FIELD DETERMINATION
OF
THE CRITICAL NUTRIENT
CONCENTRATIONS
FOR
CLADOPHORA IN STREAMS
AND THEIR IMPORTANCE
IN WASTE LOAD MANAGEMENT

Ontario

Ministry
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Environment

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Deputy Minister

FIELD DETERMINATION OF THE CRITICAL NUTRIENT CONCENTRATIONS

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AND THEIR IMPORTANCE IN WASTE LOAD MANAGEMENT

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ABSTRACT

Many streams in Southern Ontario are seriously infested with excessive seasonal growth of aquatic plants such as *Cladophora* and *Potamogeton*. A direct relationship, with a regression coefficient of 0.87, was observed between ambient P concentration in the water and P content of plant tissue in six rivers. Critical or growth controlling total P concentration of 60 µg/L in stream water and 1.6 mg/g dry weight in plant tissue were determined. Unlike P, no significant correlation was observed between N content of plant tissue and N concentration in water. The correlation of total P with plant growth can be used to estimate the waste load which would result in maximum growth rate of *Cladophora*.

INTRODUCTION

Assessment of the enrichment status of streams based on specific aspects such as benthic fauna diversity or dissolved oxygen content will provide only limited control devices for better watershed management. Even though these are good indices of water quality, they yield only descriptive data while serving to define problem areas. Deterioration of water quality resulting from increased nutrient supply, leading to nuisance weed growth, is a serious problem and makes evaluation of critical nutrient levels and the determination of the permissible waste load very important.

The dissolved oxygen fluctuation of a river often reflects its capacity to support a balanced aquatic habitat. One expects a greater fluctuation as the plant biomass increases. By controlling the excess nutrient supply, it is hoped that the oxygen fluctuations can be maintained within the desirable range.

Due to the fact that macrophytes and larger algae such as *Cladophora* and *Chara* have a great intracellular food storage capacity, it is uncertain that lowering the available nutrients in the water medium will reduce the weed growth accordingly. Gerloff and Krombholz (1966), in their studies with several species of angiosperms, showed good correlation between plant yield and tissue content below the critical or growth controlling level. However, Wilson (1972) suspected that lowering the nutrient concentration in water would not have any effect in controlling the spread of plant biomass since increased growth did not correspond to loss of nutrients in laboratory experiments.

Since the nutrients in water are generally accepted as the best point of control in natural systems as opposed to light energy, turbidity or water depth; a close examination of weed growth as affected by the external nutrient supply became important. Unfortunately, as indicated by many researchers in their recent bioassay experiments (Fogg, 1965; Likens, 1972) the evaluation of nutrient-plant relationships based on the nutrient content of water has many complications such as element interactions and variations in nutrient supply due to flow etc. Thus, an alternative approach to the problem is to examine the elements themselves, (both in the plant tissue and in the water) assuming that a correct evaluation of growth response can be derived by the tissue analysis technique as recommended by Gerloff and his collaborators (1957, 1966).

Critical level determinations for aquatic weeds from both intracellular and external supplies have been reported in many laboratory bioassay studies (Pitcairn and Hawkes, 1973; Mackenthun, 1968, and Gerloff and Krombholz, 1966), yet the range of phosphorus concentrations found to be the critical level by different workers has led to much argument. Therefore, our objective in this study is to evaluate the effect of phosphorus and nitrogen on *Cladophora*, our most troublesome weed species in streams, and attempt to determine critical nutrient concentrations using a direct approach at the field level.

STUDY AREAS

Our field survey was initiated on a 20 km stretch of the North Thames River in Ontario during the summer of 1973. The reach has an average width of 40 m and the flow ranges from 2.7 to 1.4 m^3/sec. Mats of *Cladophora* and *Potamogeton* covered most of the reaches during their peak growth in summer; individual filaments of *Cladophora* being as long as six meters. Aside from its effect on the recreational value of the river, the respiratory demand of such dense biomass may cause serious deoxygenation at night. Daily oxygen fluctuations with maximum concentrations of more than 25 mg/L at noon and a minimum of 3 mg/L at night are common at times of low flow.

In order to determine the critical nutrient level which facilitates maximum growth of plants, our attention was turned, in the summer of 1974, to six river systems; namely, the Avon, Middle Maitland, Bayfield, Nith, Conestogo and Thames, all within a 100 mile radius of Stratford, Ontario (figure 1). River sections chosen were restricted to areas of similar substrate and were all subjected to low municipal and industrial effluent discharges, with the hope that covering a broad range of nutrient loads would provide us with some low nutrient effect data. Table 1 illustrates some general characteristics of the six rivers studied.

Cladophora glomerata and *Potamogeton pectinatus* are the dominant species in these shallow lotic communities. Generally, the *Cladophora* reaches a peak in June and is succeeded by *Potamogeton* in July lasting until the end of August when *Cladophora* usually reappears (figure 2). the succession of these two species is believed to be due to morphological developments which are brought on by physical changes in the environment such as water temperature and photoperiod (Whitton, 1971, and Bellis and McLarty, 1967).
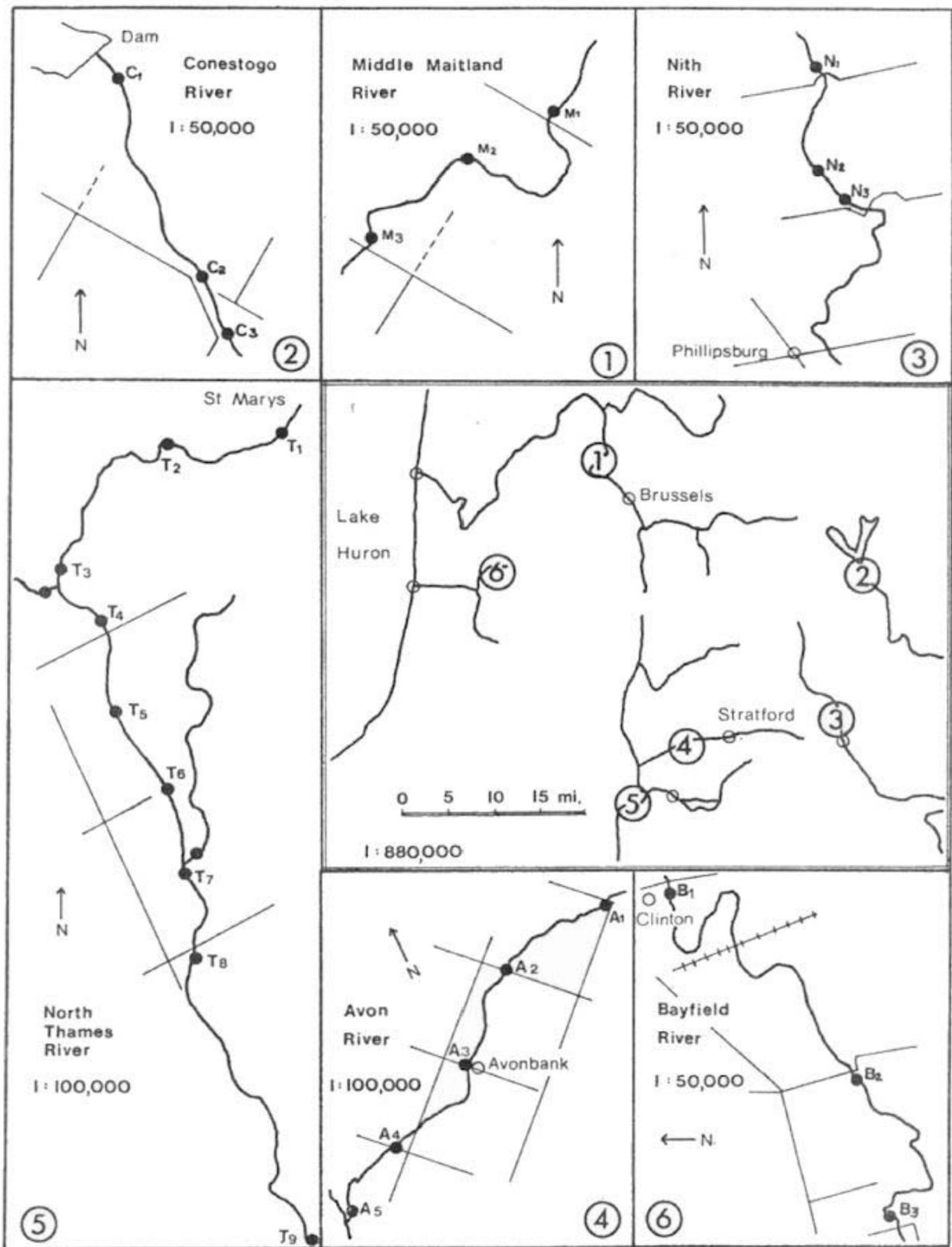
Figure 1:   Location of six rivers studied in 1974.

C - *CLADOPHORA*

P - *POTAMOGETON*

F - *FONTINALIS*

Figure 2: Seasonal succession of the dominant plant species in the six rivers studied.

Since large amounts of *Cladophora* appear to be a greater nuisance than a similar biomass of *Potamogeton* and since the rapid vigorous growth of *Cladophora* has been a source of public complaint for many years, our work stressed this important species.

METHODS

Sample stations divided the rivers into three or more one mile sections depending on road access and the physical characteristics of the river. Each river was visited twice a month and on each visit, five days of continuous water sampling and productivity studies were carried out.

Water samples for total phosphorus were collected in duplicate 2-3 times daily at all stations on the river throughout the 5 days. Uniform mixing of suspended particles was verified by determining the extinction coefficient with a LI-COR quantum sensor across transects at all stations. Exactly 50 ml of samples collected from mid-stream was immediately pipetted into 250 ml acid washed Erlenmeyer flasks and later digested in the same flasks (Dillon, 1974).Total nitrogen samples were collected in polyethylene bottles at the same time. All water samples were kept refrigerated and analyzed by the chemistry laboratory in London, Ontario, Ministry of the Environment.

Plant samples were collected twice within 20 days by removing all vegetation, including roots in the case of *Potamogeton*, with a Surber sampler. Four random quadrats were cropped along each of four transects between stations. Plant materials were washed thoroughly and blotted dry. After the addition of dry ice and steel balls the plant samples were ground for five minutes in a ball mill which was fastened in a paint shaker. Subsample were taken for both dry weight determinations (105°C to constant weight) and tissue total phosphorus and total nitrogen content.

Community metabolism was measured using methods described by Odum (1956) and Armstrong *et al* (1968). A continuous record of diurnal oxygen fluctuation at upstream and downstream stations was obtained by using EIL oxygen meters coupled with Rustrak recorders.

Solar energy was measured with a Weather Measure R401 pyranometer and underwater light energy was measured with a LI-COR quantum sensor model LI-185. Other physical measurements are described in a separate report on productivity in streams (in preparation).

The total phosphorus concentrations in different watersheds on the Thames River were measured throughout the year by the water quality branch and the annual phosphorus export from the Fish Creek headwater was calculated and used in the following discussion.

RESULTS

The immediate effects of flow, runoff and effluent discharges may cause local variations in nutrient concentrations over short periods of time (fig. 3). In addition, plant biomass acts as a good filtering mechanism thus diminishing the concentration to a greater extent between stations (fig. 4). In view of the frequent sampling needed to obtain a daily representative value, we collected at least 50 total P samples for each 20 day interval.

Even though a small percentage of unhealthy plants may produce some error in the tissue analyses (Gerloff, 1966), we feel that the entire quadrat sample will give a good representation of tissue content and eliminate the prejudice involved with choosing healthy plants.

Random quadrats were individually analysed to determine the concentration of P and N in plant tissue. An average monthly value was derived by averaging the results from two visits (table 2).

Relationship between nutrients in plant tissue and nutrients in water

Figure 5 shows the relationships between total phosphorus concentrations in water and in plant tissue for the two dominant species, *Cladophora* and *Potamogeton* in six river systems. Individual regression lines for *Cladophora* and *Potamogeton* exhibit a similar slope with regression coefficients of 0.80 and 0.84 respectively. When the data were pooled as shown in figure 5, the regression coefficient (r^2) improved slightly to 0.87. The calculated regression line is:

$$[P]_{tissue} = 16\,[P]_{water} + 0.67$$

Table 1: Some general characteristics of the six rivers studied in 1974.

| River	Substrate	Max. Mean Daily Temp.	Ave. 1% light depth	Flow May - Sept. m /sec	Alk. meg/L	[P] mg/L mean monthly min. summer avg. mean monthly max.	[N] mg/L mean monthly min. summer avg. mean monthly max.
Thames	Rubble	24°C	2.8 m	max 9.2 min 2.2	3.6	0.034 0.155 0.210	1.41 1.54 1.85
Avon	Rubble	24°C	4.0 m	max 0.83 min 0.26	4.5	0.010 0.064 0.083	N.A.
Nith	Silt & Rubble	24°C	1.8 m	max 9.8 min 0.7	3.6	0.069 0.082 0.099	1.45 1.62 1.79
Conestogo	Rubble	21°C	1.2 m	max 4.5 min 0.8	2.9	0.028 0.082 0.099	1.39 1.47 1.59
Maitland	Bedrock & Rubble	24°C	3.5 m	max 5.0 min 0.3	4.2	0.028 0.045 0.064	0.89 1.48 2.50
Bayfield	Rubble	26°C	3.9 m	dries up in July	4.0	0.062 0.069 0.075	N.A.
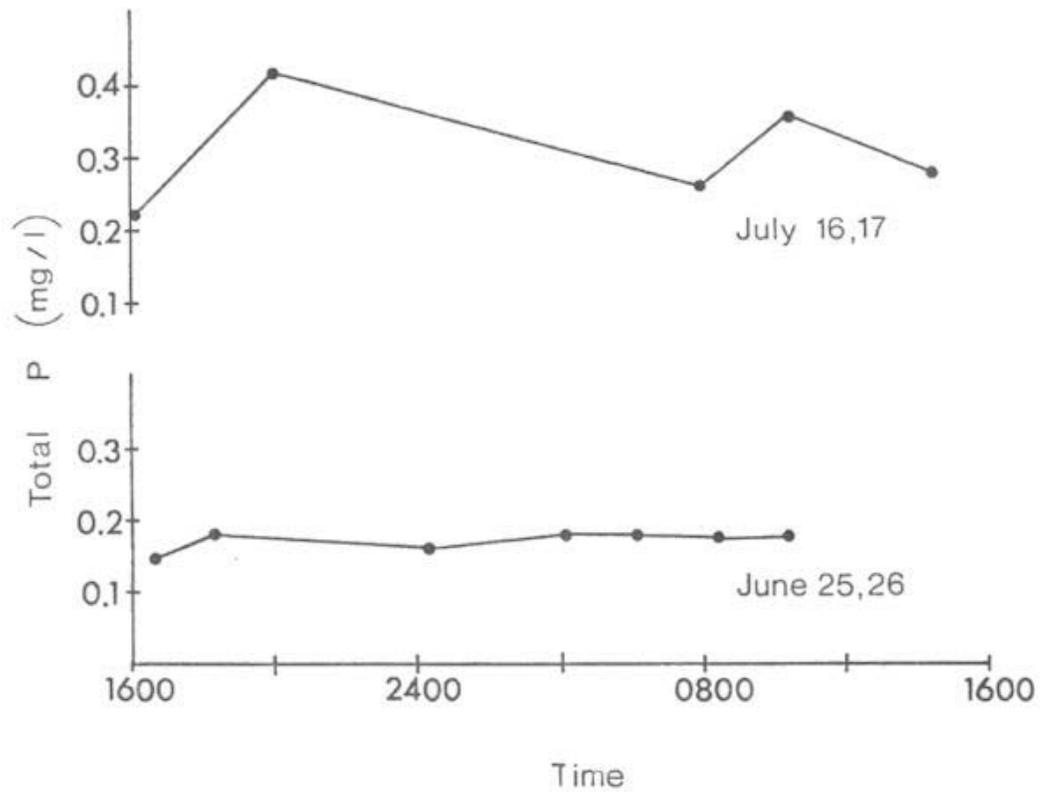
Figure 3: Variations in total P concentrations on the Thames River at T_2 over two separate 24 hr. periods.
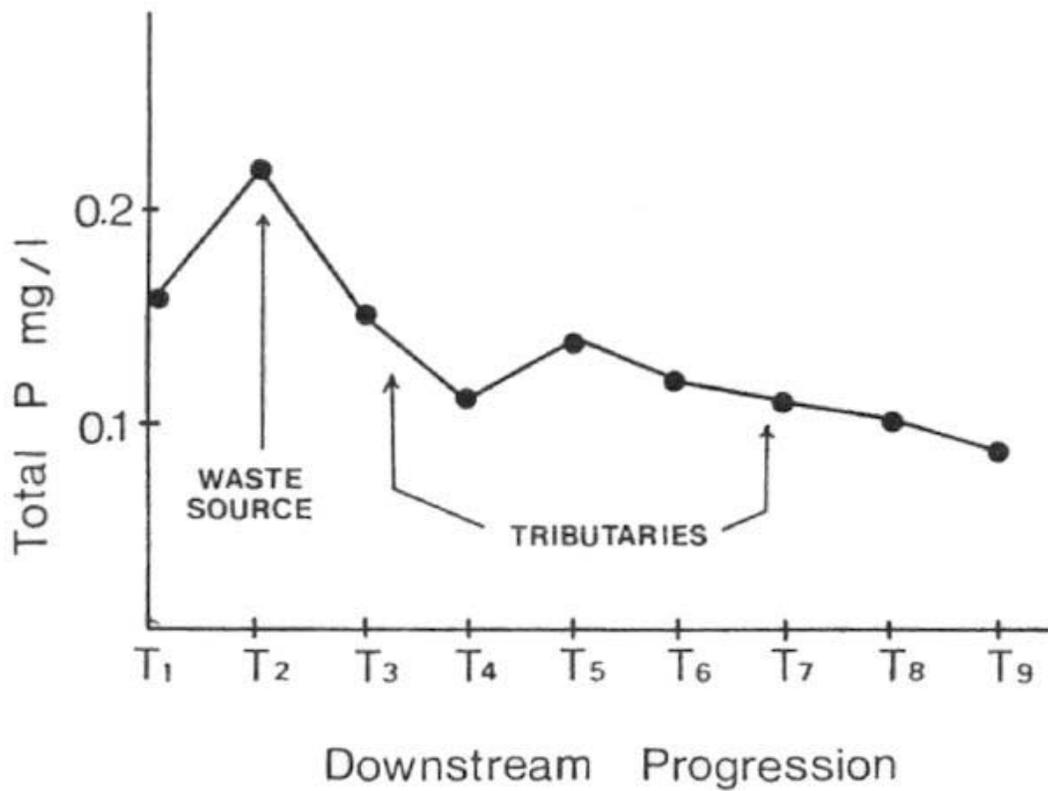
Figure 4:   Changes in total P with progression downstream over a short period of time.

Table 2: Total Phosphorus and Nitrogen content of *Cladophora* from June 7 to 26, 1973 (these results were obtained from four transects between station 1 and 2 on the Thames River).
Zeroes indicate that no plant material was found in the quadrat.

THAMES RIVER - BETWEEN STATION 1 & 2

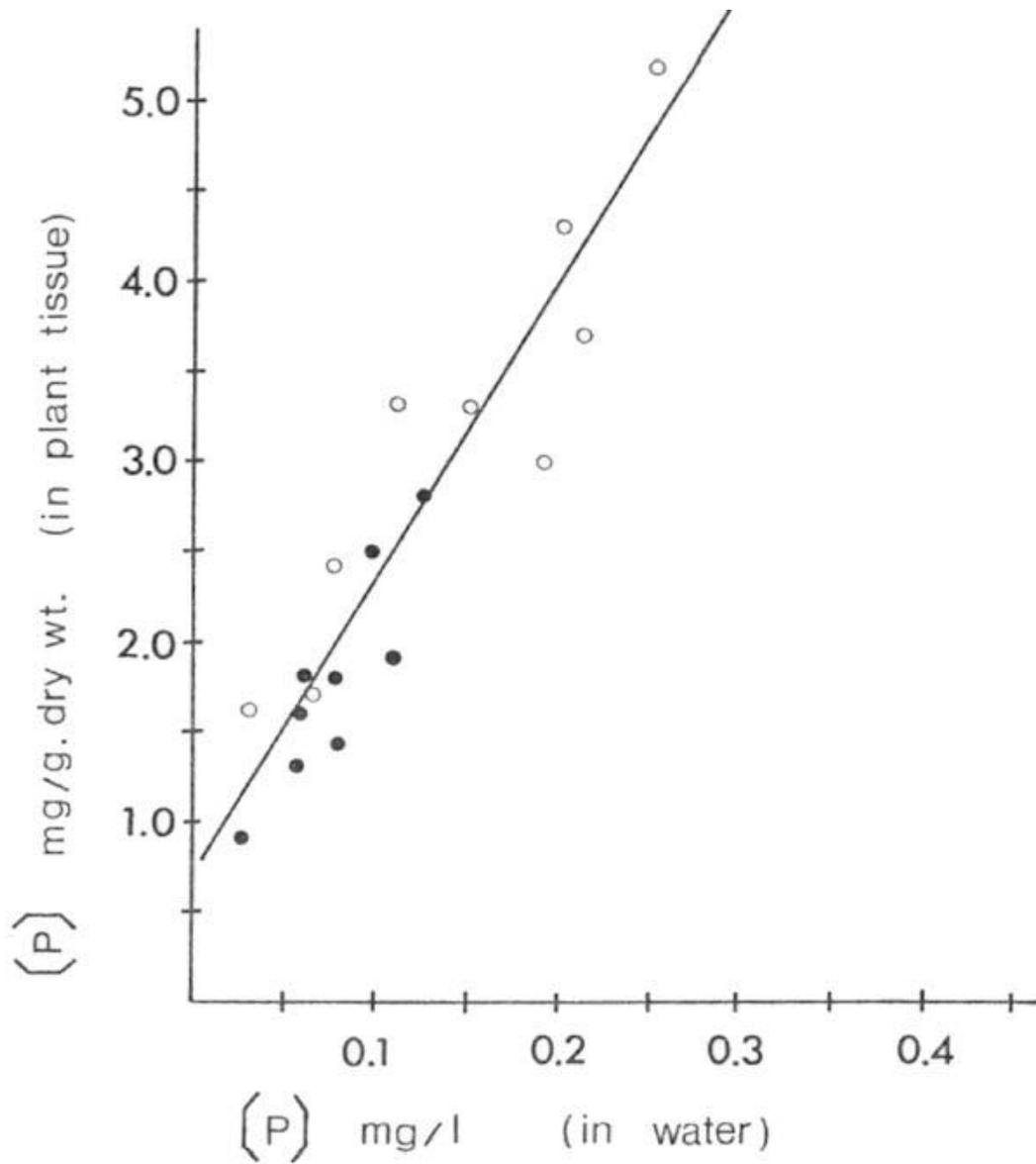| DATE	Transect	Quadrat	P content µg/g .d.w.	N content µg/g. d.w.	Date	Transect	Quadrat	P content µg/g. d.w.	N content µg/g. d.w	Mean Tissue Content For Sampling Period P µg/g.d.w	N µg/d.w.
June 7, 1973		1	2.6	21	June 26, 1973		1	1.4	5.7		
	1	2	0	0		1	2	1.2	6.3		
		3	3.1	20			3	0	0		
		4	0	0			4	1.6	14.0		
		5	2.6	13			5	1.3	16.0		
	2	6	2.8	27		2	6	1.5	9.4		
		7	2.9	22			7	1.6	14.0		
		8	0	0			8	0	0		
		9	0	0			9	1.5	14.0		
	3	10	2.9	24		3	10	1.6	11.0		
		11	0	0			11	1.8	12.0		
		12	0	0			12	1.9	13.0		
		13	2.5	21			13	1.7	11.0		
	4	14	1.8	12		4	14	1.3	12.0		
		15	0	0			15	1.5	13.0		
		16	2.6	20			16	1.8	21.0		
$\overline{X}$			2.64	20.0				1.55	12.31	2.10	16.16
$S\overline{x}$			0.37	4.8				0.21	3.83		
$S\overline{x}$			0.12	1.6				0.06	1.02		

Figure 5: Relationship between Phosphorus in the plant tissue and Phosphorus in water for *Cladophora* (closed circle) and *Potamogeton* (open circle).

Since the levels of stored P in the plant tissues are related to the concentrations of P in the external medium, this relationship can be best explained by the theories of a high demand for phosphorus (Schwoeibel and Tillmanns, 1968) and the efficient use of phosphorus (Bigler, 1969) in the plant community.

This relationship, however, differs from that found between P in plant tissue and P in water for 25 impoundments in Southern Ontario (Wile and McCombie, 1972) in that the slope of the calculated regression line found in the present study is less by a factor of 6 than that found in the pond study. This may have resulted because a) uptake of P through the root systems is probably much more important in the fertile ponds than in the rivers; b) only healthy plants were analyzed in the pond study and their P content would have been higher than that of dying or dead plant material (Caines, 1965) included in the river study, and c) the plant species investigated in the pond study were different from those in the river study with one exception.

Unlike phosphorus, there was no relationship between total kjeldahl nitrogen in plant biomass and total nitrogen (NO_3 + NO_2 + TKN) in water (Fig. 6).Very similar data have been reported by other authors (Gerloff and Skoog, 1957; Golterman, 1960 and Krauss, 1953). Due to rapid uptake and slow turnover, high storage of nitrogen beyond its optimal range may actually reduce the potential of plants to utilize nitrogen efficiently. From the lack of any relationship of total nitrogen between the two media, it is apparent that a change of total nitrogen content in solution may not have a direct effect on weed growth during times when excess nitrogen exists in the plant biomass. Accordingly any interpretation of N:P ratios from water medium in relation with aquatic plant growth may be misleading.

Prediction of P concentration in water

Since growth response to nutrient supply is a long-term effect, addition of nutrient to flowing water does not give an instantaneous increase in plant biomass. Because of the unpredictable variations in nutrient concentrations due to fluctuating waste load input and other physical parameters, a representative available nutrient value for a certain time interval requires a great number of analyses. Another approach is to make a simple prediction based on the empirical relationship of tissue content.
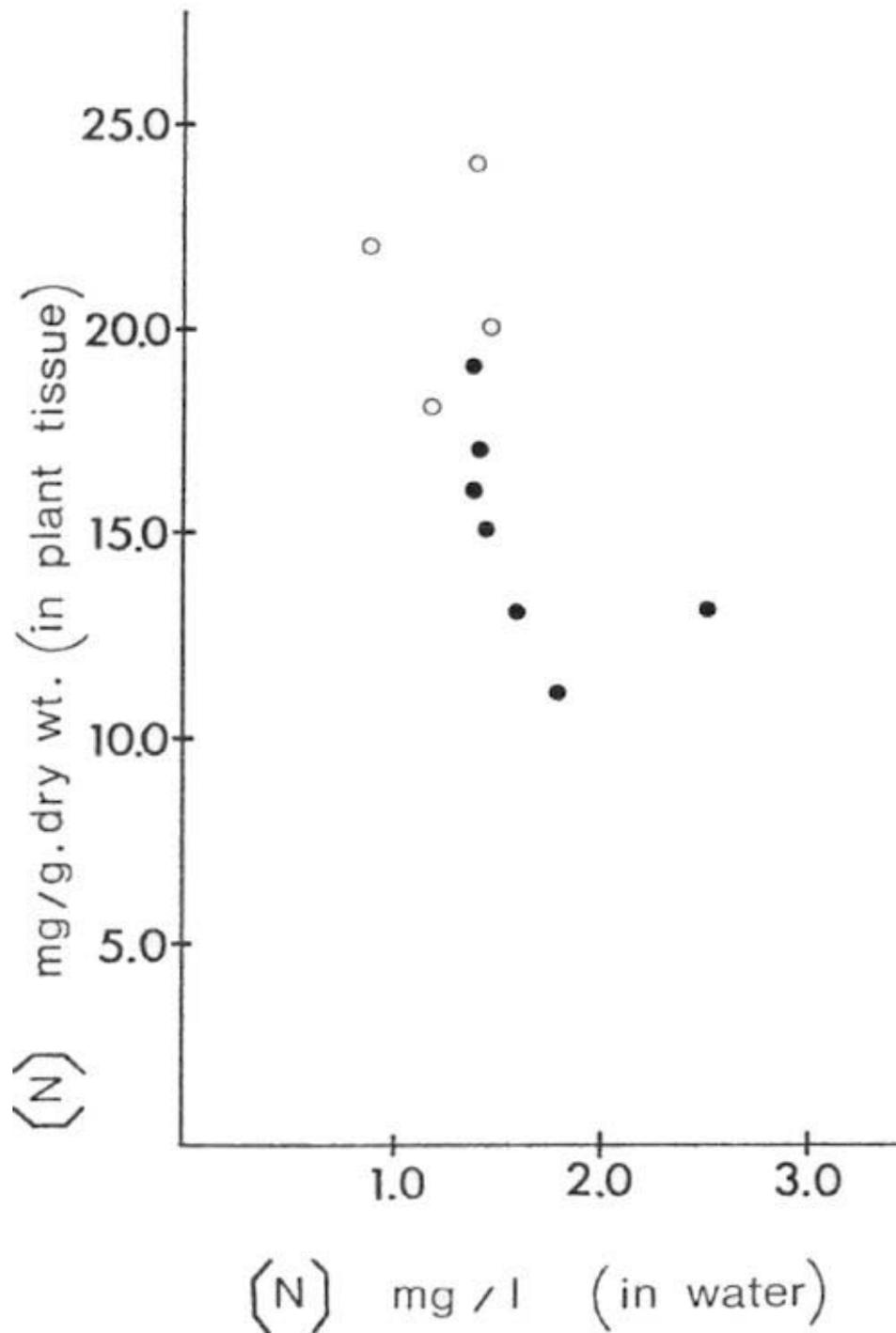
Figure 6: Relationship between total Nitrogen in the plant tissue and total nitrogen in the water for *Cladophora* (closed circle) and *Potamogeton* (open circle). Nitrogen analysis were not carried out in 3 rivers. Similar results were obtained using inorganic nitrogen in place of total nitrogen.

Nutrients in plant tissue are subject to less fluctuation and remain comparatively more stable. Quadrat samples along a given transect can be pooled and five to ten random transects in a one mile stretch will give us a representative mean P in plant tissue value. By using average nutrient in plant tissue data from two separate croppings (table 2) we can predict the average P concentration in water for the corresponding interval by using the following formula:

$$[P]_{water, predicted} = 0.050 \ [P]_{tissue, measured} - 0.020$$

where [P] water is in mg/L and [P] tissue is in mg/g dry wt.

Although application of the above empirical relationship requires further testing for other plant species, it would allow us to minimize the consideration of physical effects such as flow change and water depth on average nutrient concentration values.

<u>Field determinations of critical levels from tissue content</u>

The critical level of an element in plant tissue is defined as the minimum concentration of this element required to promote maximum plant growth. Determination of critical levels were confined exclusively to laboratory studies in the past. A tissue content of 1.3 mg P/g dry wt. proposed by Gerloff and Krombholz has been generally accepted as the critical concentration for aquatic weeds. From our regression line in figure 5, this concentration in the plant tissue is equivalent to 0.042 mg/L in water. On the other hand, if we consider Mackenthun's 0.10 mg/L as the critical phosphorus concentration in water, the corresponding plant tissue concentration would be 2.3 mg/g dry wt. which is almost twice the concentration of Gerloff and Krombholz. Other critical phosphorus level proposals such as that of Pittcairn and Hawkes (1.0 mg/L in water) and Wilson (0.06 to 0.08 mg/g dry wt. in plant tissue) make us feel that an evaluation of this phosphorus level for our species under natural stream conditions is necessary.

Evaluation of aquatic weed growth is difficult due to the affects and interactions of physical factors such as available light energy, turbidity, shading, change in biomass due to loss and decay and the uneven biomass distribution. Therefore the detection of critical levels by using nutrient concentration growth response relationships has not been attempted under natural conditions.

Plant growth in most enrichment studies is often expressed as the change in biomass from plant harvesting. The inaccuracy of using dry weight to determine the productive development of aquatic plants has been pointed out by Wetzel (1964) in his study of higher plant productivity. Crop yield determination always underestimates net production of *Cladophora* due to the large amount of detached, drifting plant material which cannot be accounted for. We collected an average of 3.5 kg fresh wt. of drifting *Cladophora* from the Thames River on a one square meter screen over a period of six hours during peak growth.

In addition to these problems we know that the change in biomass is not the result of nutrient effects alone since light energy also plays a major role in plant production. In order to permit comparison of external effects on growth the direct measurement of productivity was employed and growth per unit population was expressed in the relative units pl/p_{max} as suggested by Ryther (1956) where pl is the daily photosynthesis for the corresponding light intensity at the plant depth and p_{max} is the maximum photosynthesis at or near light saturation.

Since daily production is governed by nutrient supply and the amount of light energy available, the nutrient effect on growth is apparent only when the influence of light energy has been considered. Figure 7 shows the relationship between photosynthetically available radiation at plant depth (PAR) and the daily relative photosynthesis measured by the diurnal oxygen fluctuation technique. Details are included in a subsequent report (Wong and Clark, in preparation). By using the empirical relationship in figure 7 we can arrive at a correction factor which when applied, will allow us to observe the relative daily production independent of the effect of light. This correction factor will enable us to adjust our daily production figures such that they all appear to occur under maximum light conditions. Our data shows the maximum light intensity (PAR) observed for a cloudless summer day to be 160 g cal/cm^2/day, allowing 80% surface light transmission to plant depth. Using this figure, the correction factor, Rd on the daily pl/p_{max} can be computed from the equation

$$Rd = k \times \ln (I_{max} / I)$$

where Rd is in relative units, k is 1.85 (the empirical slope), I_{max} is 160 g cal/cm^2/day and I is the measured PAR at plant depth. Rd is then added to the measured pl/p_{max} to give a result corrected to standard light regime.

Figure 7: Relationship between Photosynthetically-available radiation at plant depth (PAR) and the daily relative photosynthesis (pl/p $_{max.}$ x hrs. daylight).

Figures 8 and 9 show the relationship between P and N in the plant tissue and mean relative growth at a PAR level of 160 g cal/cm /day. From figure 8 we can observe that below approx. 1.6 mg P/g dry wt. the relative growth of *Cladophora* decreases. The low points on the curve are represented by healthy plants observed during the growing season, May to June, when measured production was high therefore the low nutrient in plant tissue results do not represent dying plants.

Since figure 9 shows a critical level to lie between 12 to 15 mg/g dry wt. for N in the plant tissue it is difficult to determine whether the low points on figure 8 are indicative of a Phosphorus limiting effect or whether they represent a partial N limiting effect or a combination. The extreme low point on figure 8 (1) corresponds to a high N concentration of 16 mg/g dry wt., and if we accept Gerloff's 13 mg N/g dry wt. as the critical level for N in plant tissue then the other 3 low points on figure 8 would correspond to N levels near or above the critical level. If 1.6 mg P/g dry wt. is taken as the critical level from field observations then 0.06 mg P/L becomes our critical level in water when referring to the regression line in figure 5.This lies between Machenthum's value of 0.10 mg/L and Gerloff's projected value of 0.042 mg/L. Because there is no satisfactory relationship between total N in water and plant tissue, no critical N levels in water can be estimated.

With the exception of one survey, our data from tissue analysis indicate that nitrogen is always in excess. Thus a similar curve of phosphorus content in solution in relation to growth should be expected regardless of the fluctuation of nitrogen levels in the external medium. Figure 10 shows the familiar curve with the breaking point at around 0.07 mg P/L.

Estimation of maximum P waste loading capacity

To avoid nuisance growth of *Cladophora*, phosphorus must be maintained well below the calculated critical level of 0.06 mg/L. Figures 11 and 12 show the typical situation where P levels are well above 0.06 mg/L, whereas figures 13 and 14 typify streams where the P concentrations are below the critical level. Because *Cladophora* production is correlated to phosphorus concentration in the water only below this level, (production remaining constant above 0.060 mg/L) that level must be reached before control is initiated. Of all possible sources of P input, only industrial and domestic sewage has been controlled to any extent.
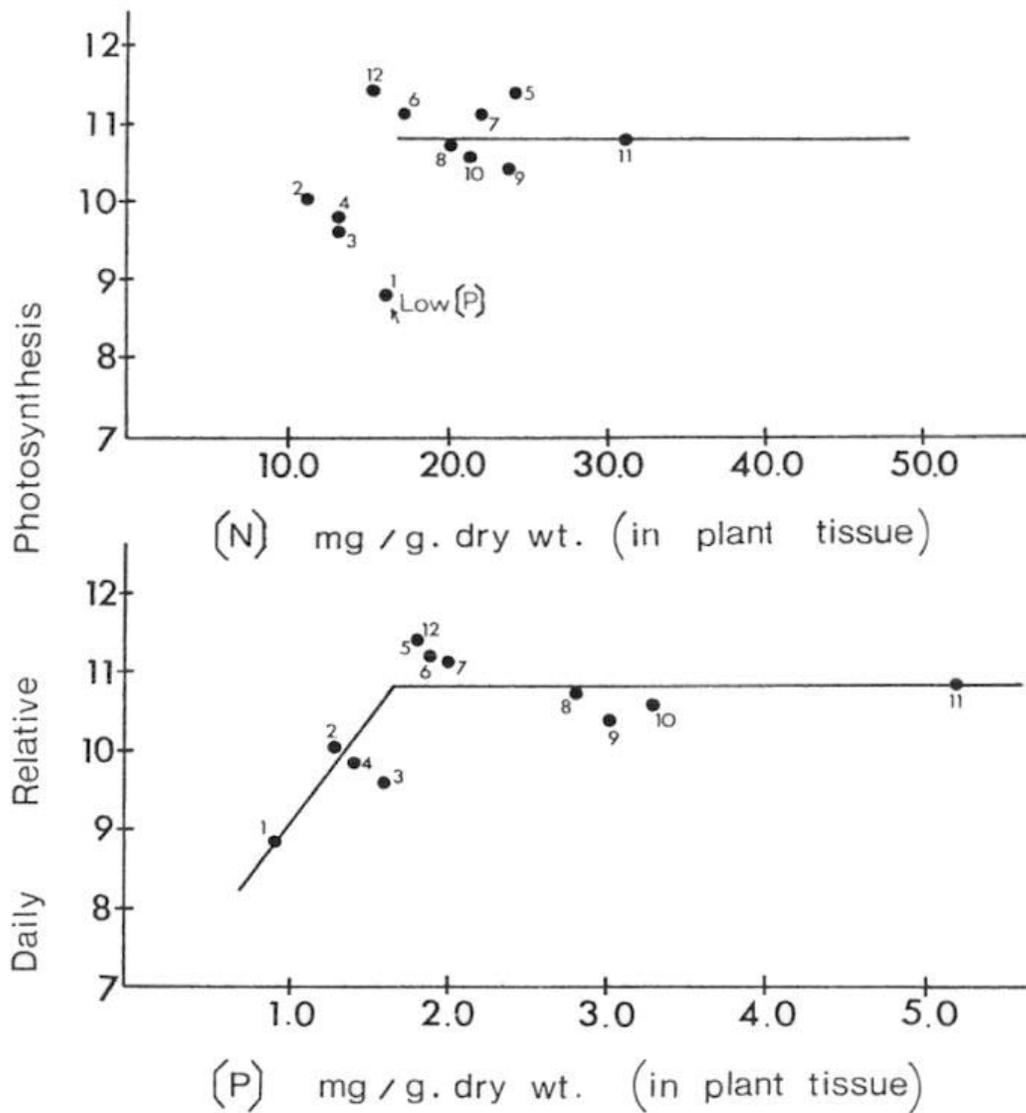
FIGURE 8 and 9: Figure 8 shows the relationship between P in *Cladophora* and the daily relative photosynthesis.

Figure 9 shows the relationship for N in *Cladophora* and the daily relative photosynthesis.
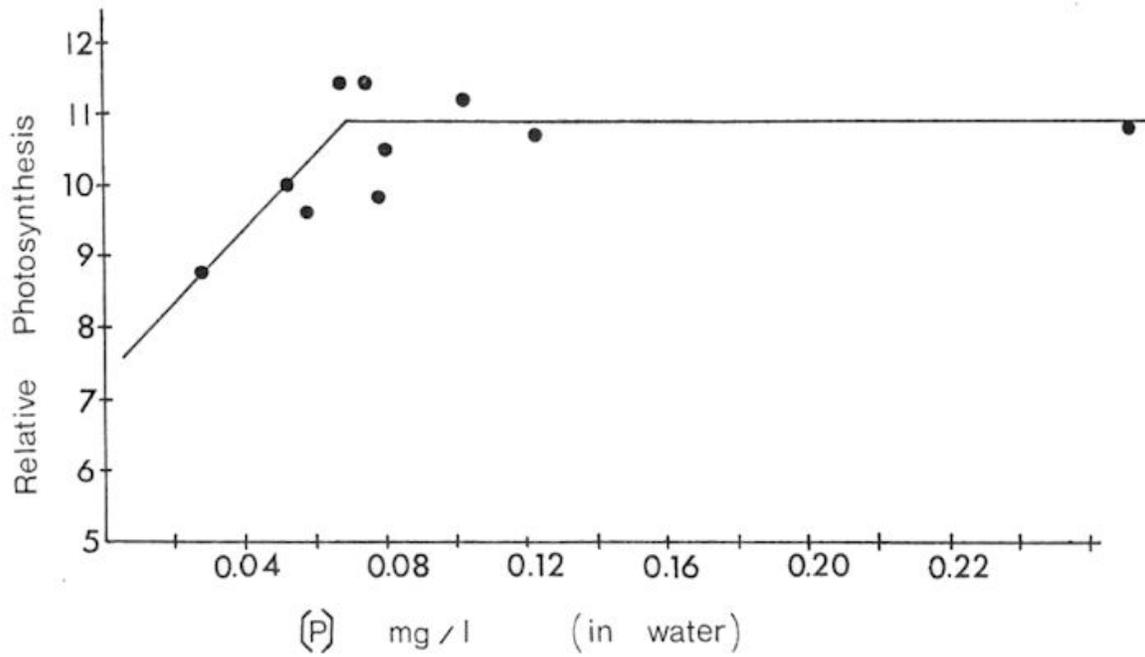
Figure 10: Relationship between P in water and the daily Relative Photosynthesis of *Cladophora.*

Figures 11 (top) and 12 show excessive growth of *Cladophora* on the Avon River.

Figures 13 (top) and 14   show a section of the Maitland which has not been choked by aquatic plants.

Input such as groundwater and forest runoff and other diffuse sources such as agricultural runoff are difficult to control. If the headwater land usage is comparable to similar polluted areas downstream then we can use the export figures from the headwater region to estimate the background P loading from diffuse sources in the problem area. The background P concentration can be derived by the equation

$$[P]_b \; = \; Es \; x \; Ad/R \tag{1}$$

where $[P]_b$ is the background concentration in mg/L; Es is the export coefficient in $mg/m^2/yr$; Ad is the drainage area in m^2; and R is the total runoff in liter/year.

The minimum waste load that would result in the maximum growth rate of *Cladophora* can be computed from the following equation

$$L_w \; = \; ([P]_c - [P]_b) \; x \; Q \tag{2}$$

where L_w is the waste loading in kg; $[P]_c$ is the critical concentration of 60 µg/L; and Q is the measured annual flow.

For example, the N. Thames River, with 90% agricultural land, has a drainage area of 1,092 km^2. The measured total P export coefficient was 18 mg/m^2/yr (Thames River Report, Ministry of the Environment, unpublished data) and a mean runoff of 3.77 x 10^{11} L/yr, derived by applying 1.0 cfs/sq/ mile (from Coulson's long term prediction in Southern Ontario watersheds, 1956) to the drainage area. Thus a background P concentration of 0.050 mg P/L is derived using equation 1. From a mean annual flow of 57 cfs (1.6 m^3/sec), measured in the basin, the minimum waste load that would result in the maximum growth rate of *Cladophora* would be 500 kg/yr in addition to the background loading.

Seasonal phosphorus export coefficients are not available for the growing period of aquatic macrophytes (i.e. May to October), so an annual export coefficient was applied in the simple example above. However, the incorporation of seasonal export coefficients in the above equations will derive a more accurate background P concentration and should be used in practical applications.

CONCLUSIONS

The determination of the actual concentration of phosphorus below which the specific growth rate of *Cladophora* is reduced is difficult to determine, particularly under field conditions; nevertheless, it can be asserted from the existing data that the level is approximately 1.6 mg P/g dry wt. in tissue and 0.06 mg/L in water. Unlike P, no significant correlation was observed between N content of plant tissue and N concentration in water. The correlation of total P with plant growth can be used to estimate the waste load which would result in maximum growth rate of *Cladophora*. However, this has no predictive value with respect to total biomass oxygen fluctuations and further investigation is necessary to determine the ambient phosphorus concentration required to control these factors.

Since the phosphorus content in plant tissue is less affected by daily fluctuations in the ambient phosphorus concentration, it can be used to predict the average ambient nutrient concentration for a reach over a desired time period with less sampling required.

FUTURE CONSIDERATIONS

Research to date has provided us with critical levels in water and in plant tissue for *Cladophora*. These critical levels indicate the phosphorus concentration in water which will yield maximum growth of *Cladophora* and below which the specific growth of *Cladophora* will be decreased. This information does not indicate the effect of such a reduction on the total biomass or the subsequent resulting oxygen fluctuations. Future research will move in these directions and provide information which will link oxygen fluctuation more closely to the nutrient load of specific rivers.

APPENDIX

P_{max} -   Maximum hourly photosynthetic rate, at light saturation, observed for that particular day.
g O_2/m^2/hr.

pl   -   Average hourly photosynthetic rate observed for that day.
g O_2/m^2/hr.

pl/p_{max} -   is a ratio with no units.

Therefore daily relative photosynthesis is pl /p_{max} x hours of photoperiod.

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