

# RELATIONS OF BIOLOGICAL INDICATORS TO NUTRIENT DATA FOR LAKES AND STREAMS IN PENNSYLVANIA AND WEST VIRGINIA, 1990-98

by Robin A. Brightbill and Edward H. Koerkle

## ABSTRACT

The Clean Water Action Plan of 1998 provides a blueprint for federal agencies to work with states, tribes, and other stakeholders to protect and restore the Nation's water resources. The plan includes an initiative that addresses the nutrient-enrichment problem of lakes and streams across the United States. The U.S. Environmental Protection Agency (USEPA) is working to set nutrient criteria by nationwide nutrient ecoregions that are an aggregation of the Omernik level III ecoregions. Because low levels of nutrients are necessary for healthy streams and elevated concentrations can cause algal blooms that deplete available oxygen and kill off aquatic organisms, criteria levels are to be set, in part, using the relation between chlorophyll *a* and concentrations of total nitrogen and total phosphorus.

Data from Pennsylvania and West Virginia, collected between 1990 and 1998, were analyzed for relations between chlorophyll *a*, nutrients, and other explanatory variables. Both phytoplankton and periphyton chlorophyll *a* concentrations from lakes and streams were analyzed separately within each of the USEPA nutrient ecoregions located within the boundaries of the two states. These four nutrient ecoregions are VII (Mostly Glaciated Dairy), VIII (Nutrient Poor, Largely Glaciated Upper Midwest and Northeast), IX (Southeastern Temperate Forested Plains and Hills), and XI (Central and Eastern Forested Uplands).

Phytoplankton chlorophyll *a* concentrations in lakes were related to total nitrogen, total phosphorus, Secchi depth, concentration of dissolved oxygen, pH, water temperature, and specific conductivity. In nutrient ecoregion VII, nutrients were not significant predictors of chlorophyll *a* concentrations. Total nitrogen, Secchi depth, and pH were significantly related to phytoplankton chlorophyll *a* concentrations in nutrient ecoregion IX.

Lake periphyton chlorophyll *a* concentrations from nutrient ecoregion XI were related to total phosphorus rather than total nitrogen, Secchi depth, and pH. In all cases, Secchi depth was inversely related to the chlorophyll *a* concentrations in a lake. Nutrient ecoregion VIII had too few samples for any type of analysis.

Streams within the different nutrient ecoregions had many variables that were significantly related to periphyton chlorophyll *a* concentrations. These variables consisted of total nitrogen, total phosphorus, drainage area, percent forest cover, several macroinvertebrate indices, pH, basin slope, total residue, total suspended solids, and water temperature. Nutrients were not significantly related to periphyton chlorophyll *a* in streams within nutrient ecoregions VII or IX but were in nutrient ecoregion XI. Drainage area, percent forest cover, and several invertebrate indices were significant variables in nutrient ecoregion VII. Percent forest cover and several invertebrate indices had a negative relation with chlorophyll *a* concentrations in these streams. Percent forest cover and basin slope had a negative effect on periphyton in nutrient ecoregion IX streams. Light availability was more critical to periphyton growth in streams than nutrients.

Ecoregion XI had enough samples to do seasonal analyses. Summer-season periphyton chlorophyll *a* concentrations in nutrient ecoregion XI streams were positively related to total phosphorus and drainage area but negatively related to percent forest cover. Summer-season phytoplankton in streams was related to different variables within the same nutrient ecoregion. Both total nitrogen and total phosphorus were positively related with chlorophyll *a* concentrations as well as basin slope, total residue, and total suspended solids but negatively related to pH. The winter stream phytoplankton chlorophyll *a* concentrations were related to water temperature only.

## INTRODUCTION

A presidential initiative called the Clean Water Action Plan, released in 1998, provides a blueprint for federal agencies to work with states, tribes, and other stakeholders to protect and restore the Nation's water resources. The plan includes an initiative to address the nutrient-enrichment problem of lakes and streams across the United States. Emphasis is on the development of nutrient water-quality criteria for every geographic region in the country (U.S. Environmental Protection Agency, 1998). The premise was to compile water-quality, physical, biological, and chlorophyll *a* data in each state, create a working database, analyze the data, and report on the findings. Lakes and streams were to be analyzed separately. The U.S. Geological Survey (USGS), in cooperation with the Pennsylvania Department of Environmental Protection (PaDEP) and the U.S. Environmental Protection Agency (USEPA), conducted an analysis of data gathered from state and federal studies in both Pennsylvania and West Virginia in support of the USEPA nutrient-criteria program.

Nitrogen and phosphorus are nutrients needed for algal growth. These algae then become a food source for other organisms. Excess nutrients (nitrogen and phosphorus) can cause algal blooms that affect the taste and esthetic quality of water, and can deplete oxygen in the water, causing fish kills in streams, lakes, or ponds (Stevenson and others, 1996; Stevenson and Lowe, 1986; Sze, 1986). Point-source discharges of nutrients, which are fairly constant in load, are controlled by USEPA regulations (Buck and others, 2000). Nonpoint-source nutrients from fertilization of crops and grazing cattle have become a problem in some agricultural areas (Manahan, 1984). Some of these nutrients are released to the atmosphere and are returned to waterways in the form of rainfall (Buck and others, 2000).

Nutrient and algal criteria are to serve as benchmarks for the evaluation of the relative success of any nutrient-management effort (Buck and others, 2000). USEPA recommends using chlorophyll *a* as an indicator of the amount of nutrients in a stream or lake. Chlorophyll *a* is used as the algal-biomass indicator because it is a principal photosynthetic pigment in all algae (Sze, 1986; Collins and Weber, 1978). In cases in which nutrients are the limiting factor (essential for algal growth), chlorophyll *a* should increase with an increase of

nutrients to the system. The trend should be similar for each nutrient ecoregion (designated areas of similar characteristics); however, these thresholds may be different depending on the underlying geology. Other factors also may complicate this relation, such as light attenuation (Secchi depth and turbidity), temperature, alkalinity, pH, specific conductance, and aquatic invertebrates. A correlation between chlorophyll *a* and nutrients must be established and statistically valid if chlorophyll *a* is to be used as an indicator of nutrient enrichment.

Laboratory stream studies have shown that concentrations of chlorophyll *a* greater than 0.10 to 0.15  $\mu\text{g}/\text{m}^2$ , or a cover greater than 20 percent by filamentous algae, are unacceptable because they are harmful to stream biota (Horner and others, 1983). Other studies tend to suggest similar limits on the maximum level of biomass to maintain healthy streams. The Water Management Branch in British Columbia, Canada, suggests less than 0.05  $\mu\text{g}/\text{m}^2$  of chlorophyll *a* for recreational waters and less than 0.10  $\mu\text{g}/\text{m}^2$  to protect aquatic life (Nordin, 1985). New Zealand's Ministry for the Environment has recommended that biomass should not exceed 0.10  $\mu\text{g}/\text{m}^2$  of chlorophyll *a* for recreational waters (Zuur, 1992). However, a more recent study in New Zealand suggests that the maximum algal biomass should be 0.20  $\mu\text{g}/\text{m}^2$  (Biggs, 2000), which is higher than the original standard.

### **Purpose and Scope**

This report describes the relations among chlorophyll *a*, total nitrogen, total phosphorus, other water-quality constituents (turbidity, Secchi depth, specific conductance, pH, temperature, and alkalinity), habitat, topographic and land-use characteristics, and macroinvertebrate indices in the lakes and streams of four nutrient ecoregions of Pennsylvania and West Virginia. Data from Pennsylvania and West Virginia collected between 1990 and 1998 were used to show the influence of the explanatory variables on chlorophyll *a* concentrations. The concentrations of chlorophyll *a* and water-quality constituents, including nutrients, were determined from grab samples, and the macroinvertebrate indices used were generated using the USEPA Rapid Bioassessment Protocols (RBP). Lake and stream site maps were generated to indicate where more data are needed to fully describe the relations within a given nutrient ecoregion.

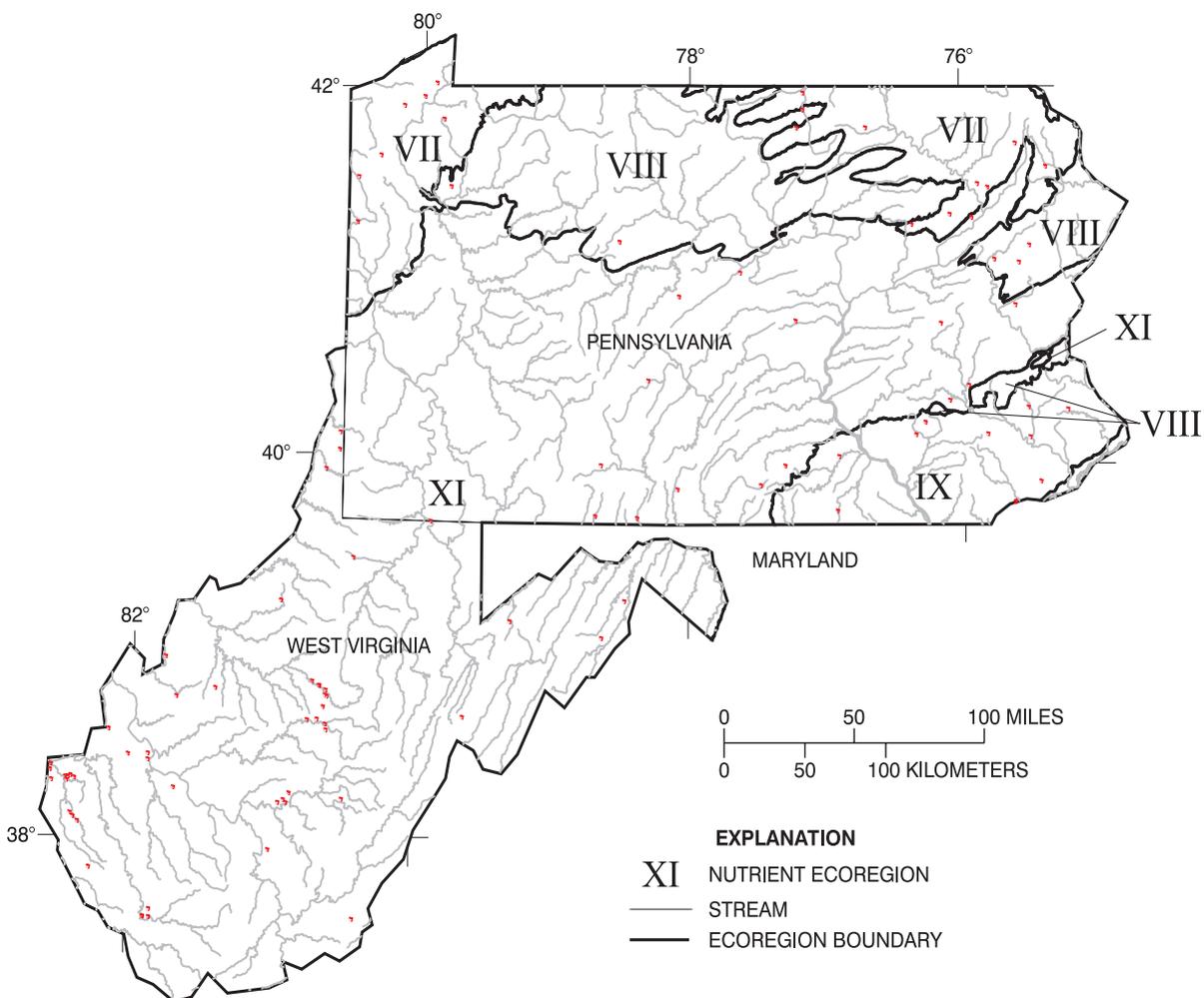
### Study Area

The study area includes the states of Pennsylvania and West Virginia. The states are divided into four nutrient ecoregions that are an aggregation of the Omernik (1987) level III ecoregions. The nutrient ecoregions are regions VII - Mostly Glaciated Dairy, VIII - Nutrient Poor, Largely Glaciated Upper Midwest and Northeast, IX - Southeastern Temperate Forested Plains and Hills, and XI - Central and Eastern Forested Uplands. All of West Virginia and a large part of Pennsylvania are in nutrient ecoregion XI (U.S. Environmental Protection Agency, 2000). Nutrient ecoregions VII, VIII, and IX are not located in West Virginia.

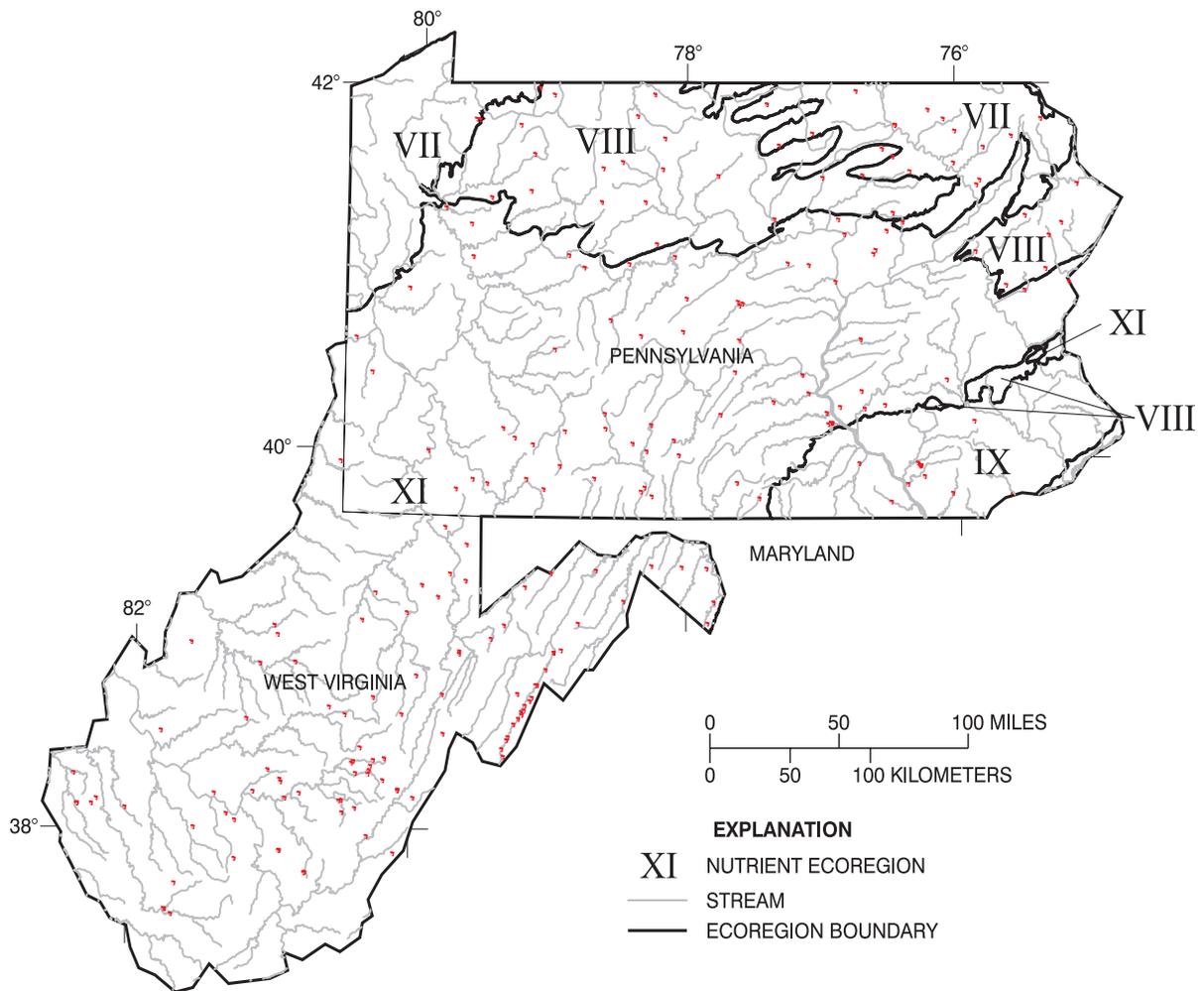
Nutrient ecoregion VII is a combination of the Omernik level III Northern Appalachian Plateau and Uplands, and the Erie Drift Plains Eco-

regions. Nutrient ecoregion VIII is the Northern Central Appalachians Ecoregion, and nutrient ecoregion IX is the Northern Piedmont Ecoregion (U.S. Environmental Protection Agency, 2001). Nutrient ecoregion XI contains all of West Virginia and part of Pennsylvania and is a combination of the Blue Ridge, the Ridge and Valley, the Central Appalachian, and the Western Allegheny Plateau ecoregions (U.S. Environmental Protection Agency, 2001).

Chlorophyll *a* and nutrient data were available for 18 lakes and 20 streams in nutrient ecoregion VII, 6 lakes and 27 streams in nutrient ecoregion VIII, 11 lakes and 12 streams in nutrient ecoregion IX, and 67 lakes and 170 streams in nutrient ecoregion XI. The locations of lake and stream sites are shown in figures 1 and 2, respectively.



**Figure 1.** Lake sites for which chlorophyll *a* and nutrient data were available within the nutrient ecoregions of Pennsylvania and West Virginia, 1990-98. (From U.S. Environmental Protection Agency, 2001.)



**Figure 2.** Stream sites for which chlorophyll a and nutrient data were available within the nutrient ecoregions of Pennsylvania and West Virginia, 1990-98. (From U.S. Environmental Protection Agency, 2001.)

### **Acknowledgments**

Many colleagues have contributed to the data aggregation and presentation of this report. Data aggregation was provided by Rodney Kime and Barbara Lathrop (Pennsylvania Department of Environmental Protection (PaDEP)), Michael Arcuri (West Virginia Department of Environmental Protection), Donna Beatsch (Ohio River Valley Water Sanitation Commission), Robert Edwards (Susquehanna River Basin Commission), Brian Hill (U.S. Environmental Protection Agency (USEPA)), and Gregory Wacik (U.S. Army Corps of Engi-

neers). Many others provided information about laboratory methods. Colleague reviewers were Ifeyinwa Davis, who also coordinated this effort, and others from USEPA, Charles McGarrell (PaDEP) and Kimberly Miller (U.S. Geological Survey (USGS)). Technical assistance was provided by Michael Bilger, James Bubb, Scott Hoffman, Leona McLanahan, Katherine Paybins, Terriann Preston, Jennifer Rasberry, Marla Stuckey, and Naomi Langland, all of the USGS. Technical expertise was provided by J. Kent Crawford (water-quality specialist) and Kim Otto (reports specialist) (USGS).

## **APPROACH**

Data for this effort were compiled from sources in Pennsylvania and West Virginia. Several agencies aided the effort to have as complete a data set of water-quality data from 1990 to 1998 as possible, including nutrients, chlorophyll *a*, and macroinvertebrates. These data were then assembled into a single database, which was provided to USEPA, PaDEP, and West Virginia Department of Environmental Protection (WVDEP) for their use in setting nutrient criteria. The USGS used the information to examine relations between chlorophyll *a*, water-quality constituents including nutrients, and macroinvertebrates.

### **Data Sources**

Chlorophyll *a*, nutrients and other water-quality constituents, and habitat and macroinvertebrate indices were compiled for lakes and streams in Pennsylvania and West Virginia for the years 1990 through 1998. Major sources of information were the USGS National Water Quality Information System (NWIS), PaDEP, WVDEP, Susquehanna River Basin Commission (SRBC), Delaware River Basin Commission (DRBC), U.S. Army Corps of Engineers (COE), the USEPA Environmental Monitoring and Assessment Program (EMAP), the USEPA Storage and Retrieval (STORET) database, the National Park Service (NPS), and Ohio River Valley Sanitation Commission (ORSANCO). A large part of the macroinvertebrate data were collected according to the USEPA Rapid Bioassessment Protocol technique (Plafkin and others, 1989) with some variations on the level of identification. Land use, drainage-basin area, and slope were calculated from Geographical Information System (GIS) data. Land use was derived from USGS Multi-Resolution Land Characteristics 2000 (MRLC2000) data (U.S. Geological Survey, 2001). MRLC2000 land-use classes were simplified into three classes: forested (MRLC classes 8, 9, 10, 11), urban (MRLC classes 2, 3, 4), and agriculture (all remaining MRLC classes). Laboratory methods are listed in the appendix.

### **Data Analysis**

Data were assembled into a Microsoft ACCESS database as requested by the USEPA. Data were analyzed using S-Plus. The GIS data that were created were added to the ACCESS database and were used as explanatory variables.

## **Algae**

Data from lakes and streams were analyzed separately. Lake-water samples were characterized by use of phytoplankton chlorophyll *a* except in some West Virginia lakes where periphyton chlorophyll *a* data were collected in some streams. Stream-water samples were characterized using periphyton chlorophyll *a* except in West Virginia where phytoplankton chlorophyll *a* data were collected in some streams. The West Virginia stream phytoplankton chlorophyll *a* data were analyzed separately using the same explanatory variables as used for the periphyton chlorophyll *a* data. Lake data from Pennsylvania, West Virginia, and the EMAP program were combined, where appropriate, to form a more complete data set. The same approach was used in the compilation of the stream data. Differences in data collection and laboratory methods among these groups were not great enough to prevent grouping the data. Data were removed from the final data set when the methods were not identified clearly or sampling was completed by some unknown method. Phytoplankton and periphyton chlorophyll *a* lake data were examined separately because they represent different environments, sampling techniques, and concentration reporting units.

The final stream data set used for this report includes chlorophyll *a*, water quality, basin characteristics, habitat characteristics, and aquatic macroinvertebrate indices. The lake data set included chlorophyll *a* and other water-quality characteristics. Not all types of data were available for all sampling points. At a minimum, data for chlorophyll *a*, nutrients, and basin characteristics were available. Following Gibson and others (2000), lake data were considered independent across sampling locations and years. The stream data, however, were considered independent across sampling locations but not years. This was done to prevent biasing the data set because of an unbalanced number of years of data for the various sampling locations. Where sites were in close proximity along a stream and no significant tributaries were present that could potentially alter water quality among these sites, the data were combined using a mean for the stream length. This provided one data point and reduced the potential for bias in the data set. A GIS procedure was used to determine the proximity of sites to each other.

Seasonal means of the data were used in the analysis of lakes and streams. The seasons consist of summer (May through September) and winter (October through April). If multiple samples for a season were not available, single-sample data were substituted for seasonal means. Winter-season lake data were limited to six or less points in nutrient ecoregions VII, VIII, and IX; therefore, no winter-season analysis was done for these nutrient ecoregions. Nutrient ecoregion XI had sufficient data for winter-season lake data analysis. Summer-season data for streams included both periphyton and phytoplankton chlorophyll *a*. Winter-season stream data were limited or nonexistent for nutrient ecoregions VII, VIII, and IX, so no analysis was completed in these nutrient ecoregions. Analysis of winter-season stream data for nutrient ecoregion XI was completed for both periphyton and phytoplankton chlorophyll *a*.

Data reduction was used to minimize the number of potential explanatory variables and make the largest possible data set for analysis. Individual explanatory variables were, in many cases, populated with data from multiple parameter codes. Field and laboratory parameter codes were compiled under one parameter for specific conductance (00094, 00095) and pH (00400, 00403). Secchi depth data were compiled from meters (00078), feet (48701), or inches (00077) parameter codes and converted to meters, if required. A mean value was used per lake per year using a similar approach as the other potential explanatory variables. Instantaneous and mean streamflow (00061, 00060) were compiled into one parameter. Alkalinity and acid neutralizing capacity (ANC) data were reduced into one parameter compiled from four parameter codes (00410, 29801, 39086, 39087). Because very little total nitrogen data (parameter code 00600) were available, various component species of nitrogen were combined to produce estimates of total nitrogen. The various combinations used were: total Kjeldahl nitrogen (00625) + total nitrite plus nitrate (00630); total Kjeldahl nitrogen (00625) + total nitrite (00615) + total nitrate (00620); total Kjeldahl nitrogen (00625) + dissolved nitrite plus nitrate (00631); total organic nitrogen (00605) + total ammonia (00610) + total nitrite plus nitrate (00630). Combining of phosphorus species was not required. The term nutrients throughout the text will refer to total nitrogen and total phosphorus. Phytoplankton chlorophyll *a* values were combined to show the mean for a lake regardless of the sampling depth. Invertebrate indices were reduced

using the same approach. The indices used in the calculations were generated from data collected using the RBP method or a variation of that method. A mean of the indices was calculated where appropriate, but this was infrequent because there were rarely overlapping studies at the same site using different methods of sampling.

USEPA is interested in several key constituents for both lakes and streams. If data for those constituents were available, they were included in the final regression analysis. Some of the constituents are response variables, but for this analysis, all were used as causative variables. For lakes, USEPA was most interested in total nitrogen, total phosphorus, total organic carbon, suspended solids, transparency, turbidity, direct algal counts, and biochemical oxygen demand (Gibson and others, 2000). For streams, total nitrogen, total phosphorus, total suspended solids, transparency, turbidity, flow, and velocity were key characteristics (Buck and others, 2000). Information on these constituents was included in the multiple-regression analysis.

Across nutrient ecoregions, statistical comparisons were made using a nonparametric multiple-stage Kruskal-Wallis test (Helsel and Hirsch, 1995). Chlorophyll *a*, total nitrogen, total phosphorus, specific conductance, alkalinity or acid-neutralizing capacity (ANC), pH, Secchi depth, and turbidity were examined for significant differences among the four nutrient ecoregions.

Within each nutrient ecoregion, multiple linear regression was used to select water-quality constituents and environmental indicators that were significant in explaining chlorophyll *a* concentrations. Prior to regression analysis, correlation coefficients among the explanatory variables were examined. For highly correlated ( $r \geq 0.9$ ) pairs, the variable less commonly cited as an indicator variable was deleted from the list of potential explanatory variables. Highly correlated variables in a multiple linear regression will exhibit multicollinearity. The existence of multicollinearity can result in incorrect sign, incorrect magnitude, and inflated variance in the coefficient estimates (Freund and Littell, 1986). The explanatory variables available for chlorophyll *a* regressions within each nutrient ecoregion are listed in table 1 for lakes and in table 2 for streams. In nutrient ecoregions VII, VIII, and IX, an explanatory variable that was limited in number of data to less than 75 percent of the total number of data for that nutrient

**Table 1.** Explanatory variables examined for significance in regressions of the log of chlorophyll a concentrations for nutrient ecoregions VII, VIII, IX, and XI lakes, Pennsylvania and West Virginia, 1990-98

[ANC, acid neutralizing capacity]

	Nutrient ecoregion VII	Nutrient ecoregion VIII	Nutrient ecoregion IX	Nutrient ecoregion XI
Explanatory variables	alkalinity, ANC	alkalinity, ANC	alkalinity, ANC	alkalinity, ANC
		dissolved oxygen	dissolved oxygen	dissolved oxygen
	pH	pH	pH	pH
	Secchi depth	Secchi depth	Secchi depth	Secchi depth
	specific conductivity	specific conductivity	specific conductivity	specific conductivity
	total nitrogen	total nitrogen	total nitrogen	total nitrogen
	total phosphorus	total phosphorus	total phosphorus	total phosphorus
	water temperature	water temperature	water temperature	water temperature

**Table 2.** Explanatory variables examined for significance in regressions of the log of chlorophyll a concentrations for nutrient ecoregions VII, VIII, IX, and XI streams, Pennsylvania and West Virginia, 1990-98

[pct., percent; ANC, acid neutralizing capacity; HBI, Hilsenhoff's biotic index; EPT, Ephemeroptera-Plecoptera-Trichoptera]

	Nutrient ecoregion VII	Nutrient ecoregion VIII	Nutrient ecoregion IX	Nutrient ecoregion XI
Explanatory variables	alkalinity, ANC	alkalinity, ANC		alkalinity, ANC <sup>1</sup>
	basin slope	basin slope	basin slope	basin slope <sup>1</sup>
			dissolved oxygen	
	drainage area	drainage area	drainage area	drainage area <sup>1</sup>
	Ephemeroptera	Ephemeroptera		Ephemeroptera
	Ephemeroptera taxa	Ephemeroptera taxa		Ephemeroptera taxa
	Ephemeroptera taxa richness	Ephemeroptera taxa richness		Ephemeroptera taxa richness
	EPT: Chironomidae ratio	EPT: Chironomidae ratio		EPT: Chironomidae ratio
	EPT index	EPT index		EPT index
	EPT taxa	EPT taxa		EPT taxa
	HBI	HBI		HBI
	pct. agriculture land use	pct. agriculture land use	pct. agriculture land use	pct. agriculture land use <sup>1</sup>
	pct. collector gatherers	pct. collector gatherers		pct. collector gatherers
	pct. dominant taxa	pct. dominant taxa		pct. dominant taxa
	pct. filterer collectors	pct. filterer collectors		pct. filterer collectors
	pct. forest land use	pct. forest land use	pct. forest land use	pct. forest land use <sup>1</sup>
	pct. Plecoptera individuals	pct. Plecoptera individuals		pct. Plecoptera individuals
	pct. scrapers	pct. scrapers		pct. scrapers
	pct. scraper filterers	pct. scraper filterers		
	pct. Trichoptera individuals	pct. Trichoptera individuals		pct. Trichoptera individuals
	pct. tolerant individuals	pct. tolerant individuals	pct. tolerant individuals	pct. tolerant individuals
	pct. urban land use	pct. urban land use	pct. urban land use	pct. urban land use <sup>1</sup>
	pH	pH	pH	pH <sup>1</sup>
	Plecoptera taxa richness	Plecoptera taxa richness	Plecoptera taxa richness	Plecoptera taxa richness
	specific conductivity	specific conductivity	specific conductivity	specific conductivity <sup>1</sup>
			stream discharge	
			suspended sediment	
	taxa richness	taxa richness		taxa richness
	tolerant taxa richness	tolerant taxa richness		tolerant taxa richness
	total residue	total residue		total residue <sup>1</sup>
	total nitrogen	total nitrogen	total nitrogen	total nitrogen <sup>1</sup>
	total phosphorus	total phosphorus	total phosphorus	total phosphorus <sup>1</sup>
				total suspended solids <sup>2</sup>
Trichoptera taxa richness	Trichoptera taxa richness		Trichoptera taxa richness	
turbidity	turbidity		turbidity	
		water temperature	water temperature <sup>2</sup>	

<sup>1</sup> Explanatory variables examined for periphyton and phytoplankton regression relations.

<sup>2</sup> Explanatory variables examined for phytoplankton regression relations only.

ecoregion was not considered for model inclusion. The regression procedure trims the available data for all variables to that of the variable with the smallest number, including variables with less than 75 percent of the total available number of data. This would result in a loss of acceptable data. Because the data set for streams in nutrient ecoregion XI is much larger than for streams in the other nutrient ecoregions, subsets as small as 24 samples were used to allow inclusion of as many explanatory variables as possible. Chlorophyll *a* concentrations and explanatory variables were log transformed, if required, to satisfy any statistical assumptions. Statistical significance was set at  $\alpha = 0.05$ .

The final regression equations were selected on the basis of minimizing the Mallows CP statistic, the PRESS statistic and the absence of pathologies such as multicollinearity, high influence, and coefficient signs and magnitudes contrary to known system behaviors. Multicollinearity was diagnosed with eigenvalues and the variance inflation factor. Multicollinearity was resolved by deleting the explanatory variable as described above. Outliers and high-influence points were diagnosed with partial leverage plots, standardized residuals, Cook's D, and Studentized residuals. Outliers and

high-influence points were deleted from regression data sets if, as a result of an F test comparison, their inclusion was shown to significantly alter regression coefficients. The decision to delete outliers with high influence was made considering the unknown quality of much of the available data. In most cases, only one and at most two data points were deleted from any one data set. In this manner, individual data points of unknown quality were prevented from significantly altering regressions that, in their absence, better represented the bulk of the data.

### **Macroinvertebrates**

Macroinvertebrate data were available for streams but not for lakes. Correlations among macroinvertebrate indices were examined. As with other explanatory variables, correlation coefficients equal to or greater than 0.90 resulted in eliminating one of the correlated indices from the data analysis. Other reasons for selecting an index for deletion included indicators not typically reported and small numbers of available data. The remaining indices were entered as potential explanatory variables in the regression procedure.

## RELATIONS AMONG CHLOROPHYLL *a*, NUTRIENTS, OTHER WATER-QUALITY CHARACTERISTICS, AND HABITAT AND MACROINVERTEBRATE INDICES

Algae are primary producers in lakes and streams. They are a food source and shelter for animals, and chemical modulators transforming many inorganic chemicals into their organic forms (Stevenson and others, 1996). Algae show active uptake of nitrate, ammonia, and other nutrients during the day (Triska and others, 1989), are an important sink for nutrients (Wetzel, 1996), and help to stabilize the substrata by decreasing the effects of scour (Biggs, 1996). Too much algae, however, especially filamentous green algae, in a lake or stream can have a detrimental affect on water-supply usability, aesthetic appeal, and in-water recreational use (Stevenson and others, 1996; Wharfe and others, 1984).

Nutrients—nitrogen and phosphorus—are required in moderate quantities in aquatic and terrestrial ecosystems (Stevenson and others, 1996). Major inputs of nutrients to streams are from rainfall and runoff in the summer and heavy rains or snowmelt in the winter (Goldman and Horne, 1983). Streams provide the largest input of nitrogen to lakes, even though the lake surface is in constant contact with the air, which is an inexhaustible nitrogen reservoir (Goldman and Horne, 1983). Large influxes of nutrients into a stream usually

will cause the biomass of plants and animals to increase downstream of the inflow. Even though uptake by plants may buffer the amount of nitrate in the water, it is commonly so abundant that only a small fraction is removed (Casey, 1977). Nitrogen gas is the most common form of nitrogen in lakes and typically shows no seasonal or depth variations except those resulting from temperature changes (Goldman and Horne, 1983).

In the northern half of the United States, phosphorus is typically more limiting than nitrogen (Stevenson and others, 1996; Sze, 1986; Smith, 1980). Phosphorus has a high affinity for soils and little phosphorus is released into the water column unless the soil is eroded; nitrogen in the form of nitrate moves freely through soils (Goldman and Horne, 1983). Nitrogen may be limiting during periods of low streamflow when the denitrification process may reduce the nitrogen supply (Lohman and others, 1991).

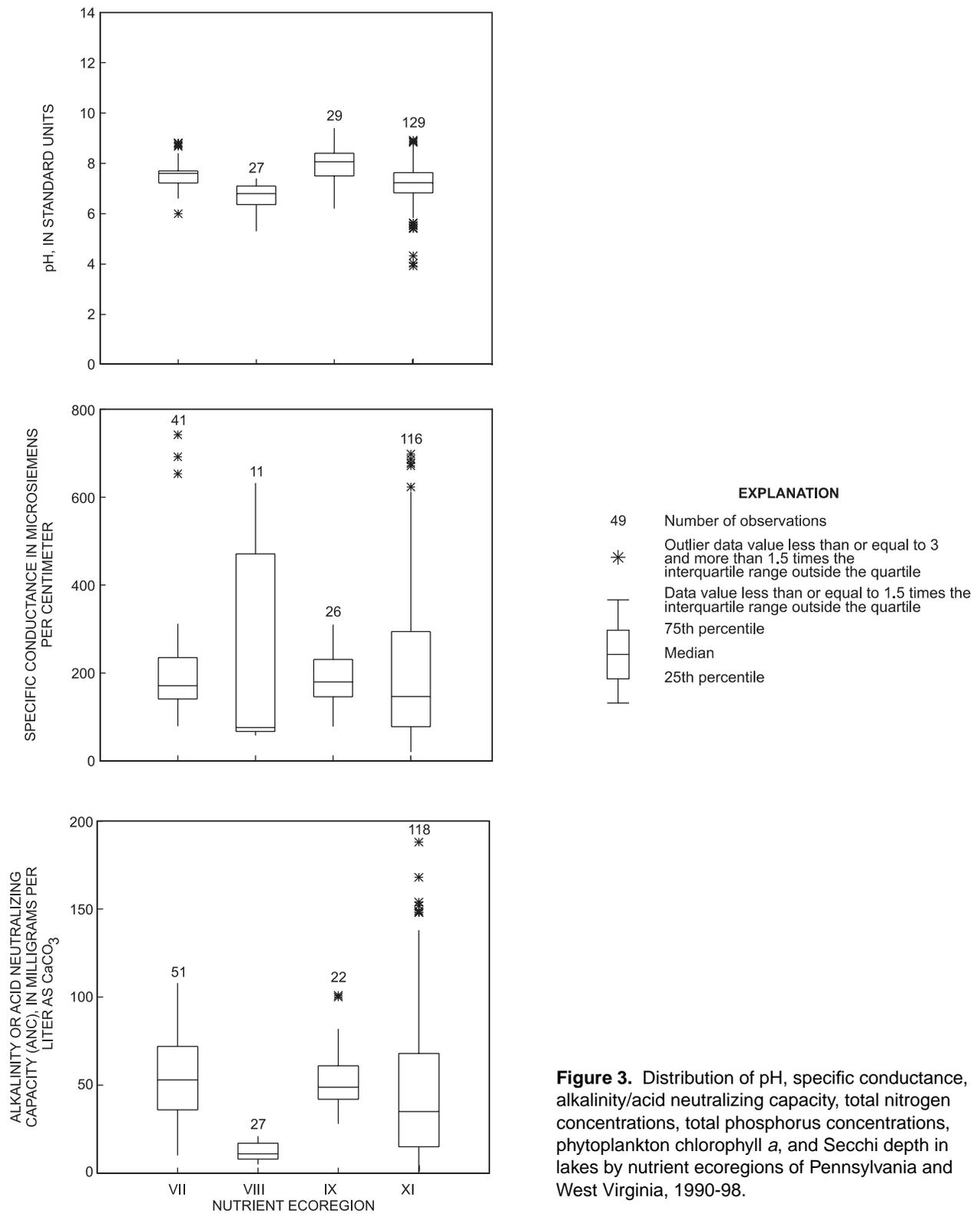
### Comparison Among Nutrient Ecoregions

**Lakes.**—Median concentrations of phytoplankton chlorophyll *a* and water-quality constituents except for specific conductance and alkalinity show significant differences among the nutrient ecoregions (fig. 3) (table 3). Lake sites in West Virginia, where periphyton data were collected, were not used in figure 3 and table 3.

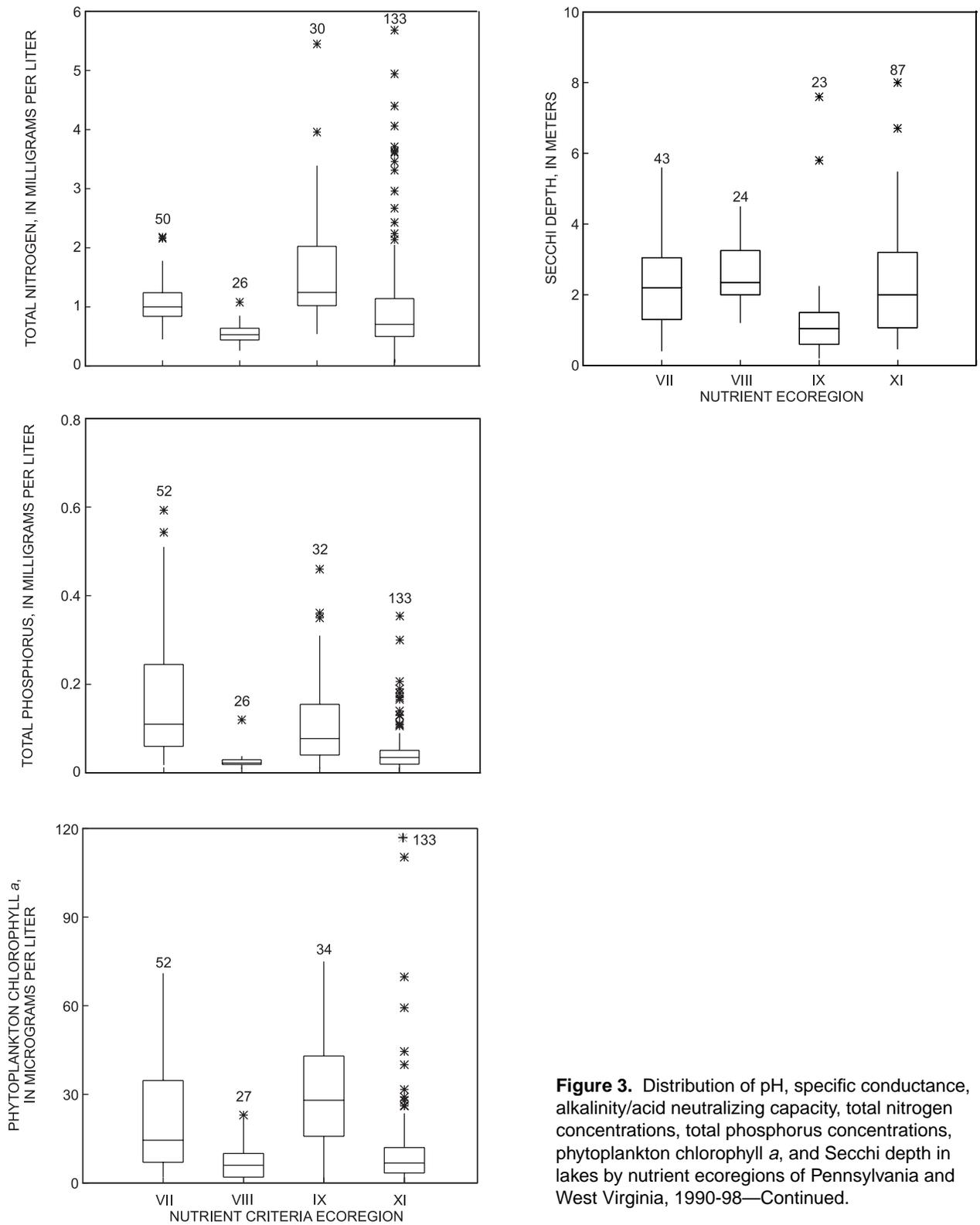
**Table 3.** Results of multiple-stage Kruskal-Wallis test for significant differences in median values of water-quality constituents data from lakes in nutrient ecoregions VII, VIII, IX, and XI, Pennsylvania and West Virginia, 1990-98

[Median values contained in shaded blocks are not significantly different. Median values in unshaded blocks are significantly different from all other values.  $\mu\text{S}/\text{cm}$ , microsiemens per centimeter;  $\text{mg}/\text{L}$ , milligrams per liter;  $\mu\text{g}/\text{L}$ , micrograms per liter; m, meters]

Water-quality constituent	Median values			
	Nutrient ecoregion VII	Nutrient ecoregion VIII	Nutrient ecoregion IX	Nutrient ecoregion XI
pH (units)	7.6	6.8	8.1	7.2
Specific conductance ( $\mu\text{S}/\text{cm}$ )	171	76	180	143
Alkalinity (acid neutralizing capacity) ( $\text{mg}/\text{L}$ )	51	27	48	30
Phytoplankton chlorophyll <i>a</i> ( $\mu\text{g}/\text{L}$ )	14.5	6.00	28.0	6.76
Total nitrogen ( $\text{mg}/\text{L}$ )	1.00	.53	1.24	.63
Total phosphorus ( $\text{mg}/\text{L}$ )	.110	.023	.078	.032
Secchi depth (m)	2.20	2.35	1.04	2.00



**Figure 3.** Distribution of pH, specific conductance, alkalinity/acid neutralizing capacity, total nitrogen concentrations, total phosphorus concentrations, phytoplankton chlorophyll *a*, and Secchi depth in lakes by nutrient ecoregions of Pennsylvania and West Virginia, 1990-98.



**Figure 3.** Distribution of pH, specific conductance, alkalinity/acid neutralizing capacity, total nitrogen concentrations, total phosphorus concentrations, phytoplankton chlorophyll a, and Secchi depth in lakes by nutrient ecoregions of Pennsylvania and West Virginia, 1990-98—Continued.

Median concentrations of phytoplankton chlorophyll *a* (28 µg/L), total nitrogen (1.24 mg/L), and pH (8.1) are greatest in nutrient ecoregion IX and decrease in order from nutrient ecoregion VII, XI, to VIII. Nutrient ecoregion IX also had the lowest median value for Secchi depth (1.04), which was significantly lower than the other three nutrient ecoregions. Nutrient ecoregion VII had the greatest median concentration of total phosphorus (0.110 mg/L) and alkalinity/acid-neutralizing capacity (51 mg/L as CaCO<sub>3</sub>) although neither were significantly greater than those in nutrient ecoregion IX. Nutrient ecoregion VIII had the lowest median values for phytoplankton chlorophyll *a* (6.0 µg/L), concentration of total nitrogen (0.53 mg/L), concentration of total phosphorus (0.023 mg/L), specific conductance (76 µS/cm), alkalinity/acid-neutralizing capacity (27 mg/L as CaCO<sub>3</sub>), and pH (6.8) of all nutrient ecoregions but had the highest median value for Secchi depth (2.35). However, phytoplankton chlorophyll *a*, total nitrogen, Secchi depth, alkalinity, and specific conductance were not significantly lower than in nutrient ecoregion XI. Median values of specific conductance were not significantly different among any of the nutrient ecoregions, nor was water temperature with a maximum difference in median water temperature of less than 2°C.

In general, concentrations of chlorophyll *a* and water quality tend to separate the nutrient ecoregions into two groups, nutrient ecoregions VII and IX and nutrient ecoregions VIII and XI. Nutrient ecoregions VII and IX have the highest median values and nutrient ecoregions VIII and XI have the lowest. Where median concentrations of chlorophyll *a* are highest, Secchi depths are lowest, and where median concentrations of chlorophyll *a* are lowest, Secchi depths are highest. Notably, nutrient ecoregion XI had the largest sample con-

centrations for phytoplankton chlorophyll *a* (160 µg/L), total nitrogen (5.66 mg/L), and alkalinity/ANC (168 mg/L as CaCO<sub>3</sub>) but some of the lowest median concentrations. The substantially larger data set and broader areal coverage of nutrient ecoregion XI likely contributed to these maximum individual sample values.

**Streams.**—Median values for the water-quality constituents tended to be greatest in nutrient ecoregion IX streams and least in nutrient ecoregion VIII streams (fig. 4 and table 4). Significantly higher concentrations of periphyton chlorophyll *a* (24 mg/m<sup>2</sup>), total nitrogen (6.22 mg/L), total phosphorus (0.143 mg/L) and specific conductance (579 µS/cm) were measured in nutrient ecoregion IX. Significantly higher alkalinity/acid-neutralizing capacity (876 mg/L as CaCO<sub>3</sub>) and pH (8.2) were measured in nutrient ecoregion VII. Nutrient ecoregion VIII had the lowest median values for all variables presented. Chlorophyll *a* was not significantly lower than nutrient ecoregions VII and XI, while alkalinity was not significantly different from nutrient ecoregions IX and XI. The median turbidity was significantly lower in nutrient ecoregion VIII and highest in XI. Data from sites where phytoplankton data were collected in West Virginia streams are not shown in figure 4 and table 4.

The distribution of periphyton chlorophyll *a* among the nutrient ecoregions tends to increase positively with the distribution of instream nutrients (fig. 4). The substantially greater median concentration of periphyton chlorophyll *a* measured in nutrient ecoregion IX (fig. 4) coincides with higher concentrations of total nitrogen and total phosphorus. A similar relation can be observed in nutrient ecoregion VIII for the lowest median concentrations of chlorophyll *a* and nutrients.

**Predictive Relations Between Chlorophyll a and Nutrients**

Predictive relations between concentrations of chlorophyll a and nutrients have been reported previously. For benthic algae (periphyton), Dodds and others (1997) suggested the following general regression equations if more locale specific relations are not available:

$$\log(\text{mean chlorophyll } a) = 0.011173 + 0.5949(\log \text{ total nitrogen}) \quad (1)$$

$$\log(\text{mean chlorophyll } a) = 1.091 + 0.2786(\log \text{ total phosphorus}) \quad (2)$$

These regression equations lack strong predictive power, particularly if total phosphorus is the explanatory variable. The  $r^2$  for the total nitrogen regression equation (eqn. 1) is 0.35 and for the total phosphorus regression equation (eqn. 2) is

0.089. Dodds and others (1997) combined total nitrogen and total phosphorus for an increase in predictive power (eqn. 3) ( $r^2 = 0.43$ ).

$$\begin{aligned} \log(\text{mean chlorophyll } a) = & -3.233 \\ & + 2.826(\log \text{ total nitrogen}) \\ & - 0.43(\log \text{ total nitrogen})^2 \\ & + 0.255(\log \text{ total phosphorus}) \end{aligned} \quad (3)$$

where (eqns. 1, 2, and 3)

Chlorophyll a is concentration, in milligrams per square meter;

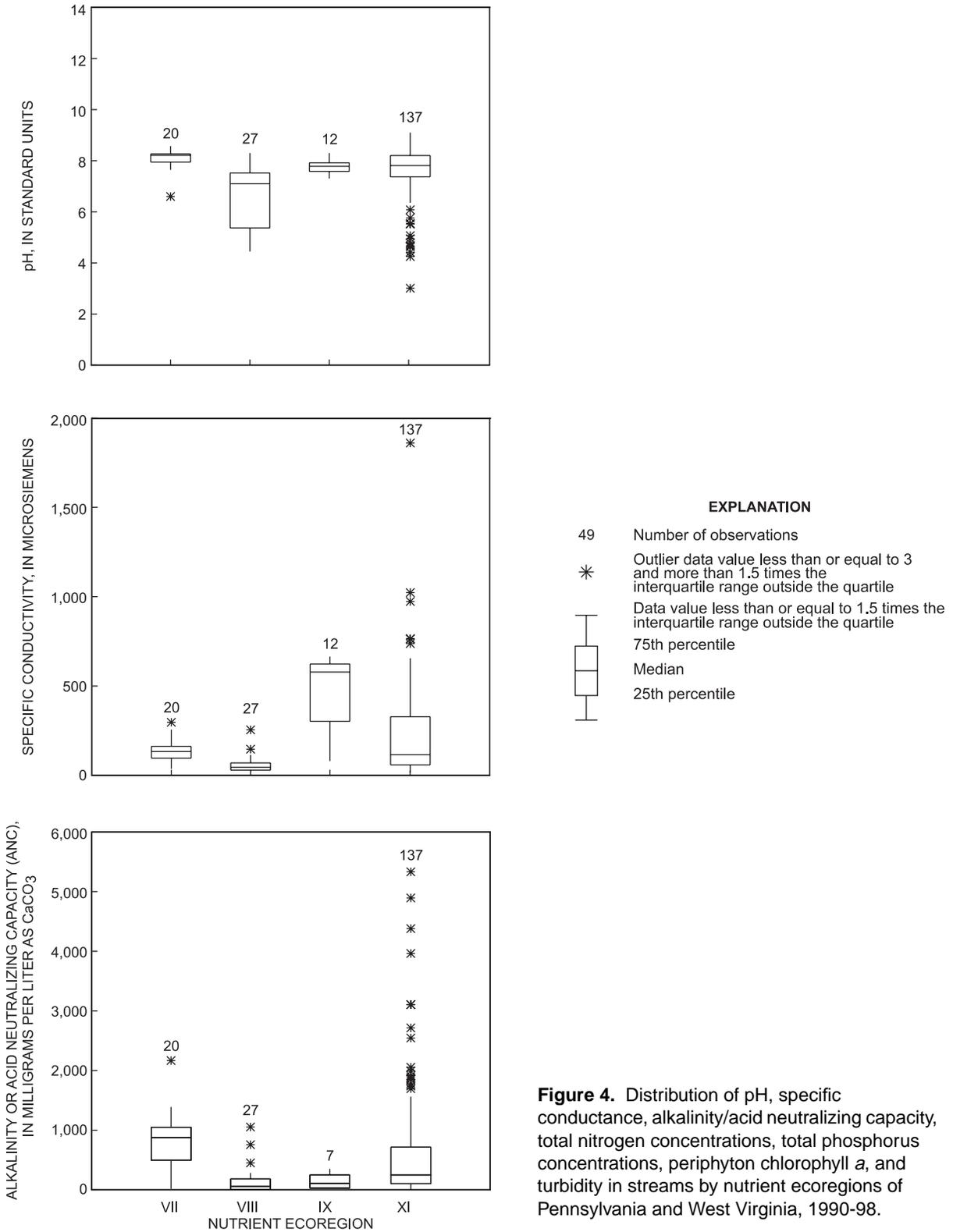
Total nitrogen is concentration, in micrograms per liter; and

Total phosphorus is concentration, in micrograms per liter.

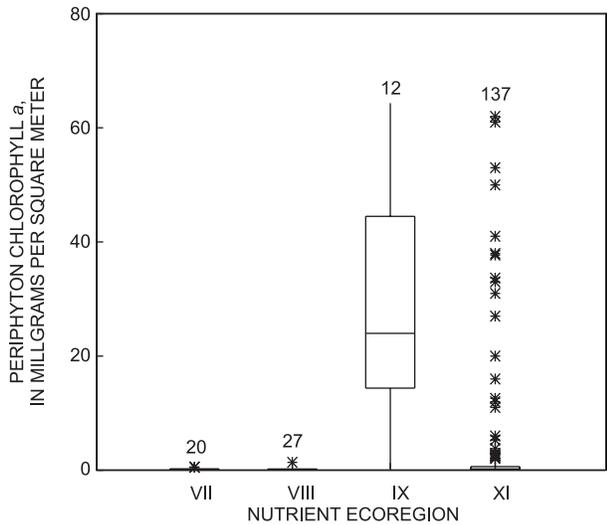
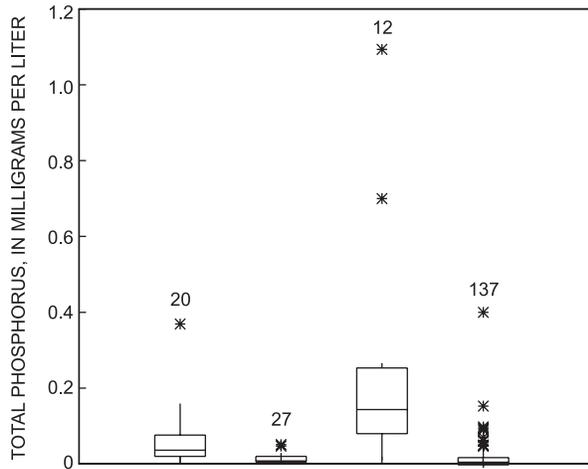
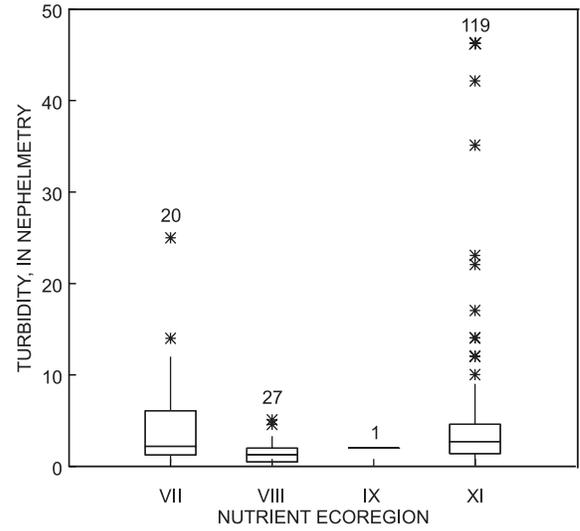
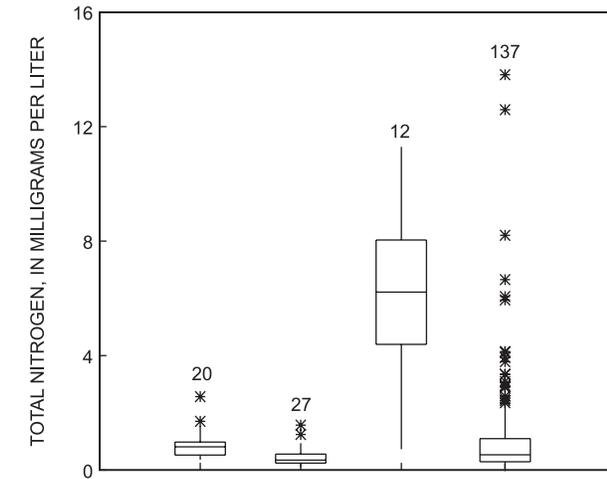
**Table 4.** Results of multiple-stage Kruskal-Wallis test for significant differences in median values of water-quality constituent data from streams in nutrient ecoregions VII, VIII, IX, and XI, Pennsylvania and West Virginia, 1990-98

[Median values contained in shaded blocks are not significantly different. Median values in unshaded blocks are significantly different from all other values; ND, no data;  $\mu\text{S}/\text{cm}$ , microsiemens per centimeter;  $\text{mg}/\text{L}$ , milligrams per liter;  $\text{mg}/\text{m}^2$ , milligrams per meter squared; NTU, turbidity units]

Water-quality constituent	Median values			
	Nutrient ecoregion VII	Nutrient ecoregion VIII	Nutrient ecoregion IX	Nutrient ecoregion XI
pH (units)	8.2	7.1	7.8	7.8
Specific conductance ( $\mu\text{S}/\text{cm}$ )	134	46	579	116
Alkalinity (acid neutralizing capacity) ( $\text{mg}/\text{L}$ )	876	62	109	247
Periphyton chlorophyll a ( $\text{mg}/\text{m}^2$ )	.13	.01	24.0	.18
Total nitrogen ( $\text{mg}/\text{L}$ )	.81	.35	6.22	.57
Total phosphorus ( $\text{mg}/\text{L}$ )	.036	.008	.143	.015
Turbidity (NTU)	2.2	1.3	ND	2.7



**Figure 4.** Distribution of pH, specific conductance, alkalinity/acid neutralizing capacity, total nitrogen concentrations, total phosphorus concentrations, periphyton chlorophyll *a*, and turbidity in streams by nutrient ecoregions of Pennsylvania and West Virginia, 1990-98.



**Figure 4.** Distribution of pH, specific conductance, alkalinity/acid neutralizing capacity, total nitrogen concentrations, total phosphorus concentrations, and periphyton chlorophyll a, and turbidity in streams by nutrient ecoregions of Pennsylvania and West Virginia, 1990-98—Continued.

For suspended algae (phytoplankton) in streams, Van Nieuwenhuysse and Jones (1996) reported the following equation:

$$\begin{aligned} \log(\text{chlorophyll}) = & \\ -1.65 + 1.99(\log \text{ total phosphorus}) & \\ - 0.28(\log \text{ total phosphorus})^2 & \end{aligned} \quad (4)$$

where

Chlorophyll is concentration, in milligrams per cubic meter; and

Total phosphorus is concentration, in milligrams per liter.

The predictive power of equation 4 ( $r^2 = 0.67$ ) is considerably better than those suggested by Dodds and others (1997) for benthic algae. By incorporating drainage-basin area into the Van Nieuwenhuysse and Jones equation (1996) (eqn. 4), the resulting equation 5 has a slightly increased predictive power ( $r^2 = 0.73$ ).

$$\begin{aligned} \log(\text{chlorophyll}) = & \\ -1.92 + 1.96(\log \text{ total phosphorus}) & \\ - 0.30(\log \text{ total phosphorus})^2 & \\ + 0.12(\log \text{ drainage-basin area}) & \end{aligned} \quad (5)$$

where

Chlorophyll is concentration, in milligrams per cubic meter;

Total phosphorus is concentration, in milligrams per liter; and

Drainage-basin area is area, in square kilometers.

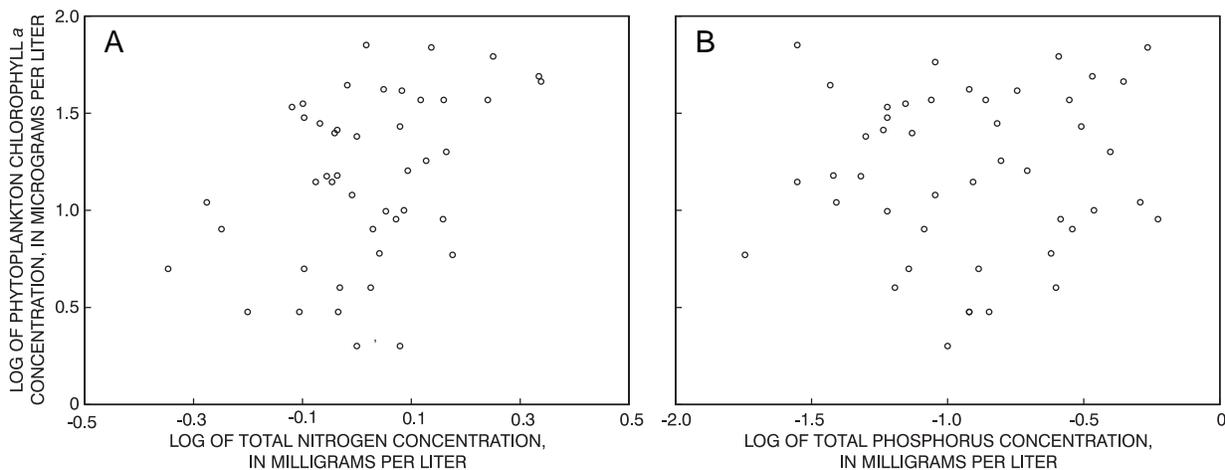
All five equations are general in scope and stronger predictive relations should be expected from nutrient ecoregion specific data (U.S. Environmental Protection Agency, 2000). Several studies have indicated that in multiple regression equations, constituents such as water velocity and discharge may explain as much or more of the variation in benthic algal biomass as nutrients (Welch and others, 1988; Biggs and Close, 1989; Duncan and Blinn, 1989), or that phosphorus input into streams is positively related to the average slope of the drainage basin (Kirchner, 1975).

#### **Predictive Relations Between Chlorophyll *a* and Nutrients for Nutrient Ecoregions VII, VIII, IX, and XI**

The previous section discussed general predictive equations that could be applied to all nutrient ecoregions as a whole. By looking at each nutrient ecoregion separately, better and more precise predictive equations relating chlorophyll *a* to nutrients should be attainable.

#### **Nutrient Ecoregion VII**

Lakes.—The data set consisted of 47 samples from 18 lakes. The relation between concentrations of phytoplankton chlorophyll *a* and concentrations of total nitrogen and total phosphorus (fig. 5) show considerable scatter. The data for total nitrogen suggest a positive correlation (fig. 5A), but no relation is discernible in the data for total phosphorus (fig. 5B).



**Figure 5.** Relation between log of phytoplankton chlorophyll *a* concentrations and the log of concentrations of total nitrogen (A) and total phosphorus (B) in lakes within nutrient ecoregion VII, Pennsylvania, 1990-98.

In the absence of other explanatory variables, concentrations of total nitrogen had a low but significant predictive power ( $r^2 = 0.16$ ) (eqn. 6) (table 5) for phytoplankton chlorophyll *a*. Concentrations of total phosphorus had no significant predictive power. When other water-quality data were entered into the regression analysis, a substantial increase in the predictive power of the resulting equation ( $r^2 = 0.75$ ) was noted (eqn. 7) (table 5), although the data set was smaller. Secchi depth and the concentration of dissolved oxygen combined to explain about three-quarters of the variation in the log of concentrations of phytoplankton chlorophyll *a* (fig. 6). Concentrations of total nitrogen were not significant in this regression equation.

Secchi depth and chlorophyll *a* concentrations are negatively related. Secchi depth, which is measured by lowering a black and white colored disk into the water and determining how far down in the water column the disk can be seen, is used to determine the water clarity in a lake. Secchi depth can be used as a causative or an explanatory variable. As turbidity increases in the water column from sediment, chlorophyll *a* concentrations can decrease. However, in this relation, Secchi depth is

a response variable, and as chlorophyll *a* concentrations increased, meaning the bloom is greater, the Secchi depth decreased and the water became more turbid.

Concentrations of dissolved oxygen also can be a response variable to the amount of algae in a lake. Algae produces oxygen during the day and can raise the amount of dissolved oxygen in the lakes. At night, the algae respire and use the oxygen. Large algal blooms can deplete oxygen supplies in the water and cause fish kills.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) \quad (6)$$

$$\log(\text{Chlorophyll } a) = I + B(\text{Secchi depth}) + C(\text{Dissolved oxygen}) \quad (7)$$

where

Chlorophyll *a* is concentration, in micrograms per liter;

Total nitrogen is concentration, in milligrams per liter;

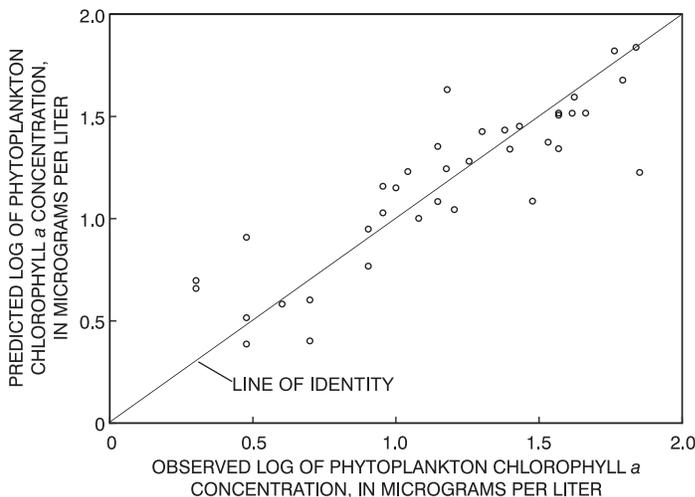
Secchi depth is depth, in meters; and

Dissolved oxygen is concentration, in milligrams per liter.

**Table 5.** Regression equation statistics for phytoplankton chlorophyll *a* concentrations in nutrient ecoregion VII lakes, Pennsylvania, 1990-98

[—, not used in equation; <, less than; A, B, and C are regression coefficients]

Equation	Intercept		Total nitrogen		Secchi depth		Dissolved oxygen		n	$r^2$	Residual standard error
	I	p-value	A	p-value	B	p-value	C	p-value			
6	1.143	<0.001	1.191	0.007	—	—	—	—	45	0.16	0.403
7	1.551	<.001	—	—	-0.345	<0.001	0.078	0.003	35	.75	.223



**Figure 6.** Relation between observed and predicted logs of phytoplankton chlorophyll *a* concentrations in lakes within nutrient ecoregion VII, Pennsylvania, 1990-98. (Predicted concentrations were computed using equation 7.)

**Streams.**—No significant regression equations for periphyton chlorophyll *a* and nutrients were noted when modeled in the form suggested by Dodds and others (1997) (eqns. 1 and 2). The data set for streams in nutrient ecoregion VII consisted of 20 samples. Basin areas above the sampling sites ranged from 0.32 to 26.9 mi<sup>2</sup>. Median basin slope was 488 ft/mi. The predominant land use was forest; the median cover was 67 percent. The relations between chlorophyll *a* and concentrations of total nitrogen and total phosphorus show scatter with no discernible linearity (fig. 7). Of the potential explanatory variables evaluated (table 2), water temperature, stream discharge, dissolved oxygen, suspended sediment, and all habitat indicators were not included. These variables had either no or limited (<10 points) available data. Variables describing drainage-basin characteristics and water quality (including nutrients) were entered in the regression analysis, but resulted in no significant relation. In the absence of other explanatory variables, the benthic-invertebrate indices were not significantly related to concentrations of chlorophyll *a*. However, when combined with the site characteristics of drainage-basin area and percent forest cover, the indices Ephemeroptera, Plecoptera, Trichoptera index (EPT index), percent collector/gatherer, percent filterer/collector, percent Plecoptera individuals, and percent Trichoptera individuals showed a strong relation

to chlorophyll *a* ( $r^2 = 0.85$ ) (eqn. 8) (table 6 and fig. 8). Percent forest cover, EPT index, percent collector/gatherer, and percent filterer/collector are negatively related to concentrations of periphyton chlorophyll *a* in this nutrient ecoregion VII.

$$\begin{aligned} \log(\text{Chlorophyll } a) = & I + A(\text{drainage area}) \\ & + B(\text{percent forest cover}) + C(\text{EPT Index}) \\ & + D(\text{percent collector-gatherer}) \\ & + E(\text{percent filterer-collector}) \\ & + F(\text{percent Plecoptera individuals}) \\ & + G(\text{percent Trichoptera individuals}) \end{aligned} \quad (8)$$

where

Chlorophyll *a* is concentration, in milligrams per square meter;

Drainage area is area, in square miles;

Percent forest cover is in percent;

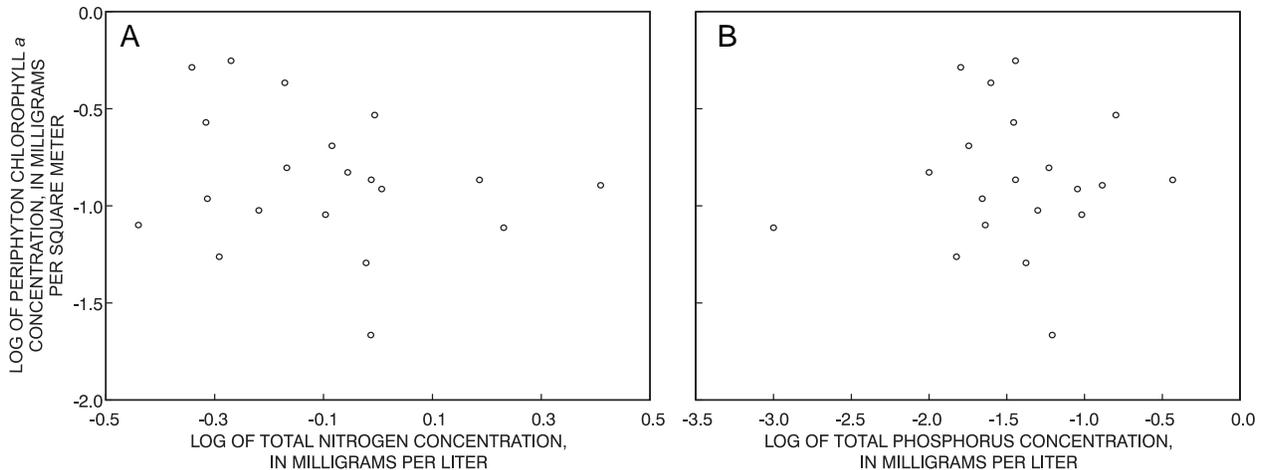
EPT index is the total number of distinct taxa within the orders Ephemeroptera, Plecoptera, and Trichoptera compared to the total number of taxa present;

Percent collector-gatherer is in percent;

Percent filterer-collector is in percent;

Percent Plecoptera individuals is in percent; and

Percent Trichoptera individuals is in percent.



**Figure 7.** Relation between log of periphyton chlorophyll *a* concentration and the log of concentrations of total nitrogen (A) and total phosphorus (B) in streams within nutrient ecoregion VII, Pennsylvania, 1990-98.

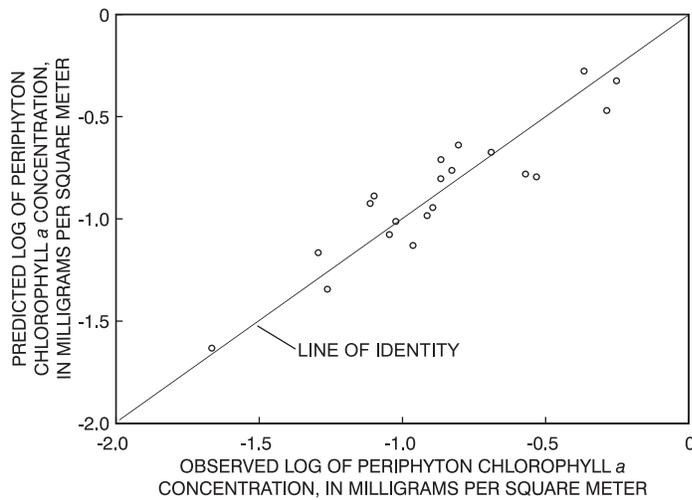
**Table 6.** Regression equation statistics for periphyton chlorophyll *a* concentrations in nutrient ecoregion VII streams, Pennsylvania, 1990-98

[A, B, C, D, E, F, and G are regression coefficients]

Equation	Intercept		Drainage area		Percent forest cover		EPT index		Percent collector/gatherer	
	I	p-value	A	p-value	B	p-value	C	p-value	D	p-value
8	1.3734	0.0035	0.0405	0.003	-0.0281	0.0001	-0.045	0.0298	-0.0251	0.001

Percent filterer/collector		Percent Plecoptera individuals		Percent Trichoptera individuals		n	r <sup>2</sup>	Standard error of estimate
E	p-value	F	p-value	G	p-value			
-0.016	0.0054	0.0405	0.0025	0.101	0.0002	20	0.847	0.173



**Figure 8.** Relation between observed and predicted logs of periphyton chlorophyll *a* concentrations in streams within nutrient ecoregion VII, Pennsylvania, 1990-98. (Predicted concentrations were computed using equation 8.)

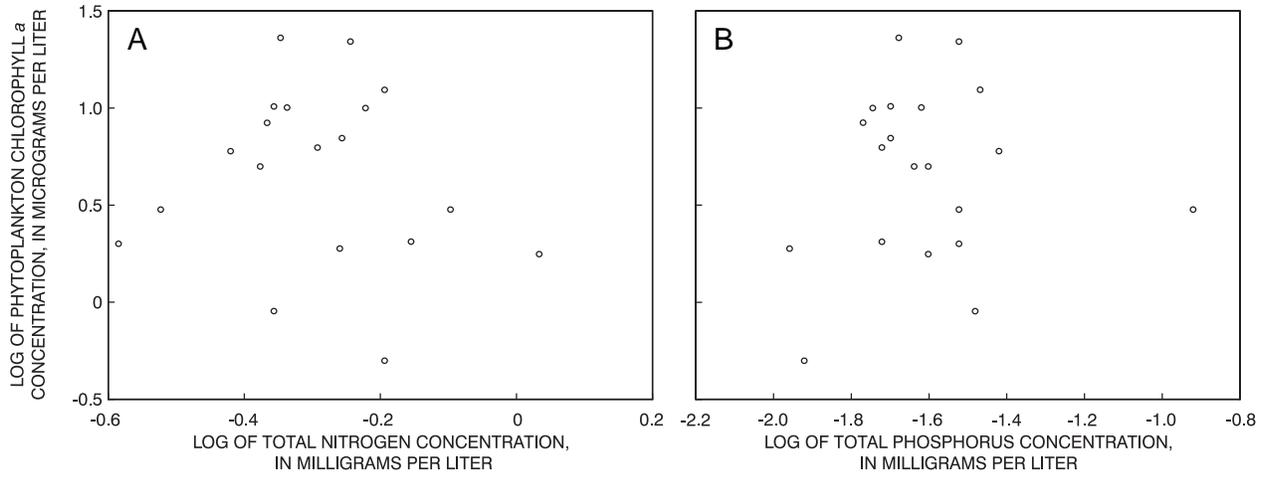
Examination of the coefficients for the benthic-invertebrate indices (table 6) coupled with the small size of the data set suggests that the relation may be a chance occurrence rather than a functional relation between concentration of periphyton chlorophyll *a* and these explanatory variables. With a data set of less than 30 samples, it is easy to draw the incorrect conclusions because the sample size is not large enough to truly reflect the total population. To satisfy the Central Limit Theorem, a sample size of at least 30 is needed to approximate a normal distribution with little variation from the population mean (Ott, 1993) and to achieve adequate information in sampling the binomial populations (Helsel and Hirsch, 1995).

Grazing of algae by benthic macroinvertebrates can offset or lessen the increase in biomass caused by nutrient enrichment, and the effects will not be noted until the invertebrate population begins to increase in response to the increase in algal growth (Mundie and others, 1991; Hill and others, 1992; Rosemond and others, 1993; Peterson and others, 1993). Grazing can cause either a decrease or an increase in algal growth. Where nutrient concentrations are low, grazers actually cause nutrient regeneration from within the periphyton community, allowing more algal cells to grow (McCormick and Stevenson, 1991). Temperature and light also can affect nutrient uptake by algae (Stevenson and others, 1996; Borchardt and others, 1994; Dortch and others, 1991; Winterbourn, 1990; Hill and Knight, 1988), suggesting other factors sometimes may be more important in determining algal composition than nutrients alone (Stevenson and others, 1996).

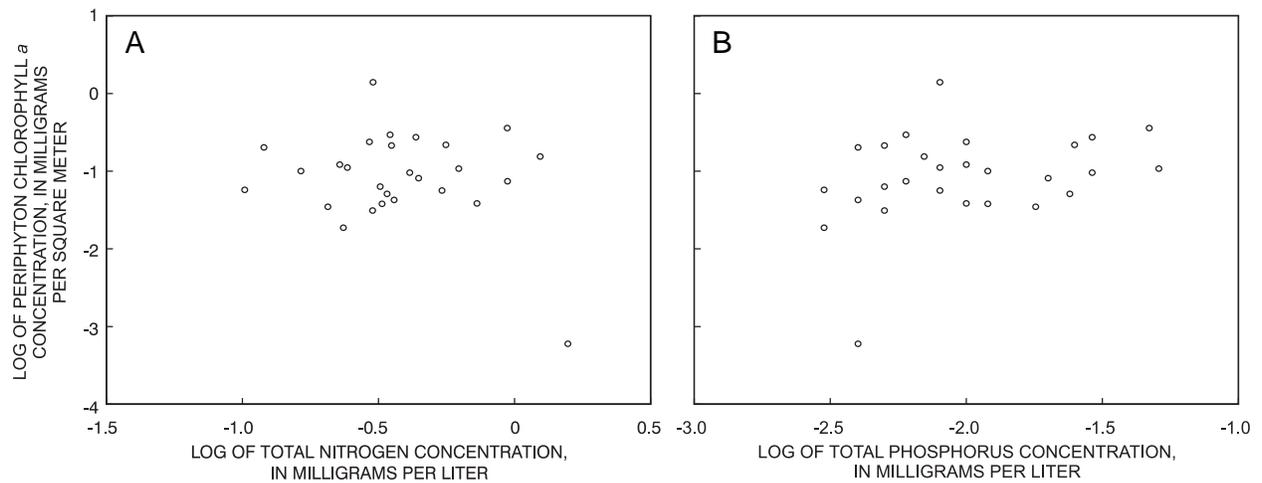
## Nutrient Ecoregion VIII

Lakes.—The data set consisted of 28 samples from 6 lakes. No significant regression equations for phytoplankton chlorophyll *a* resulted from analysis of the available data. The relation between logs of phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen and total phosphorus showed no recognizable linear relation (fig. 9).

Streams.—No significant regression equations for periphyton chlorophyll *a* resulted from analysis of the available data. The data set for streams in nutrient ecoregion VIII consisted of 27 samples. Basin areas above the sampling sites ranged from 0.12 to 590 mi<sup>2</sup>. Median basin slope was 418 ft/mi. The predominant land use was forest; the median cover was 96 percent. The logs of periphyton chlorophyll *a* concentrations show little or no change with changes in the logs of concentrations of total nitrogen or total phosphorus (fig. 10) with the exception of one outlier point (periphyton chlorophyll *a* <0.001 mg/m<sup>2</sup> [log -3.22]). Because this outlier could strongly influence any regression equation, data were analyzed with and without the outlier. No significant regression equations resulted either with or without the outlier. Regression analysis of potential explanatory variables other than nutrients (table 2) also resulted in no significant equations. Water temperature, stream discharge, dissolved oxygen, suspended sediment, and all habitat indicators were not included as explanatory variables. These indicators had either no or limited (< 10 points) available data.



**Figure 9.** Relation between logs of phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in lakes within nutrient ecoregion VIII, Pennsylvania, 1990-98.



**Figure 10.** Relation between log of periphyton chlorophyll *a* concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in streams within nutrient ecoregion VIII, Pennsylvania, 1990-98.

### Nutrient Ecoregion IX

**Lakes.**—The data set consisted of 29 samples from 11 lakes. The relation between the logs of phytoplankton chlorophyll *a* concentrations and logs of total nitrogen concentrations shows a possible positive linear correlation (fig. 11A); a similar plot for concentrations of chlorophyll *a* and total phosphorus (fig. 11B) does not.

When nutrients alone were evaluated, concentrations of total nitrogen were determined to have significant although low ( $r^2 = 0.16$ ) predictive power (eqn. 9) (table 7). The addition of other water-quality constituents to the total nitrogen equation resulted in no significant improvement but showed Secchi-depth data from one of the lakes (Lake Marburg) had a significant influence on the regression coefficients. Although the Lake Marburg data were not suspect, deleting these data resulted in a significant and much improved regression equation ( $r^2 = 0.78$ ) (eqn. 10) (table 7 and fig. 12). A comparison with equation 7 shows

the Secchi-depth coefficients, which are the most significant, are nearly identical and lends support to the validity of the equation 10 coefficients. The relation between predicted and observed logs of phytoplankton chlorophyll *a* concentrations (fig. 12) shows good agreement. In this equation, both Secchi depth and pH were inversely related to chlorophyll *a* concentrations.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) \quad (9)$$

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) + B(\text{Secchi depth}) + C(\text{pH}) \quad (10)$$

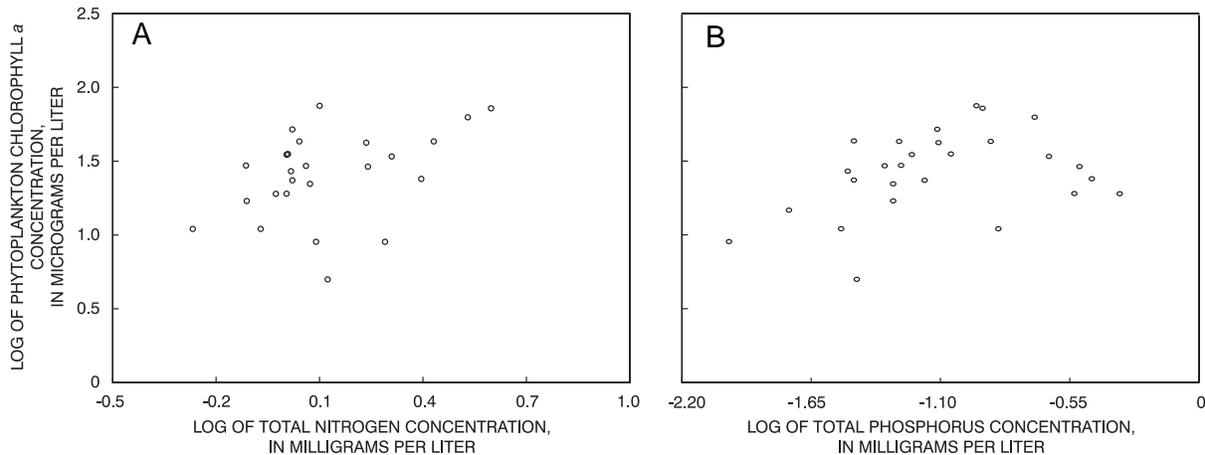
where

Chlorophyll *a* is concentration, in micrograms per liter;

Total nitrogen is concentration, in milligrams per liter;

Secchi depth is depth, in meters; and

pH is in standard units.

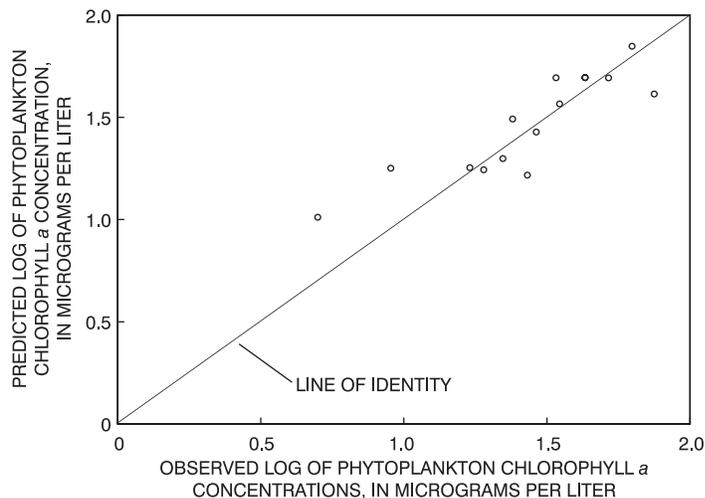


**Figure 11.** Relation between log of phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in lakes within nutrient ecoregion IX, Pennsylvania, 1990-98.

**Table 7.** Regression equation statistics for phytoplankton chlorophyll *a* concentrations in nutrient ecoregion IX lakes, Pennsylvania, 1990-98

[—, not used in equation; <, less than; A, B, and C are regression coefficients]

Equation	Intercept		Total nitrogen		Secchi depth		pH		n	$r^2$	Standard error of estimate
	I	p-value	A	p-value	B	p-value	C	p-value			
9	1.338	<0.001	0.575	0.044	—	—	—	—	25	0.16	0.278
10	3.181	<.001	.480	.025	-0.332	0.001	-0.179	0.010	15	.78	.144



**Figure 12.** Relation between observed and predicted logs of phytoplankton chlorophyll *a* concentrations in lakes within nutrient ecoregion IX, Pennsylvania, 1990-98. (Predicted concentrations were computed using equation 10.)

**Streams.**—Regression analysis of summer-season stream data from nutrient ecoregion IX resulted in one significant regression equation for the log of periphyton chlorophyll *a* concentrations. The data set for nutrient ecoregion IX (12 samples) was the smallest of the stream data sets. Basin areas above the sampling sites ranged from 1.10 to 261 mi<sup>2</sup>. Median basin slope was 271 ft/mi. The predominant land use was agriculture; the median cover was 76 percent. Comparisons of chlorophyll *a* and concentrations of total nitrogen and total phosphorus (fig. 13) show no discernible relation and also reveal an outlier point similar to nutrient ecoregion VII. Data were analyzed with and without the outlier. The presence of the outlier did affect the significance of the regression results; deleting the outlier resulted in a significant regression equation with good predictive power ( $r^2 = 0.74$ ) (eqn. 11) (table 8). The relation between

the observed and predicted logs of periphyton chlorophyll *a* concentrations is shown in figure 14. In this equation, both percent forest cover and pH have a negative effect on periphyton chlorophyll *a* concentrations.

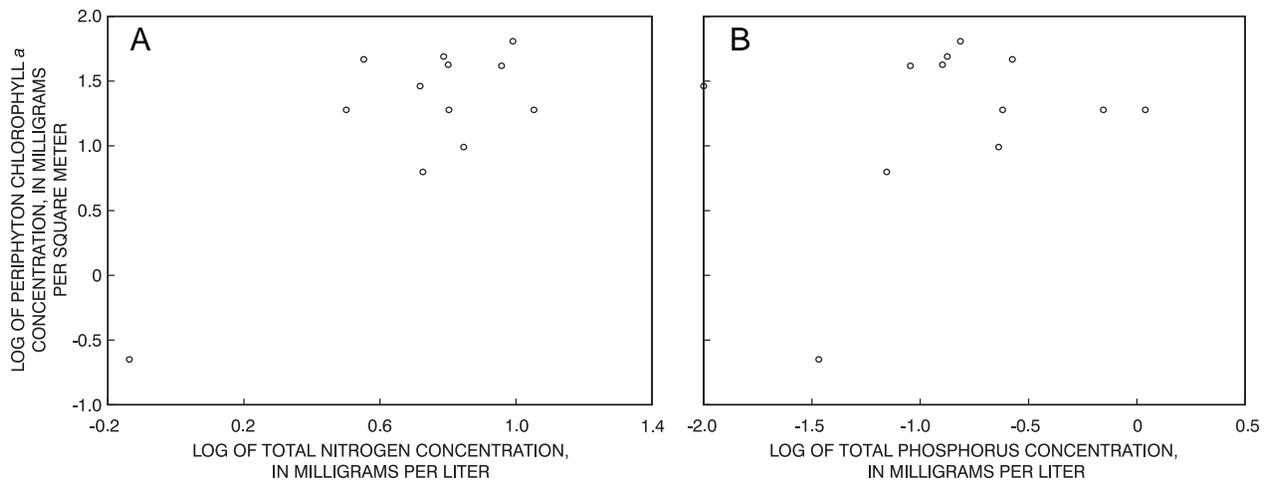
$$\log (\text{Chlorophyll } a) = I + A (\text{Percent forest cover}) + B (\text{pH}) \quad (11)$$

where

Chlorophyll *a* is concentration, in milligrams per square meter;

Percent forest cover is in percent; and

pH is in standard units.

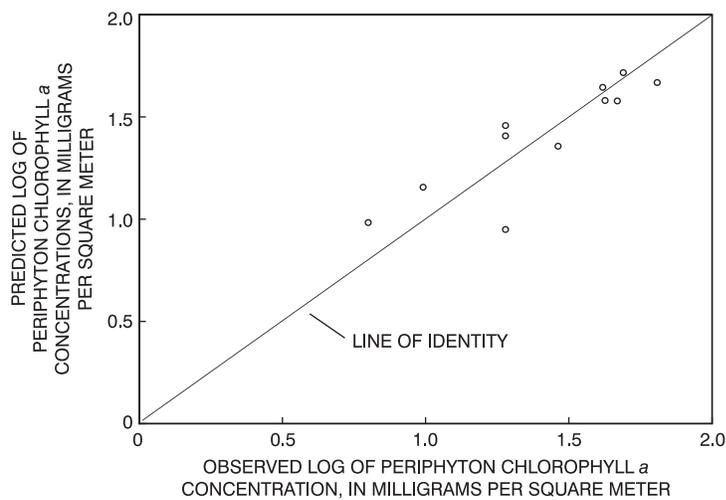


**Figure 13.** Relation between logs of periphyton chlorophyll *a* concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in streams within nutrient ecoregion IX, Pennsylvania, 1990-98.

**Table 8.** Regression equation statistics for summer-season periphyton chlorophyll *a* concentrations in nutrient ecoregion IX streams, Pennsylvania, 1990-98

[A and B are regression coefficients]

Equation	Intercept		Forest cover		pH		n	r <sup>2</sup>	Standard error of estimate
	I	p-value	A	p-value	B	p-value			
11	6.109	0.002	-0.016	0.012	-0.570	0.014	11	0.74	0.181

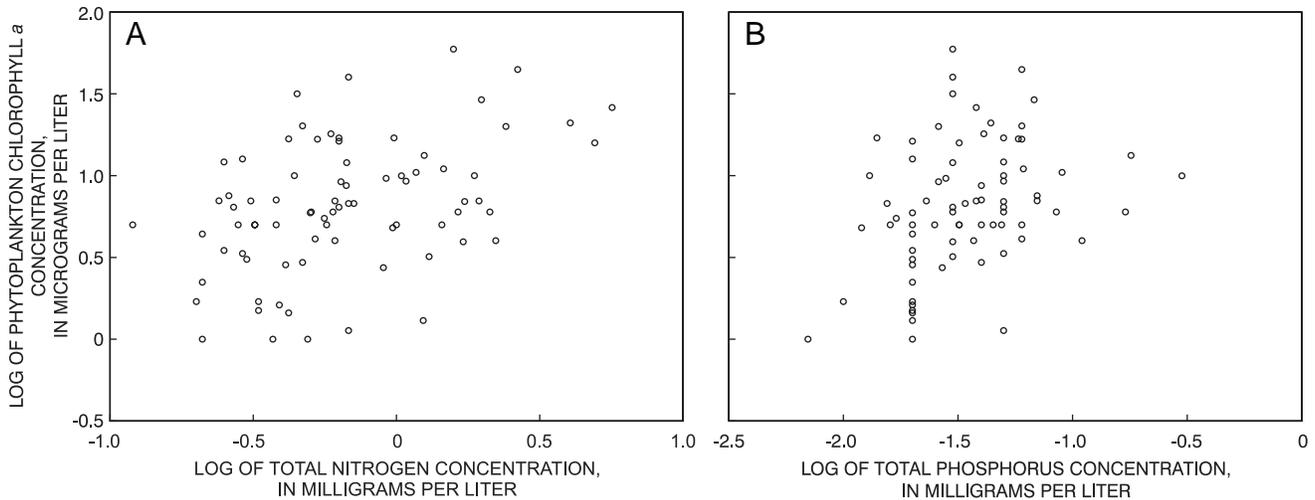


**Figure 14.** Relation between observed and predicted logs of periphyton chlorophyll *a* concentrations in streams within nutrient ecoregion IX, Pennsylvania, 1990-98. (Predicted concentrations were computed using equation 11.)

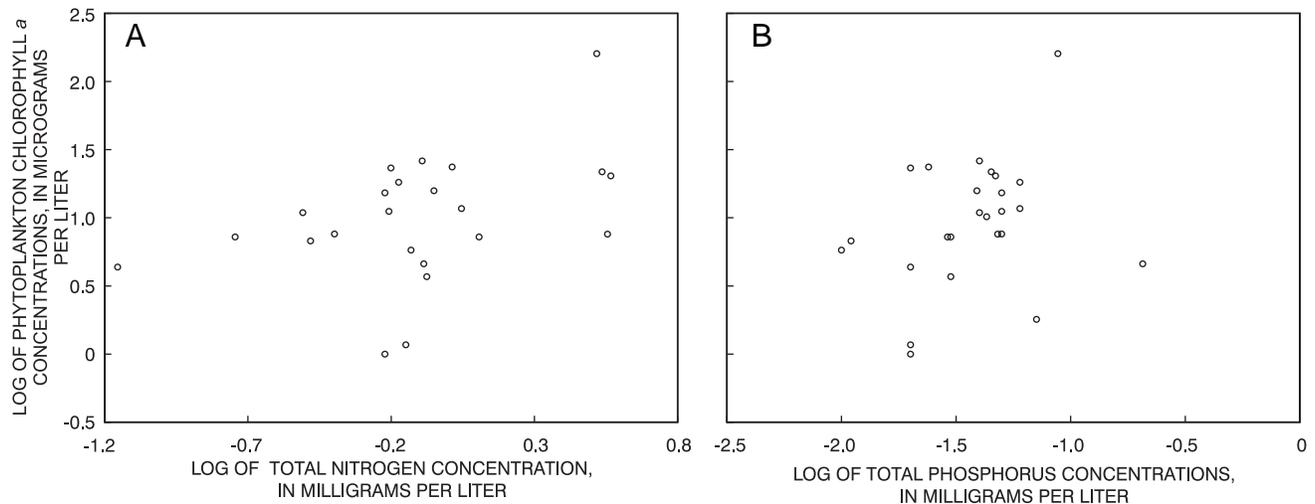
### Nutrient Ecoregion XI

**Lakes.**—The data set of phytoplankton chlorophyll *a* concentrations consisted of 78 summer-season samples from 43 lakes and 25 winter-season samples from 19 lakes. The relations between logs of summer-season phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen and total phosphorus

(fig. 15) visually suggest positive correlations but contain large amounts of scatter. The relations between winter-season phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen and total phosphorus (fig. 16) suggest a positive correlation for concentrations of total nitrogen but no discernible correlation for total phosphorus.



**Figure 15.** Relation between log of phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in lakes during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98.



**Figure 16.** Relation between log of phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in lakes during winter months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98.

Nutrient concentrations alone had minimal explanatory power for summer-season concentrations of phytoplankton chlorophyll *a* in nutrient ecoregion XI lakes. In the absence of other explanatory variables, concentrations of total nitrogen alone explained about 18 percent of the variation in chlorophyll *a* concentrations ( $r^2 = 0.18$ ) (eqn. 12) (table 9) and concentrations of total phosphorus alone explained 12 percent of the variation ( $r^2 = 0.12$ ) (eqn. 13) (table 9). Concentrations of total phosphorus were not significant in combination with total nitrogen. When all available explanatory variables were included in the regression analysis, total nitrogen and Secchi depth (negative relation) provided the best equation but explained less than half of the variation in logs of phytoplankton chlorophyll *a* concentrations ( $r^2 = 0.44$ ) (eqn. 14) (table 9). Although this two-variable equation was statistically significant, it exhibited poor linearity (fig. 17). In particular, the equation underestimates chlorophyll *a* concentrations at greater concentrations of total nitrogen, possibly because of under specification of the equation or,

equally likely, limiting behavior from a variable other than total nitrogen controlling phytoplankton production. Once an excess of nutrients are reached, the bio-mass of algae in a lake changes very little; however, at this point, the species composition can change significantly. Several studies have shown biomass stays fairly constant as nutrients increase, but the community composition changes from species that fix nitrogen to species that are nutrient tolerant, such as *Cladophora*, and the species composition may even decrease in number (Carrick and others, 1988; Keithan and others, 1988; Winder and Duthie, 2000).

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) \quad (12)$$

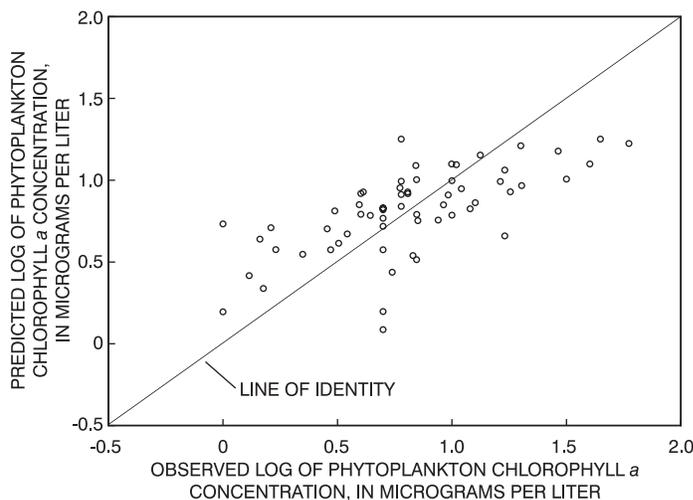
$$\log(\text{Chlorophyll } a) = I + B \log(\text{Total phosphorus}) \quad (13)$$

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) + C(\text{Secchi depth}) \quad (14)$$

**Table 9.** Regression equation statistics for summer-season phytoplankton chlorophyll *a* concentrations in nutrient ecoregion XI lakes, Pennsylvania and West Virginia, 1990-98

[—, not used in equation; <, less than; A, B, and C are regression coefficients]

Equation	Intercept		Total nitrogen		Total phosphorus		Secchi depth		n	$r^2$	Standard error of estimate
	I	p-value	A	p-value	B	p-value	C	p-value			
12	0.901	<0.001	0.491	<0.001	—	—	—	—	78	0.18	0.363
13	1.543	<.001	—	—	0.501	0.002	—	—	78	.12	.376
14	1.235	<.001	.384	.002	—	—	-0.156	<0.001	62	.44	.297



**Figure 17.** Relation between observed and predicted logs of phytoplankton chlorophyll *a* concentrations in lakes during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98.

where

Chlorophyll *a* is concentration, in micrograms per liter;

Total nitrogen is concentration, in milligrams per liter;

Total phosphorus is concentration, in milligrams per liter; and

Secchi depth is depth, in meters.

The best regression equation for winter-season lake data used different variables than the summer-season model. In absence of other explanatory variables, the concentration of total nitrogen was evaluated as a significant explanatory variable. However, the total-nitrogen equation (eqn. 15) (table 10) had minimal explanatory power ( $r^2 = 0.18$ ) and failed the normality of residuals test. The concentration of total phosphorus was not significant. Entering all variables into the regression analysis improved the predictive power ( $r^2 = 0.69$ ) and resulted in an equation with water temperature, specific conductance (negative relation), and pH as significant variables (eqn. 16) (table 10). Concentration of total nitrogen was no longer significant to algal growth when the other variables were entered into the equation. The vari-

ables deemed important to algal growth in the winter are not the same variables deemed important to algal growth over the summer. Algal needs change with the season and the water temperatures.

At lower concentrations of chlorophyll *a* there is more scatter in the plot (fig. 18) meaning that at lower concentrations of phytoplankton chlorophyll *a* water temperature, specific conductivity, and pH are not strong indicators of algal biomass. These variables become more critical to algal biomass as algal biomass increases.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) \quad (15)$$

$$\log(\text{Chlorophyll } a) = I + B(\text{Water temperature}) + C(\text{Specific conductance}) + D(\text{pH}) \quad (16)$$

where

Chlorophyll *a* is concentration, in micrograms per liter;

Total nitrogen is concentration, in milligrams per liter;

Water temperature is in degrees Celsius;

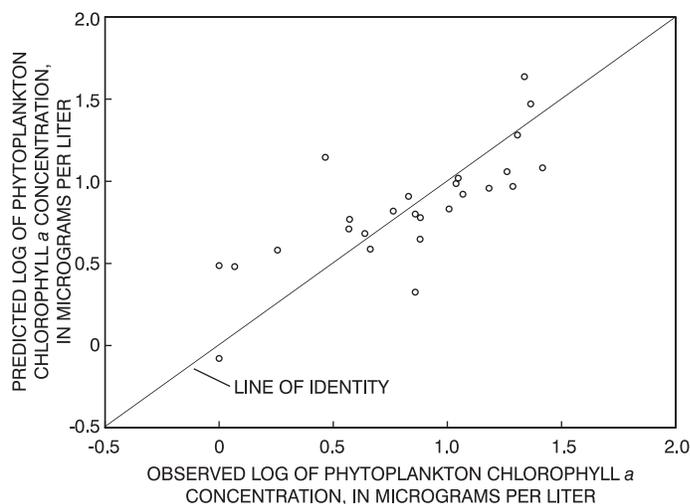
Specific conductance is in microsiemens per centimeter; and

pH is in standard units.

**Table 10.** Regression equation statistics for winter-season phytoplankton chlorophyll *a* concentrations in nutrient ecoregion XI lakes, Pennsylvania and West Virginia, 1990-98

[—, not used in equation; <, less than; A, B, C, and D are regression coefficients]

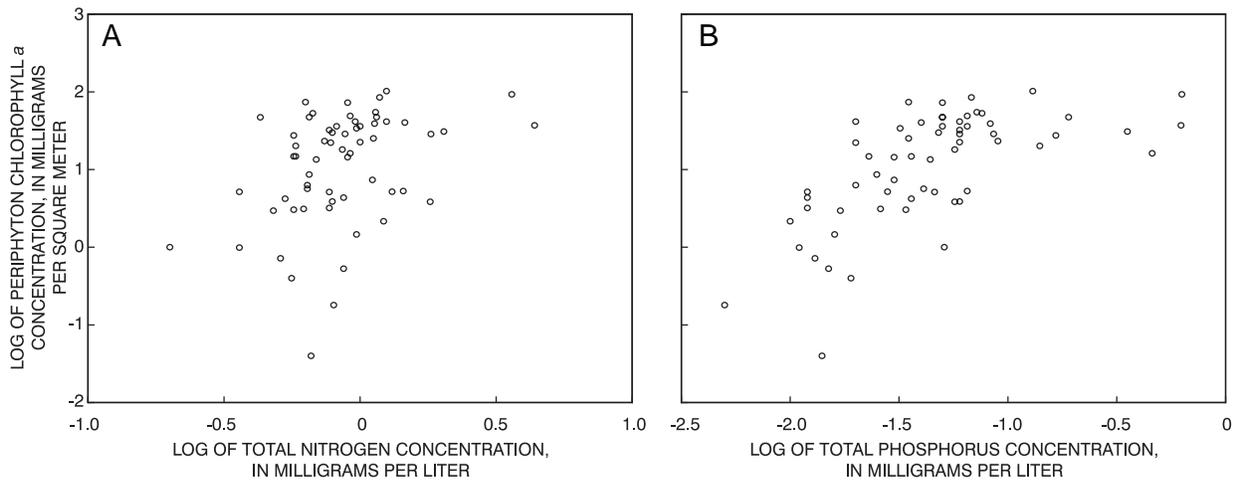
Equation	Intercept		Total nitrogen		Water temperature		Specific conductance		pH		n	R <sup>2</sup>	Standard error of estimate
	I	p-value	A	p-value	B	p-value	C	p-value	D	p-value			
15	1.044	<0.001	0.470	0.047	—	—	—	—	—	—	23	0.18	0.430
16	-2.991	.002	—	—	0.057	<0.001	-0.001	0.005	0.439	0.001	27	.69	.289



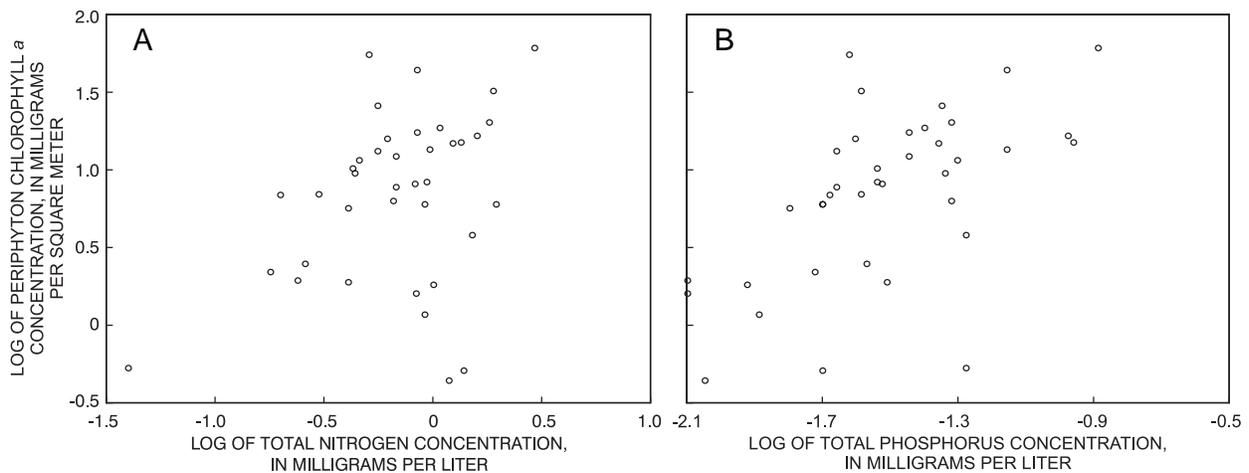
**Figure 18.** Relation between observed and predicted phytoplankton chlorophyll *a* concentrations in lakes during winter months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98. (Predicted concentrations were computed using equation 16.)

Nutrient ecoregion XI lakes in West Virginia were sampled by WVDEP for periphyton rather than phytoplankton. The data set of nutrient ecoregion XI periphyton chlorophyll a concentrations consisted of 63 summer-season samples from 22 lakes and 41 winter-season samples from 18 lakes. The relation between logs of summer-season periphyton chlorophyll a concentrations and logs of total nitrogen concentrations (fig. 19) is mostly scattered. However, the relation between periphyton chlorophyll a concentrations and logs of total phosphorus concentrations has a positive correlation up to chlorophyll a concentrations of

about 0.1 mg/L (-1.0 log units). Concentrations of total phosphorus above 0.1 mg/L show no further increase of chlorophyll a concentrations. The relation between winter-season periphyton chlorophyll a concentrations and logs of total nitrogen concentrations is similar to the summer-season relation. The relation between periphyton chlorophyll a concentrations and total phosphorus is positive (fig. 20). If the 0.1 mg/L breakpoint present in the summer samples exists in winter, the limited range of measured concentrations of total phosphorus does not show this relation.



**Figure 19.** Relation between log of periphyton chlorophyll a concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in lakes during summer months within nutrient ecoregion XI, West Virginia, 1990-98.



**Figure 20.** Relation between log of periphyton chlorophyll a concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in lakes during winter months within nutrient ecoregion XI, West Virginia, 1990-98.

Large or filamentous periphyton taxa can be correlated positively with total water-column phosphorus (Cattaneo, 1987). Typically, the response between periphyton in lakes and nutrients in the water column is weak, but periphyton does play a significant role in the release of sediment-associated phosphorus (Riber and Wetzel, 1987; Carlton and Wetzel, 1988). This is one possible explanation why the relation between periphyton and concentrations of total phosphorus is stronger than the relation between phytoplankton and total phosphorus.

Logs of total phosphorus concentrations, alone and in combination with other explanatory variables, and logs of total nitrogen concentrations alone were significant predictors of logs of summer-season periphyton chlorophyll *a* concentrations in nutrient ecoregion XI lakes (table 9). In the absence of other explanatory variables, the log of total phosphorus concentrations was the strongest predictor and explained almost 40 percent of the variation in logs of chlorophyll *a* concentrations

( $r^2 = 0.40$ ) (eqn. 17) (table 11). The log of total nitrogen concentrations alone was a significant but weaker predictor of logs of periphyton chlorophyll *a* concentrations ( $r^2 = 0.15$ ) (eqn. 18) (table 11). The total-nitrogen equation also failed the normality of residuals test. When water temperature and Secchi depth (negative relation) were entered into the total-phosphorus equation, the predictive power increased ( $r^2 = 0.66$ ) (eqn. 19) (table 11). The equation using total phosphorus, water temperature, and Secchi depth also underpredicts concentrations of periphyton chlorophyll *a* at greater values (fig. 21) but less so than the model using only total phosphorus.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total phosphorus}) \quad (17)$$

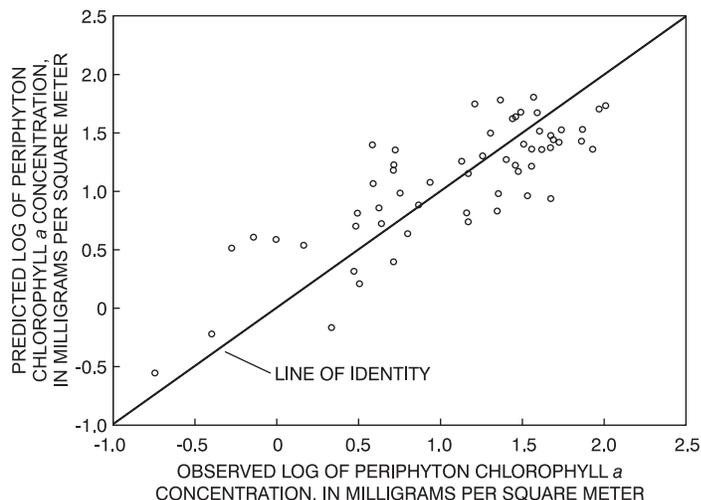
$$\log(\text{Chlorophyll } a) = I + B \log(\text{Total nitrogen}) \quad (18)$$

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total phosphorus}) + C(\text{Water temperature}) + D(\text{Secchi depth}) \quad (19)$$

**Table 11.** Regression equation statistics for summer-season periphyton chlorophyll *a* concentrations in nutrient ecoregion XI lakes, West Virginia, 1990-98

[—, not used in equation; <, less than; A, B, C, and D are regression coefficients]

Equation	Intercept		Total phosphorus		Total nitrogen		Water temperature		Secchi depth		n	$r^2$	Standard error of estimate
	I	p-value	A	p-value	B	p-value	C	p-value	D	p-value			
17	2.484	<0.001	1.067	<0.001	—	—	—	—	—	—	61	0.40	0.560
18	1.128	<.001	—	—	1.276	0.002	—	—	—	—	61	.15	.668
19	1.598	<.001	.556	<.001	—	—	0.031	0.031	-0.230	<0.001	58	.66	.386



**Figure 21.** Relation between observed and predicted logs of periphyton chlorophyll *a* concentrations in lakes during summer months within nutrient ecoregion XI, West Virginia, 1990-98. (Predicted concentrations were computed using equation 19.)

where

Chlorophyll *a* is concentration, in milligrams per square meter;

Total phosphorus is concentration, in milligrams per liter;

Total nitrogen is concentration, in milligrams per liter;

Water temperature is in degrees Celsius; and Secchi depth is depth, in meters.

Logs of winter-season periphyton chlorophyll *a* concentrations were predicted best by the log of total phosphorus concentrations and Secchi depth (negative relation). Regression analysis of nutrients and periphyton chlorophyll *a* resulted in an equation containing only concentrations of total phosphorus ( $r^2 = 0.46$ ) (eqn. 20) (table 12). Alone or in combination with total phosphorus, total nitrogen was not a significant explanatory variable. Secchi depth was the remaining significant explanatory variable. Logs of total phosphorus concentrations and Secchi depth explained more than half the variation in logs of periphyton chlorophyll *a* concentrations ( $r^2 = 0.65$ ) (eqn. 21) (table 12). The

relation between observed and predicted logs of periphyton chlorophyll *a* concentrations is shown in figure 22.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total phosphorus}) \quad (20)$$

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total phosphorus}) + B(\text{Secchi depth}) \quad (21)$$

where

Chlorophyll *a* is concentration, in milligrams per square meter;

Total phosphorus is concentration, in milligrams per liter; and

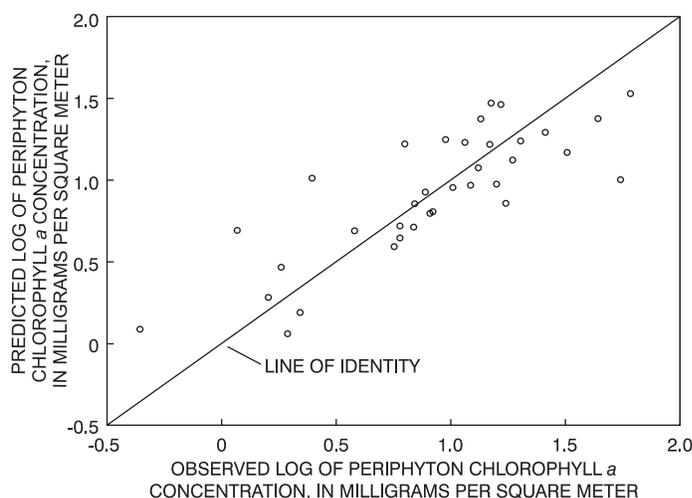
Secchi depth is depth, in inches.

**Streams.**—Regression analysis of stream data for nutrient ecoregion XI resulted in a number of significant periphyton regression equations. The stream data set of nutrient ecoregion XI periphyton consisted of 136 summer-season samples. Basin areas above the sampling sites ranged from 0.04 to 6,860 mi<sup>2</sup>. Median basin slope was 668 ft/mi. The predominant land use was forest; the median cover was 83 percent, although agricultural and urban

**Table 12.** Regression equation statistics for winter-season periphyton chlorophyll *a* concentrations in nutrient ecoregion XI lakes, West Virginia, 1990-98

[—, not used in equation; <, less than; A and B are regression coefficients]

Equation	Intercept		Total phosphorus		Secchi depth		n	r <sup>2</sup>	Standard error of estimate
	I	p-value	A	p-value	B	p-value			
20	2.692	<0.001	1.193	<0.001	—	—	37	0.46	0.386
21	2.082	<.001	.541	.028	-0.242	0.001	62	.65	.292

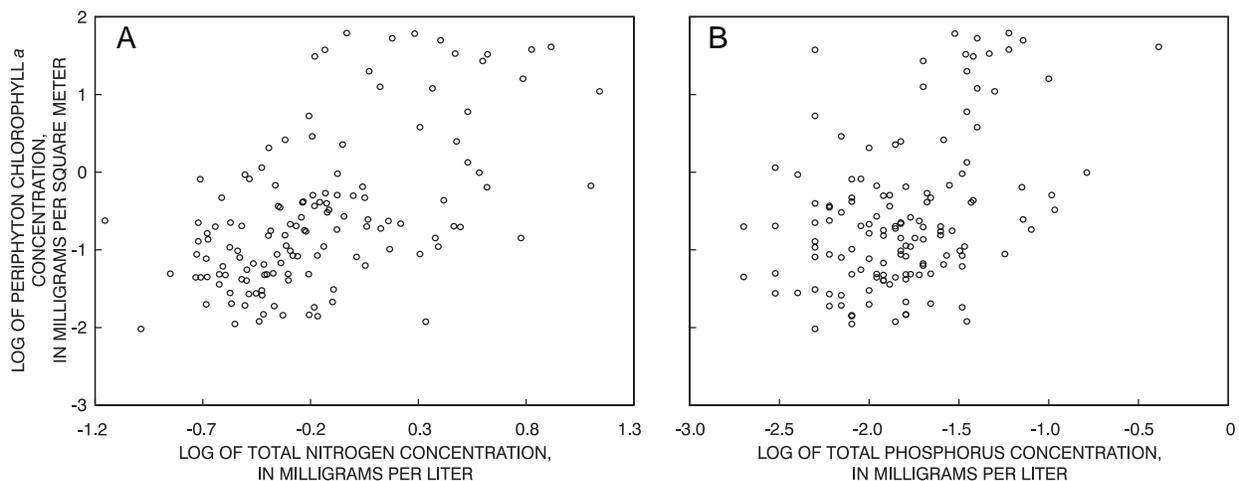


**Figure 22.** Relation between observed and predicted logs of periphyton chlorophyll *a* concentrations in lakes during winter months within nutrient ecoregion XI, West Virginia, 1990-98. (Predicted concentrations were computed using equation 21.)

land use were as great as 95 and 89 percent, respectively, in individual basins. The relations between logs of periphyton chlorophyll *a* concentrations and logs of concentrations of total nitrogen and total phosphorus (fig. 23) are scattered but suggest a positive correlation in both cases.

Logs of concentrations of total phosphorus alone and total nitrogen alone were significant predictors of summer-season concentrations of periphyton chlorophyll *a* in nutrient ecoregion XI streams. The log of total nitrogen concentrations explained about 30 percent ( $r^2 = 0.32$ ) (eqn. 22) (table 13) and the log of total phosphorus concentrations explained slightly less than 20 percent ( $r^2 = 0.18$ ) (eqn. 23) (table 13) of the variation in chlorophyll *a*. However, both regression equations failed the residuals normality test. Including the

drainage-basin characteristics—drainage area and percent forest cover—with total phosphorus in the regression analysis resulted in the strongest equation ( $r^2 = 0.54$ ) (eqn. 24) (table 13). Phosphorus input into streams is correlated positively to average slope of the drainage basin (Kirchner, 1975). Percent forest cover had a negative relation with chlorophyll *a* concentrations. As percent forest cover increases, less light reaches the stream. Because algae are primary producers and need the sunlight to grow, less light to the stream causes algae to grow slower. In this situation, light is more critical to algal growth than are nutrients. A stream can contain excess nutrients, but without light, algae still cannot grow. No benthic-invertebrate or habitat indices were significant explanatory variables.



**Figure 23.** Relation between log of periphyton chlorophyll *a* concentrations and the logs of concentrations of total nitrogen (A) and total phosphorus (B) in streams during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98.

**Table 13.** Regression equation statistics for summer-season periphyton chlorophyll *a* concentrations in nutrient ecoregion XI streams, Pennsylvania and West Virginia, 1990-98

[—, not used in equation; <, less than; A, B, C, and D are regression coefficients]

Equation	Intercept		Total nitrogen		Total phosphorus		Drainage area		Percent forest cover		n	$r^2$	Standard error of estimate
	I	p-value	A	p-value	B	p-value	C	p-value	D	p-value			
22	-0.365	<0.001	1.239	<0.001	—	—	—	—	—	—	135	0.32	0.796
23	1.287	<.001	—	—	1.028	0.001	—	—	—	—	135	.18	.874
24	.941	<.001	—	—	.531	.003	0.528	<0.001	-0.012	<0.001	134	.54	.661

Limestone streams within nutrient ecoregion XI may be phosphorus limited. Excess phosphorus can be removed from wastewaters by applying lime (Manahan, 1984) and the same process may be happening in limestone streams. A future step in the process may be to analyze the limestone streams separately. This may yield stronger relations between concentrations of total phosphorus and chlorophyll *a* concentrations for limestone streams.

Equation 24 tends to overpredict lower concentrations of periphyton chlorophyll *a* and underpredict higher concentrations (fig. 24). The prediction biases do not appear to arise from non-linearity in the data. Outliers or high-influence points, which commonly cause fitting problems, were not identified.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) \quad (22)$$

$$\log(\text{Chlorophyll } a) = I + B \log(\text{Total phosphorus}) \quad (23)$$

$$\log(\text{Chlorophyll } a) = I + B \log(\text{Total phosphorus}) + C \log(\text{Drainage area}) + D (\text{Percent forest cover}) \quad (24)$$

where

Chlorophyll *a* is concentration, in milligrams per square meter;

Total nitrogen is concentration, in milligrams per liter;

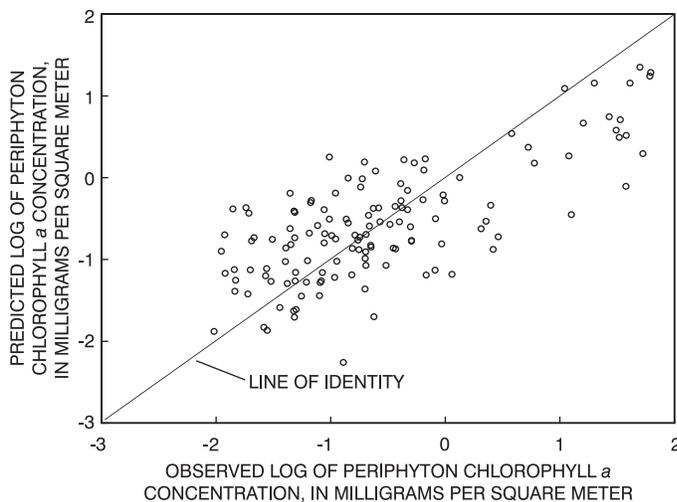
Total phosphorus is concentration, in milligrams per liter;

Drainage area is area, in square miles; and

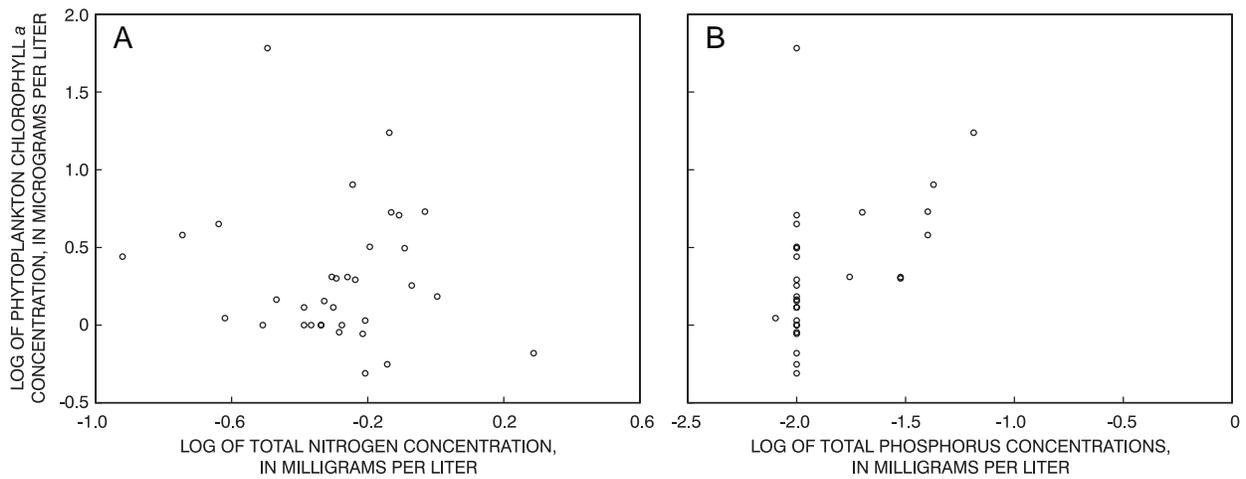
Percent forest cover is in percent.

Only one winter-season periphyton chlorophyll *a* concentration was available for nutrient ecoregion XI. No data analysis was done.

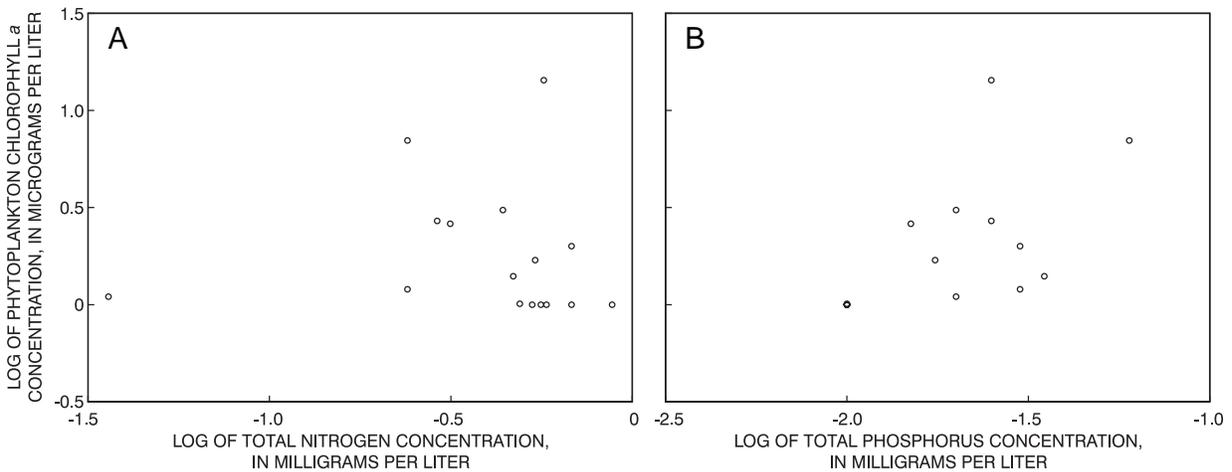
Several nutrient ecoregion XI streams in West Virginia were sampled for phytoplankton, not periphyton, chlorophyll *a*. The phytoplankton data set consisted of 34 summer-season and 17 winter-season samples. Basin areas above the sampling sites ranged from 2.80 to 910 mi<sup>2</sup>. Median basin slope was 989 ft/mi. The predominant land use was forest; the median cover was 92 percent. The relations between logs of summer-season phytoplankton chlorophyll *a* concentrations and logs of concentrations of total nitrogen and total phosphorus (fig. 25) show a weak negative correlation for total nitrogen and a weak positive correlation for total phosphorus. The total phosphorus correlation is weak partly because a majority of the concentrations are at the 0.01 mg/L (-2.0 log units) reporting limit for total phosphorus that restricts the range over which a linear relation can be developed. Logs of winter-season phytoplankton chlorophyll *a* concentrations show no apparent relation with logs of total nitrogen concentrations but show a positive correlation with logs of total phosphorus concentrations (fig. 26).



**Figure 24.** Relation between observed and predicted logs of periphyton chlorophyll *a* concentrations in streams during summer months within nutrient ecoregion XI, Pennsylvania and West Virginia, 1990-98. (Predicted concentrations were computed using equation 24.)



**Figure 25.** Relation between log of phytoplankton chlorophyll a concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in streams during summer months within nutrient ecoregion XI, West Virginia, 1990-98.



**Figure 26.** Relation between log of phytoplankton chlorophyll a concentrations and logs of concentrations of total nitrogen (A) and total phosphorus (B) in streams during winter months within nutrient ecoregion XI, West Virginia, 1990-98.

Regression analysis of nutrient ecoregion XI summer-season phytoplankton chlorophyll *a* data resulted in significant regression equations with total phosphorus alone and in combination with other explanatory variables. The equation using only the log of total phosphorus concentration (eqn. 25) (table 14) explained approximately half ( $r^2 = 0.48$ ) the variation in log of chlorophyll *a* concentrations. Total nitrogen by itself and in combination with total phosphorus was not a significant explanatory variable. However, total nitrogen was significant in the strongest equation ( $r^2 = 0.95$ ) that included total phosphorus and four other explanatory variables (eqn. 26) (table 14). All relations were positive except for pH. This is similar to the results from nutrient ecoregion IX. The relation between predicted and observed concentrations for eqn. 26 are shown in figure 27.

$$\log(\text{Chlorophyll } a) = I + B \log(\text{Total phosphorus}) \quad (25)$$

$$\begin{aligned} \log(\text{Chlorophyll } a) = & I + A \log(\text{Total nitrogen}) \\ & + B \log(\text{Total phosphorus}) + C(\text{Basin slope}) \\ & + D(\text{pH}) + E(\text{Total residue}) \\ & + F(\text{Total suspended solids}) \end{aligned} \quad (26)$$

where

Chlorophyll *a* is concentration, in milligrams per square meter;

Total nitrogen is concentration, in milligrams per liter;

Total phosphorus is concentration, in milligrams per liter; and

Basin slope is slope, in feet per mile;

pH is in standard units;

Total residue is concentration, in milligrams per liter; and

Total suspended solids is concentration, in milligrams per liter.

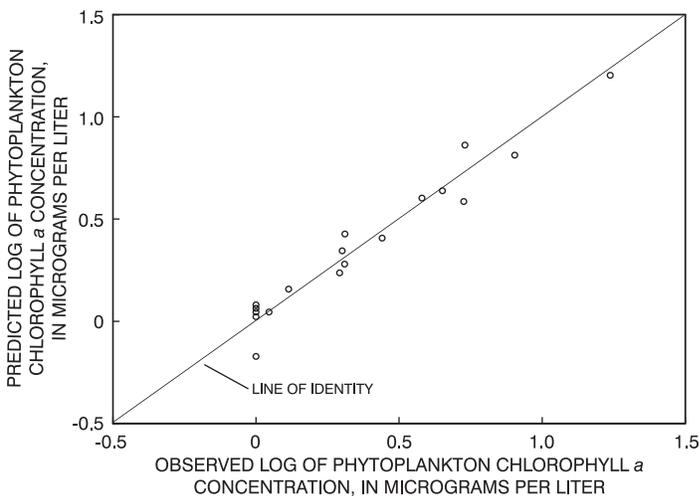
**Table 14.** Regression equation statistics for summer-season phytoplankton chlorophyll *a* concentrations in nutrient ecoregion XI streams, West Virginia, 1990-98

[—, not used in equation; <, less than; A, B, C, D, E, and F are regression coefficients]

Equation	Intercept		Total nitrogen		Total phosphorus		Basin slope		pH	
	I	p-value	A	p-value	B	p-value	C	p-value	D	p-value
25	2.146	<0.001	—	—	1.007	<0.001	—	—	—	—
26	7.531	<.001	0.542	0.005	.428	.008	0.0005	0.003	-1.016	<0.001

Equation	Total residue		Total suspended solids		n	$r^2$	Residual standard error
	E	p-value	F	p-value			
25	—	—	—	—	33	0.48	0.256
26	0.001	0.038	0.020	0.005	18	.95	.101



**Figure 27.** Relation between observed and predicted logs of phytoplankton chlorophyll *a* concentrations in streams during summer months within nutrient ecoregion XI, West Virginia, 1990-98. (Predicted concentrations were computed using equation 26.)

Regression analysis of the data on winter-season phytoplankton chlorophyll *a* concentrations for streams in nutrient ecoregion XI resulted in several regression equations judged significant but of poor form. Analysis of total nitrogen alone ( $r^2 = 0.41$ ) (eqn. 27) (table 15) and total phosphorus alone ( $r^2 = 0.55$ ) (eqn. 28) (table 15) resulted in significant equations but the residuals from the total-nitrogen equation exhibited curvature and non-constant variance when plotted in relation to the predicted data. The relation between concentrations of total nitrogen and phytoplankton chlorophyll *a* was negative. The total-phosphorus regression equation failed the normality of residuals test. Concentrations of total nitrogen and total phosphorus were not significant in combination. Entering basin-characteristic and water-quality constituents into the regression analysis resulted in water temperature alone explaining the greatest ( $r^2 = 0.74$ ) (eqn. 29) (table 15) proportion of variance in the logs of phytoplankton chlorophyll *a* concentrations. Residuals from equation 29 also exhibited curvature when plotted in relation to the predicted data. The relation between predicted and observed concentrations are shown in figure 28.

$$\log(\text{Chlorophyll } a) = I + A \log(\text{Total nitrogen}) \quad (27)$$

$$\log(\text{Chlorophyll } a) = I + B \log(\text{Total phosphorus}) \quad (28)$$

$$\log(\text{Chlorophyll } a) = I + C(\text{Water temperature}) \quad (29)$$

where

Chlorophyll *a* is concentration, in micrograms per liter;

Total nitrogen is concentration, in milligrams per liter;

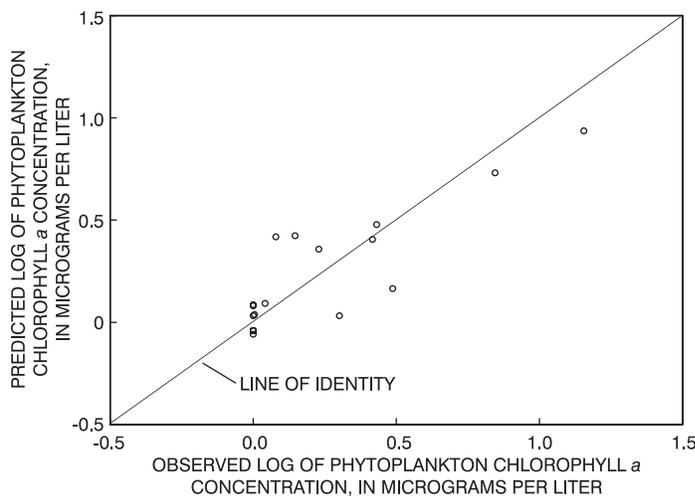
Total phosphorus is concentration, in milligrams per liter; and

Water temperature is in degrees Celsius.

**Table 15.** Regression equation statistics for winter-season phytoplankton chlorophyll *a* concentrations in nutrient ecoregion XI streams, West Virginia, 1990-98

[—, not used in equation; <, less than; A, B, and C are regression coefficients]

Equation	Intercept		Total nitrogen		Total phosphorus		Water temperature		n	$r^2$	Standard error of estimate
	I	p-value	A	p-value	B	p-value	C	p-value			
27	-0.111	0.348	-0.947	<0.010	—	—	—	—	15	0.41	0.202
28	1.494	<.001	—	—	0.739	0.001	—	—	16	.55	.173
29	-.245	.013	—	—	—	—	0.060	<0.001	17	.74	.178



**Figure 28.** Relation between observed and predicted logs of phytoplankton chlorophyll *a* concentrations in streams during winter months within nutrient ecoregion XI, West Virginia, 1990-98. (Predicted concentrations were computed using equation 29.)

## ECOLOGICAL INDICATORS OF NUTRIENT ENRICHMENT

Nutrient enrichment of lakes and streams is difficult to describe using algal biomass. Interpreting algal responses to nutrient limitation on the basis of chlorophyll *a* commonly is misleading, because algae are sensitive to a number of different stresses, including light, predation (Keithan and others, 1988; Bourassa and Cattaneo, 1998), iron, water color, and the percentage of the watershed in urban and suburban land use (Hill and others, 2000). Yet, some generalizations about algae can be made. Filamentous algae in lakes and streams are correlated positively with total water-column phosphorus (Cattaneo, 1987); filamentous chlorophytes typically become more abundant when nitrogen and phosphorus levels are relatively high and light is sufficient. Some diatoms and cyanobacteria dominate when there is a sufficient amount of phosphorus and not enough nitrogen in the system (Elwood and others, 1981; Fairchild and others, 1985; Stockner and Shortreed, 1978; Hill and Knight, 1988). Phytoplankton communities typically will become dominated with cyanobacteria—nitrogen-fixing bacteria—when phosphorus enrichment occurs (Stevenson and others, 1996).

Nutrient levels low enough to prevent impaired stream water quality may not be possible to attain. But even in areas where agriculture is the dominant land use, riparian buffer strips may help reduce nutrient input to the stream and provide shading to reduce the algal growth (Biggs, 2000). On the basis of results of this study, shading had a greater effect on algal growth than did nutrient availability.

In nutrient ecoregion VII lakes, nitrogen is a limiting factor for algal growth. When other explanatory variables were added to the equation, total nitrogen was no longer dominant; rather, Secchi depth and concentrations of dissolved oxygen had a much stronger relation with chlorophyll *a* concentrations in the lake. Streams in this nutrient ecoregion showed no significant relation between concentrations of chlorophyll *a* and nutrients. The most influential variables on periphyton chlorophyll *a* concentrations are drainage area, percent forest cover, EPT index, percent collector/gatherer, percent filterer/collector, percent Plecoptera individuals, and percent Trichoptera individuals (tables 16 and 17).

Neither lake nor stream chlorophyll *a* data in nutrient ecoregion VIII showed a discernible relation with any environmental indices available for this analysis. Sample sizes for both lakes and streams were less than optimal (tables 16 and 17).

Like nutrient ecoregion VII, the lake chlorophyll *a* in nutrient ecoregion IX showed a weak relation with total nitrogen concentration (tables 16 and 17). When other variables were added to the equation, total nitrogen was still an important variable as well as Secchi depth and pH. Because this equation is similar to nutrient ecoregion VII, combining these two nutrient ecoregions may define a better relation for lakes. The stream data within this nutrient ecoregion generated no significant relations with nutrients although there was a relation between periphyton chlorophyll *a*, percent forest cover, and pH. The data set contained only 12 samples. More data are necessary to determine the validity of this equation in this nutrient ecoregion. Combining data from nutrient ecoregion VII and IX could more accurately represent what is happening in this area.

Nutrient ecoregion XI had seven separate analyses performed with the data. Periphyton and phytoplankton data were available for the lakes and streams and sufficient winter and summer samples were available to run seasonal analyses, except for winter-stream periphyton. A large area of Pennsylvania and all of West Virginia is in this nutrient ecoregion. WVDEP sampled different algal communities than the other agencies within Pennsylvania and West Virginia; therefore, the data could not be combined into the larger nutrient ecoregion XI data set. The analysis of the WVDEP data was a separate exercise.

Summer-season lake phytoplankton chlorophyll *a* concentrations show a relation with concentrations of both total nitrogen and total phosphorus; total nitrogen has the greater predictive value (table 16). When combined with the other explanatory variables, the strongest equation included total nitrogen concentration and Secchi depth; concentration of total phosphorus was not significant. Winter-season phytoplankton chlorophyll *a* concentrations also showed a relation with total nitrogen concentrations, but when other variables were added to the equation, total nitrogen was no longer significant. During winter, water temperature, specific conductivity, and pH have a stronger relation with algal growth than concentration of total nitrogen.

**Table 16.** Summary of influential lake explanatory variables related to lake chlorophyll *a* through regression analysis for each nutrient ecoregion and season [mg/L, milligrams per liter; m, meters; °C, degrees Celsius; μS, microsiemens; --, no relation]

Explanatory variable to chlorophyll <i>a</i>	Nutrient ecoregion VII		Nutrient ecoregion VIII		Nutrient ecoregion IX		Nutrient ecoregion XI							
	Summer lake phytoplankton chlorophyll <i>a</i>		Summer lake phytoplankton chlorophyll <i>a</i>		Summer lake phytoplankton chlorophyll <i>a</i>		Summer lake phytoplankton chlorophyll <i>a</i>		Winter lake phytoplankton chlorophyll <i>a</i>		Summer lake periphyton chlorophyll <i>a</i>		Winter lake periphyton chlorophyll <i>a</i>	
	Equation value	p-value	Equation value	p-value	Equation value	p-value								
Total nitrogen (mg/L) (nutrients only)	1.191	0.007	--	--	0.575	0.044	0.491	<0.001	0.471	0.047	1.276	0.002	--	--
Total phosphorus (mg/L) (nutrients only)	--	--	--	--	--	--	.501	.002	--	--	1.067	<.001	1.193	<0.001
Total nitrogen (mg/L) (with other variables)	--	--	--	--	.480	.025	.384	.002	--	--	--	--	--	--
Total phosphorus (mg/L) (with other variables)	--	--	--	--	--	--	--	--	--	--	.556	<.001	.541	.028
Secchi depth (m)	-0.345	<.001	--	--	-.332	.001	-.156	<.001	--	--	-.230	<.001	-.242	.001
Dissolved oxygen (mg/L)	.078	.003	--	--	--	--	--	--	--	--	--	--	--	--
pH	--	--	--	--	-.179	.010	--	--	.439	.001	--	--	--	--
Water temperature (°C)	--	--	--	--	--	--	--	--	.057	<.001	.031	.031	--	--
Specific conductivity (μS)	--	--	--	--	--	--	--	--	-.001	.005	--	--	--	--

**Table 17.** Summary of influential stream explanatory variables related to stream chlorophyll *a* through regression analysis for each nutrient ecoregion and season [mg/L, milligrams per liter; sq. mi, square mile; EPT, Ephemeroptera, Plecoptera, Trichoptera; ft/mi, feet per mile; °C, degrees Celsius; --, no relation]

Explanatory variable to chlorophyll <i>a</i>	Nutrient ecoregion VII		Nutrient ecoregion VIII		Nutrient ecoregion IX		Nutrient ecoregion XI							
	Summer stream periphyton chlorophyll <i>a</i>		Summer stream periphyton chlorophyll <i>a</i>		Summer stream periphyton chlorophyll <i>a</i>		Summer stream periphyton chlorophyll <i>a</i>		Winter stream periphyton chlorophyll <i>a</i>		Summer stream phytoplankton chlorophyll <i>a</i>		Winter stream phytoplankton chlorophyll <i>a</i>	
	Equation value	p-value	Equation value	p-value	Equation value	p-value								
Total nitrogen (mg/L) (nutrients only)	--	--	--	--	--	--	1.239	<0.001	--	--	--	--	-0.947	<0.010
Total phosphorus (mg/L) (nutrients only)	--	--	--	--	--	--	1.028	.001	--	--	1.0070	<0.001	.739	.001
Total nitrogen (mg/L) (with other variables)	--	--	--	--	--	--	--	--	--	--	.5420	.005	--	--
Total phosphorus (mg/L) (with other variables)	--	--	--	--	--	--	.531	.003	--	--	.4280	.008	--	--
Drainage area (sq. mi)	0.040	0.0030	--	--	--	--	.528	<.001	--	--	--	--	--	--
Percent forest cover	-.028	.0001	--	--	-0.016	0.012	-.012	<.001	--	--	--	--	--	--
EPT index	-.045	.0298	--	--	--	--	--	--	--	--	--	--	--	--
Percent collector/gatherer	-.025	.0010	--	--	--	--	--	--	--	--	--	--	--	--
Percent filterer/collector	-.016	.0054	--	--	--	--	--	--	--	--	--	--	--	--
Percent Plecoptera individuals	.040	.0025	--	--	--	--	--	--	--	--	--	--	--	--
Percent Trichoptera individuals	.101	.0002	--	--	--	--	--	--	--	--	--	--	--	--
pH	--	--	--	--	-.570	.014	--	--	--	--	-1.0160	<.001	--	--
Basin slope (ft/mi)	--	--	--	--	--	--	--	--	--	--	.0005	.003	--	--
Total residue (mg/L)	--	--	--	--	--	--	--	--	--	--	.0010	.038	--	--
Total suspended solids (mg/L)	--	--	--	--	--	--	--	--	--	--	.0200	.005	--	--
Water temperature (°C)	--	--	--	--	--	--	--	--	--	--	--	--	.060	<.001

Summer-season lake periphyton chlorophyll *a* concentrations are controlled more by concentrations of total phosphorus than total nitrogen but both are influential to algal growth (table 16). The most influential regression equation for predicting log of chlorophyll *a* concentrations included concentration of total phosphorus, water temperature, and Secchi depth. Winter-season chlorophyll *a* concentrations were not related to total nitrogen but were related to total phosphorus. Concentration of total phosphorus was a significant explanatory variable both alone and in combination with Secchi depth.

Periphyton in streams from summer-season samples show concentrations of total nitrogen and total phosphorus are influential to chlorophyll *a* concentrations when analyzed alone (table 17). When other variables are introduced, total phosphorus concentrations, drainage area, and percent forest cover best explained the chlorophyll *a* concentrations. Total nitrogen concentrations are no longer influential to chlorophyll *a* concentrations.

Only one winter periphyton chlorophyll *a* sample was available, and so no analysis could be completed.

Summer-season stream phytoplankton chlorophyll *a* samples are influenced by total phosphorus, but not total nitrogen (table 17). When combined with other variables, both concentrations of total nitrogen and total phosphorus, along with basin slope, pH, total residue, and total suspended solids are part of the final equation for predicting log of chlorophyll *a* concentrations of stream phytoplankton. Winter-season samples show concentrations of total nitrogen and total phosphorus being indicative of chlorophyll *a* concentrations, but the best predictor is water temperature. When water temperature is added to the equation, concentrations of total nitrogen and total phosphorus became insignificant.

As determined by this work, periphyton and phytoplankton are influenced by different environmental factors. Nutrient ecoregion plays a role in determining the most significant variables for chlorophyll *a* production. Sometimes, environmental indicators are more influential than the amount of nutrients in the water. Many variables play a role in algal reproduction and biomass other than just nutrients and all must be added into the total equation.

Percent forest cover was significant for stream periphyton chlorophyll *a* concentrations in all nutrient ecoregions. The higher the percent forest cover, the lower chlorophyll *a* concentrations no matter what the nutrient concentrations were. In these streams, light needed for algal growth was more critical to the process than nutrient availability.

Secchi depth was significant in lakes in all nutrient ecoregions. As Secchi depth increased, phytoplankton chlorophyll *a* concentrations decreased. This is more a response to the algal concentrations in the lake than a causative response. As algal biomass increases, the light attenuation decreases. For phytoplankton samples, total nitrogen is more significant to algal growth than total phosphorus but is not the strongest explanatory variable in the equation. Periphyton lake concentrations are related more to concentration of total phosphorus and Secchi depth than total nitrogen.

## CONSIDERATIONS FOR FUTURE RESEARCH

In general, lakes tend to separate into two groups based on water quality. The groups consist of nutrient ecoregions VII and IX and nutrient ecoregions VIII and XI. Given the fairly strong grouping of the water-quality characteristics among nutrient ecoregions, the data indicate that no additional information is gained by specifying four rather than two nutrient ecoregions for lakes in Pennsylvania and West Virginia. The data set analyzed was limited, and perhaps more data would show that a different approach would be better and that smaller groups of lakes should be studied rather than putting two nutrient ecoregions together.

Nutrient ecoregion IX, although similar to nutrient ecoregion VII, has greater concentrations of chlorophyll *a*, total nitrogen, and total phosphorus and specific conductance than the other three nutrient ecoregions. The other water-quality constituents are not different, but these four may put nutrient ecoregion IX in its own group. Better trends can be seen between chlorophyll *a* and nutrients by combining nutrient ecoregions VII, VIII, and XI for analysis and analyzing nutrient ecoregion IX by itself. A clustering procedure may shed valuable insight on the question of grouping the nutrient ecoregions into larger areas for criteria development.

If more samples were collected within nutrient ecoregions VII, VIII, and IX, different results than presented here are possible or they may reinforce what has been seen in the analyses made here. As always, a larger sample size would give a more accurate representation of nutrient-biological relations in the lakes and streams of Pennsylvania and West Virginia.

Nutrient ecoregion XI has a variety of different lithologies. Limestone-type streams, in particular, may be phosphorus limiting. Nutrient criteria work may be enhanced if the limestone streams were analyzed separately from the remaining streams in nutrient ecoregion XI. The USGS National Water-Quality Assessment Program study showed that wells in carbonate areas and areas where soils and aquifers consist of sand and gravel have higher nitrate concentrations than other areas where soils are less permeable and that total phosphorus concentrations in ground water are similar in urban and agricultural settings while nitrates are higher in agricultural than urban settings (Gilliom and others, 2002). Further research may show these groupings are better at explaining the role of nutrients in surface water than nutrient ecoregions are.

Many variables affect algal growth in streams. Commonly, the nutrient enrichment of a stream is not noticed until the streams flow into a small pond or lake. The quiet waters allow algal colonies to establish and bloom. The nitrates and nitrites in the water must be converted to ammonium for utilization by the algae (Sze, 1986); therefore, ammonia concentrations may be a better constituent to measure than total nitrogen and a stronger relation may be seen.

A relation also exists between concentrations of total phosphorus and chlorophyll *a*. The relation is linear until elevated concentrations of total phosphorus are reached. At this point, concentrations of total phosphorus and algae no longer correlate, indicating a possible threshold for total phosphorus. The same pattern was seen by Van Nieuwenhuysse and Jones (1996). Orthophosphate is the form of phosphorus that is most readily used by algae (Sze, 1986), and future work may lead to sampling for orthophosphate instead of total phosphorus.

Other researchers have found that algal species data may be a better predictor of nutrient levels than chlorophyll *a* alone. As nutrients become more available, the algal community shifts and the

biomass may stay the same (Stevenson and others, 1996). Filamentous chlorophytes typically become more abundant when nitrogen and phosphorus levels are relatively high and light is sufficient, diatoms and cyanobacteria typically dominate when there is sufficient phosphorus but not nitrogen, and phytoplankton communities typically become dominated with cyanobacteria when phosphorus enrichment occurs (Stockner and Shortreed, 1978; Elwood and others, 1981; Fairchild and others, 1985; Cattaneo, 1987; Hill and Knight, 1988). Species data appear more closely related to nutrient concentrations than algal biomass.

In addition to nutrients, other environmental indices, such as canopy cover, turbidity, flow, drainage area, and aquatic invertebrates and fish that eat algae, can deter algal growth—thereby preventing a bloom even when nutrients are abundant in stream waters. These factors generally are not significant in lakes but can be quite influential in streams. Understanding all the factors that influence algal blooms or the lack of algal blooms will take more study.

More rigorous statistics and models can be used to look at these relations. Future work could include an approach described as the General Additive Models that estimates stresser-response relations for scaled metrics (scaled to reference conditions) as suggested by Danielle Tillman (U.S. Environmental Protection Agency, written commun., September 2002). Another possible model to use with the data is the Vollenweider model, as suggested by David Flemer (U.S. Environmental Protection Agency, written commun., September 2002).

## SUMMARY

A presidential initiative called the Clean Water Action Plan was released in February 1998. This provides a blueprint for federal agencies to work with states, tribes, and other stakeholders to protect and restore the Nation's water resources. The plan includes an initiative to address the nutrient enrichment problem of lakes and streams across the United States. Developing more effective controls for polluted runoff and promoting the protection of water quality within a watershed are the goals. USEPA is working to develop nutrient criteria using nutrient ecoregions to help prevent excessive nutrients in lakes and streams. Low levels of nutrients are necessary for healthy lake and stream communities; however, high nutrient con-

centrations can cause algal blooms that, in turn, can cause oxygen depletion in the system which leads to the death of aquatic animals.

This study involved gathering available data within Pennsylvania and West Virginia in support of the USEPA nutrient-criteria program. Many agencies were asked to provide data consisting of chlorophyll *a*, nutrients, water quality constituents (Secchi depth, turbidity, pH, specific conductance, etc.), invertebrate indices, and habitat indices. These data were compiled into a database and those samples where both chlorophyll *a* and nutrient data existed were used in the final analysis. Other consistently sampled variables were added into the analysis.

Each of the four nutrient ecoregions (formed by combining level III ecoregions) of Pennsylvania and West Virginia were analyzed for relations between chlorophyll *a*, and nutrients (total nitrogen and total phosphorus) and other environmental indicators. Within each nutrient ecoregion, both lakes and streams data were analyzed. Results varied among the four nutrient ecoregions, but nutrients alone were related to chlorophyll *a*, or in combination with other explanatory variables. In some cases, nutrients alone were still most strongly related to chlorophyll *a* concentrations, and, in some cases, other variables explained more of the chlorophyll *a* variation. Each nutrient ecoregion showed different variables influencing chlorophyll *a* concentrations.

Concentrations of phytoplankton chlorophyll *a* in nutrient ecoregion VII lakes were negatively related to Secchi depth and positively related to concentration of dissolved oxygen; periphyton streams were positively related to drainage area, percent Plecoptera individuals, and percent Trichoptera individuals, and negatively to percent forest cover, the EPT index, percent collector/gatherer, and percent filterer/collector. Nutrients were not statistically significant as being influential to algal chlorophyll *a* within this nutrient ecoregion.

Concentrations of chlorophyll *a* in nutrient ecoregion VIII lakes and streams had too few samples, and so no regressions could be determined.

Nutrient ecoregion IX lake phytoplankton chlorophyll *a* concentrations were positively influenced by total nitrogen, both alone and when combined with other environmental data. Secchi depth and pH had a negative relation with algal growth.

This equation is similar to results from nutrient ecoregion VII, and these nutrient ecoregions possibly could be combined for future analysis. The stream periphyton data generated no significant relations with nutrients, but forest cover and pH were negatively related to chlorophyll *a* concentrations.

Nutrient ecoregion XI had a varied lake data set. There were winter and summer, periphyton and phytoplankton lake data. Summer phytoplankton was positively related to total nitrogen and negatively related to Secchi depth. Winter phytoplankton was related to total nitrogen until other variables were added to the equation. Phytoplankton was then positively related to pH and water temperature and negatively related to specific conductance. Summer-lake periphyton concentrations were positively related to total phosphorus and water temperature, and negatively to Secchi depth. Winter periphyton were positively related to total phosphorus and negatively to Secchi depth.

The streams data for nutrient ecoregion XI were broken into three groups for analysis—periphyton summer, phytoplankton summer, and phytoplankton winter. Summer-season periphyton chlorophyll *a* concentrations were positively related to total phosphorus and drainage area and negatively related to percent forest cover. The summer-season phytoplankton chlorophyll *a* concentrations were positively related to total nitrogen, total phosphorus, basin slope, total residue, and total suspended solids and negatively to pH. The winter-season phytoplankton chlorophyll *a* concentrations were positively related to water temperature.

Different variables influenced the concentrations of chlorophyll *a* in streams among the nutrient ecoregions. Sometimes nutrients were significant variables in algal biomass. Some equations, however, showed other environmental indicators were more significant predictors of chlorophyll *a* concentrations than nutrients were. In some instances, the environmental indicators were so influential in predicting the chlorophyll *a* concentrations that nutrients were deemed insignificant. Analysis of stream samples showed percent forest cover was a significant predictive variable. Where there is not sufficient light for primary algal growth, nutrient overloads do not matter. In these instances, nutrients can be sufficient for algal growth but light is the controlling factor.

Secchi depth was a key predictor for most phytoplankton lake samples. This can be both a causative and response variable. In most cases, it appears to be a response variable. As algal biomass increases, the light penetration decreases. Nutrient concentrations were significant in predicting phytoplankton biomass only in nutrient ecoregions IX and XI. Secchi depth was negatively related to chlorophyll *a* concentrations in all nutrient ecoregions.

More research is needed for a better determination of the response of algal blooms to environmental indicators. Nutrients do contribute to algal blooms, but in certain nutrient ecoregions, other variables are more controlling to algal blooms even when nutrients are available.

## REFERENCES CITED

- Biggs, B.J.F., 1996, Patterns in benthic algae of streams, *in* Stevenson, R.J., Bothwell, M.L., and Lowe, R.L. (eds.), 1996, *Algal ecology—Freshwater benthic ecosystems*: San Diego, Calif., Academic Press, p. 31-56.
- 2000, Eutrophication of streams and rivers—Dissolved nutrient-chlorophyll relationships for benthic algae: *Journal of the North American Benthological Society*, v. 19, no. 1, p. 17-31.
- Biggs, B.J.F., and Close, M.E., 1989, Periphyton biomass dynamics in gravel bed rivers—The relative effects of flows and nutrients: *Freshwater Biology*, v. 22, p. 209-231.
- Borchardt, M.A., Hoffmann, J.P., and Cood, P.W., 1994, Phosphorus uptake kinetics of *Spirogyra fluviatilis* (Charophyceae) in flowing water: *Journal of Phycology*, v. 30, p. 403-417.
- Bourassa, N., and Cattaneo, A., 1998, Control of periphyton biomass in Laurentian streams (Quebec): *Journal of the North American Benthological Society*, v. 17, no. 4, p. 420-429.
- Buck, S., Dodds, W., Fisher, J., Hart, D., Parker, A., Stevenson, J., Watson, V., and Welch, E., 2000, *Nutrient criteria technical guidance manual, rivers and streams*: U.S. Environmental Protection Agency, Office of Water, EPA-822-B-00-002, p. 151 + appendix.
- Carlton, R.G., and Wetzel, R.G., 1988, Phosphorus flux from lake sediments—Effect of epilimnetic oxygen production: *Limnology and Oceanography*, v. 33, p. 562-570.
- Carrick, H.J., Lowe, R.L., and Rotenbury, J.T., 1988, Guilds of benthic algae along nutrient gradients—Relationships to algae community diversity: *Journal of the North American Benthological Society*, v. 7, no. 2, p. 117-128.
- Casey, H., 1977, Origin and variation of nitrate nitrogen in the Chalk Springs, Streams and Rivers in Dorset and its utilization by higher plants: *Progressive Water Technology*, v. 8, 14 p.
- Cattaneo, A., 1987, Periphyton in lakes of different trophy: *Canadian Journal of Fisheries and Aquatic Sciences*, v. 44, p. 296-303.
- Collins, G.B., and Weber, C.I., 1978, Phycoperiphyton (algae) as indicators of water quality: *Transactions American Microscopical Society*, v. 97, no. 1, p. 36-43.
- Dodds, W.K., Smith, V.H., and Zander, B., 1997, Developing nutrient targets to control benthic chlorophyll levels in streams—A case study of the Clark Fork River: *Water Resources*, v. 31(7), p. 1,738-1,750.
- Dortch, Q., Thompson, P.A., and Harrison, P.J., 1991, Variability in nitrate uptake kinetics in *Thalassiosira pseudonona* (Bacillariophyceae): *Journal of Phycology*, v. 27, p. 35-39.
- Duncan, S.W., and Blinn, D.W., 1989, Importance of physical variables on the seasonal dynamics of epilithic algae in a highly shaded canyon stream: *Journal of Phycology*, v. 25, p. 455-461.
- Elwood, J.W., Newbold, J.D., Trimble, A.F., and Stark, R.W., 1981, The limiting role of phosphorus in a woodland stream ecosystem—Effects of P enrichment on leaf decomposition and primary producers: *Ecology*, v. 62, p. 146-158.
- Fairchild, G.W., Lowe, R.L., and Richardson, W.B., 1985, Algal periphyton growth on nutrient-diffusing substrates—An *in situ* bioassay: *Ecology*, v. 66, p. 465-472.
- Freund, R.J., and Littell, R.C., 1986, *SAS system for regression*: Cary, N.C., SAS Institute Inc., 164 p.

## REFERENCES CITED—CONTINUED

- Gibson, G., Carlson, R., Simpson, J., Smeltzer, E., Gerritson, J., Chapra, S., Heiskary, S., Jones, J., and Kennedy, R., 2000, Nutrient criteria technical guidance manual, lakes and reservoirs (1st ed.): Washington, D.C., U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, EPA-822-B00-001.
- Gilliom, R.J., Mueller, D.K., Zogorski, J.S., and Ryker, S.J., 2002, A national look at water quality: Water Resources IMPACT, v. 4, no. 4, p. 12-16.
- Goldman, C.R. and Horne, A.J., 1983, Limnology: New York, McGraw-Hill Book Company, p. 464.
- Helsel, D.R., and Hirsch, R.M., 1995, Statistical methods in water resources: Elsevier Science B.V., 529 p.
- Hill, B.H., Herlihy, A.T., Kaufmann, P.R., Stevenson, R.J., McCormick, F.H., and Burch Johnson, C., 2000, Use of periphyton assemblage data as an index of biotic integrity: Journal of the North American Benthological Society, v. 19, no. 1., p. 50-67.
- Hill, W.R., Boston, H.L., and Steinman, A.D., 1992, Grazers and nutrients simultaneously limit lotic primary productivity: Canadian Journal of Fisheries and Aquatic Sciences, v. 49, p. 504-512.
- Hill, W.R., and Knight, A.W., 1988, Nutrient and light limitation of algae in two northern California streams: Journal of Phycology, v. 24, p. 125-132.
- Horner, R.R., Welch, E.B., and Veenstra, R.B., 1983, Development of nuisance periphytic algae in laboratory streams in relation to enrichment and velocity, in Wetzel, R.G., 1983, Periphyton of freshwater ecosystems: The Hague, Dr. W. Junk Publishers, p. 121-134.
- Keithan, E.D., Lowe, R.L., and DeYoe, H.R., 1988, Benthic diatom distribution in a Pennsylvania stream—Role of pH and nutrients: Journal of Phycology, v. 24, p. 581-585.
- Kirchner, W.B., 1975, An examination of the relationship between drainage basin morphology and the export of phosphorus: Limnology and Oceanography, v. 20, p. 267-270.
- Lohman, K., Jones, J.R., and Baysinger-Daniel, C., 1991, Experimental evidence for nitrogen limitation in a northern Ozark stream: Journal of the North American Benthological Society, v. 19, p. 14-23.
- Manahan, S.E., 1984, Environmental chemistry (4th ed.): Boston, PWS Publishers, 612 p.
- McCormick, P.V., and Stevenson, R.J., 1991, Grazer control of nutrient availability in the periphyton: Oecologia, v. 86, p. 287-291.
- Mundie, J.H., Simpson, K.S., and Perrin, C.J., 1991, Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm: Canadian Journal of Fisheries and Aquatic Sciences, v. 48, p. 2,061-2,072.
- Nordin, R.N., 1985, Water quality criteria for nutrients and algae (technical appendix): Victoria, Canada, Water Quality Unit, Resource Quality Section, Water Management Branch, British Columbia Ministry for the Environment, 104 p.
- Omernik, J.M., 1987, Ecoregions of the conterminous United States: Annals of the Association of American Geographers, v. 77, p. 118-125.
- Ott, R.L., 1993, An introduction to statistical methods and data analysis (4th ed.): Belmont, Calif., Duxbury Press, 1,051 p. + appendix.
- Peterson, B.J., Deegan, L., Helfrich, J., Hobbie, J.E., Hullar, M., Moller, B., Ford, T.E., Hershey, A., Hiltner, A., Kipphut, G., Lock, M.A., Fiebig, D.M., McKinley, V., Miller, M.C., Vestal, J.R., Ventullo, R., and Volk, G., 1993, Biological responses of a tundra river to fertilizations: Ecology, v. 74, p. 653-672.
- Plafkin, J.L., Barbour, M.T., Porter, K.D., Gross, S.K., and Hughes, R.M., 1989, Rapid bioassessment protocols for use in streams and rivers—Benthic macroinvertebrates and fish: Washington, D.C., U.S. Environmental Protection Agency, Office of Water Regulations and Standards, EPA 440-4-89-001, 128 p. + 4 appendices.

## REFERENCES CITED—CONTINUED

- Riber, H.H., and Wetzel, R.G., 1987, Boundary layer and internal diffusion effects on phosphorus fluxes in lake periphyton: *Limnology and Oceanography*, v. 32, p. 1,181-1,194.
- Rosemond, A.D., Mulholland, P.J., and Elwood, J.W., 1993, Top-down and bottom-up control of stream periphyton—Effects of nutrients and herbivores: *Ecology*, v. 74, p. 1,264-1,280.
- Smith, R.L., 1980, *Ecology and field biology* (3d ed.): New York, Harper and Row Publishers, 835 p.
- Stevenson, R.J., Bothwell, M.L., and Lowe, R.L., eds., 1996, *Algal ecology—Freshwater benthic ecosystems*: San Diego, Calif., Academic Press, 753 p.
- Stevenson, R.J., and Lowe, R.L., 1986, Sampling and interpretation of algal patterns for water quality assessments, in Isom, B.G., ed., *Rationale for sampling and interpretation of ecological data in the assessment of freshwater ecosystems*: Philadelphia, American Society for Testing and Materials, ASTM STP 894, 118-149 p.
- Stockner, J.G., and Shortreed, K.R.S., 1978, Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island: *Journal of Fisheries Resource Board of Canada*, v. 35, p. 28-34.
- Sze, P., 1986, *Algae* (2nd ed.): Dubuque, Iowa, Wm. C. Brown Publishers, 259 p.
- Triska, F.J., Kennedy, V.C., Avanzino, R.J., Zellweger, G.W., and Bencala, K.E., 1989, Retention and transport of nutrients in a third-order stream-channel processes: *Ecology*, v. 70 (6), p. 1,877-1,892.
- U.S. Environmental Protection Agency, 1998, *National strategy for the development of regional nutrient criteria*: Washington D.C., Office of Water, EPA-822-F-98-002, 47 p.
- \_\_\_\_\_, 2000, Draft aggregations of Level III ecoregions for the National nutrient strategy, accessed March 1, 2001, at URL <http://www.epa.gov/OST/standards/ecomap.html>.
- \_\_\_\_\_, 2001, Ecoregional nutrient criteria documents for rivers and streams, accessed February 28, 2001, at URL <http://www.epa.gov/waterscience/criteria/nutrient/ecoregions/rivers/>.
- U.S. Geological Survey, 2001, Multi-resolution land characteristics 2000 (MRLC2000), accessed January 17, 2001, at URL <http://edcw2ks15.cr.usgs.gov/lccp/mrlc2k/>
- Van Nieuwenhuysse, E.E., and Jones, J.R., 1996, Phosphorus-chlorophyll relationship in temperature streams and its variation with stream catchment area: *Canadian Journal of Fisheries and Aquatic Science*, v. 53, p. 99-105.
- Welch, E.B., Jacoby, J.M., Horner, R.R., and Seeley, M.R., 1988, Nuisance biomass levels of periphytic algae in streams: *Hydrobiologia*, v. 157, p. 161-168.
- Wetzel, R.G., 1996, Benthic algae and nutrient cycling in lentic freshwater ecosystems, in Stevenson, R.J., Bothwell, M.L., and Lowe, R.L., eds., 1996, *Algal ecology—Freshwater benthic ecosystems*: San Diego, Calif., Academic Press, p. 641-667.
- Wharfe, J.R., Taylor, K.S., and Notgomery, H.A.C., 1984, The growth of *Cladophora glomerata* in a river receiving sewage effluent: *Water Resources*, v. 18, p. 971-979.
- Winder, J.G., and Duthie, H.C., 2000, Epilithic diatoms as indicators of stream total N and total P concentration: *Journal of the North American Benthological Society*, v. 19, no. 1, p. 32-49.
- Winterbourn, M.J., 1990, Interactions among nutrients algae and invertebrates in a New Zealand mountain stream: *Freshwater Biology*, v. 23, p. 463-474.
- Zuur, B., ed., 1992, *Water quality guidelines number 1—Guidelines for the control of undesirable biological growths in water*: Wellington, New Zealand, New Zealand Ministry for the Environment, 60 p.

**APPENDIX—LABORATORY METHODS USED BY  
DIFFERENT GOVERNMENTAL AGENCIES**

**Delaware River Basin Commission**

Parameter name: Residue, total nonfilterable (00530)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Gravimetric, dried at 103-105°C (EPA 160.2)  
Method summary: A well mixed sample is filtered through a standard glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C  
Reporting limits: 0.2, 1, 2 milligrams per liter  
Years sampled: 1992-1997

Parameter name: Nitrogen, total as N (00600)<sup>2</sup>  
Unit of measure: milligrams per liter  
Method name:  
Method summary: no method provided  
Reporting limits: Not listed  
Years sampled: 1997

Parameter name: Nitrogen, total organic as N (00605)<sup>2</sup>  
Unit of measure: milligrams per liter  
Method name:  
Method summary: no method provided  
Reporting limits: 0.16, 0.39, 0.44, 0.78, 0.84, 0.85, 0.9, 0.97, 1.7 milligrams per liter  
Years sampled: 1992-1996

Parameter name: Nitrogen, total ammonia as N (00610)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Colorimetric, automated phenate (EPA 350.1)  
Method summary: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.  
Reporting limits: 0.01, 0.02 milligrams per liter  
Years sampled: 1992-1997

Parameter name: Nitrite, total as N (00615)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Spectrophotometric (EPA 354.1)  
Method summary: The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer.  
Reporting limits: Not listed  
Years sampled: 1992-1997

## Delaware River Basin Commission—Continued

Parameter name: Nitrate, total as N (00620)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, cadmium reduction, and Spectrophotometric (EPA 353.2 minus 354.1)

Method summary: A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-dthylenediamind dihydrochloride to form a highly colored azo dye which is measured colorimetrically. (minus) The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer at 540 nm.

Reporting limits: Not listed

Years sampled: 1992-1997

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, semi-automated block digester, AAI (EPA 351.2)

Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.

Reporting limits: 1 milligram per liter

Years sampled: 1992-1997

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>5</sup>

Unit of measure: milligrams per liter

Method name:

Method summary: no method provided

Reporting limits: 0.02 milligrams per liter

Years sampled: 1991-1994

Parameter name: Phosphorus, total as P (00665)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, ascorbic acid (EPA 365.1)

Method summary: Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration. Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by manual sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by manual persulfate digestion. The developed color is measured automatically on the AutoAnalyzer.

Reporting limits: 0.01 milligrams per liter

Years sampled: 1992-1997

## Delaware River Basin Commission—Continued

Parameter name: Phosphorus, dissolved orthophosphate as P (00671)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, ascorbic acid (EPA 365.1)

Method summary: Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration. This is measured automatically on the AutoAnalyzer.

Reporting limits: Not listed

Years sampled: 1991 and 1997

Parameter name: Orthophosphorus, total as P (70507)<sup>5</sup>

Unit of measure: milligrams per liter

Method name:

Method summary: no method provided

Reporting limits: 0.01, 0.02, 0.05, 0.07 milligrams per liter

Years sampled: 1991-1994

## National Park Service

Parameter name: Nitrogen, total ammonia as N (00610)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Automated phenate method (Standard method 4500-NH<sub>3</sub>-H)

Method summary: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color is intensified with sodium nitroprusside.

Reporting limits: 0.02 milligrams per liter

Years sampled: 1991-1995

Parameter name: Nitrite, total as N (00615)<sup>6</sup>

Unit of measure: milligrams per liter

Method name: Determination of inorganic anions by ion chromatography (EPA 300.0)

Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into an ion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time.

Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.

Reporting limits: 0.004, 0.012 milligrams per liter

Years sampled: 1991-1995

Parameter name: Nitrate, total as N (00620)<sup>6</sup>

Unit of measure: milligrams per liter

Method name: Determination of inorganic anions by ion chromatography (EPA 300.0)

Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into an ion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time.

Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.

Reporting limits: Not listed

Years sampled: 1991-1995

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, semi-automated block digester, AAI (EPA 351.2)

Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination.

Reporting limits: 0.2 milligrams per liter

Years sampled: 1991-1995

Parameter name: Phosphorus, total as P (00665)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Automated ascorbic acid reduction (Standard method 4500-P-F)

Method summary: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which yields an intense blue color suitable for photometric measurement.

Reporting limits: Not listed

Years sampled: 1991-1995

## Ohio Environmental Protection Agency

Parameter name: Residue, total nonfilterable (00530)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Gravimetric, dried at 103-105°C (EPA 160.2)

Method summary: A well mixed sample is filtered through a standard glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C

Reporting limits: 2 milligrams per liter

Years sampled: 1994

Parameter name: Nitrogen, total ammonia as N (00610)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated phenate (EPA350.1)

Method summary: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

Reporting limits: 0.05 milligrams per liter

Years sampled: 1994

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, semi-automated block digester, AAII (EPA 351.2)

Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.

Reporting limits: Not listed

Years sampled: 1994

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, cadmium reduction (EPA 353.2)

Method summary: A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically.

Reporting limits: Not listed

Years sampled: 1994

Parameter name: Phosphorus, total as P (00665)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, block digester AA II (EPA 365.4)

Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub> and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted to 25 mL and placed on the Auto Analyzer for phosphorus determination.

Reporting limits: Not listed

Years sampled: 1994

## Ohio River Valley Water

Parameter name: Nitrogen, total ammonia as N (00610)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated phenate (EPA350.1)

Method summary: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

Reporting limits: 0.03 milligrams per liter

Years sampled: 1990-1998

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, cadmium reduction (EPA 353.2)

Method summary: A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically.

Reporting limits: 0.02 milligrams per liter

Years sampled: 1990-1998

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, semi-automated block digester, AAI (EPA 351.2)

Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.

Reporting limits: 0.05 milligrams per liter

Years sampled: 1990-1992

Parameter name: Phosphorus, total as P (00665)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, ascorbic acid, single reagent

Method summary: Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Reporting limits: 0.05 milligrams per liter

Years sampled: 1990-1998

## Pennsylvania Department of Environmental Protection

Parameter name: Residue, total nonfilterable (00530)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Gravimetric, dried at 103-105°C (EPA 160.2)  
Method summary: A well mixed sample is filtered through a standard glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C  
Reporting limits: 2 milligrams per liter  
Years sampled: 1992-1996

Parameter name: Nitrogen, total as N (00600)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name:  
Method summary: Calculated value - typically 00625 (total kjeldahl) + 00630 (nitrite+nitrate)  
Reporting limits: Not listed  
Years sampled: 1995-1996

Parameter name: Nitrogen, total organic as N (00605)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Calculated value (EPA351.2 - EPA350.1)  
Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination. (minus) Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.  
[nitrogen, kjeldahl, total]-[nitrogen, ammonia]  
Reporting limits: 0.01 milligrams per liter  
Years sampled: 1994

Parameter name: Nitrogen, total ammonia as N (00610)<sup>4</sup>  
Unit of measure: milligrams per liter  
Method name: Automated phenate method (Standard method 4500-NH<sub>3</sub>-H)  
Method summary: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color is intensified with sodium nitroprusside.  
Reporting limits: 0.02 milligrams per liter  
Years sampled: 1991-1996

Parameter name: Nitrite, total as N (00615)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Determination of inorganic anions by ion chromatography (EPA 300.0)<sup>6</sup>  
Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into an ion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time. Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.  
Reporting limits: 0.004, 0.04 milligrams per liter  
Years sampled: 1991-1994

## Pennsylvania Department of Environmental Protection—Continued

Parameter name: Nitrate, total as N (00620)<sup>6</sup>

Unit of measure: milligrams per liter

Method name: Determination of inorganic anions by ion chromatography (EPA 300.0)

Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into an ion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time.

Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.

Reporting limits: 0.04 milligrams per liter

Years sampled: 1991-1994

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, semi-automated block digester, AAI (EPA 351.2)

Method summary: The sample is heated in the presence of sulfuric acid,  $K_2SO_4$ , and  $HgSO_4$  for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination.

Reporting limits: 0.2 milligrams per liter

Years sampled: 1991-1994

Parameter name: Phosphorus, total as P (00665)<sup>4</sup>

Unit of measure: milligrams per liter

Method name: Automated ascorbic acid reduction (Standard method 4500-P-F)

Method summary: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which yields an intense blue color suitable for photometric measurement.

Reporting limits: Not listed

Years sampled: 1991-1996

Parameter name: Phosphorus, dissolved as P (00666)<sup>4</sup>

Unit of measure: milligrams per liter

Method name: Automated ascorbic acid reduction (Standard method 4500-P-F)

Method summary: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which yields an intense blue color suitable for photometric measurement.

Reporting limits: Not listed

Years sampled: 1991-1995

Parameter name: Orthophosphate, dissolved as P (00671)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Automated ascorbic acid reduction (Standard method 4500-P-F)

Method summary: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which yields an intense blue color suitable for photometric measurement.

Reporting limits: 0.002 milligrams per liter

Years sampled: 1995

## Pennsylvania Department of Environmental Protection—Continued

Parameter name: Phytoplankton, chlorophyll a, spectrophotometric, uncorrected (32230)<sup>3</sup>

Unit of measure: micrograms per liter

Method name:

Method summary:

Reporting limits: Not listed

Years sampled: 1991-1996

Parameter name: Orthophosphorus, total as P (70507)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Automated ascorbic acid reduction (Standard method 4500-P-F)

Method summary: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which yields an intense blue color suitable for photometric measurement.

Reporting limits: 0.002 milligrams per liter

Years sampled: 1991-1994

## Susquehanna River Basin Commission

PaDEP laboratory is used for all data analysis. All parameters follow the same methods procedure as listed for PaDEP.

## U.S. Army Corps of Engineers - Huntington

Parameter name: Residue, total (00500)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Total solids dried at 103-105°C (SM2540B)

Method summary: A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 - 105°C. The increase in weight over that of the empty dish represents the total solids.

Reporting limits: Not listed

Years sampled: 1990-1998

Parameter name: Residue, total nonfilterable (00530)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Total suspended solids dried at 103-105°C (SM2540D)

Method summary: A well mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, the difference between the total solids and the total dissolved solids may provide an estimate of the total suspended solids.

Reporting limits: 1, 5 milligrams per liter

Years sampled: 1990-1998

Parameter name: Nitrogen, dissolved ammonia as N (00608)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Ammonia-selective electrode method using known addition (SM4500-NH<sub>3</sub>F)

Method summary: The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia is converted to NH<sub>3</sub>(aq) by raising pH to above 11 with a strong base. NH<sub>3</sub>(aq) diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

Reporting limits: 0.02 milligrams per liter

Years sampled: 1996-1998

Parameter name: Nitrogen, kjeldahl dissolved as N (00623)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric; titrimetric; potentiometric (EPA 351.3)

Method summary: The sample is heated in the presence of concentrated sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub>, and evaporated until SO<sub>3</sub> fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by nesslerization, titration or potentiometry.

Reporting limits: Not listed

Years sampled: 1997-1998

## U.S. Army Corps of Engineers - Huntington—Continued

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric; titrimetric; potentiometric (EPA 351.3)

Method summary: The sample is heated in the presence of concentrated sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub>, and evaporated until SO<sub>3</sub> fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by nesslerization, titration or potentiometry.

Reporting limits: 0.02, 0.1 milligrams per liter

Years sampled: 1992-1998

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>6</sup>

Unit of measure: milligrams per liter

Method name: Determination of inorganic anions by ion chromatography (EPA 300.0)

Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into anion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time.

Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.

Years sampled: 1991-1998

Parameter name: Nitrite plus nitrate, dissolved as N (00631)<sup>6</sup>

Unit of measure: milligrams per liter

Method name: Determination of inorganic anions by ion chromatography (EPA 300.0)

Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into anion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time.

Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.

Reporting limits: Not listed

Years sampled: 1998

Parameter name: Phosphorus, total as P (00665)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Digestion method and Ascorbic acid method (SM4500-P B and SM4500-P E)

Method summary: Convert the phosphorus form of interest to dissolved orthophosphate and use colorimetry to determine concentration. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes. And, ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to intensely colored molybdenum blue by ascorbic acid.

Reporting limits: 0.01, 0.02, 0.05 milligrams per liter

Years sampled: 1992-1998

## U.S. Army Corps of Engineers - Huntington—Continued

Parameter name: Phosphorus, orthophosphate, dissolved as P (00671)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Ascorbic acid method (SM4500-P E)

Method summary: Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to intensely colored molybdenum blue by ascorbic acid.

Reporting limits: 0.01, 0.02, 0.05 milligrams per liter

Years sampled: 1992-1998

Parameter name: Chlorophyll a, phytoplankton, spectrophotometric acid (32211)<sup>2</sup>

Unit of measure: microgram per liter

Method name: Spectrophotometric determination of chlorophyll (SM10200H-2)

Method summary: With a narrow band width between 0.5 and 2.0 nm the spectral band shows the amount of chlorophyll a present in the sample

Reporting limits: 1 microgram per liter

Years sampled: 1991-1998

## U.S. Army Corps of Engineers - Philadelphia

Parameter name: Nitrogen, total ammonia as N (00610)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated phenate (EPA350.1)

Method summary: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

Reporting limits: 0.01 milligrams per liter

Years sampled:

Parameter name: Nitrite, total as N (00615)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Spectrophotometric (EPA 354.1)

Method summary: The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer.

Reporting limits: 0.01 milligrams per liter

Years sampled:

Parameter name: Nitrate, total as N (00620)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, cadmium reduction (EPA 353.2)

Method summary: A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-dthylenediamind dihydrochloride to form a highly colored azo dye which is measured colorimetrically.

Reporting limits: 0.05 milligrams per liter

Years sampled:

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, semi-automated block digester, AAI (EPA 351.2)

Method summary: The sample is heated in the presence of sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub> for two and one half hours. The residue is cooled, diluted, and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.

Reporting limits: 0.1 milligrams per liter

Years sampled:

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>6</sup>

Unit of measure: milligrams per liter

Method name: Addition of EPA 354.1 and EPA 353.2 methods

Method summary: The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer.

(plus) A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-dthylenediamind dihydrochloride to form a highly colored azo dye which is measured colorimetrically.

Reporting limits: 0.05 milligrams per liter

Years sampled:

## U.S. Army Corps of Engineers - Philadelphia—Continued

Parameter name: Phosphorus, total as P (00665)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Phosphorus, colorimetric, phosphomolybdate, automated-segmented flow

Method summary: All forms of phosphorus, including organic phosphorus, are converted to orthophosphate by an acid-persulfate digestion.

Reporting limits: 0.0177 milligrams per liter

Years sampled:

Parameter name: Phosphorus, dissolved orthophosphate as PO<sub>4</sub> (00666)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, ascorbic acid, single reagent

Method summary: Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. Ascorbic acid is added to reduce the complex and an intense blue color is formed.

The color is proportional to the orthophosphate concentration.

Reporting limits: 0.050 milligrams per liter

Years sampled:

Parameter name: Phytoplankton, chlorophyll a<sup>6</sup>

Unit of measure: milligrams per cubic meter

Method name: APHA 10200 H-2

Method summary: Quantitative filtration (glass fiber) in field, extraction of filter into acetone; analysis by spectrophotometry (monochromatic)

Reporting limits: 0.0679 milligrams per cubic meter

Years sampled:

## U.S. Environmental Protection Agency

Parameter name: Residue, solids, total evaporation (00500)<sup>6</sup>  
Unit of measure: milligrams per liter  
Method name: Gravimetric, dried at 103-105°C (EPA 160.3)  
Method summary: A well mixed aliquot of the sample is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103-105°C  
Reporting limits: 0.1 milligrams  
Years sampled: 1993-1995

Parameter name: Nitrogen, total as N (00600)<sup>6</sup>  
Unit of measure: milligrams per liter  
Method name: Determination of total nitrogen (EPA 353.2 modified)  
Method summary: Alkaline persulfate digestion with determination of nitrate by cadmium reduction and determination of nitrite by automated colorimetry.  
Reporting limits: 1 micrograms per liter  
Years sampled: 1993-1995

Parameter name: Nitrogen, total ammonia as N (00610)<sup>6</sup>  
Unit of measure: milligrams per liter  
Method name: Determination of ammonium - photometric method (EPA 350.7)  
Method summary: Alkaline phenol and hypochlorite react with ammonia to form an amount of indophenol blue that is proportional to the ammonium concentration. The blue color intensifies with sodium nitroprusside.  
Reporting limits: 0.02 milligrams per liter  
Years sampled: 1993-1995

Parameter name: Nitrite, total as N (00615)<sup>5</sup>  
Unit of measure: milligrams per liter  
Method name: Spectrophotometric (EPA 354.1)  
Method summary: The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer.  
Reporting limits: Not listed  
Years sampled: 1993-1995

Parameter name: Nitrate, total as N (00620)<sup>6</sup>  
Unit of measure: milligrams per liter  
Method name: Determination of nitrate by ion chromatography (EPA 300.6)  
Method summary: Ion chromatography combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into anion chromatograph and run through columns to separate the anions. The separated anions are measured using a conductivity cell. Anion identification is based on retention time. Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards.  
Reporting limits: 0.03 milligrams per liter  
Years sampled: 1993-1995

## U.S. Environmental Protection Agency—Continued

Parameter name: Phosphorus, total as P (00665)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Phosphorus, colorimetric, phosphomolybdate, automated-segmented flow

Method summary: All forms of phosphorus, including organic phosphorus, are converted to orthophosphate by an acid-persulfate digestion.

Reporting limits: 1 microgram per liter

Years sampled: 1993-1995

Parameter name: Periphyton, chlorophyll a (70955)<sup>6</sup>

Unit of measure: milligrams per square meter

Method name: APHA 10200 H-2

Method summary: Quantitative filtration (glass fiber) in field, extraction of filter into acetone; analysis by spectrophotometry (monochromatic).

Reporting limits: 1 microgram per liter

Years sampled: 1993-1995

Reporting limits: 0.03 milligrams per liter

Years sampled:

## U.S. Geological Survey

Parameter name: Residue, total nonfilterable (00530)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Solids, residue at 105°C, suspended, gravimetric

Method summary: The unfiltered sample is mixed thoroughly and an appropriate volume is rapidly poured into a graduated cylinder. The suspended solids are collected on a glass-fiber filter, and the insoluble residue is dried and weighed.

Reporting limits: Not listed

Years sampled: 1993-1995

Parameter name: Nitrogen, total as N (00600)<sup>2</sup>

Unit of measure: milligrams per liter

Method name: Calculated value - Negatives are eliminated by taking the max using zero on the left

Method summary: Add parameter 00625 and parameter 00630.

Reporting limits: Not listed

Years sampled: 1993 and 1998

Parameter name: Nitrogen, total organic as N (00605)<sup>2</sup>

Unit of measure: milligrams per liter

Method name: Calculated value - Negatives are eliminated by taking the max using zero on the left.

Method summary: Subtract parameter 00610 from parameter 00625.

Reporting limits: Not listed

Years sampled: 1998

Parameter name: Nitrogen, dissolved organic as N (00607)<sup>2</sup>

Unit of measure: milligrams per liter

Method name: Calculated value - Negatives are eliminated by taking the max using zero on the left.

Method summary: Subtract parameter 00608 from parameter 00623.

Reporting limits: Not listed

Years sampled: 1998

Parameter name: Nitrogen, dissolved ammonia as N (00608)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Nitrogen, ammonia, low ionic-strength water, colorimetry, salicylate-hypochlorite, automated-segmented flow

Method summary: Ammonia reacts with hypochlorite and salicylate ions in the presence of ferricyanide ions to form the salicylic acid analog of indophenol.

Reporting limits: 0.002, 0.02 milligram per liter (regular line, low level line)

Years sampled: 1993-1998

Parameter name: Ammonia, total as N (00610)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Nitrogen, ammonia, colorimetric, salicylate-hypochlorite, automated-segmented flow

Method summary: Ammonia reacts with sodium salicylate, sodium nitroprusside, and sodium hypochlorite to form an intense color compound that is directly proportional to the concentration of ammonia present.

Reporting limits: 0.01, 0.02 milligram per liter

Years sampled: 1993-1995

## U.S. Geological Survey—Continued

Parameter name: Nitrite, total as N (00615)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Nitrogen, nitrite, colorimetric, diazotization, automated-segmented flow

Method summary: Nitrite ion reacts with sulfanilamide under acidic conditions to form a red compound and the absorbance is measured colorimetrically.

Reporting limits: 0.1 milligram per liter

Years sampled: 1993-1995

Parameter name: Nitrate, dissolved as N (00618)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Nitrogen, nitrate, ion-exchange chromatographic, automated

Method summary: Nitrate is determined by ion-exchange chromatography.

Reporting limits: 0.01 milligrams per liter

Years sampled: 1993 and 1998

Parameter name: Nitrate, total as N (00620)<sup>1</sup>

Unit of measure: milligrams per liter

Method name:

Method summary: Calculated value.

Reporting limits: Not listed

Years sampled: 1993-1995

Parameter name: Nitrogen, dissolved kjeldahl as N (00623)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Nitrogen, ammonia plus organic, colorimetry, block digester-salicylate-hypochlorite, automated-segmented flow

Method summary: Organic nitrogen compounds are reduced to the ammonium ion by digestion with sulfuric acid in the presence of mercuric sulfate and potassium sulfate. The ammonium ion produced is determined by reaction with sodium salicylate, sodium nitroprusside, and sodium hypochlorite in an alkaline medium. The resulting color is directly proportional to the concentration of ammonia present.

Reporting limits: 0.10 milligrams per liter

Years sampled: 1993-1996

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Nitrogen, ammonia plus organic, colorimetry, block digester-salicylate-hypochlorite, automated-segmented flow

Method summary: Organic nitrogen compounds are reduced to the ammonium ion by digestion with sulfuric acid in the presence of mercuric sulfate and potassium sulfate. The ammonium ion produced is determined by reaction with sodium salicylate, sodium nitroprusside, and sodium hypochlorite in an alkaline medium. The resulting color is directly proportional to the concentration of ammonia present.

Reporting limits: 0.10 milligrams per liter

Years sampled: 1993-1998

## U.S. Geological Survey—Continued

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>1</sup>  
Unit of measure: milligrams per liter  
Method name: Nitrogen, nitrite plus nitrate, colorimetric, cadmium reduction-diazotization, automated-segmented flow  
Method summary: An acidified sodium chloride extraction procedure is used to extract nitrate and nitrite from bottom material for this determination.  
Reporting limits: 0.2 milligrams per liter  
Years sampled: 1993-1995

Parameter name: Nitrite plus nitrate, dissolved as N (00631)<sup>1</sup>  
Unit of measure: milligrams per liter  
Method name: Nitrogen, nitrite plus nitrate, colorimetric, cadmium reduction-diazotization, automated-segmented flow  
Method summary: An acidified sodium chloride extraction procedure is used to extract nitrate and nitrite from bottom material for this determination.  
Reporting limits: 0.05 milligrams per liter  
Years sampled: 1993-1998

Parameter name: Orthophosphate, dissolved as PO<sub>4</sub> (00660)<sup>2</sup>  
Unit of measure: milligrams per liter  
Method name: Calculated value - Negatives are eliminated by taking the max using zero on the left.  
Method summary: Multiply parameter 00671 by 3.06618.  
Reporting limits: Not listed  
Years sampled: 1998

Parameter name: Phosphorus, total as P (00665)<sup>1</sup>  
Unit of measure: milligrams per liter  
Method name: Phosphorus, colorimetric, phosphomolybdate, automated-segmented flow  
Method summary: All forms of phosphorus, including organic phosphorus, are converted to orthophosphate by an acid-persulfate digestion.  
Reporting limits: 0.004, 0.05 milligram per liter  
Years sampled: 1993-1998

Parameter name: Phosphorus, dissolved as P (00666)<sup>1</sup>  
Unit of measure: milligrams per liter  
Method name: Phosphorus, colorimetric, phosphomolybdate, automated-segmented flow  
Method summary: All forms of phosphorus, including organic phosphorus, are converted to orthophosphate by an acid-persulfate digestion.  
Reporting limits: 0.004, 0.05 milligrams per liter  
Years sampled: 1993-1998

## U.S. Geological Survey—Continued

Parameter name: Orthophosphate, dissolved as P (00671)<sup>1</sup>

Unit of measure: milligrams per liter

Method name: Phosphorus, orthophosphate, colorimetry, phosphomolybdate, automated-segmented flow

Method summary: Orthophosphate ion reacts with ammonium molybdate in acidic solution to form phosphomolybdic acid and is reduced with ascorbic acid to produce an intensely blue color. Antimony potassium tartrate is added to increase the rate of reduction.

Reporting limits: 0.01 milligrams per liter

Years sampled: 1993-1996

Parameter name: Periphyton chlorophyll *a* (70957)<sup>1</sup>

Unit of measure: milligrams per square meter

Method name: Chlorophyll in periphyton by chromatography and fluorometry

Method summary: A periphyton sample is obtained and the chlorophylls *a* and *b* are extracted from the algal cells. The chlorophylls are separated from each other and chlorophyll *a* is eluted and measured using a spectrofluorometer.

Reporting limits: 0.1 milligram per meter square

Years sampled: 1993-1998

## West Virginia Division of Environmental Protection

Parameter name: Residue, total nonfilterable (00530)<sup>3</sup>

Unit of measure: milligrams per liter

Method name: Gravimetric, dried at 103-105°C (EPA 160.2)

Method summary: A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C.

Reporting limits: 0.5, 5 milligrams per liter

Years sampled: 1993-1997

Parameter name: Nitrogen, total ammonia as N (00610)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric; titrimetric; potentiometric - distillation procedure (EPA 350.2)

Method summary: The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds and is then distilled into a solution of boric acid. The ammonia in the distillate can be determined colorimetrically by nesslerization, titrimetrically with standard sulfuric acid with the use of a mixed indicator, or potentiometrically by the ammonia electrode.

Reporting limits: 0.01, 0.1, 0.5, 0.6 milligrams per liter

Years sampled: 1993-1996

Parameter name: Nitrogen, total kjeldahl as N (00625)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric; titrimetric; potentiometric (EPA 351.3)

Method summary: The sample is heated in the presence of concentrated sulfuric acid, K<sub>2</sub>SO<sub>4</sub>, and HgSO<sub>4</sub>, and evaporated until SO<sub>3</sub> fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by nesslerization, titration or potentiometry.

Reporting limits: 0.01, 0.1, 0.5 milligrams per liter

Years sampled: 1993-1995

Parameter name: Nitrite plus nitrate, total as N (00630)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, automated, cadmium reduction (EPA 353.2)

Method summary: A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically.

Reporting limits: 0.01, 0.05, 0.1, 0.5 milligrams per liter

Years sampled: 1993-1997

Parameter name: Phosphorus, total as P (00665)<sup>5</sup>

Unit of measure: milligrams per liter

Method name: Colorimetric, ascorbic acid, two reagent (EPA 365.3)

Method summary: Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Reporting limits: 0.02, 0.1 milligrams per liter

Years sampled: 1993-1997

## West Virginia Division of Environmental Protection—Continued

Parameter name: Periphyton, chlorophyll *a* (32223)<sup>3</sup>  
Unit of measure: milligrams per square meter  
Method name: Spectrophotometric determination of chlorophyll (Standard method 10200)  
Method summary: With a narrow band width between 0.5 and 2.0 nm the spectral band shows the amount of chlorophyll *a* present in the sample.  
Reporting limits: 0.5 milligrams per square meter  
Years sampled: 1993 -1994

Parameter name: Periphyton, chlorophyll *a* (32223)<sup>3</sup>  
Unit of measure: milligrams per square meter  
Method name: Fluorometric determination of chlorophyll *a* (Standard method 10200)  
Method summary: Optimum sensitivity for chlorophyll *a* extract measurements is obtained at an excitation wavelength of 430 nm and an emission wavelength of 663 nm.  
Reporting limits: 1, 6 milligrams per square meter  
Years sampled: 1995-1997

<sup>1</sup>Fishman, M.J., and Friedman, L.C., eds., 1989, Methods for determination of inorganic substance in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations of the United States Geological Survey, book 5, chap. A1, p. 545.

<sup>2</sup>Fishman, M.J., ed., 1993, Methods of analysis of by U.S. Geological Survey national water quality laboratory—Determination of inorganic and organic constituents in water fluvial sediments: U.S. Geological Survey Open-File Report 93-125, p. 217.

<sup>3</sup>Greenberg, A.E., Clesceri, L.S., and Eaton, A.D., 1992, Standard methods for the examination of water and wastewater: Washington, D.C., American Public Health Association, p. 981.

<sup>4</sup>U.S. Environmental Protection Agency, 1974, Methods for chemical analysis of water and wastes: Cincinnati, Ohio, EPA/625/6-74/003, p. 297.

<sup>5</sup>U.S. Environmental Protection Agency, 1979, Methods for chemical analysis of water and wastes: Cincinnati, Ohio, EPA/600/4-79/020, p. 552.

<sup>6</sup>U.S. Environmental Protection Agency, 1987, Handbook of methods for acid deposition studies—Laboratory analysis for surface water chemistry: Washington, D.C., EPA/600/4-87/026, p. 376.