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Seasonal Patterns in Nutrients, Carbon, and Algal Responses in Wadeable Streams within Three Geographically Distinct Areas of the United States, 2007–08

Scientific Investigations Report 2012–5086

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

Front cover. Cedar Creek near East Bethel, Minnesota, photograph by John B. Greene, U.S. Geological Survey (USGS), 2008.

Back cover. Upper: Andrew Berg making a streamflow measurement under ice at the Mississippi River near Vern, Minnesota on February 5, 2007, photograph by John Greene, USGS. Middle: John Greene making a streamflow measurement at Moran Creek near Staples, Minnesota, May 28, 2008, photograph by Andrew Berg, USGS. Lower: Andrew Berg checking the stage sensor at the Mississippi River near Vern, Minnesota, on June 3, 2007, photograph by John Greene, USGS.

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By Kathy E. Lee, David L. Lorenz, James C. Petersen, and John B. Greene

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KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

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Foreword

The U.S. Geological Survey (USGS) is committed to providing the Nation with reliable scientific information that helps to enhance and protect the overall quality of life and that facilitates effective management of water, biological, energy, and mineral resources (*http://www.usgs.gov/*). Information on the Nation's water resources is critical to ensuring long-term availability of water that is safe for drinking and recreation and is suitable for industry, irrigation, and fish and wildlife. Population growth and increasing demands for water make the availability of that water, measured in terms of quantity and quality, even more essential to the long-term sustainability of our communities and ecosystems.

The USGS implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to support national, regional, State, and local information needs and decisions related to water-quality management and policy (*http://water.usgs.gov/nawqa*). The NAWQA Program is designed to answer: What is the quality of our Nation's streams and groundwater? How are conditions changing over time? How do natural features and human activities affect the quality of streams and groundwater, and where are those effects most pronounced? By combining information on water chemistry, physical characteristics, stream habitat, and aquatic life, the NAWQA Program aims to provide science-based insights for current and emerging water issues and priorities. From 1991 to 2001, the NAWQA Program completed interdisciplinary assessments and established a baseline understanding of water-quality conditions in 51 of the Nation's river basins and aquifers, referred to as Study Units (*http://water.usgs.gov/nawqa/studies/study_units.html*).

National and regional assessments are ongoing in the second decade (2001–2012) of the NAWQA Program as 42 of the 51 Study Units are selectively reassessed. These assessments extend the findings in the Study Units by determining water-quality status and trends at sites that have been consistently monitored for more than a decade, and filling critical gaps in characterizing the quality of surface water and groundwater. For example, increased emphasis has been placed on assessing the quality of source water and finished water associated with many of the Nation's largest community water systems. During the second decade, NAWQA is addressing five national priority topics that build an understanding of how natural features and human activities affect water quality, and establish links between sources of contaminants, the transport of those contaminants through the hydrologic system, and the potential effects of contaminants on humans and aquatic ecosystems. Included are studies on the fate of agricultural chemicals, effects of urbanization on stream ecosystems, bioaccumulation of mercury in stream ecosystems, effects of nutrient enrichment on aquatic ecosystems, and transport of contaminants to public-supply wells. In addition, national syntheses of information on pesticides, volatile organic compounds (VOCs), nutrients, trace elements, and aquatic ecology are continuing.

The USGS aims to disseminate credible, timely, and relevant science information to address practical and effective water-resource management and strategies that protect and restore water quality. We hope this NAWQA publication will provide you with insights and information to meet your needs, and will foster increased citizen awareness and involvement in the protection and restoration of our Nation's waters.

The USGS recognizes that a national assessment by a single program cannot address all water-resource issues of interest. External coordination at all levels is critical for cost-effective management, regulation, and conservation of our Nation's water resources. The NAWQA Program, therefore, depends on advice and information from other agencies—Federal, State, regional, interstate, Tribal, and local—as well as nongovernmental organizations, industry, academia, and other stakeholder groups. Your assistance and suggestions are greatly appreciated.

William H. Werkheiser
USGS Associate Director for Water

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Conversion Factors and Datums

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square meter (m^2)	10.76	square foot (ft^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
milliliter (mL)	0.03382	ounce, fluid (fl. oz)
	Flow rate	
meter per second (m/s)	3.281	foot per second (ft/s)
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
	Mass	
kilogram (kg)	2.205	pound avoirdupois (lb)
	Application rate	
kilogram per square kilometer per year [(kg/km^2)/yr]	0.008921	pounds per acre per year [(lb/acre)/yr]

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Altitude, as used in this report, refers to distance above the vertical datum.

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L). Concentrations of benthic algal biomass are given either in milligrams per square meter (mg/m^2) or grams per square meter (g/m^2).

Symbols, Abbreviations, and Acronyms

<	less than
ρ	Spearman correlation coefficient; rho
E	estimated
GIS	geographic information system
LRL	laboratory reporting level
LT-MDL	long-term method detection level
MDL	method detection limit
MRL	minimum reporting level
NAWQA	National Water-Quality Assessment
NEET	Nutrient Enrichment Effects Team
NWQL	National Water Quality Laboratory
OZRK	Ozark Plateaus
p-value	probability value
PVC	polyvinyl chloride
UMIS	Upper Mississippi River Basin
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
USNK	Upper Snake River Basin

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Abstract

The U.S. Geological Survey determined seasonal variability in nutrients, carbon, and algal biomass in 22 wadeable streams over a 1-year period during 2007 or 2008 within three geographically distinct areas in the United States. The three areas are the Upper Mississippi River Basin (UMIS) in Minnesota, the Ozark Plateaus (ORZK) in southern Missouri and northern Arkansas, and the Upper Snake River Basin (USNK) in southern Idaho. Seasonal patterns in some constituent concentrations and algal responses were distinct. Nitrate concentrations were greatest during the winter in all study areas potentially because of a reduction in denitrification rates and algal uptake during the winter, along with reduced surface runoff. Decreases in nitrate concentrations during the spring and summer at most stream sites coincided with increased streamflow during the snowmelt runoff or spring storms indicating dilution. The continued decrease in nitrate concentrations during summer potentially is because of a reduction in nitrate inputs (from decreased surface runoff) or increases in biological uptake. In contrast to nitrate concentrations, ammonia concentrations varied among study areas. Ammonia concentration trends were similar at UMIS and USNK sampling sites with winter peak concentrations and rapid decreases in ammonia concentrations by spring or early summer. In contrast, ammonia concentrations at OZRK sampling sites were more variable with peak concentrations later in the year. Ammonia may accumulate in stream water in the winter under ice and snow cover at the UMIS and USNK sites because of limited algal metabolism and increased mineralization of decaying organic matter under reducing conditions within stream bottom sediments. Phosphorus concentration patterns and the type of phosphorus present changes with changing hydrologic conditions and seasons and varied among study areas. Orthophosphate concentrations tended to be greater in the summer at UMIS sites, whereas total phosphorus concentrations at most UMIS and USNK sites peaked in the spring during runoff and then decreased through the remainder of the sampling period. Total phosphorus and orthophosphate concentrations in OZRK streams peaked during summer indicating a runoff-based source of both nutrients. Orthophosphate concentrations may increase in streams in the late summer when surface runoff composes less of total streamflow, and when groundwater containing orthophosphate becomes a more dominant source in streams during lower flows.

Seston chlorophyll *a* concentrations were greatest early in the growing season (spring), whereas the spring runoff events coincided with reductions in benthic algal chlorophyll *a* biomass likely because of scour of benthic algae from the channel bottom that are entrained in the water column during that period. Nitrate, ammonia, and orthophosphate concentrations also decreased during that same period, indicating dilution in the spring during runoff events.

The data from this study indicate that the source of water (surface runoff or groundwater) to a stream and the intensity of major runoff events are important factors controlling in-stream concentrations. Biological processes appear to affect nutrient concentrations during more stable lower flow periods in later summer, fall, and winter when residence time of water in a channel is longer, which allows more time for biological uptake and transformations. Management of nutrient conditions in streams is challenging and requires an understanding of multiple factors that affect in-stream nutrient concentrations and biological uptake and growth.

Introduction

Understanding how and why nutrient concentrations are changing with time in streams and rivers is essential for effectively managing and protecting these water resources. Two of the major nutrients of concern are nitrogen and phosphorus. According to the U.S. Environmental Protection Agency (USEPA; 2007), nutrients are the fifth leading pollutant in streams nationally and are the leading pollutant in lakes and reservoirs. The USEPA Wadeable Streams Assessment

identifies nitrogen and phosphorus as the two most widespread stressors contributing to diminished biological quality in flowing waters across the Nation (U.S. Environmental Protection Agency, 2006).

Nitrogen and phosphorus are essential for the development and maintenance of aquatic food webs. Nitrogen and phosphorus occur in multiple forms in streams (table 1). Algae are the base of this food web and include photosynthetic nonvascular aquatic plants (for example, diatoms, green algae, and red algae) and photosynthetic bacteria (for example, blue green algae). Vascular plants in aquatic systems grow in or near water and are submergent, emergent, or floating. Algae and aquatic plants convert dissolved inorganic forms of nitrogen (nitrate, nitrite, and ammonium) and phosphorus (orthophosphate) into biomass that higher trophic organisms utilize for food and shelter.

Elevated concentrations of nutrients can lead to excessive growth of algae and plants. High algal and plant biomass can cause large diurnal fluctuations in dissolved oxygen and pH because of production and respiration and can generate excessive decaying organic material during senescence (Welch and others, 1988; Allan, 2004; Mallin and others, 2006). Decaying aquatic algae and plants cause decreased oxygen concentrations in streams because of microbial respiration (Allan, 2004). Decreased oxygen concentrations can be lethal to fish and invertebrates and can affect organism behavior through changes in energy expenditure during respiration leading to reduced growth and potential predation (Nebecker and others, 2004; Kramer, 1987). Excessive algal or plant growth also can lead to reductions in preferred invertebrate and fish habitat, reductions in visibility through increased turbidity for sight predators, and changes in the hydrologic regime of a stream reach as increasing algal and plant biomass slows current velocity (Dodds and Biggs, 2002). In addition, small streams convey a large percentage of nitrogen to main-stem streams. Nitrogen cycling and retention processes are small compared with transport in large main-stem streams so nitrogen is transported downstream (Alexander and others, 2000; Richardson and others, 2004).

Sources of nitrogen and phosphorus to aquatic ecosystems are natural and anthropogenic. Nitrogen and phosphorus can occur naturally in soils, rocks, vegetation, and biota and can be delivered to streams in a variety of ways including microbial fixation of nitrogen in terrestrial and aquatic ecosystems, dissolution of phosphorus-bearing rocks or minerals, and by the decay of biota. Anthropogenic sources include fertilizer runoff, groundwater discharge, point-source discharges, and atmospheric deposition. The sources of nutrients vary geographically across the country partially because of land use. Fertilizer is a dominant source in agricultural areas where extensive row cops are planted, whereas manure is a dominant source in grazing areas (Dubrovsky and others, 2010).

Nitrogen and phosphorus transport to streams is affected by climate, hydrology, biological processing, and landscape characteristics such as geology, geomorphology, soil composition, and land cover (Cirmo and McDonnell, 1997; Fallon and

Table 1. Description of nitrogen and phosphorus forms described in this report (Hem, 1985; Stumm and Morgan, 1996; Wetzel, 2001).

Nutrient	Description
Ammonium	Ammonia is a dissolved inorganic form of nitrogen in streams generated by bacteria as the end product of decomposition of decaying organic matter (mineralization). Ammonia is not stable in most stream environments. It exists as the ionized form (ammonium) in streams with pH values less than 9. It is immobilized as organic material (amino acid synthesis) when assimilated by plants.
Nitrate plus nitrite	Nitrate is a dissolved inorganic form of nitrogen that is highly soluble in water and is stable over a variety of environmental conditions. It is readily transported to groundwater and streams. In this report, nitrate refers to nitrate plus nitrite because nitrite concentrations generally were negligible.
Organic nitrogen	Organic nitrogen includes particulate and dissolved forms. Particulate nitrogen is the organic nitrogen incorporated in algal cells and coarse organic matter entrained in the water column. Dissolved organic matter is not a single compound but a mixture of compounds ranging from simple amino acids to complex humic substances. Organic nitrogen can originate within the stream (autochthonous) from the growth and decay of algae and plants or from sources outside the stream (allochthonous) such as atmospheric deposition and land-surface runoff.
Total nitrogen	Total nitrogen is a measure of all forms of organic and inorganic nitrogen including ammonia, nitrate, nitrite, and organic nitrogen.
Orthophosphate	Most of the dissolved phosphorus in streams is in the form of orthophosphate, which is moderately soluble relative to nitrate. Algae can readily utilize orthophosphate by converting it to particulate and dissolved organic phosphorus.
Total phosphorus	Total phosphorus is a measure of dissolved and particulate phosphorus including live or decaying algal cells and mineral phases that usually are bound to sediment. Erosion can transport considerable amounts of particulate phosphorus into streams, and much of the phosphorus is bound to sediment in streams.

McNellis, 2000; Burt and Pinay, 2005; Demars and Edwards, 2007). Dominant hydrologic characteristics, such as runoff or groundwater discharge, and soil properties in a watershed have a substantial effect on nutrients delivered to streams (Green, Fisher, and Bekins, 2008; Green, Puckett, and others, 2008; Tesoriero and others, 2009). In temperate areas with seasonal snow and ice cover, spring snowmelt transports nutrients that have accumulated during the winter. As much as 50 percent of the nitrogen and 20 percent of the phosphorus applied to the landscape annually is delivered to streams through runoff from land surfaces during snowmelt and precipitation events (Mueller and Spahr, 2006).

Nitrogen and phosphorus are expected to have different transport pathways. Ammonia is sorbed to soil and does not transport from soils easily. Because nitrate does not sorb to soil particles or aquifer sediments, nitrate enters streams through runoff and through groundwater sources. Particulate phosphorus typically is sorbed to soils and enters streams as particulates carried by runoff from areas with phosphorus-rich soils or where phosphorus has been applied to soils (Hem, 1985; Gburek and Sharpley, 1998). Orthophosphate, in contrast, can enter streams through shallow groundwater (Tesoriero and others, 2009).

Once in a stream or lake, nutrients can be deposited within sediments, utilized as nutrients by biota, exported through streamflow from the watershed, leached to groundwater, or lost through processes such as volatilization and denitrification. Factors that affect algae and plant uptake and metabolism include hydrologic disturbance, light availability, stream temperature, and removal of algae through grazing organisms (Biggs and Kilroy, 2000).

Nutrient concentrations vary seasonally in streams because of changes in nutrient sources, climate, hydrology, residence time, and aquatic algae and plant uptake. Seasonal patterns vary regionally and locally. Dubrovsky and others (2010) reported that phosphorus concentrations in streams in the western United States were greater during snowmelt, whereas the greatest nitrogen concentrations occurred during the winter when streamflow was lower; this is in contrast to most streams in the eastern United States. Management of nutrient conditions in streams is a challenge that requires an understanding of the physical, chemical, and biological factors that affect in-stream nutrient concentrations.

Study Objectives

In 1987, the U.S. Geological Survey (USGS) began a study of more than 50 river basins and aquifers across the Nation as part of the National Water-Quality Assessment (NAWQA) Program. As part of the USGS NAWQA Program, a Nutrient Enrichment Effects Team (NEET) was implemented in 2001 (Brightbill and Munn, 2008). A major goal was to assess nutrient-biota interactions in agriculturally affected streams in different geographic regions of the United States. Three geographically distinct NAWQA study areas were selected to address temporal patterns of nutrient concentrations and algal responses among agricultural streams in different environmental settings: the Upper Mississippi River Basin (UMIS), the Ozark Plateaus (OZRK), and Upper Snake River Basin (USNK). The objectives of the study described in this report were (1) to determine seasonal patterns in nutrients, carbon, and algal responses; and (2) to determine the effect of geographic location and associated physical characteristics on seasonal patterns.

Purpose and Scope

The purpose of this report is to describe seasonal patterns in nutrients, carbon, and algal response variables for the 22 sites sampled monthly for 1 year during 2007 or 2008 and to describe how relations between nutrients and biological responses vary seasonally. Samples were collected during 2007 in the OZRK study area and during 2008 in the UMIS and USNK study areas. The scope of this report is limited to describing the seasonal pattern trends observed during the 1 year of sampling in each of the three NAWQA study areas.

Study Design and Approach

The NAWQA NEET (*http://wa.water.usgs.gov/neet/*) assessed nutrient-biota interactions in eight NAWQA study areas by sampling multiple sites (approximately 30) within each study area during a single growing season. This present study described in this report was done during 2007 and 2008 to address the temporal component of nutrient-biota interactions in three of the study areas: UMIS, OZRK, and USNK (fig. 1). Each selected study area contained a variety of land cover, agricultural land uses, and nutrient characteristics. The UMIS study area (fig. 1) drains Minnesota, Wisconsin, and small parts of Iowa, North Dakota, and South Dakota, from the headwaters of the Mississippi River in the northern part of the area to its confluence with the St. Croix River. The Ozark Plateaus study area includes parts of Arkansas, Kansas, Missouri, and Oklahoma (Petersen and others, 1998; Adamski and others, 1995). The USNK study area is located in southeastern Idaho and northwestern Wyoming and includes small parts of Nevada and Utah.

Sampling sites within each study area were selected using a targeted design to include sites with high, medium, and low nutrient concentrations (Brightbill and Munn, 2008). A combination of geographic information system (GIS) methods, field reconnaissance, and monitoring data were used to select sites. For the seasonal study described within this report, 7 sites were selected within each of the OZRK and UMIS study areas, and 8 sites were selected in USNK study area, for a total of 22 sites (table 2). Sampling sites in the UMIS and OZRK study areas have drainage areas ranging from 75 to 340 square kilometers (km^2); sampling sites in USNK study area have drainage areas ranging from 21 to 2,002 km^2 (table 2).

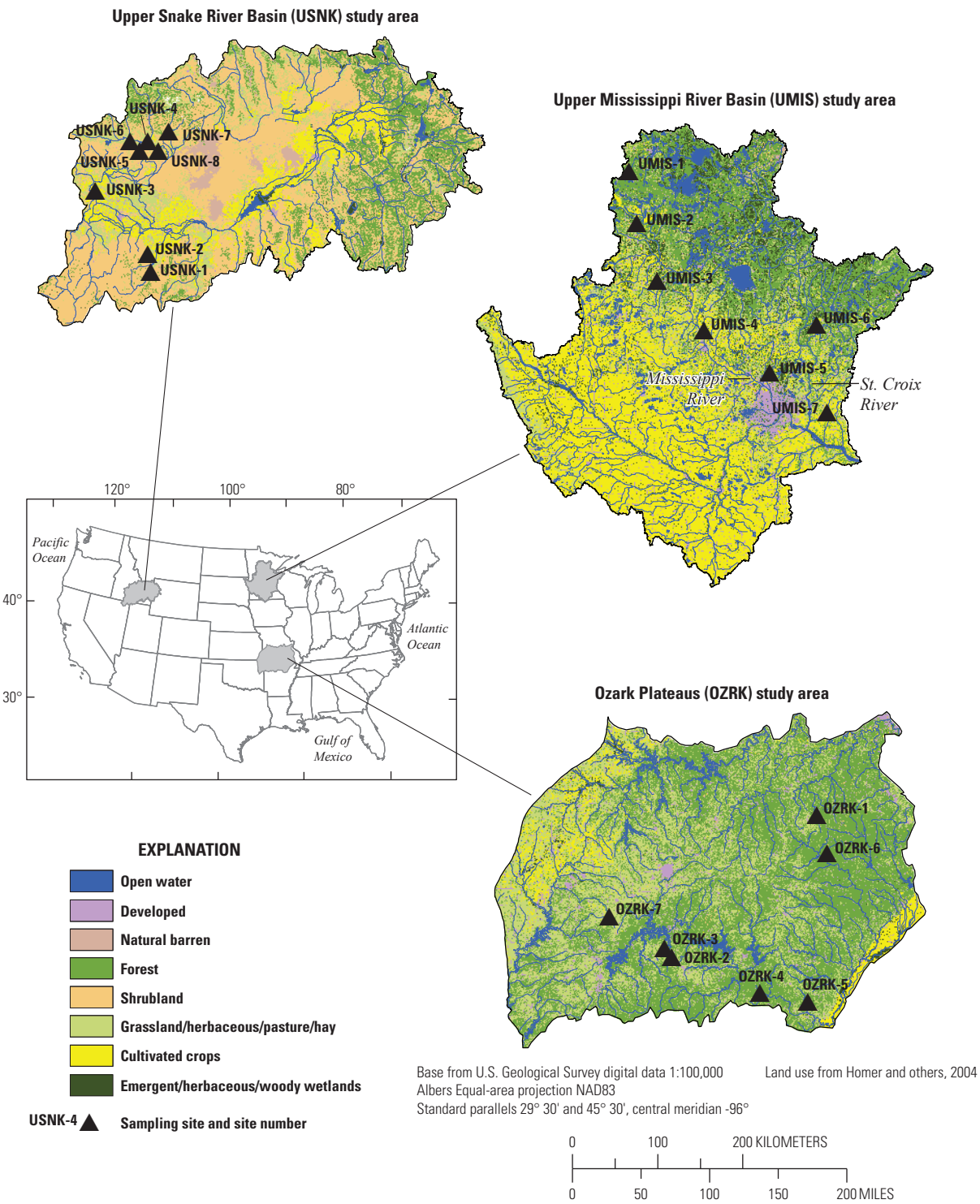

Figure 1. Location and land use of study areas and sampling sites.

Table 2. Characteristics of sites sampled during 2007 or 2008.

[USGS, U.S. Geological Survey; km², square kilometers; NAVD 88, North American Vertical Datum of 1988. Samples were collected during 2007 in the OZRK study area and during 2008 in the UMIS and USNK study areas]

USGS site number (shown on fig. 1)	USGS station identification number	USGS station name	Latitude	Longitude	Drainage area (km²)	Altitude (meters above NAVD 88)
Upper Mississippi River Basin (UMIS) study area						
UMIS-1	05200170	Mississippi River near Vern, Minn.	471932	0951330	199	481
UMIS-2	05243200	Shell River near Horton, Minn.	464851	0950717	277	472
UMIS-3	05245295	Moran Creek near Staples, Minn.	461510	0945030	177	405
UMIS-4	05268700	Little Rock Creek at Rice, Minn.	454548	0941215	174	354
UMIS-5	05286297	Cedar Creek near East Bethel, Minn.	451958	0931803	159	280
UMIS-6	05338955	Wood River at North Williams Road near Grantsburg, Minn.	454707	0923752	340	309
UMIS-7	05341854	Kinnickinnic River at Steeple Drive near Hammond, Wis.	445522	0923154	136	325
Ozark Plateaus (OZRK) study area						
OZRK-1	07010335	Meramec River above Cook Station, Mo.	374119	0912530	243	377
OZRK-2	07053203	Long Creek southeast of Denver, Ark.	362150	0931613	255	457
OZRK-3	07053250	Yocum Creek near Oak Grove, Ark.	362715	0932121	136	394
OZRK-4	07060710	North Sylamore Creek near Fifty Six, Ark.	355930	0921250	151	385
OZRK-5	07060894	Sullivan Creek near Sandtown, Ark.	355315	0913829	75	182
OZRK-6	07065040	Big Creek at Mauser Mill, Mo.	371847	0911900	108	343
OZRK-7	07186670	Shoal Creek near Wheaton, Mo.	364637	0940126	112	439
Upper Snake River Basin (USNK) study area						
USNK-1	13082500	Goose Creek above Trapper Creek Near Oakley, Id.	420734	1135608	1,640	1,840
USNK-2	13088510	Cottonwood Creek near Oakley, Id.	421739	1140118	80	2,041
USNK-3	13134640	Billingsley Creek below Vader Grade near Hagerman, Id.	424754	1145203	204	1,087
USNK-4	13140800	Big Wood River at Stanton Crossing near Bellevue, Id.	431945	1141909	2,002	2,251
USNK-5	13140900	Willow Creek near Spring Creek Ranch near Bellevue, Id.	431922	1141927	21	1,527
USNK-6	13141500	Camas Creek near Blaine, Id.	431958	1143231	1,638	1,713
USNK-7	13147900	Little Wood River above High Five Creek near Carey, Id.	433122	1141827	645	2,181
USNK-8	13150200	Stalker Creek near Gannett, Id.	431844	1141058	39	1,535

Field Collection and Laboratory Analytical Methods

Continuous hydrologic conditions (streamflow and stage), field properties (dissolved oxygen, water temperature, specific conductance, turbidity, and pH), water chemistry (nutrients, carbon, suspended sediment, and alkalinity), water column algae (sestonic algal chlorophyll *a*), benthic algae (benthic algal chlorophyll *a,* ash-free dry mass, and identification), macrophyte/attached algal cover, in-stream and near-stream (riparian) physical habitat characteristics, and basin characteristics were collected or measured at all 22 sites. Samples were collected during 2007 in the OZRK study area and during 2008 in the UMIS and USNK study areas.

Hydrologic and Water Temperature Measurements

Streamflow and water temperature were measured following USGS protocols (Rantz and others, 1982a, 1982b; Turnipseed and Sauer, 2010) at each site during each sampling event. Stream sensors were used to record stage and temperture at 15-minute intervals at each location. Transducers were installed inside polyvinyl chloride (PVC) pipes that were anchored to the bank and streambeds with fence posts or mounted to bridge piers. An arbitrary datum was obtained for each site using USGS surveying equipment and methods described in Kennedy (1990).

Water Sampling and Analyses

Water samples were collected during 2007 and 2008 at approximately monthly intervals beginning in February and ending in November at each of the 22 sampling sites. A full suite of field properties, water chemistry samples, and sestonic and benthic algae samples was collected during each sampling event.

Field properties of dissolved oxygen, water temperature, specific conductance, turbidity, and pH were measured in the field at the time of sampling using a multiparameter meter. The meter was calibrated according to U.S. Geological Survey (variously dated) and manufacturer's specifications before and after sampling to ensure accurate measurements.

Water samples were collected using integrated width-and depth-sampling techniques to ensure that the samples collected were representative of water flowing in the entire stream cross section (Edwards and Glysson, 1988; U.S. Geological Survey, variously dated). All water samples were collected by use of the equal-width-increment method with a hand-held DH-59 depth-integrating sampler (Edwards and Glysson, 1988), except when stream conditions were not appropriate; that is, stream velocity was less than approximately 0.45 meter per second (m/s), or maximum depth was less than 0.15 meter (m). For either of these circumstances, a grab sample was collected with an open bottle at the center of the flow. Samples were then split using a churn splitter into appropriate bottles for laboratory analysis and analyzed for constituents listed in table 3.

Samples to be analyzed for dissolved constituents (ammonia, nitrate plus nitrite, and orthophosphate) were filtered in the field through a 0.45-micrometer glass fiber filter, chilled and maintained at 4 degrees Celsius (°C), and immediately shipped to the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colo. At the NWQL, samples were analyzed according to methods in Fishman (1993) and Patton and Kryskalla (2003). Unfiltered total nitrogen (ammonia + nitrite + nitrate + organic nitrogen) samples were acidified in the field with 1 milliliter (mL) of 4.5 normality sulfuric acid, chilled and maintained at 4°C, and immediately shipped to the NWQL for analyses according to Patton and Kryskalla (2003). Unfiltered total phosphorus samples were acidified in the field with 1 mL of 4.5 normality sulfuric acid, chilled and maintained at 4°C, and immediately shipped to the NWQL for analyses according to to the U.S. Environmental Protection Agency (1993).

Seston algae also were sampled from one of the splits obtained with the churn splitter. For seston algae collection, a water sample was filtered through a 47-millimeter (mm) glass fiber filter. The filter was folded into quarters, wrapped in aluminum foil, placed in a labeled petri dish, placed in a plastic bag, and frozen on dry ice for shipment to NWQL (Moulton and others, 2002). Chlorophyll *a* was analyzed by the NWQL using protocols outlined in Arar and Collins (1997).

Samples for analysis of dissolved organic carbon were filtered using a SUPOR filter. The filtered water sample was placed in a 125-mL amber glass bottle. The sample was acidified to a pH of less than 2 with sulfuric acid, chilled and maintained at 4°C, and immediately shipped to the NWQL for analysis (Brenton and Arnett, 1993).

Water was filtered through 25-mm glass fiber filters for analysis of particulate organic carbon and total particulate nitrogen. These filters were folded in half, wrapped in aluminum foil, placed in a labeled Petri dish, placed in bags, chilled and maintained at 4°C, and immediately shipped to the NWQL for analysis. Laboratory analysis was completed according to guidelines as stated in the Office of Water Quality Technical Memorandum 2000.08 (U.S. Geological Survey, 2000).

Water samples from the churn splitter also were collected for suspended-sediment analysis. Concentrations of suspended sediments were analyzed at USGS sediment laboratories according to methods described by Guy (1969) and the American Society for Testing and Materials (2007).

Benthic Algal Sample Collection and Laboratory Analyses

Benthic algal samples were collected during all sampling periods except during winter ice cover and during spring high-flow conditions. Samples were collected from five locations at each site throughout the sampling reach and composited for each sampling date. Samples were collected from gravel substrate in UMIS streams and from cobble substrate in OZRK and USNK streams using protocols outlined in Moulton and others (2002). Each benthic algal sample was split into aliquots for identification and enumeration, measurement of chlorophyll *a*, and ash-free dry mass determinations. Samples collected at UMIS sites were processed to remove benthic algae from coarse substrate by a series of elutriations. For the elutriations, approximately 100 mL of tap water was poured into a container with the sample, and the slurry was stirred to loosen algae from the substrate. The sample slurry was poured through a 500-micrometer sieve at least three times to catch the bed material, and an additional rinse with tap water was used as a last rinse. The algal-water mixture was decanted into a clean 1-liter (L) plastic container, taking care

Table 3. Constituents analyzed in stream samples, detection limits, sample medium, and analytical method references.

[mg/L, milligrams per liter; --, not applicable; µg/L, micrograms per liter; USEPA, U.S. Environmental Protection Agency; mg/m^2, milligrams per square meter; g/m^2, grams per square meter]

Constituent	Method detection limit	Sample medium	Analytical method
Ammonia as nitrogen, dissolved	0.01 mg/L	Filtered water	Patton and Kryskalla, 2003; Fishman, 1993
Nitrate plus nitrite, as nitrogen, dissolved[1]	.008 mg/L	Filtered water	Fishman, 1993
Total particulate nitrogen	.02 mg/L	On filter	U.S. Geological Survey, 2000
Total nitrogen	.008 mg/L	Whole water	Patton and Kryskalla, 2003
Orthophosphate as phosphorus, dissolved	.003 mg/L	Filtered water	Fishman, 1993
Total phosphorus	.008 mg/L	Whole water	U.S. Environmental Protection Agency, 1993
Particulate organic carbon	.12 mg/L	On filter	U.S. Geological Survey, 2000
Dissolved organic carbon	.2 mg/L	Filtered water	Brenton and Arnett, 1993
Suspended sediment	--	Whole water	Guy, 1969; American Society for Testing and Materials, 2007
Seston chlorophyll *a*	.1 µg/L	On filter	USEPA 445.0; Arar and Collins, 1997
Benthic algal chlorophyll *a* biomass	.1 mg/m^2	On filter	USEPA 445.0; Arar and Collins, 1997
Benthic algal biomass, ash-free dry mass	.1 g/m^2	On filter	Britton and Greeson, 1997
Benthic algal identification and enumeration	--	Bottom substrate	Charles and others, 2002

[1]Referred to as nitrate throughout the report.

not to introduce sand into the clean container. Benthic algal samples at OZRK and USNK sites were processed by placing a PVC tube of a known diameter on the cobble, removing and discarding all algal growth around the tube with wire brushes, and then removing the algal growth within the tube and washing it into a 1-L sample container. The elutriated sample was then homogenized by shaking the algal-water mixture in the 1-L container, and then a 2- to 5-mL subsample was withdrawn from the mixture using a syringe and filtered for analysis of chlorophyll *a* concentrations and ash-free dry mass calculations as described in Moulton and others (2002). If relatively few solids were present on the filter surface, then the filtering process was repeated until a thin, pigmented film was deposited on the filter. The filter was then removed and processed as described in Moulton and others (2002), frozen on dry ice, and sent to the NWQL for analysis (Arar and Collins, 1997; Britton and Greeson, 1987). During three of the sampling periods (spring, summer, and fall), a sample was preserved with 4 percent formalin and transferred to the Academy of Natural Sciences of Philadelphia for identification and enumeration processing (Charles and others, 2002).

Basin and Near-Stream Characteristics

Digital datasets were aggregated by the NAWQA GIS team (Naomi Nakagaki, U.S. Geological Survey, written commun., 2008). Basin characteristics (landscape characteristics, which include ecoregions, drainage area, altitude, and geology; land use; nutrient use; climatic characteristics; land management and soil characteristics) were calculated for each site using a nationally consistent approach described in Brightbill and Frankforter (2010). Near-stream characteristics (for example, riparian land use/land cover and channel shading) were determined at the reach according to Johnson and Zelt (2005).

In-Stream Physical Characteristics

Stream physical habitat data were collected three times (spring, summer, and fall) along each sampling reach at 11 equidistant transects oriented perpendicular to streamflow and established throughout the reach according to Fitzpatrick and others (1998). Wetted channel width and bankful width were measured at each transect. Water depth, water velocity, and substrate size (bedrock, boulder, cobble, gravel, sand, and silt) were characterized at three to five points across each transect. Channel shading was determined with a concave spherical densitometer at 30 centimeters (cm) above the water surface at the water's edge along both sides of the stream and in the center of the channel at each transect. At five points along each of the 11 transects, the percentage of submerged macrophytes or filamentous macroalgae cover or the presence of both was determined according to a modification of Biggs and Kilroy (2000). A 0.09-square-meter (m^2) quadrat (a measured and marked rectangle used to isolate a sampling area for the purpose of counting the population of different species in that area) was placed at each sampling point. The cover of macrophytes or filamentous algae greater than 3 cm in length was estimated to the nearest 10 percent. These five values along the 11 transects were then averaged to obtain an estimate of the mean percentage of cover by a combination of macroalgae and macrophytes for the site. In addition, channel width, channel shading, and macrophyte cover were measured during each sampling visit at the UMIS and USNK sites and during selected sampling dates at the OZRK sites.

Data Analyses Methods

The initial datasets for nutrient, carbon, and algal concentrations were extracted from the USGS NAWQA Data Warehouse (*http://water.usgs.gov/nawqa/data)*. The datasets went through a series of quality-assurance steps including verification of correct dates, times, and station locations.

Data Preparation

Proper censoring levels were determined before data analyses. Censored data (less-than values) can present challenges to analyses if there are multiple censoring levels or if a large percentage of the data are censored. Within this study's dataset, these conditions were encountered for several of the constituents. For example, 31 percent of the ammonia concentrations for samples collected within the UMIS study area were censored, whereas more than 50 percent were censored in samples from the USNK study area (table 4).

Two different types of reporting levels were used by the NWQL for data used in this report. Currently (2010), the NWQL uses the laboratory reporting level (LRL) for most constituents. The LRL protects against false negatives (reporting a value as a censored value when its concentration is actually greater than the stated value). The LRL also protects against false positives (reporting a value without a censoring indicator when the actual concentration is zero). The LRL corresponds to the quantitation limit terminology proposed by the USEPA (U.S. Environmental Protection Agency, 2004). In conjunction with reporting censored values at the LRL, concentrations measured at less than the method detection limit (MDL) are reported as a value with an associated "E" (estimated) remark code by the NWQL. The NWQL previously used the minimum reporting level (MRL) that protects only against false positives and corresponds to the detection limit terminology in U.S. Environmental Protection Agency (2004). No special E-coded values are used in conjunction with the MRL. The reporting for field-determined values is similar to the use of MRLs by the NWQL.

The use of the LRL and associated E-coded values can lead to an unintentional bias when interpreting data (Helsel, 2005). Helsel (2005) described three methods to deal with unintentional bias; the first method was used in the data analyses for this report. For this method, the first step was to determine the reporting level type. All of the censoring values in the original dataset were checked to determine what type of reporting level (LRL or MRL) was listed in the initial file. All LRLs in the initial dataset were recoded as less than (<) the long-term method detection level (LT-MDL) for the respective detection limit. All other reporting levels were retained as in the original retrieved data because they represent the detection limit. These recoded data were used for summary statistics using left-censored methods as needed. The method of simple substitution was used when analyzing the data for general trends (loess and Spearman correlations). The value used for substitution was one-half of the detection limit or the median of all values less than the detection limit for elevated detection limits.

Seasonal Comparisons within Study Areas

The wide range in nutrient and chlorophyll *a* concentrations among different sites made it difficult to discern seasonal patterns within a study area. Therefore, concentrations used for comparison among seasons within a study area were normalized to better accentuate seasonal patterns. Concentrations of a constituent were normalized at each site to highlight the concentration of a constituent during one sampling date relative to the mean concentrations for that constituent at the site over the whole sampling period according to the following equation:

$$\textit{Normalized value} = \frac{\textit{Concentration for one sampling date}}{\textit{Mean concentration over whole sampling period}}$$

This process was useful to show how the concentrations within a particular season deviated from the mean concentrations at a site, therefore highlighting the seasonal trend. A Kruskal-Wallis non-parametric test (Helsel and Hirsch, 2002)

Table 4. Summary of censored data.

[All values in milligrams per liter (mg/L), except for seston chlorophyll *a*, which is in micrograms per liter. OZRK, Ozark Plateaus; UMIS, Upper Mississippi River Basin; USNK, Upper Snake River Basin; <, less than; NA, not applicable]

Constituent	UMIS		OZRK		USNK		Substitution value
	Censoring values	Fraction censored	Censoring values	Fraction censored	Censoring values	Fraction censored	
Ammonia as nitrogen, dissolved	0.01	19 / 61	0.01	24 / 63	0.01	45 / 70	0.005
	NA	NA	NA	NA	.002	1 / 70	[1].0013
Nitrate plus nitrate as nitrogen, dissolved	.008	9 / 57	NA	0 / 62	.008	17 / 70	[2].004
Total particulate nitrogen	.02	2 / 61	.02	7 / 63	.02	8 / 70	[3].011
	NA	NA	.022	33 / 63	.01	1 / 70	[3].011
Total nitrogen	NA	0 / 61	NA	0 / 63	NA	0 / 70	None
Orthophosphate as phosphorus, dissolved	NA	0 / 61	.003	9 / 63	.003	4 / 70	.0015
Total phosphorus	NA	0 / 61	.008	20 / 63	NA	0 / 70	.004
Particulate organic carbon	NA	0 / 61	.12	34 / 63	.12	4 / 70	.06
Dissolved organic carbon	NA	0 / 61	NA	0 / 63	NA	0 / 70	None
Seston chlorophyll *a*	NA	0 / 61	.1	2 / 63	NA	0 / 70	.05
Benthic algal chlorophyll *a* biomass	NA	0 / 47	NA	0 / 63	NA	0 / 70	None
Benthic algal biomass, ash-free dry mass	NA	0 / 47	NA	0 / 63	NA	0 / 70	None

[1]The median value for all of the data (0.0013 mg/L) less than the censored value for site USNK-5 (02/21/2008 at 9:30 AM) was used as the substitution value.

[2] A substitution value of <0.008 mg/L was used for site USNK-2 (9/23/2008 at 1455 PM) because a different analytical method was used that had greater detection limits for this sample. Three missing values at site UMIS-7 were estimated as 97 percent of the total nitrogen concentration.

[3] If the value for this constituent was less than 0.022 mg/L or censored, then 0.011 mg/L was used as the substitution value.

followed with a multiple comparison test on ranks (Helsel and Hirsch, 2002) was used to determine differences among seasons within a study area. Differences were considered to be statistically significant at probability values (p-values) less than (<) 0.05. The p-value is a measure of the confidence that what is observed in the sample is true for the population. Loess-smoothed plots (Tibco Spotfire S+8.1, 2008) of the concentrations at individual sites also are presented to demonstrate the variability in seasonal patterns within each study area.

Correlation Analyses

Spearman rank correlation (Helsel and Hirsch, 2002) was used to describe the strength of the relation between selected variables. The strength of the relation is reported as the value for rho (ρ); a hypothesis test was performed, and a critical value was established at a p-value of <0.05.

Principal Components Analyses

Multivariate principal components analyses (Shaw, 2003) were used to characterize or determine site groupings based on physical characteristics. Principal components analysis is a standard technique for finding optimal linear combinations of the variables. The accumulative variance explained by each principal component and the loading for each variable on each principal component is reported.

Basin, Near-Stream, and In-Stream Characteristics Among Study Areas

This section briefly describes the basin-level (landscape characteristics, which include drainage area, basin altitude, and soils; land use, which includes water use; nutrient use, which includes nitrogen and phosphorus sources; and climate) and the near- or in-stream (hydrology, temperature, in-stream habitat, and riparian) characteristics among study areas. The derivation of these characteristics is detailed in Brightbill and Frankforter (2010). These characteristics affect sources of nutrients to streams and within stream processing and may affect the seasonal patterns of nutrients in streams.

Landscape Characteristics

The UMIS sites were located primarily within the Northern Lakes and Forests, and Northern Central Hardwood Forests, with one site, UMIS-7, partially within the Western Cornbelt Plains ecoregions (U.S. Environmental Protection Agency, 2009). The landscape in this area developed through

a series of glaciations and retreating ice sheets that deposited a complex pattern of moraines, outwash plains, drumlins, and lake plains (U.S. Department of Agriculture, 2006; Stark and others, 1996). Glacial deposits have a mixed provenance, with intermixtures of sandy deposits sourced from Precambrian igneous and metamorphic rocks, and more clay-rich, calcareous glacial deposits sourced from carbonate and shale bedrock (Ruhl, 1987; Grimley, 2000). Slopes are nearly level to gently undulating, and the altitudes of sites range from 280 to 481 m above the North American Vertical Datum of 1988 (NAVD 88; U.S. Geological Survey, 2003; table 2). Soils in the drainage basins upstream from the sites are well drained and predominantly composed of sand-sized particles followed by silt- and clay-sized particles (table S1).

The OZRK sites are within the Ozark Highlands ecoregion (U.S. Environmental Protection Agency, 2009). Basement igneous rocks of Precambrian age are overlain by as much as 1,500 m of gently dipping sedimentary rocks throughout much of the area (Adamski and others, 1995). The igneous and sedimentary rocks that underlie the OZRK study area are extensively fractured, and karst features, including caves, springs, and spring-fed streams, are common. Topography in the OZRK study area is mostly gently rolling and land-surface altitudes at sampling sites range from 182 to 457 m above NAVD 88 (U.S. Geological Survey, 2003; table 2). Soils in the drainage basins upstream from the sites are predominantly composed of silt- and clay-sized particles followed by sand-sized particles (table S1).

The USNK sites are within the Snake River Plain and Central Basin and Range ecoregions (U.S. Environmental Protection Agency, 2009) and are characterized by alluvial fans, plateaus, buttes, and scattered mountains. The surficial geology of this area is primarily composed of Columbia River basalts and silicic volcanic and plutonic rocks of the Idaho Batholith (Maupin, 1995; U.S. Department of Agriculture, 2006). Altitudes at sampling sites range from 1,087 to 2,251 m above NAVD 88 (U.S. Geological Survey, 2003) at the sites (table 2). Most soils at the sampling sites generally are well drained and composed primarily of silt- and sand-sized particles (table S1).

Land Use

Land use estimated for 2001 varies among and within the drainage areas upstream from the sites in the three study areas (table S2). In the UMIS study area, land use/land cover is primarily a mosaic of wet forested areas with coniferous species and dry forested prairie areas with maple/basswood forests (Fandrei and others, 1988) and cultivated cropland. The percentage of cultivated croplands upstream from UMIS sites varied from less than 1 to 61.5 percent and consists primarily of corn and soybeans. Among the three study areas, the UMIS study area has the highest percentage of cultivated cropland, wetlands, and open water (table S2).

Forest cover was the dominant land use followed by pasture and hay in the drainage basins upstream from sampling sites in OZRK study area (table S2). Common tree species within the forested areas are oak, eastern red cedar, hickory, and shortleaf pine (Adamski and others, 1995). Most of the agricultural land in the OZRK study area is pastureland associated with production of poultry and cattle. Pastureland in areas with gentle slopes primarily is fescue. Other open areas have warm-season grasses such as big bluestem, Indian grass, little bluestem, and dropseeds (U.S. Department of Agriculture, 2006). The southwestern part of the study area has many large poultry farms (Adamski and others, 1995).

Land use in the USNK study area is primarily rangeland that supports shrub-grassland vegetation (table S2) characterized by big sagebrush or low sagebrush and by blue bunch wheatgrass, western wheatgrass, or Idaho fescue (U.S. Department of Agriculture, 2006). The primary agriculture in the study area is irrigated agriculture for hay production in the Snake River Plain and grazing (Maupin, 1995). A large percentage of the alluvial valleys bordering the Snake River are in agriculture, with sugar beets, potatoes, and vegetables being the principal crops (Maupin,1995). Grazing, cattle feedlots, dairy operations, and fish hatcheries also are common in the river plain (Maupin, 1995).

Water-use practices such as irrigation varied among the study areas. The area in each basin with irrigation source wells during 1997 (Brightbill and Frankforter, 2010; Nakagaki, 2008) varied among study areas. The area of land with irrigation source wells was least in the OZRK (ranged from 0 to 0.87 km^2 among sites) and UMIS (ranged from 0 to 2 km^2 among sites) study areas and was greatest in the USNK study area (ranged from 0.02 to 54 km^2 among sites).

Nitrogen and Phosphorus Sources

Estimates of the types and amounts of nitrogen and phosphorus used in the drainage basins upstream from the sampling sites varied within and among study areas (fig. 2; table S3). Sources of nitrogen and phosphorus, including atmospheric deposition, farm and nonfarm applications (fertilizer), and manure from confined and unconfined animal feedlots for 2002 (Brightbill and Frankforter, 2010), varied among sites and study areas depending on local land use. The UMIS study area had the greatest median nitrogen use, whereas the OZRK study area had the greatest median phosphorus use. The USNK study area had the lowest median nitrogen and phosphorus use.

Most of the estimated nitrogen and phosphorus use at UMIS sites was from farm applications that occur in both the spring and fall (Minnesota Department of Agriculture, 2010; Kroening and Andrews, 1997) and can be spread on snow in the winter (Fallon and McNellis, 2000). The freeze-thaw cycle in the UMIS study area can release nutrients in the soil to spring streamflow when the snow melts (Honeycutt, 1995). Secondarily, atmospheric deposition is a source of nitrogen in

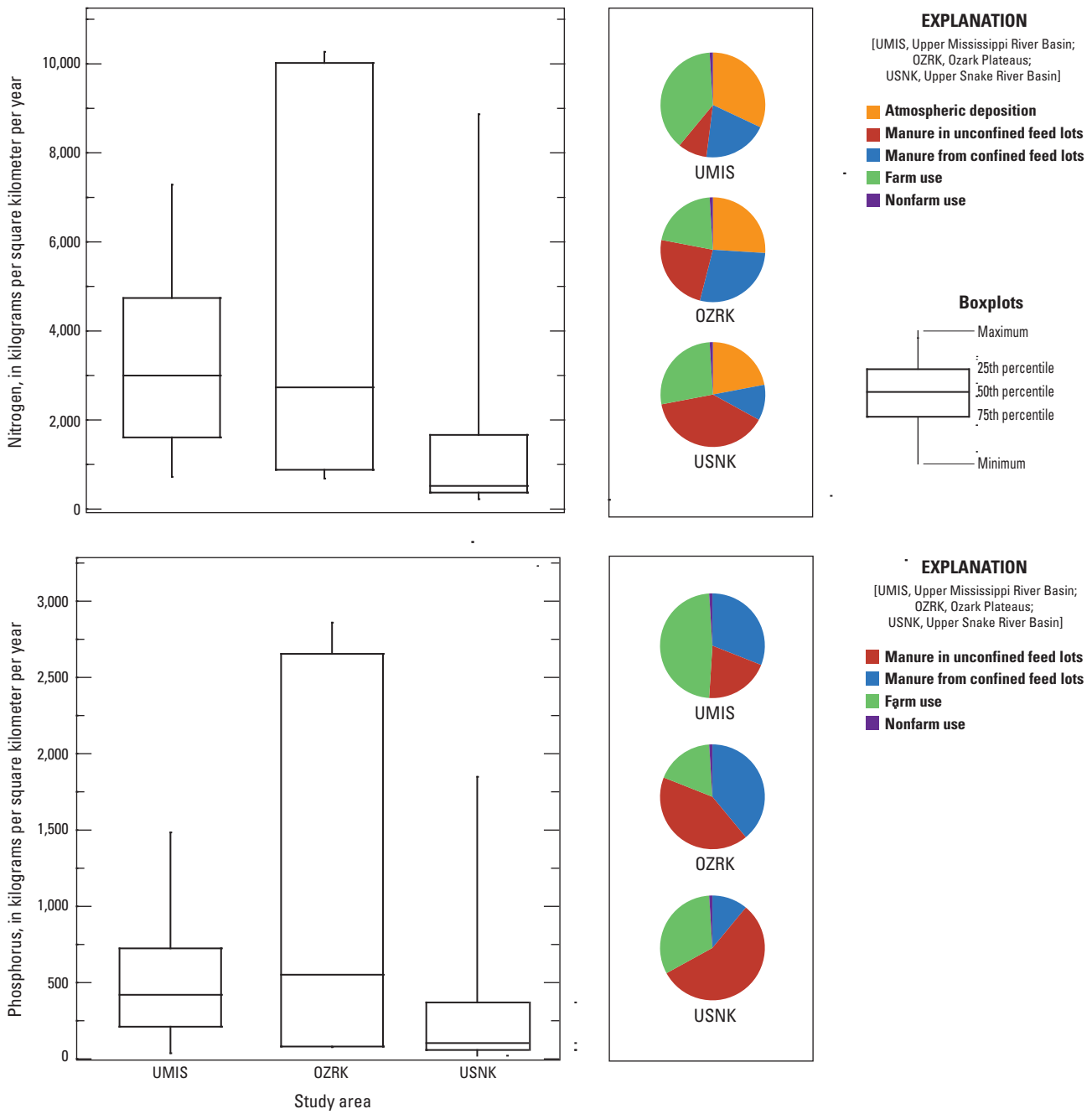

Figure 2. Estimates of nitrogen and phosphorus use during 2002 for each study area and pie diagrams showing the distribution of use among various sources (data from Brightbill and Frankforter, 2010).

the UMIS study area (fig. 2; table S3; Kroening and Andrews, 1997). Nitrogen supplied from precipitation averages 494–1,235 kilograms per square kilometer per year in Minnesota (Buman and others, 2010). Coarse textured soils such as those in the UMIS study area have a low water holding capacity and have a high leaching potential (Buman and others, 2010).

In contrast to the UMIS sites, the OZRK sites had nitrogen and phosphorus sources that primarily were confined and unconfined feedlots, with confined poultry and beef cattle as major sources of nutrient loading in parts of the study area (table S3; Davis and Bell, 1998). Atmospheric deposition also is a source of nitrogen in the OZRK sites. Fertilizer application in the OZRK study area primarily is poultry litter supplemented by commercial fertilizer. Nitrogen and phosphorus content in manure varies by animal, type of manure handling, and animal diet. Poultry manure has the greatest percentage of nitrogen and phosphorus content compared to swine, dairy, and beef (Indiana Cooperative Extension Service, 2010;

Blanchett and Schmidt, 2010). Most of the nitrogen in manure is in the organic form and breaks down to inorganic nitrogen (ammonium) and is transformed to nitrite and nitrate, which are relatively mobile and can leach into groundwater or run off from the land surface (Indiana Cooperative Extension Service, 2010; Blanchett and Schmidt, 2010). Nitrogen and phosphorus sources for the USNK sites primarily were from unconfined feed lots (primarily cattle grazing) and farm application (table S3).

Climate

The UMIS and OZRK study areas have humid climates. The Gulf of Mexico is the main source of moisture for the UMIS and OZRK study areas, and major weather systems normally move from west to east during the fall, winter, and spring seasons (Adamski and others, 1995; Stark and others, 1996). In contrast, the climate in the USNK study area is semiarid and affected predominantly by eastward-moving air masses from the Pacific Ocean (Maupin, 1995). The USNK and UMIS study areas have similar estimated mean annual air temperatures at sampling sites ranging from about 2 to 10°C (table S4), whereas the temperatures in the OZRK study area are much warmer ranging from about 12 to 15°C. The annual pattern of air temperature is similar among all three study areas (fig. 3).

The amount and timing of precipitation varied among study areas. Mean annual precipitation in the drainage basins upstream from the sampling sites was greatest in the OZRK study area (118 to 130 cm), followed by the UMIS study area (69 to 86 cm), and least in the USNK study area (27 to 70 cm) (table S4). In the UMIS study area, most precipitation falls as rain during the 5-month growing season (May through September), and the remainder falls as snow (Stark and others, 1996; U.S. Department of Agriculture, 2006). Winters are cold in the UMIS study area, and substantial amounts of snow can accumulate. Within the OZRK study area, precipitation generally is greatest in the late spring and least in late winter (Adamski and others, 1995; U.S. Department of Agriculture, 2006). The climate in the OZRK study area is characterized by intense rainfall, thunderstorms, and rare tornadoes; snow is uncommon. In the USNK study area, the amount of precipitation is lowest from midsummer to early fall, rainfall occurs in spring and sporadically in summer, and the precipitation in winter is mainly snow (Maupin, 1995).

Hydrology

The hydrology within study streams varied among study areas, among streams, and seasonally (fig. 4). Seasonal variations in streamflow and stream stage primarily are the result of seasonal differences in precipitation and evapotranspiration and the dominant pathways of water to a stream. Relatively quick pathways of water to streams are through runoff from land surfaces after precipitation events and through tile drains; slow pathways are through groundwater discharge to streams. Estimated runoff was greatest in the OZRK streams, followed by the UMIS streams, and then the USNK streams with fairly large site-to-site variability (table S4). The relative importance of groundwater discharge is indicated by the estimated base-flow index, which is a percentage of streamflow derived from base flow or groundwater discharge. The base-flow index was lowest in the OZRK study area and greatest in the USNK study area (table S4).

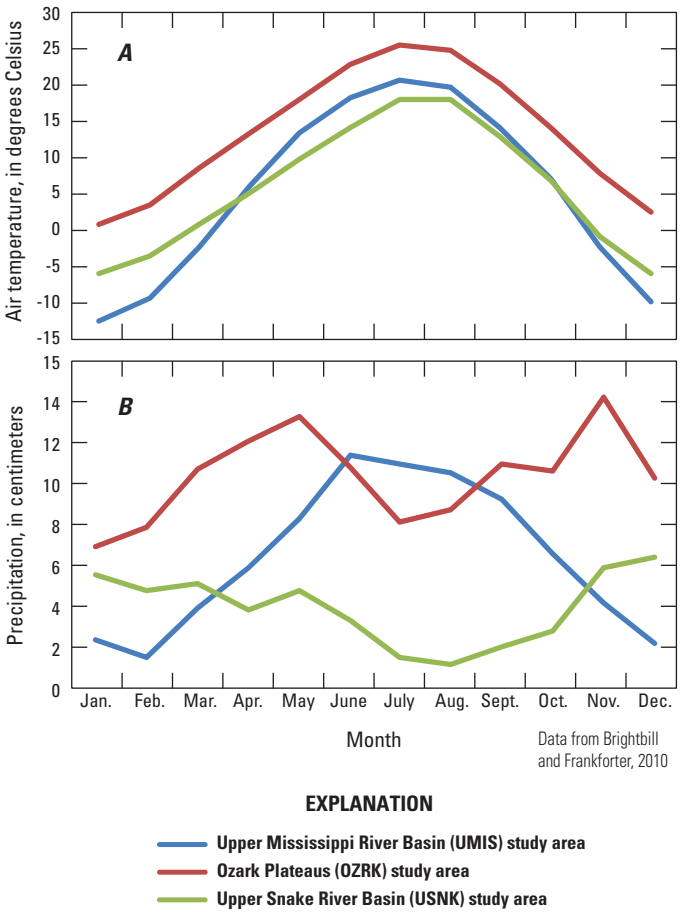

Figure 3. Air temperature and precipitation for sampling sites in the three study areas. *A*, mean monthly temperature and *B*, mean monthly precipitation.

Spring snowmelt and early spring precipitation events are the dominant hydrologic events in the two northern study areas (USNK and UMIS) from March through May resulting in delivery of large quantities of water from surface runoff during that period relative to runoff during late summer and winter. Stream stage remained high through July at most UMIS and USNK sites as a result of melting snow, rains falling on melting snow, or heavy rains falling on saturated or frozen soils (Stark and others, 1996). Following spring runoff, most water in streams in the USNK and UMIS study areas comes from groundwater discharge and springs (Maupin, 1995; Stark and others, 1996). Precipitation events during June and October resulted in stream stage increases at most UMIS sites. Stream

stage for site UMIS-7 was different from these general stage trends at other UMIS sites because the stage did not change appreciably during the year and slowly increased during the sampling period as macrophyte growth became more dominant in the channel (fig. 4). Streamflow at site UMIS-7 also was remarkably steady over the sampling period, indicating that the macrophytes were acting as a channel control and increasing river stage over the sampling period. Beaver dam construction throughout 2008 at site UMIS-2 complicated the stage trends.

In the OZRK study area, minimum monthly streamflows typically occur in summer and fall (July through October), whereas maximum monthly streamflows typically occur in spring, March through May (Adamski and others, 1995). During 2007, stream stage indicated precipitation events in January, February, May, and September (fig. 4). In contrast to stages at the UMIS and USNK sites, stage increased and receded rapidly (within 5–7 days) at most OZRK sites. The stage at site OZRK-3 was unique in that it experienced an increase in stage starting in July that persisted until October because of a hydrologic modification downstream from the site.

Stream Temperature

Stream temperatures for all measured sampling sites increased predictably during the spring and summer and then decreased into the subsequent fall and winter (fig. 5). At UMIS sites, daily temperatures fluctuated approximately 2–5°C at each site. One exception was at site UMIS-7, which had low temperatures throughout most of the year and less seasonal and daily fluctuations in temperature potentially because the main source of water to this stream is groundwater discharge. The water temperature at OZRK sampling sites had the same general pattern as temperatures at UMIS sites. Temperatures at USNK sampling sites also followed the same general pattern as temperatures at sites in the other two study areas, but variability was great among USNK sites. Temperature fluctuations at site USNK-3 varied little seasonally and daily because streamflow at this site is dominated by groundwater discharge, similar to site UMIS-7.

In-Stream Characteristics

In-stream characteristics such as width, depth, water-surface gradient, velocity, streamflow, and geomorphic units (pools, riffles, and runs) provide information about water residence time in a stream and potential areas for algal growth. These factors are important for understanding potential in-stream processes that will affect nutrient cycling. The UMIS streams generally had lower gradients and lower width-to-depth ratios and were characterized by fairly uniform runs where water is moved rapidly through the channel with few pools (table S5). The OZRK and USNK streams tended to have higher gradients and more diversity in geomorphic units (table S5).

Riparian Characteristics and Channel Shading

Riparian characteristics such as channel shading are important because they are an indication of light penetration and local land use that can affect the water quality of the stream. Woody vegetation in the 50-m buffer surrounding each stream segment and channel shading at the banks was greatest in UMIS and OZRK streams (table S6). Shading in the center of the channel was greatest in OZRK streams. Wetlands were prominent in the riparian area of UMIS and USNK streams; however, USNK sampling sites had few upstream wetlands, and all of which were constrained to riparian zones along streams. More than one-half of the UMIS sites and two USNK sites had cropland in riparian zones (table S6). Grassland and shrubland composed a substantial percentage of land cover at USNK sites.

Site Grouping Based on Basin, Near-Stream, and In-Stream Characteristics

Seasonal patterns in nutrient concentrations are affected by factors at many scales including climate, fertilizer use, hydrology, land use, stream temperature, light availability, and in-stream biological processing. Although it is not possible to directly link physical factors to chemical concentrations and biological responses on the basis of data collected in this study, the relations between constituents and physical factors can provide evidence of potential factors that affect nutrient cycling and responses in streams.

A principal components analyses of climatic, basin-level, near-stream, and in-stream physical factors grouped sites fairly consistently into three groups that coincided with study areas (table 5; fig. 6). The sampling sites from each study area plot in the same general area in the principal components analyses but with some variability among their locations. The streams are in unique settings within each study area as illustrated in figure 7. Streams within the USNK study area are primarily located within basins dominated by grassland and rangeland, whereas streams in the UMIS study area primarily are within areas dominated by row crop agriculture with some forested and wetlands cover. The OZRK streams are within basins that are primarily forested with pasture and poultry production. The variability among sites within a study area was not always because of nutrient use. Based on the clear distinction among sites by study area shown in the principal components analyses, seasonal patterns in nutrients, carbon, and algal responses were expected to differ among study areas and between streams within a study area.

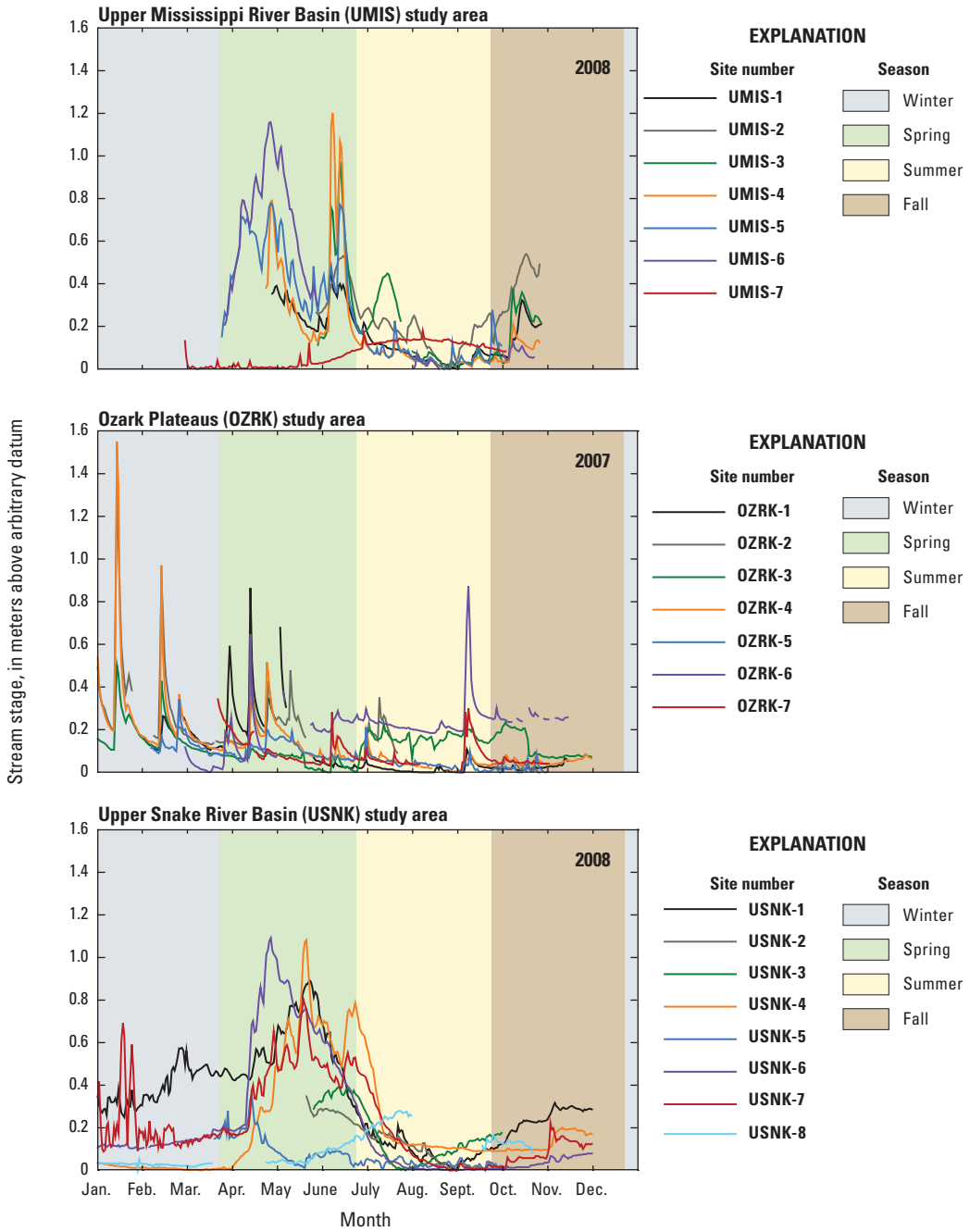

Figure 4. Stream stage measured continuously with stage recording instrumentation and manually during sampling dates.

Seasonal Trends in Nutrients, Carbon, and Algal Responses

This section of the report describes seasonal patterns in nutrients (nitrogen and phosphorus), carbon, and algal responses. For each constituent, concentrations are characterized by season within each study area. The differences in normalized values of each constituent are used to describe general seasonal trends in seasons by study area. Normalized values alleviate the problem of showing patterns in streams with wide ranges in nutrient concentrations, which obscure patterns when all sites are combined. Concentrations were normalized relative to site mean concentrations

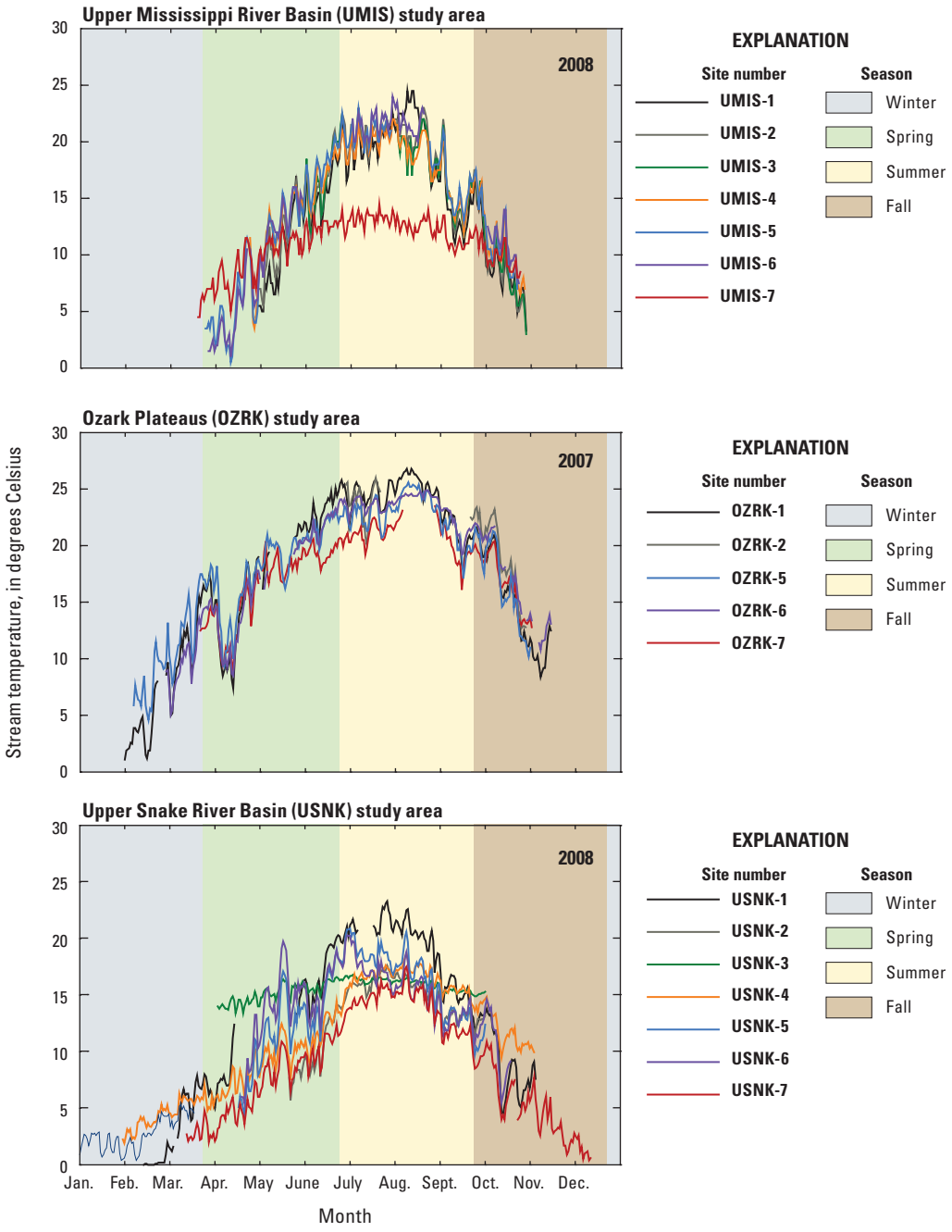

Figure 5. Stream temperature measured continuously.

(concentration at a site on one sampling date divided by the mean of the concentrations for all sampling periods at a site) to better highlight the changes in concentrations through the seasons relative to site means (table 6). Seasonal patterns for each constituent at each site within a study area also are shown to highlight the unique patterns among study areas and sites.

Nitrogen

Nitrogen concentrations varied widely in streams within each study area ranging from relatively low concentrations to relatively high concentrations (table S7). Overall, total nitrogen and nitrate concentrations were greatest at UMIS and OZRK sites and least at USNK sites (table S7). Particulate

Table 5. Statistical summary of principal components cumulative proportion of variance explained and component loadings for each physical factor.

[km^2, square kilometers; --, not applicable; NAVD 88, North American Vertical Datum of 1988; $(kg/km^2)/yr$, kilogram per square kilometer per year; m^3/s, cubic meters per second; m/s, meters per second; °C, degrees Celsius; cm, centimeter]

Physical factor	Principal component 1	Principal component 2	Principal component 3
Cumulative proportion of variance explained	0.28	0.48	0.62
Landscape characteristics			
Drainage area (km^2)	0.25	--	--
Basin altitude (meters above NAVD 88)	.32	0.16	--
Land use/land cover in drainage basin			
Percentage open water in basin	--	-0.19	-0.31
Percentage developed in basin	-0.22	-.21	.17
Percentage basin forest in the basin	-.17	.10	-.39
Percentage shrubland and grassland in basin	.32	.14	.10
Percentage pasture and hay in the basin	-.23	--	.29
Percentage cropland in basin	--	-.29	.19
Percentage wetland in basin	--	-.37	--
Land use/land cover in riparian area and channel shading			
Percentage open water in riparian area	0.12	-0.13	--
Percentage urban in riparian area	--	--	--
Percentage woody vegetation in the riparian area	-.29	.10	-0.17
Percentage shrubland and grassland in riparian area	.19	--	.21
Percentage cropland in riparian area	--	-.16	.24
Percentage wetland in riparian area	.22	-.12	--
Mean percent channel shading	-.25	--	-.18
Soil characteristics in drainage basin			
Percentage silt	--	0.28	0.24
Percentage sand	--	-.38	-.16
Percentage clay	-0.15	.37	--
Nutrient-use characteristics in drainage basin			
Total nitrogen use per area [$(kg/km^2)/yr$]	-0.18	--	0.35
Total phosphorus use per area [$(kg/km^2)/yr$]	-.18	--	.35
Water-use characteristics in drainage basin			
Number of irrigation wells per area	0.19	--	0.10
Hydrology			
Mean water-surface gradient (dimensionless)	0.35	-0.10	0.22
Mean streamflow (m^3/s)	.22	--	-.11
Mean stream velocity (m/s)	--	-.16	.27
Climate			
Mean annual temperature (°C)	-0.27	0.19	0.11
Mean annual precipitation (cm)	-.31	.14	-.12

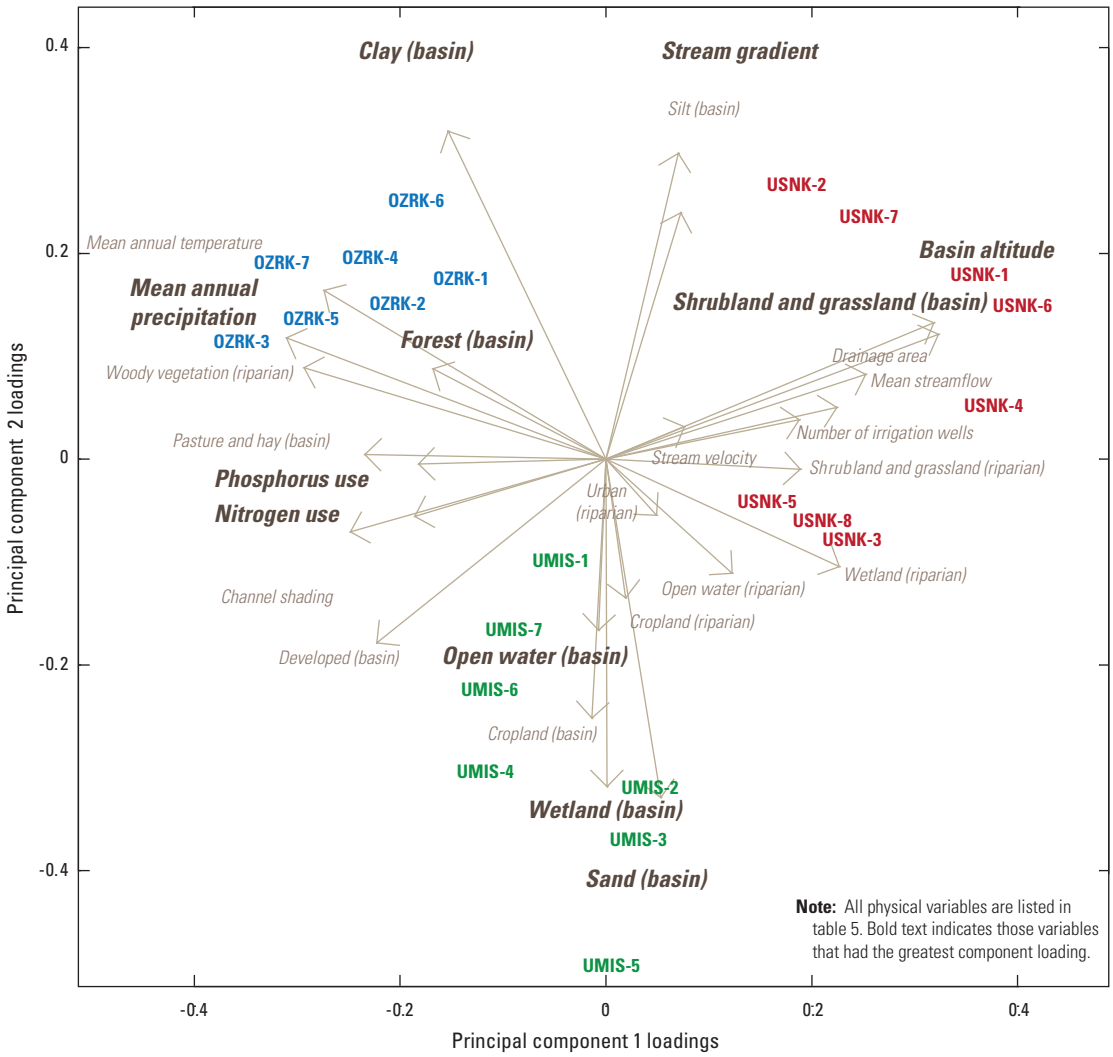

Figure 6. Principal components of the axis loading scores for principal components 1 and 2 for physical factors for the drainage basin (basin) or riparian area (riparian) at sampling sites.

nitrogen concentrations, in contrast, were lowest at OZRK sites (table S7), and ammonia concentrations were greatest at UMIS sites. The sites with the greatest total nitrogen and nitrate concentrations had the greatest total nitrogen use estimates (table S3: UMIS-4, UMIS-7, OZRK-3, OZRK-7, USNK-3, and USNK-8).

Nitrate

In general, nitrate (includes both dissolved nitrate and nitrite as nitrogen) concentrations were greatest in the winter and declined during summer and fall at most stream sites. Normalized nitrate concentrations in winter were on average 2.70, 1.66, and 1.71 times greater than mean site concentrations for the UMIS, OZRK, and USNK study areas, respectively (fig. 8, fig. 9, table 6), and were significantly different from concentrations in other seasons (p-value<0.05; Kruskal-Wallis).

Nitrate in streams during the winter likely is from groundwater because runoff generally is reduced during the winter period. Nitrate also can occur in streams from nitrification (conversion of ammonia in groundwater to nitrite and nitrate); however, nitrification rates generally are reduced in the winter (Duff and others, 2002) particularily in the main channels of streams within the USNK and UMIS study areas that are ice and snow covered. The relatively greater concentrations of nitrate in snow-covered streams in the winter may be because of a reduction in denitrification rates that decrease because of low stream temperatures and snow cover (Richardson and others, 2004). In addition, the reduction in light and cooler temperatures slows algal uptake and metabolism of nitrate (Reynolds, 1990). In streams with no ice cover (OZRK study area), limited primary production does occur in the winter (Strauss and others, 2006) because of benthic algal growth. Benthic algal oxygen production creates oxic conditions that are favorable for nitrification (conversion of

Upper Mississippi River Basin study area
Moran Creek near Staples, Minnesota
Photograph by John Greene
U.S. Geological Survey
August 19, 2008

Upper Snake River Basin study area
Camas Creek near Blaine, Idaho
Photograph by Chris Mebane
U.S. Geological Survey
September 28, 2006

Ozark Plateaus study area
Big Creek at Mauser Mill, Missouri
Photograph by James C. Peterson
U.S. Geological Survey
September 29, 2005

Figure 7. *A*, Moran Creek near Staples, Minnesota; *B*, Big Creek at Mauser Hill, Missouri; and *C*, Camas Creek near Blaine, Idaho.

ammonia to nitrate) in the sediments below the oxic layer. In OZRK streams, nitrification of ammonia may be occurring as indicated by the relatively lower concentrations of ammonia in the winter and relatively higher concentrations of nitrate in the winter at most OZRK sites than during the other seasons.

In general, nitrate concentrations decreased over the growing season (May through September) at most sites. Decreases in nitrate concentrations in the spring are coincident with or follow shortly after snowmelt and high streamflow (table 6) or stage (fig. 4) in the UMIS and USNK study areas or after runoff events in the OZRK study area likely because of dilution during snowmelt runoff or spring storms. Continued decreases in nitrate concentrations at some sites through the spring and summer after streamflow has receded indicate reduced nitrate inputs (less surface runoff) and increases in algal metabolism of nitrate and conversion to algal biomass. Increases in nitrate concentrations during fall potentially are from additional input of nitrate during runoff from the land surface where assimilation of nitrate by terrestrial plants is reduced because of harvest, or potentially from runoff of late fall fertilizer applications. Additionally, a reduction in nitrate metabolism by algae also could contribute to an increase in in-stream concentrations.

The seasonal trends of nitrate concentrations were not identical at all sites (fig. 9), which is not unexpected as the hydrologic conditions, local land-use practices, and assimilation vary among sites resulting in unique seasonal patterns. For example, the relatively low nitrate concentrations decrease rapidly at three UMIS sites (UMIS-1, UMIS-2, and UMIS-3) that have low percentages of agricultural land use in contrast to the four remaining UMIS sites where nitrate concentrations remain relatively constant. The rapid decrease in nitrate

Table 6. Mean normalized values for constituents by study area and by season with Kruskal-Wallis probability (p-value) statistics for differences among the seasons.

[Bold values indicate statistically significant differences (p-value less than 0.05); <, less than]

Constituent	Mean normalized values[1]				p-value
	Winter	Spring	Summer	Fall	
Upper Mississippi River Basin (UMIS) study area					
Nitrate plus nitrite, dissolved	2.70a	0.90b	0.62b	0.75b	**0.0042**
Ammonia as nitrogen, dissolved	3.23a	1.06b	.28b	.34b	**.0001**
Total particulate nitrogen	1.16ab	1.55a	.66b	.66b	**.0008**
Total nitrogen	1.22a	1.08ab	.89b	.85b	**.0016**
Orthophosphate as phosphorus, dissolved	1.10b	.91b	1.22a	.86ab	**.0101**
Total phosphorus	1.17ab	1.21a	.96ab	.72b	**.0052**
Dissolved organic carbon	1.07a	1.22a	.96b	.74b	**.0035**
Particulate organic carbon	1.25ab	1.58a	.63b	.60b	**.0006**
Seston chlorophyll *a*	.60b	1.67a	.66b	.80b	**<.0001**
Benthic algal chlorophyll *a* biomass	.06a	1.54a	.57a	1.04a	**.0451**
Benthic algal biomass, ash-free dry mass	.45	1.35	.71	.86	.14
Macrophyte and macroalgal cover	.10	.93	1.13	.98	.14
Streamflow	.50b	1.74a	.68b	.81b	**<.0001**
Ozark Plateaus (OZRK) study area					
Nitrate plus nitrite, dissolved	1.66a	0.91b	0.74b	0.90b	**0.0002**
Ammonia as nitrogen, dissolved	.80	1.14	.80	1.26	.22
Total particulate nitrogen	.88	1.08	1.06	.86	.21
Total nitrogen	1.39a	1.01b	.82b	.87b	**.0002**
Orthophosphate as phosphorus, dissolved	.79b	.78b	1.37a	.92ab	**.0001**
Total phosphorus	.80b	.98ab	1.13a	1.00ab	**.0216**
Dissolved organic carbon	.89b	1.13a	1.02ab	.84b	**.0003**
Particulate organic carbon	.66b	1.11a	1.12a	.91ab	**.0048**
Seston chlorophyll *a*	.65b	1.34a	.90b	.87b	**.0013**
Benthic algal chlorophyll *a* biomass	.67b	.83b	.94a	1.77b	**.0008**
Benthic algal biomass, ash-free dry mass	.68b	.88b	1.04a	1.50ab	**.0003**
Macrophyte and macroalgal cover	.47	1.13	1.08	.91	.45
Streamflow	1.52a	1.65a	.42b	.37b	**<.0001**
Upper Snake River Basin (USNK) study area					
Nitrate plus nitrite, dissolved	1.71a	1.15b	0.70ab	0.70ab	**0.0367**
Ammonia as nitrogen, dissolved	1.99a	.91ab	.68b	.73b	**.0003**
Total particulate nitrogen	.58b	1.86a	.76b	.69b	**.0001**
Total nitrogen	.92	1.30	.91	.81	.0653
Orthophosphate as phosphorus, dissolved	.93	1.23	.87	1.03	.0704
Total phosphorus	.74b	1.78a	.74b	.67b	**<.0001**
Dissolved organic carbon	.71b	1.37a	.90b	.93ab	**.0004**
Particulate organic carbon	.63b	1.75a	.81b	.69ab	**.0010**
Seston chlorophyll *a*	.73b	1.38a	.86b	1.05ab	**.0119**
Benthic algal chlorophyll *a* biomass	1.57a	.66c	.94abc	1.14b	**.0106**

Table 6. Mean normalized values for constituents by study area and by season with Kruskal-Wallis probability (p-value) statistics for differences among the seasons.—Continued

[Bold values indicate statistically significant differences (p-value less than 0.05); <, less than]

Constituent	Mean normalized values[1]				p-value
	Winter	Spring	Summer	Fall	
Upper Snake River Basin (USNK) study area—Continued					
Benthic algal biomass, ash-free dry mass	1.56a	0.66c	1.02ab	0.96abc	**0.0005**
Macrophyte and macroalgal cover	1.40ab	.28a	1.18b	1.26b	**.024**
Streamflow	.50b	2.25a	.53b	.49b	**<.0001**

[1]Constituent values were normalized by dividing a sample concentration at a single site and sampling date by the mean concentration of the constituent for that site for all sampling periods to arrive at a value that represents the times greater or less than the site mean. The numbers shown in this table are means of the normalized values for all sites in a study area by season. The letters following the numbers show the statistical groupings for statistically significant differences.

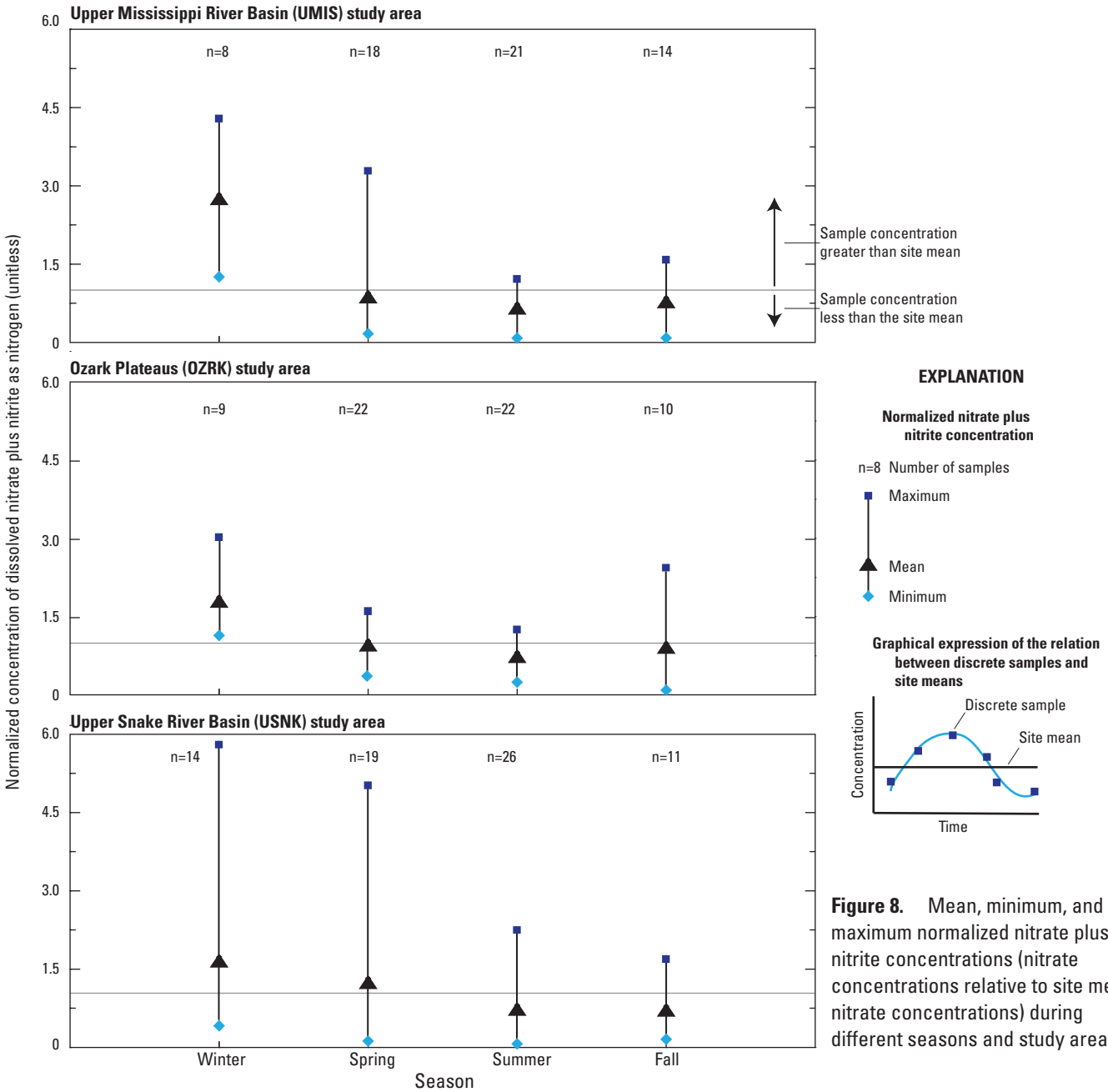

Figure 8. Mean, minimum, and maximum normalized nitrate plus nitrite concentrations (nitrate concentrations relative to site mean nitrate concentrations) during different seasons and study areas.

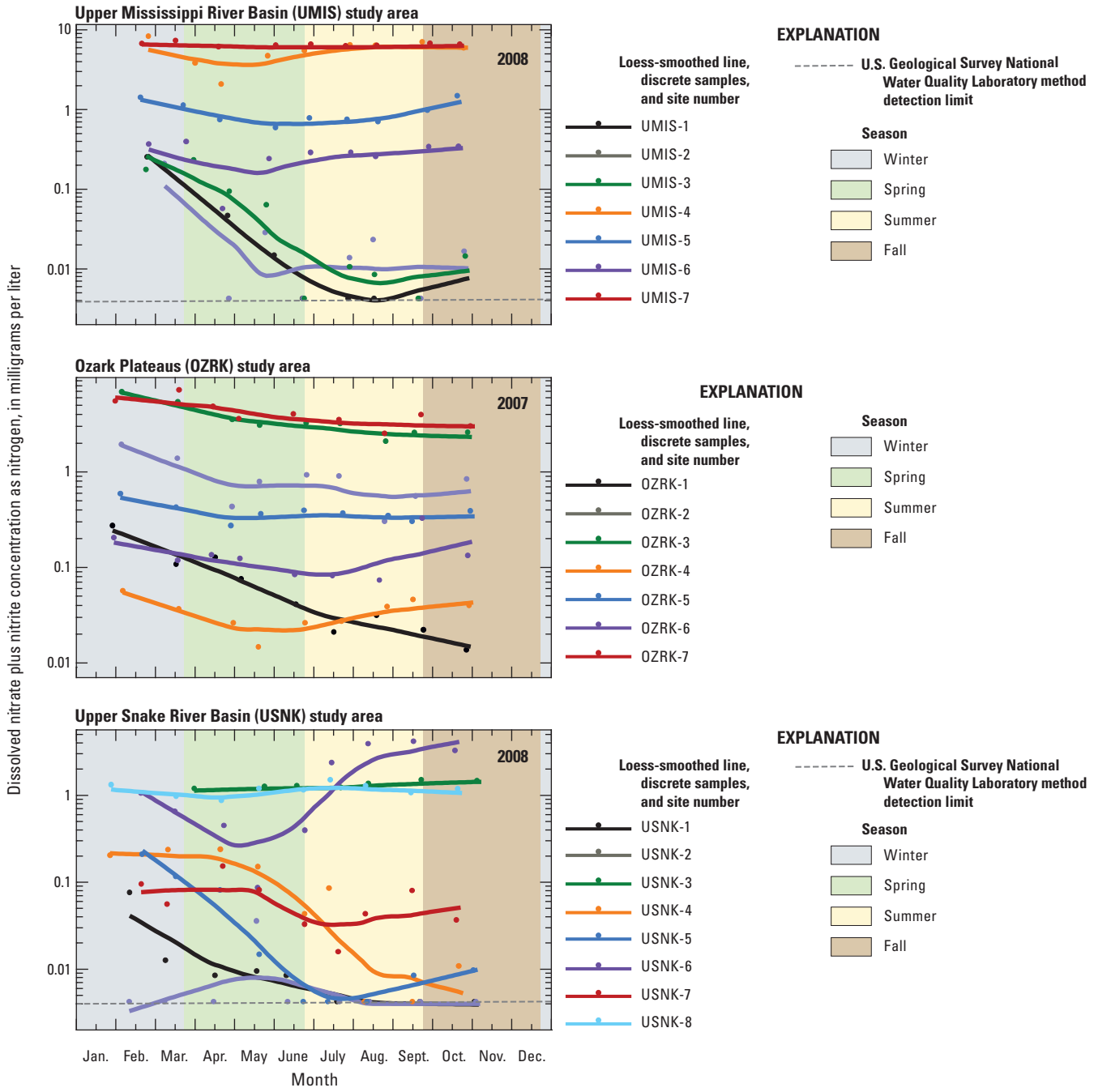

Figure 9. Concentrations of nitrate plus nitrite in streams over the 1-year sampling periods.

concentrations at the three UMIS sites following spring to near detection limits indicates that rapid uptake and dilution and denitrification are occurring. The nitrate concentrations at the remaining four sites that have greater agricultural land use decline less rapidly in the spring and remain fairly constant throughout the remainder of the sampling period indicating that a more constant groundwater or surface-runoff source of nitrate is available. Nitrate concentrations were remarkably consistent for the entire sampling period at sites UMIS-7, USNK-3, and USNK-8 potentially from groundwater discharge or from a constant upstream source. Site USNK-6, in contrast, had a unique and substantial increase in nitrate concentrations in the summer and fall potentially from a groundwater source during the summer low streamflow period (fig. 9).

The OZRK sites, in general, had less variable seasonal patterns in nitrate concentrations among sites than did the UMIS and USNK sites. The long-term (1999–2007) seasonal patterns in nitrate concentrations at site OZRK-3 (high percentage of agricultural land use and nitrogen use) declined

steadily and were remarkably consistent with the patterns observed during 2007 indicating that the nitrate concentration patterns observed in this study are representative of the long-term conditions for this site (fig. 10). Although the exact nutrient dynamics are unkown, the nitrate trend at this site potentially reflects that dilution is a factor in the spring and continued reductions through the summer are from metabolism followed by increases in nitrate from runoff in the fall during more modest increases in streamflow.

Ammonia

Ammonia and its ioinized form, ammonium, can co-occur in streams depending on pH and temperature conditions. Seasonal trends of ammonia (primarily the ionized form of ammonium) concentrations were similar at UMIS and USNK sites but unique at OZRK sites (fig. 11). Normalized ammonia concentrations (dissolved as nitrogen) in the winter were on average 3.23 and 1.99 times greater than mean site concentrations for the UMIS and USNK study areas, respectively, and were significantly different from concentrations in other seasons (p-value<0.05; Kruskal-Wallis; table 6). Ammonia concentrations in the summer and fall were less than mean concentrations in the UMIS and USNK study areas (table 6). Concentrations periodically decreased to less than MDLs at most UMIS sites throughout the sampling period. Most of the USNK sites had ammonia concentrations greater than MDLs only in the winter and spring with the exception of sites USNK-3 and USNK-8. Site USNK-3 had consistently high concentrations of ammonia throughout the sampling period with little seasonal change, indicating a constant source of ammonia (potentially a fish hatchery located approximately 3 km upstream) at relatively high concentrations to this stream. In contrast to UMIS and USNK sites, ammonia concentrations tended to be greater during the spring and fall, but were not significantly different among sampling seasons at the OZRK sites (fig. 11; table 6).

Unique ammonia patterns in individual streams indicate differences in nitrogen sources and in-stream processing. During the winter, ammonia likely is entering streams from groundwater because precipitation events and surface runoff are reduced. Ammonia may accumulate in stream water in the winter under snow-covered ice at the UMIS and USNK sites because of low nitrification (conversion of ammonia to nitrite and nitrate) rates in the winter (Duff and others, 2002) and limited algal assimilation because of colder water temperatures and snow cover that results in light limitation (Reynolds, 1990). Mineralization of decaying organic matter to ammonium in reducing conditions within stream-bottom sediments likely contributes a small amount of ammonia in USNK and UMIS streams because mineralization also is reduced in the winter (Wetzel, 2001).

The rapid decline in ammonia concentrations in the spring at the UMIS and USNK sites coincides with increased streamflow (table 6) indicating that dilution may be a factor in ammonia concentration declines. In addition, biological processes, such as ammonia assimilation into organic material by seston and benthic algae, are occurring as water temperatures warm up and light availability increases (Reynolds, 1990). During summer, realtively low ammonia concentrations at USNK and UMIS sites continue likely because of a combination of algal assimilation and nitrification whereby most of the ammonia from groundwater likely is oxidized to nitrate in the stream-bottom sediments (Wetzel, 2001; Tesoriero and others, 2009).

The un-ionized form of ammonia can be toxic to aquatic plants, invertebrates, and fish at concentrations less than 1 milligram per liter (mg/L) depending on temperature and pH conditions (U.S. Environmental Protection Agency, 1999; Newton and Bartsch, 2007). Ammonia concentrations in

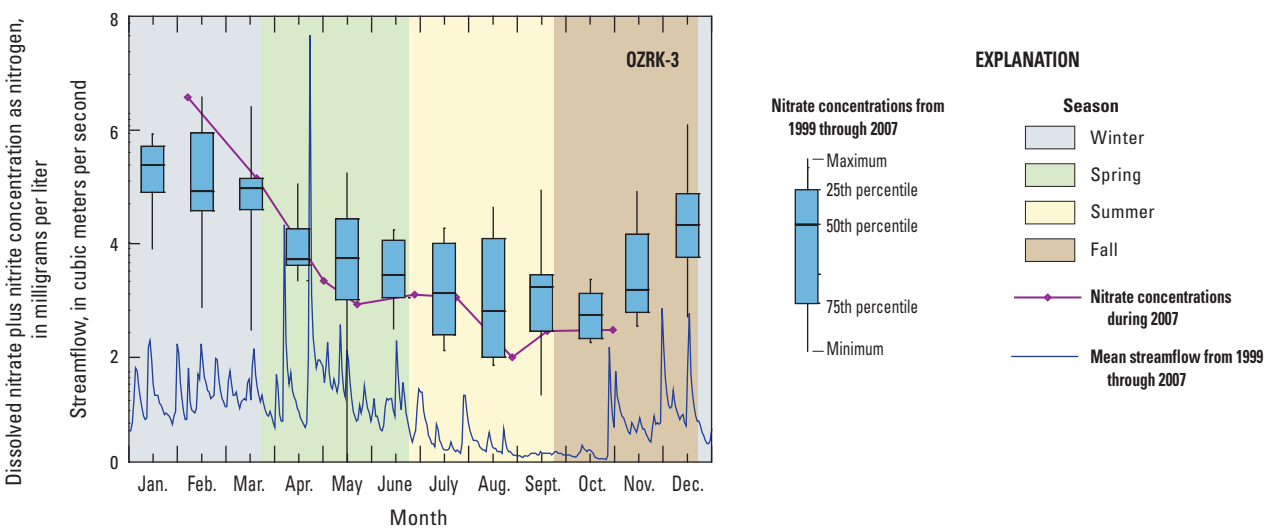

Figure 10. Nitrate plus nitrite concentrations for the period from 1999 through 2007 for site 3 in the Ozark Plateaus study area.

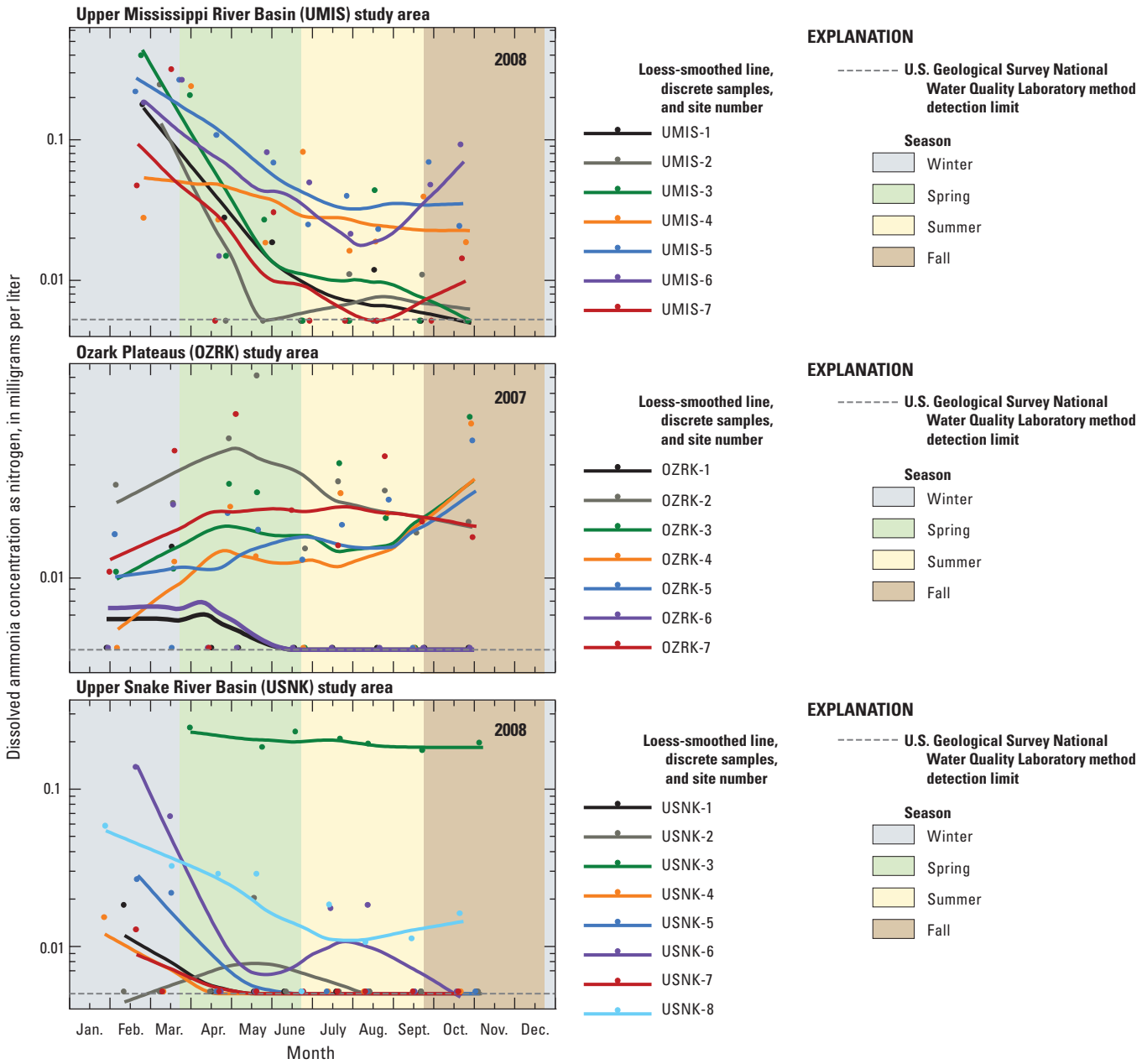

Figure 11. Concentrations of ammonia in streams over the 1-year sampling periods.

stream samples were compared with aquatic-health criteria for acute and chronic effects using guidelines established by the USEPA for protection of aquatic life (U.S. Environmental Protection Agency, 1999). The acute criteria for ammonia concentrations in water range from 2.1 to 44.6 mg/L of total ammonia for pH values of 6.7 to 8.5 and water temperatures of 0 to 32°C (conditions observed at sampling sites). The chronic criteria range from 0.4 to 6.4 mg/L of total ammonia for the same pH and water temperature ranges (U.S. Environmental Protection Agency, 1999). None of the water samples collected during this study had un-ionized ammonia concentrations that were greater than the USEPA criteria.

Total Particulate Nitrogen

Concentrations of total particulate nitrogen, which includes algal cells and other particulate living and nonliving material, were greatest in the spring and summer at most sites. Normalized concentrations of total particulate nitrogen in the spring were on average 1.55 and 1.86 times greater than mean site concentrations for the UMIS and USNK study areas, respectively, and were significantly different from concentrations in other seasons (p-value<0.05; Kruskal-Wallis; table 6). Seasonal patterns of total particulate nitrogen concentrations over the sampling period were similar among the UMIS and

USNK sites with a spring peak concentration, late summer lows, and a resurgence in the fall (fig. 12). The total particulate nitrogen concentrations at the OZRK sites were not significantly different among seasons (table 6), and the seasonal trends at OZRK sites were not as pronounced as those for the UMIS and USNK sites (fig. 12).

In the UMIS and USNK study areas, concentrations of total particulate nitrogen, particulate organic carbon, and seston chlorophyll *a* had similar seasonal patterns and concentration peaks occurring at approximately the same period for all constituents. In the UMIS and USNK study areas, the spring peak in total particulate nitrogen coincided with peaks in streamflow (table 6; fig. 5). Total particulate nitrogen and particulate organic carbon were positively correlated during all seasons for all study areas with the exception of the OZRK study area in the winter based on Spearman rank correlations (table 7). Concentrations of total particulate nitrogen, particulate organic carbon, and seston chlorophyll *a* were positively correlated for the summer period in all study areas and in the spring and summer in the OZRK and USNK study areas based on Spearman rank correlations (table 7). This indicates that most of the total particulate nitrogen and particulate organic carbon during the summer in all study areas and during the summer and spring in the OZRK and USNK was composed of seston or benthic algae entrained in the water column.

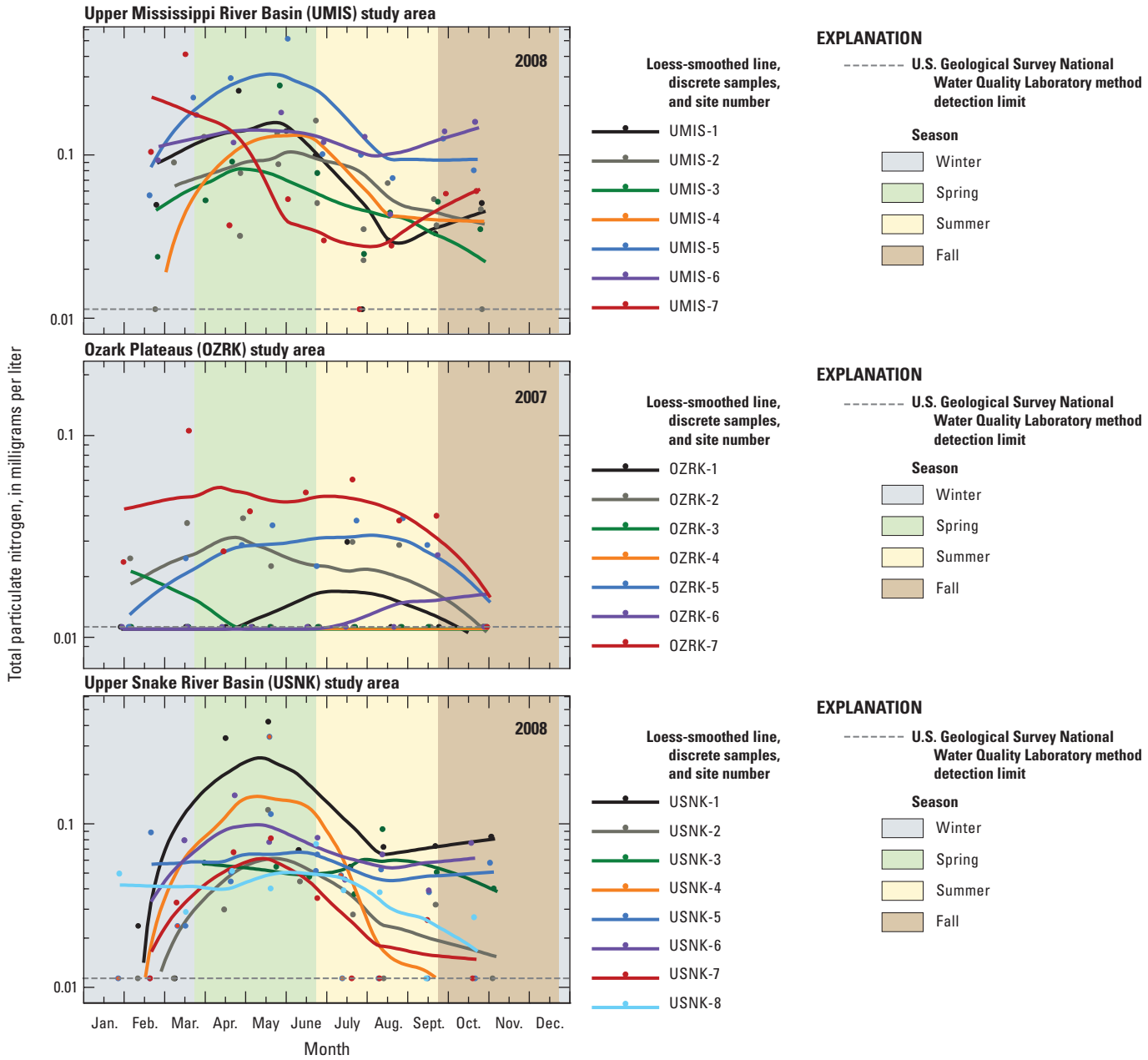

Figure 12. Concentrations of total particulate nitrogen in streams over the 1-year sampling periods.

Table 7. Spearman rank correlations among total particulate nitrogen, particulate organic carbon, and seston chlorophyll *a* concentrations by season and study area.

[Correlations are for all sites within a study area and season. Values are Spearman's rho; the values in bold text and shaded cells represent those correlations that were significant (p-values less than 0.05). Significance is dependent on the number of samples; the fewer the samples, the greater the rho has to be to achieve the desired 0.05 significance level. UMIS, Upper Mississippi River Basin study area; OZRK, Ozark Plateaus study area; USNK, Upper Snake River Basin study area; --, not applicable]

Season	Total particulate nitrogen			Particulate organic carbon		
	UMIS	OZRK	USNK	UMIS	OZRK	USNK
Seston chlorophyll *a*						
Winter	**0.71**	**0.81**	0.31	0.51	**0.71**	0.28
Spring	.39	**.85**	**.72**	.30	**.86**	**.69**
Summer	**.51**	**.50**	**.67**	**.54**	**.55**	**.61**
Fall	.36	.47	.42	.30	**.79**	.31
Particulate organic carbon						
Winter	**0.95**	0.65	**0.88**	--	--	--
Spring	**.97**	**.92**	**.86**	--	--	--
Summer	**.96**	**.94**	**.87**	--	--	--
Fall	**.92**	**.69**	**.95**	--	--	--

Total Nitrogen

Seasonal differences among normalized total nitrogen concentrations were significant (p-value<0.05; Kruskal-Wallis; table 6) for the UMIS and OZRK study areas, whereas seasonal differences were not significant for the USNK study area. Total nitrogen concentrations in the winter were 1.22 and 1.39 times greater on average than mean site concentrations for the UMIS and OZRK study areas, respectively (table 6).

Patterns in total nitrogen concentrations varied by study area. Total nitrogen concentrations were fairly consistent among UMIS sites over the sampling period, with the greatest concentrations in February–March and the lowest in August and September (fig. 13). The greater concentrations in February–March prior to snowmelt runoff may be because of several factors including contributions of nitrogen from groundwater, lower microbial metabolism in the streambed, and lower assimilation from the water column. Trends in total nitrogen concentrations for OZRK sites were similar to trends for UMIS sites in that concentrations gradually declined over the sampling year with a slight increase at the end of the year in the fall (fig. 13). In contrast, total nitrogen concentrations in the USNK study area were highly variable among and within streams compared with concentrations in the UMIS and OZRK study areas.

In contrast to concentrations of individual forms of nitrogen, total nitrogen concentrations did not change much among the seasons at individual streams within the UMIS and OZRK study areas, likely because seasonal differences in total nitrogen can be obscured because of differences in the composition of the organic and inorganic nitrogen forms present. Most of the total nitrogen at the OZRK sites was in the form of nitrate with very little organic nitrogen during all sampling periods (median of 72 percent nitrate among all OZRK sites compared to 30 and 31 percent nitrate at the UMIS and USNK sites, respectively). Nitrate concentrations at low-intensity agricultural sites in the UMIS study area (sites UMIS-1, UMIS-2, and UMIS-3) and in the USNK study area (sites USNK-1, USNK-2, and USNK-5), composed a relatively small percentage of the total nitrogen concentration, whereas organic nitrogen was dominant at these sites during the entire year.

Phosphorus

Phosphorus concentrations varied widely in streams within each study area ranging from relatively low concentrations to relatively high concentrations (table S7). Orthophosphate (dissolved as phosphorus) and total phosphorus concentrations were greatest at UMIS sampling sites and least at OZRK sites (table S7). Orthophosphate and total phosphorus concentrations were near MDLs, which in some cases resulted in a detection of orthophosphate but not in a detection of total phosphorus in the same sample. Orthophosphate concentrations were low at most OZRK sites with the exceptions of sites OZRK-3 and OZRK-7, which had the greatest percentage of pastureland in upstream drainage basins, the greatest number of poultry houses, and the greatest number of cattle produced per square kilometer among OZRK sites (Justus and others, 2010).

Orthophosphate

Normalized orthophosphate concentrations were significantly different (p-value<0.05; Kruskal-Wallis; table 6) among seasons for the UMIS and OZRK study areas. Normalized orthophosphate concentrations in the summer were on average 1.22 and 1.37 times greater than the mean site concentrations for the UMIS and OZRK study areas, respectively, whereas seasonal differences for the USNK study area were not significant (table 6).

Orthophosphate concentrations at most UMIS and OZRK sampling sites had a distinct trend of low concentrations in the spring and peak concentrations in the late summer followed by decreases in the fall (fig. 14). The seasonal peaks of orthophosphate concentrations at UMIS sites occurred later in the summer compared to peaks in total phosphorus concentrations, whereas the orthophosphate and total phosphorus

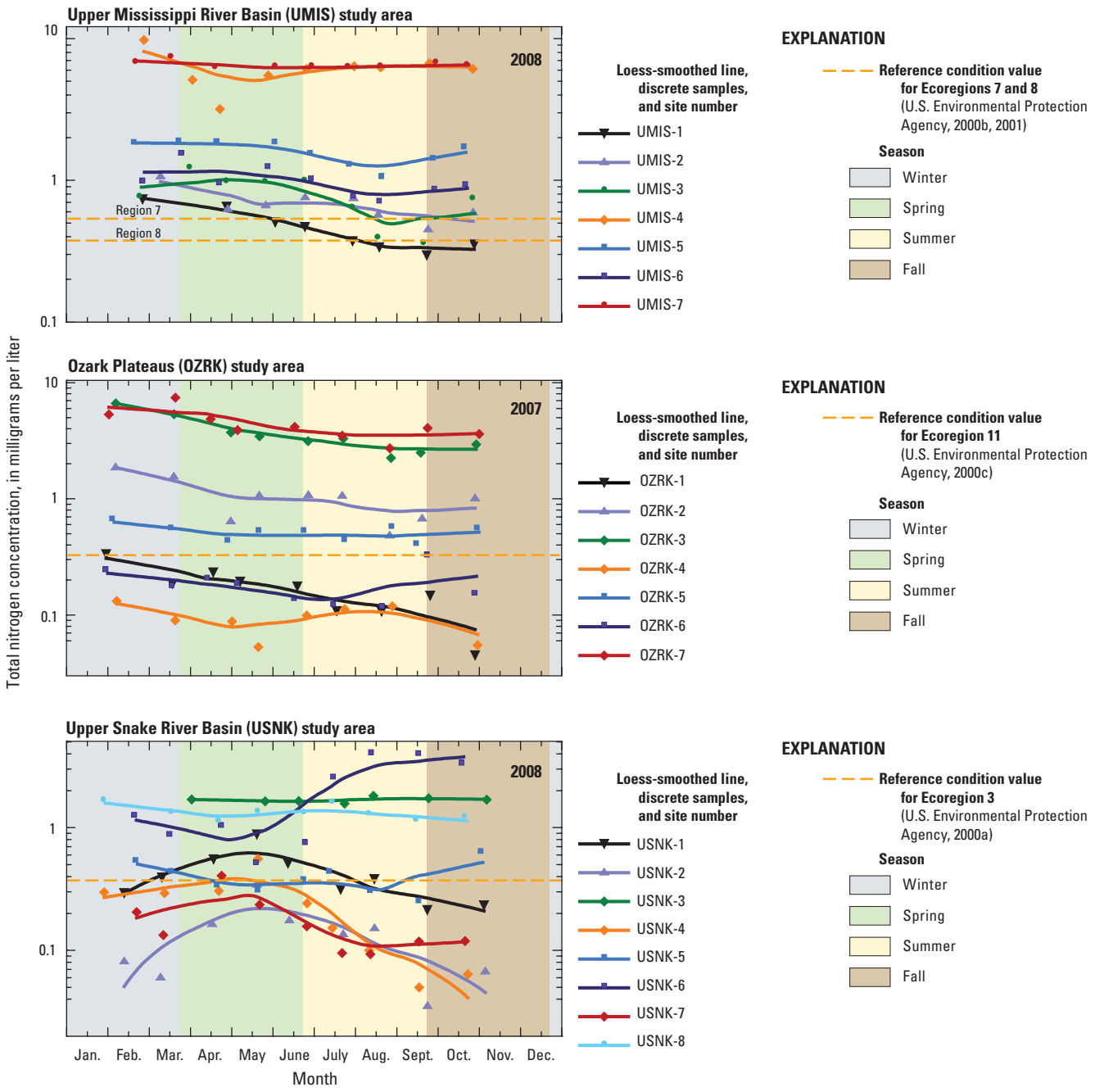

Figure 13. Concentrations of total nitrogen in streams over the 1-year sampling periods.

concentrations both peaked in the summer at the OZRK sites (figs. 14 and 15). Seasonal changes in orthophosphate concentrations likely are becaue of a combination of changes in algal metabolism and changes in sources from surface runoff in the spring and early summer to groundwater in the summer. Tesoriero and others (2009) and Dubrovsky and others (2010) measured orthophosphate concentrations in groundwater and found that the contributions to streams varied depending on local oxygen conditions at the stream and groundwater interface. Orthophosphate may increase in streams in the late summer when surface runoff composes less of total streamflow and when groundwater containing orthophosphate becomes a more dominant source in streams during lower flows. The continued downward trend in orthophosphate concentrations during the summer likely is because of biological uptake. Seasonal patterns of orthophosphate concentrations varied more among USNK sites than UMIS and OZRK sites (fig. 14). The variability among USNK sites indicates potential differences in sources, geochemistry, hydrologic characteristics, or biological uptake.

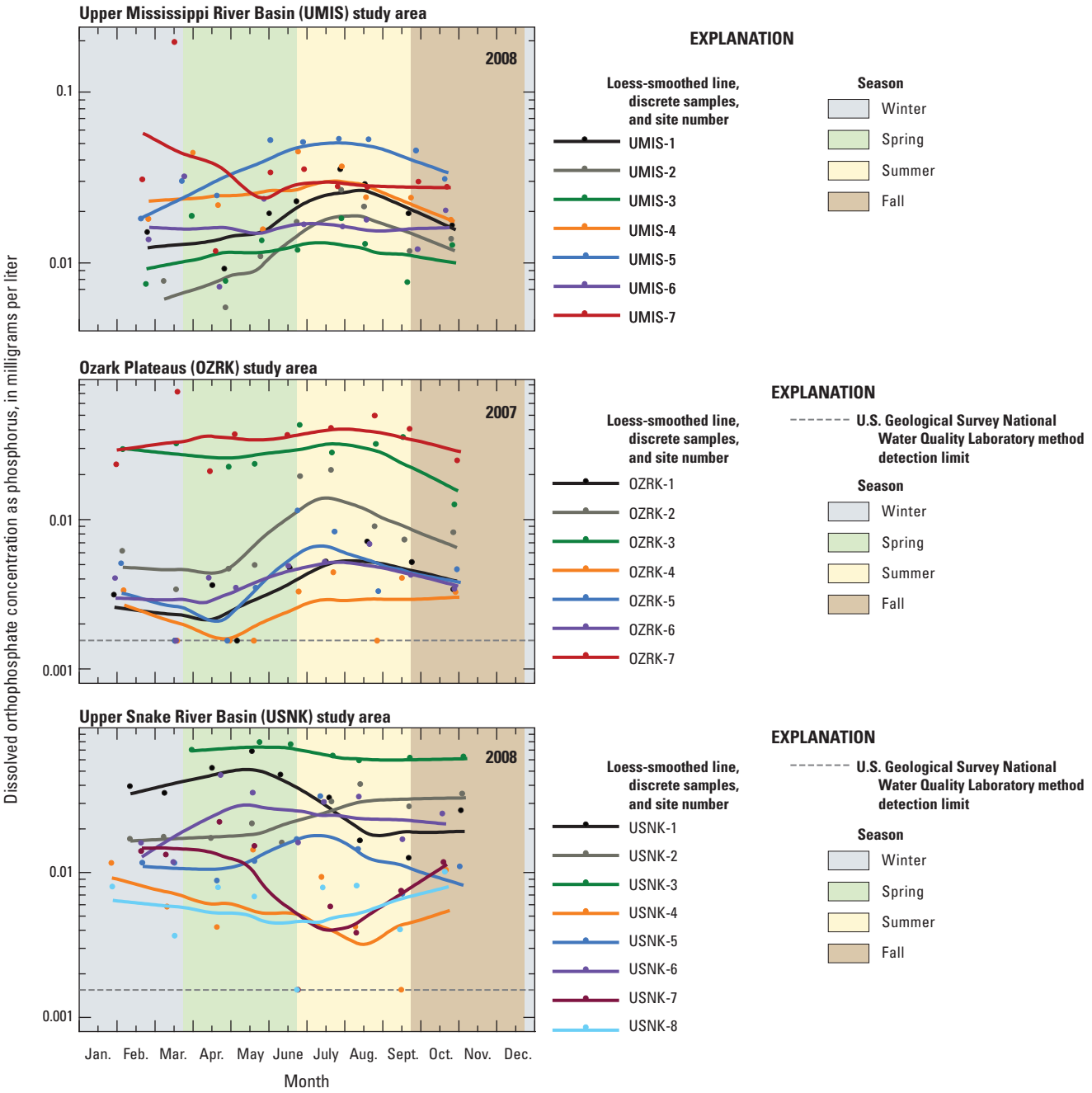

Figure 14. Concentrations of orthophosphate in streams over the 1-year sampling periods.

Total Phosphorus

Normalized concentrations of total phosphorus were significantly different (p-value<0.05; Kruskal-Wallis; table 6) among seasons for all three study areas. Normalized concentrations in the spring were on average 1.21 and 1.78 times greater than mean site concentrations for the UMIS and USNK study areas, respectively (table 6). In contrast, normalized total phosphorus concentrations in the summer were 1.13 times greater than mean site concentrations for the OZRK study area (table 6).

Trends in total phosphorus concentrations at most UMIS and USNK sites were consistent with a peak concentration in the spring (fig. 15) coinciding with snowmelt runoff and then a decrease through the remainder of the sampling period or with a slight increase in the fall. High total phosphorus concentrations in the spring may be because of runoff transporting phosphorus from the land surface to streams. Alternatively, total phosphorus also is a measure of seston algae and may be an

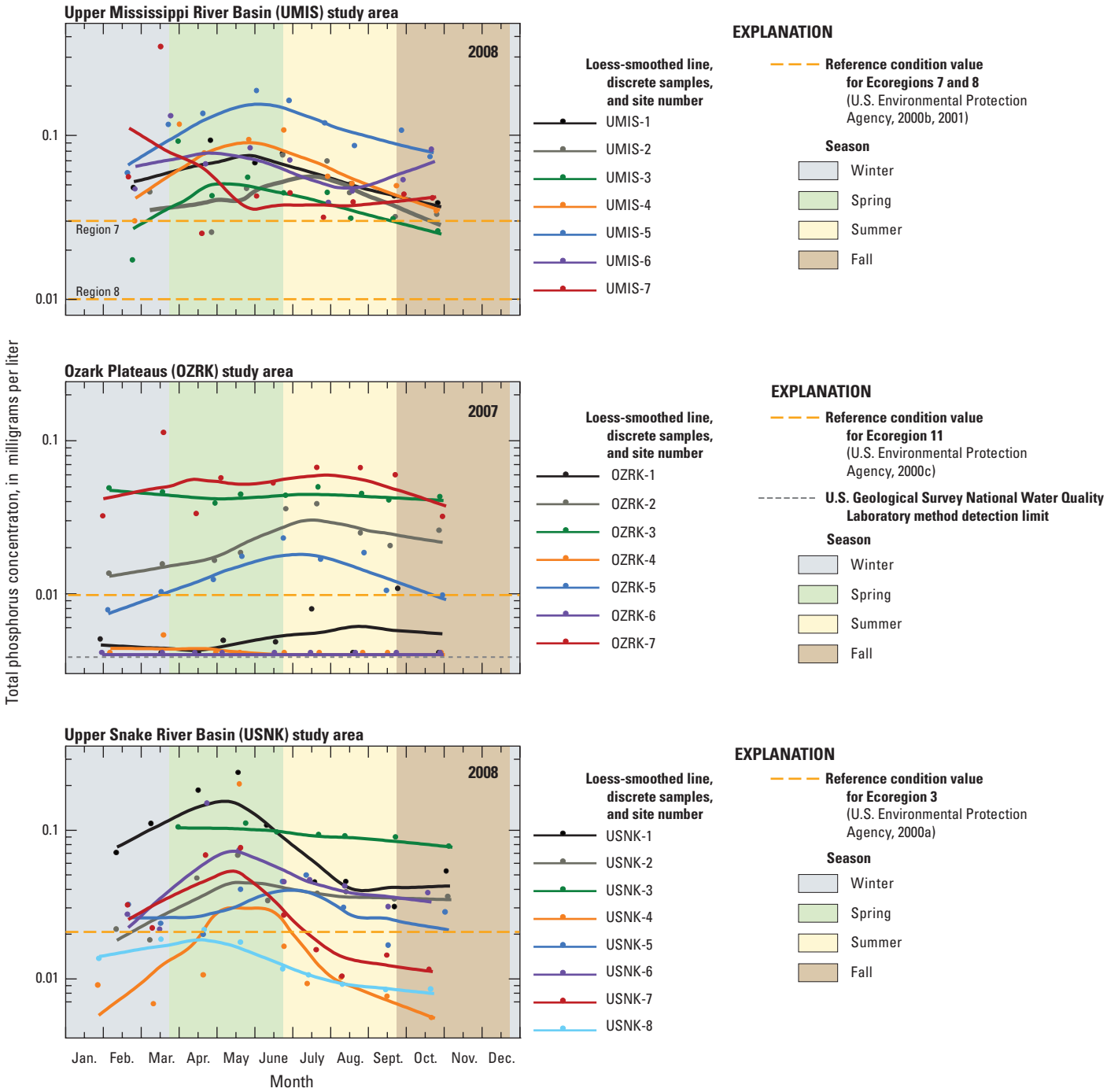

Figure 15. Concentrations of total phosphorus in streams over the 1-year sampling periods.

indication of increased seston algal growth as water temperatures warm and light is less restricted by riparian vegetation. In contrast to concentrations in UMIS and USNK streams, total phosphorus concentrations in OZRK streams did not peak in the early spring, but rather peaked during summer similar to the peak orthophosphate concentrations potentially because of the later season application of manure and fertilizers in the OZRK study area. Total phosphorus concentrations at five OZRK sites were greater than MDLs during the summer and fall (fig. 15).

Carbon

Concentrations of dissolved and particulate organic carbon varied among study areas and among sampling sites (table S7). Concentrations of dissolved and particulate organic

carbon were greater in UMIS and USNK streams than in OZRK streams. Dissolved organic carbon and particulate organic carbon include carbohydrates, proteins, peptides, amino acids, fats, waxes, resins, and humic substances, which are the largest fraction of natural organic matter in water (Thurman, 1985). Natural sources of organic carbon primarily are from soil and terrestrial plants. Sources within streams include excretion from actively growing algae or the decomposition of dead algae and macrophytes. Other sources include animal waste and septic tank discharge.

Dissolved Organic Carbon

Normalized concentrations of dissolved organic carbon were significantly different (p-value<0.05; Kruskal-Wallis; table 6) among sampling seasons at all three study areas. Normalized concentrations of dissolved organic carbon in the spring were on average 1.22, 1.13, and 1.37 times greater than mean site concentrations for UMIS, OZRK, and USNK study areas, respectively (table 6).

Trends in dissolved organic carbon concentrations varied among sites, but concentrations were greatest during the spring and early summer at most sites (fig. 16) and were correlated with the seston chlorophyll *a* concentrations in summer at USNK and OZRK sites (Spearman rho values of 0.45 and 0.78 for the USNK and OZRK sites, respectively, p-value<0.05). Correlations between dissolved organic carbon and seston chlorophyll *a* at the UMIS sites were not significant. The greater concentrations of dissolved organic carbon in the spring and the positive correlation with seston chlorophyll *a* at OZRK and USNK sites indicate that a predominant source of dissolved organic carbon may be from algal cells that release carbon (Wetzel, 2001). The lack of correlations between dissolved organic carbon and seston chlorophyll *a* at the UMIS sites may indicate that dissolved organic carbon in these streams is exported from the relatively greater percentage of wetland and upstream lakes rather than being predominantely from in-stream production.

Particulate Organic Carbon

Particulate organic carbon comprises living and nonliving material. Seasonal differences in particulate organic carbon were significant (p-value<0.05; Kruskal-Wallis; table 6) for the three study areas. Normalized concentrations of particulate organic carbon in the spring were 1.58 and 1.75 times greater than mean site concentrations for the UMIS and USNK study areas, respectively. Normalized concentrations of particulate organic carbon were greatest during the spring and summer at OZRK sites (table 6).

Concentrations of particulate organic carbon varied among the study areas. Concentrations were low at OZRK sites with detectable concentrations at only four sites. When detected at OZRK sites, concentrations of particulate organic carbon generally peaked in the spring and summer and were low in the winter and fall. Trends in particulate organic carbon concentrations were similar among the UMIS and USNK sites and similar to total particulate nitrogen concentrations (fig. 12), with a peak concentration in the spring, low concentrations in late summer, and a resurgence in the fall. Seasonal concentration trends for total particulate nitrogen, seston chlorophyll *a*, and particulate organic carbon are similar for most study areas and are correlated (Spearman rank; table 7) indicating that they are all a measure of the same organic carbon source, which may be seston algae or benthic algae entrained in the water column.

Algal Responses

Algae (seston and benthic) and macrophytes are organisms that primarily obtain energy from sunlight and are present in streams (Allan, 1995). Seston, in the context of this report, refers to algae that are suspended in the water column. Benthic algae are found on the surfaces of most substrates in streams and are common in streambeds of small rivers such as those sampled for this study. Chlorophyll *a*, the primary pigment of all photosynthetic organisms, is present in sestonic and benthic algae and macrophytes and macroalgae (Wetzel, 2001). Chlorophyll *a* is used as an estimate of algal biomass and accounts for between 0.9 and 3.9 percent of ash-free dry mass (Reynolds, 1990). Algae affect the presence of nutrients and dissolved oxygen through nutrient assimilation, transformation, and release (Stumm and Morgan, 1996). Seston algae develop sustainable populations in streams under certain conditions including sufficient residence time to allow for biomass accrual above transport rates.

Macrophytes (vascular plants) and macroalgal cover generally are found in flowing waters of moderate stream velocity and at depths where light penetration is sufficient (Allan, 1995). Macrophytes include various types of plants including emergent, floating leaved and attached, and submerged taxa. Macroalgae include filamentous algae (for example, *Cladophora* and *Spirogyra*) that are attached to stream substrate or to macrophytes. Macrophytes and macroalgae provide cover for fish and substrate for aquatic invertebrates and produce oxygen in streams. However, overabundance can result from high nutrient concentrations and may interfere with stream functioning, hydrology, and recreational activities (such as swimming, fishing, and boating), and detract from the aesthetic appeal of the stream (U.S. Environmental Protection Agency, 2010).

Seston Chlorophyll *a*

Seston chlorophyll *a* concentrations were greater at UMIS and USNK sites than at OZRK sites (table S7). Normalized concentrations of seston chlorophyll *a* in the spring were 1.67, 1.34, and 1.38 times greater than mean site concentrations for the UMIS, OZRK, and USNK sites, respectively, and were significantly different from concentrations for other

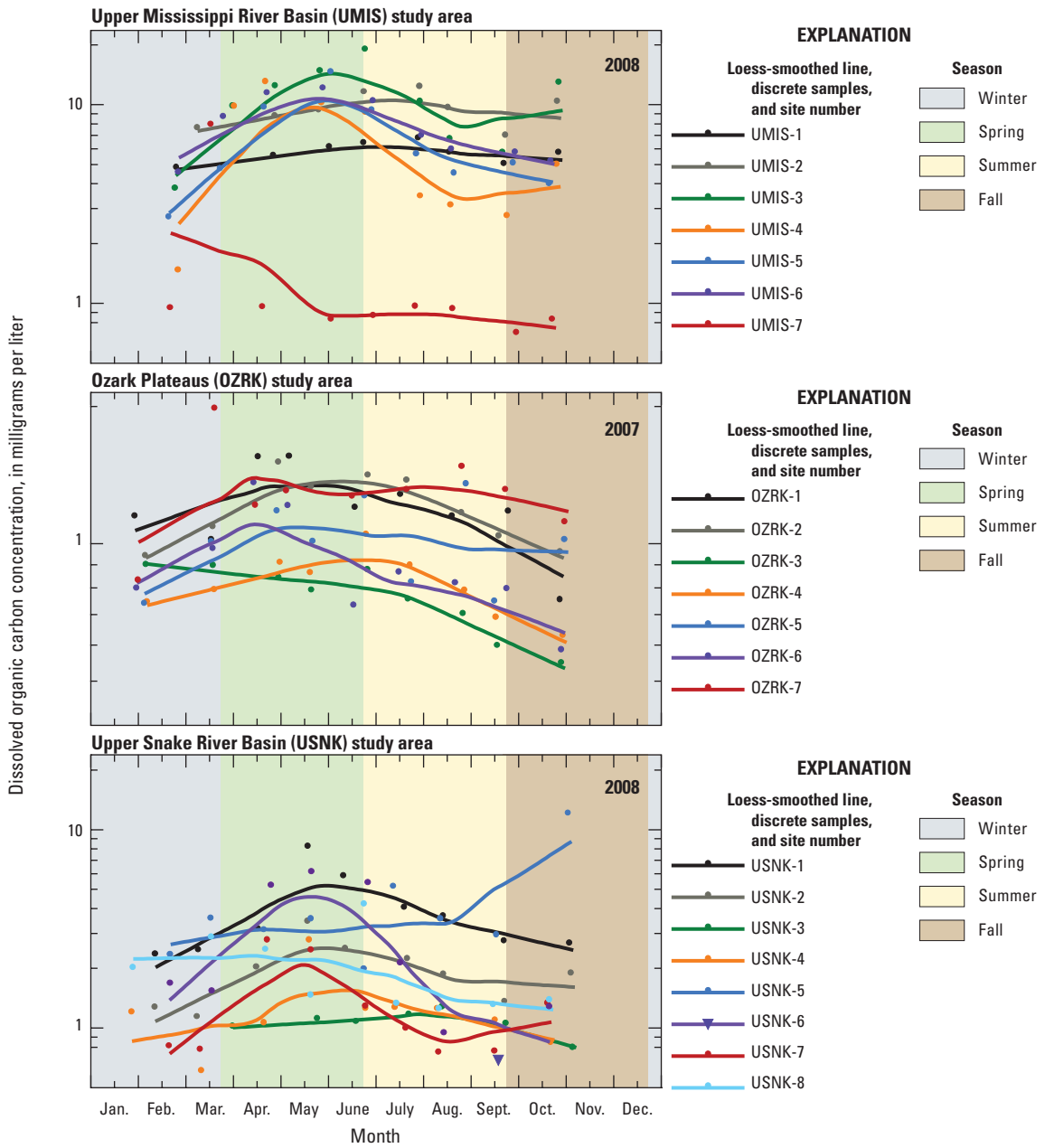

Figure 16. Concentrations of dissolved organic carbon in streams over the 1-year sampling periods.

seasons (p-value<0.05; Kruskal-Wallis; table 6). In general, seston chlorophyll *a* concentrations were greatest early in the growing season at most sites (fig. 17). Similar to other constituents, the range in seston chlorophyll *a* concentrations at individual sites was greater at UMIS and USNK sampling sites than at OZRK sampling sites. Seston chlorophyll *a* concentrations peaked a little earlier in the year at most OZRK sites than at UMIS and USNK sites potentially because of warmer temperatures in the OZRK study area earlier in the year (figs. 3 and 17).

The interpretation of seasonal patterns in seston chlorophyll *a* concentrations is complicated because of seasonal changes in streamflow and biological assimillation. For example, an increase in seston chlorophyll *a* concentrations during the spring can be because of an increase in seston algal growth when temperatures warm and no shading is present to inhibit growth (Allan, 1995; Wetzel, 2001). This interpretation is supported by reductions in dissolved inorganic nitrogen and orthophosphate concentrations during that same period. Alternatively, decreases in dissolved inorganic nitrogen and orthophosphate concentrations also may be because of dilution

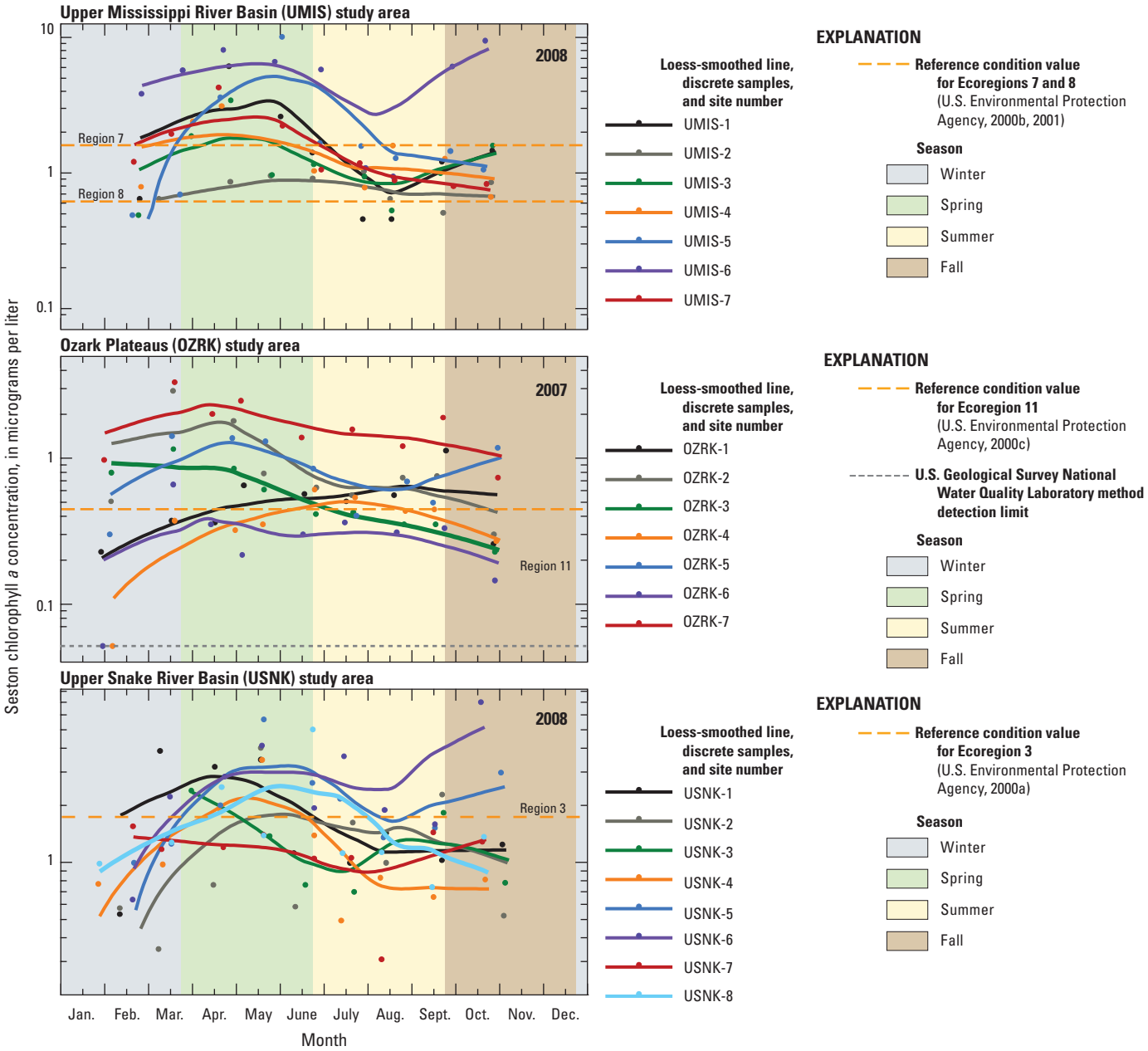

Figure 17. Seston chlorophyll *a* concentrations in streams over the 1-year sampling periods.

in the water column during high streamflow during this period, and the increased seston chlorophyll *a* concentrations at that time are because of entrained benthic algae from runoff events that scoured the channel or from transport of algae from upstream wetlands and lakes. In all three study areas, concentrations of seston chlorophyll *a*, particulate organic carbon, and total particulate nitrogen were positively correlated during summer (Spearman rank; table 7), indicating that particulate organic carbon and particulate organic nitrogen in those streams primarily is composed of seston algae or benthic algae entrained in the water column.

Generally, seston chlorophyll *a* concentrations increased as total phosphorus and total nitrogen concentrations increased in all three study areas; however, the strength and significance of the correlations varied among study areas and seasons (fig 18; table 8). Spearman rank correlations between concentrations of total phosphorus and seston chlorophyll *a* were significant (p-value<0.05) during winter and spring for the OZRK study area. Seston chlorophyll *a* concentrations also were positively correlated with dissolved nutrients (orthophosphate, nitrate, and ammonia) during the spring for the OZRK study area (table 8).

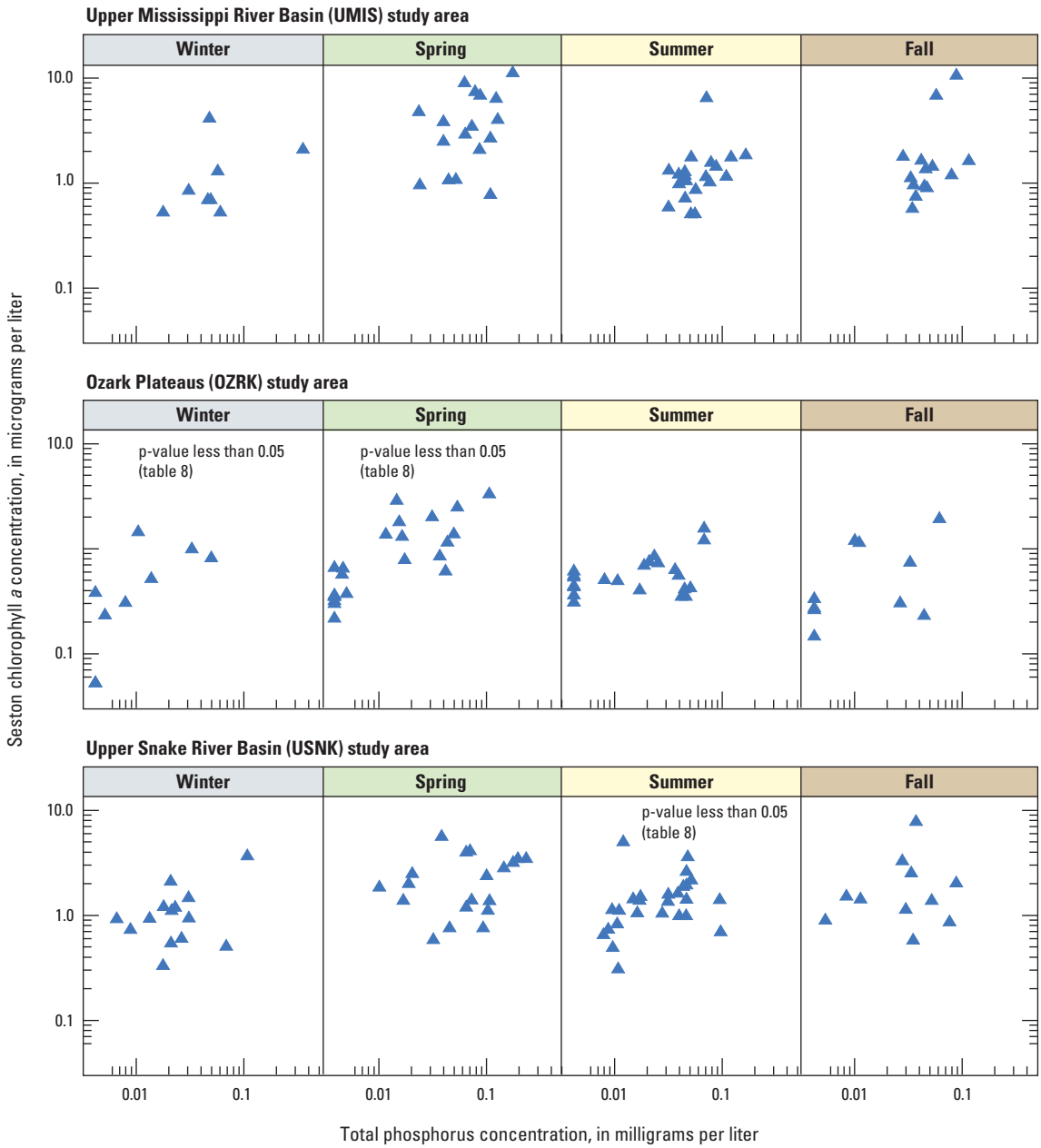

Figure 18. Relation between concentrations of total phosphorus and seston chlorophyll *a* within each study area among all four seasons.

Benthic Algal Chlorophyll *a* and Ash-Free Dry Mass

Benthic algal chlorophyll *a* biomass was greatest at OZRK sites followed by USNK sites and then UMIS sites (table S7). The community structure of the benthic algae varied among study areas. Diatoms were prevalent at UMIS sites, whereas blue-green algae were prevalent at most OZRK and USNK sites (table S8). Blue-green algae are common in environments where nitrogen and phosphorus are elevated (Downing and others, 2001); however, blue-green algae also are expected in environments with low nutrient concentrations, such as those measured at OZRK and USNK sites, because these algae are able to fix atmospheric nitrogen in the absence of nitrogen in the water column (Stevenson and others, 1996; Reynolds,1990).

Seasonal patterns in concentrations of benthic algal chlorophyll *a* biomass (fig. 19) and benthic algal ash-free dry mass were more variable among sites than trends for other constituents. Benthic algal chloropyll *a* biomass and ash-free dry mass were the most variable among sites within a study area during April through June potentially because of differences in

Table 8. Spearman rank correlations between selected nutrient concentrations and biological responses by season and study area.

[Correlations are for all sites within a study area and season. Values are Spearman's rho; the values in bold text and shaded cells represent those correlations that were significant (p-values less than 0.05). Significance is dependent on the number of samples; the fewer the samples the greater the rho has to be to achieve the desired 0.05 significance level. UMIS, Upper Mississippi River Basin study area; OZRK, Ozark Plateaus study area; USNK, Upper Snake River Basin study area; --, not enough data to compute]

Season	Total nitrogen			Dissolved nitrate, as nitrogen			Dissolved ammonia, as nitrogen			Total phosphorus			Dissolved, orthophosphate, as phosphorus		
	UMIS	OZRK	USNK	UMIS	OZRK	USNK	UMIS	OZRK	USNK	UMIS	OZRK	USNK	UMIS	OZRK	USNK
Seston chlorophyll *a*															
Winter	0.36	**0.68**	0.38	0.15	**0.68**	0.20	-0.31	0.27	0.15	0.29	**0.78**	0.26	0.40	0.19	-0.27
Spring	.06	**.79**	.07	.08	**.77**	-.05	.04	**.55**	-.14	.34	**.79**	.22	.11	**.45**	-.09
Summer	**.44**	.14	**.49**	.35	.08	.16	.35	.35	-.03	.41	.25	**.49**	.11	.08	.26
Fall	-.18	.42	.37	-.11	.30	.34	.36	.07	-.12	.41	.43	.009	.01	.50	-.22
Benthic algal chlorophyll *a* biomass															
Winter	--	0.05	0.02	--	0.05	0.04	--	-0.01	0.22	--	-0.01	0.13	--	0.40	0.16
Spring	0.42	**.84**	.17	0.30	**.81**	.26	0.03	**.49**	**.52**	-0.07	**.82**	**-.60**	-0.07	**.66**	-.25
Summer	**.51**	**.46**	**.58**	**.54**	**.45**	.30	.04	.23	**.46**	-.21	**.43**	**.45**	**.43**	.35	**.45**
Fall	.39	.41	.45	.36	.28	.46	.51	-.27	.59	.15	.38	.32	--	.44	.26
Macrophyte and macroalgal cover															
Winter	--	0.30	0.29	--	0.30	0.28	--	0.72	0.52	--	0.1	-0.25	--	1.0	-0.24
Spring	0.18	.14	.11	0.10	.09	.21	-0.24	.45	.40	-0.05	.03	-.33	-0.24	.03	.05
Summer	-.08	.11	**.56**	-.10	.06	.26	-.33	.31	**.56**	**-.52**	.19	.08	**-.72**	.06	.28
Fall	-.04	-.10	.35	-.12	-.08	.58	-.37	-.07	.57	-.18	.15	-.02	-.09	.07	-.06

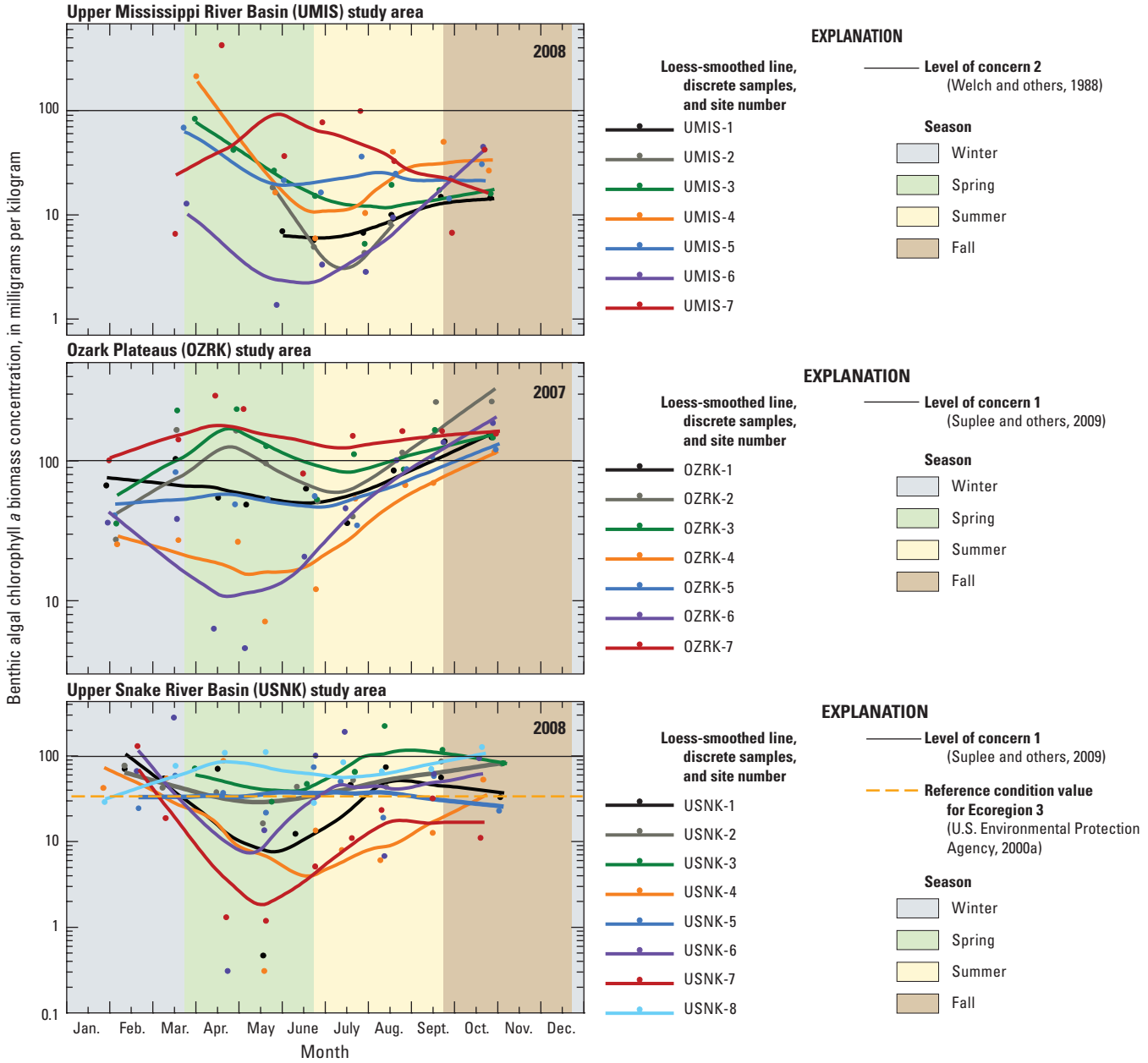

Figure 19. Concentrations of benthic algal chlorophyll *a* biomass in streams over the 1-year sampling periods.

nutrient inputs and and streamflow. The potential importance of this greater difference in the spring and early summer is that it indicates this period may be the best time to measure differences among sites.

Normalized concentrations of benthic algal chlorophyll *a* biomass were 1.54 times greater at UMIS sites in the spring, 1.77 times greater at the OZRK sites in the fall, and 1.57 times greater at USNK sites in the winter than mean site concentrations for respective study areas and were significantly different from concentrations for other seasons (p-value<0.05; Kruskal-Wallis; table 6). This variability likely is because of differences in streamflow, particle size of substrate, light penetration, grazing, and nutrient limitation (Allan, 1995), and also may be because of removal by herbivorous fish and invertebrates and through scour during runoff events.

The major spring runoff events at USNK and UMIS streams coincide with reductions in benthic algal chlorophyll *a* biomass. Concentrations of benthic algal chlorophyll *a* biomass and seston chlorophyll *a* had contrasting trends during runoff events at many of the USNK and UMIS sites. During periods when streamflow was greater in the USNK study area, concentrations of benthic algal chlorophyll *a* biomass were low (table 6). For example, the concentration of benthic algal chlorophyll *a* biomass at site USNK-4 decreased when

stream stage, streamflow, and seston chlorophyll *a* concentrations were increasing (table S7). This trend occurred at other USNK sites (USNK-2, USNK-6, USNK-7, and USNK-8). This same trend also was observed at UMIS sites, but early winter samples could not be obtained at UMIS sites because of thick ice cover so the trends for the UMIS sites are estimated for a shorter season. This trend was not consistent among the OZRK sites. The contrasting trends between concentrations of seston chlorophyll *a* and benthic algal chlorophyll *a* biomass at UMIS and USNK sites indicate that extreme events such as snowmelt runoff have scouring potential to remove benthic chlorophyll *a* from the bottom of the channel, which then is entrained in the water column and measured as seston chlorophyll *a*. Positive and significant correlations between benthic algal chlorophyll *a* biomass and most nutrient concentrations occurred relatively consistently during the spring and summer among all three study areas (table 8), indicating that benthic algal growth increases when concentrations of available nutrients increase and hydrologic conditions are more stable.

Macrophytes and Macroalgae

Macrophyte and macroalgal cover was greater at UMIS and USNK sites than at the OZRK sites (table S7). Macrophyte and macroalgal cover increased at most UMIS and USNK sites during the summer period (fig. 20) but a significant difference among seasons (p-value<0.05) was only indicated for the USNK sites (Kruskal-Wallis; table 6). The OZRK sites had less than 25 percent macrophyte and macroalgal cover at most sites, and trends among OZRK sites were not consistent.

Correlations between macrophyte and macroalgae cover and in-stream nutrient concentrations (table 8) generally were not significant with the exception of the summer period. Significant correlations for the summer period were negative with total phosphorus and orthophosphate concentrations at the UMIS sites, and positive with total nitrogen and ammonia concentrations at USNK sites. The negative correlations could indicate that macrophytes and macroalgae or epiphytic diatoms remove nutrients from the water column or create conditions that make nutrient removal more favorable. Macrophytes have direct and indirect effects in nutrient removal from the water column (Greenway, 2003; Wetzel, 2001). Submergent and floating macrophytes tend to remove nutrients from the water through leaf surfaces, whereas emergent macrophytes obtain nutrients from the interstitial water in sediments through the roots (Wetzel, 2001; Greenway, 2003). Macrophytes reduce water velocity, which aids in sedimentation, and provide surface area for epiphytic algal growth, which can enhance microbial nitrification and denitrification (Wetzel, 2001; Greenway, 2003). The effects of macrophyte growth on hydrologic conditions were observed at site UMIS-7 where streamflow did not increase predictably with stream stage increases, indicating that the macrophyte cover was effectively damming up the stream over the growing period. Macrophytes also release oxygen from their roots into the rhizosphere (area surrounding the roots in the bottom sediments), which aids in nitrification, and by the direct uptake of nutrients (Wetzel 2001; Greenway, 2003).

Relations Between In-Stream Nutrient Concentrations and Nutrient Use

The relation between in-stream total nitrogen and phophorus concentrations with nitrogen and phosphorus use was assessed to determine the effects of nutrient use on in-stream concentrations. Spearman rank correlations were determined between the median total nitrogen and phosphorus concentrations (computed from all samples collected during this study) and the total nitrogen and phosphorus use estimates from fertilizer (farm and nonfarm application), manure, and atmospheric deposition at each site.

The correlation between in-stream total nitrogen concentrations and nitrogen use was greater for streams in the OZRK study area (rho = 0.96) than for streams in the UMIS (rho = 0.86) and USNK (rho = 0.76) study areas (fig. 21). The rho value for the USNK study area was affected substantially by the median concentration and nitrogen use at one site (USNK-3). The UMIS sites with the two highest nitrogen use values (sites UMIS-4 and UMIS-7) had the greatest median in-stream total nitrogen concentrations. These positive correlations indicate that in-stream total nitrogen concentrations are related to fertilizer and manure applications and subsequent overland runoff and movement into streams through groundwater discharge.

The relation between in-stream total phosphorus concentrations and phosphorus use was stronger for the OZRK study area (rho = 0.95) than for the UMIS (rho = -0.18) and USNK (rho=0.05) study areas (fig. 22). In contrast to nitrogen, the relation between in-stream total phosphorus concentrations and phosphorus use for UMIS streams was nonexistent potentially because of the complicated phosphorus cycling and storage in streams. As a result, estimates of phosphorus use do not correlate well with median in-stream total phosphorus concentrations. Most streams in the USNK study area had estimates of phosphorus use that were low and that did not correlate well with in-stream total phosphorus concentrations. An exception is the relation between concentrations and use at site USNK-3, which had the greatest in-stream concentrations and phosphorus use. The relatively high correlation of median in-stream total phosphorus concentrations in streams and phosphorus use estimates in the OZRK study area likely is because of rapid movement of nutrients from the land surface through the karst systems with little processing in the subsurface.

The timing of fertilizer application is a potential factor affecting in-stream concentrations of nutrients. Fertilizers that contain nitrogen generally are applied in the spring and late fall in the UMIS study area (Kroening and others, 1997). Manure also is applied to land surfaces in the UMIS study area in the fall and winter (Fallon and McNellis, 2000), which is followed by snowmelt in the spring. In the OZRK study area,

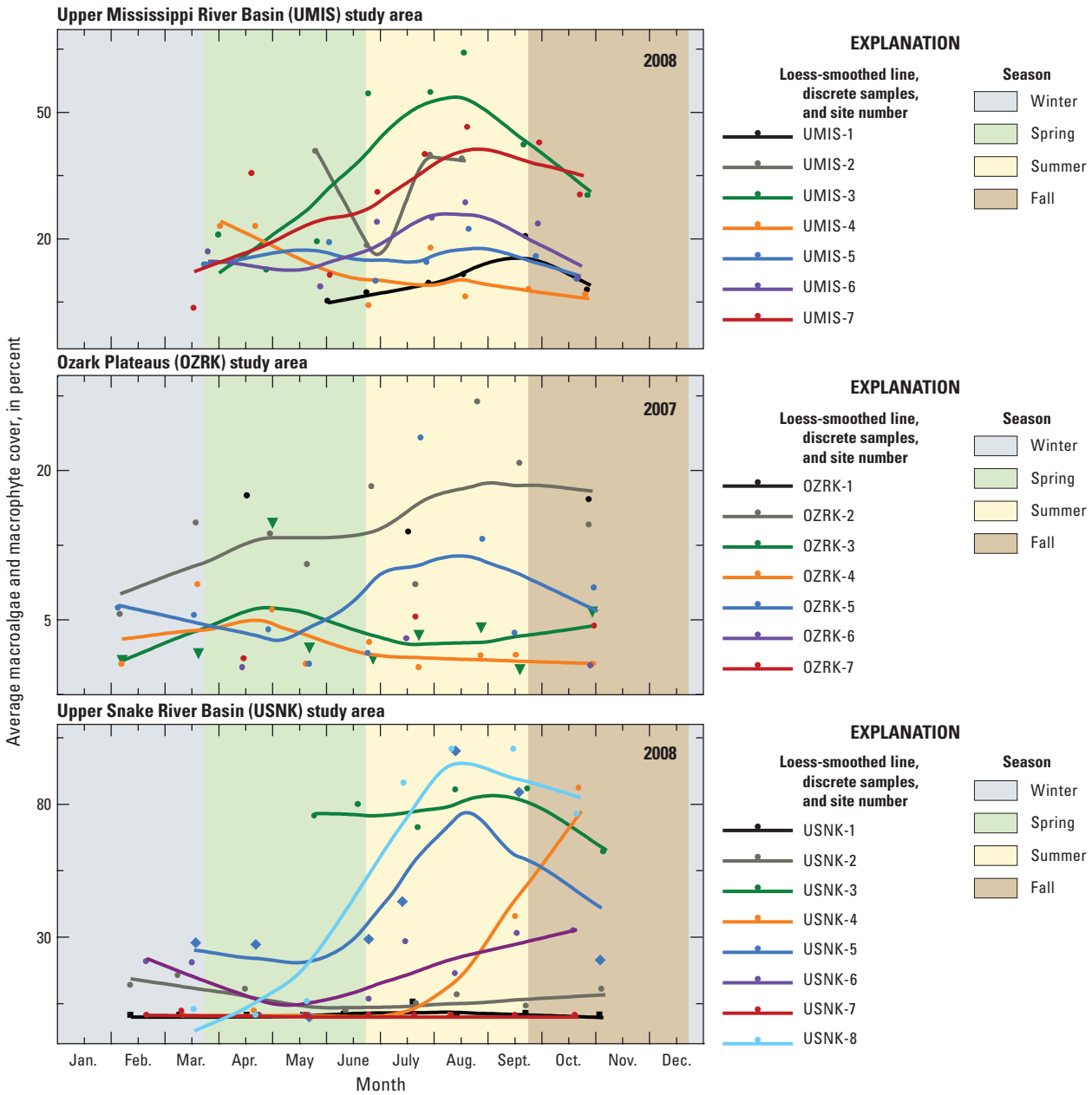

Figure 20. Graphs showing percentage of macrophyte and macroalgal cover in streams over the 1-year sampling periods.

poultry litter generally is applied in April, and commercial fertilizers are applied in summer following the spring application of manure. In the OZRK study area, if only commercial fertilizer is used, then it generally is applied February through March (Fulhage, 2009; Minor and others, 2009). This application period coincides with dry periods followed by precipitation events (summer and fall storms). In the USNK study area, split applications of nitrogen fertilizer are used in pasture fertilization and phosphorus is added in the fall (University of Idaho Extension, 2010).

Factors Affecting Seasonal Patterns and Implications

Nutrients, particularly nitrogen and phosphorus, have been identified as an important water-quality issue because of their role in the eutrophication of streams, lakes, and coastal waters (U.S. Environmental Protection Agency, 1998). More recently, nitrogen and phosphorus were identified as common stressors in streams in the United States (U.S. Environmental Protection Agency, 2006). Excessive concentrations of nitrogen or phosphorus can lead to substantial growth of aquatic vegetation in streams, resulting in problems associated

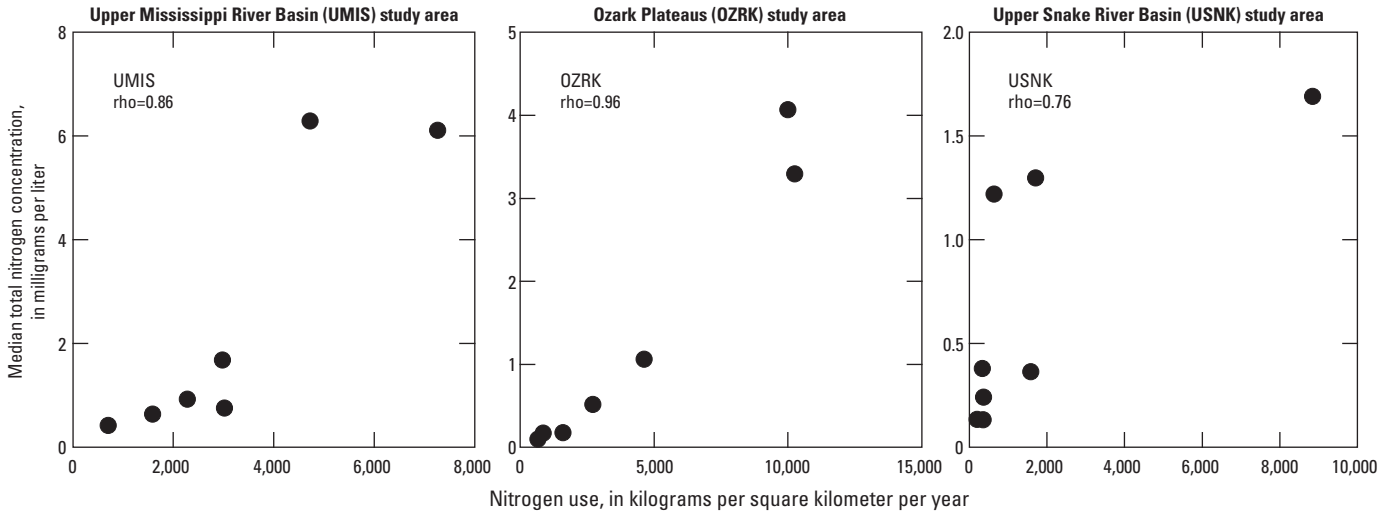

Figure 21. Plots showing relation between median in-stream total nitrogen concentrations and estimates of phosphorus use for each study area.

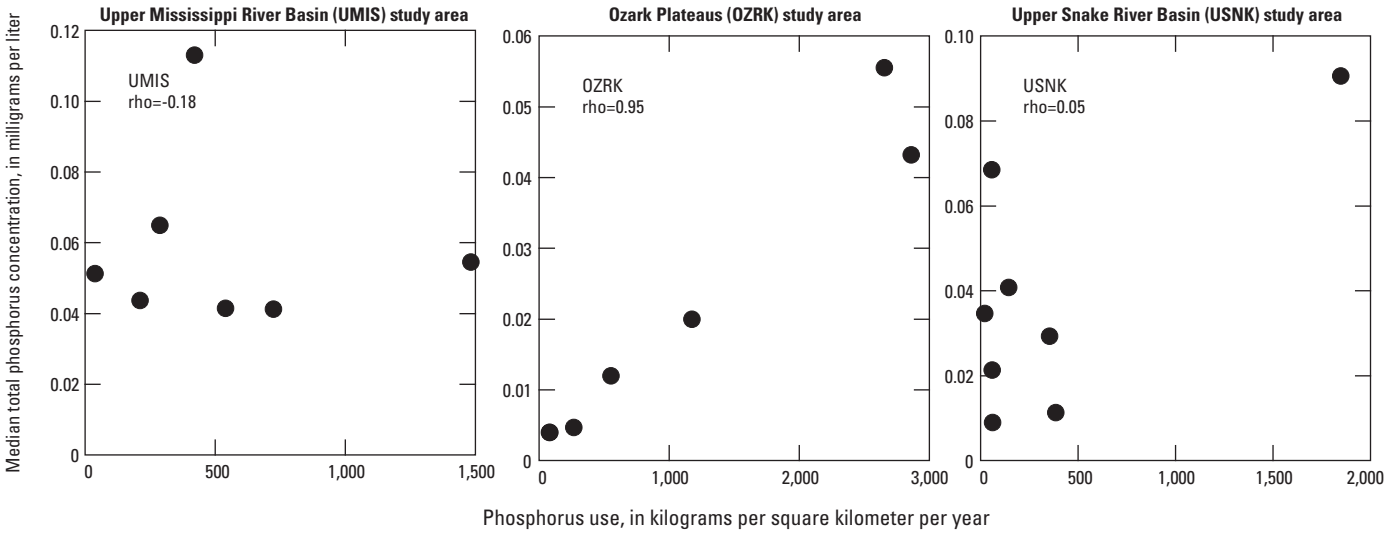

Figure 22. Plots showing relations between median in-stream total phosphorus concentrations and estimates of nitrogen use for each study area.

with water quality including wide ranges in dissolved oxygen and reductions in overall biological conditions (Heiskary and others, 2008). Although excessive algal growth is a known stressor in aquatic environments, little is known about the seasonal variability or factors that affect algal growth in different environmental settings. An understanding of the seasonal patterns in algal growth is important to effectively target land-management practices to potentially reduce excessive algal growth.

Seasonal trends in nutrient concentrations and algal responses were distinct among study areas and among sites, indicating that nutrient inputs and processing in streams are dynamic. The variations in seasonal patterns are because of numerous factors that involve timing, magnitude, source (atmospheric deposition, surface runoff, or groundwater) of inputs, changes in hydrology (dilution and concentration), and complex chemical and biological in-stream interactions.

Although it is not possible to directly link physical factors to nutrient concentrations and biological responses, the data from this study indicate that the source of water and nutrients to a stream and the intensity of large runoff events are important factors controlling in-stream concentrations.

Biological processes appear to affect nutrient concentrations during more stable low-flow periods when residence time of water in a channel is longer, thus allowing more time for biological uptake.

Seasonal variations in precipitation and streamflow affect nutrient concentrations by increasing runoff or enhancing dilution and affecting biological growth and metabolism. During high-flow events, like snowmelt runoff in the spring (UMIS and USNK study areas) and summer storm runoff (OZRK study area), constituents are transported from the land surface into streams, and in-stream concentrations may be decreased because of dilution. During this period, groundwater is discharging to streams but is a relatively small component of the overall flow in the stream. Following runoff of the spring snowmelt in the USNK and UMIS study areas, streams are primarily spring-fed or receive water through groundwater discharge for the remainder of the season. The USNK sites also are affected more by hydrologic modifications from irrigation practices than are sites in the other two study areas.

Following high-flow events, nutrient inputs from surface runoff decline. Additionally, as streamflow declines, the source of incoming water changes to groundwater discharge, and the stream water takes on the characteristics of local groundwater. The extent to which nutrients accumulate in shallow aquifers and, subsequently, discharge to streams is dependent on the hydrology and geochemistry of its basin; the amount of streamflow that comes from groundwater affects the amount of nutrients entering streams from groundwater sources. In-stream processes such as denitrification also will affect nutrients in streams by converting nitrate in groundwater to nitrogen gas if anaerobic conditions are present in the streambed (Richardson and others, 2004).

Streams with a large component of groundwater discharge throughout the year maintain fairly stable conditions with respect to nutrient concentrations because the characteristics of the streams are dominated by the characteristics of the groundwater. For example, nutrient concentrations at sites UMIS-7 and USNK-3 are relatively constant potentially because of a high percentage of streamflow from groundwater throughout summer, fall, and winter. During these more stable flow periods, biological uptake may increase, which may result in lower in-stream nutrient concentrations as residence time is increased, thus allowing for uptake from the water column. The continued low nutrient concentrations during the summer and fall at many sites and increases in benthic algal chlorophyll *a* biomass during that same time indicate that some uptake and metabolism of nutrients is occurring during the more stable streamflow conditions.

The quality of incoming groundwater also controls in-stream concentrations during low-flow periods of the year, whereas surface-runoff dynamics control concentrations during runoff events. Sites with elevated nutrient concentrations from groundwater pose a particular challenge because the length of time that the groundwater will maintain high concentrations may be extended and depends on subsurface flow-path length and complexity and on microbial and physical conditions (Tesoriero and others, 2009).

Stream hydrology, nutrient sources, and other factors such as temperature and shading also affect algal uptake that converts inorganic nutrients to biomass. Intense precipitation events result in scour of benthic algae and entrainment in the water column and flushing of upstream lakes and wetlands, whereas benthic algal growth is supported during more stable streamflow periods. The interrelation of nutrient sources and transport mechanisms determines the timing of nutrient delivery to streams and, in turn, affects algal communities in streams. In cases where nutrient concentrations are high in the winter when algal growth is limited by temperature, the effects of nutrients on excessive growth may not be an issue; however, the contribution of the relatively high nutrient concentrations in the spring and winter to later algal and macrophyte growth could not be estimated and remains a lingering question.

Management of nutrient conditions in streams is a challenge that requires an understanding of multiple factors that affect in-stream nutrient concentrations and biological uptake and growth. The results of this study indicate that prevention of runoff through various management practices, such as installation of riparian buffers that slow overland flow, could reduce overall concentrations in streams during runoff periods. In addition, land-management practices, such as the timing and locations of application of manure, may be critical to reduce runoff or infiltration that ends up in streams. For example, the application of manure on snow or frozen ground may result in runoff during the spring rather than reaching the target crops (Fallon and McNellis, 2000). Restoration of sinuosity, natural hydrologic conditions, and natural geomorphic characteristics may provide longer residence time in a stream, which increases denitrification or nutrient uptake in the streambed (Ensign and Doyle, 2005). The results of this study also indicate that the timing of sample collection can result in unique conclusions in the relations between nutrient concentrations and algal growth. For example, the relation between nutrient concentraions and algal growth varies seasonally in the OZRK study area so different conclusions could be drawn if the sampling was done during the spring or winter rather than in the summer and fall.

Relation to U.S. Environmental Protection Agency Ecoregion Criteria

The USEPA has developed recommended criteria for total nitrogen, total phosphorus, and seston chlorophyll *a* for streams within 14 nutrient ecoregions in the United States for the protection of aquatic life. The criteria are based on the 25th percentile of the data that were available at the time of analyses. The recommended criteria are not regulations but are termed guidance for States and tribes to use in development of water-quality standards (U.S. Environmental Protection Agency, 2000a, 2000b, 2000c, 2001). The data for this study

are shown in relation to the recommended nutrient criteria for context relative to other sites sampled within each of the four ecoregions in which the study sites are located.

Individual sample and site-median total nitrogen concentrations in many streams measured for this study were greater than the USEPA reference criteria established for aggregate nutrient ecoregions for the protection of aquatic life based on the 25th-percentile values of available data (U.S. Environmental Protection Agency, 2000a, 2000b, 2000c, 2001) (fig. 13). Individual total nitrogen concentrations at most of the UMIS sites were greater than the reference condition values for USEPA aggregate ecoregions 7 (mostly glaciated dairy region) and 8 (nutrient-poor, largely glaciated, Upper Midwest and Northeast) during the entire sampling period. The median total nitrogen concentration for 89 percent of the UMIS sites were greater than the reference concentrations for ecoregions 7 and 8. Approximately 40–50 percent of the OZRK and USNK sites had individual total nitrogen concentrations that were greater than the reference condition values for USEPA nutrient ecoregions 11 (central and eastern forested uplands) and 3 (xeric west), respectively. Median total nitrogen concentrations for sites were greater than the reference criteria in 57 and 38 percent of the OZRK and USNK sites, respectively. The median total nitrogen concentration at three OZRK sites (OZRK-1, OZRK-4, and OZRK-6) had median concentrations that were less than the reference concentrations. Sites draining agricultural land use were selected along a gradient of nutrient use for this study; therefore, it is not unexpected that the concentrations in the streams were greater than reference conditions at some sites.

Total phosphorus concentrations in many streams sampled for this study were greater than the USEPA reference condition values established for aggregate nutrient ecoregions for the protection of aquatic life based on the 25th-percentile values of available data (U.S. Environmental Protection Agency, 2000a, 2000b, 2000c, 2001). Total phosphorus concentrations in more than 90 percent of the UMIS samples, 46 percent of the OZRK samples, and 75 percent of the USNK samples were greater than reference condition values. Median total phosphorus concentrations for all samples at individual sites were greater than the reference criteria in 100, 43, and 63 percent of the UMIS, OZRK, and USNK sites, respectively.

Seston chlorophyll *a* concentrations in many streams for this study were greater than the USEPA reference condition values established for aggregate nutrient ecoregions for the protection of aquatic life based on the 25th-percentile values of available data (U.S. Environmental Protection Agency, 2000a, 2000b, 2000c, 2001) (fig. 17). Concentrations greater than reference condition values most commonly occurred during the winter and spring in the UMIS study area, during all seasons in the OZRK study area, and predominantly in the spring in the USNK study area.

Currently (2011), consistent national or regional values or criteria are not available for “acceptable” levels of benthic algal biomass in streams partially because of the effects of the interrelation and changes in streamflow, nutrient concentrations, and biological uptake rates. However, several studies have reported that algal biomass concentrations greater than 100 milligrams per square meter (mg/m^2) can be a concern for stream health (Welch and others, 1988) or are perceived as an issue (Suplee and others, 2009). Concentrations of benthic algal chlorophyll *a* biomass ranged from 0.3 to 406 mg/m^2 among all sites (table S7). Two sites (2 samples) in the UMIS study area, all sites in the OZRK study area (25 samples), and 5 sites in the USNK study area (8 samples) had concentrations that were greater than this value of concern (100 mg/m^2). Concentrations at the OZRK sites generally were greater than the level of concern during the spring and fall, whereas concentrations at the USNK sites were greater than the level of concern throughout the year.

Summary

Understanding how and why nutrient concentrations are changing with time in streams and rivers is essential for effectively managing and protecting water resources. Elevated concentrations of nutrients can lead to excessive growth of algae and plants that can result in decreased oxygen or variable concentrations in streams because of microbial respiration, coverage of preferred invertebrate and fish habitat, reductions in visibility for sight predators through increased turbidity, and changes in the hydrologic regime of a stream reach as increasing algal and plant biomass slows current velocity. In addition, streams convey a large percentage of nitrogen to main-stem rivers where nitrogen cycling and retention processes are small compared with transport.

In 2007 and 2008, the U.S. Geological Survey implemented the Nutrient Enrichment Effects Topical team as part of the National Water-Quality Assessment (NAWQA) Program to determine how nutrients are changing in agriculturally affected streams over time in different geographic regions of the United States. Wadeable streams in three geographically distinct NAWQA study areas were selected for sampling to address temporal patterns of nutrient and carbon concentrations and biological responses among agricultural streams in different environmental settings. These three study areas were the Upper Mississippi River Basin (UMIS) in Minnesota, Ozark Plateaus (OZRK) in southern Missouri and northern Arkansas, and Upper Snake River Basin (USNK) in southern Idaho. The objectives of this study were to determine (1) seasonal patterns in nutrients, carbon, and algal responses; and (2) the effect of geographic location and associated physical characteristics on seasonal patterns. For this study, 22 sites were sampled monthly for 1 year during 2007 or 2008.

The streams are in unique settings within each study area. Streams within the USNK study area primarily are located within basins dominated by grassland and rangeland, whereas those in the UMIS study area primarily are located within areas dominated by row crop agriculture with some forested and wetlands cover. The OZRK streams are located within

basins that are primarily forested and are dominated by pasture and poultry production. Spring snowmelt and early spring precipitation events are the dominant hydrologic events in the two northern study areas (USNK and UMIS) from March through May resulting in delivery of large quantities of water from surface runoff during that period relative to runoff during late summer and winter. In the OZRK study area, minimum monthly streamflows typically occur in summer and fall (July through October), whereas maximum monthly streamflows typically occur in spring (March through May).

Some patterns of nutrients, carbon, and algal responses were similar among study areas. For example, nitrate concentrations were greatest during the winter in all study areas potentially because of a reduction in algal assimmilation of nitrate. Decreases in nitrate concentrations during the spring and summer at most stream sites coincided with increased streamflow during snowmelt runoff or spring storms indicating dilution. The continued decrease in nitrate concentrations during summer is because of some other process such as a reduction in nitrate inputs (from decrased surface runoff) or increases in in-stream assimilation of nitrate with conversion to algal biomass.

Ammonia concentration trends were similar at UMIS and USNK sampling sites with winter peak concentrations and rapid decreases in ammonia concentrations by spring or early summer. In contrast, trends in ammonia concentrations at OZRK sampling sites were more variable with peak concentrations occurring later in the year. Ammonia may accumulate in stream water in the winter under ice and snow cover because of limited algal assimilation from colder water temperatures and ice cover and because of increased mineralization of decaying organic matter under reducing conditions within stream bottom sediments. Low ammonia concentrations throughout the remainder of the sampling period also could be because of ammonia assimilation by seston and benthic algae as water temperatures warm and light availability increases in the spring and summer periods.

Seasonal patterns of total particulate nitrogen, which include algal cells and other particulate living and nonliving material, were similar among the UMIS and USNK sites with a spring peak concentration, late summer lows, and a resurgence in the fall. Total particulate nitrogen concentrations at the OZRK sites were not significantly different among the seasons, and the seasonal trends at OZRK sites were not as pronounced as those for the UMIS and USNK sites. In contrast to concentrations of individual forms of nitrogen, total nitrogen concentrations did not change much among the seasons at individual streams within the UMIS and OZRK study areas, likely because seasonal differences in total nitrogen can be obscured because of differences in the composition of the organic and inorganic nitrogen forms present.

Phosphorus concentrations and the type of phosphorus present change with changing hydrologic conditions. Orthophosphate concentrations tended to be greater in the summer at UMIS sites, whereas total phosphorus concentrations at most UMIS and USNK sites peaked in the spring during runoff and then declined through the remainder of the sampling period. Total phosphorus and orthophosphate concentrations in OZRK streams peaked during summer. Orthophosphate may increase in streams in the late summer when surface runoff composes less of total streamflow and when groundwater containing orthophosphate becomes a more dominant source in streams during lower flows.

Patterns in dissolved organic carbon concentrations varied among sites, but concentrations were greatest during the spring and early summer at most sites and were correlated with the seston chlorophyll *a* concentrations in summer at USNK and OZRK sites. The positive correlation with seston chlorophyll *a* concentrations at OZRK and USNK sites indicates that a predominant source of dissolved organic carbon may be from algal cells that release carbon. The lack of correlations between dissolved organic carbon and seston chlorophyll *a* concentrations at the UMIS sites may indicate that dissolved organic carbon in these streams is exported from the relatively greater percentage of wetland and upstream lakes rather than being predominately from in-stream production.

Seston chlorophyll *a* concentrations also were similar among most sites and were greatest early in the growing season (spring) at most sites. This trend also was observed for particulate organic carbon and particulate nitrogen indicating that much of the particulate nitrogen was composed of sestonic algal cells or benthic algal cells entrained in the water column. The trend in seston chlorophyll *a* concentrations potentially may be because of an increase in seston algal growth during the spring when temperatures warm and shading is not available. This pattern is supported by reductions in inorganic forms of nitrogen (nitrate and ammonia), and orthophosphate concentrations during that same period at many sites. Alternatively, ammonia and other constituents are diluted in the spring during runoff events, and the seston chlorophyll *a* in the water column is benthic algae scoured from the high flow and entrained in the water column.

The major spring and summer runoff events coincide with reductions in benthic chlorophyll *a* concentrations, indicating scour of benthic algae from the channel bottom during that period for most sites. Seasonal patterns in concentrations of benthic algal chlorophyll *a* biomass and benthic ash-free dry mass were more variable than trends for other constituents measured. Concentrations tended to be greater at UMIS sites in the spring, at OZRK sites in the fall, and at USNK sites in the winter.

Concentrations of total nitrogen, total phosphorus, and seston chlorophyll *a* measured at some sites in this study were greater than U.S. Environmental Protection Agency (USEPA) ecoregion-based nutrient criteria established for the protection of aquatic life. This reflects the original study design for which sites were selected over a gradient of nutrient concentrations. More than one-half of the sites within each study area had total nitrogen and total phosphorus concentrations that were greater than the corresponding USEPA nutrient reference condition criteria during the sampling period.

Seston chlorophyll *a* concentrations were greater than USEPA criteria primarily in the winter and spring at UMIS, during all seasons in the OZRK sites, and primarily in the spring at the USNK sites. Consistent national or regional values or criteria are not available for "acceptable" levels of benthic algal biomass in streams; however, several studies have reported that algal biomass concentrations greater than 100 milligrams per square meter can be a concern for stream health. Two sites in the UMIS study area (2 samples), all sites in the OZRK study area (25 samples), and 5 sites in the USNK study area (8 samples) had concentrations that were greater than this value of concern. Concentrations at the OZRK sites generally were greater than the level of concern during the spring and fall, whereas concentrations at the USNK sites were greater than the level of concern throughout the year.

Seasonal trends were distinct for some chemical concentrations and algal responses among study areas and among sites, indicating that nutrient inputs and processing in streams are dynamic. The variations in seasonal trends are because of numerous factors that involve timing, magnitude, and source (atmospheric deposition, surface runoff or groundwater) of inputs, changes in hydrology (dilution and concentration), and complex chemical and biological in-stream interactions.

Although it is not possible to directly link physical factors to chemical concentrations and biological responses, the data from this study indicate that the source of water and nutrients to a stream and the intensity of major runoff events are important factors controlling in-stream conditions. Biological processes appear to affect nutrient concentrations during more stable low-flow periods during late summer, fall, and winter when residence time of water in a channel is longer and allows more time for biological uptake.

Management of nutrient conditions in streams is a challenge that requires an understanding of multiple factors that affect in-stream nutrient concentrations and biological uptake and growth. An understanding of the seasonal patterns in algal growth can assist management decisions related to sample collection timing. The results of this study indicate that the timing of sample collection can result in unique conclusions. For example, the relation between nutrient concentraions and algal growth varies seasonally in the OZRK study area so different conclusions could be drawn if the sampling was done during the spring or winter rather than in the summer and fall. The results of this study also indicate that prevention of runoff through various management practices, such as installation of riparian buffers that slow overland flow, could reduce overall concentrations in streams during runoff periods. In addition, land-management practices, such as the timing and locations of application of manure, may be critical to reduce runoff or infiltration that ends up in streams. For example, the application of manure on snow or frozen ground may result in runoff during the spring rather than reaching the target crops. Restoration of sinuosity, natural hydrologic conditions, and natural geomorphic characteristics may provide longer residence time in a stream, which increases denitrification or nutrient uptake in the streambed.

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Supplemental Tables

Table S1. Soil characteristics in the drainage basin upstream from each sampling site.

[Data from Brightbill and Frankforter (2010); USGS, U.S. Geological Survey; cm/h, centimeter per hour]

USGS site number (shown on fig. 1)	Average permeability (cm/h)	Average clay content (percent)	Average silt content (percent)	Average sand content (percent)
Upper Mississippi River Basin (UMIS) study area				
UMIS-1	4.9	14	30	56
UMIS-2	7.9	10	21	69
UMIS-3	3.2	10	25	65
UMIS-4	7.7	8	23	69
UMIS-5	10.5	6	11	83
UMIS-6	6.2	13	29	58
UMIS-7	2.8	13	51	36
Ozark Plateaus (OZRK) study area				
OZRK-1	2.4	32	38	30
OZRK-2	1.8	32	39	30
OZRK-3	1.7	36	41	23
OZRK-4	1.8	36	38	27
OZRK-5	1.8	30	35	35
OZRK-6	1.9	41	40	20
OZRK-7	1.2	40	44	17
Upper Snake River Basin (USNK) study area				
USNK-1	2.6	20	41	40
USNK-2	0.8	33	44	23
USNK-3	3.7	15	43	42
USNK-4	3.1	16	44	40
USNK-5	5.9	17	44	38
USNK-6	2.0	22	37	41
USNK-7	1.9	24	44	32
USNK-8	4.0	18	52	30

Table S2. Land-use characteristics of the drainage basin upstream from each sampling site estimated for 2001.

[Data from Brightbill and Frankforter (2010)]

Site number (shown on fig. 1)	Land-use percentages							
	Open water	Developed	Natural or barren	Forest	Shrub and grassland	Pasture and hay	Cultivated cropland	Wetland
Upper Mississippi River Basin (UMIS) study area								
UMIS-1	8.2	1.4	0.0	83.1	2.2	1.9	0.2	2.9
UMIS-2	7.8	2.7	.0	56.4	4.6	4.4	13.7	10.5
UMIS-3	.6	4.1	.0	22.5	9.2	28.6	16.5	18.6
UMIS-4	.1	3.4	.0	8.2	2.4	26.1	50.9	8.9
UMIS-5	1.9	8.2	.0	27.9	4.2	12.1	29.2	16.5
UMIS-6	4.3	4.5	.0	43.6	3.9	20.2	11.2	12.3
UMIS-7	.2	6.2	.0	7.7	1.5	22.2	61.5	.7
Ozark Plateaus (OZRK) study area								
OZRK-1	0.1	3.2	0.1	80.0	1.3	15.1	0.2	0.1
OZRK-2	.0	4.0	.2	58.3	1.7	35.4	.1	.3
OZRK-3	.0	5.3	.1	23.8	.4	70.2	.0	.2
OZRK-4	.1	2.8	.0	94.9	.7	1.5	.0	.1
OZRK-5	.0	4.6	.0	64.4	3.4	27.4	.3	.0
OZRK-6	.1	3.7	.0	87.8	4.5	3.7	.1	.1
OZRK-7	.0	5.4	.3	13.0	.2	80.8	.1	.1
Upper Snake River Basin (USNK) study area								
USNK-1	0.0	0.0	0.0	9.9	88.4	1.4	0.0	0.3
USNK-2	.0	.8	.0	12.0	87.2	.0	.0	.0
USNK-3	.1	3.0	.0	0.0	67.8	7.3	21.8	.0
USNK-4	.0	1.8	.4	43.0	51.9	.7	.5	1.5
USNK-5	.0	4.9	.0	0.4	42.0	24.0	23.2	5.6
USNK-6	.2	1.2	.1	6.6	89.1	1.1	.9	.9
USNK-7	.0	.1	.1	25.6	70.8	1.1	.1	1.2
USNK-8	.3	3.9	.1	0.8	49.6	15.9	25.5	4.0

Table S3. Estimated nitrogen and phosphorus use during 2002 in the drainage basin upstream from each sampling site.

[Data from Brightbill and Frankforter, (2010); USGS, U.S. Geological Survey; DEP, deposition; UCAF, unconfined feed lot; CAF, confined feed lot]

USGS site number (shown on fig. 1)	Nitrogen use, in kilograms per square kilometer per year						Phosphorus use, in kilograms per square kilometer per year				
	Total from all sources	Atmospheric DEP	Manure in UCAF	Manure in CAF	Farm	Non-Farm	Total from all sources	Manure in UCAF	Manure in CAF	Farm	Nonfarm
Upper Mississippi River Basin (UMIS) study area											
UMIS-1	701.3	542.3	47.6	27.0	84.1	0.4	37.3	14.3	7.4	15.4	0.1
UMIS-2	1,589.1	576.0	77.9	219.5	714.4	1.3	211.1	19.1	60.6	130.9	.5
UMIS-3	3,019.5	574.8	429.7	791.3	1,222.6	1.2	540.3	116.1	199.8	224.0	.5
UMIS-4	7,264.9	656.4	653.2	2,554.8	3,399.5	1.0	1,483.9	178.1	682.7	622.7	.4
UMIS-5	2,977.5	914.0	124.3	268.2	1,599.8	71.2	420.1	31.7	67.5	293.1	27.8
UMIS-6	2,280.3	764.3	267.6	466.4	776.6	5.3	287.3	75.7	90.0	120.9	.6
UMIS-7	4,719.7	826.5	610.2	1,336.3	1,928.0	18.7	724.8	162.8	259.5	300.2	2.2
Ozark Plateaus (OZRK) study area											
OZRK-1	1,605.9	522.0	454.9	49.2	579.7	0.1	264.5	143.1	15.1	106.3	0.0
OZRK-2	4,634.0	368.1	1,391.3	1,966.8	904.1	3.7	1,172.7	427.0	619.2	125.2	1.3
OZRK-3	10,248.2	380.4	2,326.7	6,063.3	1,460.9	16.8	2,858.8	695.7	1,954.9	202.4	5.9
OZRK-4	665.6	349.7	100.0	125.2	90.4	.3	81.3	31.5	37.1	12.5	.1
OZRK-5	2,711.8	395.7	798.1	793.5	724.4	.0	551.5	233.0	218.2	100.3	.0
OZRK-6	859.9	564.7	153.1	18.5	118.6	4.9	77.2	48.7	5.5	21.7	1.3
OZRK-7	10,000.2	401.0	2,714.2	4,684.6	2,186.5	13.9	2,654.5	841.6	1,408.4	400.8	3.6
Upper Snake River Basin (USNK) study area											
USNK-1	339.8	140.6	145.7	16.2	37.3	0.0	57.1	42.9	4.1	10.2	0.0
USNK-2	201.1	123.4	70.9	1.8	5.1	.0	21.0	19.4	.4	1.3	.0
USNK-3	8,847.0	90.1	1,997.3	4,170.2	2,583.0	6.5	1,848.2	470.1	726.7	649.9	1.4
USNK-4	365.5	92.4	229.1	1.8	28.5	13.7	62.8	52.5	.2	7.2	2.9
USNK-5	1,579.3	94.0	582.5	53.0	849.8	.0	354.4	133.4	7.2	213.8	.0
USNK-6	633.6	82.7	51.2	187.1	312.1	.5	144.1	17.5	48.0	78.5	.1
USNK-7	355.7	94.0	243.3	1.1	17.2	.2	60.4	55.9	.1	4.3	.0
USNK-8	1,707.7	94.0	616.3	58.6	938.8	.0	385.4	141.2	8.0	236.2	.0

Table S4. Climate and hydrologic statistics for sampling sites.

[Data from Brightbill and Frankforter (2010); USGS, U.S. Geological Survey; °C, degrees Celsius; cm, centimeter; mm/yr, millimeter per year; mm, millimeter]

USGS site number (shown on fig. 1)	Mean annual temperature for 1980–97 (°C)	Mean annual precipitation for 1980–97 (cm)	Mean runoff for 1990–2002 (mm/yr)	Base-flow index (percent)	Mean annual potential evapotranspiration for 1971–90 (mm)
Upper Mississippi River Basin (UMIS) study area					
UMIS-1	3.8	71	164	62	550
UMIS-2	4.1	69	169	67	581
UMIS-3	5.2	71	141	67	598
UMIS-4	5.8	74	148	58	613
UMIS-5	6.5	86	173	55	631
UMIS-6	5.7	82	274	58	595
UMIS-7	6.5	86	267	57	635
Ozark Plateaus (OZRK) study area					
OZRK-1	12.6	125	336	26	805
OZRK-2	13.6	122	427	35	813
OZRK-3	13.8	118	429	37	814
OZRK-4	14.3	130	451	38	839
OZRK-5	15.1	129	476	37	864
OZRK-6	12.8	126	504	42	796
OZRK-7	13.3	121	366	47	784
Upper Snake River Basin (USNK) study area					
USNK-1	6.6	44	26	75	547
USNK-2	5.3	60	25	77	498
USNK-3	9.5	27	58	73	667
USNK-4	2.1	70	167	74	448
USNK-5	6.3	40	169	75	542
USNK-6	5.1	51	75	69	524
USNK-7	2.9	63	250	75	471
USNK-8	6.3	39	245	76	540

Table S5. Width, depth, velocity, water-surface gradient, and geomorphic units for sampling sites.

[Mean values determined for sampling periods: 2007 for OZRK study area and 2008 for UMIS and USNK study areas. USGS, U.S. Geological Survey; m^3/s, cubic meters per second; m/s, meters per second; CV, coefficient of variation]

USGS site number (shown on fig. 1)	Mean wetted width (meters)	Mean depth (meters)	Mean percent pools	Mean percent riffles	Mean percent runs	Mean streamflow (m^3/s)	Mean stream velocity (m/s)	Mean stream velocity (CV)	Mean water-surface gradient (dimensionless)
Upper Mississippi River Basin (UMIS) study area									
UMIS-1	7.6	0.31	0	33	67	1.10	0.48	43.3	0.0025
UMIS-2	11.9	.49	4	9	86	1.57	.33	55.5	.0013
UMIS-3	8.6	.39	13	7	80	.47	.15	74.5	.0004
UMIS-4	8.0	.33	0	0	100	.62	.18	61.5	.0004
UMIS-5	7.0	.48	0	0	100	.88	.28	39.9	.0004
UMIS-6	10.0	.59	0	0	100	1.93	.25	43.7	.0007
UMIS-7	8.9	.47	0	0	100	.66	.24	51.7	.0005
Ozark Plateaus (OZRK) study area									
OZRK-1	10.7	0.27	11	23	66	0.67	0.30	91.6	0.0035
OZRK-2	11.5	.26	18	16	66	.65	.13	117.6	.0028
OZRK-3	8.6	.27	31	21	49	.37	.19	105.2	.0018
OZRK-4	9.6	.34	32	21	48	.28	.05	128.5	.0024
OZRK-5	8.7	.28	35	22	44	.46	.17	120.9	.0033
OZRK-6	9.6	.46	27	29	44	.99	.36	95.8	.0034
OZRK-7	7.2	.38	51	28	21	.79	.33	59.7	.0032
Upper Snake River Basin (USNK) study area									
USNK-1	6.3	0.56	22	24	54	1.2	0.39	58.7	0.0050
USNK-2	3.5	.24	9	38	50	.39	.23	89.2	.0050
USNK-3	7.2	.61	0	9	91	.97	.33	64.5	.0019
USNK-4	16.9	.30	11	20	69	6.03	.22	71.9	.0014
USNK-5	3.4	.32	0	18	82	.12	.27	87.9	.0020
USNK-6	9.0	.21	0	34	66	3.87	.15	84.3	.0030
USNK-7	12.4	.31	0	50	50	3.28	.54	34.4	.0062
USNK-8	20.4	.80	0	0	100	.64	.05	91.0	.0003

Table S6. Percentage of land cover and land use within a 50-meter buffer along either side of the stream sampling reach and channel shading.

[USGS, U.S. Geological Survey]

USGS site number (shown on fig. 1)	Barren land	Cropland	Farmstead	Grassland	Open water	Shrubland	Urban/ built-up land	Wetland	Woody vegetation	Other	Mean channel shading (percent)
Upper Mississippi River Basin (UMIS) study area											
UMIS-1	0	3	1	3	0	3	1	4	85	0	29
UMIS-2	0	3	1	4	8	28	1	41	14	0	39
UMIS-3	0	12	0	5	9	49	0	17	8	0	28
UMIS-4	0	0	0	2	0	2	0	0	96	0	49
UMIS-5	0	0	2	0	8	0	1	74	15	0	31
UMIS-6	0	0	0	1	5	0	0	0	94	0	24
UMIS-7	0	14	1	17	0	2	0	0	66	0	30
Ozark Plateaus (OZRK) study area											
OZRK-1	13	0	0	18	7	0	0	0	62	0	21
OZRK-2	0	0	0	8	7	0	0	0	79	6	33
OZRK-3	2	0	0	19	5	0	0	0	74	0	51
OZRK-4	0	0	0	0	7	0	0	0	93	0	43
OZRK-5	0	0	0	6	0	0	0	0	94	0	58
OZRK-6	0	0	0	5	5	0	1	0	67	12	36
OZRK-7	0	0	0	51	3	0	0	0	46	0	38
Upper Snake River Basin (USNK) study area											
USNK-1	0	0	0	0	0	19	0	81	0	0	9
USNK-2	0	0	0	3	0	45	0	0	52	0	26
USNK-3	0	31	7	2	5	4	1	50	0	0	1
USNK-4	44	0	0	2	19	4	1	17	13	0	11
USNK-5	0	0	0	62	0	0	23	15	0	0	10
USNK-6	0	0	0	65	0	0	0	35	0	0	6
USNK-7	6	0	0	18	0	9	0	19	48	0	30
USNK-8	0	4	1	31	16	0	1	47	0	0	9

Table S7. Water-quality data used for analyses of seasonal patterns in streams.

The Excel file can be accessed at *http://pubs.usgs.gov/sir/2012/5086/downloads/tableS7.xlsx*.

Table S8. Summary statistics for benthic algal community taxa.

[USGS, U.S. Geological Survey]

USGS site number (shown on fig. 1)	Diatoms (percent abundance)			Green algae (percent abundance)			Blue-green algae (percent abundance)			Red algae (percent abundance)		
	Minimum	Median	Maximum	Minimum	Median	Maximum	Minimum	Median	Maximum	Minimum	Median	Maximum
Upper Mississippi River Basin (UMIS) study area												
UMIS-1	47	55.3	63	0	1.4	2	29	32.0	51	0.0	3.1	15
UMIS-2	29	41.5	54	1	1.5	2	32	49.3	66	3	6.2	9
UMIS-3	44	45.3	76	1	1.3	1	18	50.6	55	.0	1.3	4
UMIS-4	35	59.1	87	.0	.5	3	11	38.1	64	.0	.0	2
UMIS-5	34	57.6	63	.0	.0	3	34	38.5	62	1	2.6	4
UMIS-6	54	57.6	71	.0	12.2	14	27	29.9	32	.0	.0	.0
UMIS-7	54	65.0	75	.0	.2	3	21	34.5	45	.0	.0	1
Ozark Plateaus (OZRK) study area												
OZRK-1	1	13.0	19	13	16.1	53	46	62.4	74	0.0	0.0	3
OZRK-2	.0	.6	14	1	1.2	1	85	98.4	98	.0	.0	.0
OZRK-3	.0	.5	6	.0	.6	10	90	94.2	99	.0	.0	.2
OZRK-4	20	27.0	34	1	7.2	14	65	65.6	66	.0	.2	.3
OZRK-5	3	9.8	12	1	3.6	6	80	81.2	95	.3	2.6	5
OZRK-6	1	4.4	22	3	6.2	12	77	87.5	92	.0	.0	.02
OZRK-7	.0	.2	2	1	1.4	2	96	98.2	99	.0	.1	.3
Upper Snake River Basin (USNK) study area												
USNK-1	20	31.0	46	0.0	1.3	6	48	63.1	74	0.0	4.4	6
USNK-2	16	21.4	27	3	4.9	7	69	71.0	74	.0	.0	6
USNK-3	46	46.8	56	3	5.6	8	41	46.0	48	.0	.0	.0
USNK-4	27	36.0	40	9	22.4	26	34	46.3	51	.0	.0	4
USNK-5	10	28.7	33	1	2.1	11	60	67.1	74	2	5.7	6
USNK-6	11	38.0	46	2	4.0	5	50	57.1	87	.0	.0	1
USNK-7	8	11.9	25	.0	6.0	7	55	81.0	92	.0	.0	.0
USNK-8	10	16.7	22	1	2.6	3	73	79.1	86	2	2.7	3

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