Nutrients, Eutrophic Response, and Fish Anomalies in the Little Miami River, Ohio[1]

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ABSTRACT. We documented the eutrophic and chemical environment in the Little Miami River (LMR) to better understand the interaction between eutrophication, eutrophic response variables, and the health of aquatic organisms. Total phosphorus (TP) and soluble reactive phosphorus (SRP), nitrogen, dissolved oxygen (DO), benthic and sestonic algal biomass, benthic phosphorus storage, aqueous trace metals (Cd, Cr, Cu, Se, Zn), heavy metals (Al, Fe, Mn) and major cations (Ca, K, Mg, Na, Si) were analyzed at twelve sites over two summers. Results showed excess TP (>70 ug/L, $p<0.05$) and SRP (≥62.5 ug/L, $p<0.05$), borderline nuisance benthic algal biomass (mg/L chlorophyll *a*/m²) (periphyton: mean = 73.8 +/- 74.2, n = 125; *Cladophora*: mean = 216.7 +/- 380.7, n = 54), excess benthic phosphorus storage (mg P/m²) (periphyton: mean = 45.5 +/- 23.2, n = 64; *Cladophora*: mean = 129.3 +/- 224, n = 52), and high daytime DO (mean = 9.1 +/- 1.5 mg/L, n = 132). Previous studies showed aqueous phosphorus concentration and diurnal DO swings were positively correlated with fish anomalies (OEPA 1995, 2000). In this study, however, periphyton phosphorus (P) was the only eutrophic response variable to correlate with the distribution of fish anomalies reported by OEPA in 1995 and 2000, and the association was negative (R^2 = 0.143, p = 0.002, m = -1.634, df = 1, 62). We concluded that aqueous nutrients, eutrophic response variables, and/or water chemistry alone did not explain the occurrence of fish anomalies in the LMR.

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INTRODUCTION

The impact of both point and non-point source pollution can be detrimental to the biological integrity of aquatic systems and currently appears to be affecting water quality in the Little Miami River (LMR) in Ohio. The Ohio Environmental Protection Agency (OEPA) has reported high occurrences of fish deformities, fin erosions, lesions, and tumors (that is, DELT anomalies) (OEPA 1995, 2000). In healthy fish communities less than 1 to 2% of fish are afflicted with such conditions (Reash and Berra 1989); in sections of the LMR, up to 10% of fish are affected by one or more anomalies (OEPA 1995, 2000).

As well as having a high incidence of DELT anomalies, parts of the river fail to meet full attainment of exceptional warm water habitat (EWH) criteria based on diversity and abundance of fish and macroinvertebrate communities. A 1993 survey found only 41% of the LMR in full attainment of EWH criteria while 56% was in partial attainment and 3% was in non-attainment (OEPA 1995). The portion of the river in non-attainment of EWH criteria increased to 12.7% by 1998 (OEPA 2000).

Nutrient enrichment has been associated with nuisance algal growth (Chetelat and others 1999; McCormick and O'Dell 1996; Welch and others 1989) that decreases habitat availability and impairs diversity and abundance of aquatic species (Carpenter and others 1998). High algal biomass can cause severe diurnal DO and pH swings (Correll 1998) that release toxic metals and other substances from sediments, thereby contaminating the aquatic environment (Brick and Moore 1996). DELT anomalies like those found in LMR fish have been associated with exposure to, and accumulation of, metals that interfere with nutrition, development (Bengtsson and Larsson 1986; Meteyer and others 1988; Prageesthwaran and others 1987), and immune system function (Hetrick and others 1979; Knittel 1981; MacFarlane and others 1986; Reash and Berra 1989; Sharples and others 1994). Variation in DO and pH enhances metal availability (Brick and Moore 1996), uptake (Bentley 1992; Livonen and others 1992), and toxicity to aquatic organisms (Freeman 1980; Hughes and Flos 1978; Kane and Rabeni 1987; Schubauer-Berigan and others 1993). DELT anomalies in the LMR were correlated with cumulative effluent load and total phosphorus (in 1993) and large diurnal dissolved oxygen (DO) swings characterized by supersaturated daytime conditions and very low nighttime concentrations (in 1998) (OEPA 1995, 2000).

Surface water eutrophication due to excess nitrogen (N) and/or phosphorus (P) was the focus of research and publications used by the United States Environmental Protection Agency (USEPA) to develop nutrient and eutrophic response variable criteria (USEPA 2000a,b). These criteria are specific to streams with similar geology, topography, land use, and nutrient concentrations, and serve as a starting point for states to delineate their own criteria. USEPA analyzed available data from each subecoregion and developed recommendations for N, P, sestonic, and benthic algal biomass (that is, primary eutrophic response variables), DO, and phosphorus storage in benthic algae (that is, secondary response variables). Recommended criteria for southwest Ohio streams, including the LMR (that is, Level III Ecoregion VI, Subecoregion 55), are summarized in Table 1. To date, there have been no published comparisons of LMR data to these criteria.

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TABLE 1

Recommended summer average nutrient and response variable criteria for streams in level III subecoregion 55 (USEPA 2000a,b).

Variable	Recommended Summer Mean
Nutrients	
Total Phosphorus (TP) ug/L	70.0
Soluble Reactive Phosphorus (SRP) ug/L	62.5
Total Nitrogen (TN) mg/L	3.5
Primary Eutrophic Response Variables	
Chlorophyll *a* (ug/L)	5.37
Chlorophyll *a* (mg/m^2)*	150
Secondary Eutrophic Response Variables	
Dissolved Oxygen (DO) mg/L	7.9
Phosphorus (mg/m^2)*	20

*Denotes concentration in benthic algae.

Two objectives are addressed in this paper. First, to document the extent of eutrophication in the LMR by comparing nutrient and eutrophic response data to USEPA recommended criteria (2000a,b). Null hypotheses to be tested relative to this objective were: 1) nutrients (P and N) do not exceed recommended concentrations for prevention of nuisance algal growth, 2) sestonic and benthic algal biomass, the primary response variables associated with eutrophication, do not exceed USEPA criteria, and 3) dissolved oxygen concentration and benthic algal phosphorus accumulation, secondary response variables associated with eutrophication, do not exceed USEPA criteria. The second objective was to analyze aqueous trace metals, heavy metals, and major cations in LMR water samples. The null hypothesis tested with these data was that there is no relationship between eutrophication and aqueous metals and the occurrence of DELT anomalies in the LMR.

MATERIALS AND METHODS

Site Description

The LMR is a meandering 172 km-long north temperate river that begins in North-central Ohio and ends in Cincinnati, OH, where it merges with the Ohio River (Fig. 1). Land use throughout the 4,549-km^2 watershed is primarily agricultural, especially in the northern half where non-point source pollution creates concern for water quality (OEPA 1995, 2000). The southern half of the watershed supports densely populated areas where development pressure, urban runoff, and wastewater treatment plant (WWTP) effluent are significant sources of LMR pollution (Fig. 1) (Manhart 1998; OEPA 1995, 2000; Staley 1997).

Despite the fact that agriculture dominates land use, point sources (that is, WWTPs) may have a significant impact on LMR water quality. OEPA estimated that during low flow periods the volume of WWTP effluent running through some segments of the LMR is as much as 30 to 70% of the total water flow (OEPA 1995). Manhart (1998), using historical river discharge and WWTP effluent release data from two sites on the mainstem, Spring Valley and Milford (Fig. 1), determined that 50% of the river flow consisted of WWTP effluent between 17 and 22% of the time, respectively. Phosphorus, mainly from WWTP effluent, is the primary nutrient associated with water quality issues in the LMR (Manhart 1998; OEPA 1995, 2000; Staley 1997).

Sampling Strategy

Eleven riffle sites (Fig. 1) were sampled on 6 dates (that is, 3 times per year) during the summer of 1996 and 1997. Sampling dates in mid-June (13^{th} to 17^{th}), late-July (27^{th} to 30^{th}), and early-September (8^{th} to 11^{th}) of each year were chosen by anticipating that algal response to nutrient enrichment would be most dramatic over the summer, low-flow season (Lohman and others 1992). A sampling date consisted of two consecutive field days in which six sites in the northern half of the LMR (that is, upstream of km 82) were sampled on day one, and five sites in the southern half (that is, downstream of km 82) were sampled on day two (Fig. 1). Sampling sites were selected based on access to the river and the presence of wadeable riffles found during the spring flows of April and May 1996.

Water Sample Collection

Dissolved oxygen (mg/L) and pH were measured in the field using a YSI® brand Model 58 portable oxygen meter (Yellow Springs, OH) and a HACH® brand pH meter (Loveland, CO), respectively. Meters were standardized according to the manufacturer's instructions.

Water samples were collected in 1.0 L amber polypropylene bottles (that is, one bottle per site, per sampling event) and stored on ice in closed coolers for transport back to the laboratory. Duplicate unfiltered water samples were removed for total phosphorus (TP) determination (APHA 1992). Remaining water was filtered through ashed 0.8 um, A/E Type 47 mm glass fiber filters (Wetzel and Likens 1991). Filters, containing phytoplankton, were placed in 90% acetone and kept in the dark at 4° C for 24 hours to extract chlorophyll *a* from algal cells (APHA 1992; Wetzel and Likens 1991). Duplicate aliquots of the filtrate were collected for soluble reactive phosphorus (SRP), ammonia (NH_4), nitrate (NO_3), trace metal (Cd, Cr, Cu, Se, Zn), heavy metal (Al, Fe, Mn), and major cation (Ca, K, Mg, Na) analysis (APHA 1992). Trace metal, heavy metal, and major cation samples were preserved with trace metal grade HNO_3. Nutrient samples were frozen for up to four weeks prior to analysis (APHA 1992).

Analysis of Water Samples

Phosphorus (TP and SRP) concentration (ug/L) was measured using method 4500-P described in *Standard*

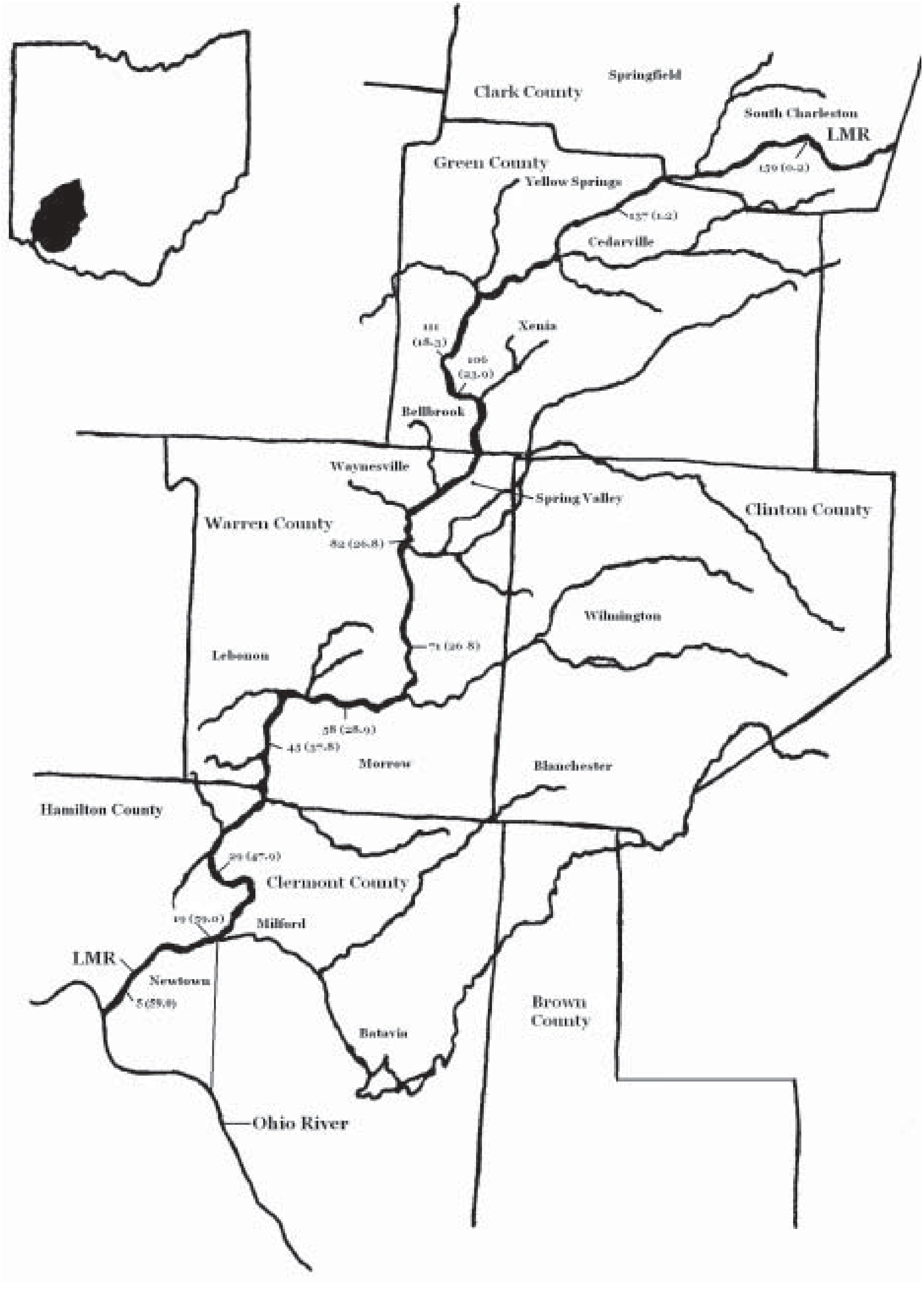

FIGURE 1. Diagram of the Little Miami River watershed modified from OEPA (1995). Whole numbers represent river kilometer upstream from the Ohio River and approximate sampling location. Values in parentheses are average cumulative total wastewater treatment plant effluent loading in million gallons per day (MGD) between 1995 and 1998 (OEPA 2000). County boundaries and populated cities and towns are identified for reference purposes only.

Methods for the Examination of Water and Wastewater (APHA 1992). TP samples were treated with potassium persulfate ($K_2S_2O_8$) and autoclaved to digest particulate bound and organic phosphates prior to analysis (APHA 1992). TP and SRP samples were reduced with an ascorbic acid reagent that produces a molybdenum blue color as a measure of orthophosphate concentration. Absorbance was measured at 880 nm using a HACH® DR4000 spectrophotometer (HACH 1995). Sample concentrations were determined against an external curve of 4 known concentrations (ug/L) (APHA 1992).

Ammonia and nitrate samples were treated with HACH® brand reagents (that is, ammonia cyanurate and ammonia salicilate for ammonia and nitraver 5 for nitrate, respectively) (HACH 1995). Absorbance was measured at 655 nm and 400 nm for ammonia and nitrate, respectively. As with phosphorus, sample absorbance readings were converted to concentration (mg/L) using the slope and y-intercept of the standard curve. Total dissolved inorganic nitrogen (DIN) was calculated by computing and adding the amount of elemental nitrogen measured as NH_4 and NO_3. This is appropriate for estimating DIN given the assumption that NO_3 comprises most of the total nitrogen in surface waters (Moss 1989).

A Thermo Jerrall Ash® (Franklin, MA) inductively coupled plasma atomic emission spectrophotometer (ICP-AES) was used to analyze dissolved trace and heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Se, Zn) and major cations (Ca, K, Mg, Na, Si). Variance during initial reading of the standards was higher than desired. A majority of the variation was in the ug/L (ppb) standards but some variation occurred at all concentrations. A review of protocol and preparation of new standards did not eliminate the variation. To control this, the machine was re-standardized every one to two hours and standards were treated as samples and put through the machine immediately before and after each re-standardization. Generally, 25-50 samples were analyzed between the standard readings. This produced "blocks" of data containing initial standard readings, 25-50 samples, and a final measurement of the standards. Sample data were corrected with the corresponding standard curve for each block. Curve corrected standard concentrations were nearly identical to the known concentration.

Sestonic algal biomass was measured as a function of chlorophyll *a* concentration (ug/L) (APHA 1992; Wetzel and Likens 1991). Absorbance of the chlorophyll *a* extract was measured before (665 nm and 750 nm) and after (664 nm and 750 nm) acidification with two drops of 0.1 N HCl to reduce analytical error associated with pheophytin *a* (APHA 1992; Wetzel and Likens 1991).

Benthic Algae Collection

To measure benthic algal biomass, rocks were randomly collected from each site using a blind location technique. The collector walked diagonally across the river, bent down after every other step, and selected the first rock touched by the forefinger. Rocks were randomly selected until the bottom area (750 cm^2) of a pan was covered without overlapping rocks. Attached algae were scrubbed off the rocks with hard plastic brushes. The algal slurry in the collection pan was drained through a 250-μm sieve. Debris remaining in the sieve was collected and determined to be primarily *Cladophora*, a common filamentous alga in eutrophic rivers (Chetelat and others 1999). Total volume of the sieved slurry (that is, periphyton) was recorded and well-mixed samples were collected for laboratory analysis. Two rock scrubs were collected from each site on each sampling date.

Analysis of Benthic Algae Samples

Periphyton and *Cladophora* were analyzed for dry weight, ash free weight, chlorophyll *a*, and phosphorus concentration. Duplicate sub-samples of wet *Cladophora* and periphyton were collected, weighed, and processed for analysis of chlorophyll *a* and TP concentration. Remaining samples were dried at 100° C, cooled, weighed for dry weight, ashed at 500° C, cooled, and weighed to calculate ash-free dry weight per m^2.

Benthic algal samples for chlorophyll *a* and phosphorus determination were analyzed using the same methods as aqueous samples. Chlorophyll *a* was extracted in 90% acetone. After a 24-hour extraction period, chlorophyll *a* concentration (ug/L) in filamentous algae and periphyton was measured as described above for sestonic algae, including the acidification step (APHA 1992; Wetzel and Likens 1991). Samples for TP measurement (mg/L) were treated with 0.5 g potassium persulfate, digested, and analyzed as described for aqueous samples. Chlorophyll *a* (ug/L) and TP (mg/L) concentrations in benthic algae were computed as mg/m^2 based on the area of the sampling pan.

Statistical Analyses

Prior to analysis, skewed data were transformed using the natural logarithm to normalize variances (Zar 1996). Statistical analyses performed included basic descriptive statistics (that is, range, mean, median, standard error, and standard deviation), one sample *t*-tests, analysis of variance (ANOVA), and multiple and simple linear regression analysis using the statistical package SYSTAT 7.0. Results are reported as mean concentration +/- one standard error. One sample *t*-tests of mean differences were used to compare average values of TP, SRP, DIN, sestonic and benthic algal chlorophyll *a*, daytime DO concentration, and benthic algal phosphorus to USEPA recommended summer criteria established for subecoregion 55 (2000a,b). ANOVA was used to test for significant variation in metal and cation concentrations between upstream and downstream sampling sites. Multiple and simple linear regression analysis was used to determine if the variation in nutrients, eutrophic response variables, and/or aqueous metals and cations could explain the distribution of DELT anomalies in the LMR. The distribution of DELT anomalies was previously documented by OEPA (2000).

RESULTS

Nutrients

TP and SRP concentration increased downstream of km 120 with the onset of WWTP effluent and increasing urbanization, and remained high with little variation throughout the rest of the river (Fig. 2). TP concentration averaged 466 +/- 203 ug/L throughout the river and was significantly higher than the recommended 70 ug/L (t >1.796; p <0.05; df = 11; CI = 95%) at all sampling sites. SRP concentration averaged 179 +/- 110 and exceeded recommended standards (that is, 62.5 ug/L) at all sites downstream of km 120 (t >1.833; df = 9; p >0.05; CI = 95%).

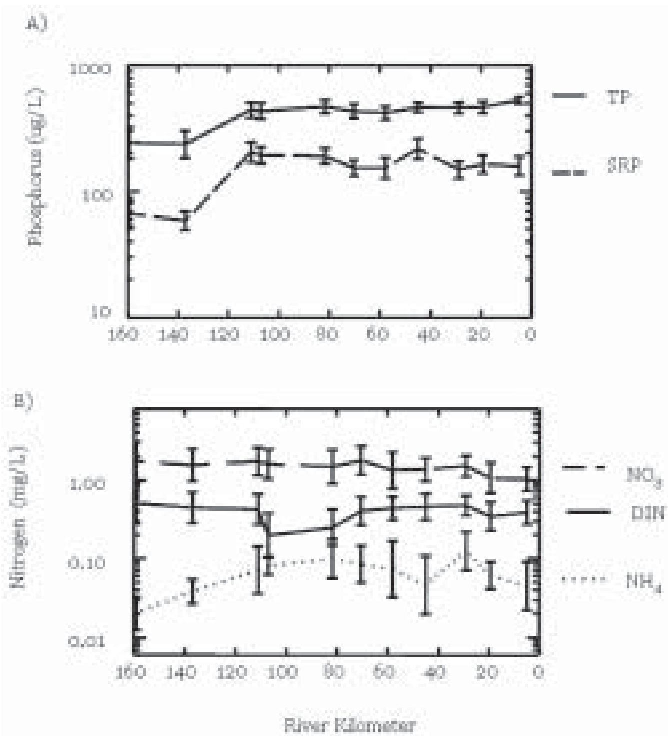

FIGURE 2. Nutrient concentrations in LMR water samples. Total Phosphorus (TP) and Soluble Reactive Phosphorus (SRP) are shown in panel A. Ammonia (NH_4), Dissolved Inorganic Nitrogen (DIN), and Nitrate (NO_3) are shown in panel B. DIN was calculated by adding the concentration of N as NO_3 and N as NH_4. USEPA recommended limits are 70 ug/L, 62.5 ug/L, and 3.5 mg/L for TP, SRP, and DIN, respectively. Data are expressed on logarithmic scale as average concentration +/- one standard error.

Nitrogen, measured in 1997 only, exhibited little variation between sites and was below the USEPA recommended limit of 3.5 mg/L for total nitrogen (Fig. 2). Ammonia (NH_4) averaged 0.02 +/- 0.014 mg/L and was too low for detection in 18 of 66 samples (27%). Samples with non-detectable ammonia concentrations all occurred in the July and September 1997 sampling events. Nitrate averaged 2.06 mg/L (+/- 1.6) and, unlike ammonia, increased over the summer sampling period. Calculated DIN (mean = 0.56 +/- 0.44 mg/L) was significantly less than 3.5 mg/L at all sampling sites (t >2.015; p = 0.05; df = 5; CI = 95%).

Primary Eutrophic Response Variables – Algal Biomass

Sestonic chlorophyll *a* (ug/L) did not exceed the recommended upper limit of 5.37 ug/L except in the lower 40 km of the river (Fig. 3a). Average summer chlorophyll *a* was less than 5.37 ug/L upstream of km 30 (t >1.796; p = 0.05; df = 12; CI = 95%). Sestonic chlorophyll *a* at sites downstream of km 40 averaged 14.63 +/- 8.2 ug/L, exceeding the recommended 5.37 ug/L.

Periphyton and *Cladophora* biomass exceeded the recommended 150 mg chlorophyll a/m^2 at the most upstream sampling site (km 159) but was at or below the recommended limit throughout most of the river (Fig. 3b). Average periphyton at km 159 was 184 +/- 123 mg chlorophyll a/m^2 while periphyton biomass at all remaining sties averaged 62 +/- 52 mg chlorophyll a/m^2. *Cladophora* was the dominant benthic macroalgae at km 159 (mean = 421 +/- 283). When present downstream of km 159, *Cladophora* biomass ranged from 0.310 to 1,958 mg chlorophyll a/m^2 (mean = 101 +/- 118).

Secondary Eutrophic Response Variables – pH, DO, and Benthic Phosphorus Storage

Though no limit on average daytime pH was identified by USEPA, pH is a secondary eutrophic response variable (USEPA 2000b). Average pH ranged between 7.8 and 8.0 in the upper two thirds of the river and

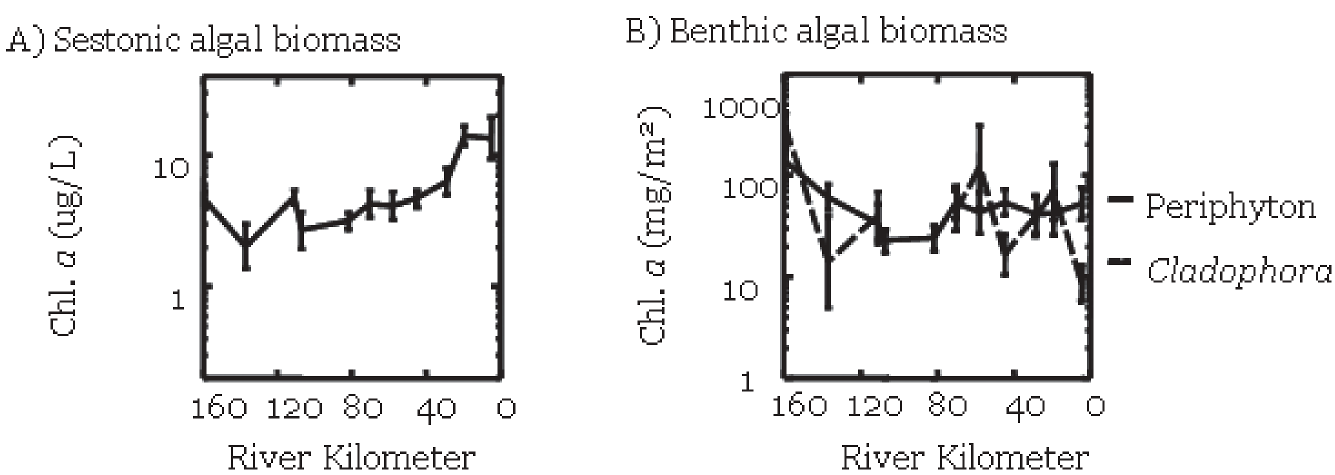

FIGURE 3. Primary eutrophic response variables in the Little Miami River. Sestonic and benthic chlorophyll *a*, shown in panels a and b, were at or below USEPA recommended criteria, 5.37 (ug/L) and 150 (mg/m^2), respectively, throughout most of the river. Data are expressed on a logarithmic scale as average values +/- one standard error.

increased to 8.2 at the two most downstream sampling sites (p = 0.008; df = 10, 119).

Dissolved oxygen (DO) concentration was highest in the upper and lower thirds of the river and exceeded USEPA recommended criteria (that is, 7.9 mg/L) at most sampling sites (Fig. 4a). DO ranged between 7.1 and 13.9 mg/L (mean = 9.1 +/- 1.5 mg/L; n = 132). At two sites, km 71 and km 58, average DO was not significantly different from a mean of 7.9 mg/L: 8.2 +/- 0.6 mg/L and 8.1 +/- 0.5 mg/L, respectively.

A limit on phosphorus storage in benthic algae (mg/m^2) was not defined by USEPA. However, some studies have suggested a limit of 20 mg P/m^2 to prevent nuisance algal growth (Chetelat and others 1999; USEPA 2000b). Phosphorus storage in benthic algae ranged between 3.2 and 115.8 mg/m^2 (mean = 45.5 +/- 23.2; n = 64) for periphyton and 2.2 to 1287.1 mg/m^2 (mean = 129.3 +/- 224; n = 52) for *Cladophora.* The average P concentration in benthic algae was greater than 20 mg P/m^2 throughout most of the river (Fig. 4b).

Aqueous Metals and Cations

Trace metals, heavy metals, and cations were detected in concentrations similar to those reported by OEPA in 1995 and 2000. Despite some variable downstream patterns, average element concentrations (Table 2) did not exceed normal reported ranges. There was no significant upstream to downstream variation in Cd, Cr, Cu, Mn or Zn concentrations (p >0.05). Aqueous Al and Fe significantly increased downstream while Se decreased (p <0.05) (Fig. 5). Ca and Mg progressively decreased downstream in the river while Na and K increased (p <0.05) (Fig. 6).

DELT Anomalies in LMR Fish

The relationship between nutrients, primary and secondary eutrophic response variables, aqueous chemistry (that is, trace and heavy metals and cations), and the distribution of DELT anomalies in the LMR (Fig. 7) was analyzed by multiple and simple linear regression. The interaction of periphyton biomass, periphyton phosphorus, and aqueous Zn and Ca explained 15.8% of the variation in DELT anomalies (p = 0.035; df = 4, 59; R^2 = 0.158). However, when these variables were analyzed separately, periphyton biomass, Zn, and Ca were not significantly related to the variation in DELT anomalies (p >0.05). Periphyton phosphorus alone explained 14.3% of the variation in DELT anomalies (p = 0.002; df = 1, 62; R^2 = 0.143) and was the only variable measured in this study to correlate significantly, albeit a negative correlation (m = -1.634), with the variation in DELT anomalies.

TABLE 1

Recommended summer average nutrient and response variable criteria for streams in level III subecoregion 55 (USEPA 2000a,b).

Variable	Recommended Summer Mean
Nutrients	
Total Phosphorus (TP) ug/L	70.0
Soluble Reactive Phosphorus (SRP) ug/L	62.5
Total Nitrogen (TN) mg/L	3.5
Primary Eutrophic Response Variables	
Chlorophyll *a* (ug/L)	5.37
Chlorophyll *a* (mg/m^2)*	150
Secondary Eutrophic Response Variables	
Dissolved Oxygen (DO) mg/L	7.9
Phosphorus (mg/m^2)*	20

*Denotes concentration in benthic algae.

DISCUSSION

In the absence of reference reach data for streams, the 25th percentile of existing data is used as a starting point

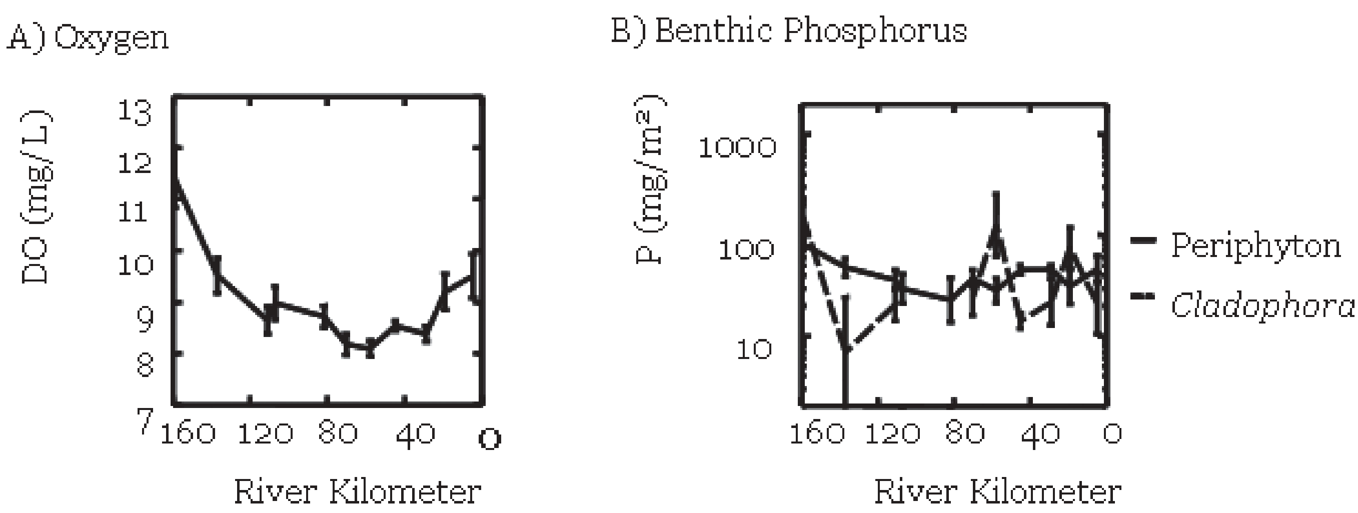

FIGURE 4. Secondary eutrophic response variables in the Little Miami River. Oxygen and benthic phosphorus storage, shown in panels a and b, were above recommended criteria, 7.9 (mg/L) and 20 (mg/m^2), respectively, throughout most of the river. Data are expressed on a logarithmic scale as average values +/- one standard error.

TABLE 2

Average metal concentrations in Little Miami River water samples.

Metal	Mean (ug/L)	S.E.
Al	212	19
Cd	0.66	0.06
Cr	10.67	0.58
Cu	10.89	0.82
Fe	93.5	11.0
Mn	18.5	1.3
Se	6.79	0.27
Zn	39.62	1.26

in developing nutrient criteria (USEPA 2000a). In this assessment, the 25th percentile of USEPA tabulated summer data for streams in subecoregion 55 were used. While these criteria are not specific to the LMR, they are currently the best starting point for assessing the extent of eutrophication and its consequences in this anthropogenically impacted watershed. Based on these criteria the LMR is burdened with excess phosphorus, benthic algal biomass that borders on nuisance growth, excess benthic phosphorus storage, and extremely high daytime DO concentration.

Nutrients

The range of TP, SRP, NH_4, NO_3, and DIN concentrations measured in this study were similar to LMR data reported by others (Manhart 1998; OEPA 1995, 2000; Staley 1997). Excess TP and SRP concentrations coupled with low nitrogen indicate the LMR is, as previously reported, a phosphorus-dominated river (OEPA 1995, 2000; Staley 1997). Excess phosphorus has not been directly linked to toxicity or health problems in either ani mals or humans (Carpenter and others 1998). The concern about high phosphorus is that excess available P can be stored intra-cellularly, as seen here in the excess P concentration in benthic algae, mobilized during times when aqueous P is low, and contribute to continued algal production after the immediate nutrient availability has ceased (Davis and others 1990). High *Cladophora* biomass was noted in the LMR during winter months when biologically available P was near a seasonal minimum because of high water flow (Shelton and Miller 2002); perhaps thriving on stored P and growing even at low temperatures with little tree canopy present to inhibit light penetration to the river.

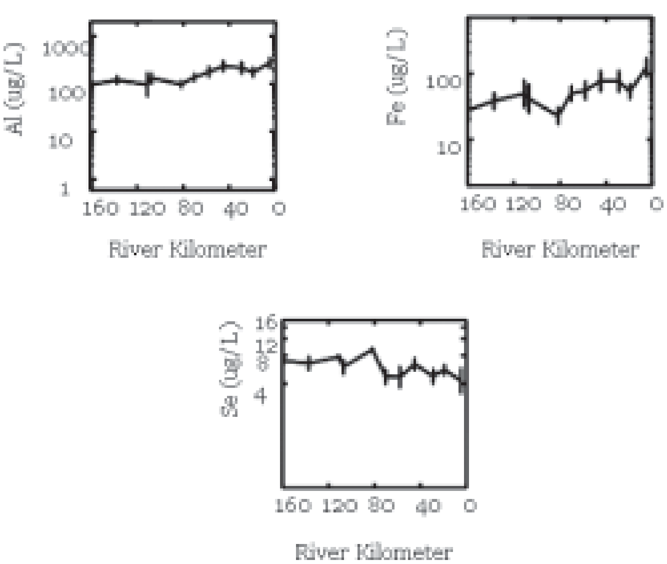

FIGURE 5. Dissolved aluminum, iron, and selenium concentrations in the Little Miami River during 1996 and 1997. Data are expressed on a logarithmic scale as average concentration +/- one standard error.

Primary Eutrophic Response Variables – Algal Biomass

Sestonic and benthic algae biomass were below standards recommended to prevent impairment risk associated with nuisance algal growth throughout most of the river (Fig. 3 a, b). The increase in sestonic algae occurred in the lower 40 km of the river where the channel becomes wider, deeper, and visibly more turbid. Deep water limits light infiltration to the bottom thereby limiting benthic algal growth and allowing sestonic algae to better compete for available nutrients near the water surface (Vannote and others 1980). Sestonic algae in the upper two-thirds of the LMR was probably limited by competition with benthic algae for available nutrients. Low benthic algal biomass is likely a result of light limitation at some forested sites and N-limitation given the low N and high P concentrations recorded throughout the river (Fig. 2).

Secondary Eutrophic Response Variables – pH, DO, and Benthic Phosphorus Storage

Higher than recommended daytime DO at all but two sites (that is, km 159 and km 137) on the river was consistent with OEPA findings (2000). The sites which did not exceed oxygen standards, km 71 and km 58, were consistently the first two sites sampled on the second day of each sampling event. They were sampled one to two hours earlier than other sites before photosynthetic oxygen accumulation could occur.

Supersaturated daytime DO and very low (for example, <5.0 mg/L) nighttime DO was one of the factors suspected by OEPA to be associated with DELT anomalies. Low DO can cause redox-based release of toxic metals and other substances from sediment making them more biologically available (Brick and Moore 1996). This can result in degraded habitat and reduced biodiversity of aquatic organisms including fish (Brick and Moore 1996). No published literature was found that directly links DO concentration with DELT anomalies.

The association between DO and DELT anomalies might be explained by the fact that metal absorption by, and toxicity to, aquatic organisms is often dependent upon DO and pH. Changing the oxygen concentration of water shifts the carbon dioxide content and the

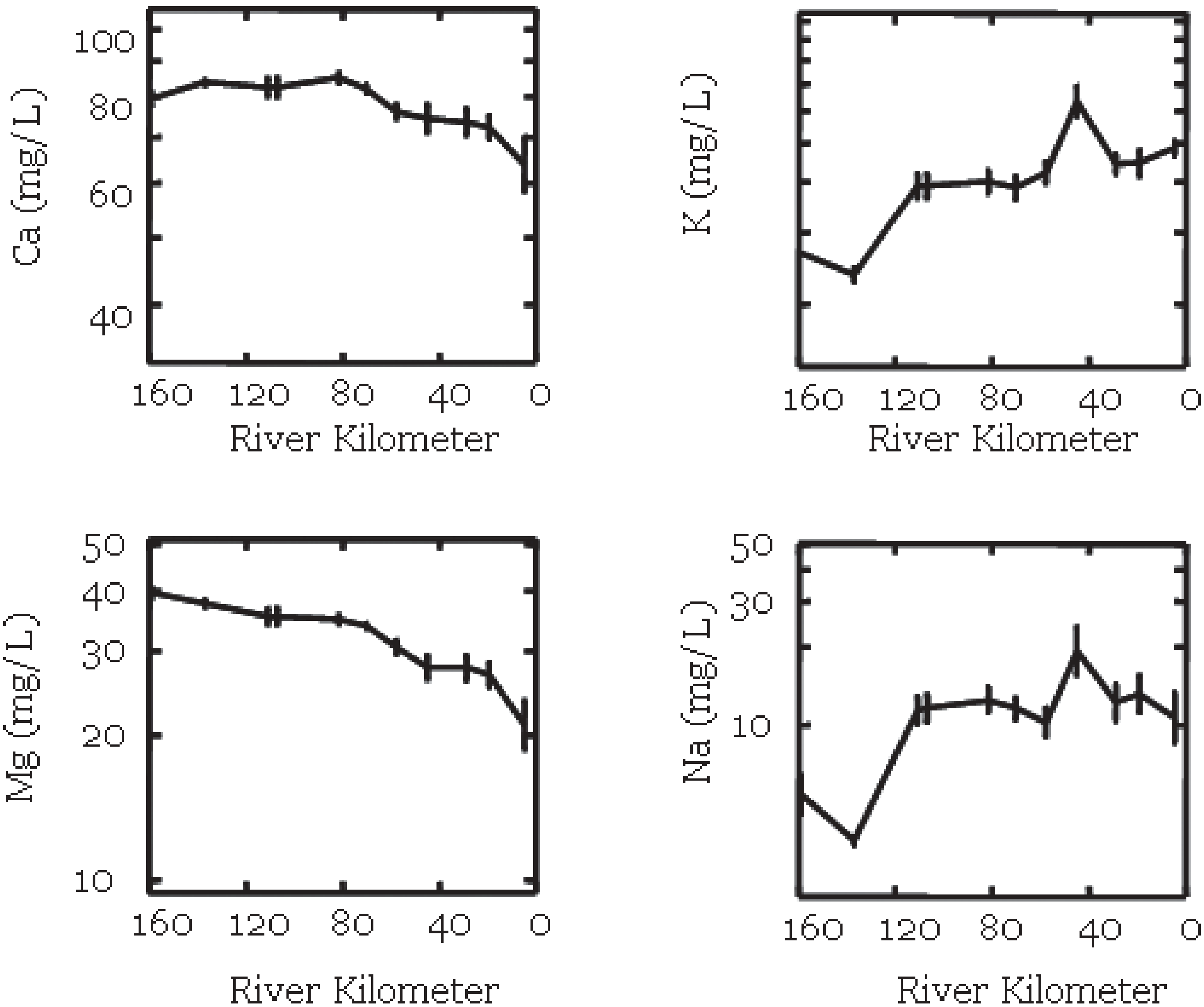

FIGURE 6. Dissolved cations in the Little Miami River during 1996 and 1997. Data are expressed on a logarithmic scale as average concentrations +/- one standard error.

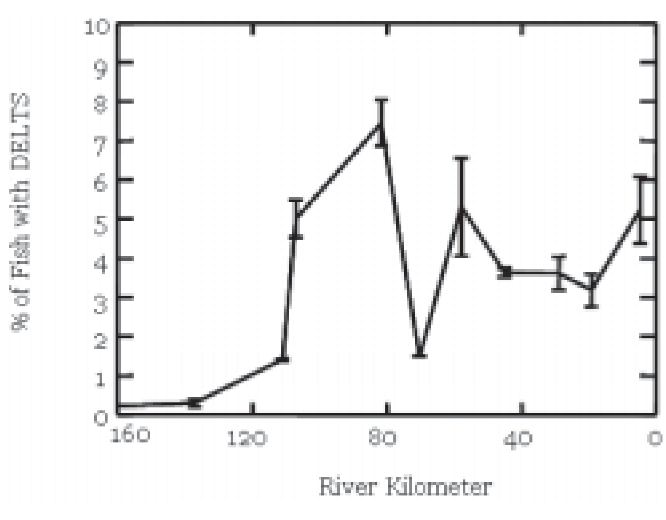

FIGURE 7. Average percent of Little Miami River fish with DELT anomalies in 1993 and 1998. Data were extrapolated from Figure 108 (OEPA 2000).

process is associated with a change in pH. Photosynthesis increases pH (more alkaline) while respiration decreases pH (more acidic). In fact, the downstream increase in average daytime pH recorded in this study was correlated with sestonic algal biomass that exceeded recommended criteria in the lower third of the river (Fig. 3). Though photosynthesis may only change the pH by 0.5 units at midday (Allen 1995) the diurnal change is likely to be more significant. DO and pH affect the absorption and toxicity of metals. Aluminum concentrations >180 ug/L were toxic to smallmouth bass at pH 5.1, while at pH 6.1 and 7.5 mortality was lower regardless of aluminum concentration (Kane and Rabeni 1987). Aluminum averaged 212 ug/L in the LMR during this study and average daytime pH was above 7.8. However, diurnal fluctuation in metal concentrations and primary response variables was not recorded and speculation regarding potential metal accumulation and/or toxicity resulting from diurnal variations is not possible. Total zinc body burden of channel catfish increased as pH decreased below 7.3 (Bentley 1992). Low DO increased the uptake of cadmium, chromium, and lead by the gills of bluegill sunfish (Freeman 1980) while Hughes and Flos (1978) found that hypoxic conditions reduced the rate of uptake of zinc by gill tissue in rainbow trout.

Aqueous Metals and Cations

The purpose of trace metal, heavy metal and major cation analysis in this study was to document the chemical environment in which LMR fish live. DELT anomalies have been linked to metal exposure. Exposure to cadmium has been shown to be correlated with skeletal deformities (Meteyer and others 1988; Prageesthwaran and others 1987). Fourhorn sculpin exposed to an effluent containing As, Cd, Cu, Hg, Pb, and Zn had an increased incidence of skeletal deformities (Bengtsson and Larsson 1986). Elements such as Cu, Ni, Pb, and Zn have been implicated as causing or acting synergistically with other agents to cause liver cancer in suager (*Stizostedion canadense*) and walleye (*Stizostedion vitreum*) (Black and others 1982). Fin erosion was prevalent in gold fish exposed to sediments containing metals (Sharples and others 1994). Metals decrease immune system function leading to susceptibility to bacterial infections that cause fin rot and lesions (Hetrick and others 1979; Knittel 1981; MacFarlane and others 1986). Creek chubs with increased body burdens of Cu and Zn also had higher incidence of fin rot as compared to creek chubs from unpolluted sites (Reash and Berra 1989).

Variation in dissolved trace and heavy metal concentrations throughout the river did not significantly correlate with the variation in occurrence of DELT anomalies measured in 1993 or 1998 by OEPA. Fish anomalies were most prevalent between km 100 and km 50 in 1993 and 1998, sometimes affecting as much as 10% of the fish community (Fig. 7). Ultimately, the lack of a correlation between dissolved metal concentrations in this study and the distribution of DELT anomalies was not surprising; analysis of water, sediment, and whole fish samples from the LMR resulted in no direct correlation between fish anomalies and metals or organic pollutants (OEPA 1995).

Land use in the LMR watershed probably influenced the significant downstream variations in Al, Fe, Se, Na, and K concentrations. Se concentration was highest in the upper half of the river where agriculture dominates land use (Fig. 5). Sedimentary and phosphate rocks that produce agricultural soils and phosphate fertilizers, respectively, are recognized sources of Se (Harte and others 1991). Al, Fe, Na, and K are common elements in rural and urban runoff and domestic sewage effluent (Harte and others 1991; Vymazal 1995). The increasing downstream concentration of these elements reflects the cumulative effects of agriculture, urbanization, and WWTP effluent in the watershed (Figs. 1, 5, 6).

The impact of land use on water quality and chemistry has been well documented. Urban development increased total phosphorus loads to surface waters (Jacoby and others 1997; May and others 1997). Deforestation, riparian zone destruction, and increased impervious surface associated with development alter stream habitat and geomorphology and contribute to higher sedimentation and reduced species diversity (May and others 1997). Agricultural runoff has long been a recognized source of nutrients, sediments, pesticides, and metals (USEPA 2000a).

Bedrock and topographic features likely account for the decrease in Ca and Mg concentration from upstream to downstream. The northern half of the watershed is characterized by rolling topography with limestone and dolomite bedrock. Limestone is a source of Ca and dolomite is composed of Ca and Mg. Gently rolling hills in the northern half of the watershed, as opposed to steep slopes in the southern half, result in longer water residence time and, thus, increased weathering of basin elements. Interestingly, some researchers have found that low Ca and Mg induces higher Pb and Cd accumulation (Livonen and others 1992). Fish from lakes with 400 ueq/L of Ca and Mg had the lowest Pb and Cd accumulation, while fish from lakes with 56 ueq/L of Ca and Mg accumulated the most Pb and Cd. Livonen and others (1992) hypothesized a competition between Ca and Mg and divalent metals for binding sites on gill tissue as a mechanism to explain the higher metal accumulation associated with low Ca and Mg concentrations.

DELT Anomalies in LMR Fish

The linear relationship between periphyton phosphorus and DELT anomalies provides little support of a foodweb connection between surface water eutrophication and the health of aquatic organisms. Though statistically significant, the linear relationship between periphyton phosphorus and DELT anomalies was negative and explained only 14.3% of the variation in DELT anomalies. Though phosphorus has not been linked to toxic effects in humans, animals, or fish (Carpenter and others 1998), when considered along with the fact DELT anomalies were positively correlated with aqueous phosphorus and diurnal DO swings (OEPA 1995, 2000), the relationship between periphyton phosphorus and DELT anomalies shown in this study may signify a connection between benthic algal response to eutrophication and DELT anomalies.

This study documents the eutrophic and chemical environment in which fish live. Results show the LMR is burdened with excess phosphorus, benthic algal biomass that borders on nuisance growth, excess benthic phosphorus storage, and high daytime DO concentration. The association between DELT anomalies and periphyton phosphorus adds to the information reported by OEPA that suggests a correlation between surface water eutrophication and the health of aquatic organisms. Published literature yields little insight into the association between eutrophication and DELT anomalies without coming to the conclusion that benthic algal metal accumulation may be involved.

The aquatic environment is a complicated and open system. An important step in identifying the source of frequent DELT anomalies in LMR fish, and understanding their apparent association with eutrophic parameters, is to analyze metal accumulation in benthic algae to determine if, in fact, there is a relationship between eutrophication and metal accumulation at the base of the foodweb.

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