A LOTIC ECOSYSTEM TROPHIC STATUS INDEX

USING THE PERIPHYTIC COMMUNITY AS

A BIO-INDICATOR

By

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER 1

INTRODUCTION

Sources of potable fresh water are declining globally as a result of human activities on land and in the water (Food and Agriculture Organization, 1993). Water resources in the United States are also being stressed by human activities and increased demand (Francko and Wetzel, 1983). Of the water on Earth, 97.61 percent is in the oceans, 2.08 percent in polar ice glaciers, 0.29 percent is in ground water, 0.009 percent is in freshwater lakes and reservoirs, and 0.00009 percent is in rivers and streams, with the remainder existing as soil moisture and atmospheric water vapor (Vallentyne, 1972). In the United States, 86.4 percent of our fresh water is in ground water, 13.0 percent is in lakes and reservoirs, 0.03 percent is in rivers and streams, and the remainder is in soil moisture, water vapor, and glaciers (Francko and Wetzel, 1983). We rely on surface waters for more than 75 percent of our water needs nationally. On average, each human in the U.S. consumes, directly and indirectly, 1500 liters of water per day, or nearly 383 billion liters nationally per day. Of that, 82 percent is used for agriculture, 15 percent for individual needs, and three percent for

residential needs (Francko and Wetzel, 1983). Agricultural demand for water resources exceeds 313 billion liters daily.

The Clean Water Act of 1972 established the goal of protecting the physical, chemical, and biological integrity of our Nation's waters. In the twenty years since these objectives were established, water quality in the United States has improved significantly (EPA, 1987). However, the United States Environmental Protection Agency's (EPA) 1986 National Water Quality Inventory Report to Congress ranked nonpoint sources such as runoff from agricultural areas, as the leading contributors of pollution to lakes, streams and estuaries (EPA, 1987). The report cited nonpoint sources as the leading cause of pollution for 75 percent of polluted lakes, 65 percent of polluted streams, and 45 percent of polluted estuarine areas.

Abatement of nonpoint source pollution requires knowledge of the sources of pollution for a given stream reach, the effect that pollution has on the biota of a stream reach, and the spatial and temporal loading of the pollutant to the stream. The terrestrial, geo-morphological, hydrological, meteorological, and aquatic characteristics of lotic ecosystems in the U.S. vary dramatically between and within major drainage basins. Abatement and assessment of these nonpoint source pollution problems will require the development of novel methods for large-scale monitoring of rivers and streams.

Problem Statement

Nonpoint source (NPS) loading of nutrients to rivers and streams (lotic ecosystems) represents a major source of uncontrolled pollution in the United States (EPA, 1992). Due to the magnitude of the area and the diversity of land-use practices within each regulatory region across the U.S., prioritization and targeting of NPS pollution sources is necessary for effective implementation of remediation or restoration programs. Directly measuring NPS nutrient loading to a stream is a difficult and expensive process. Most stream nutrient monitoring is performed using discrete, or grab, samples from a given place at a given time.

Nutrients such as phosphorus and nitrogen are typically transported to the stream with sediment or in solution in surface runoff. The resulting high concentration of nutrient import to the stream ecosystem occurs over a very brief period of time. Discontinuous monitoring, such as grab sampling, often misses these events (Hughes et al., 1990; Round, 1991). The more expensive alternative is continuous, flow-weighted sampling using automatic samplers. While this approach provides a more accurate estimate of nutrient loading, the peak loadings are still diluted in the sample aliquot, resulting in underestimation of the potential for cultural eutrophication. Another problem inherent in chemical monitoring is the fact that this method does not consider biologic availability.

The physical and chemical properties of the water in a lotic ecosystem are determined by the characteristics of the terrestrial watershed (Lotspeich, 1980; Vannote et al., 1983). The structure and productivity of the biological community in a lotic ecosystem directly reflects the chemical and physical properties of the water (Douglass, 1958; Fjerdingstad, 1964; Patrick, 1977; Lange-Bertalot, 1979; Evenson et al., 1981; Gotah and Negoro, 1986; Keitham et al. 1988; Hughes et al., 1990; Round, 1991). Measuring the response of the biological community in a given stream reach should provide information on the degree of terrestrial and aquatic perturbation existing within the watershed.

Research Objectives

My principle objective with this research effort was to develop a method for characterizing watershed ecosystem impact from human activities on primary productivity in the aquatic ecosystem at the watershed level. This research program had three distinct objectives:

1. To develop a method for determining the limiting nutrient in a lotic ecosystem;
2. To apply this method to measure the limiting nutrient in three watersheds within a basin;
3. To determine the periphytic trophic status of lotic ecosystems in specific watersheds within a sensitive basin;

This dissertation represents my attempt to accomplish these objectives. The work presented here is a chronology of three years effort. This information is presented sequentially, beginning with a literature review, followed by a discussion of the methods, results, and discussions for each of the aforementioned objectives, then closing with a general discussion, conclusions, and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

Lotic Ecosystems

Lotic ecosystems are aquatic systems characterized by flowing water, in contrast with lentic ecosystems, which are characterized by non-flowing water (Round, 1981). Lotic ecosystems throughout the world have common structures and components (Minshall et al., 1985). A simple model of lotic ecosystem component interactions is presented in Figure 1. This model illustrates the interactions of functional groups in carbon cycling, but does not consider spatial or temporal variability in quantitative interactions. When the interactions are quantified, the model becomes quite complex, with fundamental feedback loops becoming apparent (Figure 2). The functional groups referred to in Figure 2 are defined in the following section. Modeling the energy flow through a river provides a more intuitive linear progression from primary producers to top predators (Figure 3).

Figure 1: Simple Model of Lotic Ecosystem Interactions (after Cummins, 1974).

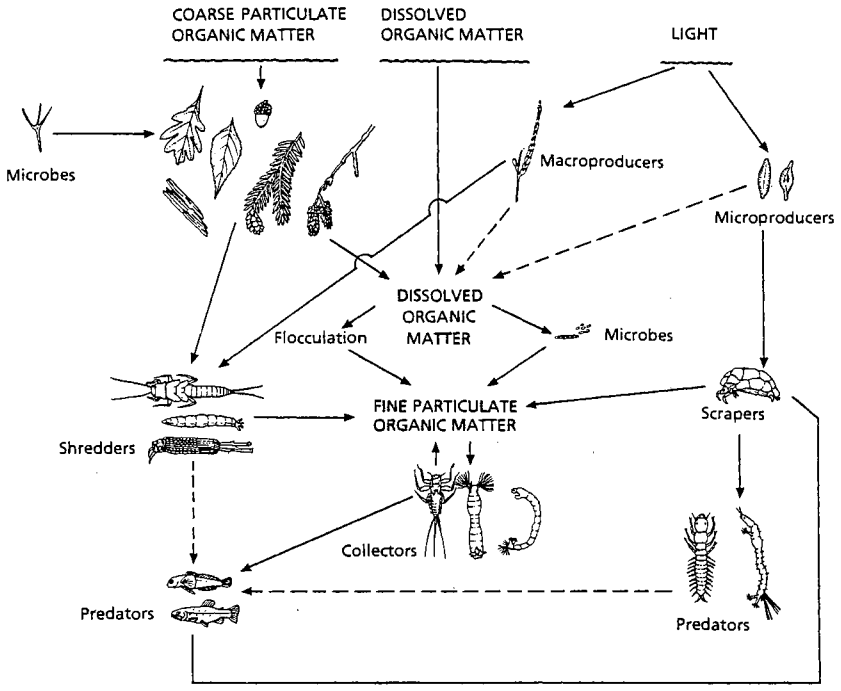


Figure 2: Stream Ecosystem Model with Variable Environmental States. Units are dry mass m^{-2} yr^{-1} (from Calow and Petts 1992).

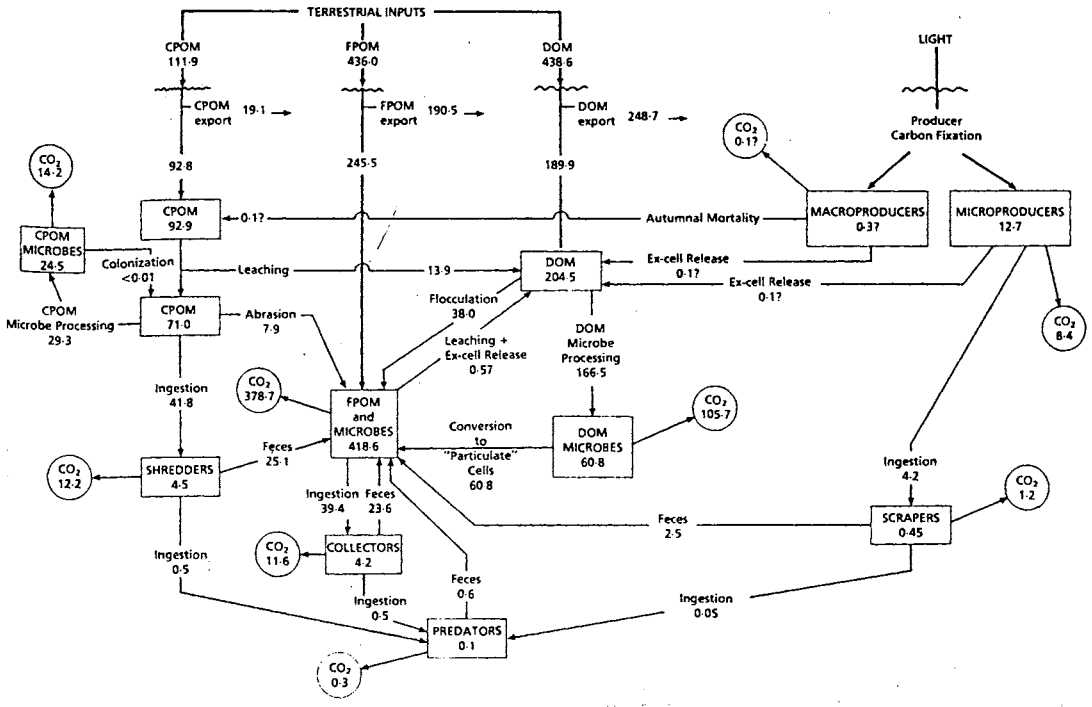
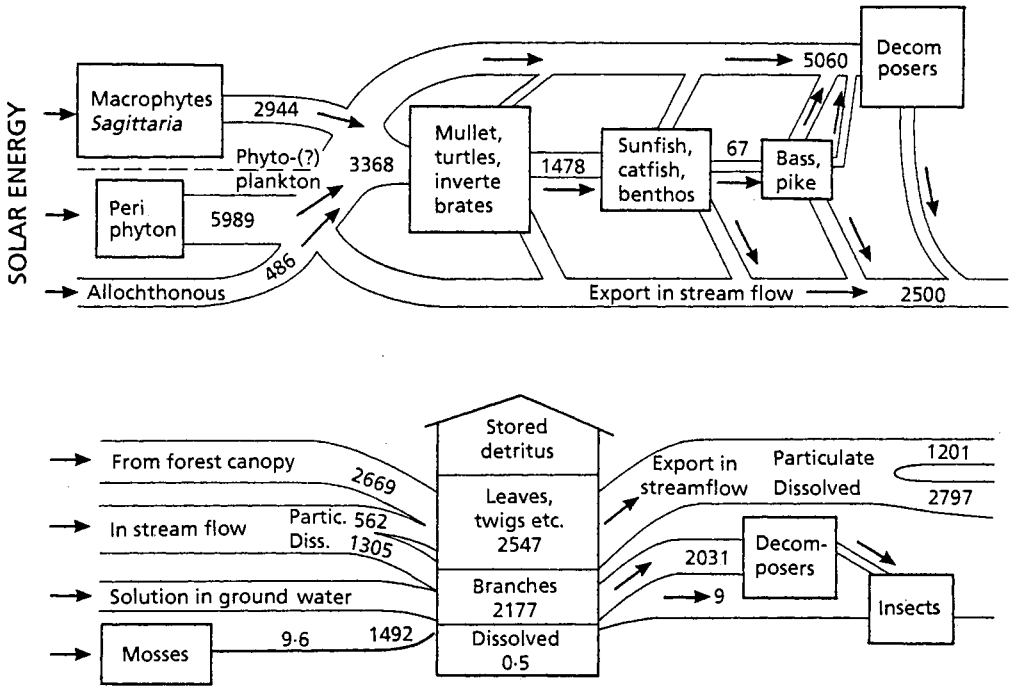


Figure 3: Energy Flow Through Two Rivers.
Units are kcal m^{-2} yr^{-1} (from Calow and Petts 1992).



River Continuum Concept

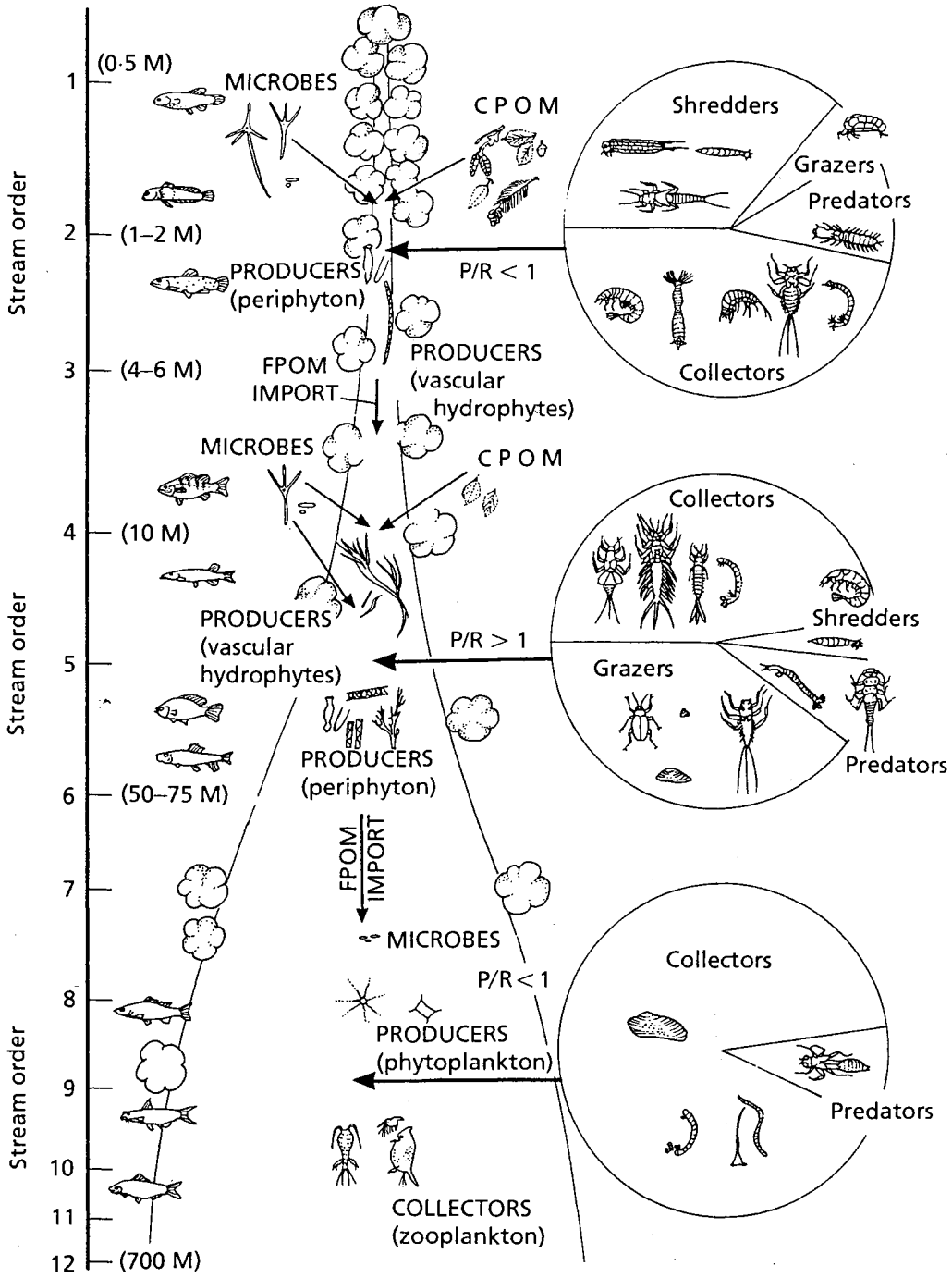
Lotic ecosystems are best understood as a spatial and temporal continuum of physical, chemical, and biotic components (Vannote et al., 1980). This river continuum concept (RCC) has become the standard paradigm for lotic ecosystem function (Figure 4). The RCC specifically states that understanding the biological strategies and stream dynamics requires consideration of the gradient of physical factors formed by the drainage network (Vannote et al., 1980). Based on the RCC, lotic ecosystems are classified as **headwaters** (stream orders 1-3), **medium-sized streams** (stream orders 4-6), and **large rivers** (stream orders >6).

Headwaters

Headwaters are generally influenced strongly by riparian vegetation. The canopy cover over a first, second, or third order stream tends to reduce autocthonous production (carbon fixed within the stream) by shading. However, the contribution of allocthonous detritus (carbon fixed outside the stream) from the riparian zone tends to be significant (Vannote et al., 1980). Allocthonous production is generally in the form of course particulate organic matter (CPOM), or leaf, stem, and root materials from the terrestrial ecosystem. The gross primary productivity to community respiration ratio (P/R) is generally less than one in lower order streams (Vannote et al., 1980).

Figure 4: An Illustration of the River Continuum Concept

(from Calow and Petts 1992).



Medium-sized Streams

As streams increase in size, the influence of allocthonous productivity decreases, while autocthonous primary productivity increases. The distinction between headwaters and medium-sized streams is generally when P/R>1 (Vannote et al., 1980). By definition, algal growth (primary productivity) in headwater streams is limited by light; when the stream reaches a size such that the riparian canopy shading no longer restricts algal growth, the stream is classified as medium sized. This may occur in a first-order stream in xeric regions, or in a third-order stream in a dense conifer forest or canyon, depending on the degree of shading (Minshall, 1978).

Large Rivers

Large rivers receive significant amounts of fine particulate organic matter (FPOM) from upstream. This FPOM is processed CPOM from the headwaters (Vannote et al., 1980). Autocthonous primary production may be limited by turbidity from suspended sediment or depth of the photolytic zone. Under these conditions, P/R<1 (Vannote et al., 1980).

Biotic Components of Lotic Ecosystems

The biotic component of lotic ecosystems can be divided into heterotrophic microbes, algae, macrophytes, invertebrates, and vertebrates (Calow and Petts, 1992). The biotic component of primary interest for this investigation is algae;

this section briefly describes the other biotic components and provides a more comprehensive review of algae in rivers. It should be noted that the divisions of these biotic components is based largely on morphological and taxonomic classifications. These components exist in a dynamic flux, and should be considered parts of a greater continuum.

Heterotrophic Microbes

Heterotrophic microbes (fungi and bacteria) are the primary decomposers in streams. This component of the biota in aquatic ecosystems has been largely ignored in energy flux investigations (Pomeroy, 1991). However, recent evidence suggests that much of the respiration that occurs in marine ecosystems is microbial, and in some ecosystems, may exceed primary production (Smith and Mackenzie, 1987). Microbial biomass in sediment provides a food source for macro-faunal grazers (Van de Bund et al., 1994).

Macrophytes

Aquatic macrophytes are macroscopic flora including aquatic spermatophytes (seed-bearing plants), pteridophytes (ferns and fern allies) and bryophytes (mosses and liverworts) (Fox, 1992). Macrophytes compete for three resources: light, space, and nutrients (Grime, 1979). In lowland rivers, nutrients are often available in excess (Ladle and Casey, 1971), making light the dominant limiting resource for macrophytic communities. The competitive characteristics for

macrophytes include canopy formation, use of bicarbonate for dissolved inorganic carbon, use of carbon dioxide in the air, a low light compensation point resulting in early seasonal and daily growth, low root/shoot ratio, and high litter production (Grime, 1979).

Invertebrates

Invertebrates are an ecological link between algae and heterotrophic microbes (their food resource) and fish (their predators) (Cummins, 1992). Aquatic invertebrates are classified as micro-invertebrates (generally < 0.5 mm) and macro-invertebrates (generally > 0.5 mm) (Cummins, 1975). Micro-invertebrates, also referred to as zooplankton (free-swimming species) or meiofauna (sessile species) are predominantly collectors or filter feeders, while macro-invertebrates are composed of many functional feeding groups, including scrapers, shredders, predators, and collectors (Figure 4) (Cummins, 1974). Taxonomic identification of freshwater invertebrates has been focused in Europe and North America (Cummins, 1992). This class of aquatic biota is very poorly documented globally; in fact, due to the global scale of environmental alteration of running waters, many species will become extinct without ever having been identified or characterized (Wilson, 1988).

Vertebrates

The principle vertebrates in lotic ecosystems are fish. Amphibians and reptiles, avians and mammals are also present and part of the lotic community, but fish comprise the dominant portion of biomass of lotic vertebrates. There are roughly 8500 species of freshwater fish; most of these species occur in rivers or connected floodplains (Lowe-McConnell, 1987). Fish species guilds include primary, secondary, and final predators, detrivors and herbivores.

Algae

Algae are generally responsible for autocthonous production in lotic ecosystems. The conditions in which algae have evolved in lotic ecosystems vary dramatically, resulting in intense inter- and intra-species competition for resources (Reynolds, 1992). This high level of selective pressure has resulted in extremely diverse survival strategies and high levels of speciation among algae. In spite of this diversity, algae are among the most cosmopolitan classes of organisms (Cairns, 1991).

Ecological Terminology

Clarification of terminology is warranted at this point, as generalizations are being made about ecological organizational levels. *Communities* are functionally defined as collections of species living together and recurring in spatially

separated habitats (Round, 1981). *Populations* are collections of individuals of one species living within a defined area or volume. An example would be the population of elk in the Central Rocky Mountains. A collection of populations classified by some functional characteristic is an *assemblage*. The shredder assemblage in streams is composed of 10 to 20 populations of macro-invertebrates. An *association* is an assemblage of species that recurs under comparable ecological conditions in different places, characterized by a few dominant populations (Hutchinson, 1967). An example of an assemblage is the oak-hickory forest, which is identified by the dominant species, but consists of many other implied populations, including both flora and fauna. By this definition, an assemblage is a specialized definition of a community (Round, 1981).

Classification of algal habitats is difficult since algae occur in virtually every place on Earth that is exposed to sunlight (Round, 1981). Even separating algal communities into freshwater and marine classes is artificial since water and algal species associated with it varies continuously from rainwater through freshwaters, oceans, to hypersaline landlocked seas (Round, 1981). Distinguishing between lotic and lentic algal communities, and even attached and unattached algal communities within lotic ecosystems, is even more arbitrary.

However, classification is essential for conceptual thinking and clarification of ecological concepts (Round, 1981). Care should be taken to remind oneself that the classification of algal communities by habitat characteristics is functional at best, and holds no ecological significance to the algal species themselves. With this in mind, algae are segregated into *plankton* and *benthos*. The term plankton refers to open water ecosystems; planktonic algae are called *phytoplankton*. The phytoplankton are further divided into *euplankton* (permanent community of the open water) and *pseudoplankton* (algae caught up in water currents or washed into the open water). Benthos are associated with the bottom of the water column and submerged objects. The algal communities growing in the benthos have been classified based on the growth form of the algae (Round, 1981). Algae growing on vegetation are called *epiphyton*. Algae growing on rock surfaces are called *epilithon*. *Epipelon* are algae growing in sediment and sand (Round, 1981).

The term "Periphyton" is commonly used to describe the combined epiphytic, epipelic, and epilithic communities (Round, 1981). This rather imprecise term was originally used to describe algal growth on artificial substrates, and should probably be limited to that definition, but it is now commonly taken to include all attached algal growth. We will classify the algae in lotic ecosystems as attatched communities (periphyton, or aufwuchs) and unattached communities (phytoplankton).

Autocthonous Primary Production In Lotic Ecosystems

The algal assemblage within the periphytic community is responsible for the greater part of autocthonous (self-generated) primary productivity in lotic ecosystems (Hill et al., 1992). Aquatic bryophytes, macrophytes, and phytoplankton may contribute to autocthonous primary productivity, and in very limited spatial and temporal zones may be the major primary producers in streams; however, these assemblages generally represent a small portion of the overall primary production of streams (Reynolds, 1992). Primary productivity, or carbon fixation, is generally measured as an increase in biomass or phytopigment concentration (Sand-Jensen, 1983). Changes in algal biomass is a function of growth rate, colonization, mechanical detachment, and grazing by macro-invertebrates and fish. This relationship can be described as:

$$\Delta B = (G + C) - (Gr + M) \qquad (1)$$

where ΔB is the change in algal biomass (or phytopigment) over a specific time interval, **G** is the growth or division rate, **C** is the colonization rate, **Gr** is the rate of grazing, and **M** is the rate of mechanical detachment (Sand-Jensen, 1983).

Human Impact On Lotic Ecosystems

Nutrient loading, specifically phosphorus and nitrogen, to rivers and streams often limits the uses of the affected bodies of water (Beaulac and Reckhow, 1982). Nutrient loading results in ecological resource enrichment, and can lead to significant disturbance of the ecological health of a system (Cairns et al.,

1992). Nutrients originate from point (end of pipe,or discernable channalized conveyance) and nonpoint (no discernable channel or conveyance) sources in a watershed. Point sources of pollution are generally more quantifiable, monitorable, and controllable than nonpoint sources (Beaulac and Reckhow, 1982). Much progress has been made in reducing point source loading under the Clean Water Act of 1972 (Environmental Protection Agency, 1987).

Nonpoint source pollution (NPS), on the other hand, is very difficult to quantify, monitor, and control (Beaulac and Reckhow, 1982). The USEPA has estimated that as much as 65 percent of stream and 79 percent of lake designated use impairment is from nonpoint sources (Environmental Protection Agency, 1987). The factors that influence NPS nutrient loading are land use, soil characteristics, climate, topography, and land cover practices (Beaulac and Reckhow, 1987). Estimates of nutrient loadings from specific land uses exhibit considerable uncertainty, making temporal and spatial monitoring of water quality critical to pollution abatement (Beaulac and Reckhow, 1982).

Lotic ecosystems have an assimilative capacity for nutrients within a range that is consistent with evolutionary conditions (Cairns and Pratt, 1990). This natural pollutant buffering capacity of streams is being degraded by removal of natural vegetation within the stream and in the riparian zone, stream channelization, and increased sedimentation (Environmental Protection Agency, 1977). These

cumulative impacts increase the demand for temporal and spatial monitoring of specific lotic ecosystems to assess their potential for degradation.

Lotic Ecosystem Responses to Disturbance/stress

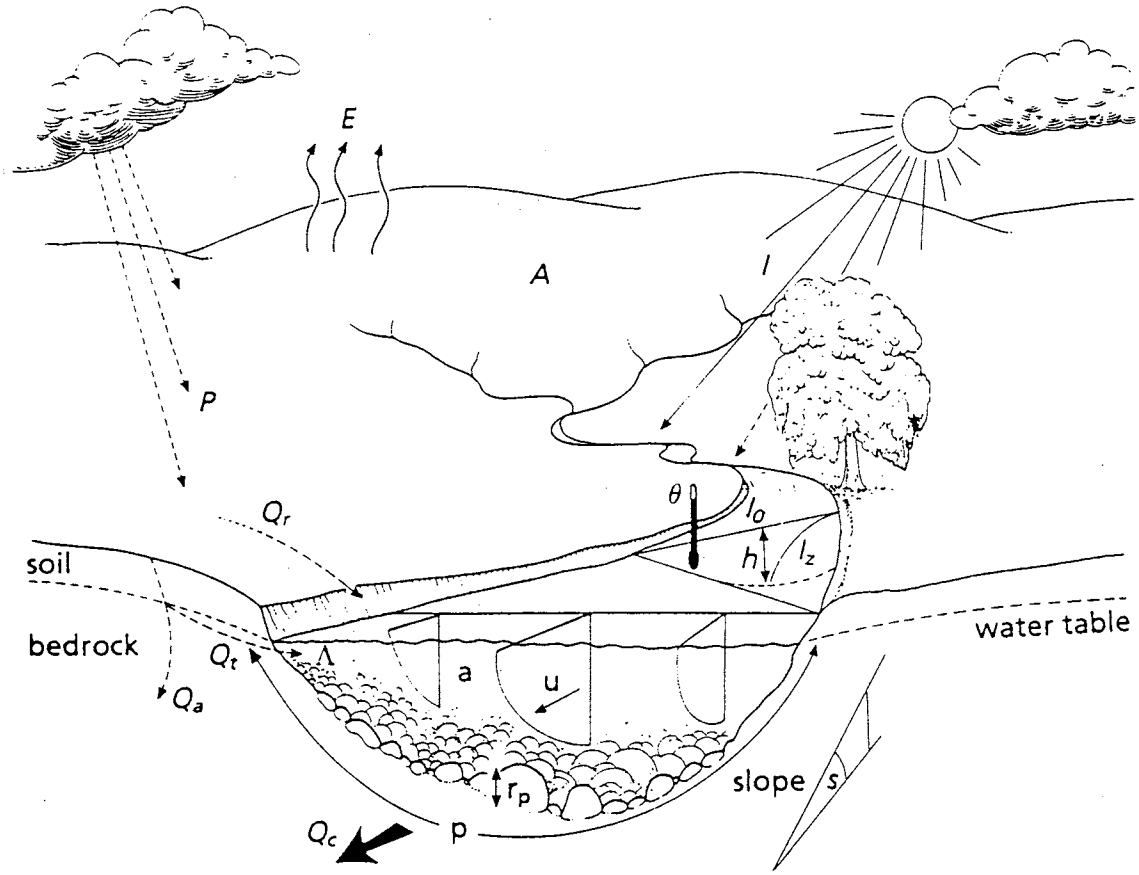
The periphytic community exists in an environment governed by extremes (Calow and Petts, 1992). The environmental characteristics of rivers and watersheds that influence the periphytic community are presented in Figure 5, and are summarized below (Calow and Petts, 1992). The driving forces for lotic ecosystems are predominantly hydrologic; net discharge (**Q**) is a function of the balance between all forms of precipitation (**P**) and the evaporative losses (**E**) per unit of area in the drainage catchment (**A**). Net discharge is equal to the flow across ground surface (Q_r) plus lateral percolation, or interflow (Q_t), and percolation to the groundwater (Q_a). The channel flow yield (Q_c) is the sum of Q_r and Q_t. The total load of solutes (Λ) is a function of Q_c, expressed as:

$$\Lambda = k\, Q_c^{\,f} \qquad (2)$$

where ***k*** is a solute availability/solubility constant, and **f** is a dilution factor, generally less than one (Meybeck et al., 1989). The downstream velocity (**u**) is a function of Q_c, the wetted perimeter (**p**), the slope of the river bed (**s**), the area of flow (**a**), and the perimeter roughness coefficient (r_p, from Manning's Equation, Haan et al., 1994). The exact relationships between these variables are scale dependent, and are discussed in detail elsewhere (Haan et al., 1994).

Figure 5: Environmental Variables Of Lotic Ecosystems

(from Calow and Petts 1992).



Light penetration into the water column at depth **z** (I_z) is a function of surface reflection (I_o) and the coefficient of light extinction (ε):

$$I_z = I_o e^{-\varepsilon z} \quad (3)$$

The coefficient of monochromatic light extinction is a function of the water itself, dissolved color, suspended algae, and suspended particles. Seasonal variables affecting the lotic ecosystem include temperature (θ), depth of the stream (***h***), shading by the riparian vegetation, and daylength.

The periphytic community is a microcosm of autotrophic and heterotrophic assemblages within a self-generated boundary layer (Sand-Jensen, 1983). The processes that govern the periphytic community response to resource utilization include selective competition, pollution-induced community tolerance, competitive exclusion, symbiosis, parasitism, predation, and cooperation (Tilman, 1982). These trophic interactions are the major determinants of the diversity patterns in a community (Hutchison, 1967).

In spite of the complexity and controversy regarding the mechanisms of community interaction, the fundamental process governing community structure is competition for limiting resources (Tilman, 1982). A resource is any substance or factor which can lead to increased growth as its availability in the environment is increased, and which is consumed by an organism (Tilman, 1982). Species compete with each other through depression of resource levels caused by

consumption. For a pair of essential resources, the growth rate of a species will be determined by either one or the other resource, whichever is most limiting. Rhee (1980) demonstrated that phosphate and nitrate are non-interactive essential resources for freshwater algae.

In fluvial systems, attached algal communities generate an extended laminar flow region over the substrate to which they attach. The transport processes within the periphytic community occur by passive diffusion within this laminar flow boundary layer (Sand-Jensen, 1983; Riber and Wetzel, 1987). The movement of water in fluvial systems results in exposure of algae to fresh media and continual removal of extra-cellular products (Round, 1981).

The Rothamsted experiments of Lawes and Gilbert (1880) demonstrated the dramatic effect of enrichment of a habitat with a limiting resource on plant community structure. Plant communities exposed to high resource levels declined in species richness from 40 species to about 3 to 4 species. Similar effects were observed in aquatic plant communities (Kilham and Kilham, 1981). Liebig's concept of a single limiting nutrient in terrestrial systems has been applied to lotic ecosystems with general success. However, the potential exists for competitive and co-limitation of nutrients in aquatic systems. Primary productivity of a lotic ecosystem may be limited by a combination of macro- and micro-nutrient availability and light intensity (Roos, 1983). Competitive exclusion

of nutrients has also been demonstrated between pelagic and periphytic algae (Roos, 1983).

The response of lotic ecosystems to nutrient enrichment have been well described and documented (Douglas, 1958; Evenson et al., 1981; Wetzel, 1983). Nutrient enrichment has been greatly accelerated in many lotic ecosystems as a result of increased nutrient loading from human activities, or cultural eutrophication (Patrick, 1977; Beaulac and Reckhow, 1982; Lowe et al., 1986). Periphytic community carbon fixation constitutes the major part of autocthonous primary production in lotic ecosystems (Roos, 1983).

Determining the limiting resource in a lotic ecosystem is a difficult task due to the high temporal variability in water chemical resource concentration, the high spatial variability of habitat, and the complexity of community resource competition. One approach to determine the limiting nutrient in a stream has been to continuously add a concentrated source of the nutrients of concern to the stream and monitor the response. These continuous flow resource enrichment systems have been successfully applied to determine the limiting resources of streams, but do not provide quantifiable enrichment concentrations due to variability in dilution effects associated with flow conditions (Peterson et al., 1983; Lewis et al., 1993).

Passive diffusion resource enrichment systems using clay pots or tiles as the diffusion regulator and periphyton growth substrate have been developed and applied to lotic and lentic ecosystems (Marks and Lowe, 1993). This approach has been criticized because the diffusion rate of nutrients through the clay varies spatially and between pots and tiles. The disturbance rate, or rate of supply of a limiting resource/nutrient, strongly influences the species composition and diversity of communities and sessile organisms (Tilman, 1982). In addition, the periphyton colonizing the clay pot or tile systems imbed themselves in the substrate, requiring an extraction process (usually scraping with a toothbrush), which results in increased variability between samples. In lotic ecosystems, sessile algae are attatched to the substratum in such a way as to preclude effective extraction by brushing or scraping. Estimates of periphyton recovery efficiencies from semi-porous media range from 50 to 80 percent, resulting in a significant loss of the sample community (Cattaneo and Reberg, 1991).

Periphyton As An Indicator of Ecosystem Stress

Algal assemblage structures are valuable indicators of stream trophic status, but measuring them requires expertise in algal taxonomy (Patrick, 1977). Measuring chlorophyll production of the algal assemblage in a river community is a less intensive alternative to measuring assemblage structure (Dixit et al., 1992). However, the only methods available to date involve measuring primary productivity (algal growth) and applying a generalized trophic index to determine

the trophic status (Aloi, 1990). This approach provides no information on the pristine condition of a river; assessing the degree of impact with no standard for measure is ineffective (Karr et al., 1986; Hughes and Larsen, 1988).

Lotic ecosystems undergo a definite shift in population composition in response to resource enrichment (Harris, 1994). The response of primary producers to resource enrichment within a community drives this shift (McCormick et al., 1994). Previous methods for investigating the response of lotic communities to resource enrichment have been impractical to apply at a large scale, or inconsistent in delivery of the resource to the growth surface (Aloi, 1990). Many variables must be considered when investigating periphytic assemblages; grazing by macro-invertebrates and fish, turbulent and laminar scouring, light limitations, sediment deposition, and high flow events can increase replicate variability within and between treatments.

Indices of Lotic Ecosystem Pollution Stress

Lotic ecosystem responses to stress, including anthropogenic pollution, have been the subject of scientific inquiry for more than a century. Patrick and others recognized as early as 1949 that there was a quantifiable relationship between the physical and chemical stress of lotic ecosystems and characteristics of the submerged attached micro-community, or periphyton (Patrick, 1949; Cholnoky, 1960; Kolkwitz and Marsson, 1967). The efficacy of the periphytic assemblage

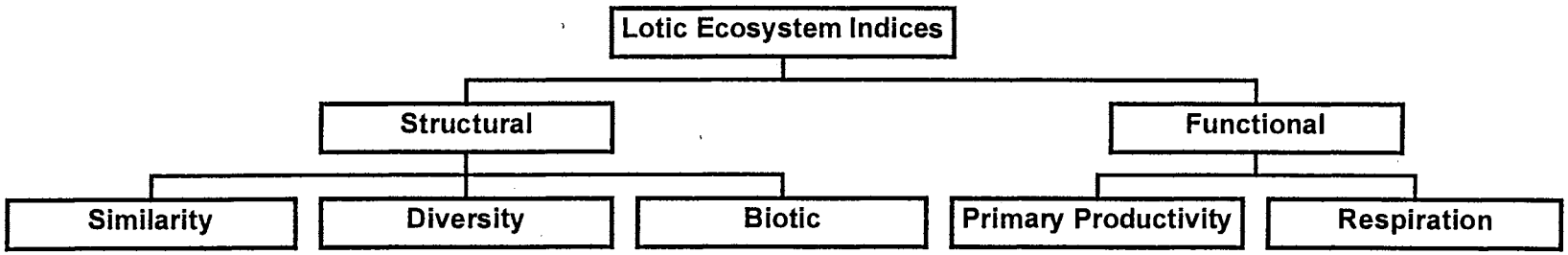
(also referred to as the periphytic micro-community) as bio-indicators is due to their cosmopolitan distribution (Cairns, 1991), the high complexity and diversity within the micro-community (Wetzel, 1975), and the principle opportunity this micro-community has to accumulate and retain dissolved nutrients and toxicants (Rodgers et al., 1979). A plethora of lotic ecosystem pollution status indices have been developed over the last half century, incorporating many of these characteristics.

The approaches investigators have taken over the years to develop lotic ecosystem pollutant stress indices have been influenced by their predecessors and contemporaries. It stands to reason, then, that several distinct approaches to characterizing lotic ecosystem responses to pollutants have arisen. I have reviewed and classified the current indices of lotic ecosystem pollution stress (Figure 6). This classification scheme was influenced heavily by the works of Rodgers et al. (1979) and Washington (1984).

Ecosystems are, by definition, composed of structural components that form functional units (Tansley, 1935; Rodgers et al., 1979). Odum (1962) elaborated on Tansly's definition of ecosystem structure and function by defining the terms as follows:

- Structure: The composition of the biological community including species,

Figure 6: Classification of Lotic Ecosystem Indices



number, biomass, life history, and distribution in space of populations; the quantity and distribution of abiotic components such as nutrients, water, etc.; and the range or gradient of conditions of existence.

- Function: The process of biological energy flow through the ecosystem, including processes of production, respiration (at individual, population, and community levels), process of material or nutrient cycling, and biological/ecological processes of regulation.

While by definition it is impossible to consider the function or structure of ecosystems independently, lotic ecosystem indices generally rely on one or the other as a primary indicator of stress.

Structural Lotic Indices

Assessments of the degree to which a lotic ecosystem is stressed have historically focused on evaluating the structural characteristics of communities (Cairns et al., 1973). Structural characteristics are biotic or abiotic components of the system that relate to the quantity, composition, arrangement, and distribution or pattern of organization at any point in time (Rodgers et al., 1979). The structural approach generally has used identification of species, abundance and/or total number of individuals, and indicator species or groups to define degrees of water quality degradation (Lowe, 1974; Rodgers et al., 1979). The most common structural characteristics related to aquatic ecosystem perturbation are: (1) diversity, (2) similarity, and (3) biotic (Washington, 1984). A

detailed survey of indices of perturbation using each structural characteristic follows.

Diversity Indices

Diversity indices are commonly used to evaluate the effects of pollution on lotic communities (Washington, 1984). Diversity is generally considered the structural component of community stability, or biological integrity (Cairns, 1977). However, many ecologists are questioning this relationship, as the definition of ecological stability evolves (Washington, 1984). Diversity is defined in terms of species or communities when applied to ecology. Species diversity is a function of the number of species present and the evenness of their distribution (Hurlbert, 1977). Community diversity has been defined by Pielou (1966) as "... the degree of uncertainty attatched to the specific identity of any randomly selected individual. The greater the number of species and the more nearly equal their proportions, the greater the uncertainty and hence the diversity." Margalef (1958) proposed that indices of diversity should incorporate the distribution and number of species (***S***) and the abundance of individuals within the species (***N***). In general, the diversity indices in use today follow these guidelines. Some of the diversity indices in use today are summarized in Table 1.

Table 1: Summary of Diversity Indices

Diversity Index	Equation*	Citation
Simpson's D	$D = \frac{\sum_{i=1}^{S} n_i(n_i - 1)}{n(n-1)}$	Simpson (1949)
Kothe's Species Deficit	$SDef = \frac{A_1 - A_x}{A_1} \times 100$	Kothe (1962)
Gleason's Index	$D = \frac{S}{\ln N}$	Gleason (1922)
Margalef's Index	$D = \frac{S-1}{\ln N}$	Margalef (1958)
Menhinick's Index	$D = \frac{S}{\sqrt{N}}$	Menhinick (1964)
Motomura's Geometric Series	$y = Ac^{(x-1)}$	Motomura (1932)
Fisher's a	$S_1 = \alpha \ln\left(1 + \frac{N}{\alpha}\right)$	Fisher et al. (1943)
Modified Yule's Characteristic	Yule's C = $\frac{n^2}{\sum n(n-1)}$	Williams (1964)
Preston's Log-normal *a*	$y = y_0 \exp(-aR)^2$	Preston (1948)
Brillouin's *H*	$H = \frac{1}{N} \ln \frac{N!}{\prod_{i=1}^{S} N_i!}$	Brillouin (1951)

Table 1: Summary of Diversity Indices (Continued).

Diversity Index	Equation*	Citation
Shannon's *H'*	$H' = -\sum_{i=1}^{S} \frac{n_i}{n} \ln \frac{n_i}{n}$	Shannon (1949)
Evenness	$\varepsilon = \frac{H'}{H'_{max}}$	Lloyd and Ghelardi (1964)
McIntosh's M	$M = \frac{n - \sqrt{\sum_{i=1}^{S} n_i^2}}{n - \sqrt{n}}$	McIntosh (1967)
Cairns' SCI	$SCI = \overline{DI_I} \times No.Taxa$	Cairns and Dickson (1968)
	$\overline{DI_I} = \frac{\sum \frac{no.runs}{no.specimens}}{no.statist.signif.runs}$	
Keefe's TU	$TU = 1 - \left(\frac{n}{n-1}\right)\left\{\sum_{i=1}^{K} p_i^2 - \frac{1}{n}\right\}$	Keefe and Bergersen (1976)

***List of terms:**

S = number species in a sample or population.
n = number of individuals in a sample from a population.
n_i = number of individuals in species i.
K = number of taxa in either sample or population.
N = number of individuals in a population or community.
N_i = number of individuals in species i of a population or community.
$p_i = n_i/n$
$\pi_i = N_i/N$
A1 = number of species occurring upstream of a waste discharge.
Ax = number of species occurring downstream of a waste discharge.

As the reader can discern from Table 1, diversity indices rely on relationships of taxonomic groups to characterize a sample site. They then rely on the characterization of some reference site (usually upstream of some point-source pollutant) to classify the degree of perturbation of the water body. Diversity indices have been used with varying success in identifying point sources of pollution in lotic ecosystems (Washington, 1984). Simpson's D has been used to characterize the impact of oil refinery wastes on benthic macro-invertebrates in receiving streams in Oklahoma (Wilhm and Dorris, 1966; Wilhm, 1967). However, Shannon-Wiener's H' is the most commonly used diversity index in lotic ecosystem studies (Washington, 1984).

Similarity Indices

Similarity indices are measures of similarity of the structure and/or composition of two or more lotic ecosystem communities (Washington, 1984). Similarity indices cannot give a value for one site alone, as they are quantified comparisons of sites. It stands to reason, therefore, that the use of similarity indices dictates the use of reference or control sites (Pratt and Smith, 1991). As a result, similarity indices are of particular value when assessing the impact of a point source on a lotic ecosystem, but are difficult to apply for characterizing the effects of a non-point source (Washington, 1984). A brief review of commonly used similarity indices is presented in Table 2.

Table 2: Summary of Similarity Indices for Lotic Ecosystem Assessment.

Similarity Index	Equation*	Citation
Jaccard's Index	$JI = 100 \times \frac{n_c}{n_i + n_j}$	Jaccard (1908)
Percent Similarity Index	$PSC = 100 - 0.5\sum_{i=1}^{K}\lvert a - b\rvert$	Whittaker (1952)
Brey-Curtis Index	$D_{ij} = \frac{1}{2}\sum_{i=1}^{S}\lvert p_{it} - p_{jt}\rvert$	Brey and Curtis (1957)
Euclidian Distance Index	$d = \left[\sum_{i=1}^{n}\left(X_{ij} - X_{ik}\right)^2\right]^{1/2}$	Sokal (1966)
Pinkham and Pearson's Index	$B = \frac{1}{K}\sum_{i=1}^{K}\frac{\min(X_{ia}, X_{ib})}{\max(X_{ia}, X_{ib})}$	Pinkham and Pearson (1976)

*** List of Terms**

n_c = number of species common to quadrats i and j.
n_i = number of species in quadrat i.
n_j = number of species in quadrat j.
a = percent of total sample A that a given species represents.
b = percent of total sample B that a given species represents.
p_{ij} = prominence value of i at j (Wilhm 1967).
p_{jt} = prominence value of j at t (Wilhm 1967).
X_{ia} = the number of individuals in the *i*th taxon for station a.
X_{ib} = the number of individuals in the *i*th taxon for station b.

Biotic Indices

Biotic indices utilize indicator organisms to characterize water pollution (Beak, 1965). Biotic indices are likely to be specific for one or two particular types of pollution, since the indicator organisms are generally not sensitive to all types of pollution (Washington, 1984). The biotic indices developed for water quality are summarized below.

Saprobiensystem. The saprobic system of zones of organic enrichment classifies lotic ecosystems based on the protozoan species that survive there (Kolkowitz and Marsson, 1908; Myslinski and Ginsburg, 1977). The zones of degradation are:

1. Polysaprobic: zone of gross pollution with little or no dissolve oxygen (D.O.), detritivours only.
2. Alpha-mesosaprobic: zone where some oxidation takes place, with more types of animals than polysaprobic.
3. Beta-mesosaprobic: zone where decomposition products approach mineralization.
4. Oligosaprobic: zone of recovery, dominant in pure water. High oxygen content, wide range of animals and plants.

Patrick's Histogram. Patrick's Biological Measure of Stream Condition (1949) is composed of a biotic index and a diversity index. This index is based on a ratio of sample site and control site number of species from eight taxonomic groups in a stream (Table 3). The comparative ratios of these taxa classes are used to asses stream impact from pollution. Patrick's five stream classes range from healthy (histograms greater than 50 percent on Taxa Classes IV, VI, and VII) to semi-healthy, polluted, very polluted, then atypical.

Table 3: Patrick's Seven Taxonomic Groups (Patrick, 1950).

Taxa Class	Description
I.	Blue-green algae, genera of green algae, and the bdelloid rotifers.
II.	Oligocheates, leeches, and puhmonate snails.
III.	Protozoa.
IV.	Diatoms, red algae, and most of the green algae.
V.	All rotifers not included in Column I, plus clams, prosobranch snails and trichadid worms.
VI.	All insects and crustacea.
VII.	All fish.

Palmer's Biotic Index Palmer's biotic index uses algae, rather than the more common use of macroinvertebrates (Palmer, 1969). Palmer ranked twenty algal

taxa based on their tolerance to pollution. More than 50 individuals of one of these indicator taxa per milliliter in a sample constituted a rating; the sum of the ratings were compared with a standard index. A rating of 20 is indicative of high organic pollution.

Beck's Biotic Index Beck (1955) developed an index of stream water quality based on macroinvertebrates in an attempt to report biological results in an easy form to individuals in other fields. Beck's biotic index is designed to be a simple index of the "cleanliness" of a portion of a stream or lake (Washington, 1984). Beck's index is expressed as:

$$\mathbf{BI = 2(S \times Class\ 1) + (S \times Class\ 2)} \qquad (4)$$

where **S** is the total number of species present at the site, **Class 1** is the number of clean water species present at a site, and **Class 2** is the number of moderately pollutant-tolerant species present at a site. Beck's index ranges from 0-40; a value of 10 or greater is considered clean, 1.0 to 6.0 is considered moderately polluted, and less than 1.0 is grossly polluted.

Beak's River Index Beak (1956) derived a biotic index based on six years of data on benthic macroinvertebrates from the Canadian River in Oklahoma. Beak's river index (Table 4) ranges from zero for severely polluted waters to six for unpolluted waters. This index incorporates the entire benthic

macroinvertebrate fauna into the index, and can be calculated with results from any statistically sound sampling program.

Table 4: Beak's River Index (Washington, 1984).

Pollution Status	Biotic Index	Macroinvert. Commun. Type
Unpolluted	6	Sensitive, facultative and tolerant predators, herbivores, filter and detritus feeders all represented
Slight to moderate pollution	4-5	Sensitive predators and herbivores reduced in population density or absent. Facultative predators, herbivores, and possibly filter and detritus feeders well developed and increasing in numbers as index decreases
Moderate pollution	3	All sensitive species absent and facultative predators absent or scarce. Pelopiinae and Tendipedidae present in large numbers
Moderate to heavy pollution	2	Facultative and tolerant species reduced in numbers if pollution toxic; if organic, few species insensitive to low oxygen present in large numbers
Heavy pollution	1	Only most tolerant detritus feeders present in large numbers
Severe pollution	0	No macroinvertebrates present

Graham's Index. Graham (1965) developed a biotic index based on the Trent Index (Woodiwiss, 1960). This index uses benthic macroinvertebrate species composition as indicators of water quality (Table 5); the index ranges from 1.0 (cleanest water) to 6.0 (most polluted).

Table 5: Graham's Index of Stream Quality

Graham's Index	Taxa Description	No. Groups
1	Stoneflies and non-baetid mayflies present	10+
2		0-9
2	One or both of the above absent, caddis and	10+
3	shrimp present	0-9
3	Stoneflies, non-baetic mayflies and caddis absent;	10+
4	*Baetis*, shrimps, Asellus, snails, or leaches present.	0-9
5	All above groups absent; fauna restricted to *Tubifex, Nais,* midge larvae or blood worms.	---
6	No macroinvertebrates found.	---

Hilsenhoff's Index Hilsenhoff (1977) developed a stream water quality index based on the work of Chutter (1972). Hilsenhoff modified Chutter's work by using North American arthropods in his index, and adjusting the range of the index to 0 - 5 instead of 0 - 10 (Table 6). This index is expressed as:

$$HI = \sum_{i=1}^{K} \frac{n_i Q_i}{n} \qquad (5)$$

where **K** is the number of taxa in a sample, n_i is the number of individuals in species i, n is the total number of individuals, and Q_i is quality value for species i.

Table 6: Hilsenhoff Biotic Index

Biotic Index	Water Quality	State of the Stream
< 1.75	Excellent	Clean, undisturbed
1.75 - 2.25	Good	Some enrichment or disturbance
2.25 - 3.00	Fair	Moderate enrichment or disturbance
3.00 - 3.75	Poor	Significant enrichment or disturbance
> 3.75	Very poor	Gross enrichment or disturbance

Biological Criteria The US Environmental Protection Agency has encouraged the development of formal biological criteria for characterizing lotic ecosystem status (USEPA, 1990). Generally, the biological criteria incorporate biotic indices with diversity indices to generate a combined assessment of water quality. Plafkin et al. (1989) developed the Rapid Bioassessment Protocol (RBP) for assessing rivers and streams in this way. The RBP incorporates fish and benthic macroinvertebrate biotic indices with habitat indices to provide a matrix for comparing the degree of impact of a site with a reference, or control site. This method represents the current state of the art in characterizing stream impairment (Barbour and Stribling, 1991).

Functional Lotic Indices

Function refers to rate processes of the lotic ecosystem or its components (Rodgers et al., 1979). Functional lotic indices are based on some measure of a rate process in the lotic ecosystem. A relatively recent shift in the research emphasis of lotic ecosystem from structural, or descriptive, relationships to functional levels of organization has resulted in development of innovative methods for measuring functional indices of perturbation (Cummins, 1974; Rodgers et al., 1979). However, practicality of application governs the functionality of any assessment tool. Odum (1977) designated primary productivity and respiration as the metabolic processes most efficacious for use as indicators of ecosystem status. A discussion of the methods employed to measure lotic primary productivity and respiration follows.

Primary productivity, or the rate of assimilation of the products of photosynthesis, represents a fundamental functional characteristic of lotic ecosystems (Hynes, 1970). Measuring primary productivity in streams is difficult, due to the variability of input variables (light, CO_2 concentration, temperature, substrate, flow, etc.). The periphytic micro-community is responsible for varying amounts of primary productivity as streams mature, further complicating these estimates (Naiman, 1983). Measuring respiration of the periphytic micro-community is also complicated by these factors. The following methods have been employed to

measure lotic primary productivity and respiration with relative degrees of success.

Dissolved Oxygen Production/Consumption

Measuring the dissolved oxygen production/consumption upstream and downstream of a point source has been used to characterize the effects of point source loadings (Odum, 1956; Vollenweider, 1974; APHA, 1976). However, the effects of dark respiration and diurnal fluxes of oxygen complicate the interpretation of the data for estimating primary productivity (Owens, 1965; Hynes, 1970; Wetzel, 1975). This method is not applicable to systems with dissolved oxygen concentration at or near saturation, a serious limitation for lotic ecosystem investigations.

Incubation Chambers

Enclosing periphytic micro-communities in closed or flow-through chambers, both *in situ* and *in vivo*, has been used to characterize primary productivity and respiration with some success (Wetzel, 1964; Loeb, 1981). These methods use dissolved oxygen production/consumption, pH change, and carbon dioxide consumption/production as indicators of the rates of photosynthesis and respiration (Robinson, 1983). Naiman (1983) placed Plexiglas chambers on rocks in streams and measured oxygen changes over 24 hours to determine

relative rates of primary productivity, and ranked the streams based on impact from the watershed.

Carbon Dioxide Uptake (^{14}C Method)

Measuring the consumption of the substrate of photosynthesis (CO_2) is an alternative to measuring the bi-product of photosynthesis (dissolve oxygen) for estimating primary productivity. This method, developed by Duthie and Hamilton (1983), uses ^{14}C radio-labeled CO_2 uptake and assimilation as a direct measure of the rate of photosynthesis within a defined volume. There are many variants of this method, ranging from crude exposure and uptake in natural periphytic communities to controlled mass-balance investigations in artificial streams. *In-situ* closed-chamber uptake of ^{14}C probably represents the most accurate method of estimating periphyton primary productivity available today (Reynold, 1992). However, the use of radio-isotopes is not practical for broad surveys of streams due to the regulatory limitations of isotope releases into the environment.

Biomass

Rates of accumulation of biomass on artificial substrate have been used for estimating primary productivity (Rodgers et al., 1979; Klapwijk et al., 1983). Biomass is often measured gravimetrically as ash-free dry weight (AFDW) (Weitzel, 1979; Ridley-Thomas and Austin, 1989).

Chlorophyll *a* Production

Chlorophyll *a* production has been used successfully as an indicator of primary production (Rodgers et al., 1979). This method has severe limitations, however; colonization must be relatively rapid, grazing must not be a factor, and the incubation period must be a period of instantaneous growth (Kavern et al., 1966).

Biochemical Indicators

Biochemical indicators have been developed for determining the rates of primary production and respiration at the population and community level (Antoniette, 1983; Fitgerald and Nelson, 1966). Measures of adenosine tri-phosphate (ATP), alkaline phosphotase, and various genetic components (DNA, RNA, mRNA) may become standard tools for ecological monitoring.

Proposed Periphyton Lotic Ecosystem Trophic Status Index

The index that I propose uses the ratio of baseline periphyton primary production to maximum potential primary production (MPP) to characterize stream ecosystem status. The measured MPP represented the rate of periphytic growth when nutrients are not limiting. Presumably, the factors limiting growth of a community at MPP are light, substrate, or metabolic kinetics. The ratio of baseline growth to MPP, by definition a functional index, provides a classification

tool for lotic ecosystem trophic status. This approach is limited to streams that are nutrient limited and have perennial flow.

The closest analogue to the proposed BPP/MPP index in the literature is the algal growth potential test (AGPT) using the *Selenastrum capricornutum* bottle assay in lakes (Raschke and Schultz, 1987). This assay measures the growth *of S. capricornutum* in bottles filled with lake water (control) and nutrient-enriched media (MPP) to determine the ratio of the baseline growth to MPP. As with the index I propose, Raschke and Schultz's assay is based on the premise that the maximum yield is proportional to the amount of nutrient which is present and biologically available in minimal quantity with respect to the growth requirement of algae (APHA, 1985).

A trophic status index for lentic ecosystems was developed based on the AGPT, and has been applied to lakes and reservoirs in the south-eastern US for over a decade (Vollenweider, 1974; Raschke and Schultz, 1987). However, the only analogous index of trophic status for lotic ecosystems in the literature (based on the discoveries of this review) was that of Vollenweider (1971). In this land-mark report, Vollenweider states that "virtually no satisfactory quantitative analysis have yet been made of the invasion of lakes by ...littoral algae..." Vollenweider (1971) developed a lotic trophic status index based on nutrient (N and P) loading (Table 7), but recognized that this "tentative classification...is admittedly not

rigorous enough to meet the demands of theoretical limnology and obviously can not be followed to the letter."

Table 7: Vollenweider's N and P Concentration Trophic Index

Trophic Characteristics	Total P (mg/m^3)	Inorganic N (mg/m^3)
Ultra-oligotrophic	<5	<200
Oligo-mesotrophic	5-10	200-400
Meso-eutrophic	10-30	300-650
eu-polytrophic	30-100	500-1500
polytrophic	>100	>1500

Conclusions

The study of lotic ecosystem management is still in its infancy. The advances in lotic ecosystem indices of pollution impact have been made by limnologists with ecological and taxonomic backgrounds. As a result, most of the indices in use today are structural in nature, relying on rigorous taxonomic identification of algal, benthic macroinvertebrate, or fish species. While this requirement should not be an impedance to scientific inquiry, it does present logistic problems to ecosystem managers (generally state and federal agencies) who do not have the professional expertise or resources to perform these assays.

Functional indices have been developed to complement structural indices, providing a more holistic ecosystem approach. Based on this literature review, there does not exist in the professional literature a trophic index for lotic ecosystems analogous to the Vollenweider Index for reservoirs (Vollenweider, 1971). The periphyton lotic ecosystem trophic status index I propose is a lotic analogue for the current lentic trophic status index developed by Rascke and Schult (1987). It is my assertion that the reason this approach has not been developed prior to now has been a limitation of practical methods of nutrient enrichment of the periphytic community.

CHAPTER 3

THE MATLOCK PERIPHYTOMETER: A QUANTITATIVE PASSIVE DIFFUSION METHOD FOR MEASURING THE EFFECTS OF NUTRIENT ENRICHMENT IN LOTIC ECOSYSTEMS

Introduction

Nutrient enrichment and cultural eutrophication have been greatly excellerated in many lotic ecosystems as a result of increased nutrient loading from human activity (Patrick, 1977; Beaulac and Reckhow, 1982; Wetzel, 1983; Dixit et al., 1992). As discussed in the previous chapter, determining the limiting nutrient in a lotic ecosystem is difficult (Aloi, 1990). The most direct measure of lotic limiting nutrients is obtained by measuring the response of the primary producer community (generally periphyton) to direct nutrient enrichment. Nutrient enrichment by continuous addition, usually from stock reservoirs with metering pumps, has been used to determine stream limiting nutrients (Peterson et al., 1983; Pringle, 1987; Hill et al., 1992; Lewis et al., 1993). This method can be impractical, however, due to logistics and apparatus requirements (metering pumps, electrical power, secure area for stock reservoirs, etc.). In addition, the concentration of nutrient enrichment can only

be quantified with this system if the metering pump flow rates are adjusted to stream flow rates over the exposure period (Pringle, 1987).

Passive diffusion nutrient enrichment systems using nutrient-enriched agar with clay pots, tiles, or sand as periphyton growth substrate have been developed and successfully applied to determine limiting nutrients in both lotic and lentic ecosystems (Fairchild and Lowe, 1984; Fairchild et al., 1985; Lowe and Webster, 1986; Pringle et al., 1986; Chessman et al., 1992; Fairchild and Sherman, 1993; Marks and Lowe, 1993; Hepinstall and Fuller, 1994). However, these methods do not provide a constant and uniform rate of nutrient enrichment to the growth surface (Pringle, 1987; Aloi, 1990). In addition, the periphyton colonizing the clay pot or tile systems imbed themselves in the substrate, requiring an extraction process (usually scraping with a toothbrush). Estimates of periphyton recovery efficiencies from semi-porous media range from 50 to 80 percent, resulting in a significant loss of sample and increased variability between samples (Cattaneo and Reberg, 1991).

A quantitative passive diffusion method has been determined for measuring in situ the periphytic community response to nutrient enrichment. The apparatus was designed to be easy to build and deploy for surveying multiple sites over multiple seasons. This chapter describes the apparatus and its application in determining the limiting nutrient in a temperate woodland stream.

Quantitative Passive Diffusion Periphytometer

The quantitative passive diffusion nutrient enrichment apparatus (Matlock periphytometer) was constructed of a cellulose semi-permeable dialysis membrane (Spectra-Por® 08-667B -25mm, Spectrum Medical Industries, Inc., Los Angeles, CA), a glass fiber filter (Whatman® 934-AH, 37 mm, Whatman International Limited, Maidstone, England), and a one liter (L) low-density polyethylene flexible nutrient reservoir (Cubitainer®, Texberry Container Corp., Houston, TX) (Figure 7). The dialysis membrane (12 to 14 kilodalton (kD) pore size) regulates diffusion, and the glass fiber filter (1.5 µm pore size) serves as a growth substrate. A series of Matlock periphytometers can be supported in rigid racks and secured to the substrate in a stream (Figure 8).

Each Matlock periphytometer sampling unit was constructed by filling the nutrient reservoir with a nutrient solution, cutting a 2.85 cm diameter hole in the reservoir cap, slicing a hydrated 4 cm length of dialysis membrane tubing along one side (making a 4 cm square), placing a glass fiber filter on the membrane across the bottle opening, and carefully placing the lid onto the container (Figure 7).

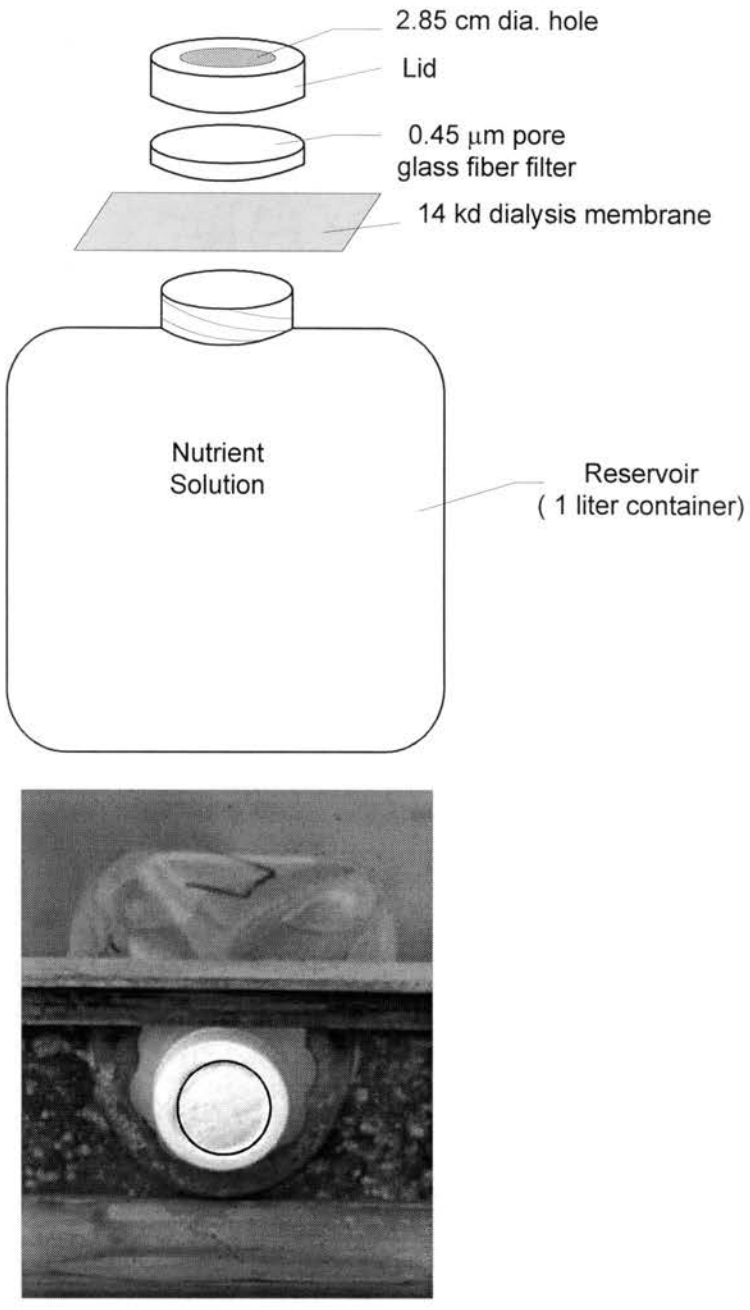


Figure 7: Diagram of Matlock Periphytometer and illustration of growth surface.

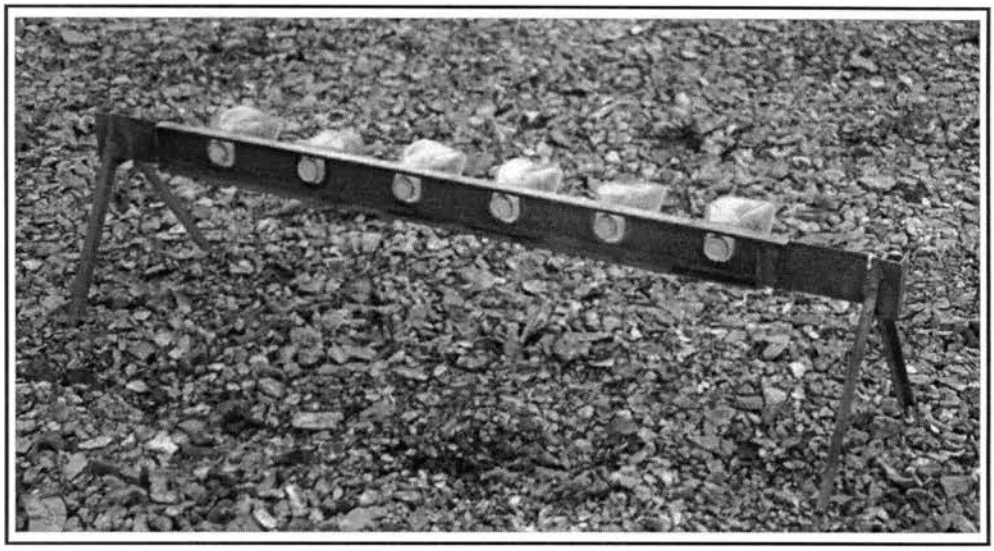


Figure 8: Matlock periphytometer support rack and orientation in the stream.

Theoretical and Observed Rates of Diffusion for Phosphate and Nitrate Ions

Methods

The diffusion of ions across the semi-permeable membrane in the Matlock periphytometer can be approximated by Fick's Law, which describes passive diffusion of ions in aqueous solutions (Weast and Astle 1981). Fick's Law is expressed as:

$$m = \Delta A \frac{(d_2 - d_1)t}{h} \quad (6)$$

where **m** is the mass of substance which diffuses through the cross sectional area **A** in time **t**, $\mathbf{d_1}$ and $\mathbf{d_2}$ are the concentration of ions at the membrane surface, **h** is the thickness of the membrane, and Δ is the diffusion coefficient. For this application, $\mathbf{d_1}$ represents an assumed constant concentration of ions in the stream and $\mathbf{d_2}$ represents the concentration of ions inside the sampling unit, which decreases over time.

The diffusion coefficient for relatively small ions with this dialysis membrane was determined empirically to be 0.40 $cm^2\ hr^{-1}$. This was accomplished by placing a Matlock periphytometer with 500 mg l^{-1} potassium chloride into 80 liters of deionized water and measuring the rate of diffusion over a 21 day period. The data was plotted and Fick's law was solved for Δ.

I determined the rate of diffusion of phosphate and nitrate ions across the 12 to 14 kD semi-permeable membrane and glass fiber filter empirically by measuring the change in electrical conductivity (EC) of nutrient solutions over time in Matlock periphytometer reservoirs placed in a constant flow trapezoidal flume. The flume was 1.0 m wide at the top, 0.25 m wide at the bottom, 0.7 m deep, and 30 m long, with water flowing at 0.05 m s^{-1}. The initial concentrations of the nutrient solutions were 415 and 690 mg l^{-1} K_2HPO_4 (phosphate) and $NaNO_3$ (nitrate), respectively. The flume, located at the United States Department of Agriculture Hydrology Laboratory in Stillwater, Oklahoma, was in full sun; the EC of the flume stream was 374 μS cm^{-2}, the temperature of the stream was 26° C, and the pH was 7.9. The water source for the flume was Lake Carl Blackwell, a rural flood control reservoir with relatively low nutrient levels. I placed 18 replicates of each ion treatment in the flume and measured the conductivity of each solution and ambient water eight times over a 27 day period. The diffusion rates for the observed and theoretical data were determined by regression analysis.

Results

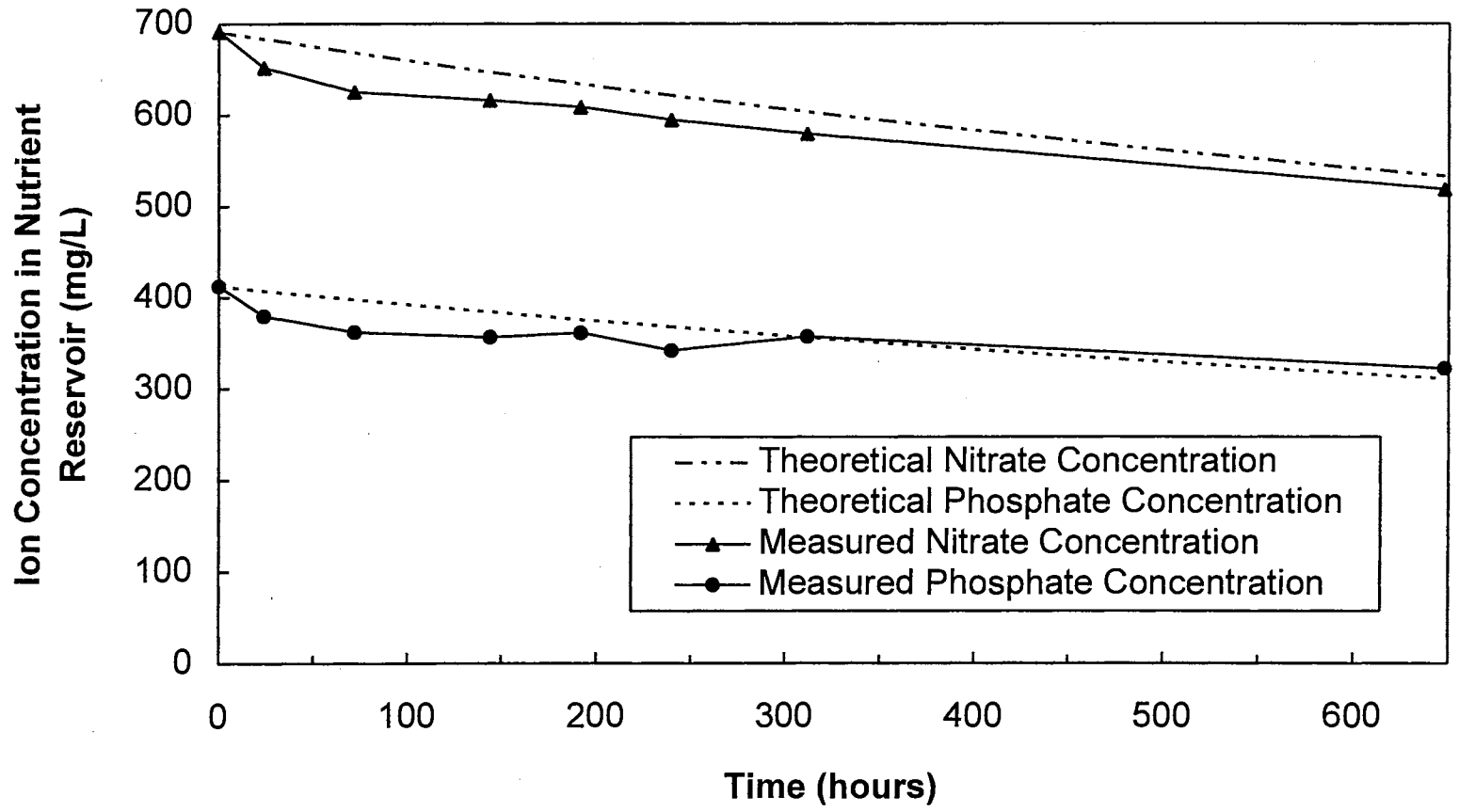
The calculated (theoretical) rates of diffusion determined using a diffusion coefficient of 0.40 cm^2 hr^{-1} and observed rates of diffusion are compared in Figure 9. The observed rates of diffusion for phosphate and nitrate ions across the semi-permeable membrane and glass fiber filter were very close to the

theoretical rates (Figure 9). The observed diffusion rate of phosphate was 17 $\mu g\ cm^{-2}\ hr^{-1}$ with a standard error of 16 $\mu g\ cm^{-2}\ hr^{-1}$ and a coefficient of determination of 0.69. The observed diffusion rate of nitrate was 27 $\mu g\ cm^{-2}\ hr^{-1}$ with a standard error of 17 $\mu g\ cm^{-2}\ hr^{-1}$ and coefficient of determination of 0.91. The difference in the rates of diffusion between the two ions was attributable to the differences in their initial concentrations. In 27 days, 22 and 25 percent of the initial concentrations of phosphate and nitrate, respectively, diffused out of the one liter resource reservoirs.

Discussion

Fick's law describes the diffusion of a solute in solution, while the apparatus uses a semi-permeable membrane to restrict nutrient diffusion. However, the nominal pore size of the membrane is over 100 times larger than the molecules diffusing through it, resulting in a near-linear diffusion rate over time (Figure 9). The concentration of nutrients in the Cubitainers® decreased approximately 15 percent in 14 days and 25 percent in 27 days, but the concentrations of nitrogen and phosphorus enrichment were relatively constant over the 27 day exposure time. The investigator can predetermine the rate of substrate nutrient enrichment by adjusting the initial nutrient concentration.

Figure 9: Theoretical phosphate and nitrate concentrations and ion concentrations measured as EC for the Matlock periphytometer in a constant flow flume for 21 days.



The rate of diffusion across the semi-permeable membrane can be controlled by changing the nominal pore size of the membrane and/or adjusting the concentration of stock nutrient solution in the reservoir. The diffusion coefficient of a molecule is dependent on the size and polarity of the molecule, and should be determined empirically for specific applications.

Determining the Minimum Number of Replicates

Methods

The minimum number of replicates of the Matlock Periphytometer required for a 95 percent confidence with a precision of ± 25 percent of the periphytic community mean chlorophyll *a* concentration for any given treatment was emperically determined. I placed 36 replicates of a total algal nutrient treatment described by Weber et al. (1989) in Peacheater Creek, a tributary to the Illinois River in northeast Oklahoma, on January 23, 1994 and retrieved them on February 5, 1994. The stream temperature was 8°C, and the pH was 7.2. The glass fiber filters from each replicate were then placed in 3 ml 90 percent acetone saturated with magnesium carbonate at 5° C, wrapped in aluminum foil to shield from light, and transported to the laboratory. Chlorophyll *a* was extracted from each replicate and concentrations were determined fluorometrically using the methods described in Standard Method 10200H.3 (APHA, 1989). The data from this analysis are presented in Appendix 1.

Results

The minimum replicate number estimated from the initial field data collection required to obtain a 95 percent confidence and a precision of ±25 percent of the mean value for any given treatment was five (Table 8). This minimum replicate number is consistent with other periphyton and phytoplankton sampling procedures (Steel and Torrie, 1980; Krebs, 1989; Morin and Catteneo, 1992). Based on these results, six replicates were used in the experimental design for measuring the response of periphyton to nutrient enrichment in streams.

Table 8. Minimum Replicate Calculation for Chlorophyll *a* Production ($\mu g\ cm^{-2}$).

Mean	2.5
Standard Deviation	0.7
Count (n)	36
Minimum n:	**5**
Assumptions	
Relative Error (%):	25
t alpha for 95% confidence level:	2
Coefficient of Variation:	0.28

Determining Limiting Nutrient of a Lotic Ecosystem

Methods

I used the Matlock periphytometer to determine the limiting nutrient in Battle Creek, a tributary of the Illinois River in northeast Oklahoma from December 22, 1994 through January 5, 1995. The Battle Creek watershed covers 2200 ha in the Uplands Ecoregion of Oklahoma, characterized by relatively high rainfall (122 cm/yr), hilly terrain, expansive forests and savannahs. The predominant land uses are pasture and woodland. There are over fifty farms with an average size of less than 65 hectares in the watershed. The Battle Creek watershed was selected for this study because it is characteristic of watersheds throughout eastern Oklahoma and the Illinois River Basin.

The sample station was placed above a riffle in a run 0.3 m deep. I used a randomized block design with a series of three treatments per block and six replicates per site. The three treatments were as follows:

1. Control, consisting of deionized water, with a nominal conductivity of 30 $\mu S\ cm^{-2}$,
2. Nitrate, consisting of a 4.3 mM (5 ppm) solution of $NaNO_3$ in deionized water, and
3. Phosphate, consisting of a 2.9 mM (5 ppm) solution of K_2HPO_4 in deionized water.

The nutrient reservoirs (one L Cubitainers®) were filled with the treatment solutions, placed on aluminum racks oriented perpendicular to the channel bottom and parallel to stream flow, and secured to the stream substrate for 14 days (Figure 8). The algal growth surfaces were protected from fish and macro-invertebrate grazing by placing an aluminum screen (8 mesh, or approximately 3 wires per cm, 0.7 mm diameter wire) over the face of the racks, approximately 5 cm from the algal growth surfaces. Although the screen reduced light incident on the growth surfaces slightly, this effect was the same across all treatments.

At the end of the growth period, the colonized filters were removed from the bottles, placed in 3 ml of 90 percent acetone solution saturated with magnesium carbonate at 5°C, wrapped in aluminum foil, and transported to the laboratory for analysis. The chlorophyll *a* was extracted from the filters for direct measurement in the laboratory using EPA Standard Method 10200H.3 (APHA 1989). The chlorophyll *a* data from each sample site was expressed as micrograms (μg) per exposed surface area of the filter (6.6 cm^2) and analyzed to determine if the treatment means were significantly different using multiple comparison analysis for $\alpha = 0.05$ (Krebs, 1989).

Results

The summary statistics of the Control, Nitrogen, and Phosphorus treatments are presented in Table 9, and the data are presented in Appendix 1. The sampling design and power were analyzed as described by Morin and Catteneo (1992). A three-level one-way analysis of variance comparison of the chlorophyll *a* concentrations indicated the variability between treatments was greater than the variability within treatments ($\alpha = 0.05$).

Table 9: Control, nitrogen, and phosphorus enriched treatment chlorophyll *a* concentrations collected using Matlock periphytometers in Battle Creek, Oklahoma, December 22, 1994 through January 5, 1995.

Treatment	Replicate Number	Mean ($\mu g\ cm^{-2}$)	Standard Deviation ($\mu g\ cm^{-2}$)	Coeficient of Variation (%)
Control	6	1.46	0.71	48
Nitrogen	6	1.29	0.85	66
Phosphorus	6	3.44	2.66	78

Multiple Comparison Analyses (Krebs, 1989) of the chlorophyll *a* concentrations were performed to determine which treatment means were significantly different at $\alpha = 0.05$. The results of the Multiple Comparison analysis for the three treatments are presented in Table 10. There was no significant difference between the control and nitrogen enriched treatments, but the variance between the phosphorus enriched treatment and both the control and nitrogen enriched treatments were greater than the variance within the treatments.

Phosphorus enrichment resulted in a significant increase in periphyton chlorophyll *a* production (3.44 μg cm^{-2}) compared to the nitrogen enriched (1.29 μg cm^{-2}) and the control (1.46 μg cm^{-2}) treatments. Nitrogen enrichment resulted in no significant increase in chlorophyll *a* production relative to the control treatment. I conclude that phosphorus was the limiting nutrient for Battle Creek for late December 1994 through early January, 1995.

Table 10: Multiple comparison analysis of variances between control, nitrogen, and phosphorus enriched treatment chlorophyll *a* concentrations (μg cm^{-2}) collected using the Matlock periphytometer in Battle Creek, Oklahoma, December 22, 1994 through January 5, 1995.

Treatment Comparison	$F_{critical}$	F_{sample}	$P(F_{crit}<F)$	Conclusions*
Nitrogen -- Control	5.05	1.44	0.350	No significant difference
Phosphorus -- Control	5.05	14.15	0.006	Significantly different
Nitrogen -- Phosphorus	0.20	9.85	0.013	Significantly different

* Based on $\alpha = 0.05$

Discussion

As with any broadly applied measure of community productivity, there are many sources of variability that must be recognized and addressed when using this method. These sources include grazing, turbulent or laminar scouring, light limitation, siltation, temporal fluctuations in stream velocities, and nutrient availability. All treatment blocks were placed in similar light environments to reduce variability associated with direct and indirect light exposure. In this application, the growth surfaces were protected from fish and macro-

invertebrate grazing by placing an aluminum screen over the surface of the treatment blocks. The screen reduced direct light uniformly for all treatments. Sites were selected to avoid scouring under normal (base) flow, and high flow events were avoided as much as possible by sampling during low rainfall seasons. The aluminum screen reduced flow across the growth surfaces, which also reduced scouring. Siltation was minimized by orienting the growth surfaces perpendicular to the stream surface.

Periphytic growth is a function of propagule concentration, colonization composition and rate, irradiance, temperature, and limiting resource competition. Irradiance and temperature were standardized across experimental blocks. Composition and rate of colonization, and to a lesser degree competition for limiting resources, are stochastic processes; they were assumed to be responsible for the major part of the treatment variability. The number of replicates was selected to provide an acceptable degree of confidence in the treatment response.

Morin and Cattaneo (1992) reported periphyton field studies "...will only detect differences in periphyton abundance or productivity where the means differ by a factor of 2 or more." The periphytic chlorophyll *a* concentration resulting from phosphorus enrichment was twice the nitrogen enriched and control chlorophyll *a* concentrations, which is consistent with Morin and Cattaneo's (1992) analyses of the variability inherent to periphytic sampling methods. The

sensitivity of this method could likely be enhanced significantly by increasing the replicate number to 20 or more (Morin and Cattaneo, 1992). Periodic deployments of the Matlock periphytometer throughout the year and over multiple years could be used to detect seasonal and inter-annual changes in resource limitation.

CHAPTER 4

DEVELOPMENT AND APPLICATION OF A LOTIC ECOSYSTEM TROPHIC STATUS INDEX

Introduction

Lotic primary productivity, or the amount of carbon fixed per unit time and area in a stream, is the defining functional characteristic of the lotic ecosystem (Naiman, 1983). Periphytic chlorophyll *a* production has been broadly applied as an indicator of primary production (Rodgers et al., 1979; Peterson et al., 1983; Fairchild and Sherman, 1993; Lewis et al., 1993). My approach in developing the lotic ecosystem trophic status index was to quantify the response of periphytic communities in three similar streams to nutrient enrichment, and to develop an index for comparing these responses. This index characterizes stream ecosystem status as the ratio of baseline periphyton primary production to maximum potential primary production (MPP). This chapter describes the lotic ecosystem trophic status index and its application to three streams over two seasons.

Methods

Site Description

The Upper Illinois River Basin covers approximately 400,000 hectares in northwest Arkansas and northeast Oklahoma (Figure 10). The Illinois River is a designated scenic river in Oklahoma, is a significant recreational resource for the state. Water quality in the Illinois River has been degrading at an accelerated rate for more than 20 years (Gakstatter and Katko, 1986). The primary source of degradation is nutrient enrichment; 95 percent of nutrient loading to the Illinois River is from non-point sources (Gakstatter and Katko, 1986).

The poultry industry represents a potential source of increased nutrient loading to the Illinois River; more than 200 million broiler chickens are reared in the Upper Illinois River Basin annually (SCS, 1992). Litter produced by poultry production is often applied to permanent pasture at rates based on crop nitrogen demand, which may result in excess phosphorus application and soil phosphorus build-up.

The Illinois River Basin has been the subject of intensive investigation for more 10 years, yet relatively little information is available in the literature regarding these investigations, with the exception of Gakstatter and Katko (1986). Historical water quality data and biological background information is scarce.

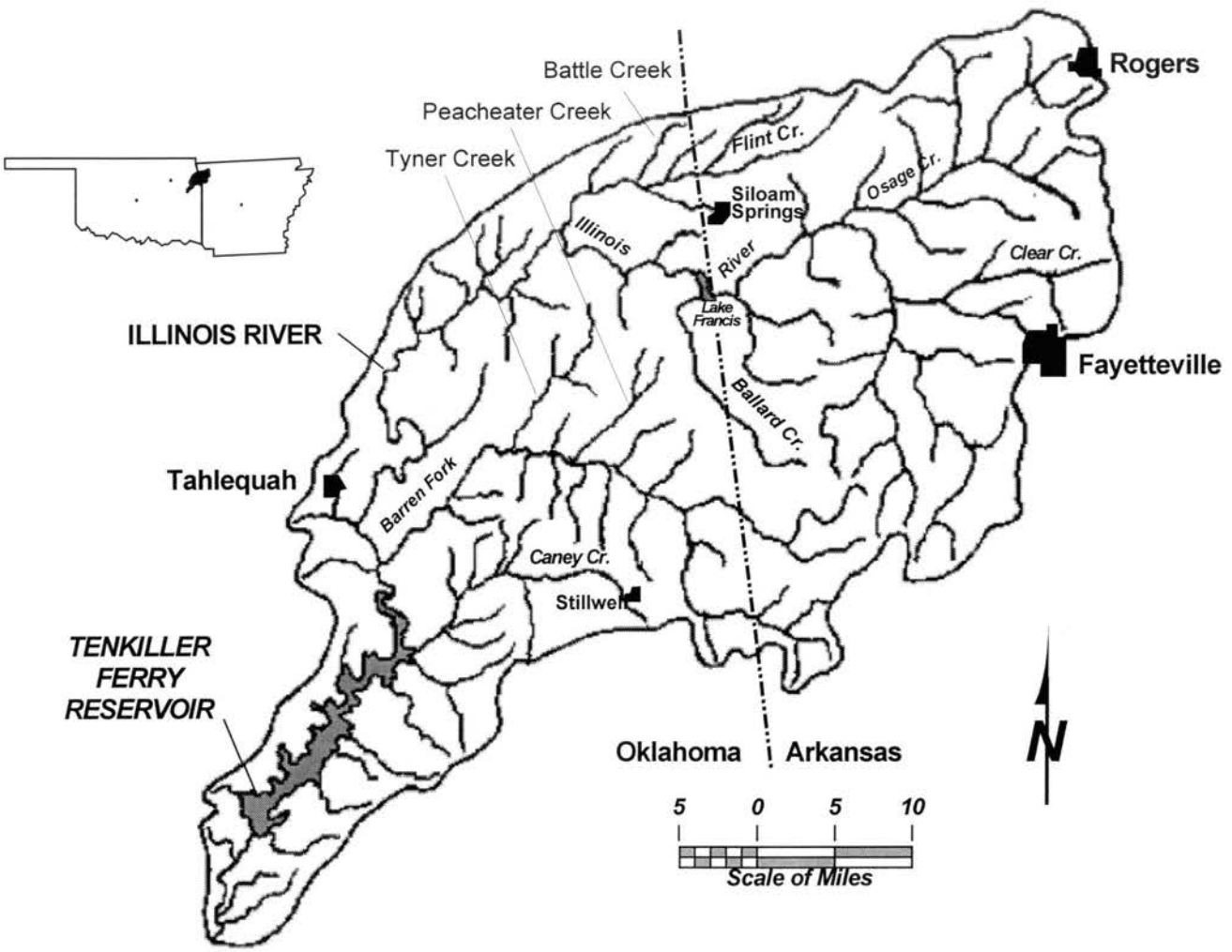


Figure 10: Location of Battle, Peacheater, and Tyner Creeks in the Illinois River Basin.

However, based on the limited information available, the three most impacted streams in the Upper Illinois River Basin (Battle, Peacheater, and Tyner Creeks) were selected for study. Historical water quality data from these streams are presented in Table 11. These data were compiled from US Geological Survey Water Resources Data from water years 1991 through 1994 (USGS, 1991-1994), and from unpublished data provided by the Oklahoma Conservation Commission (personal communication, OCC, 1995). The study streams were sampled using the Matlock periphytometer in April and October, 1995 to determine their limiting nutrient(s) and trophic status (Figure 10).

Table 11: Historical water quality data from Battle, Tyner, and Peacheater Creeks, expressed as means, minimums (Min), and maximums (Max) (USGS, 1991-1994; OCC, 1995).

Water Quality Parameter	Battle Creek 1991-1994			Peacheater Creek 1993			Tyner Creek 1991		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Nitrate-Nitrite Nitrogen (mg l^{-1})	2.16	3.90	0.81	2.27	3.10	1.50	1.98	3.60	0.00
Ammonia Nitrogen (mg l^{-1})	0.02	0.03	0.01	0.02	0.03	0.01	--	--	--
Total Phosphorus (mg l^{-1})	0.13	0.46	0.08	0.07	0.28	0.02	0.04	0.01	0.11
Ortho-Phosphorus (mg l^{-1})	0.11	0.34	0.06	0.05	0.20	0.02	--	--	--

Battle Creek Watershed

Battle Creek is a tributary of the Illinois River. The Battle Creek watershed covers 2,236 ha in the northern portion of the Illinois River Basin (Figure 10). The watershed is in the Uplands Ecoregion of Oklahoma, characterized by relatively high rainfall (122 to 127 cm annually), hilly terrain, expansive forests and savannahs (Jarman, 1984). The average temperatures in July range from 24 °C to 26 °C. The predominant land uses are pasture and woodland. There are over fifty farms with an average farm size of less than 65 hectares in the watershed. The sample site was located at 94°41'30" lattitude, 36°12'45" longitude.

Peacheater Creek Watershed

Peacheater Creek watershed covers about 6,560 ha and is located in the central portion of the basin. The mean annual rainfall in this watershed is 106 to 112 cm, with average temperatures in July ranging from 25 °C to 27 °C. Vegetative and geologic characteristics are similar to those in the Battle Creek watershed. The sample site was located at 94°41'15" lattitude, 35°57'15" longitude.

Tyner Creek Watershed

Tyner Creek watershed covers about 6,475 ha and is adjacent to Peacheater Creek watershed on the eastern side (Figure 10). These watersheds are similar in physical characteristics. Both streams are forth order, with roughly the same number of dairies, poultry houses, and residences. The predominant land uses

are pasture and woodland, with increasing numbers of concentrated animal feeding operations (predominantly poultry). The watershed land uses are summarized in Table 12. The sample site was located at 94°43'30" lattitude, 36°1'45" longitude.

Table 12: Summary of land use by area (hectares) for Battle, Tyner, and Peacheater Creek Watersheds in the Illinois River Basin in Eastern Oklahoma.

Land Use Description	Battle Creek		Peacheater Creek		Tyner Creek	
	ha	%	ha	%	ha	%
Pasture and Range	1414	63	4172	64	4328	68
Forest	752	34	2337	35	2054	32
Crop	8	--	1	--	6	--
Urban, Homestead and Transportation	64	3	43	1	8	--
Total	2238	100	6553	100	6396	100

Primary Productivity and Limiting Nutrient Measurements

Limiting nutrients for the streams were determined using Matlock periphytometers as described in the previous chapter. Six nutrient enrichment treatments were used (Appendix 1):

1. Nitrate, consisting of a 4.3 mM (5 ppm) solution of $NaNO_3$ in deionized water;
2. Phosphate, consisting of a 2.9 mM (5 ppm) solution of K_2HPO_4 in deionized water;
3. Nitrate and phosphate, consisting of treatments 1 and 2 combined;
4. Micro-nutrients, from Weber et al. (1989) at 200 times concentration;

5. Total nutrients, consisting of treatments 3 and 4 combined; and
6. Control, consisting of deionized water, with a nominal conductivity of 30 $\mu S\ cm^{-2}$.

We sampled each site using a randomized block design consisting of a treatment array of six treatments per block, and six replicates of each block per site (Figure 11, Appendix 1). Each treatment block of six Matlock periphytometers (Figure 11) was supported in a rigid aluminum frame so that the growth surfaces were oriented perpendicular to the channel bottom and parallel to stream flow. The treatment arrays were secured to the stream substrate in a run 0.3 m deep in the stream above a riffle for 14 days (Figure 12, Appendix 2). The algal growth surfaces were protected from fish and macro-invertebrate grazing by placing an aluminum screen (8 mesh, or approximately 3 wires per cm, 0.7 mm diameter wire) over the face of the racks, approximately 5 cm from the glass fiber filter growth surfaces.

At the end of the growth period, the colonized filters were removed from the bottles, placed in 3 ml of 90 percent acetone solution saturated with magnesium carbonate at 5°C, wrapped in aluminum foil, and transported to the laboratory for analysis. The chlorophyll was extracted from the filters for direct measurement in the laboratory using EPA Standard Method 10200H.3 (APHA, 1989). The chlorophyll *a* data from each sample site were normalized to the

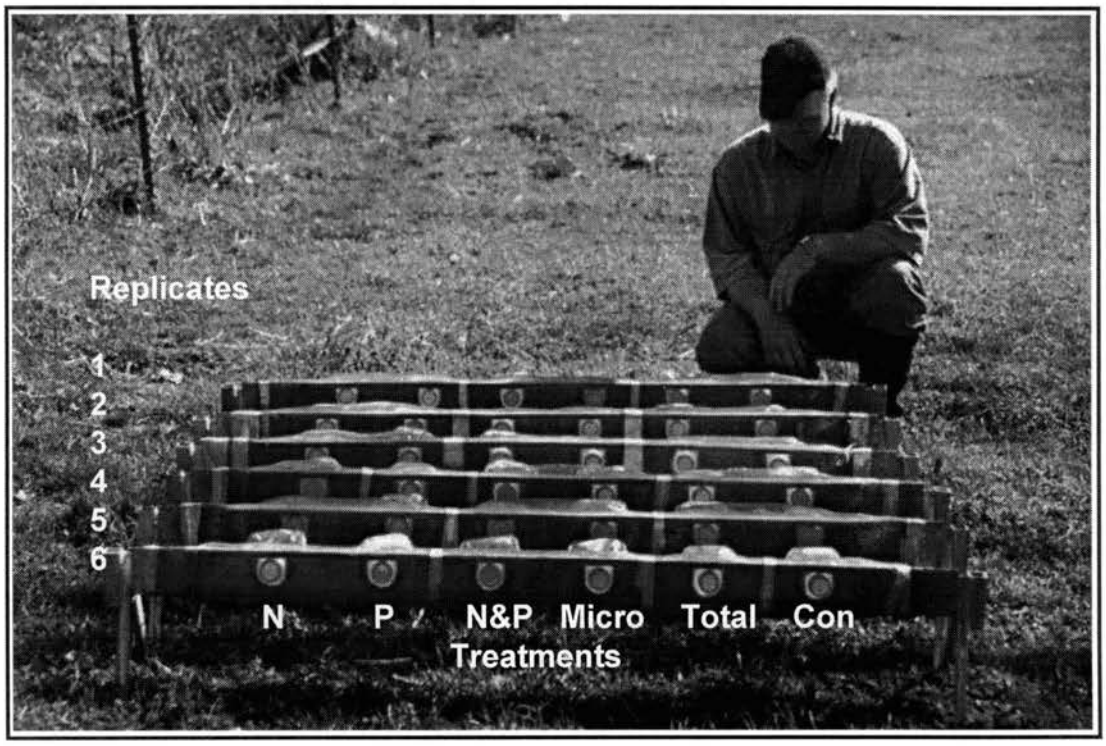


Figure 11: Treatment array for Matlock periphytometers.

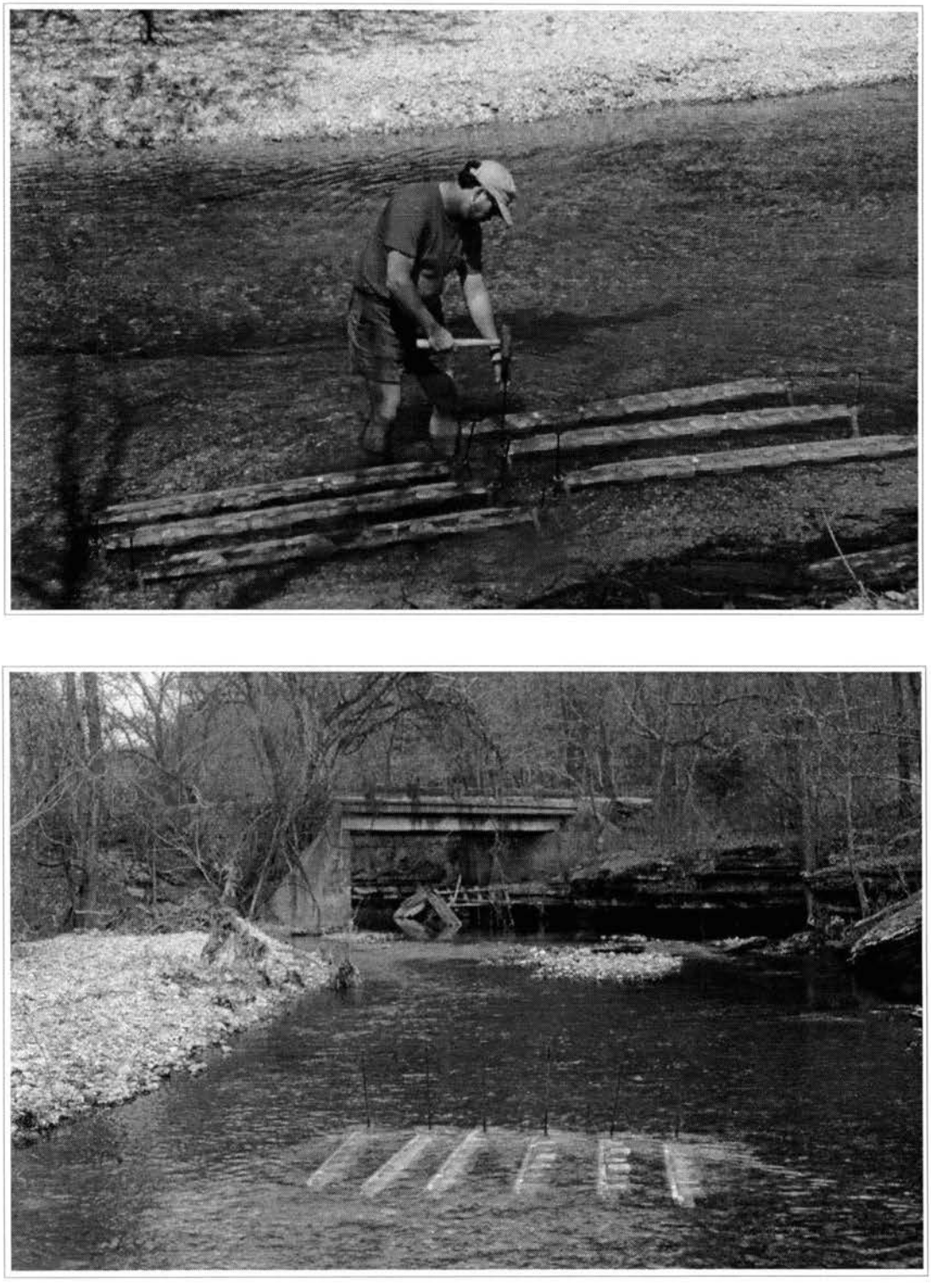


Figure 12: Matlock periphytometers
as deployed in the stream.

exposed surface area of the filter (6.6 cm^2) for comparison. The mean chlorophyll *a* concentrations for each treatment were compared using the Waller-Duncan K-Ratio Multiple Comparison Test using SAS/STAT© (SAS Institute Inc., Cary, NC). The upper and lower 80 percent confidence intervals ($\alpha = 0.20$) were calculated for direct comparison of treatment mean chlorophyll *a* concentration at each site.

Lotic Ecosystem Trophic Status Index

The lotic ecosystem trophic status index (LETSI) was developed as a tool for making comparisons of stream impact from nutrients between watersheds. The underlying assumption of this index is that the Matlock periphytometer total nutrient treatment provides a measurement of the maximum potential productivity (MPP) of a stream at a given site over a given time period. The MPP, therefore, represents the level of periphytic primary productivity (measured as chlorophyll *a* production) that will occur when nutrients are not limiting. The lotic ecosystem trophic status index is the ratio of the baseline primary productivity (control treatment) to the (total nutrient treatment). This ratio represents the proportion of maximum potential productivity manifested at a site in the stream, and under the environmental conditions that occurred over the time period sampled.

It stands to reason that if a single nutrient is limiting primary productivity in a stream, the ratio of an enriched treatment of that nutrient to the total nutrient treatment should approach 1.0. We evaluated the phosphorus, nitrogen, and phosphorus plus nitrogen enrichment responses using the LETSI concept. The phosphorus LETSI (P-LETSI) is the ratio of the phosphorus enriched treatment from the Matlock periphytometer to the MPP. Likewise, the nitrogen LETSI (N-LETSI) and nitrogen plus phosphorus LETSI (NP-LETSI) are the ratios of the nitrogen enriched treatment and nitrogen plus phosphorus enriched treatments to the MPP, respectively. We calculated these indices for each data set.

Results

Watershed Land Use Comparison

Peacheater and Tyner Creek watersheds were similar in size and larger than Battle Creek watershed, though the primary land-use distribution were similar (Table 12). The predominant land uses in the watersheds were pasture and range (63 to 68 percent), with substantial forest cover (32 to 36 percent). The principal difference in land uses between the three watersheds was the impact from anthropogenic activity.

Primary Productivity and Limiting Nutrient Measurements

The mean chlorophyll *a* concentrations, with standard deviations and coefficient of variations, from nutrient enrichment treatments using the Matlock

periphytometer in April and October 1995 for Battle, Peacheater and Tyner Creeks are presented in Tables 13 and 14, respectively. Sample replicate numbers less than six indicate loss of samples. High flow events occurred in Battle Creek during both sampling periods, resulting in the loss of replicates due to scouring of the filter papers. This loss of replicates reduced the sensitivity of the method. Comparisons of the treatment means using the Waller-Duncan K-ratio t test (α = 0.20) for Battle, Tyner, and Peacheater Creeks for April and October, 1995, are presented in Tables 15 through 20, respectively. The means and corresponding confidence intervals (α = 0.20) for the three sample sites for April and October, 1995, are presented in Figures 13 and 14, respectively. The chlorophyll *a* data are presented in Appendix 4.

Spring Sampling Results

The April 1995 Battle Creek results showed a significant increase (α = 0.20) in chlorophyll *a* production for the nutrient enriched treatments (Waller group A, Table 15). The phosphorus and nitrogen plus phosphorus enriched treatments were not significantly different than the total nutrient treatment, yet they were significantly different than the control. While the nitrogen treatment was not significantly different (α = 0.20) from the total nutrient treatment, neither was it significantly different from the control. Therefore, it is not possible to say whether the nitrogen response was truly the result of nitrogen enrichment,

Table 13: Chlorophyll *a* concentrations for nitrogen (N), phosphorus (P), nitrogen plus phosphorus (N and P), micro-nutrients, total nutrients, and control treatments using the Matlock periphytometer in Battle, Peacheater, and Tyner Creeks, Oklahoma during the period of April 8 - 21, 1995.

Site	Treatment	Replicate Number	Mean Chl. *a* ($\mu g\ cm^{-2}$)	Standard Deviation ($\mu g\ cm^{-2}$)	Coefficient of Variation (%)
Battle Creek	N	5	1.16	0.64	60
	P	1	1.61	--	--
	N and P	5	1.67	0.60	36
	Micro-nutrients	5	0.48	0.76	160
	Total Nutrients	2	1.98	0.39	19
	Control	6	1.05	0.30	28
Peacheater Creek	N	6	1.05	0.42	40
	P	6	1.38	0.44	32
	N and P	6	1.61	0.72	45
	Micro-nutrients	6	0.35	0.10	28
	Total Nutrients	6	1.66	0.69	20
	Control	6	0.51	0.23	46
Tyner Creek	N	6	0.31	0.17	57
	P	6	0.20	0.08	42
	N and P	5	0.28	0.11	40
	Micro-nutrients	6	0.20	0.15	77
	Total Nutrients	6	0.33	0.10	29
	Control	6	0.21	0.14	65

Table 14: Chlorophyll *a* concentrations for nitrogen (N), phosphorus (P), nitrogen plus phosphorus (N and P), micro-nutrients, total nutrients, and control treatments using the Matlock periphytometer in Battle, Peacheater and Tyner Creeks, Oklahoma during the period of September 20 - October 3, 1995.

Site	Treatment	Replicate Number	Mean Chl. *a* ($\mu g\ cm^{-2}$)	Standard Deviation ($\mu g\ cm^{-2}$)	Coefficient of Variation (%)
Battle Creek	N	4	0.33	0.05	17
	P	2	0.24	0.26	109
	N and P	4	0.63	0.36	56
	Micro-nutrients	2	0.21	0.09	42
	Total Nutrients	4	0.57	0.14	25
	Control	4	0.28	0.17	62
Peacheater Creek	N	6	0.55	0.18	33
	P	6	0.35	0.06	16
	N and P	6	0.55	0.55	49
	Micro-nutrients	6	0.23	0.23	24
	Total Nutrients	6	0.69	0.69	50
	Control	6	0.28	0.04	11
Tyner Creek	N	6	1.09	0.43	40
	P	6	1.06	0.20	19
	N and P	5	1.01	0.24	24
	Micro-nutrients	5	0.45	0.21	46
	Total Nutrients	6	0.98	0.40	41
	Control	6	0.55	0.19	35

Table 15: Waller-Duncan K-ratio t test (α=0.20) for Battle Creek chlorophyll *a* collected using the Matlock Periphytometer from April 8 - 21, 1995.

Waller Grouping*		Treatment	Mean ($\mu g\ cm^{-2}$ Chl. *a*)	Number of Replicates
	A	Total	1.98	2
	A	N&P	1.67	5
	A	P	1.61	1
B	A	N	1.16	5
B		Control	1.05	6
B		Micro	0.48	5

*Means with the same letter are not significantly different.

Table 16: Waller-Duncan K-ratio t test (α=0.20) for Peacheater Creek Chlorophyll *a* collected using the Matlock Periphytometer from April 8 - 21, 1995.

Waller Grouping*		Treatment	Mean ($\mu g\ cm^{-2}$ Chl. *a*)	Number of Replicates
	A	Total	1.66	6
	A	N&P	1.61	6
B	A	P	1.38	6
B		N	1.05	6
	C	Control	0.51	6
	C	Micro	0.35	6

*Means with the same letter are not significantly different.

Table 17: Waller-Duncan K-ratio t test (α=0.20) for Tyner Creek Chlorophyll *a* collected using the Matlock Periphytometer from April 8 - 21, 1995.

Waller Grouping*		Treatment	Mean ($\mu g\ cm^{-2}$ Chl. *a*)	Number of Replicates
	A	N	0.31	6
	A	P	0.20	6
	A	N&P	0.28	6
	A	Total	0.33	6
	A	Control	0.21	6
	A	Micro	0.20	6

* The Waller-Duncan k-ratio t test could not be performed on the Tyner Creek data collected in the spring due to a very low value of F (less than 1.0), meaning no significant difference in the means was detected.

Table 18: Waller-Duncan K-ratio t test (α=0.20) for Battle Creek Chlorophyll *a* collected using the Matlock Periphytometer from September 20 - October 3, 1995.

Waller Grouping*		Treatment	Mean ($\mu g\ cm^{-2}$ Chl. *a*)	Number of Replicates
	A	N&P	0.63	4
B	A	Total	0.57	4
B	A	N	0.33	4
B	A	Control	0.28	4
B	A	P	0.24	2
B		Micro	0.21	2

*Means with the same letter are not significantly different.

Table 19: Waller-Duncan K-ratio t test (α=0.20) for Peacheater Creek Chlorophyll *a* collected using the Matlock Periphytometer from September 20 - October 3, 1995.

Waller Grouping*		Treatment	Mean ($\mu g\ cm^{-2}$ Chl. *a*)	Number of Replicates
	A	Total	0.69	6
	A	N&P	0.55	6
B	A	N	0.55	6
B		P	0.35	6
	C	Control	0.28	6
	C	Micro	0.23	6

*Means with the same letter are not significantly different.

Table 20: Waller-Duncan K-ratio t test (α=0.20) for Tyner Creek Chlorophyll *a* collected using the Matlock Periphytometer from September 20 - October 3, 1995.

Waller Grouping*		Treatment	Mean ($\mu g\ cm^{-2}$ Chl. *a*)	Number of Replicates
	A	N	1.09	6
	A	P	1.06	6
	A	N&P	1.01	5
	A	Total	0.98	6
B		Control	0.55	6
B		Micro	0.45	5

*Means with the same letter are not significantly different.

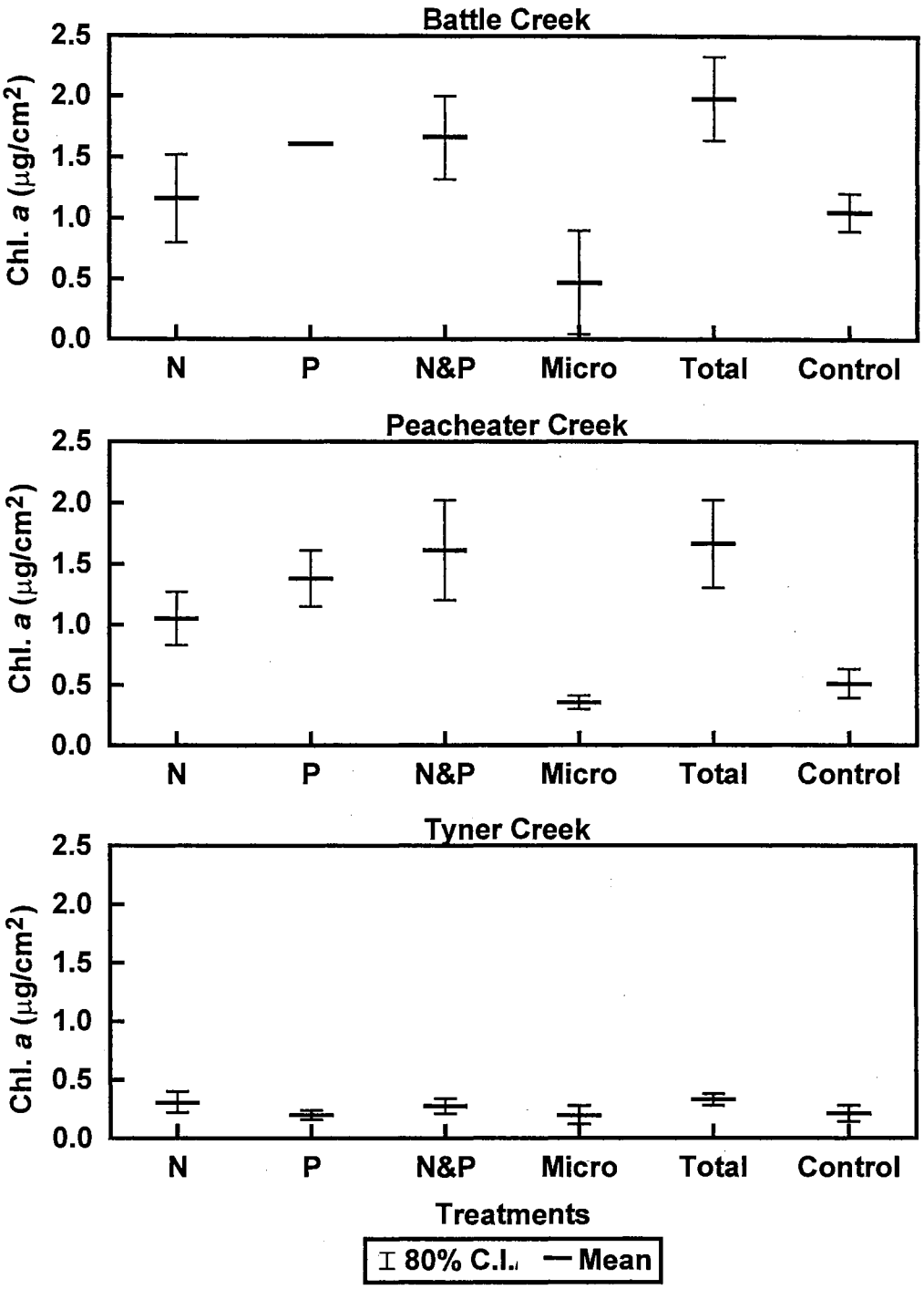


Figure 13: Comparison of treatment mean chlorophyll *a* production, with 80 percent confidence intervals (α=0.20) from Battle, Peacheater, and Tyner Creeks, April 1995.

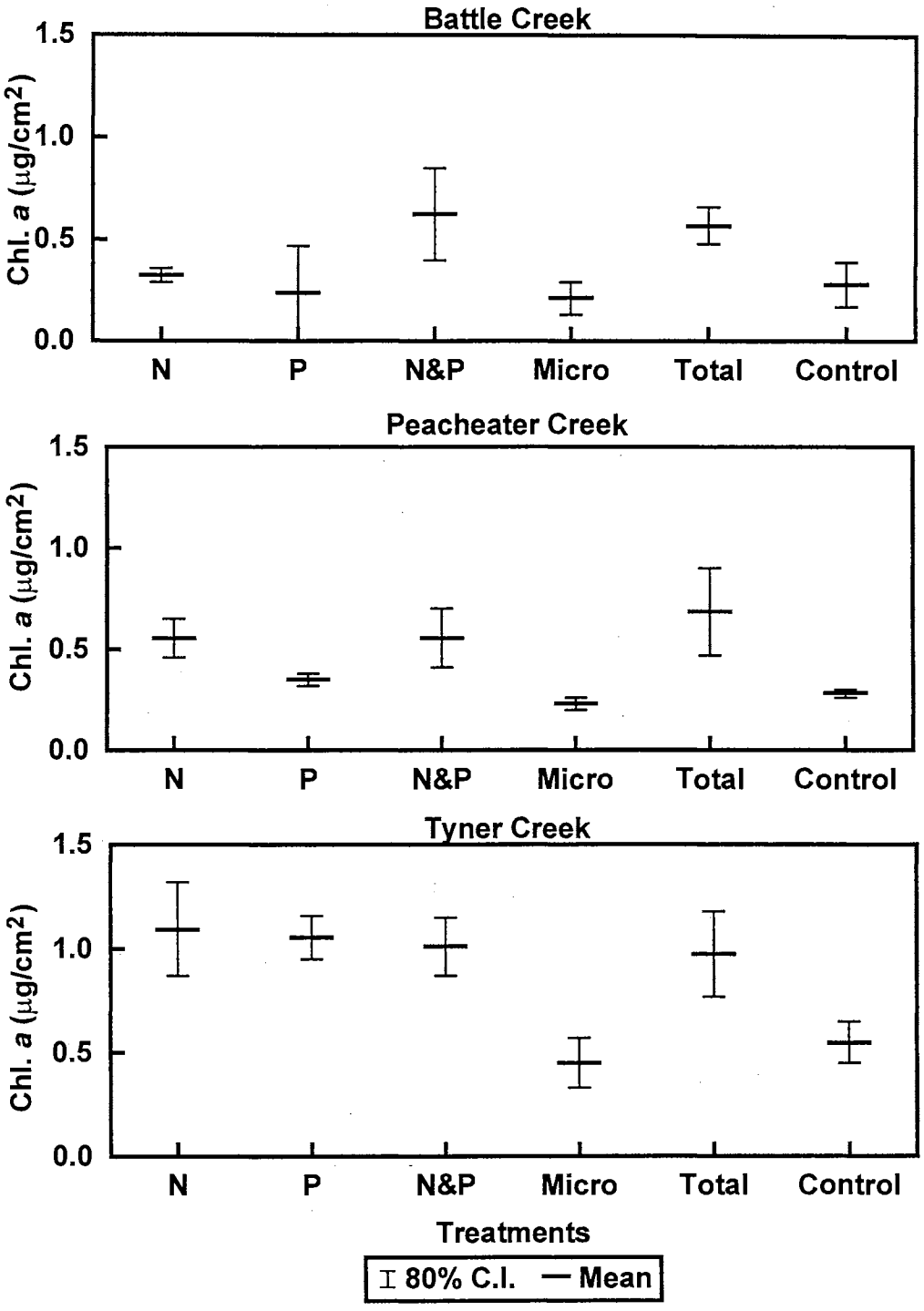


Figure 14: Comparison of treatment mean chlorophyll *a* production, with 80 percent confidence intervals (α=0.20) from Battle, Peacheater, and Tyner Creeks, September 20 - October 3, 1995.

or a product of the inherent variability of the sampling system. Based on these results, Battle Creek was probably phosphorus limited in the spring.

The April 1995 Peacheater Creek results suggested a potentially co-limited system (Table 16). The total, nitrogen plus phosphorus, and phosphorus nutrient treatment chlorophyll *a* concentrations at this site (Waller group A) were significantly higher (α = 0.20) than micro-nutrient and control treatments (Waller group C). However, the nitrogen enriched treatment was also significantly higher than the control and micro-nutrient treatments (Waller group B). Adding nitrogen and/or phosphorus to this system increased the periphytic community's production of chlorophyll *a*. As with Battle Creek, the data indicate phosphorus was principally responsible for limiting primary production, since the phosphorus treatment was the same as the total nutrient treatment. Nitrogen, however, was secondarily limiting primary production, since nitrogen enrichment increased chlorophyll *a* production relative to the control.

The Tyner Creek data for the spring sampling period showed no significant difference (α = 0.20) in the response of the periphytic community to nutrient enrichment (Table 17). The implication is that some factor other than nutrients was limiting the periphytic primary production in the stream. The most probable

limiting factor is light, although the possibility exists that some micro-nutrient or vitamin not present in the total or micro-nutrient treatments was limiting growth.

Fall Sampling Results

The October 1995 Battle Creek showed no significant difference (α = 0.20) in the response of the periphytic community to nutrient enrichment (Table 18). While there were two Waller groups (A and B), the only difference in the groups were the inclusion of micro-nutrients or nitrogen plus phosphorus treatments. The implication is that some factor other than nutrients was limiting the periphytic primary production in the stream. However, the loss of replicates in all treatments due to high flow has compromised the statistical inferences of these data. While it is possible light is the limiting factor, it is more likely that repeated sampling will detect a nutrient limitation.

The October 1995 Peacheater Creek results were similar to the April results, with the exception that nitrogen was the primary limiting nutrient and phosphorus was the secondary limiting nutrient (Table 19). The nitrogen treatment response was not significantly different (α = 0.20) than the total or nitrogen plus phosphorus treatments, while the phosphorus treatment was significantly different. However, the phosphorus enriched treatment was also significantly higher than the control and micro-nutrient treatments (Waller group

B). Adding nitrogen and/or phosphorus to this system increased the periphytic community production of chlorophyll *a*, suggesting a co-limited system.

The October Tyner Creek data showed significant increases in chlorophyll *a* concentration resulting from nutrient enrichment (Table 20). There was no detectable increase in primary production resulting from micro-nutrient enrichment (Waller group B). Nitrogen and/or phosphorus was limiting primary production in Tyner Creek during the sample period in apparently equal proportions.

Comments

With the exception of Tyner Creek in April, 1995, it is apparent from Figures 13 and 14 that nutrient enrichment with nitrogen and/or phosphorus increased chlorophyll *a* production in all the streams in April and October, 1995. However, given this data set alone, it is difficult to assess which nutrient (nitrogen or phosphorus) is exerting the most influence on primary productivity. The data suggest clearly that micro-nutrients are not limiting in these lotic ecosystems.

Lotic Ecosystem Trophic Status Indices

Lotic ecosystem trophic status indices (LETSI, N-LETSI, P-LETSI, and N&P-LETSI) for Battle, Peacheater, and Tyner Creeks for April and October, 1995, are presented in Table 21. The baseline productivity (BP) of the streams was

expressed as the concentration of chlorophyll *a* extracted from the control treatments at each site, expressed as μg chlorophyll *a* cm^{-2}.

The LETSI represents the proportion of maximum potential productivity manifested in the stream during the sample period. The theoretical LETSI ranges from 0 to 1, from lowest to highest degree of impact from nutrient loading. A stream with a LETSI of 0.50 can be said to be at 50 percent of its MPP, or at half the potential growth based on nutrient availability. A LETSI of 1.0 suggests that the stream is at its maximum potential productivity, and adding nutrients will not increase chlorophyll *a* production in the periphytic community.

Analyzing limiting nutrients using the LETSI differs from previous methods by comparing responses to nutrient enrichment to maximum potential responses, providing a perspective for comparison. The ratio of a nutrient enrichment response to the total provides a comparative analysis of the role of that nutrient in limiting primary productivity. A nitrogen LETSI (N-LETSI), for example, of 1.0 suggests the nitrogen enriched treatment response was the same as the total nutrient enrichment response. In this case, nitrogen would be the limiting nutrient. Limiting nutrients as determined by the LETSI are presented in Table 22.

Table 21: Lotic Ecosystem Trophic Status Indices (LETSI's) reflecting the ratios of the control, nitrogen (N), and phosphorus (P) enriched treatment chlorophyll *a* concentrations with the total nutrient concentrations for Matlock Periphytometer samples collected from Battle, Peacheater, and Tyner Creeks, Oklahoma, in spring (April 8 - 21) and fall (September 20 - October 3) 1995.

Season	Sample Site	BP[1] ($\mu g\ cm^{-2}$)	MPP[2] ($\mu g\ cm^{-2}$)	LETSI	P - LETSI	N - LETSI	N&P - LETSI
Spring	Battle Creek	1.05	1.98	0.60	0.92	0.66	0.84
	Peacheater Creek	0.51	1.66	0.30	0.83	0.63	0.97
	Tyner Creek	0.21	0.33	0.64	0.60	0.92	0.83
Fall	Battle Creek	0.28	0.57	0.49	0.42	0.57	1.10
	Peacheater Creek	0.28	0.69	0.41	0.51	0.81	0.81
	Tyner Creek	0.55	0.98	0.56	1.08	1.12	1.04

[1] Baseline Productivity.
[2] Maximum Potential Productivity.

Table 22: Summary of limiting nutrient status of Battle, Peacheater, and Tyner Creeks in the Spring and Fall of 1995, based on LETSI analysis.

Season	Site	Limiting Nutrient(s)*		
		P	N	N&P
Spring	Battle Creek	**Pr**		
	Peacheater Creek	**Pr**	**S**	
	Tyner Creek		**Pr**	
Fall	Battle Creek			**Pr**
	Peacheater Creek	**S**	**Pr**	
	Tyner Creek	**Pr**	**Pr**	

* Pr=Primary limiting nutrient.
S=Secondary limiting nutrient.

Spring LETSI Results

In the spring (April 8 - 21, 1995) Battle and Tyner Creeks were at approximately 60 percent of MPP, while Peacheater Creek was at 30 percent MPP (Table 21).

However, Battle and Peacheater Creeks had similar MPP values (1.98 and 1.66 μg cm^{-2} chlorophyll *a*, respectively), while Tyner Creek MPP was much lower (0.33 μg cm^{-2}).

Comparison of the nutrient treatment LETSI's for both Battle and Peacheater Creeks suggested phosphorus was the nutrient primarily responsible for limiting growth of the periphytic community during the sample period. The LETSI analysis suggests nitrogen was secondarily limiting chlorophyll *a* production in Peacheater Creek (Table 21); this analysis is supported by the Waller-Duncan mean comparison test (Table 15). Tyner Creek was nitrogen limited, though the results of the Waller Duncan comparison (Table 17) demonstrated that the means of all six treatments were not significantly different.

Fall LETSI Results

In the fall (September 20 - October 3, 1995) Peacheater Creek was at 41 percent MPP, while Battle and Tyner Creeks were at 49 and 56 percent MPP, respectively. Battle and Peacheater Creeks had similar BP and MPP, while Tyner Creek BP and MPP were considerably higher (Table 21). Peacheater Creek was primarily nitrogen limited in the fall, with secondary phosphorus limitation. Battle and Tyner Creeks appeared to be co-limited. These results are consistent with the Waller-Duncan comparison of the means (Tables 18 - 20).

In the fall Battle Creek responded to both N and P enrichment, but not to N or P individually. While this phenomenon might be an artifact, it might also be the result of low-level community co-limitation by nitrogen and phosphorus. When the periphytic community was enriched with nitrogen or phosphorus alone, a limitation in the alternate nutrient may have been induced. Enrichment with both nitrogen and phosphorus resulted in increased chlorophyll *a* production, suggesting both nutrients were limiting growth.

Nutrient enrichment of Tyner Creek in the fall elicited a significant response, though the differences between individual nutrient enrichment treatments were not significant. Tyner Creek periphyton responded similarly to nutrient enrichment with nitrogen, phosphorus, nitrogen plus phosphorus, and total nutrients. This response suggests the Tyner Creek periphyton community responded facultatively to the nutrients that were enriched. It is possible some components of the periphytic community had stored molecular nitrogen and others has stored molecular phosphorus (presumably during luxury consumption), so when one or the other nutrient was present the respective periphytic community component could respond accordingly. The response suggests that components of the periphytic community interact in such a way as to collectively increase primary production through population-level selective

uptake and sequestering of nutrients. This form of community guild has been observed among higher organisms, but not algae.

Discussion

Comparison of Watersheds

The LETSI was designed as a tool for comparing watersheds with respect to the impact of nutrient enrichment on periphytic productivity. While considerably more data must be collected before generalizations may be made regarding the significance of the LETSI's in a basin, some speculation is possible and perhaps useful. In the spring sampling period, Battle and Tyner Creeks had the same LETSIs (60 percent of MPP), yet Tyner Creek baseline productivity (0.21 $\mu g\ cm^{-2}$) was 20 percent of Battle Creek's baseline productivity (1.05 $\mu g\ cm^{-2}$) and Tyner Creek's MPP (0.33 $\mu g\ cm^{-2}$) was less than 17 percent of Battle Creek's MPP (1.98 $\mu g\ cm^{-2}$). It would be inaccurate to assert that these two streams were equally affected by nutrient loading. A speculative interpretation of these data would be that Tyner Creek was less productive than Battle Creek due to variables other than nutrient loading, and was proportionally affected by nutrients. An alternative hypothesis is that the periphytic community in Battle Creek has evolved with higher resource availability, resulting in a higher primary productivity, while Tyner Creek periphyton are less efficient at utilizing episodic increases in nutrient availability.

It is possible to conclude, however, that for the spring sample period, Battle Creek was more productive and generally more enriched by nutrients than Peacheater and Tyner Creeks.

During the fall sampling period, Battle and Peacheater Creeks were equally productive and nutrient enriched. Tyner Creek was twice as productive, yet the degree of impact was proportionally similar to the other two streams. However, caution must be used in comparing these data. If nothing else, this data illustrates the temporal variability in nutrient loading and stream responses to environmental conditions. Many other factors such as light, temperature, flow rate, and suspended sediment concentration may be more influential to periphyton growth than nutrients. This work does suggest that the LETSI might be useful in assessing the most sensitive season for nutrient loading to the stream. However, additional confirmation is appropriate before direct conclusions may be drawn from the watershed comparison.

Co-Limitation

Classic nutrient limitation theory was based on the response of individual organisms or monocultures of plants to nutrient limitations, and states that only one nutrient limits the growth of a plant at a time (Laws and Gilbert, 1895). The periphytic community is not a monoculture, however, and responds to nutrient enrichment in a more complex manner. The periphytic community in an

episodically nutrient enriched stream may have populations of algae that sequester nutrients during times of excess, with luxury consumption, releasing these nutrients to the community during times of nutrient stress. This could explain the co-limitation observed in Tyner Creek during the fall sampling period.

However, the co-limitation of nutrients observed in Battle Creek during the fall sampling period suggested that both nitrogen and phosphorus were simultaneously limiting. When phosphorus was added in excess, a nitrogen limitation was immediately induced. When nitrogen alone was added in excess, a phosphorus limitation was immediately induced. However, when nitrogen and phosphorus were added in excess simultaneously, neither was limiting, and primary productivity increased. These conclusions must be tempered by considering that nutrient limitations are being induced by providing another nutrient in excess; the response of the periphytic community to nutrient stimulation may be considerably different than to the absence of a nutrient.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

Discussion

Our knowledge of lotic ecosystem energy flow processes is small compared to our knowledge of lentic ecosystem processes. Perhaps the most obvious reason for our limited knowledge is lotic primary production is orders of magnitude more temporally and spatially variable than lentic systems (Hepinstall and Fuller, 1994) and may be influenced by factors including but not limited to light, temperature, and nutrients (Chessman et al., 1992; Hill et al., 1992). In fact, the primary producers in rivers and streams may change as the stream order increases. At the headwaters, allocthonous carbon supply may be responsible for up to 60 percent of primary production, while in a lower order stream periphyton may be responsible for more than 80 percent of primary production. As the stream order increases, flow rate decreases and the influence of riparian shading decreases, resulting in increased potential primary production from phytoplankton.

For large watersheds (greater than 20,000 km^2), Naiman (1983) showed that periphyton may only account for a small, but conspicuous, amount of primary

production (11 - 19 percent). The remainder of carbon contribution to the lotic ecosystem was from FPOM (fine particulate organic material), or allocthonous sources. Streams with more frequent critical flow events, and thus a higher rate of substrate scouring, may have higher mean nutrient concentrations and lower primary productivity due to the purging effects of the high flow events. These variables confound standardized characterizations of periphytic trophic status in lotic ecosystems.

Periphyton Measurement Variability

Many difficulties were encountered during this investigation. A detailed chronology of the development of the Matlock periphytometer is presented in Appendix 5. Theft and vandalism of the Matlock periphytometer arrays were constant problems. High flow events during the exposure period eroded the filter paper; optimizing exposure duration and nutrient concentrations took multiple sample events; grazing from benthic macro-invertebrates and minnows resulted in loss of a full sample set, and two others were lost while testing for an appropriate screen.

The difficulties encountered while measuring lotic primary productivity often result in high variability in the data. Morin and Cattaneo (1992) reported periphyton field studies "...will only detect differences in periphyton abundance or productivity where the means differ by a factor of 2 or more." The results

presented in Chapter 4 were consistent with this assessment (Figures 13 and 14). Comparison of data in Chapter 4 using an alpha value of 0.20 is consistent with comparisons in the literature (Morin and Cattaneo, 1992). Some investigators have used an alpha of 0.25 to characterize periphytic responses (Krebs, 1989).

The sensitivity of the Matlock periphytometer, and by extension the LETSI method, could be enhanced by increasing the replicate number. However, Morin and Cattaneo (1992) suggested that increasing replicate numbers to more than 20 would not result in decreased sample variance, due to the stochastic nature of the system being measured. This inherent variability often results in contradictory results, and has perhaps been the major detractor in the application of periphyton as an ecological indicator.

Nutrient enrichment very likely increases variability of the responses as well (Morin and Cattaneo, 1992). In fact, Tilman (1982) concluded that community responses to nutrient enrichment were highly variable within similar communities, depending on the historical conditions experienced by the community.

Periphyton Nutrient Co-Limitation

Perhaps the most perplexing result in this set of experiments was the indication that at times periphyton growth may be limited by multiple nutrients (Table 22).

Each of the study streams were co-limited by nitrogen and phosphorus at one time during the study. Figures 13 and 14 clearly demonstrate that either nitrogen or phosphorus enrichment resulted in similar increases in periphyton growth as nitrogen plus phosphorus and total nutrient enrichment.

The process of community competition for limited resources (nutrients) is at the heart of the debate on community function. The conventional theory of evolution holds that individuals within populations within communities compete for limited resources, resulting in a community mosaic that is characteristic of the resource status. When the limiting resource is no longer limited, or supplied in excess, as in the nutrient enrichment treatments, the individuals within the community undergo niche release, and a dramatic shift in community energetics occurs. The individuals within the populations that can most efficiently utilize the newly available resource become more prevalent in the community.

The conventional view of community dynamics is being challenged by a relatively new paradigm of community interaction often referred to as the Gaia hypothesis. This paradigm suggests that communities respond to resource limitation and enrichment in a synergistic manner. The response of individuals within the community is governed by internal community-level feed-back mechanisms which have evolved to produce the most resilient community, not individual. This

is not the same as saying the communities respond in an intentional manner, as the Gaian view is often romanticized to suggest.

While this argument may seem tangential to this dissertation, the co-limited phenomenon observed in Figures 13 and 14 are the result of this fundamental life process. In fact, disturbance ecology theory suggests that disturbance, or extreme events, define community characteristics rather than median conditions. By inference, one could speculate that the periphytic community in a stream that experiences frequent episodes of nutrient enrichment might respond more efficiently to nutrient enrichment *vis-à-vis* the Matlock periphytometer than the periphytic community in a stream that is not nutrient enriched. Put another way, communities that have evolved under a given set of conditions are more apt to thrive under those conditions than communities that have not.

Understanding the mechanisms involved at the community level may provide insight into the fundamental question of evolution: "Why are there so many species?" If competition alone governed species survival, then evolution would tend towards a few highly successful "super-species." In fact, there are between 10 and 100 million species on Earth at this time, though we have described only about 12,000 (Wilson, 1988).

Analysis of LETSI's

Analyzing the LETSI's from different sites at different times may provide valuable insight into the nutrient processes within a stream, but caution is warranted in placing statistical significance in the quantitative differences in the ratios. The trophic status indices presented in Table 14 are a ratio of means, and do not reflect the variability inherent in the data used to derive them. The LETSI is constructed of a ratio of statistics that reflect populations, and therefore are stochastic and have underlying distributions. Assessing the stochastic distribution of the LETSI, and thus any confidence intervals, resulting from the distribution of the component populations is not a trivial statistical concept, and is not practical at this time (personal communication, L. Claypool, Ph.D., Chair, Oklahoma State University Statistics Department, Stillwater, Oklahoma, 1995). The LETSI should be viewed as reflecting trends in community responses, not absolute measures of physical characteristics.

The proposed LETSI is analogous to the algal growth potential test (AGPT) using the *Selenastrum capricornutum* bottle assay in lakes (Raschke and Schultz, 1987). This assay measures the growth *of S. capricornutum* in bottles filled with lake water (control) and nutrient-enriched media (MPP) to determine the ratio of the baseline growth to MPP. As with the LETSI, Raschke and Schultz's assay is based on the premise that the maximum yield is proportional to the amount of nutrient which is present and biologically available in minimal

quantity with respect to the growth requirement of algae (APHA, 1989). A trophic status index for lentic ecosystems based on the AGPT was developed and applied to lakes and reservoirs in the south-eastern US for over a decade (Vollenweider, 1974; Raschke and Schultz, 1987). However, no analogous index of trophic status for lotic ecosystems was discovered in the literature. Vollenweider (1971) developed a lotic trophic status index based on nutrient (N and P) loading, but recognized that this "tentative classification...is admittedly not rigorous enough to meet the demands of theoretical limnology and obviously can not be followed to the letter." Therefore, the LETSI represents a novel approach for analyzing and comparing lotic ecosystems. The concepts on which the LETSI is based are consistent with current theories of aquatic primary productivity.

Comparison of Results with Other Studies

After reviewing the large number of difficulties associated with this method, it is important to point out that the results obtained in this investigation are consistent with similar studies throughout the world. Pringle et al. (1986) measured the response of periphyton to nutrient enrichment in nutrient enriched tropical streams in the Cordillera Central mountains of Costa Rica using nutrient enriched agar and sand as a substrate. They measured periphyton chlorophyll *a* concentrations ranging from 0.5 to 3.1 $\mu g\ cm^{-2}$ after 14 days. The range of data observed in my investigation was very similar, ranging from 0.2 to 2.9 $\mu g\ cm^{-2}$

after 14 days (Tables 13 and 14). Pringle's control and total nutrient enriched values were 0.9 and 3.1 $\mu g\ cm^{-2}$, respectively, resulting in a LETSI of 0.29. The LETSI values for the three watersheds in my study ranged from 0.30 to 0.64.

After considering the plethora of stochastic variables that influence the growth of periphyton, it is profoundly astonishing that third order streams in the Cordillera Central mountains of Costa Rica produce virtually the same range of chlorophyll *a* in 14 days as third order streams in eastern Oklahoma. These streams are separated by over 30 degrees latitude, are in dramatically different ecosystems, have different hydrologic, soil, and daylength characteristics, yet the periphytic communities respond in similar fashions. This illustrates clearly the implications of a cosmopolitan distribution of species; the algae in Costa Rica may differ little from the algae in Oklahoma (Cairns, 1991b).

A lotic ecosystem classification index based on a variety of matrices, including chlorophyll *a* concentration extracted from glass rod periphytometers, has been developed for higher order rivers in the Illinois River Basin and two other adjoining basins (Lynch, 1993). However, this classification system is not appropriate for lower order streams like Battle, Peacheater, and Tyner Creeks. This index would classify each these streams as un-impacted based on chlorophyll *a* production.

Conclusions

The Matlock periphytometer is relatively simple to use, is quantitative, and allows complete recovery of algae from the growth media. This method can be modified to asses the response of the periphytic community to specific concentrations of any water soluble (polar) molecule less than 10 kD in size. The potential applications for this method include phytotoxicity studies, trophic status evaluations, seasonal and long-term nutrient status flux investigations, and quantitative ecological risk assessments.

Passive diffusion periphytometers are an appropriate tool for assessing the nutrients which limit lotic ecosystem primary productivity (Fairchild and Sherman, 1993). The ratio of baseline growth to MPP, by definition a functional index, provides an index of lotic ecosystem trophic status. This index represents the proportion of MPP currently manifested in a stream or river, and is indicative of the current or potential impact from nutrient enrichment. This approach is most appropriate in streams that are nutrient limited and have perennial flow.

Finally, the implications of these data are clear: streams in the Illinois River are impacted by nutrients, and are under temporally varying nutrient stress. In 1985, Gakstater and Katko (1986) observed that "...nuisance-level quantities of periphyton...or mats on the stream bed, were not prevalent ... in the Illinois River basin." That is not the case today, ten years later; the substrate in the main

channel above the scour zone are coated with a thick growth of periphyton from Lake Francis to Tenkiller Ferry Reservoir. The limiting nutrient in an unimpacted temperate woodland stream should be phosphorus (Wetzel, 1975). This investigation has documented temporal shifts in limiting nutrients in the three streams from nitrogen to phosphorus to co-limited conditions, therefore indicating these lotic ecosystems are enriched from nonpoint source nutrient loading.

Recommendations for Future Research

There are many potential areas for future research using the Matlock periphytometer and the Lotic Ecosystem Trophic Status Index. Several recommendations for future research are made below.

1. The Matlock periphytometer could be used to monitor the effectiveness of nutrient control best management plans (BMPs) within a watershed. If the BMPs are effective, the nutrient levels in a stream should change over time. Additional insight into the seasonal variations within the lotic ecosystems could be gained by periodic deployment of the Matlock periphytometer throughout the year and over multiple years.

2. The statistical distribution of periphytic growth on the Matlock periphytometer must be determined in order to improve comparative statistical analysis of the results. I speculate the distribution of periphytic colonization and growth can

be more appropriately approximated by a lognormal, rather than normal, distribution. Periphyton data have been historically treated as normally distributed (Morin and Catteneo, 1992). Determining the underlying distribution may require sampling more than 50 replicates of each treatment under a given set of conditions.

3. The Matlock periphytometer could be integrated into an ecological risk assessment for nutrient enrichment impact. Using a series of concentrations of the limiting nutrient in a lotic ecosystem, an investigator could determine the concentration that results in a significant biological response. This concentration would be the threshold level for the ecological risk assessment.

4. The Matlock periphytometer could also be used to investigate the fundamental processes involved in community responses to nutrient enrichment.

5. If the LETSI is going to find utility in watershed management, we must investigate the significance of the index. Specifically, paired watershed studies comparing the LETSI from impacted and non-impacted watersheds in different ecoregions would provide a baseline of data for interpreting the LETSI.

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APPENDIX 1

MATLOCK PERIPHYTOMETER CHLOROPHYLL *a*

DATA FOR CHAPTER 3

Table A-1: Chlorophyll *a* data ($\mu g\ cm^{-2}$) from Matlock periphytometer Total Nutrient Treatments placed in Peacheater Creek, a tributary of the Illinois River from January 23 through February 5, 1994.

Replicate Number	Chlorophyll *a* ($\mu g\ cm^{-2}$)	Replicate Number	Chlorophyll *a* ($\mu g\ cm^{-2}$)
1	2.33	19	4.60
2	2.37	20	1.74
3	1.22	21	2.23
4	1.01	22	2.42
5	1.95	23	2.19
6	3.01	24	2.23
7	1.90	25	3.05
8	2.51	26	2.68
9	2.56	27	2.73
10	2.16	28	2.40
11	1.95	29	2.91
12	2.40	30	3.38
13	1.88	31	3.95
14	1.97	32	3.48
15	2.14	33	2.96
16	1.97	34	2.54
17	2.09	35	2.11
18	2.65	36	3.48

Table A-2: Chlorophyll *a* data ($\mu g\ cm^{-2}$) from Matlock periphytometer Nitrogen, Phosphorus, and Control Nutrient Treatments placed in Battle Creek, a tributary of the Illinois River from December 22, 1994 through January 5, 1995.

Replicate Number	Nitrogen Treatment Chl. *a* ($\mu g\ cm^{-2}$)	Phosphorus Treatment Chl. *a* ($\mu g\ cm^{-2}$)	Control Treatment Chl. *a* ($\mu g\ cm^{-2}$)
1	0.29	1.62	1.86
2	0.42	0.51	0.45
3	1.26	4.84	1.99
4	1.89	7.99	1.09
5	2.51	3.27	1.06
6	1.33	2.42	2.33

APPENDIX 2

NUTRIENT SOLUTIONS FOR THE MATLOCK PERIPHYTOMETER

The nutrients used for enriching growth surfaces in the Matlock Periphytometer were standard algal media nutrients (Weber et al., 1984). This media was selected because it is a total nutrient growth media, and the micro-nutrients stay in solution at low temperatures (4° C). The Total Nutrients treatment was the same composition used to grow and maintain multiple generations of algae. The concentration of micro-nutrients was increased 100 times the standard concentration to insure adequate diffusion of very low concentrations of nutrients. In order to reduce the rate of bacterial degradation of the cellulose membrane, 250 $\mu g\ l^{-1}$ Penicillin G® was added to each treatment stock solution. The nutrients used in each treatment for the LETSI work are summarized in Tables A-1 and A-2.

Table A-3: Nutrient concentrations (mg l^{-1}) for each treatment in the Matlock periphytometer.

Treatment	Nutrient	Concentration (mg l^{-1})
Nitrogen	$NaNO_3$	0.30
Phosphorus	K_2HPO_4	0.50
Nitrogen plus Phosphorus	$NaNO_3$ K_2HPO_4	0.30 0.50
Micro-Nutrients	<See Table A-2>	<See Table A-2>
Total Nutrients	$NaNO_3$ K_2HPO_4 Micro-Nutrients	0.30 0.50 <See Table A-2>
Control	Reverse Osmosis Water	Conductivity=15 $\mu mhos/cm^2$

Table A-4: Nutrient concentrations (mg l^{-1}) for Micro-Nutrient treatment in the Matlock periphytometer

Nutrient	Concentration (mg l^{-1})
$MgCl \bullet 6\ H_2O$	675
$CaCl_2 \bullet 2\ H_2O$	250
$MgSO^4 \bullet 7\ H_2O$	750
H_3BO_4	10
$MnCl_2 \bullet\ H_2O$	25
$ZnCl_2$	2.5
$CoCl_2 \bullet 6\ H_2O$	2.5
$CuCl_2 \bullet 2\ H_2O$	2.5
$Na_2MoO_4 \bullet 2\ H_2O$	0.5
$FeCl_3 \bullet 6\ H_2O$	7.5
$Na_2EDTA \bullet 2\ H_2O$	15

APPENDIX 3

PHOTOGRAPHS OF SAMPLE SITES

Figure A-1: Battle Creek Sample Site

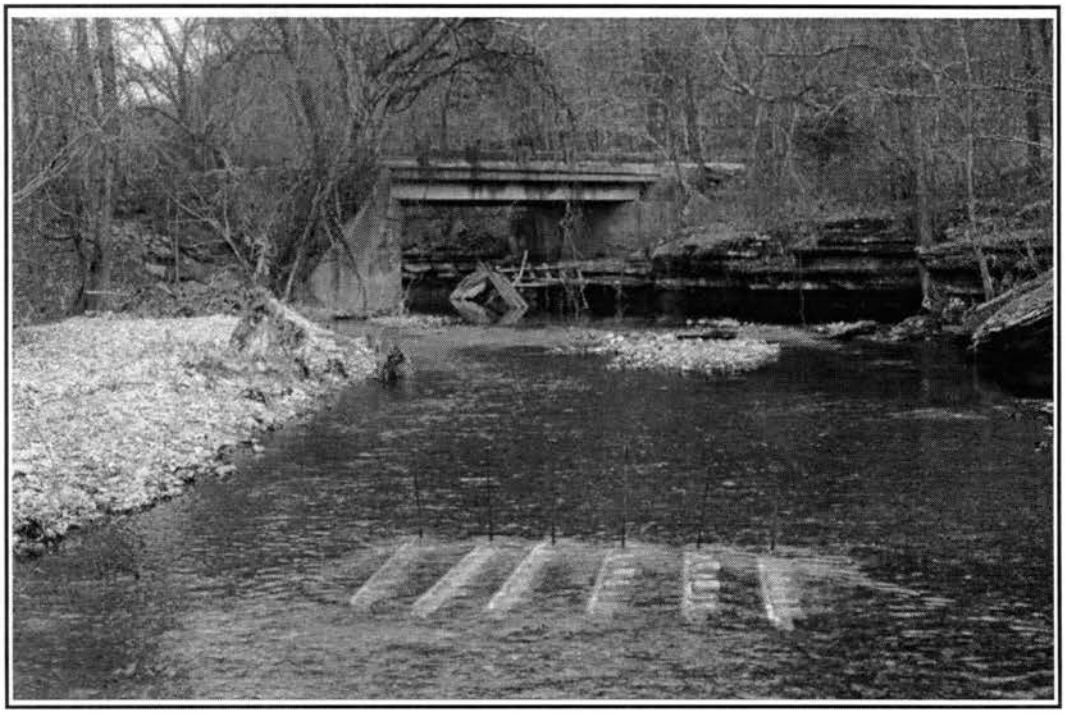


Figure A-2: Peacheater Sample Site.

Figure A-3: Tyner Creek Sample Site.

APPENDIX 4

MATLOCK PERIPHYTOMETER CHLOROPHYLL *a*

DATA FOR CHAPTER 4

Table A-5: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Battle Creek between April 8 - 21, 1995.

Replicate Number	Treatments					
	N	P	N&P	Micro	Total	Control
1	0.30	1.61	2.22	0.44	2.26	1.46
2	0.86	---	2.15	0.41	1.71	0.84
3	1.97	---	1.71	0.36	---	0.99
4	1.51	---	0.73	0.60	---	0.71
5	1.17	---	1.52	0.53	---	0.95
6	---	---	---	---	---	1.38

Figure A-4: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Battle Creek between April 8 - 21, 1995.

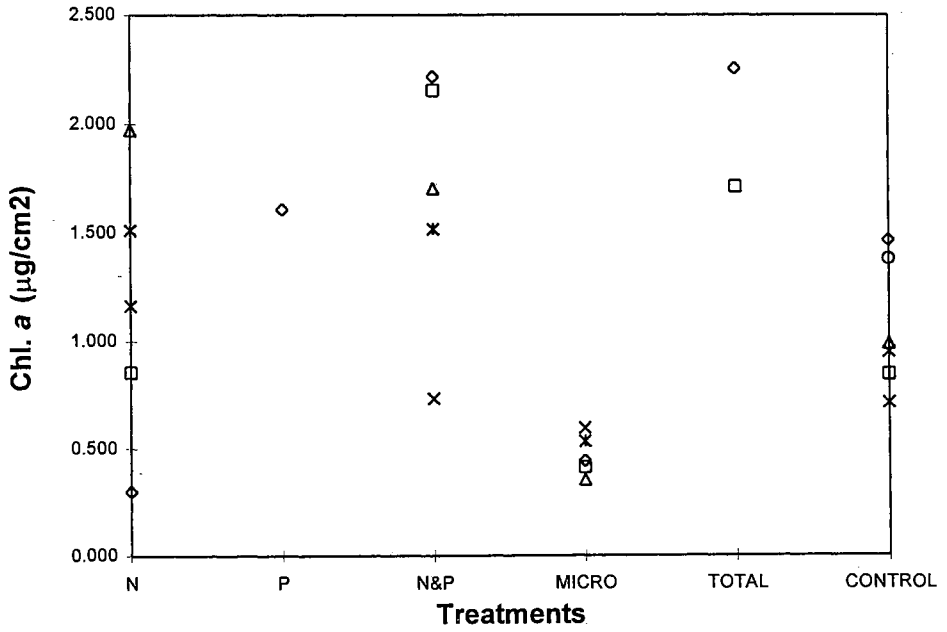


Table A-6: Matlock periphytometer chlorophyll *a* data ($\mu g\ cm^{-2}$) collected from Peacheater Creek between April 8 - 21, 1995.

Replicate Number	Treatments					
	N	P	N&P	Micro	Total	Control
1	0.57	0.36	0.22	0.33	0.24	0.18
2	0.33	0.16	0.47	0.13	0.43	0.11
3	0.40	0.21	0.22	0.23	0.32	0.17
4	0.18	0.19	0.27	0.05	0.28	0.34
5	0.07	0.16	0.21	0.41	0.47	0.05
6	0.29	0.12	---	0.03	0.26	0.41

Figure A-5: Matlock periphytometer chlorophyll *a* data ($\mu g\ cm^{-2}$) collected from Peacheater Creek between April 8 - 21, 1995.

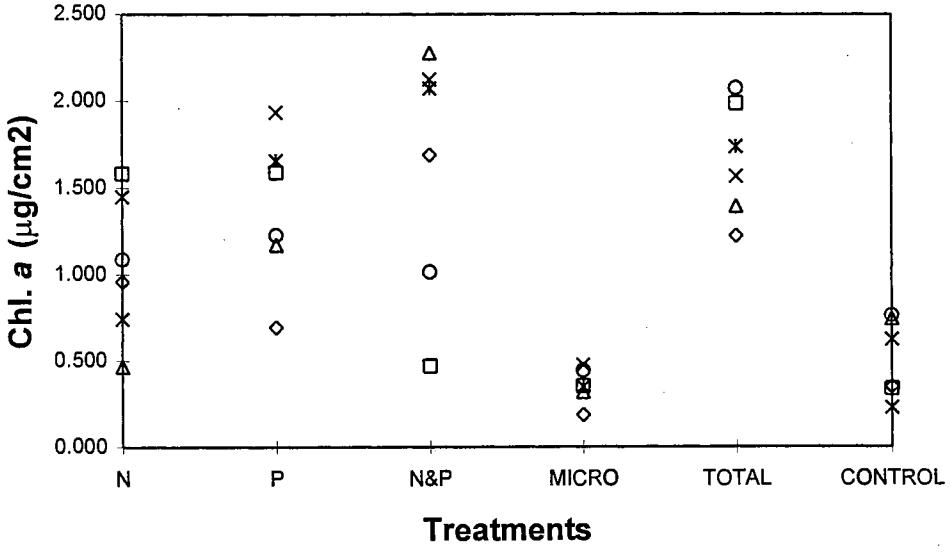


Table A-7: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Tyner Creek between April 8 - 21, 1995.

Replicate Number	Treatments					
	N	P	N&P	Micro	Total	Control
1	0.95	0.69	1.69	0.19	1.23	0.35
2	1.58	1.59	0.47	0.35	1.98	0.33
3	0.47	1.17	2.28	0.32	1.40	0.74
4	1.45	1.94	2.12	0.48	1.57	0.23
5	0.74	1.66	2.07	0.35	1.74	0.62
6	1.09	1.23	1.02	0.44	2.07	0.76

Figure A-6: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Tyner Creek between April 8 - 21, 1995.

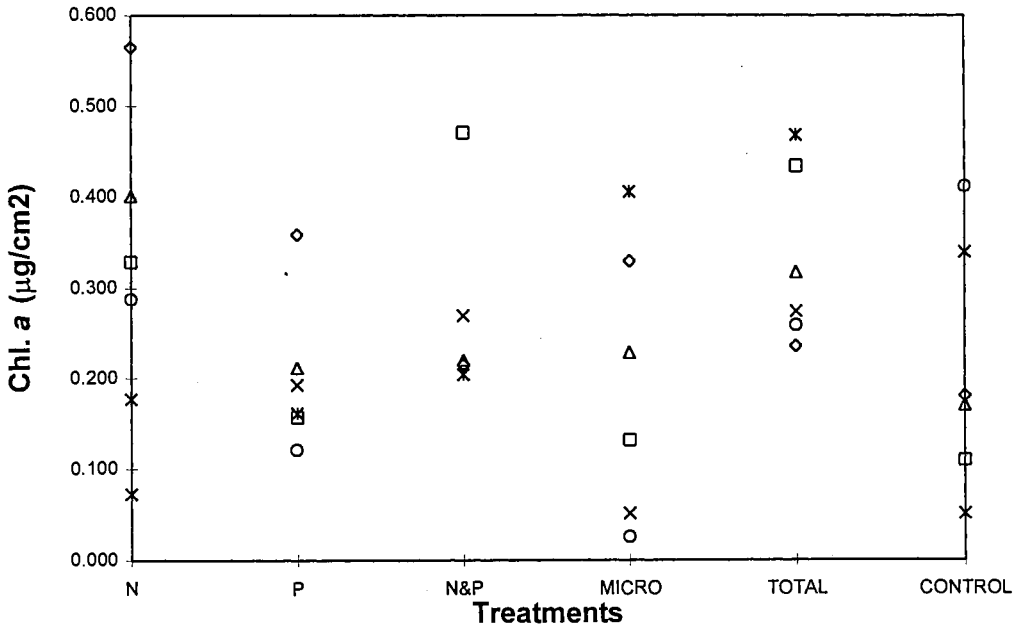


Table A-8: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Battle Creek between September 20 - October 3, 1995.

Replicate Number	Treatments					
	N	P	N&P	Micro	Total	Control
1	0.40	0.23	0.66	0.15	0.65	0.04
2	0.28	0.24	0.98	0.28	0.72	0.36
3	0.33	---	0.72	---	0.51	0.29
4	0.30	---	0.14	---	0.40	0.43
5	---	---	---	---	---	---
6	---	---	---	---	---	---

Figure A-7: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Battle Creek between September 20 - October 3, 1995.

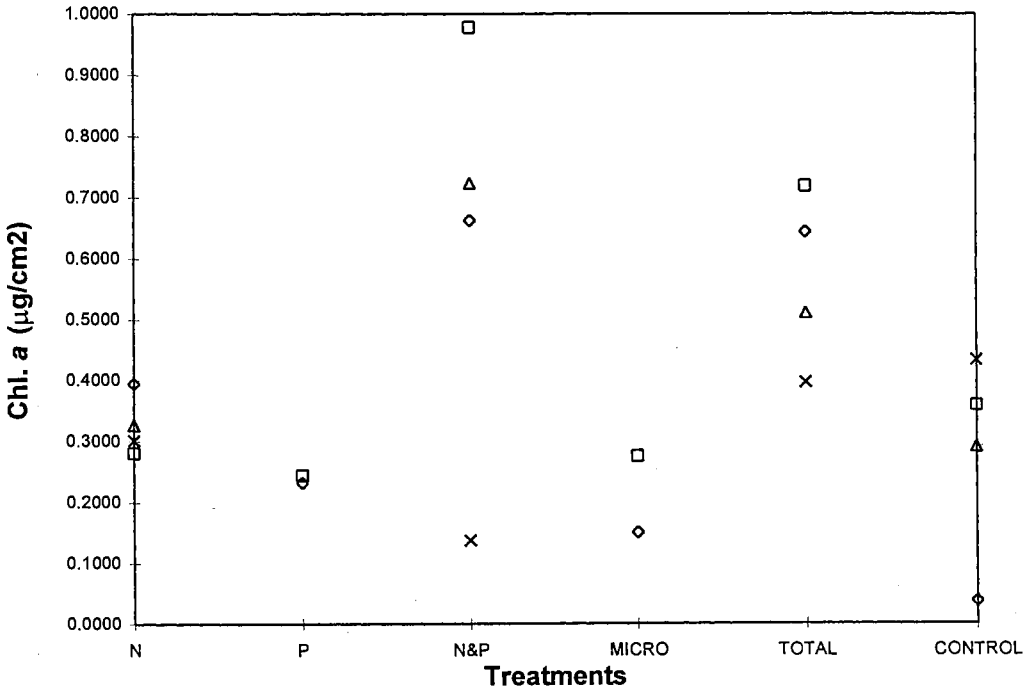


Table A-9: Matlock periphytometer chlorophyll *a* data ($\mu g\ cm^{-2}$) collected from Peacheater Creek between September 20 - October 3, 1995.

Replicate Number	Treatments					
	N	P	N&P	Micro	Total	Control
1	0.63	0.35	0.48	0.21	0.98	0.25
2	0.46	0.30	0.37	0.16	0.23	0.28
3	0.24	0.42	0.39	0.27	0.36	0.30
4	0.69	0.27	0.42	0.20	0.40	0.27
5	0.57	0.39	0.59	0.30	0.89	0.26
6	0.73	0.37	1.09	0.23	1.24	0.36

Figure A-8: Matlock periphytometer chlorophyll *a* data ($\mu g\ cm^{-2}$) collected from Peacheater Creek between September 20 - October 3, 1995.

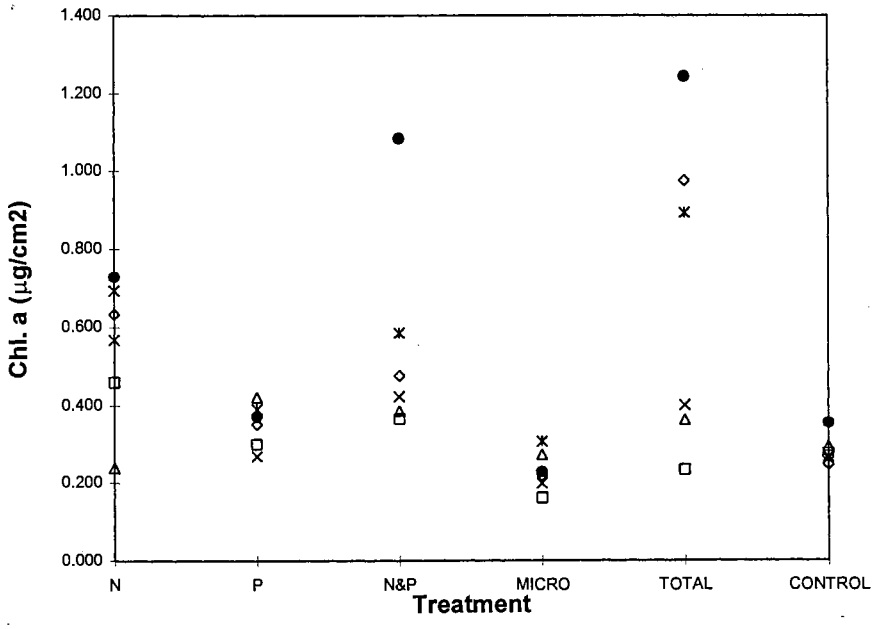
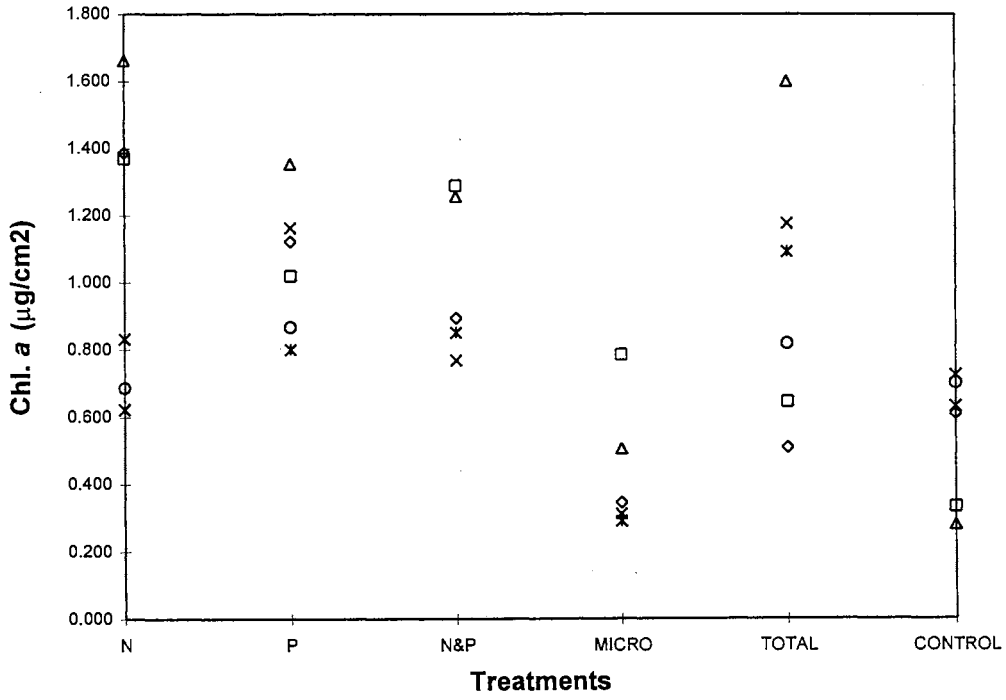


Table A-10: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Tyner Creek between September 20 - October 3, 1995.

Replicate Number	Treatments					
	N	P	N&P	Micro	Total	Control
1	1.39	1.12	0.90	0.35	0.51	0.61
2	1.37	1.02	1.29	0.79	0.65	0.33
3	1.66	1.36	1.26	0.51	1.60	0.28
4	0.83	1.16	0.77	0.31	1.18	0.73
5	0.62	0.80	0.85	0.29	1.09	0.63
6	0.69	0.87	---	---	0.82	0.70

Figure A-9: Matlock periphytometer chlorophyll *a* data (μg cm^{-2}) collected from Tyner Creek between September 20 - October 3, 1995.



APPENDIX 5

DETAILS ON DEVELOPMENT OF THE MATLOCK PERIPHYTOMETER

Summary of Modifications to the Matlock Periphytometer

The modifications made during the development of the Matlock periphytometer are summarized in this section. A chronology of the activities that resulted in these modifications is presented in the following section. These sections are presented to facilitate application of the Matlock periphytometer under conditions that require additional modification, with the hope that the reader will learn from my experiences and avoid unnecessary complications.

Support Racks

The initial support racks for the Matlock periphytometer were constructed of low-carbon steel. After two weeks immersed in a river, they became heavily oxidized, raising the concern that solubilized iron was interfering with the nutrient dynamics of the periphyton. The next set of racks were painted with an epoxy coating, which successfully decreased the amount of rust accumulating on the racks, but increased the cost of construction of each rack significantly. Finally, aluminum was selected as a construction material for the racks. The benefits of aluminum were its strength, light weight, and resistance to oxidation. The principal disadvantage of aluminum is its desirability for scrap metal, thus the increased potential for vandalism and theft.

Treatment Bottles

The first prototype Matlock periphytometers used a four liter plastic reservoir. This system worked well, but was cumbersome and required nearly 40 gallons of nutrient solution per site; six sites required 240 gallons of solution, weighing nearly a ton. This presented a logistic problem. The large size was initially selected to insure adequate nutrient solution was available for diffusion over the 14 day treatment period. After quantifying the rate of diffusion, it became clear that one liter containers would be adequate. The Matlock periphytometer currently uses one liter containers.

Semi-Permeable Membrane

The semi-permeable membrane presented several problems: the membrane degraded over time due to bacterial activity, and it was prone to tear under turbulent flow conditions when the surface of the glass fiber filter was buffeted with stream water. The first problem was addressed by adding 0.25 mg l-1 penicillin G to the nutrient solutions. The second problem was addressed by screening the racks with aluminum screen, thereby creating a laminar flow zone above the filter surfaces. Ultimately, a stronger material would make this apparatus more resilient. However, polypropylene and Teflon membranes are still in development, and not commercially available.

Glass Fiber Filters

The Matlock periphytometer was initially constructed with Whatman® 934-AH glass micro-fiber filters, 7.7 cm diameter. However, the filters and membranes continually failed for the first two years of implementation. Several other types of glass fiber filters were tested, including GF/C and GF/D, which were much thicker than the 934-AH filters. However, the thicker glass fiber filters eroded at a faster rate than the initial filters. Ultimately, solving the problems of grazing and turbulent flow were the keys to increasing the resiliency of the filters.

Grazing

The problem of grazing on the glass fiber filters was the most difficult to address. I was resistant to screening the growth surfaces, since it required covering the growth surface in such a way as to possibly induce light limitation. In addition, grazing was not a problem (nor was turbulence) in the high order main channel of the Illinois River, leading me to believe I could avoid the problem in the lower order tributaries. However, after numerous attempts to collect data were thwarted by destroyed sample growth surfaces due to grazing, I investigated screening materials. The first, and seemingly most attractive screen I tried was a clear polypropylene net with 0.3 cm openings. However, this material fouled terribly with algal growth, resulting in almost complete shading of the treatment growth surfaces. The material that worked the most effectively was aluminum wire cloth (8-mesh). This material did not foul, presumably due to the toxic effects of aluminum as a growth surface for algae.

Stream Flow

Initial field testing demonstrated that the Matlock periphytometer was susceptible to damage from high flow. This was controlled to the extent possible by placement of the racks in pools above riffles, and sampling during low flow

seasons. However, high flow events were unavoidable, and resulted in the loss of many samplers. The aluminum screen reduced these losses dramatically, presumably by creating a laminar buffer over the growth surfaces.

Chronology of Development of the Matlock Periphytometer

Development of the Matlock periphytometer took over three years, and involved a great deal of trial and error. This appendix chronicles the field implementation of the Matlock periphytometer, including problems encountered, various efforts to address the problems, and the solutions resulting from these efforts.

January 9 - 23, 1993

The alpha prototype of the Matlock periphytometer consisted of a steel rack holding four one-liter treatment bottles with the growth surface parallel to the surface of the river. Four racks holding eight replicates each of a control and total nutrient treatment (16 bottles total) were placed in the Illinois River at Horseshoe Bend (just above Lake Tenkiller) for 14 days. Sediment deposition was an obvious problem with this system, since the growth surfaces were horizontal. The membranes and filter papers were all intact, though flow velocities were relatively slow (about 0.1 m s^{-1}). Chlorophyll *a* was not analyzed because the laboratory fluorometer was not working, and required nine weeks for repair.

January 23 - February 6, 1994

The racks holding the Matlock periphytometers were modified to hold six bottles each, and so the growth surfaces were oriented perpendicular to the water surface, thus limiting the amount of sediment accumulating during exposure. I was concerned that iron solubilizing from the steel racks might induce a cation deficiency in the periphyton; therefore the steel racks were painted to reduce rust accumulation. Six racks of total nutrient treatments (36 bottles) were placed in Peacheater Creek for fourteen days to determine the minimum replicate size required. The samples were recovered and analyzed (see Chapter 3).

June 3 - 16, 1994

A series of metal racks were manufactured from aluminum angle iron to avoid the difficulties associated with iron oxidation. Nine control and nine total nutrient treatments (three racks) were placed in Cedar Hollow Creek, and six controls and six totals (two racks) were placed in Peacheater Creek in an attempt to measure the response of an un-impacted stream to nutrient enrichment. After two weeks, all five racks had been vandalized. The bottles were strewn on the stream bank, and the racks were mangled. No data were recovered.

July 1 - 16, 1994

Six target sites were selected for sampling, but only three sites were sampled with a full field implementation of six replicates of six treatments each. The sites were Peacheater Creek, Illinois River at Chewey Landing (94°47'15" lattitude, 36°01'45" longitude), and Tyner Creek. Battle Creek access was restricted by the land-owner due to the up-coming holiday, Steeley hollow flow was too low for sampling, and the Illinois River at Echota Bend (94°55'15" lattitude, 35°54'30" longitude), was too public for sampling during the high-use period. All the samples were destroyed, presumably by grazing. Handling four-liter bottles was a logistical problem in this implementation.

August 16 - September 12, 1994

Diffusion rates were quantified at the USDA hydrology lab in Stillwater (Chapter 3) to quantify diffusion from the apparatus and determine if smaller bottles could be used. Eighteen replicates each (three racks) of a potassium phosphate and sodium nitrate solution were deployed in a constant flow flume for 27 days. Their EC was measured periodically to determine the rates of diffusion of the ions from the bottles. I observed less than a 15 percent change in ion concentration in 14 days. Based on these results, I modified the design to use one-liter bottles.

August 27 - September 10, 1994

The modified Matlock periphytometer treatment array was placed in Peacheater, Tyner, Battle Creeks and the Illinois River at Chewey Landing. Sampling was successful at Chewey Landing, but high flows and grazing resulted in the loss of samples at the other sites. The data were analyzed (Table A-11), but no conclusions could be drawn due to the lack of comparative data.

December 22 - 30, 1994

The Matlock periphytometer treatment array was placed in Steeley Hollow, Peacheater, Tyner, Battle Creeks and the Illinois River at Chewey Landing and Echota Bend. The racks were covered with polypropylene screen to prevent grazing. I was concerned about secondary epiphysism due to conversations with Dr. Dick Pratt of Oregon State University. He suggested I shorten the exposure time to seven days to prevent sloughing. After seven days exposure, the samples were collected from Peacheater, Tyner, and Steely Hollow Creeks. The samples were intact, but no significant growth was observed on the filters, though the screens were fouled with algae and sediment. The remaining sites

Table A-11: Matlock periphytometer chlorophyll *a* data ($\mu g\ cm^{-2}$) with means and standard deviations (Std. Dev.) collected from August 27 - September 10, 1994.

Replicate Number	Peacheater Creek					
	N	P	N&P	Micro	Total	Control
1	13.67	---	---	---	---	2.51
Replicate Number	The Illinois River at Chewey Landing					
	N	P	N&P	Micro	Total	Control
1	1.13	0.06	0.29	0.22	1.01	0.18
2	0.34	1.16	0.22	0.40	0.35	0.13
3	1.35	0.19	1.36	0.36	0.74	0.14
4	0.38	0.82	1.14	0.29	0.72	0.17
5	1.67	0.26	1.57	0.64	0.27	0.81
6	0.44	0.34	0.61	---	---	---
Mean	0.89	0.47	0.87	0.38	0.62	0.29
Std. Dev.	0.57	0.43	0.57	0.16	0.30	0.29
Replicate Number	Battle Creek					
	N	P	N&P	Micro	Total	Control
1	0.42	0.625	---	---	1.51	0.21
2	---	---	---	---	1.50	0.24
3	---	---	---	---	2.23	0.15
4	---	---	---	---	---	0.58
5	---	---	---	---	---	0.51
Mean	0.42	0.625	---	---	1.75	0.34
Std. Dev.	---	---	---	---	0.42	0.19
Replicate Number	Tyner Creek					
	N	P	N&P	Micro	Total	Control
1	3.80	0.99	---	---	5.62	1.00

were left for another week's exposure. The data for Peacheater Creek were analyzed (Table A-12).

January 5, 1995

After fourteen days, the Matlock periphytometers from Battle Creek, Chewey Landing and Echota Bend were collected. The Illinois River had dropped in stage significantly over the two week period, leaving the Echota Bend samples high and dry. The Chewey Landing screens were completely fouled with algae, rendering the samples useless. The Battle Creek data were analyzed and reported (Chapter 3).

Table A-12: Matlock periphytometer chlorophyll *a* data ($\mu g\ cm^{-2}$) with means and standard deviations (Std. Dev.) collected from December 22 - 30, 1994.

Replicate Number	Peacheater Creek					
	N	P	N&P	Micro	Total	Control
1	0.04	0.01	0.02	0.01	0.09	0.03
2	0.02	0.06	0.01	0.01	0.00	0.02
3	0.09	0.12	0.06	0.01	0.10	0.07
4	0.06	0.02	0.02	0.01	0.01	0.03
5	0.03	0.02	0.02	0.01	0.02	0.03
6	0.01	0.02	0.01	0.01	0.01	0.01
Mean	0.04	0.04	0.02	0.01	0.04	0.03
Std. Dev.	0.03	0.04	0.02	0.00	0.05	0.02

April 8 - 21, 1995

Matlock periphytometer treatment arrays were placed in Peacheater, Tyner, Battle Creeks and the Illinois River at Chewey Landing. Aluminum screen was placed over the samplers to prevent grazing. Sampling was not performed at Steeley Hollow due to low flow condition, nor at Echota Bend due to impassable roads. I spent 5 hours digging a truck and trailer out of a bog. After two weeks exposure, the samples were retrieved from the tributaries, but the Chewey Landing samples were unretrievable due to high water. The data were analyzed and reported (Chapter 4).

September 20 - October 3, 1995

Matlock periphytometer treatment arrays were placed in Peacheater, Tyner, and Battle Creeks. Aluminum screen was placed over the samplers to prevent grazing. Sampling was not performed at Steeley Hollow due to low flow condition, nor at Echota Bend or Chewey Landing due to impassable roads. After two weeks exposure, the samples were retrieved from the tributaries. The data were analyzed and reported (Chapter 4).

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