AGRICULTURAL IMPACTS ON STREAM WATER QUALITY

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INTRODUCTION

Agricultural practices frequently disturb terrestrial ecosystems with concomitant effects on downstream aquatic ecosystems. The impact of these disturbances on lakes and reservoirs is now reasonably predictable with respect to nutrients (Vollenweider, 1968, Rast and Lee, 1978, Canfield and Bachmann, 1981). However, it is difficult to gauge the severity of these effects on rivers and streams. In running water (lotic) ecosystems, changes in discharge complicate an assessment of the effect of nutrients on the biota (Horner, et al., 1983). For this reason, study of nutrient limitation in streams has not progressed as rapidly as it has in lakes. There is a need for good methods to assess nutrient limitation in streams.

There are a number of methods for assessing nutrient limitation and each has its strengths and weaknesses. They are as follows: nutrient concentrations in water, ratios of N:P nutrients in water, ratios of N:P in algae or periphyton, a substrate technique and alkaline phosphatase activity.

Because it is easy to measure nutrient concentrations in water, it would be very useful if mere concentrations of a nutrient reflected nutrient limitation, i.e. nutrient limitation occurred at or below some threshold concentration. However, often this is not the case. The instantaneous standing quantity of a nutrient, particularly phosphate, in water does not reliably indicate how fast it is being used and recycled (Wetzel, 1983). If algae are P deficient, they will rapidly use P as fast as it is supplied.

Nutrients commonly measured are orthophosphate, nitrate and ammonia. Orthophosphate is measured as soluble reactive phosphorus (SRP), and most of SRP is thought to be usable by algae as are both nitrate and ammonia.

Ratios of N:P as nutrients could also be used to indicate potential for algal growth. Given the fact that N:P occur at a 7:1 ratio in nutrient replete algae, an increase in the N:P ratio in water to higher values <u>might</u> indicate P limitation (Vallentyne, 1974). As indicated above, the N:P content of algal cells is about 7:1 in nutrient replete algae. A shift to higher ratios in algal cells could also demonstrate limitation by P.

The substrate technique involves exposing artificial substrates which diffuse nutrients (Fairchild et al., 1985). After a suitable time, biovolume on substrates is determined and treatments are compared to controls. A significant increase of biovolume on treatments indicates limitation by the treatment nutrient.

The alkaline phosphatase activity (APA) technique measures P-limitation only (Healey and Hendzel, 1979). It has its basis in that algae have the enzyme alkaline phosphatase when P is limited, but not at other times. It is very rapid and potentially useful to screen many samples (sites).

Limitation of algae by N is relatively uncommon in freshwater (Wetzel, 1983) and thus techniques other than the substrate technique do not test for N limitation. However, two facts could lead to a screening procedure for N limitation in streams. Nitrogen fixation (NF) by autotrophs only occurs when N nutrients are very low and NF in autotrophs occurs almost exclusively in one group of blue-green algae, those with heterocysts (Wetzel, 1983).

Naturally, techniques to measure nutrient limitation must account for time. While short term limitation is interesting, the techniques should measure and integrate the effect of nutrients over the growth cycle of the organisms. Thus, time scales of weeks become appropriate. It is also important to have a measure of nutrient limitation which reflects and integrates the effects of exposure to stream water varying in velocity, nutrient concentration and discharge. The periphyton seems ideal for such tests.

This research was directed at assisting Oklahoma water managers in identifying when and where nutrient limitation occurs in streams, specifically in south-eastern Oklahoma where water quality problems are expected to result from projected expansion of the poultry industry. In McCurtain County alone, brooder houses will increase from 360 to 400 in one year. Relatively pristine streams are found in this area.

Poultry manure will be spread on agricultural land in the vicinity of the rivers. As a result, nitrogen (N) and phosphorus (P) nutrients could enter the streams. If this should occur, benthic aquatic plant growth will increase and

could lower dissolved oxygen (DO) at low discharge.

OBJECTIVES

The objective of this research was to compare methods for measuring nutrient limitation in streams. I intended to demonstrate that there is a high correlation between the results of substrate tests for P-limitation and tests for alkaline phosphatase activity (APA) and surplus P. If true, it will strengthen the conclusion that APA reliably measures P-limitation in streams. In addition, I wished to establish a relationship between results from substrate tests and dissolved nutrient ratios (nitrate + ammonia/soluble reactive P). It would seem most likely that nutrient limitation of P would occur when the N:P ratio in water was greater than 10:1 and that N-limitation would occur when the N:P ratio was less than 5:1. Between 5:1 neither could be predicted to be limiting. Testing was done seasonally: fall, winter, spring, summer.

STUDY SITE

The study site is in the Little River drainage basin (Figure 1). The study stream (Glover Creek) originates in the Quachita Mountains and flows southerly for about 90 km to its confluence with the Little River. The area of the drainage basin is 876 km^2. The stream gradient varies from 19 m/km in the upper reaches to 1 m/km at the mouth (U.S. Army Corps of Engineers, 1975). It is one of the remaining streams in Oklahoma which has not been impounded.

The watershed is largely forested, originally mixed pine and hardwoods. Current forestry practices include clear cutting, forest fertilization, and replacement of mixed hardwoods and pine with monocultures. Clear-cut regions can be found within 5 to 10 km of the study sites, but not in direct contact with the river. There are small farms in scattered clearings. Many farms contain chicken brooder houses, the basis of agriculture in the vicinity.

Initially, we chose four study sites (Figure 1), but after experience with logistics in the area we reduced the number to two (sites I and II). When additional tests for nutrient limitation were added to the repertoire, we had to

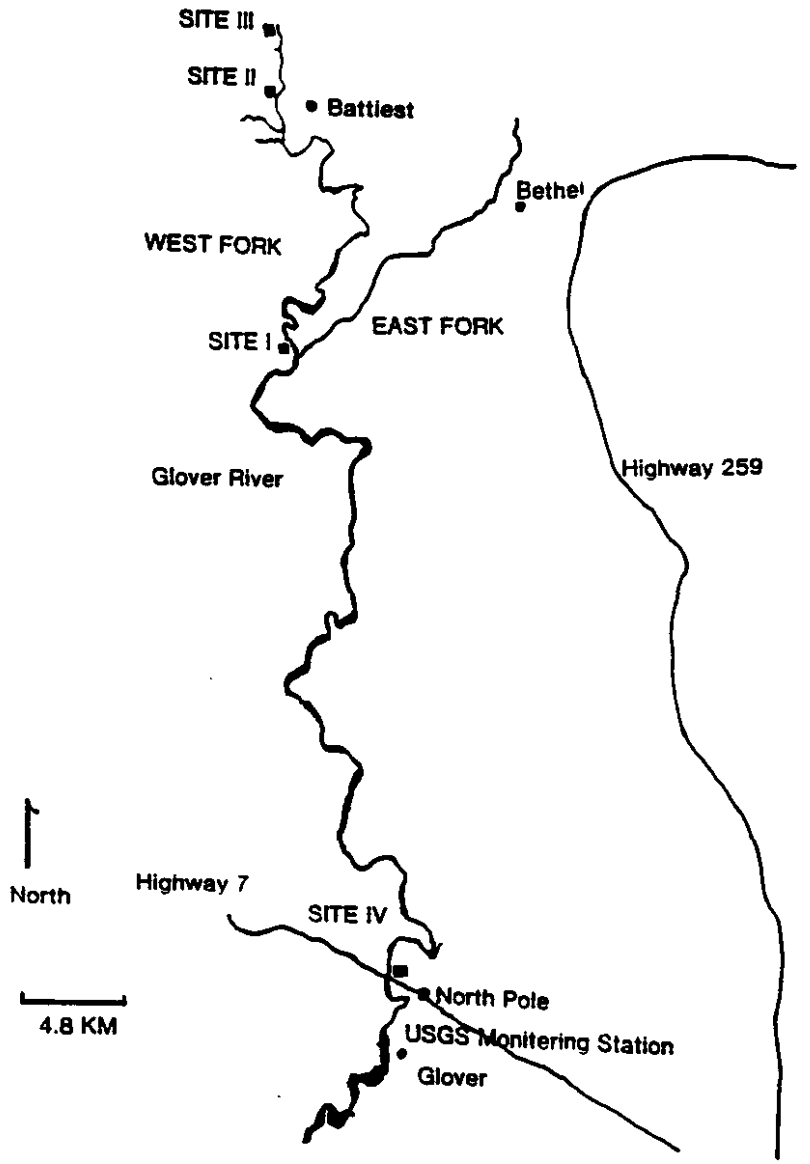

Figure 1. Location map, Glover River, showing study sites for 1989 and 1990.

focus our efforts on only one (site I). Nevertheless, nutrient data alone continued to be collected at all sites when time allowed.

At site I the river is about 30 m wide; the substrate consists almost entirely of large smoothed rocks. Turbidity was 2-23 nephelometric units.

Further upstream, at site II, the stream is about 10 m wide, but the substrate is the same. Because the substrate at both sites is not alluvium, these reaches can be classified as cascades, not pools and riffles.

Discharge can be so low that stream water is virtually motionless, especially in summer. But, extremely high discharges can occur during flood events. At sites I and II flood debris was observed resting in streamside trees up to 2 m above the stream banks. Samplers were frequently lost in such flood events, especially during the spring of 1990.

Average discharge is extremely variable with some monthly ranges exceeding 300 cubic feet per second (CFS) while others are as low as 14 CFS. August is the month of lowest discharge and April is the month of peak flow. The river is not impounded and frequently floods in the spring and early summer (U.S. Army Corps of Engineers, 1975).

At all sites the river bed is covered with rocks or a combination of large rocks and boulders. Heavy accumulations of periphyton were observed on the rocks in the fall and summer of 1988. The water depth varies from 30 centimeters to 1.5 meters depending on the season. The decrease in flow and volume of the river in fall 1988 gave site II the appearance of a series of interconnecting pools. Thick vegetation surrounds both sites with large elm trees and small understory trees and shrubs along the shoreline. The abundance of aquatic macrophytes is very low, with a few emergent and floating species at site I in the summer. Grazers such as chironmids and immature odonates are a part of the benthic community.

METHODS

Substrate Tests

I followed the technique of Fairchild et al. (1985) who used clay flower

pots as substrates diffusing nutrients. Clay flower pots were filled with 2% agar. Controls had no nutrients added (n=4). Experimental units had 0.1 M nitrate and 0.1 M phosphate added to the agar, respectively. Pots were set out with controls upstream and those with nutrients downstream. After about 30 days periphyton was removed by scrapping the pots with a razor blade. The material was analyzed for carbon content using a wet oxidation technique (Strickland and Parsons, 1968) after filtration onto precombusted 0.7 µm glass fiber filters (450 °C). Chlorophyll a was also determined on known aliquots that had been removed from substrates by filtering through Millipore filters at 0.3 atm to estimate the concentration of chl. a (pore size 0.45 µm). Chlorophyll samples were stored in paper envelopes in the dark at -5°C until analysis. Samples were either analyzed using the monochromatic method to convert absorbance in a 1-cm cell to chl. a concentrations (Wetzel and Likens 1979) and for phaeopigments or determined by fluorescence. Chlorophyll was determined with a Turner Model 111 fluorometer which had been calibrated using known concentrations of chl. a (Sigma Chemical Co.).

This work was planned so that additional tests would be developed and added to the substrate tests. Thus, surplus P was added in the fall of 1989 and APA in 1989 and 1990. These are now described.

Surplus Phosphorus

Surplus phosphorus analyses were performed on periphyton that had accumulated on the clay pot nutrient-diffusing substrates. Samples of periphyton were obtained from a series of 12-18 substrates 14 to 21 days after the substrates were placed in the river. Biomass calculations and APA analysis were concurrently performed on duplicate enrichment substrates.

The sample of periphyton used for surplus phosphorus analysis was rinsed with 40.0 ml of distilled, deionized water and gently boiled for one hour. The sample was cooled to room temperature and filtered through a pre-rinsed 1.2 µm Whatman 4.25 cm glass fiber filter. A 2.7 ml volume of supernatant was reacted in a 5 cm cuvette with 0.3 ml of mixed reagent following Strickland and Parsons

(1968) for ten minutes and then absorbed and measured at 885 nm with a Shimadzu model TB-85 spectrophotometer. Phosphorus concentration was calculated from a standard concentration curve. A 2.7 ml volume of standard was reacted with a 0.3 ml volume of mixed reagent to correct for volume in calculations of concentration.

In 1989 duplicate samples were filtered through a pre-muffled 1.2 µm Whatman 4.25 glass fiber filters and dried at 250 degrees C for 24 hours. The dried filter was then weighed. The filter was then muffled for 2 hours at 450 °C. The filter was re-weighed and the difference between the dried and muffled filter was determined. This difference provided a measurement of ash free weight of the sample.

In 1990 the subsamples were filtered through a 0.7 µm type AA Millipore filter and chlorophyll a concentration calculated as below. Surplus P was normalized to chlorophyll a concentration following Wynne and Berman (1980).

Alkaline Phosphatase Activity (APA)

This section describes APA methodology developed for use in 1989-90 which unfortunately could not be employed because of high water. It was used, however, to measure APA of water taken at the outset of one experiment which was subsequently washed away by floods.

Periphyton was removed from nutrient diffusing substrates and placed into a polyethylene bottle for transport to Stillwater frozen following Perrin et al (1987). Whole water and filtered water was analyzed following Petterson (1980) and concentrated periphyton and periphyton was analyzed for APA following Bothwell (1988). A whole water sample was analyzed for total APA. Total APA includes both a dissolved fraction and a fraction associated with particulate matter or sestonic APA. The APA analyzed was directly associated with the cells or cell membranes.

APA was measured by the hydrolysis of 100 µM 3-0-methylfluorescein phosphate (o-MFP). A 4.5 ml volume of water containing concentrated cells was placed in a fluorometer tube. Then 0.5 ml of 100 µmoles of o-MFP in 10.0

micromolar tris buffer was added to the sample to begin the reaction. The fluorometer tubes were sealed with parafilm, inverted and fluorescence read.

APA samples were measured against Tris controls. APA was measured as the average increase in fluorescence and converted to absolute units using a standard curve of fluorescence verses o-MFP concentration. Distilled, deionized water was used as a blank. Activities were expressed in micromoles MFP hydrolyzed per unit of biomass. Parallel fluorometric analysis of chl. a concentration was used to normalize APA values to biomass units (chl. a).

RESULTS

Simple t-tests were used to determine if there were significant differences between biomass on treatment pots compared to control pots (Steel and Torrie, 1980). Nutrient limitation was determined five times using the substrate technique. In the first experiment in late fall, 1988, at site I no nutrient limitation was found (Table 1). The significant difference for phosphate merely meant that diffusing P had significantly lower chlorophyll a than controls.

During March - April, 1989, both nitrate and phosphate treatments respectively, had higher particulate carbon (PC) than controls. However, when biomass was measured as chlorophyll, treatments were no different than controls.

At site II the same results were obtained as at site I in late fall, 1988 (Table 2). In contrast the results of the experiments in March - April at site II were very different form results at site I (Table 2). Nitrate treatments had significantly higher biomass as PC and chl. a, but phosphate treatments were no different than controls. After April, 1989, additional types of tests of P-limitation were developed and focus shifted entirely to site I.

No nutrient limitation was detected at site I by the substrate tests in June or October, 1989 (Table 1). However, both N and P limitation could be demonstrated in August. In experiment 4 lasting 20 days in August, nitrate treatments had higher PC and chl. a than controls; phosphate treatments had only higher chl. a than controls (Table 1).

The concentration of soluble reactive phosphate (SRP) was extraordinarily

high at all stations in late winter, 1988, possibly due to forest fertilization (Table 3). Consequently, failure to observe P limitation is not surprising. Similarly, the high concentrations of SRP in March - April 1989, should have precluded P - limitation then, although nitrate concentrations may have been low enough to cause the N limitation that was observed (Table 4).

By June 10 SRP at site I was only 3.9 micrograms P/liter and remained low or undetectable until October 19. Ammonia - N, however, was always above 5 micrograms/liter after July 11, and often above 20 micrograms/liter. Thus, it is not surprising to observe both N- or P-limitation after June 10 (Table 1). What is surprising is that it was not observed more frequently.

Data on nitrogen nutrients are given in Table 5 for sites II - IV. While data are not available for all stations after March, the concentration of nitrate was also low or undetectable after July, particularly at site IV.

Tests of surplus P were made of periphyton on nutrient diffusing substrates concurrently with substrate tests in July, September and October, 1989. Based upon criteria of (Fitzgerald and Nelson, 1966) P was not limiting.

During 1989-90 high water precluded APA testing. Three experiments were initiated, but aborted. Nevertheless, APA of stream water was tested on February and April. Fluorescence was below detection limits on both dates.

DISCUSSION

Substrate tests during the fall of 1989 failed to demonstrate any nutrient limitation. This is surprising as conditions for growth were not unlike summer. Perhaps the reason lies in the length of the experiment (30 days). Other experiments were shorter (about 20 days). It is believed sloughing of algae occurred, yielding the lower biomasses observed.

While it would be presumptive to suggest the mechanism, it is reasonably clear that nutrients are low or undetectable during the warm season at low flow. N- or P-limitation can occur at this time. Thick algal mats form during the summer and perhaps are able to take up any nutrients entering the stream, at least during low flow conditions. A still unanswered question is how much of a

role forest vegetation has in reducing nutrients in soil and ground water which may be entering the stream at this time. Further investigations might be profitable in determining the role of terrestrial vegetation in sequestering nutrients, particularly N.

ADDENDUM

This project ended June 30, 1990. However, research continued during July 1990. A comparison was made of APA, luxury P and substrate tests for nutrient limitation. The substrate tests were control, N releasing, P releasing, and N + P releasing. APA was highest on N releasing substrates and low on all others, including P releasing substrates, as expected. Luxury P was lowest on N releasing substrates by factors of 10 - 50 compared to other substrates. Thus, there is an inverse relationship between APA and luxury P as expected.

In this experiment P is apparently not limiting growth; rather N is limiting. However, when N is supplied from the N releasing substrate, P becomes limiting as evidenced by high APA.

This conclusion is based on the assumption that biomass on N-releasing pots will be higher than on other experimental pots. These data are not yet available.

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TABLE 1

Limiting nutrients and concentrations of nutrients in the Glover River at site I using particulate carbon (PC) and chlorophyll *a* accumulation on clay substrates to test for N- or P-limitation. No Data = flood destruction of substrates.

Experiment	Biomass Measure	Difference from Control P= 0.05		Duration	Days
		0.1 M Nitrate-N	0.1 M Phosphate-P		
1	PC	No	No	Nov 7-Dec 4, 1988	27
	Chl.*a*	No	Yes		
2	PC	Yes	Yes	Mar 15-Apr 4, 1989	21
	Chl.*a*	No	No		
3	PC	No	No Data	Jun 10-Jul 1, 1989	20
	Chl.*a*	No	No Data		
4	PC	Yes	No	Jul 29-Aug 19, 1989	21
	Chl.*a*	Yes	Yes		
5	PC	No	No	Sep 29-Oct 29, 1989	30
	Chl.*a*	No	No		

TABLE 2

Limiting nutrients in the Glover River at site II using particulate carbon (PC) and chlorophyll *a* accumulation on clay substrates to test for N- or P-limitation.

Experiment	Biomass Measure	Difference from Control P= 0.05		Duration	Days
		0.1 M Nitrate-N	0.1 M Phosphate-P		
1	PC	No	No	Nov 7-Dec 5, 1988	28
	Chl.*a*	No	Yes		
2	PC	Yes	No	Mar 15-Apr 8, 1989	21
	Chl.*a*	Yes	No		

TABLE 3

Concentration of soluble reactive phosphorus by site during 1988-1989 in the Glover River as micrograms P/liter. (ND = no data)

Date	Site I	Site II	Site III	Site IV
1988				
Nov 5	1,099.0	764.0	799.0	963.0
Dec 4	63.5	1,455.0	1,435.0	1,475.0
1989				
Mar 3	749.0	750.0	ND	ND
Apr 7	131.0	138.0	135.5	139.0
May 10	ND	0.8		
Jun 10	11.0	ND	0.8	0.8
Jul 1	7.5	ND	ND	ND
Jul 29	ND	Not Detected	ND	ND
Aug 19	0.2	ND	ND	ND
Sep 29	1.2	ND	ND	ND
Oct 19	0.4	ND	ND	1.2

TABLE 4

Concentration of nitrate-N and ammonia-N at site I of the Glover River during 1988-1989 as micrograms N/liter. (ND = not detected)

Date	nitrate-N	ammonia-N	nitrate-N + ammonia-N
1988			
Nov 5	19.0	85.0	104.0
Dec 4	89.0	23.5	112.5
1989			
Mar 3	28.0	85.0	113.0
Apr 7	ND	196.0	196.0
Jun 10	3.9	ND	3.9
Jul 1	ND	89.5	89.5
Jul 29	ND	22.0	22.0
Aug 19	2.2	23.5	25.7
Sep 29	0.1	19.3	19.4
Oct 19	0.2	5.2	5.4

TABLE 5

Concentration of nitrate-N and ammonia-N at sites II - IV of the Glover River during 1988-1989 as micrograms N/liter. (ND = no data)

Date	Site I		Site III		Site IV	
	nitrate-N	ammonia-N	nitrate-N	ammonia-N	nitrate-N	ammonia-N
1988						
Nov 5	11.7	44.5	14.0	28.0	12.2	31.0
Dec 3	34.5	62.5	62.0	12.0	47.0	671.0
1989						
Mar 3	28.0	85.5	ND	ND	ND	ND
Apr 7	30.0	848.0	Not Detected	245.0	Not Detected	294.0
Jun 10	0.7	160.0	Not Detected	160.0	3.6	Not Detected
Jul 1	7.6	169.0	Not Detected	498.0	ND	ND
Jul 29	Not Detected	13.5	0.6	10.6	2.0	14.4
Aug 19	ND	ND	ND	ND	0.9	24.4
Oct 21	ND	ND	ND	ND	Not Detected	56.0