Effects of Two Triazine Herbicides on the Structure and Metabolism of Freshwater Marsh Periphyton.

by

L. Gordon Goldsborough

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
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in
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ABSTRACT

Effects of the triazine herbicides simazine and terbutryn on productivity, chlorophyll content, total biovolume and community structure of haptobenthic periphytic algal communities colonizing artificial substrata within *in situ* marsh enclosures are described. Levels of inhibition were determined at each concentration tested in relation to an untreated control treatment. Comparisons of the relative efficacies of the two compounds indicated that terbutryn was at least ten times more phytotoxic than simazine. Secondary effects of herbicide treatment on enclosed water chemistry were quantified. Following incidental enclosure flooding and removal of herbicides, recovery of community function occurred at all but the highest treatment levels, while community structure was altered in favor of populations of the sessile diatom *Cocconeis placentula*. Determinations of community sensitivity to simazine (EC_{50}) showed that resistance / tolerance could be induced through short exposures, but did not persist in the absence of high herbicide concentrations. It was concluded that periphyton successional processes, which normally lead to the development of a complex three - dimensional mat, may be averted by short herbicide exposures. Aspects of the autecology and synecology of *Cocconeis placentula* are discussed.

ACKNOWLEDGMENTS

No research project is every a solitary effort, and I am indebted to many people for their provision of assistance, discussion, encouragement and instruction during the past four years. They include T.Atkins, Dr.R.Barclay, R.Garrod, N.Grift, S.Gurney, J.Hall, M.Husseini, Dr.N.Kenkel, G.Kruszynski, T.Muhsin, Dr.D.Muir, F.Schneider, Dr.J.Shay, T.Stevens, Dr.J.Stewart, M.Sumner, I.Suthers and A.Yarechewski. Unfortunately, human memory being what it is, I undoubtedly have neglected to mention all deserving parties (including various and sundry members of the Department of Botany and the University Field Station), to whom I apologize and offer a blanket acknowledgment.

Also deserving of recognition are the people of Johnston Industrial Plastics, who, despite the rather unusual application of their products, provided many ideas for the construction of enclosures.

The advice and guidance of my supervisory committee members, Drs. S.S.Badour, G.G.C.Robinson and I.N.Morrison, and the further members of my examining committee, Drs.F.deNoyelles and H.Halvorson are gratefully acknowledged. No citation can fully describe the rewarding education, encouragement and friendship I received from my supervisor, Dr.G.G.C.Robinson.

Financial support was provided in part by a contract with the Division of Biological Sciences of the National Research Council of Canada, a Postgraduate Scholarship, Strategic Grant, and Operating Grant from the Natural Sciences and Engineering Research Council of Canada, and a University of Manitoba Postgraduate Fellowship. The use of research facilities of the University of Manitoba Field Station (Delta Marsh) is gratefully appreciated. Ciba-Geigy kindly provided technical grade simazine and terbutryn used in this investigation.

Finally, I would like to thank my wife, Maria Zbigniewicz, for her perseverance in the face of my diverted attentions, and for her technical, editorial and culinary talents, the significance of any of which to the completion of this thesis cannot be overstressed.

INTRODUCTION

While assessments of the gross effects of herbicide additions to shallow, littoral waters are common, examination of the responses of periphytic algal communities, which may constitute a large proportion of total primary production, has been limited. The objectives of the present study, therefore, were three-fold:

1. to quantify effects of two triazine herbicides, simazine and terbutryn, on the productivity, biomass and community structure of freshwater marsh periphytic algal communities using *in situ* enclosures.

2. to quantify the static sensitivity of periphyton productivity to simazine, and to determine if resistance / tolerance could be induced through short herbicide exposures.

3. to quantify changes in marshwater chemistry following herbicide treatment in relation to hypothesized effects on epipelic algae.

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LITERATURE REVIEW

1.1 ENCLOSURES

While the emphasis of early research in aquatic biology was essentially descriptive, a recent trend towards experimental manipulation of micro-environmental factors offers a means of testing hypotheses (which are often the product of descriptive sampling) relating the spatial and temporal responses of populations and communities to specific causes.

Laboratory microcosms (usually small aquaria) containing a few aquatic populations in defined proportions have been widely used for such manipulations (eg. Bryfogle & McDiffett 1979; Francis & Metcalf 1981; Cunningham et al. 1984) although such systems are necessarily crude approximations of natural systems. Similarly, artificial streams (eg. Clark et al. 1979; Kosinski 1984; Yasuno et al. 1985) also sacrifice realism for ease of manipulation and replicability. Attempts have been made to avoid the inherent artificiality of the above communities by sampling natural communities for subsequent laboratory analyses (Antoine & Benson-Evans 1983; Lewis et al. 1983; Blanck 1985), or through the use of 'pseudo-natural' ponds for in situ manipulations (eg. deNoyelles et al. 1982; Scott et al. 1985).

Studies involving artificial perturbations of natural communities *in situ* have examined the responses of entire streams (Leland & Carter 1984), ditches (Scorgie 1980) and lakes (Brooker & Edwards 1973; Schindler et al. 1985). While such 'whole system' testing may provide data unobtainable from microcosm studies (Schindler et al. 1985), logistic limitations, and the need for adequate controls, often dictate that small subsets of a system be manipulated. To overcome these problems, the use of enclosures in toxicological and ecological studies of natural communities was first considered in the early 1960's (McAllister et al. 1961; Strickland & Terhune 1961; Goldman 1962; Walker 1964) and has since become widespread.

Among the many permutations of enclosure design in current use are examples which exclude contact of the chemical, physical or biological environment with the enclosed water column. 'Exclosures' have been used widely in the study of interactions between grazing pressure and algal standing crop (eg. Kesler 1981; Cattaneo 1983). Physically exclusive enclosures (usually for light - eg. Kistritz 1978) are generally less commonly used than those based on chemical exclusion (additions of nutrients or toxicants). While most enclosures either maintain sediment contact or delimit a portion of surface water only, bottomed enclosures have been employed to examine the role of sediments in aquatic nutrient cycling (Kistritz 1978; Zarini et al. 1983). Occa-

sionally, the basis for exclusion is complex. Plumley & Davis (1980), for example, used an exclosure to control the density of grazing crabs on a tidal marsh flat treated with a herbicide.

Table 1 summarizes the objectives of many experiments conducted using enclosures. Of those listed, most have been carried out in freshwater (75%) over a period of 2-12 weeks. Included in this tabulation are several integrated programs which saw the use of a series of enclosures for several concurrent experiments. The 'Lund tubes', originally deployed by J.W.G.Lund in the English Lakes District (Lund & Reynolds 1982) and subsequently copied by other British workers (eg. Leah et al. 1978) have been used extensively to examine the nutritional requirements of phytoplankton communities. MERL (Marine Ecosystem Research Laboratory) has examined aspects of marine phytoplankton ecology. Projects of a toxicological nature include MELIMEX (MEtal LIMnological EXperiment - Gachter 1979), CEPEX (Controlled Ecosystem Pollution EXperiments - Case 1978) and POSER (Plankton Observations with Simultaneous Enclosures in Rosfjorden - Brockmann et al. 1983). A wide variety of enclosures have been used in ELA (Experimental Lakes Area, Northwestern Ontario) projects to examine chemical fluxes in lakes, phytoplankton and zooplankton responses to eutrophication and small-scale impact of acidification and heavy metal pollution (Cruikshank et al. 1983).

TABLE 1

Summary of some uses of enclosures

for chemical, physical or biological exclusion in aquatic environments. ? = not specified, NA = not applicable

Reference	Purpose	Fresh/ Marine	Expt. Duration
Barica et al. 1980	nitrogen additions on algal blooms	F	3.5-4 mos
Bender & Jordan 1970	enclosure effects on primary productivity estimates	F	ca. 25 d
Borsheim & Olsen 1984	zooplankton grazing on biomass of phytoplankton & bacteria	F	8 d
Bower & McCorkle 1980	ELA - carbon cycling in epilimnion	F	18 d
Brunskill et al. 1980	ELA - effect of arsenic on microbial & phytoplankton growth	F	35-130 d
Caron & Sieburth 1981	MERL project - wall fouling processes & remedial action	M	16 d
Cattaneo 1983	effect of grazing on periphyton community structure & biomass	F	ca. 3 mos
Collins & Lane 1984	acidification on zooplankton & phytoplankton	F	90 d
Crowley et al. 1983	exclosure design for zoobenthic research	?	NA
Cruikshank et al 1983	epilimnetic enclosure design & construction	F	NA
Davies & Gamble 1979	effect of mercury on phyto- & zooplankton community structure	M	ca. 7 wk
Day et al. 1983	effect of herbicide on phyto-plankton & nutrient status	F	?
DeCosta et al. 1983	phosphorus additions on phyto- & zooplankton communities	F	57 d
Delisle et al. 1984	acidification on phyto- & zooplankton communities	F	ca. 6 wk

Table 1 continued

Dickman & Efford 1972	effect of fertilization on phytoplankton growth	F	1 yr
Eberlein et al. 1983	POSER project - phyto- & zooplankton ecology	M	ca. 3 wk
Gachter 1979	MELIMEX project - heavy metals on zoo- & phytoplankton	F	ca. 16 mos
Giekes & Kraay 1982	enclosure effect on diurnal oxygen fluctuation	M	5 d
Goldman 1962	relation of nutrients & primary productivity	F	NA
Hesslein & Quay 1973	vertical eddy diffusion & C fluxes	F	ca. 3 wk
Iseki et al. 1981	CEPEX project - effect of PCB's on phyto- & zooplankton	M	ca. 3 wk
Kattner et al. 1983	POSER project - phyto- & zooplankton ecology	M	ca. 4 wk
Kaushik et al. (unpubl.MS)	insecticide on zooplankton community structure	F	ca. 5 mos
Kemmerer 1968	fertilization on primary productivity	F	20 d
Kesler 1981	effect of grazing on periphyton community structure	F	5-6 mos
Kistritz 1978	nutrient recycling from decaying macrophytes	F	70 d
Klussman & Inglis 1968	fertilization on primary productivity	F	21 d
Kremling et al. 1978	CEPEX project - effect of Cd on phytoplankton & environmental fate	M	30 d
Kuiper 1977	phyto- & zooplankton communities in enclosures	M	39 d
Kuiper 1981	POSER project - effect of Cd on phyto- & zooplankton communities	M	ca. 3 mos
Kuiper et al. 1983b	POSER project - effect of Hg on phyto- & zooplankton	M	ca. 3 wk
Landers 1982	macrophyte senescence on water quality & phytoplankton biomass	F	ca. 4 mos

Table 1 continued

Leah et al. 1978	Lund tubes - phytoplankton ecology	F	1 yr
Lean et al. 1975	phosphorus additions on primary productivity & P fluxes	F	4 mos
LeCohu 1982	phytoplankton vertical migration	F	21 d
Lee & Takahashi 1977	CEPEX project - effect of petroleum on phytoplankton	M	19 d
Liao & Lean 1978	nitrogen & phosphorus cycling	F	14 mos
Lund & Reynolds 1982	Lund tubes - phyto-, zoo-plankton, & fish ecology	F	NA
Lynch 1979	zooplankton ecology	F	3.5 mos
McCauley & Briand 1979	effect of zooplankton grazing on phytoplankton	F	146 d
Mueller 1980	effect of acidification on periphyton growth	F	106 d
Plumley & Davis 1980	effect of herbicide on epipelic algal productivity	M	ca. 4 mos
Schindler et al. 1971	nitrogen & phosphorus additions on phytoplankton growth	F	ca. 60 d
Shires 1983	effect of pesticides on fish	F	8 d
Smyly 1976	Lund tubes - effect of enclosure on zooplankton	F	26 mos
Stephenson & Kane 1984	effect of pesticides on zoo-plankton & invertebrates	F	42 d
Twinch & Breen 1978	enclosure effects of physical & chemical environment	F	10 wk
Twinch & Breen 1981	nitrogen & phosphorus fluxes in enclosures following additions	F	ca. 13.5 mos
Uehlinger et al. 1984	epilimnetic carbon & phosphorus cycling	F	26 d
Zarini et al. 1983	effect of aluminum used as P flocculent on phyto- & zoo-plankton communities	F	22 d

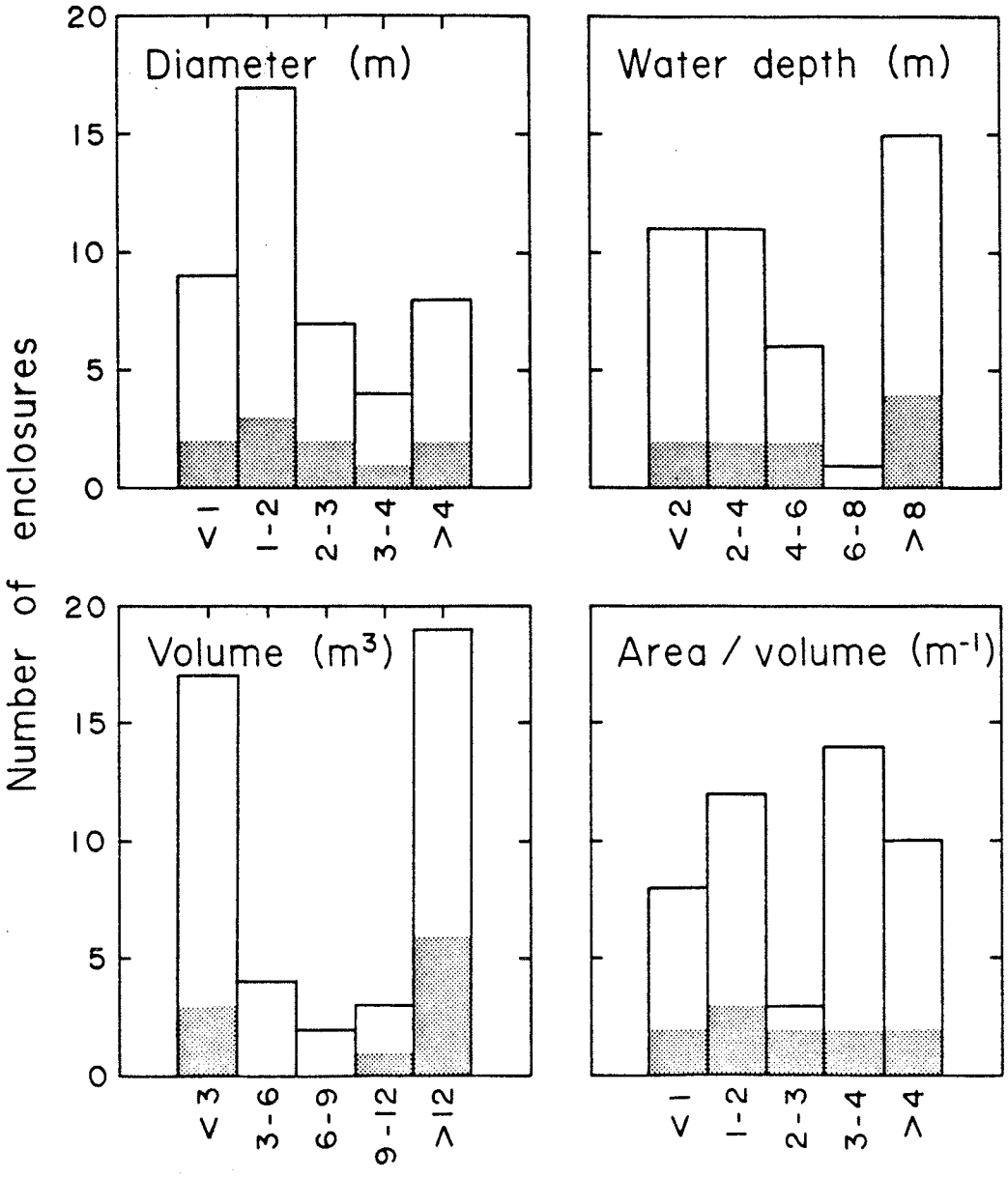
Even the most cursory examination of the literature will show the extreme diversity of design used in the construction of enclosures. Although square (eg. Dickman & Efford 1972) and triangular (eg. Hesslein & Quay 1973; Liao & Lean 1978) enclosures have been conceived, the majority have been fashioned as circular tubes or bags. Reasons for this preference include simplicity of construction (particularly when using plastics), increased structural strength and elimination of potential 'corner effects'. The range of materials used in construction has included laminated wood (Dickman & Efford 1972), steel mesh (Crowley et al. 1983), stainless steel (Shires 1983), aluminum (Kistritz 1978), rubber (Leah et al. 1978; Lund & Reynolds 1983) and a wide variety of sheet, laminate and woven plastics (eg. Goldman 1962; Kuiper 1977; Twinch & Breen 1978; Stephenson & Kane 1984; Zarini et al. 1984).

A summary of the major parameters of many enclosure designs (Figure 1) illustrates the extent to which enclosed dimensions may vary. Menzel & Steele (1978) suggested that optimal enclosure size be determined by the number of trophic levels under study, due to differences in spatial heterogeneity of each level. They proposed arbitrary enclosed volume categories for examination of phytoplankton ($<10m^3$), zooplankton ($10-1000m^3$) and fish ($>1000m^3$) communities from a subjective evaluation of literature data. This approach may be too superficial for all applications, however, and

instead may require specific knowledge of the particular community under study (Case 1978, Kuiper et al. 1983a) and the potential extent of enclosure effects (see later). The latter authors concluded that 1-2 m^3 volume enclosures were appropriate for studies of marine phytoplankton and zooplankton, although the lower densities of these communities in more oligotrophic water would necessitate the use of larger enclosures.

Lund & Reynolds (1983) proposed that the ratio of enclosure wall surface area to enclosed water volume is an important determinant of optimal enclosure size. This conclusion resulted from data obtained from the use of limnetic enclosures which showed that detachment of periphyton from walls gave erroneous phytoplankton enumeration results and that competition between periphyton and phytoplankton for nutrients can result in lower phytoplankton growth than expected in unenclosed systems (Twinch & Breen 1978; Lund & Reynolds 1983). Wall growth of periphyton has been noted within a number of enclosure designs (Figure 1), and although the extent of growth does not appear to correlate with enclosure size, the interactive effect of periphyton would have the most profound effect in enclosures with high wall surface area to enclosed water volume (A/V). Attempts have been made to alleviate this problem through physical removal of attached periphyton (Davies & Gamble 1979; Caron & Sieburth 1981).

Figure 1: Variation in dimensions of enclosure designs (chemically and/or physically exclusive) used in limnological and oceanographic research. Shading represents reported observations of periphyton wall growth within enclosures.


Number of enclosures
Diameter (m)
Water depth (m)
Volume (m³)
Area / volume (m⁻¹)
0
5
10
15
20
< 1
1-2
2-3
3-4
> 4
< 2
2-4
4-6
6-8
> 8
< 3
3-6
6-9
9-12
> 12
< 1
1-2
2-3
3-4
> 4

Though few in number, studies of periphyton growth *per se* within enclosures have involved manipulations of grazer density (eg. Plumley & Davis 1980; Kesler 1981; Cattaneo 1983), and concentration of inorganic nutrients (eg. Twinch & Breen 1978), and evaluation of some effects of pollutants (eg. Grolle & Kuiper 1980) and acid deposition (eg. Mueller 1980). Moss (1981) monitored the periphyton community structure and biomass within an unperturbed enclosure in relation to a surrounding lake and noted that typically planktonic algal taxa were often abundant in the periphyton as a result of sedimentation.

The enclosure of a natural system by walls necessarily creates an environment unlike the unenclosed system. Any changes in water quality and/or biological productivity in the absence of any experimental treatment are therefore termed 'enclosure effects' and necessitate the inclusion of unperturbed control enclosures. Inter-enclosure differences can be subtle and difficult to distinguish from treatment responses, and have been considered by relatively few workers (Kuiper 1977; Uehlinger et al. 1984).

In addition to wall growth considerations, adsorption of highly hydrophobic compounds to enclosure walls may represent a major sink (eg. Davies & Gamble 1979). At the same time, enclosure walls, particularly those made of rubber and metal, may be sources of heavy metals (eg. Gachter 1979) or inorganic nutrients. Release of plasticizing agents from

commercial plastics may also occur (Case 1978; Davies & Gamble 1979).

The relative opacity of enclosure walls may bear on *in situ* productivity. Goldman (1962) found that the Secchi depth of a water column enclosed by 0.6 m diameter tubes was less than for the adjacent lake. Increased rates of extinction of green, red and white light in 0.5 m diameter enclosures have been reported (LeCohu 1982). Klussman & Inglis (1968), on the other hand, reported no change in Secchi depth in their enclosures of similar size. Reports of increased light penetrance into larger (>2 m diameter) enclosures (Lund 1972; Twinch & Breen 1978; Landers 1982) are probably the result of reduced internal turbulence with resulting sedimentation of suspended material (Twinch & Breen 1978). Davies & Gamble (1979) cited evidence indicating that vertical eddy diffusion in marine enclosures could be decreased ten-fold and suggested that effects on relative sinking rates of enclosed phytoplankton were possible.

Similarity of temperature profiles between enclosed and unenclosed water columns (Goldman 1962; Kemmerer 1968; Klussman & Inglis 1968; Kistritz 1978; DeCosta et al. 1983) is commonly given as evidence of lack of enclosure effects on vertical fluxes (Goldman 1962). While the roles of wind-induced turbulence and mixing resulting from sampling procedures in small-volume enclosures (Kuiper et al. 1983a) are yet unknown, Boyce (1974) showed mathematically that hori-

zontal thermal conduction across enclosure walls (a function of enclosure size and wall material) could account for this similarity without necessarily implying commensurability of vertical flux rates.

Changes in water chemistry as a consequence of enclosure have been noted by some workers (eg. Leah et al. 1978; Liao & Lean 1978; Kattner et al. 1983; Stephenson & Kane 1984) and not by others (eg. Klussman & Inglis 1968; Kistritz 1978; DeCosta et al. 1983; Shires 1983). Systematic evaluation is difficult, however, as reported changes have not been universally confirmed. This may indicate that such changes depend on the specific circumstances of enclosure deployment and use. Leah et al. (1978), for instance, reported changes in phosphorus concentration in Lund tubes while noting that birds roosting on the enclosure walls sporadically introduced faeces to the enclosed water column. Several reports of reduced variability in chemical parameters relative to adjacent waters (Leah et al. 1978; DeCosta et al. 1983; Stephenson & Kane 1984) may relate to reduced internal turbulence, which may be site-specific.

DeCosta et al. (1983) observed that no change in phytoplankton chlorophyll content or carbon fixation rate occurred with enclosure in 2 m^3 bags over a 57-day period, while Bender & Jordan (1970) found that phytoplankton productivity in a 0.5 m diameter tubes was initially similar to a surrounding lake but had decreased to about 50% by the end

of a 25-day experiment. Goldsborough et al. (1986) reported that periphyon productivity within littoral enclosures (0.7 m diameter) was similar to that of an adjacent marsh for 6 weeks, after which the enclosed community was more productive. Effects of enclosure on algal detachment were postulated. Changes in phytoplankton species composition in a 5 m diameter enclosure, with diatoms assuming dominance from green algae, have been noted (Twinch & Breen 1978), while Lund (1972) reported that the phytoplankton community in Lund tubes was qualitatively similar to that of the surrounding lake (although total abundance was less). Enclosure (2-10 m diameter) of a portion of a small pothole lake (Barica et al. 1980) did not appreciably alter the timing or extent of phytoplankton bloom collapses.

1.2 ARTIFICIAL SUBSTRATA

Due to the morphology of submerged rocks and plants, quantitative sampling of periphyton communities from these substrata poses unique problems (Sladeckova 1962). These include the inability to accurately assess available colonization area (particularly of highly dissected macrophyte leaves), complex surface topography and potential disruption of adjacent substrata during collection (Tippett 1970; Lamberti & Resh 1985). Moreover, the available surface for algal colonization on dynamic substrata (eg. plants) increases with time through growth, so that closely situated surfaces may support communities in differing stages of development (eg. Delbecque 1983).

Attempts to maximize the efficiency of periphyton removal from these substrata using in situ scrubbers (eg. Douglas 1958; Stockner & Armstrong 1971; Tuchman & Stevenson 1980; Loeb 1981) or through vigorous shaking (Gough & Woelkerling 1976; Morin & Kimball 1983; Jones 1984; Goldsborough & Robinson 1985) are varyingly successful. Expression of resulting cell enumerations in terms of macrophyte stem length (Morin & Kimball 1983; Jones 1984) or weight (Gough & Woelkerling 1976; Goldsborough & Robinson 1985) may be less meaningful than areally based estimates. Some workers have resorted to direct microscopic observation of whole mounted substrata (eg. Jones 1978 - rock; Bowker & Denny 1980 - macrophyte) or in situ 'peel' techniques (eg. Margalef 1949; Cattaneo & Kalff 1978) to more accurately determine algal density. Tett et al. (1975) employed direct extraction of chlorophyll from the surfaces of rocks.

The difficulties associated with quantitative sampling of natural substrata has prompted the use of artificial substrata (Sladeckova 1962), which are operationally defined here as any substratum introduced into an aquatic habitat for the purpose of periphyton sampling. Generalizations regarding the use of such substrata are hampered, however, by the inconsistency in the choice of material, which usually results from a perceived 'superiority' of one material over another. As a result, such diverse media as slate (Dickman & Gochnauer 1978; Mueller-Haeckel & Hakansson 1978; Gale et

al. 1979), basalt (Horner & Welch 1981), sandstone (Antoine & Benson-Evans 1985), granite (Turner et al. 1983; Hamilton & Duthie 1984), wood (Mueller-Haeckel & Hakansson 1978; Putnam et al. 1981; Millie & Lowe 1983), masonite (Pringle 1985), birch leaves (Mueller-Haeckel & Hakansson 1978), paraffin (Beers & Neuhold 1968), agar (Dickman 1974; Pringle & Bower 1984), styrofoam (Putnam et al. 1981; Bothwell & Jasper 1983), baked clay (Tuchman & Stevenson 1980; Stevenson 1983; Fairchild et al. 1985), aluminum (Korte & Blinn 1983), celluloid (Eloranta & Kunnas 1979), polyethylene (Grolle & Kuiper 1977), acrylic (Gale et al. 1979), nylon (Austin et al. 1981), acetate (Hooper-Reid & Robinson 1978; Robinson & Pip 1983), polyurethane foam (Cairns et al. 1983), PVC (Higashi et al. 1981) and plexiglass (Mueller-Haeckel & Hakansson 1978; Mueller 1980; Murray 1980; Gons 1982; Hoagland et al. 1982; Korte & Blinn 1983) have been used. The prevailing popularity of glass, however, (eg. Brown & Austin 1971; Cattaneo et al. 1975; Brown 1976; Grolle & Kuiper 1977; Weitzel et al. 1979; Hoagland 1983) may be attributed to its relative inexpense, widespread availability, ease of handling, and the capability of direct microscopic observation (Herder-Brouwer 1975).

Artificial substrata can be classified into one of two groups according to their morphology. After the terminology of Austin et al. (1981), 'point' substrata permit the sampling of discretely localized communities. An example is a

glass microscope slide, which is placed in an appropriate support apparatus (for example, the Diatometer - Patrick & Reimer 1966) and anchored at a specific location in the water column. Descriptions of support structures for glass and plexiglass slides are common (eg. Sladeckova 1962; Dumont 1969; Brown & Austin 1971; Gons 1982; Hoagland 1983; Meier et al. 1983).

By contrast, a 'linear' substratum is defined as one from which spatially distinct subsamples may be collected from the same substratum. Examples of substrata which have been, or potentially could be used in linear applications include steel navigation bouys (Evans & Stockner 1972), nylon monofilaments (Austin et al. 1981), glass rods (Mason & Bryant 1975), ropes (Lang & Austin 1984), polyethylene tapes (Neal et al. 1967; Austin et al. 1981), acetate strips (Hooper-Reid & Robinson 1978; Robinson & Pip 1983) and acrylic rods (Goldsborough et al. 1986). Interesting permutations of artificial substrata which have sought to mimic the physical morphology of aquatic macrophytes have been described (Cattaneo & Kalff 1978; Fontaine & Nigh 1983; Shamess et al. 1985). Higashi et al. (1981) used PVC rods to simulate emergent reed culms.

There are several important considerations to the use of artificial substrata for periphyton monitoring. In addition to providing logistic ease, a successful artificial substratum should combine precision (low variability between repli-

cate samples) and accuracy (representativeness of constituent periphyton to that on natural substrata) (Tuchmann & Stevenson 1980). It is generally considered that artificial substratum replicates are less variable than adjacent natural substrata (eg. Tuchmann & Stevenson 1980; Lamberti & Resh 1985). Stockner & Armstrong (1971) observed that the CV (coefficient of variation - 100 x mean/standard deviation) of rock samples was internally small (ca. 20%) but was high between individual rocks (35-40%). Other reported ranges for epilithon samples of 39-82% (Wylie & Jones 1981) and 10-111% (Jones 1974) are in general agreement. A summary of studies for which the degree of precision of replicate artificial substratum samples was given or could be calculated (Table 2) indicates that replicability may depend on substratum type, the parameter being monitored, and the individual study. Cattaneo et al. (1975), for instance, reports CV's for glass slides (Table 2) greater than those of the aforementioned rocks.

Concerns have been raised of the degree to which communities developed on artificial substrata emulate natural communities. While close similarity has been reported by some (eg. Godward 1934; Brown & Austin 1971; Evans & Stockner 1972; Cattaneo et al. 1975; Tuchman & Blinn 1979; Fontaine & Nigh 1983), others find poor correlation (Tippett 1970; Brown 1976; Siver 1977; Rosemarin & Gelin 1978; Tuchmann & Stevenson 1980; Gons 1982). Eminson & Moss (1980) suggested

TABLE 2

Summary of some uses of artificial substrata

in quantitative measurements of periphyton and the variability of replicate samples. CV = coefficient of variation

Substratum	Reference	Parameter	Mean CV (%)
acetate strips	Hooper & Robinson 1976	dry weight	37
		C-fixation	31
	Hooper-Reid & Robinson 1978	protein	5
		C-fixation	20
acrylic rods	Goldsborough et al. 1986	C-fixation	26
		chlorophyll	28
		particulate-P	26
concrete blocks	Beers & Neuhold 1968	chlorophyll	35
glass coverslips	Dilks & Meier 1981	chlorophyll	25
	Meier et al. 1983	chlorophyll	13
glass disks	Blanck 1985	photosynthesis	20
glass plates	Castenholz 1961	ash-free dry wt.	25
glass slides	Hoagland 1983	algal density	65*
		light penetrance	5*
	Klapwijk et al. 1983	dry weight	105*
		algal density	68*
	Weitzel et al. 1979	ash-free dry wt.	39*
		chlorophyll	28*
	Clark et al. 1979	ash-free dry wt.	59*
		dry weight	54*
		chlorophyll	45*
		ATP	25*
	Rodgers et al. 1979	chlorophyll	50
		dry weight	50
		ash-free dry wt.	50
		ATP	41
		C-fixation	11
	Brown 1976	algal density	28

Table 2 continued

glass slides	Tilley & Haushild 1975	chlorophyll	20
	Cattaneo et al. 1975	chlorophyll	91*
		dry weight	80*
		organic carbon	91*
		organic nitrogen	103*
	Brown & Austin 1971	algal density	15
glass tubes	Meier et al. 1983	chlorophyll	8
granite plates	Turner et al. 1983	photosynthesis	19
paraffin	Beers & Neuhold 1968	chlorophyll	27

* calculated from source data

that the degree of host specificity of epiphyton (the association of particular algal taxa to specific macrophyte hosts - eg. Prowse 1959) may relate to aquatic trophic status, such that the significance of exogenous nutrient supply to epiphyton decreases with increasing oligotrophy. While the ultimate significance of host excretions to algal nutrient budgets remains in dispute (Gough & Gough 1981; Carignan & Kalff 1982), the Eminson & Moss hypothesis could explain why chemically inert artificial substrata could support qualitatively and quantitatively different periphyton communities (Shamess et al. 1985). Reported differences in algal density and community structure between live and dead host tissue (Young 1945; Grimes et al. 1980) is further evidence of an interaction between nutrient supply at the substratum surface and observed algal growth.

Cattaneo & Kalff (1978) observed that an increase in algal diversity on submerged macrophytes over closely associated plastic mimics correlated well ($r=0.95$) with the amount of marl deposited on plant surfaces as a result of photosynthetic carbon assimilation. The lack of marl on plastic plants, and its supposed importance to community architecture (Allanson 1973) may explain the qualitative dissimilarity in algal communities between substrata. Cattaneo (1978) speculated, however, that the development of marl could also reduce algal growth through shading and reduction in attachment space availability. Other possible determinants of the

representativeness of periphyton on artificial substrata (and to some extent, precision of replicates) include surface topography, substratum orientation and colonization time. Several workers have compared the communities of periphyton on substrata differing in surface texture and have concluded that coarse surfaces often support higher algal density (Rosemarin & Gelin 1978; Tuchmann & Blinn 1979; Antoine & Benson-Evans 1985). On the other hand, Herder-Brouwer (1975) found that while sandblasted glass slides supported a larger community than smooth slides, density was highest on visually less rough wood blocks. Shamess et al. (1985) found little difference in the colonization of smooth and roughened acetate strips.

The observed specificity of some taxa to substratum depressions (Dickman & Gochnauer 1978; Hamilton & Duthie 1984; Pringle 1985), the preferential distribution of periphyton on artificial and natural substratum margins (Cattaneo 1978; Hamilton & Duthie 1984) or on one side (abaxial or adaxial) of inert plastic plant 'leaves' (Cattaneo 1978) and the higher algal biomass attained on sheltered sides of substrata in lotic habitats (Beers & Neuhold 1968; Dumont 1969) indicate that hydrodynamic forces on substrata (determined in part by their orientation) may significantly affect the development of an algal community on a substratum. Brown (1976) observed that tightly adherent taxa attained higher relative density on smooth glass slides than loosely at-

tached ones. Whether these effects are due to physical abrasion by current (Stevenson 1983) or the availability of nutrients (Horner & Welch 1981) is yet unclear. Gons (1982) noted that the relatively lesser extent of self-shading by artifical substrata (as compared to dense mats of aquatic macrophytes) and the absence of shelter for invertebrates may be significant orientational effects. Mason & Bryant (1975) observed that glass rods excluded burrowing chironomid larve, which may be important grazers.

Artificial substrata are generally sampled over a short period of time after placement, whereas natural substrata are colonized throughout the growing season, often over more than one year (rocks, emergent stems), and may include varying proportions of planktonic taxa incorported into the periphyton through entanglement or sedimentation - Moss 1981). While King & Ball (1966) detected no difference in periphyton accumulation on vertically and horizontally positioned substrata over an 18-day period, Castenholz (1961) observed that horizontally positioned glass slides supported 6-12 times more 'periphyton' than vertically positioned ones, and more closely resembled adjacent epilithic community structure. Moreover, rocks which have been sterilized of all attached material have been shown to develop communities more similar to those on tiles deployed at the same time than those on unmanipulated stones (Tuchmann & Stevenson 1980; Lamberti & Resh 1985).

1.3 PERIPHYTON TOXICOLOGY

Due to the sedentary nature of periphyton communities, aquatic biomonitoring programs have long attempted to relate *in situ* algal biomass and species composition, either on artificial or natural substrata, to the relative degree of pollution of lakes and rivers (eg. Tilley & Haushild 1975; Rodgers et al. 1979). The saprobic system of Kolkowitz & Marsson (1908), which classified diatom taxa according to their degree of tolerance of organic pollutants, has undergone several modifications (Patrick 1978) and remains in current use (eg. Kobayasi & Mayama 1982). This approach has been criticized, however, on the grounds that it does not differentiate the relative impacts of different pollutants, or the ecological plasticity of individual taxa (Patrick 1978). Futhermore, such studies generally lack an unperturbed benchmark against which spatial or temporal changes are guaged.

Comparison of taxonomic proportions and total algal biomass above and downflow from a point pollutant source (eg. Besch et al. 1972; Putnam et al. 1981; Lampkin & Sommerfeld 1982; Shortreed & Stockner 1983; Austin & Deniseger 1985) is one means of offsetting the lack of controls in such studies. Any differences noted along a gradient, however, are subject to interpretation since assumed treatment effects may be confounded by inherent spatial variability (Hurlbert 1984).

Concomitant with a shift in general scientific emphasis towards experimental validation (Section 1), recent periphyton toxicological research has increasingly employed experimental manipulation of a controlled series of treatments to isolate specific effects (Table 3). Since the reported responses in these studies may be site- and toxicant-specific, generalizations are limited here to a discussion of the bases upon which impact has been assessed.

Artificial substrata have been used preferentially over natural substrata to monitor periphyton colonization (Table 3). Advantages of this technique include increased precision and ease of sampling (Grolle & Kuiper 1980; Section 2) and, in the case of epiphytic periphyton, circumvention of secondary effects of the toxicant on substratum availability and suitability, and any complicating interaction between a natural substratum and attendant periphyton. Subsequent sample analyses can be classified into two major groups. On one level, gross community responses have been evaluated through changes in biomass, as indicated by chlorophyll a content (Rodgers et al. 1979; Murray 1980; Plumley & Davis 1980; Hodgson & Carter 1982; Hodgson & Linda 1984; Leland & Carter 1985), photosynthetic rate (Rodgers et al. 1979; Plumley & Davis 1980; Kosinski & Merckle 1984; Leland & Carter 1985), carbon content (Leland & Carter 1985), ATP content (Rodgers et al. 1979), or ash-free dry weight (Rodgers et al. 1979). Derived indices of physiological condition

TABLE 3

Summary of periphyton toxicological research

Reference	Toxicant	Substratum Sampled
Anderson 1981	herbicide (hexazinone)	glass slides
Blanck 1985	aliphatic amines	circular glass disks
Cunningham et al. 1984	herbicide (atrazine)	Potamogeton perfoliatus
Dickman 1969,1974	germanium	agar-coated glass slides
Dickman & Gochnauer 1978	road salt	slate tiles
Evans & Marcan 1976	sewage	natural stones
Grolle & Kuiper 1980	mercury	glass slides & polyethylene sheets
Hodgson & Carter 1982	herbicides	glass slides
Hodgson & Linda 1984	herbicides	glass slides & Hydrilla verticilata
Kosinski 1984	herbicides	glass slides
Kosinski & Mercle 1984	herbicides	glass slides
Leland & Carter 1984,1985	copper	glass slides & natural rock cobble
Mueller 1980	acidification	plexiglass plates
Murray 1980	chlorination	glass slides
Plumley & Davis 1980	herbicide (atrazine)	natural sediments
Scorgie 1980	herbicide (cyanatryn)	glass slides

Table 3 continued

Sigmon et al. 1977	mercury	PVC strips
Sullivan et al. 1981	herbicide (glyphosate)	glass slides & natural sediments
Weber & McFarland 1981	copper	glass slides
Williams & Mount 1965	zinc	glass slides
Yasuno et al. 1985	insecticide (temephos)	natural stones

have included 'trophic state' (as either predominantly heterotrophic or autotrophic) (AFDW/chlorophyll a or ATP/chlorophyll a - Murray 1980; Leland & Carter 1985), 'physiological state' (chlorophyll a/pheophytin a - Murray 1980) or specific photosynthesis (photosynthesis per unit chlorophyll - Leland & Carter 1985). Decreases in these indices have been interpreted as indications of algal growth inhibition.

A second approach has involved enumeration of algal cells for the proportions of constituent taxa, from which total cell density (Hodgson & Carter 1982) or cell biovolume (Dickman 1974; Hodgson & Carter 1982) can be calculated. Expressions of the proportions of major taxonomic groups (Mueller 1980; Murray 1980), specific presence or absence (Grolle & Kuiper 1980), or specific/generic richness (Evans & Marcan 1976; Sigmon et al. 1977; Grolle & Kuiper 1980; Mueller 1980; Plumley & Davis 1980) may provide a crude taxonomic basis of sensitivity to the pollutant. Examinations of specific periodicity and abundance (eg. Scorgie 1980; Sullivan et al. 1981) provide detailed information, but are commonly restricted to the most numerically dominant taxa and do not necessarily imply cause and effect (Dickman 1969). Secondary effects of the pollutant on interspecific competition may also be important (Leland & Carter 1984).

Patrick et al. (1954) described a technique in which a truncated normal curve of the number of taxa in a sample residing in pre-established abundance categories is generated.

The height of the curve mode, its variance and the number of categories represented by any given sample can be interpreted in light of the effect of pollution (Patrick 1978). Unfortunately, such curves can be subject to error in the event that the primary effect of the toxicant is a reduction in biomass of all constituent taxa, and Patrick (1978) has acknowledged that the index is best combined with an indicator of total biomass. A second, and perhaps more significant drawback of this method for use in routine and survey-level studies has been that reliable results depend on the enumeration of large numbers of cells (>5000) (Weitzel 1979).

As the initial impact of a toxicant may involve the selective elimination of sensitive taxa, diversity indices are often calculated using either the Shannon-Weiner (eg. Sigmon et al. 1977; Dickman & Gochnauer 1978; Anderson 1981; Weitzel & Bates 1981) or Brouillan (eg. Weitzel & Bates 1981; Leland & Carter 1984) formula. Such calculations are insensitive to toxicological effects, however, (Plumley & Davis 1980; Kosinski 1984; Leland & Carter 1984) if changes in specific proportions are less significant that total density changes.

The use of species commonality indices may allow more complete evaluation of the effects of a toxicant on the entire algal community. Resemblance coefficients (Orloci 1978) compare two samples (treatments, communities etc.) in

terms of their respective constituent species. A wide variety of such indices exist, and the reader is referred to more lengthy discussions (eg. Orloci 1978) of their merits. It is sufficient to note that individual indices inherently stress varying aspects of data (such as the relative and absolute densities of taxa) and vary in their capability of quantifying 'ecological differences'.

Examples of resemblance coefficients used to compare algal species composition in control and treatment samples include qualitative (presence/absence) (Mueller 1980 - Sorenson's Index) and quantitative similarity measurements. The latter include SIMI (a form of the Geodesic metric) (Plumley & Davis 1980) and the Bray-Curtis Percent Similarity Index (Leland & Carter 1984). Distance coefficients (often a measure of dissimilarity - the complement of the above) have also been used. Leland & Carter (1984) used the Canberra metric, while Putnam et al. (1981) calculated Euclidean distance for use in cluster analyses.

Multivariate analyses of species datasets have been used widely in terrestrial research and to a less extent in phycological research (eg. Eminson 1978). When represented graphically (eg. Kosinski 1984 - Principal Components Analysis), the physical location of data from each treatment in relation to all others may permit a summarization of the major effect of a pollutant on algal community structure.

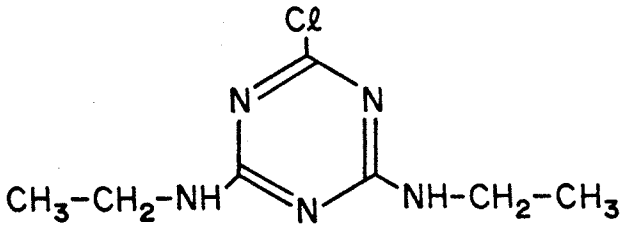
1.4 SIMAZINE AND TERBUTRYN TOXICOLOGY

Simazine and terbutryn are members of a group of organic herbicides collectively known as triazines, which consist of a central 6-membered triazine ring (alternating carbon and nitrogen atoms) with hydrocarbon chains on two of the constituent carbons (Figure 2). Simazine is further classified as a chloro-triazine due to the inclusion of a chlorine atom, while terbutryn has instead a methylthio group at the same position. Descriptions of the chemical and physical properties of each compound can be found in WSSA (1983). Both herbicides were conceived and are marketed world-wide by the Ciba-Geigy Corporation (Basle, Switzerland) in a variety of granular and powdered formulations (WSSA 1983). Simazine is currently registered in Canada for the control of terrestrial broadleaf weeds in orchard, garden and corn crops, and is one of ten herbicides recognized for aquatic use in Manitoba (Manitoba Agriculture 1985). Terbutryn has been considered for use as an aquatic herbicide (Hydamaka et al. 1977; Muir pers.comm.), but is presently not registered in Canada.

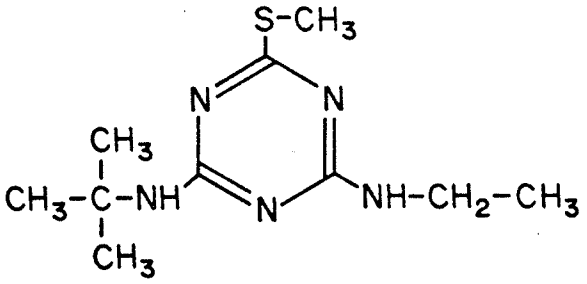
The primary mechanism of action of triazines on plant metabolism is the inhibition of photosystem II of photosynthetic electron transport (Moreland 1980). While the biochemical bases for herbicidal activity of many compounds is often unknown, studies using atrazine (a chloro-triazine closely related to simazine) -resistant plant biotypes have

Figure 2: Chemical structures of simazine (2-chloro-4,6-bis-ethylamino-s-triazine) and terbutryn (2-tert-butylamino-4-ethylamino-6-methylthio -s-triazine).

Simazine



Terbutryn



helped to elucidate the nature of triazine phytotoxicity. While it is known that triazines interfere with the transmittance of electrons from the Q to B acceptor molecules (Moreland 1980), contentions that the atrazine binding site is a 32kD protein (eg. Pfister et al. 1981) have been disputed (Gressel 1982). The basis by which members of the triazine group differ in their efficacy has not been determined, but likely relates to the binding affinity of the various sidechains to chloroplast proteins. Substituted methylthio-triazines are generally more phytotoxic than chloro-triazines (Souza-Machado et al. 1978; Radosevich et al. 1979) and phytotoxicity may be enhanced by the presence of assymetric alkylamino side groups (Radosevich et al. 1979).

Investigations using aquatic macrophytes and algae have confirmed that photosystem II activity in these organisms, as determined by oxygen evolution (eg. Zweig et al. 1963; Sutton et al. 1969; Lien et al. 1977), DCPIP dye reduction (Hendrich et al. 1976) and in vivo chlorophyll fluorescence (eg. Zweig et al. 1963), is correlated with simazine concentration. There has been comparatively little examination of the primary physiological effect of terbutryn, except to note that it is a potent inhibitor of the Hill reaction (Ciba-Geigy, unpubl.) and is generally more phytotoxic than similar levels of simazine (Johannes et al. 1973; Riemer & Trout 1980).

Secondary physiological effects of simazine (defined as any reponse other than blockage of electron flow) have included reductions in cellular RNA content of Chlorella pyrenoidosa and Anacystis nidulans at 0.2 and 2.0 $mg \cdot L^{-1}$ treatment levels (Hawxby & Mehta 1977; Mehta & Hawxby 1979), and changes in the lipid composition of Chlorella ellipsoidea at 5.0$mg \cdot L^{-1}$ simazine (Sumida & Ueda 1973). The latter may reflect a lack of precursors normally formed in the Hill reaction, mobilization of certain lipids following ATP depletion, or direct effects on the enzymes responsible for lipid synthesis (Sumida & Ueda 1973). Increases in nitrate reductase activity in Secale cereale (rye) at 0.01 and 0.16 $mg \cdot L^{-1}$ concentrations with concomitant increases in cellular protein content (Ries et al. 1967) may point to an effect of the herbicide on the rate of entry of nitrate into the cell, the movement of nitrite across the chloroplast membrane, or nitrite reductase activity in the chloroplast (Ries et al. 1967). Klepper (1979) observed a 12-fold increase in nitrite concentration in Triticum aestivum (wheat) leaf punchs without a corresponding increase in protein following exposure to 200 $mg \cdot L^{-1}$ atrazine, suggesting that these herbicides may interfere with nitrite reductase activity. The accumulation of nitrite (whether localized in the chloroplast or in the cytoplasm) would become toxic in high concentrations, possibly leading to membrane disruption. In addition, the diversion of electrons into fluorescence in response to herbicide treatment (eg. Zweig et al. 1963) sug-

gests that disruptive free radicals may be formed in the chloroplast. At concentrations of 3.0 and 5.0 $mg \cdot L^{-1}$ simazine, cessation of cell cyclosis of Elodea canadensis is followed by chloroplast migration to the cell center, loss of membrane integrity and eventual disintegration of the organelle and photosynthetic pigments (Dabydeen & Leavitt 1981). Thylakoid distortion and release of protein from polyhedral bodies in Anacystis nidulans cells exposed to 2.0 $mg \cdot L^{-1}$ simazine (Mehta & Hawxby 1979), and cell distortion coupled with significant increases in cell volume of Scenedesmus quadricauda exposed to 0.05 and 0.5 $mg \cdot L^{-1}$ (Hendrich et al. 1976) has been reported.

Sutton et al. (1971) demonstrated that a 1.0 $mg \cdot L^{-1}$ terbutryn addition stimulated the uptake of a 1.0 $mg \cdot L^{-1}$ copper solution by Hydrilla verticillata, although the biochemical basis for the interaction was not examined.

Significant algal accumulation of simazine has been implicated as an important biotic means of detoxification. Kruglov & Paromenskaya (1970) observed that cultures of Ankistrodesmus braunii and Chlorosarcina sp. assimilated 2-8% of the simazine added to a culture medium over a period of 20 days. Twenty-five to 74% of the absorbed simazine had been metabolized and bound into cellular protein, while the remainder was present as the intact molecule. Kruglov & Mikhailova (1975) discovered that 29% of simazine taken up by Chlorella vulgaris had been incorporated into protein and

that cleavage of the herbicide molecule sidechains was the primary method of metabolism. No degradation products were incorporated into pigments.

Herbicides may enter the aquatic environment in runoff from treated agricultural and commercial land, or through direct additions for water management practise. Although triazines are considered to be moderately persistent in soil (eg. Jury et al. 1984), losses due to runoff may occur if rainfall occurs soon after application. A maximum concentration of 0.3 $mg \cdot L^{-1}$ simazine has been detected in runoff occurring 2 weeks after applications of 2.24 $kg \cdot ha^{-1}$ (Wauchope & Leonard 1980) and 1.68 $kg \cdot ha^{-1}$ (Glotfelty et al. 1984), although the concentration in receiving waters would likely be lower as a result of dilution. In a stream monitoring program undertaken in Ontario, Frank et al. (1979) found that 28% of the 92 streams sampled contained simazine in concentrations exceeding 0.03 $ug \cdot L^{-1}$ (mean = 0.2 $ug \cdot L^{-1}$). Frank et al. (1982) later observed that simazine was one of three pesticides found in sampled water year-round and that annual losses arising from field use could be attributed to runoff and snowmelt (43%), and spills, drift and direct applications (56%).

Simazine has been used as a soil sterilant along irrigation canals, and contamination of intermittent flows has been reported. Smith et al. (1975) found that the herbicide concentration in water collected from a sprayed area de-

creased with each flow event, from a maximum of 0.69 $mg \cdot L^{-1}$ (8 months post-spray) to 0.04 $mg \cdot L^{-1}$ 2.5 years later, at which time 50% of the 22.4 $kg \cdot ha^{-1}$ treatment quantity remained in the soil of canal sides and bottoms. Following bank treatments of 2.25-7.43 $kg \cdot ha^{-1}$, Anderson et al. (1978) reported a maximum simazine concentration of 0.25 $mg \cdot L^{-1}$, while residual levels decreased by more than 70% within 8 km of the spray site.

Reviews of the use of herbicides in freshwater systems can be found in Mauck (1974), Brooker & Edwards (1973), Hurlbert (1975), Butler (1977) and Murphy (1982). Since the objective of aquatic applications of herbicides is usually the control of undesirable macrophytes and/or algae, concentrations resulting from such additions generally exceed those originating from incidental contamination. Simazine has been used widely in fish aquaculture and lake rejuvenation in the United States and Canada in concentrations ranging from 0.1 to 10.0 $mg \cdot L^{-1}$ (Table 4) while terbutryn has been used in Europe, Great Britain, South Africa and Australia at considerably lower levels (Table 5).

It is important to note that primary and secondary effects of triazines discussed earlier differ from definitions traditionally used by ecologists. In the latter case, primary effects (inhibition of photosynthetic organisms) may result in secondary changes in community structure of higher trophic levels and/or changes in water chemistry (Brooker & Edwards 1975; Hurlbert 1975).

TABLE 4

Summary of somes uses of simazine in aquatic management

Number preceding formulation indicates quantity (%) of active ingredient. W = wettable powder, G = granular.
? = not specified

Reference	Concentration ($mg \cdot L^{-1}$)	Expt. Duration	Formulation
Ahrens & Block 1972	1.9	summer	80W
Blackburn & Taylor 1976	0.5	4 mos	80W
Crawford 1981	4.2	6 mos	?
Ellis et al. 1976	0.25-0.5	variable	80W
Harman 1978	0.25	1 year	80W
Hydamaka et al. 1977	2.7-5.2.kg·pond^{-1}	ca. 2 mos	80W
Johannes et al. 1973	0.5-1.0	?	?
Mauck et al. 1976	0.1-3.0	2 years	80W
Patnaik & Ramachandran 1976	0.25-0.75	variable	50W
Pruss & Higgins 1975	0.1-0.3	60-79 d	80W
Schenk & Jarolimek 1966	1.0-2.0	summer	50W
Schwartz et al. 1981	0.45	2.5 years	80W
Snow 1963	2.0	1 year	80W
Snow 1964	11.3-17.0 kg·ha^{-1}	?	?
Sutton et al. 1965	0.5-3.0	28 d	80W
Tucker & Boyd 1978a	0.8	5 mos	80W
Tucker & Boyd 1978b	13.4 kg·ha^{-1} 1.5	6 mos 128 d	80W 80W
Tucker & Busch 1982	1.3	4 wk	?
Walker 1959	11.3 kg·ha^{-1}	4 wk	10G

Table 4 continued

Walker 1964	0.5-10.0	?	17G,50W,8G, 4G,20G,80W
Wile 1967	0.5-3.0	summer	50W

TABLE 5

Summary of some uses of terbutryn in aquatic management

1G = 1% granular formulation, 500FW = flowable powder, 50W = 50% wettable powder, ? = not specified.

Reference	Concentration ($mg \cdot L^{-1}$)	Expt. Duration	Formulation
Ashton et al. 1980	0.015-0.10	variable	500FW
Bowmer et al. 1979	1.0	?	500FW,1G
Elamzon 1977	0.03-0.1	?	1G
Hydamaka et al. 1977	0.18-0.28 $kg \cdot pond^{-1}$	ca. 2 mos	1G
Johannes et al. 1973	0.005-0.1	?	?
Marks 1974	0.05-0.1	6-8 wk	50W,1G
Mackenzie et al. 1983	0.05-0.4	variable	500FW,1G
Murphy et al. 1981	0.015-0.10	variable	1G
van der Weij et al. 1971	0.0125-0.10	ca. 2 mos	1G

Primary effects of simazine on aquatic systems have often been assessed qualitatively in terms of 'degree of control', with little quantitative substantiating evidence (eg. Schenk & Jarolimek 1966) or comparison with an unmanipulated control system. Moreover, it has been a common practise to classify algal taxa according to their response to treatments (eg. Ellis et al. 1976; Murphy 1982). Because no allowance is made by this approach for intraspecific variation, such crude classifications may be of limited use. Vance & Smith (1969), for example, found that growth of Chlorella pyrenoidosa was not inhibited by simazine concentrations as high as 200 $mg \cdot L^{-1}$, whereas Wells & Chappell (1965) observed total inhibition of growth of a different isolate of the same species at 1.0 $mg \cdot L^{-1}$. Senger (1977) found that the sensitivity of synchronous cultures of Scenedesmus and Chlorella varied with the physiological condition of the cell and was lowest immediately prior to cell division. Oxygen evolution of a Chlorella culture decreased by 50% at 0.5 $mg \cdot L^{-1}$ simazine during active growth but only 35% at 1.0 $mg \cdot L^{-1}$ at a later stage in the growth cycle. While the significance of this latter effect may be small in a natural population of individuals of varying phases of growth, it illustrates the potential complexity in the assessment of specific phytotoxicity.

Reductions in algal standing crop, either qualitatively or quantitatively assessed, are commonly observed following

simazine(eg. Tucker & Boyd 1978a; Schwartz et al. 1981) and terbutryn (Ashton et al. 1980) treatment. Also seen, but less well documented, are changes in phytoplankton community structure. Blue-green algal populations, notably those of the common bloom-forming genera of Anabaena, Aphanizomenon and Microcystis, are often inhibited disproportionately to other algal groups by simazine concentrations as low as 0.25 $mg \cdot L^{-1}$ (eg. Blackburn & Taylor 1976; Ellis et al. 1976), while increases in the abundance of other taxa may occur. Schwartz et al. (1981) found that Aphanizomenon flos-aquae and Anabaena circinalis blooms were succeeded by centric diatoms (primarily Stephanodiscus) and cryptomonads. Incidence of the latter appeared to coincide with elevated dissolved organic carbon levels, and the authors speculated that these populations could employ facultative heterotrophy as a primary means of nutrition. Phagotrophy has been reported in a natural population of Cryptomonas borealis (Wawrik 1970).

Elimination of blooms of Microcystis aeruginosa and Anabaena spiroides from fish ponds using 0.26-0.75 $mg \cdot L^{-1}$ simazine treatments was found to lead to increases in diverse genera such as Oscillatoria, Chlamydomonas, Scenedesmus, Euglena, Phacus, Synedra and Navicula (Patnaik & Ramachandran 1976), while Bryfogle & McDiffett (1979) observed that Chlorella sp. dominated the phytoplankton in laboratory microcosms in the presence of 0.05-0.4 $mg \cdot L^{-1}$ simazine.

There is very little evidence to determine whether simazine additions ultimately lead to the development of a resistant community. deNoyelles & Kettle (1983) reported that increases in *in vivo* chlorophyll fluorescence of phytoplankton from simazine-treated ponds (upon further herbicide additions) were of lesser magnitude than for samples from untreated ponds, implying that a physiologically resistant assemblage had developed. Moreover, certain taxa were found to be characteristic of treated ponds (deNoyelles & Kettle 1983).

Evaluations of the effect of terbutryn on algal biomass have been largely subjective (eg. Ashton et al. 1980), or have involved laboratory, unialgal cultures (van der Weij 1971; Robson et al. 1976; Lefebvre-Drouet & Calvet 1978). 'Control' of macroscopic populations of *Cladophora* sp., *Enteromorpha intestinalis*, *Rhizoclonium* spp. and *Vaucheria dichotoma* by 0.05 and 0.1 $mg \cdot L^{-1}$ treatments has been reported (Marks 1974).

The toxicological effects of atrazine on natural phytoplankton and periphyton communities, as determined by manipulative experiments, are likely analagous to those of simazine due to the close structural similarity of the two compounds (atrazine is differentiated by an isopropylamino sidechain on the triazine ring). deNoyelles et al. (1982) reported that phytoplankton productivity in man-made ponds was reduced by additions of 0.02 and 0.5 $mg \cdot L^{-1}$ atrazine for

periods ranging from 3 days (0.02) to 63 days (0.5). Increases in resistance to atrazine, and qualitative changes in phytoplankton community structure were detected, with species of Cryptomonas and Mallomonas becoming dominant at both herbicide levels. Kosinski & Merckle (1984) observed that the productivity of lotic periphyton communities exposed to 1.0 $mg \cdot kg^{-1}$ atrazine returned to that of a control community within 1-3 weeks, while a 10 $mg \cdot kg^{-1}$ treatment resulted in high levels of inhibition throughout the 3-week duration of the experiment. Notably sensitive to herbicide were populations of Phormidium spp., Rhopalodia spp., Amphora veneta, Achnanthes lanceolata and Cladophora glomerata. Periphyton exposed to a 0.01 $mg \cdot kg^{-1}$ chronic atrazine dose did not develop any resistance to the herbicide nor did any taxon proliferate in herbicide-treated systems (Kosinski 1984). Plumley & Davis (1980) determined that 10^{-5} M (ca. 2.1 $mg \cdot L^{-1}$) atrazine significantly reduced both photosynthetic activity and biomass (chlorophyll concentration) of epipelic algae in estuarine microcosms, although Cunningham et al. (1984) found only slight reductions in epiphytic and epipelic productivity in laboratory microcosms treated with 0.12 and 1.23 $mg \cdot L^{-1}$ atrazine.

Secondary effects of simazine and terbutryn on water quality have been well documented. These include decreases in dissolved oxygen (van der Weij 1971; Marks 1974; Patnaik & Ramachandran 1976; Tucker & Boyd 1978a; Wingfield & John-

son 1981; Murphy et al. 1981; Schwartz et al. 1981), redox potential (Wingfield & Johnson 1981) and pH (Sutton et al. 1965; Pruss & Higgins 1975; Ellis et al. 1976; Schwartz et al. 1981) and increases in biochemical oxygen demand (Murphy et al. 1981), free CO_2 and carbonate (Crawford 1981; Murphy et al. 1981), calcium and magnesium (Walker 1964), potassium (Walker 1964; Ashton et al. 1980), sodium (Ashton et al. 1980), organic nitrogen (Walker 1964), total nitrogen (Walker 1964; Ashton et al. 1980), ammonia (Blackburn & Taylor 1976; Murphy et al. 1981; Tucker & Busch 1982), nitrate and orthophosphate (Walker 1964) and total phosphorus (Ashton et al. 1980). Explanations for these phenomena usually invoke decreases in photosynthetic oxygen evolution (eg. Tucker & Boyd 1978a), decreased nutrient assimilation and release of labile nutrient pools from decaying plants.

Chapter II

EFFECT OF SIMAZINE AND TERBUTRYN ON PERIPHYTON PRODUCTIVITY

2.1 INTRODUCTION

Simazine (2-chloro-4,6-bis-ethylamino-s-triazine) has been widely used in lake management and fish culture for the control of undesireable macrophytic species (Walker 1964; Sutton et al. 1965; Wile 1967; Bowmer et al. 1979) and nuisance phytoplankton blooms (Blackburn & Taylor 1976; Tucker & Boyd 1978a). As a result, much is known of its efficacy and toxicology in the aquatic ecosystem (Mauck 1974). Terbutryn (2-tert-butylamino -4-ethylamino-6-methylthio -s-triazine) has recently been demonstrated to possess stronger algicidal properties than similar levels of other triazines (van der Weij 1971; Johannes et al. 1973; Lefebvre-Drouet & Calvet 1978). Registration of the latter compound for aquatic applications has been granted in some countries and considered in others, although additional evaluations of phytotoxic effects are needed.

A shortcoming of the literature dealing with effects of triazines and other herbicides on aquatic vegetation is that rarely has impact been assessed in anything but qualitative terms (for example, Schenk & Jarolimek 1966; Marks 1974; El-

lis et al. 1976; Robson et al. 1976; Elamzon 1977; Crawford 1981). Resultant plant biomass and productivity have been quantified in relatively few cases. Moreover, while field experiments have often involved treatment of shallow, unstratified ponds or ditches (Schenk & Jarolimek 1966; Ahrens & Block 1972; Pruss & Higgins 1975; Blackburn & Taylor 1976; Tucker & Boyd 1978a; Scorgie 1980; Murphy et al. 1981), few observations have been made of effects on periphyton (Scorgie 1980; Anderson 1981; Crawford 1981), even though this community may account for the largest portion of total primary production in littoral environments (Wetzel 1983). Further, while information on community recovery from a herbicide application clearly would contribute to both management and toxicological knowledge, successional events in a plant community following triazine treatment have been examined only superficially in terms of qualitative community composition (Pruss & Higgins 1975; Scorgie 1980; Crawford 1981) rather than in terms of community productivity.

The present study (experiment B, Appendix A) was initiated to examine some effects of the triazines simazine and terbutryn on dense periphyton communities in a shallow marsh environment over an extended period of time. Incidental abrupt loss of herbicide during the experiment allowed documentation of the rate of recovery of structure and function of communities. Concomitant effects on selected chemical and physical parameters of marsh water were also examined as

they may relate to the given community's response to herbicide stress.

2.2 MATERIALS AND METHODS

Experiments were conducted in the Blind Channel of the Delta Marsh on the southern end of Lake Manitoba (99° 19'W, 50° 7'N). Seven littoral enclosures (constructed by encircling 240cm x 120cm sheets of 1.5mm PVC plastic on their long axis and cementing the ends together with adhesive - Figure 3A) were placed into water of approximately 60cm depth and embedded into sediments to a depth of 45cm (mean volume = 300 L, $A/V = 5.15\ m^{-1}$).

Substrata for periphytic algal colonization consisted of 1m length and 0.62cm diameter extruded acrylic rods, scored at regular intervals with a small saw to facilitate subsampling. A number of rods were placed upright into the sediments of each enclosure in such a way that each was firmly anchored and extended from sediment surface to the water surface. Substrata were roughly equidistant from each other and no closer than 10cm from enclosure walls. Submerged macrophytes (chiefly Potamogeton spp, Myriophyllum exalbescens Fern. and Utricularia vulgaris L.) were removed from all enclosures. Since the composition of macrophytes within each enclosure was variable, removal served to minimize variable shading and abrasion of rods by plants, and to eliminate variable herbicide losses due to macrophyte uptake and effects of decay products on periphyton growth.

Figure 3: Enclosure Designs. A - Diagram of typical PVC enclosure showing handle holes (a) used in placement and removal. These holes are plugged with silicone rubber when the enclosure is in use.
A[1] - View of encircled PVC sheet prior to addition of cement showing support strips cemented to both sides of one end.
B - Diagram of modified sealed bottom enclosure showing PVC square and attached acrylic sockets used as bases for artificial substrata (b), drain holes (c), gas vents (d) and PVC floor (e) (Chapter 5).

Unformulated technical grade herbicides (98% a.i.) were dispensed into gauze sacs in quantities to yield final concentrations of 0.1, 1.0 and 5.0mg·L^{-1} simazine and 0.01, 0.1 and 1.0mg·L^{-1} terbutryn in the approximately 300L enclosure volume. Sacs were suspended in the water of individual enclosures soon after rod placement and were squeezed at regular intervals in the first 2 days of the experiment to insure complete and rapid solution of herbicide. A seventh enclosure was maintained as an untreated control.

Sampling of colonized substrata commenced 9 days after herbicide addition (week 1) and at weekly intervals for the following 5 weeks. On each sampling date, three entire rods were randomly selected from each enclosure for analysis of periphyton chlorophyll a content. Colonized portions of rods were broken into segments with pliers and placed into tubes of GF/C (Whatman) filtered water. Three additional rods were sampled from each enclosure and a 2cm segment was removed from the position on each corresponding to 30cm above the sediments. These were placed in tubes containing 20mL triple-filtered water (twice through GF/C, once through 0.45um Sartorius cellulose nitrate membrane filters) from the enclosures of substratum origin. These segments were used in CO_2-assimilation experiments.

Periphyton on rods sampled for chlorophyll was detached using a No.2 hard-bristled paintbrush into filtered water. Aliquots of the algal suspension were collected onto GF/C

filters under vacuum (<100kPa) and frozen until analysis. Pigments were extracted into 90% acetone with the use of a tissue grinder and chlorophyll a concentration measured trichromatically (Strickland & Parsons 1972) with correction for phaeopigments. Extrapolation to whole sample volume and division by total rod surface area gave estimates of mean chlorophyll a per unit substratum area. Samples for CO_2 assimilation rate determination were inoculated with 1mL standardized $NaH^{14}CO_3$ ($0.5uCi \cdot mL^{-1}$) and incubated in a laboratory growth chamber at a light intensity of approximately $50uE \cdot m^{-2} \cdot s^{-1}$ and a temperature of 20 ± 2°C for three to four hours. Samples were filtered through 0.45um filters to collect dislodged cells, and postwashed with deionized water. Filters and corresponding rod segments were fumed over concentrated HCl for one minute to remove residual inorganic ^{14}C and placed into scintillation vials containing 10mL Bray's solution (New England Nuclear). Within 24 hours, both filter and rod had dissolved completely. Sample radioactivity was determined by scintillation counting with a Picker Liquimat 220 counter and corrected for colour quenching using the channels ratio method (Wang & Willis 1965). Assimilation rate of periphyton per unit rod surface area was calculated from the formula:

$$ugC\ fixed \cdot cm^{-2} \cdot hr^{-1} = \frac{DPM(S) \times C \times 1.05}{DPM(T) \times A \times T}$$

where DPM(S) and DPM(T) are activities remaining after incubation and total added (in dpm) respectively, C is dissolved inorganic carbon present in enclosure water derived from alkalinity (APHA 1980) ($mgC \cdot 20mL^{-1}$), 1.05 is a discrimination factor to correct for uptake of ^{14}C, A is rod surface area (cm^2) and T is incubation time (hours).

Water in each enclosure was sampled daily from a depth of 10cm below the water surface and analyzed for dissolved silicon and ammonia (Stainton et al. 1977). The concentration of each herbicide in the water of treated enclosures was determined weekly using ultraviolet spectrophotometry (Mattson et al. 1970). Dissolved oxygen at 10cm depth was monitored daily using a YSI model 51B oxygen meter. Depth profiles of oxygen and temperature were also recorded weekly. Light extinction in the enclosures was measured weekly with an LI-185A Li-cor light meter with a LI-192S submersible quantum sensor.

2.3 RESULTS

Data for several physical parameters measured in the enclosures are given in Table 6. With the exception of oxygen gradient (which was steepest in the control enclosure and decreased in proportion to increasing herbicide concentration), no differences were detected which were directly related to herbicide treatment. Analysis of variance of all other variables indicated that temporal variability was sig-

nificant but spatial variability (between enclosures) was not (Table 7).

Assay for simazine in the enclosures 5 days after treatment indicated that for the two lower concentrations, actual levels were equal to or somewhat higher than the theoretical levels (Figure 4A). Actual concentration in the enclosure treated with the highest simazine level was considerably less than $5.0 mg \cdot L^{-1}$, but this was expected on the basis of a maximum water solubility of $3.5 mg \cdot L^{-1}$ at 20^{0} C for the herbicide (WSSA 1983). In the first two and a half weeks of the experiment, simazine levels in the three enclosures remained near the initial concentration. A slight decrease was noted at 0.1 and $1.0 mg \cdot L^{-1}$, indicating that loss of active herbicide in the water column was occurring. Increase in simazine at the highest application concentration over time probably indicated dissolution and/or sediment desorption of herbicide towards maximum solubility.

All initial concentrations of terbutryn were somewhat less than their theoretical maxima (Figure 4B). Maximum terbutryn solubility in water is $25.0 mg \cdot L^{-1}$ at $20^{o}C$ (WSSA 1983), so minimal residual sediment adsorption was expected. Slight decreases in concentration over time were seen at all treatment levels. Terbutryn was detectable at the lowest level ($0.01 mg \cdot L^{-1}$) only on the first sampling date. Losses resulting from degradation, biotic absorption and/or sediment adsorption decreased the concentration below the $0.01 mg \cdot L^{-1}$ level of detection.

TABLE 6

Selected physical and chemical parameters

(range and mean) measured in seven experimental enclosures over the 6-week duration of the experiment.

Time (weeks)	Light Intensity ($uE.m^{-2}.s^{-1}$)	Water Temp. (^{o}C)	Water Depth (cm)	Light Extinction ($-n.10^{2}.cm^{-1}$)	Dissolved Oxygen Gradient ($mg.L^{-1}.cm^{-1}$)
1	518-623 (565)	24.6-24.9 (24.7)	68	1.27-1.85 (1.49)	0-2.53 (1.83)
2	73-143 (87)	19.9-20.0 (20.0)	67	1.83-3.04 (2.44)	0.07-2.81 (1.22)
3	448-460 (454)	18.1-18.4 (18.2)	86	1.86-2.11 (1.95)	0.07-3.10 (1.04)
4	320-444 (405)	21.4-21.6 (21.5)	62	1.57-6.79 (4.06)	0.43-2.53 (1.05)
5	220-396 (323)	18.5-18.7 (18.6)	65	1.49-6.56 (4.10)	0.27-2.71 (1.40)
6	51-72 (64)	14.0-14.1 (14.0)	61	2.10-2.30 (2.28)	0-1.35 (0.81)

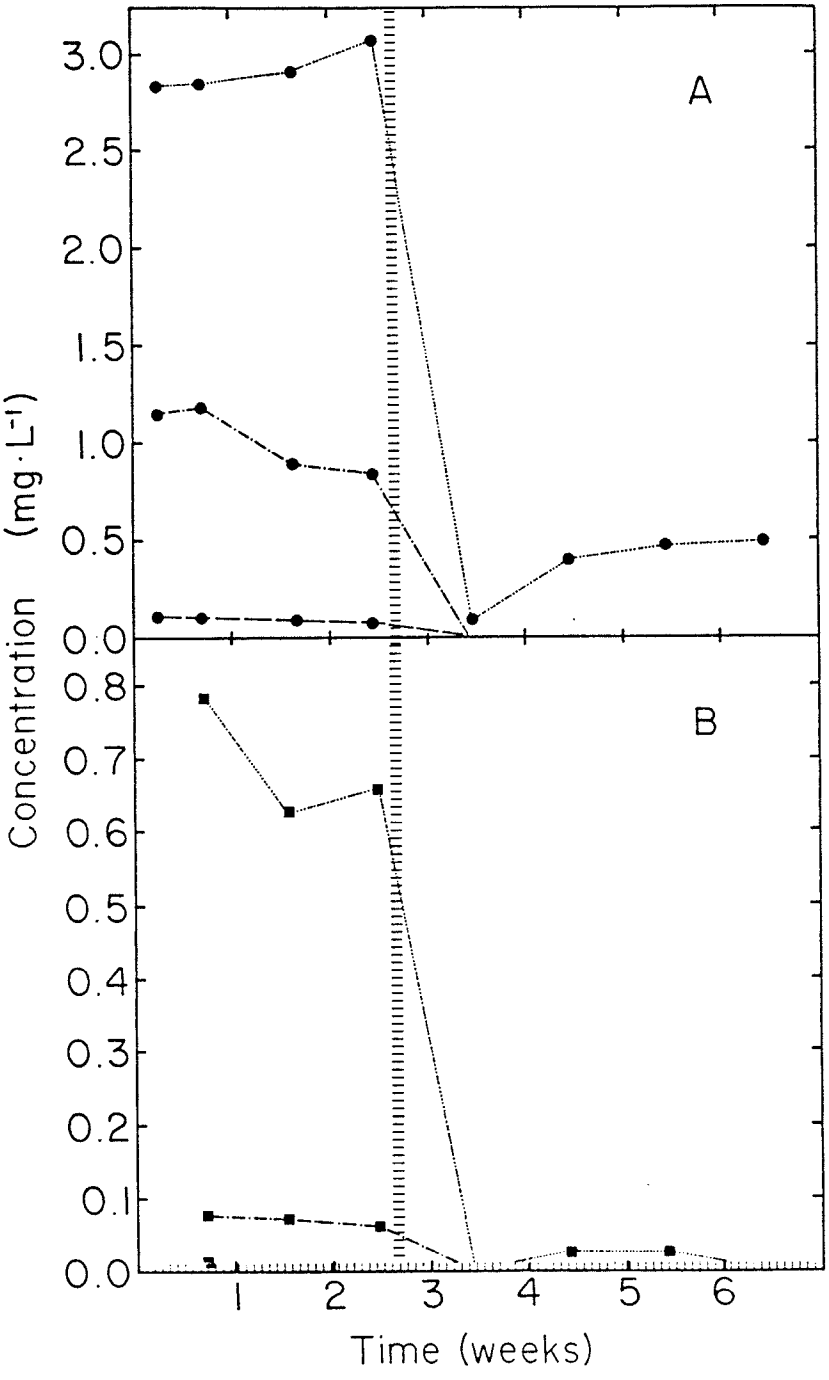
TABLE 7

Spatial and temporal variability in physico-chemical parameters

in enclosures. Two-way analysis of variance of spatial (between enclosures) and temporal (between weeks) variability in physical and chemical parameters measured in the experimental enclosures over the 6-week period of the experiment. F-ratios and p-values (in parentheses) are given.

Variable	Spatial (df = 6,30)	Temporal (df = 5,30)
Mean light intensity	0.12 (>0.5)	141.34 (<0.001)
Water temperature	1.35 (0.30)	12152.82 (<0.001)
Mean enclosure depth	1.00 (0.40)	12318.93 (<0.001)
Light extinction rate	2.22 (0.07)	7.14 (<0.001)
Oxygen gradient	3.88 (0.006)	1.00 (0.40)

Figure 4: Assayed herbicide concentrations over the 6-week period of the experiment in enclosures treated with:
A - 0.1 (●---●), 1.0 (●-·-●), and 5.0 (●···●) $mg \cdot L^{-1}$ simazine.
B - 0.01 (■---■), 0.1 (■-·-■), and 1.0 (■···■) $mg \cdot L^{-1}$ terbutryn.
Vertical bar indicates the approximate period of enclosure flooding. Horizontal bar in B indicates the limit of detection.


A
B
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Concentration (mg·L⁻¹)
1
2
3
4
5
6
Time (weeks)

On the 18th day of the experiment, a strong north wind initiated a set-up on Lake Manitoba, which connects by way of channels with the marsh. Influent lakewater caused the marsh level to rise 30cm within 24 hours. For the following 30 hour period, all enclosures were immersed under approximately 6cm of water, which was sufficient to flush most of the herbicides from the enclosures. Assays conducted the following day and 1 week later confirmed that the quantities remaining in enclosures treated with 0.1 and 1.0mg·L^{-1} simazine, and all levels of terbutryn were below the 0.01mg·L^{-1} limit of detection (Figure 4). The occurrence of this flooding of enclosures permitted examination of recovery of communities following short-term herbicide treatment from all but the highest rates of each herbicide.

Simazine remained detectable in the enclosure treated with 5.0mg·L^{-1} and increased to a maximum concentration of 0.48mg·L^{-1} within 3 weeks of flooding. This herbicide probably arose from re-equilibration between adsorbed simazine in the sediments within the enclosures and overlying water, with the result that simazine was released into solution. A similar increase to detectable levels was observed at the highest terbutryn level (Figure 4).

Chlorophyll *a* from the periphyton on rods in the control enclosure increased between sampling dates throughout the experiment (Figure 5) with the greatest rate of increase in the first 2 weeks of colonization. Accumulation was slower

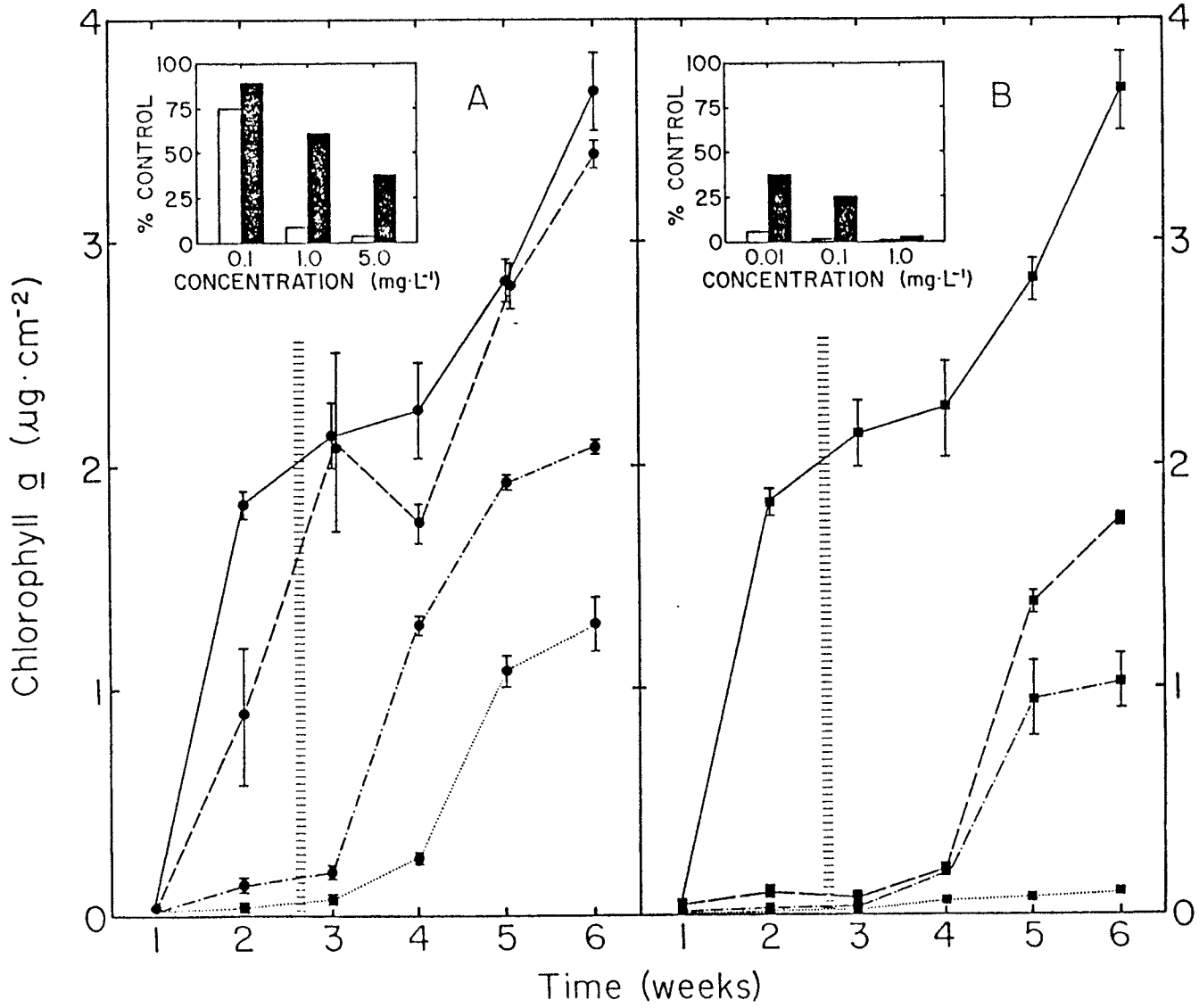
in the subsequent 2 weeks but following flooding (just prior to the third week of sampling), there was a marked increase.

At the lowest level of simazine tested, the mean chlorophyll a level was slightly less than the control at all dates (Figure 5A) but the difference was not significant ($F_{1,32}=0.88$; $p=0.40$). Following enclosure flooding, there was a reduction in chlorophyll on week 4. This may relate to the algal species composition, which consisted largely of dense, loosely attached filamentous green algae. Visual observations indicated that a certain quantity of this material was lost after flooding. Loss of similar biomass in the control may explain its slow rate of chlorophyll accumulation in this period (Figure 5).

Prior to flooding, mean chlorophyll a level at $1.0 mg \cdot L^{-1}$ simazine was approximately 10% of the control and still less (about 4%) at $5.0 mg \cdot L^{-1}$ (Figure 5A). Interpolation from these data suggests that the LC_{50} (concentration of herbicide giving 50% inhibition) of chlorophyll synthesis by the marsh periphyton must lie between 0.1 and $1.0 mg \cdot L^{-1}$ simazine. The increase in chlorophyll concentration at the highest level of herbicide tested indicates that community inhibition was not complete at this level.

Increase in periphyton chlorophyll a resulted from simazine loss due to flooding of the enclosures (Figure 5A). Rates of increase in chlorophyll a at all three herbicide

Figure 5: Periphyton chlorophyll a levels over the 6-week period of the experiment:
A - In the control enclosure (●---●) and in the enclosures treated with 0.1 (●---●), 1.0 (●-·-●), and 5.0 (●···●) $mg \cdot L^{-1}$ simazine.
B - In the control enclosure (■---■) and in the enclosures treated with 0.01 (■---■), 0.1 (■-·-■), and 1.0 (■···■) $mg \cdot L^{-1}$ terbutryn.
Insets: Mean preflood (open-bar) and postflood (shaded bar) chlorophyll a in each enclosure relative to the untreated control. Error bars are the SE of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.


Chlorophyll a (µg·cm⁻²)
Time (weeks)
A
B
% CONTROL
CONCENTRATION (mg·L⁻¹)
0.1
1.0
5.0
0.01

levels were similar to that of the control over the remaining 3 weeks of observation. The increase in simazine detectable in the highest level enclosure (Figure 4A), however, may have been related to the reduction in chlorophyll a accumulation rate in this enclosure with time. A similar increase at the intermediate herbicide level, though undetectable at the $0.01 mg \cdot L^{-1}$ limit of the methodology, may explain that enclosure's corresponding rate decline as compared to the control.

All levels of terbutryn resulted in greater than 90% reduction in chlorophyll a levels at the other two concentrations ($F_{1,32}=0.06$, $p>0.5$), level (Figure 5B). Chlorophyll a level at the lowest terbutryn concentration tested ($0.01 mg \cdot L^{-1}$) was not significantly different from chlorophyll a nor were differences in chlorophyll a between each of the higher concentrations significant ($F_{1,32}=0.00$, $p>0.5$). These data suggest that the LC_{50} for terbutryn lies below $0.01 mg \cdot L^{-1}$ and thus that terbutryn is at least ten times more inhibitory of chlorophyll a production in periphyton than simazine.

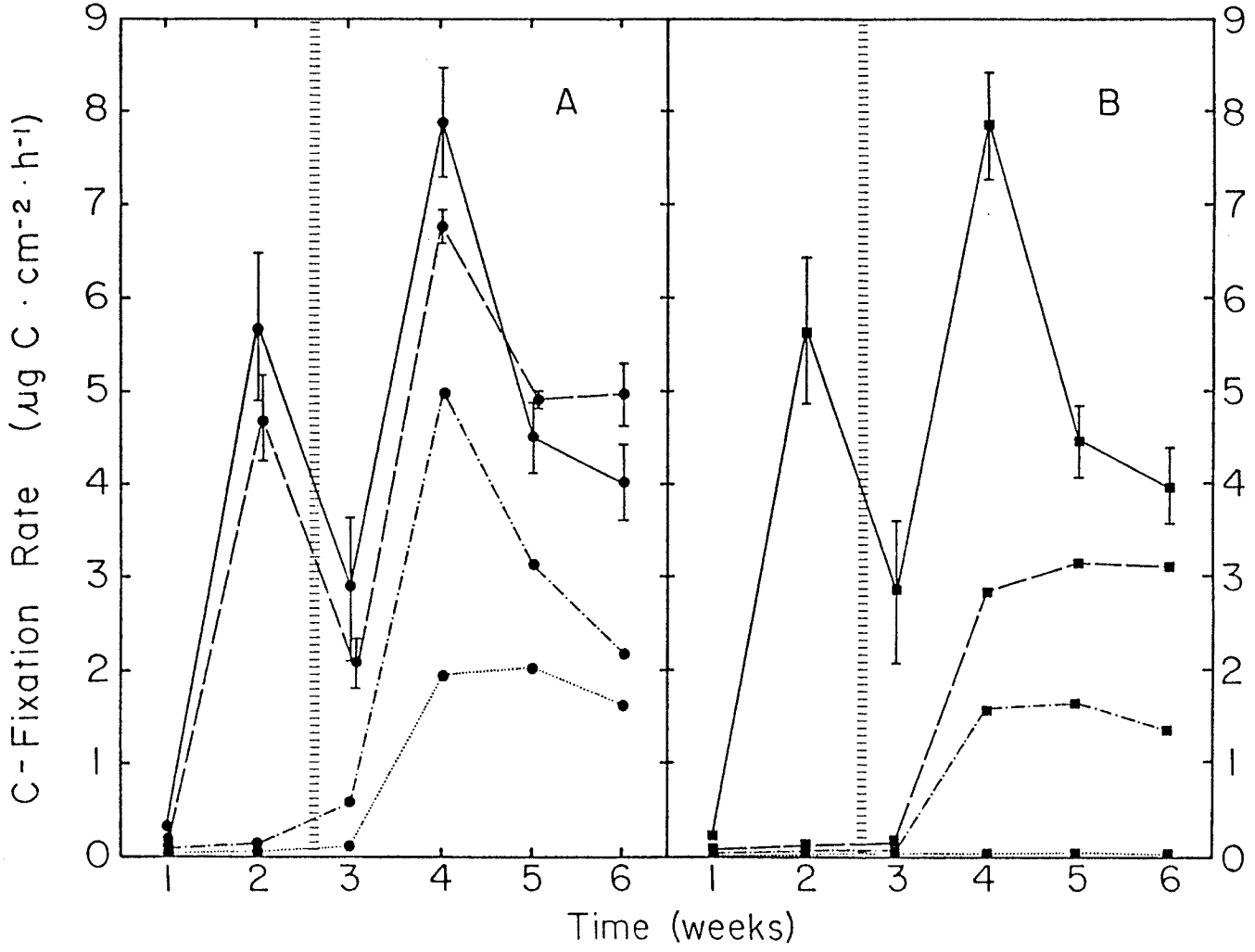
Following loss of herbicide, rapid accumulation of chlorophyll a commenced within a week at the two lower terbutryn levels (Figure 5B) with at rates exceeding that of the control. The increases were proportional to the relative herbicide exposure level and were characterized by a reduction in rate during the last weeks which was similar to that

found for simazine-treated enclosures (Figure 5A). The increase in terbutryn concentration to as high as 0.026mg·L^{-1} (Figure 4B) on week 5 in the enclosure treated with 1.0mg·L^{-1} corresponds with only slight increases in chlorophyll a following the flood. Similar concentrations in the 0.01mg·L^{-1} preflood enclosure were sufficient to inhibit chlorophyll a production to at least 10% of the control.

Photosynthetic activity of the control enclosure increased in the initial 2 weeks at a much greater rate than at 1.0 and 5.0mg·L^{-1} simazine, but was not significantly different from the 0.1mg·L^{-1} enclosure (Figure 6A). Consistent with the reduced rate of increase in chlorophyll a concentration observed in control and 0.1mg·L^{-1} enclosures following flooding, photosynthetic rates of both declined significantly (>50%) in the week following flooding. This may have related to the loss of a portion of the green algal periphytic component. Since light extinction (Table 6) was similar on the second and third sampling periods, no effect of turbidity change on photosynthesis seems likely.

Following the reduction in herbicide concentrations, marked stimulation of photosynthetic rate was observed at 1.0mg·L^{-1} preflood level. The rate of increase between weeks 3 and 4 in this enclosure paralleled that of the control and 0.1mg·L^{-1} enclosures. Photosynthetic recovery at 5.0mg·L^{-1} was less than in other enclosures but was still 300 times the preflood rate. Photosynthetic rate at all

Figure 6: Photosynthetic rates of periphyton over the 6-week period of the experiment:
A - In the control enclosure (●---●) and in enclosures treated with 0.1 (●---●), 1.0 (●-·-●), and 5.0 (●···●) $mg \cdot L^{-1}$ simazine.
B - In the control enclosure (■---■) and in enclosures treated with 0.01 (■---■), 0.1 (■-·-■) and 1.0 (■···■) $mg \cdot L^{-1}$ terbutryn.
Error bars are the SE of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.


C-Fixation Rate (µg C · cm⁻² · h⁻¹)
A
B
Time (weeks)
0
1
2
3
4
5
6
7
8
9

levels of simazine and the control peaked at week 4 and decreased at both subsequent dates. At higher herbicide levels, this was apparently manifested as slowed rates of chlorophyll *a* accumulation between weeks 5 and 6 (Figure 5A) but similar trends were not observed at either the lowest concentration or in the control.

Mean preflood photosynthetic rate of periphyton exposed to all levels of terbutryn (Figure 6B) was significantly less than the control ($F_{1,32}=32.09$, $p<0.001$). At the initial sampling period (week 1), photosynthetic rate at $0.01 mg \cdot L^{-1}$ was approximately 50% of the control but this was not maintained at subsequent dates where the former rate remained approximately constant.

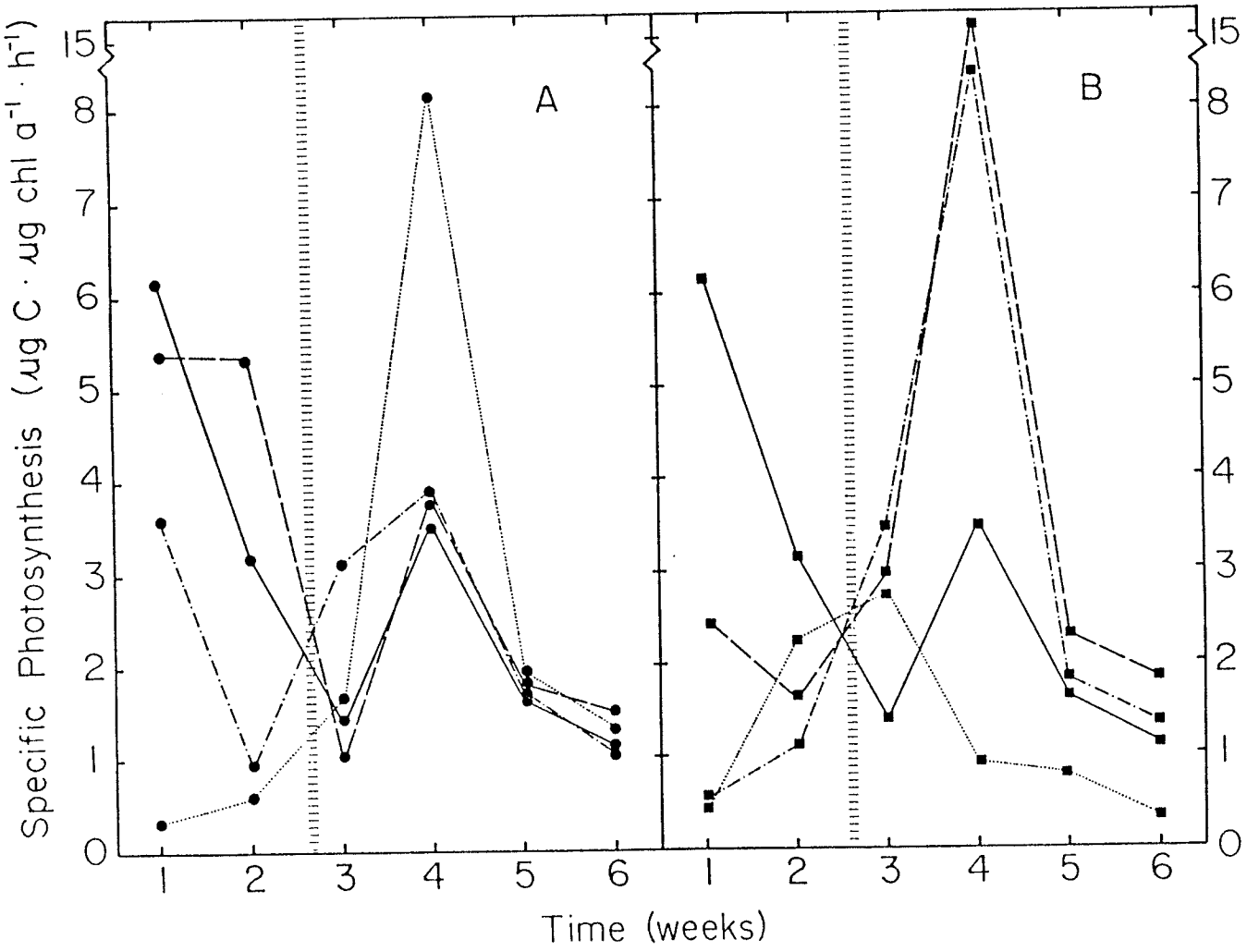
As in simazine-treated enclosures, the photosynthetic rate of the periphyton increased to 40% and 20% of the control at the lower two terbutryn levels respectively (Figure 6B) in the week after herbicide loss. The rate stabilized ($0.01 mg \cdot L^{-1}$) or decreased slightly ($0.1 mg \cdot L^{-1}$) in the subsequent 2 weeks, which corresponded with the decrease in chlorophyll *a* accumulation (Figure 5B). At $1.0 mg \cdot L^{-1}$, almost total inhibition of photosynthetic activity was observed throughout the remainder of the experiment, corresponding to residual herbicide concentrations (Figure 4B) sufficient to inhibit chlorophyll *a* synthesis (Figure 5B).

Specific productivity (carbon fixed per unit of chlorophyll) may further elucidate herbicidal effects (Figure 7). In both the control and 0.1mg·L^{-1} simazine enclosures, chlorophyll *a* increased proportionally more than photosynthetic rate in the first 2 weeks, with the result that specific productivity (SP) decreased in this period (Figure 7A). Following flooding, a short increase in SP (week 4) was followed by continued reduction in both treatments.

Specific productivity at the two higher simazine concentrations was initially a function of increasing herbicide level, although a net decrease was also observed at 1.0mg·L^{-1} (Figure 7A). Immediately following loss of herbicide (week 3), however, SP in the 1.0mg·L^{-1} enclosure (week 3) and subsequently the 5.0mg·L^{-1} enclosure (week 4) greatly exceeded (by as much as 200%) that of the control. This stimulation was not expected in light of similar rates of increase in chlorophyll *a* synthesis and photosynthetic activity to those of the control (Figures 5A and 6A) in the post-flooding period. Moreover, this period of stimulation was relatively short, with efficiency dropping to near the control by week 4 (1.0mg·L^{-1}) or week 5 (5.0mg·L^{-1}).

Preflood SP of terbutryn-treated enclosures was substantially less than the control for all concentrations (Figure 7B). Stimulation (>100% control) was observed directly following flooding at all three herbicide levels (week 3). In the case of the lower concentrations, high photosynthetic

Figure 7: Specific photosynthetic rate of periphyton over the 6-week period of the experiment:
A - In the control enclosure (●---●) and in enclosures treated with 0.1 (●---●), 1.0 (●-·-●) and 5.0 (●···●) mg·L^{-1} simazine.
B - In the control enclosure (■---■) and in enclosures treated with 0.01 (■---■), 0.1 (■-·-■) and 1.0 (■···■) mg·L^{-1} terbutryn.
Vertical bars indicate the approximate period of enclosure flooding.


Specific Photosynthesis (µg C · µg chl a⁻¹ · h⁻¹)
A
B
Time (weeks)
0
1
2
3
4
5
6
7
8
15

efficiency continued to week 4 but decreased to slightly greater than the control by week 5 (2 weeks postflood). Specific productivity in the 1.0mg·L^{-1} enclosure decreased to less than the control on all subsequent sampling dates, which corresponded with the subsequent increase of terbutryn to 0.026mg·L^{-1} in that enclosure (Figure 4B).

Ammonia concentration in enclosures treated with either herbicide was higher (Figures 8 and 9) than in surrounding water, where levels were consistently less than 0.1mg·L^{-1} throughout the experiment (data not shown). A portion of this increase may not be attributable to herbicide treatment, since the concentration in the control enclosure increased substantially in the first ten days. It is suspected that removal of macrophytic vegetation three days prior to the the beginning of the experiment disturbed the anoxic marsh sediments so that large quantities of interstitial ammonia were released. Initial enclosure placement had no effect on sediment disturbance, since ammonia levels following installation (but before macrophyte removal) were the same as levels found prior to placement. In the control enclosure, this ammonia disappeared (either through volatilization, oxidation, or biotic uptake) within 15 days (Figure 8). Concentrations at 0.1mg·L^{-1} simazine peaked at a similar level but decreased at a slower rate. At higher simazine concentrations, ammonia varied with increasing herbicide so that the amount of ammonia was much higher in these

enclosures than in the control. High levels were maintained until the enclosures flooded, when the enclosed water was replaced. Following flooding, ammonia levels in the control, 0.1, and 1.0mg·L^{-1} simazine enclosures were approximately the same throughout the remainder of the experiment, although the 5.0mg·L^{-1} enclosure increased within 20 days to near pre-flood concentrations. Thus, ammonia level appears to correlate closely with initial simazine concentration. It is unlikely that nitrogen in the simazine molecule interferred with ammonia determination, due to the high specificity of the colorimetric method used (Solorzano 1969).

Similar trends were observed for terbutryn treated enclosure (Figure 9) except that in the pre-flood phase, all concentrations of herbicide resulted in substantially higher ammonia than the control and these did not decrease until flooding. This corresponded with the near complete inhibition of chlorophyll synthesis and photosynthetic rate at all concentrations during this period. As well, return of ammonia to the water column following flooding was observed at all concentrations, although the level at the lowest terbutryn concentration decreased within 25 days of flooding to near the control (Figure 9).

Silicon concentrations in enclosures treated with simazine (Figure 8) and terbutryn (Figure 9) followed trends remarkably similar to ammonia at all concentrations. Initial high levels in all enclosures were again probably a function

Figure 8: Dissolved oxygen, ammonia, and silicon over the 6-week period of the experiment:
A - In the control enclosure (___) and in enclosures treated with 0.1 (---) 1.0 (-·-) and 5.0 (···) $mg \cdot L^{-1}$ simazine.
Vertical bar indicates the approximate period of enclosure flooding.

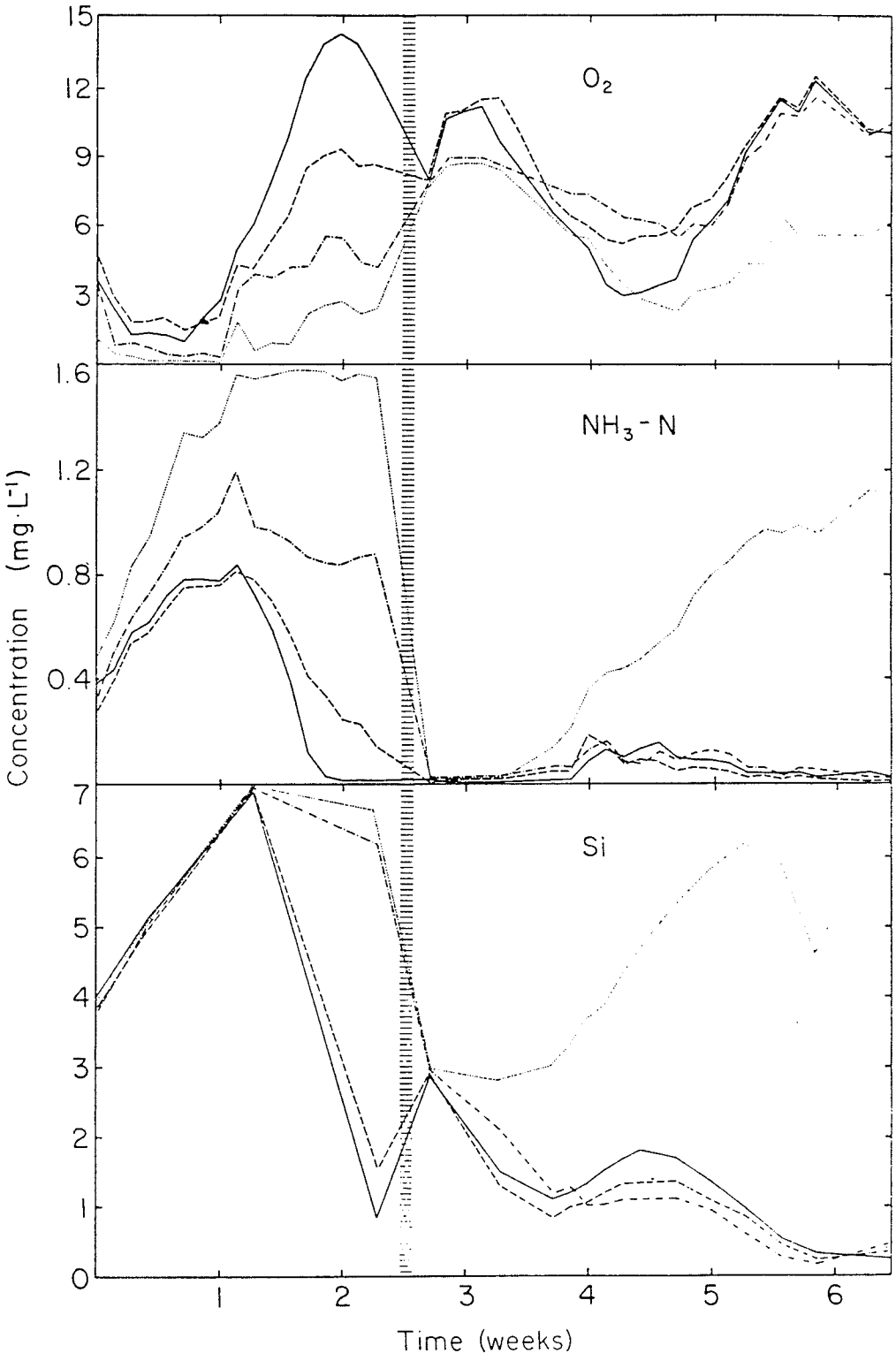
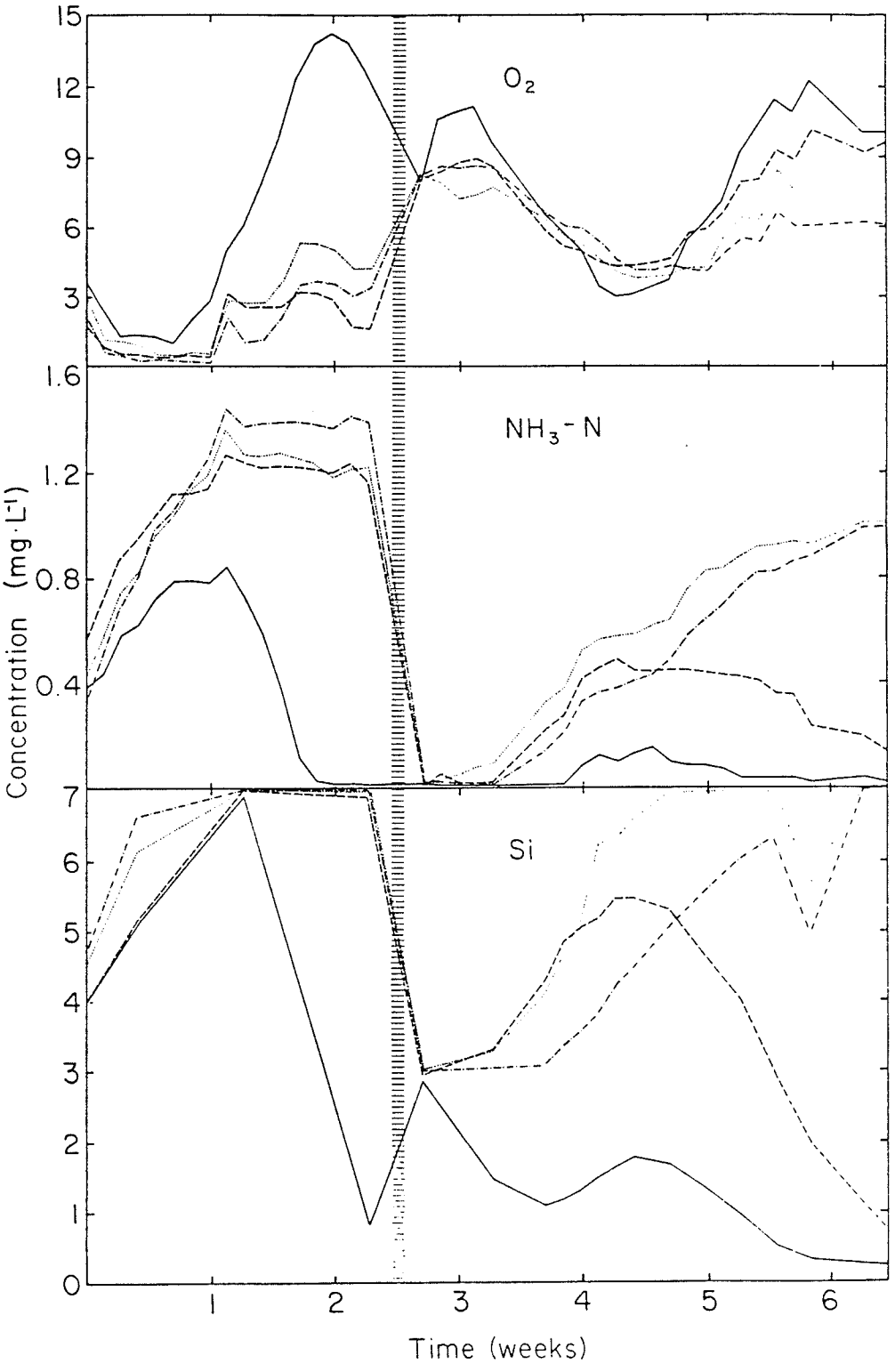

15
12
9
6
3
O_2
1.6
1.2
0.8
0.4
NH_3 - N
Concentration (mg·L⁻¹)
7
6
5
4
3
2
1
0
Si
1
2
3
4
5
6
Time (weeks)

Figure 9: Dissolved oxygen, ammonia, and silicon over the 6-week period of the experiment:
A - In the control enclosure (___) and in enclosures treated with 0.01 (---), 0.1 (-·-) and 1.0 (···) $mg \cdot L^{-1}$ terbutryn.
Vertical bar indicates the approximate period of enclosure flooding.


15
12
9
6
3
O_2
1.6
1.2
0.8
0.4
NH_3-N
Concentration (mg·L^{-1})
7
6
5
4
3
2
1
0
Si
1
2
3
4
5
6
Time (weeks)

of sediment disturbance. Correlation between the two nutrients was highly significant ($r=0.88$, $p=0.0001$), suggesting a common determinate factor. A multiple linear regression improved the correlation ($r=0.92$) by including the identity of the enclosure of sampling, and its dissolved oxygen level (which varied inversely with increasing herbicide concentration)(Figures 8 and 9).

2.4 DISCUSSION

Both herbicides exhibited clear effects on colonization of periphyton of artificial substrata, as shown by chlorophyll *a* accumulation and photosynthetic rate. In the case of simazine, reduction in growth of periphyton was observed between 0.1 and 1.0mg·L^{-1} (Figures 5A and 6A) whereas the minimum effective concentration of terbutryn was less than 0.01mg·L^{-1} (Figures 5B and 6B). Algal biomass (using chlorophyll *a* level as a crude indicator of the photosynthetically-active portion of biomass) increased over time in all treatments with the most notable increases in treated enclosures following flooding. By comparison, the photosynthetic rates of the control and 0.1mg·L^{-1} simazine treatments, while greater than those of other herbicide treated enclosure substrata, were variable. That a net increasing trend was not observed suggests that physical and chemical factors interacted to determine rates. Therefore, in order to adequately examine herbicidal effects on productivity, both photosynthetic rate and corresponding biomass should be considered.

From the preflood period, it is possible to compare the relative toxicities of the two herbicides on the basis of chlorophyll and photosynthesis levels. The ratio of mean preflood chlorophyll _a_ on substrata from the 1.0mg·L^{-1} simazine enclosure to the mean in the 1.0mg·L^{-1} terbutryn treatment indicates the greater degree to which terbutryn inhibits chlorophyll accumulation. The ratio can also be calculated at the 0.1mg·L^{-1} applications, as well as for mean preflood photosynthetic rates at each of the two concentrations. These ratios are necessarily approximations, since actual herbicide concentrations were not equal to their theoretical values. Both chlorophyll _a_ and photosynthesis measures gave similar ratios at each herbicide concentration (Table 8) pointing to the clear correspondence of the two variables. At 0.1mg·L^{-1}, terbutryn was approximately 55 times more toxic to periphyton than simazine, whereas the difference decreased to 10 times at 1.0mg·L^{-1}. Thus, while terbutryn was a more powerful algicide than simazine at low concentrations, the difference between them decreased as higher levels were used. One might predict that the ratio at 0.01mg·L^{-1} for the two herbicides would be even greater than 55.

The high level of inhibition by terbutryn at 0.01mg·L^{-1} (>90%) and at 0.025mg·L^{-1} (Figure 4B) in the 1.0mg·L^{-1} postflood enclosure, suggests that very low levels of methylthio-herbicides can achieve considerable effect on the growth

TABLE 8

Relative phytotoxicity of herbicides

Ratio of mean periphyton chlorophyll a level (and photosynthetic rate) in the 3-week preflood period at similar levels of simazine and terbutryn.

Herbicide Concentration ($mg \cdot L^{-1}$)	Chlorophyll	Photosynthesis
0.1	54.06	56.48
1.0	10.42	9.83

of periphyton. This is contrary to the findings of Robson et al. (1976), who found that terbutryn at 0.01mg·L^{-1} had no effect on the growth of the alga Vaucheria dichotoma. Lefebvre-Drouet & Calvet (1978) reported an EC_{50} value for terbutryn of 0.20mg·L^{-1} in cultures of Chlorella pyrenoidosa. The present data may explain Scorgie's(1980) observation that cyanatryn (chemically similar to terbutryn) had no significant effect on the density of periphyton on glass slides placed in two portions of a drainage ditch (one treated with herbicide and the other not). Since contamination of the control resulted in herbicide concentrations as high as 0.04mg·L^{-1}, control samples may have been as inhibited as samples from treated sites.

While close correlation between photosynthesis and chlorophyll a was observed, their ratio varied widely over time, especially following decrease in herbicide concentration as a result of flooding. The increase in specific productivity in all enclosures suggests that some sort of 'rejuvenation' occurred as a result of flooding which temporarily reversed the tendency of the communities in each to become less photosynthetically efficient with time. The marked increase in SP of communities treated with high levels of simazine and the lower two levels of terbutryn (relative to the control), however, indicates that some aspect of herbicide treatment acted to increase photosynthetic efficiency upon herbicide loss. The clear correspondence of ammonia-nitrogen and sil-

icon levels with herbicide concentration suggests that an increased supply of nitrogen and silicon to periphyton may somehow relate to changes in SP. This assertion requires the clear identification of primary (physiological) and secondary (chemical and physical) ecological effects of herbicides.

Secondary effects of herbicide treatment have been well documented (Murphy et al. 1981). A reduction in dissolved oxygen has been commonly reported (van der Weij 1971; Johannes et al. 1973; Pruss & Higgins 1975; Blackburn & Taylor 1976; Ellis et al. 1976; Tucker & Boyd 1978a; Ashton et al. 1980; Murphy et al. 1981; Wingfield & Johnson 1981) as well as a reduction in redox potential (Wingfield & Johnson 1981). Decrease in pH may occur (Sutton et al. 1965; Pruss & Higgins 1975; Ellis et al. 1976; Murphy et al. 1981) as well as increases in dissolved calcium, magnesium, potassium (Walker 1964), ammonia (Walker 1964; Blackburn & Taylor 1976; Murphy et al. 1981; Anderson 1981), nitrate and phosphate (Anderson 1981; Murphy et al. 1981).

Increases in concentrations of such compounds as phosphates and ammonia have been attributed solely to release from decaying macrophytes (Anderson 1981). While this may occur in certain situations, it is an inadequate explanation for the present data, where effects of macrophytes were eliminated by clearing of the enclosures. Instead, the sediments may represent the major source for these nutrients.

The close correspondence of dissolved oxygen and oxygen gradient with depth, ammonia, and silicon with herbicide concentration found here, and the relatively greater effect of terbutryn on these parameters than simazine suggests either a role of oxygen and redox potential affecting nutrient release from anoxic sediments (Mortimer 1941; 1942) or a direct herbicidal influence on epipelic algae which normally intercept nutrients from the sediments. Jansson(1980) showed that addition of mercuric chloride to sediment cores as a biological toxicant lead to considerably increased ammonia concentrations in overlying water. Muir et al. (1981) determined that terbutryn adsorption in upper sediment layers could result in concentrations as high as $1.4ug \cdot g^{-1}$ within 12 days of $0.1mg \cdot L^{-1}$ herbicide treatment. Thus, inhibition of the epipelon of shallow ponds, lakes and ditches by herbicides, particularly where the sediment adsorption of herbicide is high (as probably occurred at the $5.0\ mg \cdot L^{-1}$ simazine treatment level), may therefore serve to increase the quantities of nutrients supplied to the water column (Chapter 5).

Conditions in the present study may have been particularly conducive to detection of secondary effects on sediment/water nutrient flux, since their magnitude would be influenced by the degree of turbulent diffusion away from the site of liberation (which is minimized in the enclosures), the extent of anoxic, organic sediments (high in marsh envi-

ronments) and the time interval between determinations of ambient levels of nutrients. Daily monitoring of ammonia and silicon allowed detection of rapid changes in concentration which would otherwise be missed over longer periods of time. Michaud et al. (1979) observed that the magnitude of the increase in dissolved phosphorus following addition of diquat to a shallow lake was less than found by other authors using plexiglass enclosures of lake water.

In an attempt to examine the relationships between the primary effects of the two herbicides on chlorophyll a and photosynthesis, and other potential secondary effects, factor analysis was performed on the correlation matrix of the fourteen measured parameters (Table 9). Four factors were generated using Kaiser's criterion for significant factors (Dixon & Brown 1979). By association, names were assigned to each factor according to its constituent variables. Therefore, ammonia and silicon concentration, herbicide level, and dissolved oxygen became 'water chemistry', while herbicide type and identity of enclosure became 'experimental treatment'. Since the latter variable was artificially created, its inclusion may mean it is acting concomitantly for several other unquantified parameters. Variables which changed between sampling dates but not between enclosures (Table 6) became 'temporal variability', and mean light intensity and extinction were combined as 'light availability'. Clearly, these are artificial designations, and serve

only to illustrate some similarity in principle between variables in each factor. While all the above variables load reasonably clearly on only one factor, chlorophyll a and photosynthetic rate load on factors 1, 2 and 3 (chlorophyll) and 1, 2 and 4 (photosynthesis) more or less evenly, indicating that these primary herbicidal effects correlate not only with herbicide concentration but also on several other variables, among which are the herbicidally-correlative variables of ammonia, silicon, and oxygen. As a result, it may be suggested that the effect of the two herbicides was determined by a complex interaction of water chemistry and physics as well as the nature and quantity of the herbicide.

The use of herbicides in flowing water (Wile 1967; Bowmer et al. 1979; Murphy et al. 1981) and in fish culture (Tucker & Boyd 1978b), and the use of herbicide-treated water as a potable source ultimately requires examination of the persistance of herbicide residues over time. Coincident are considerations of the rate at which plant communities are reestablished (Crawford 1981), determining either the frequency of subsequent herbicide applications, or the expected duration of environmental perturbation by unintended pollutants.

Chlorophyll a and photosynthesis measures provided direct evidence of recovery in the periphyton community during the period following enclosure flooding. If the rate of in-

TABLE 9

Rotated factor loadings of variables

derived from principle components analysis (BMDP4M) of a correlation matrix between the variables. All loadings less than 0.250 have been set to zero. Proposed factor names: 1 = water chemistry, 2 = experimental treatment, 3 = temporal variability, 4 = light availability.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Ammonia	0.909	0.262	0	0	0.919
Silicon	0.809	0.478	0	0	0.940
Oxygen	-0.727	0	-0.538	0	0.877
Herbicide Concentration	0.771	0	0	0	0.625
Enclosure	0	0.941	0	0	0.942
Herbicide Type	0	0.938	0	0	0.891
Oxygen Gradient	0	-0.667	0.262	0	0.526
Light	0	0	0.899	0	0.834
Temperature	0.373	0	0.889	0	0.941
Time	-0.413	0	-0.681	0.434	0.828
Enclosure Depth	0	0	0	0.867	0.805
Light Extinction	0	0	0	0.867	0.688
Chlorophyll	-0.433	-0.597	-0.489	0	0.820
Photosynthesic Rate	-0.466	-0.581	0	0.423	0.772
Eigenvalues	3.475	3.282	2.785	1.866	11.408

crease in chlorophyll a is considered as an indicator of growth rate, then the rate of all treated enclosures except that of the highest terbutryn level is seen to nearly equal or exceed the control (Figure 2). Admittedly, the exposure period to herbicide in this experiment was relatively short, but on the basis of these data, no detrimental long-term effect on productivity of periphyton may be predicted from a single application of either herbicide. Desorption of herbicide from the sediments, if in phytotoxically significant quantities (as may have occurred in the case of the $1.0 mg \cdot L^{-1}$ terbutryn treatment) may slow the rate of recovery (as evidenced by the decreased rate of chlorophyll a accumulation over time at the higher herbicide levels in the post-flood period-Figure 5A), or prevent it altogether (Figure 5B; $1.0 mg \cdot L^{-1}$).

In conclusion, the present study is, to our knowledge, one of the first attempts to quantify effects of aquatic herbicides on periphyton by way of a controlled series of defined herbicide treatments. The use of littoral enclosures represents a simple means of simulating herbicidal effects on a small scale, with the benefit that several concentrations of one or more herbicides may be tested simultaneously, thereby minimizing the spatial and temporal variability inherent in whole lake testing (Scorgie 1980; Ashton et al. 1981; Murphy et al. 1981 and others). Since a small water volume is involved, loss of herbicide by dilu-

tion in surrounding waters provides a convenient means of monitoring recovery of communities from various chemical concentrations.

Chapter III

EFFECT OF SIMAZINE AND TERBUTRYN ON PERIPHYTON COMMUNITY STRUCTURE

3.1 INTRODUCTION

Assessment of the toxicological effects of agricultural and industrial materials on freshwater and marine periphyton communities is becoming increasingly common, as the ecological importance of this community to total aquatic primary production (Wetzel 1983) is realized. Studies have examined the relative impacts of heavy metals (Williams & Mount 1965; Dickman 1974; Sigmon et al. 1977; Grolle & Kuiper 1980), industrial effluent (Evans & Marcan 1976; Moll & Keller 1980) and pesticides (Anderson 1981; Hodgson & Carter 1982; Hodgson & Linda 1984; Kosinski 1984; Kosinski & Merckle 1984) on dry weight and pigment concentration (Hodgson & Carter 1982), physiological activity (Chapter 2) and/or species composition (Kosinski 1984; Leland & Carter 1984) of artificial (Hodgson & Linda 1984; Kosinski 1984) or *in situ* (Chapter 2) communities under a series of controlled treatments.

Recent investigations of the structure of freshwater haptobenthic periphyton using scanning electron microscopy (eg. Allanson 1973; Perkins & Kaplan 1978; Hoagland et al. 1982; Hamilton & Duthie 1984; Roemer et al. 1984) have contributed

greatly to our knowledge of the spatial and temporal complexity of this community. A usual sequence in the colonization of newly available substratum may begin with the adhesion of sessile bacteria (Jordan & Staley 1976; Roemer et al. 1984), which are followed by sessile diatoms. As horizontal space is occupied, species which possess growth habits positioning them away from the substratum (eg. mucilage stalks and tubes, basally attached cells) proliferate, thereby increasing the physical stature of the community (Patrick 1978). Multicellular filamentous, and often heterotrichous, taxa may appear as a later stage of succession and may themselves provide substratum for further colonization. If this progression continues, a community not unlike terrestrial forest is ultimately established which consists of a canopy layer underlain by 'shrub' and 'herb' layers (Hoagland et al. 1982).

It is not yet clear which of the three models of succession described by Connell & Slatyer (1977) best describes succession in haptobenthic periphyton. A 'facilitation' model, which presumes that early colonists prepare the substratum for later colonists while progressively excluding individuals of their own kind, may have a basis in an early apparent bacterial prerequisite to algal colonization (Korte & Blinn 1983; Hamilton & Duthie 1984) and the role of diatom mucilages in community architecture (Roemer et al. 1984). An 'inhibition' model, based on competitive exclusion of

later colonists by pioneer species, may be appropriate to instances where the early colonist population persists and increases in density over time (eg. Leland & Carter 1984). Finally, the 'tolerance' model provides an intermediate case in which increasing structural complexity of the developing community favors the proliferation of species better suited to prevailing environmental conditions within the community, while the pioneer community dwindles in proportion of total density.

Since periphyton community structure represents the outcome of interspecific competition between populations of varying sensitivity to a particular stress, examination of the specific responses of an entire community represents a very sensitive assay for toxicological effects. As more detailed information is gained of the autecological strategies of periphytic taxa, more detailed evaluations of toxicological effects are possible. The importance of considering the complex structure of periphyton is three-fold. Firstly, since little is known of the sensitivity of individual taxa and their importance to the creation and maintenance of structure, it is unclear whether observed structural changes are the direct result of specific toxicity or the loss of a necessary prerequisite to successful colonization. Secondly, the supposition that increases in community height result in, and are the consequence of vertical gradients of light, CO_2 and nutrients (Hoagland et al. 1982) also sug-

gests that gradients of toxicological effects, based on the inhibitor concentration at various positions within the community, variable species tolerance of the inhibitor and proximity of organisms capable of degrading or sequestering the toxicant, may exist. Lewis et al. (1983) have shown that degradation rates of 2,4-D in spatially complex periphyton communities are correlated with the velocity of medium passage across the substratum, implying that mass transport limitation may occur within dense periphyton mats. Finally, the degree and direction to which a community develops in response to xenobiotic stress may determine the availability of organic material to higher trophic levels, since taxa of differing growth habit may differ in their palatability to grazers (eg. Patrick 1970; Kesler 1981).

The study of some effects of simazine and terbutryn on the chlorophyll content and photosynthetic rate of freshwater marsh periphyton colonizing artificial substrata within in situ enclosures (Chapter 2), showed that the relative toxicity of these compounds could be estimated from observed levels of inhibition of overall community productivity. In addition, incidental enclosure flooding during the experiment provided an opportunity to monitor the extent and rate of 'recovery' of the community. It was concluded that long-term effects on periphyton communities of short (<3 weeks) exposures to these herbicides were limited. However, the degree of structural similarity of the newly developed community to that of the unperturbed control was not assessed.

The present chapter reports the findings of community structural analyses of periphyton samples collected during the previously reported experiment (experiment B, Appendix A). Aspects of the autecology and synecology of Cocconeis placentula Ehr. are discussed as they pertain to the processes involved in colonization of artificial substrata following herbicide exposure.

3.2 MATERIALS AND METHODS

Details of the experimental design are described in the preceding chapter. In brief, seven littoral enclosures (diameter = 78 cm, mean volume ca. 300 L) were situated in a shallow channel of the Delta Marsh, on the southern end of Lake Manitoba, Canada (99° 19'W, 50° 7'N). Extruded acrylic rods used as substrata for periphyton colonization and growth were positioned vertically in each enclosure, and quantities of unformulated technical grade herbicides (> 98% a.i.) were added to 6 of the 7 enclosures to yield initial concentrations of 0.1, 1.0 and 5.0mg$\cdot L^{-1}$ (simazine) and 0.01, 0.1 and 1.0mg$\cdot L^{-1}$ (terbutryn). One enclosure was maintained as an untreated control.

At weekly intervals beginning 9 days after herbicide addition and continuing over a 6-week period, 3 entire rods were randomly selected from each enclosure. Two-centimeter rod segments were used in measurements of ^{14}C bicarbonate assimilation rate, and 60 cm segments were used in measure-

ments of chlorophyll a concentration (Chapter 2). In addition, a 6 cm segment was subsampled from a position on each rod corresponding to 32-38 cm above the sediment/water interface, placed into a sample vial containing 9 mL of filtered marshwater and preserved with 1 mL of acid Lugol's solution. These samples were used in enumeration of constituent algal taxa.

Preserved periphyton on rod segments was detached with a rubber policeman into the original water sample, and the entire sample shaken for 30 s. using a Vortex Jr. Mixer to separate adherent single cells. For quantitative diatom enumeration, aliquots were removed from each sample (1-4mL depending on total density) and preservative removed by serial centrifugation and washing. The final suspension was mixed thoroughly and 0.1 mL aliquots were dispensed onto 22 mm square glass coverslips. The dried slips were ashed in a muffle furnace for 6 min. at 600°C and mounted permanently onto slides with Naphrax high-resolution diatom mountant.

Two diametric transects of each coverslip were scanned at 1000X magnification and the relative abundance of each diatom taxon determined. A total of 500-1000 frustules was counted in each sample. Taxonomy followed Patrick & Reimer (1966,1975) and Germain (1981). The absolute density of each taxon per unit of original substratum surface area was calculated from the formula:

$$\text{cells} \cdot \text{cm}^{-2} = \frac{Vt \times N \times As}{\text{---------}}$$

$$F \times Vs \times At$$

where Vt is the total volume of the original sample (ml), N is the number of cells of each taxon counted in two transects, As is the area of the sample on the coverslip (cm^2), F is the area of the coverslip examined in two transects (cm^2), Vs is the volume of the original sample used on the coverslip (mL), and At is the surface area of the original rod (cm^2).

Diatom density counts were corrected for the inclusion of dead, intact frustules present in the original samples. Cytoplasmic contents in washed cell samples were stained in an alcoholic fast-green solution (Johansen 1940) for approximately 10 minutes, and then rinsed with distilled water to remove excess stain. Aliquots of the algal suspension were placed onto coverslips, dried and mounted in Naphrax without prior ashing. The resulting preparations provided resolution under phase-contrast microscopy that was superior to similar preparations using acid fuchsin stain (Owen et al. 1978), while shrinkage of cell contents during drying was minimal. Random transects were scanned on each slide until at least 100 cells of predominant taxa were examined. The percentage of cells which had been living at the time of collection in these subsamples (assumed to be those with stained inclusions) was calculated and multiplied by the previously calculated absolute densities to give a measure of the density of living cells in the original sample. For

rare taxa, insufficient cells were observed to allow correction.

Densities of algal taxa other than diatoms were calculated as above from counts obtained from diagonal transects (200X magnification) of 0.1 mL Palmer cells containing whole preserved aliquots of each sample. Differentiation of live and dead cells was made by direct observation of cell contents. Taxonomy was based on Prescott (1962), although the fragmented nature of some filamentous green algae and the absence of sexual reproductive parts limited specific identifications of many of these taxa.

Total algal biovolume was calculated by multiplying mean cell volume (determined from measurements of several cells of each taxon, with calculation based on the nearest geometric solid) by corresponding cell densities and summing the result for all taxa.

A measure of the degree of dissimilarity of periphyton community structure in the control enclosure with that in herbicide-treated enclosures was calculated using the Euclidean distance function (Gauch 1982):

$$EDjk = [\ \Sigma\ (Aij - Aik)^2\ ]^{1/2} \qquad i = 1 \ldots n$$

where EDjk is an estimate of the dissimilarity between communities j and k, Aij and Aik are measures of the abundance of taxon i in each community, and n is the total number of taxa.

Data matrices were created for each of the two herbicides which consisted of the distances between all possible pairs of 24 communities (considering samples from the control and 3 herbicide levels over each of 6 sample dates as discrete communities). These matrices were calculated on 2 separate bases by using mean density and mean biovolume of each taxon (3 replicates / community) as measures of abundance.

A 2-dimensional graphic representation of the dissimilarity of each community to all others was derived using nonmetric multidimensional scaling (Kruskal & Wish 1978) of the Euclidean distance matrices. Calculations were performed using the algorithm ALSCAL (Takane et al. 1977) available under the Statistical Analysis System (SAS 1982) on an Amdahl 5850 computer at the University of Manitoba. Program defaults for the maximum number of computational iterations, starting configuration and convergence and minimum stress criteria were adopted.

3.3 RESULTS

Total algal biovolume on artificial substrata in the control enclosure increased substantially between the first 2 weeks of sampling (Figure 10). Following enclosure flooding between weeks 2 and 3, biovolume decreased slightly and then increased from week 4 onwards. The apparent correlation between flooding and reduction in periphyton biomass was also evident in algal chlorophyll values (Chapter 2) and is in-

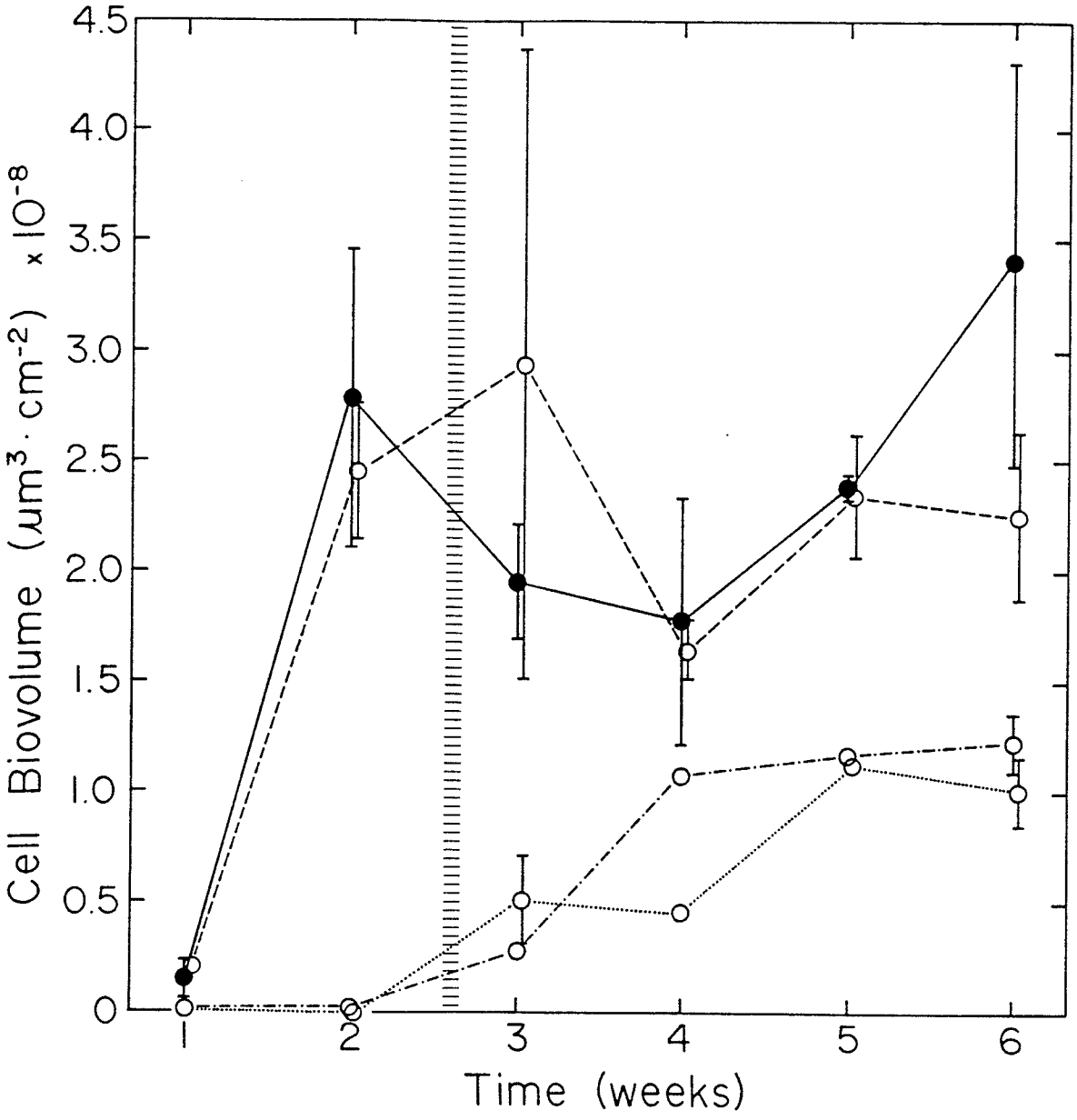
terpreted as a consequence of detachment of loosely adherent filamentous green algae during flooding.

The effects of simazine on total algal biovolume were similar to those on periphytic chlorophyll concentration and photosynthetic rate (Chapter 2). Mean biovolume over the 6-week period in the 0.1mg·L^{-1} treatment was not significantly different (natural logarithm transformed data) from the control (Duncan's multiple range test; 5% level), while means of 1.0 and 5.0mg·L^{-1} treatments were significantly different from all others. Levels of inhibition in the pre-flood period were estimated at 94% and 98% (relative to the control) in the 1.0 and 5.0mg·L^{-1} treatments respectively, and suggest that community LC_{50} (herbicide concentration yielding 50% reduction in biovolume) lies between 0.1 and 1.0mg·L^{-1} simazine.

A significant interaction between experimental treatment and sample date ($F_{15,48}$ = 9.24, p = 0.0001) was evident from the sharp increase in algal biovolume following flooding of the 1.0 and 5.0mg·L^{-1} treatment enclosures (Figure 10). Rates of increase in biovolume in these enclosures between weeks 3 and 6 were similar to that seen in the control during the corresponding time period.

Prior to enclosure flooding, high levels of inhibition (> 98%) of algal biovolume were noted at all terbutryn treatment levels (Figure 11), suggesting that the LC_{50} of marsh

Figure 10: Total algal biovolume over the 6-week period of the experiment in the control enclosure (•---•) and in enclosures treated with 0.1 (o---o), 1.0 (o-·-o) and 5.0 (o···o) $mg \cdot L^{-1}$ simazine. Error bars are the SD of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.


4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Cell Biovolume (μm³·cm⁻²) x10⁻⁸
1
2
3
4
5
6
Time (weeks)

periphyton for this herbicide lies below 0.01mg·L^{-1}. After the third week of sampling (post-flood), rates of increase in algal biovolume in the lower 2 treatment levels were similar to that of the control. Sustained low biovolume levels in the 1.0mg·L^{-1} enclosure were probably related to high post-flood residue terbutryn concentrations.

The periphytic algal community on substrata in the control enclosure was dominated throughout the experiment by sessile and erect diatoms and filamentous green algae (Figure 12). The 0.1mg·L^{-1} simazine treatment appeared to favor the incidence of diatoms (weeks 1-2), although variability between replicates suggests that the difference was not significant. Following flooding, green algae increased in abundance to proportions similar to the control.

The apparent dominance of green algae in the initial sample from the 1.0mg·L^{-1} simazine treatment is likely a result of overall low algal abundance (Figure 10) coupled with the chance appearance of sparsely distributed Chlorophyte taxa during counting procedures. Similar results were not noted in the 5.0mg·L^{-1} enclosure. The two enclosures were, however, similar in the post-flood response, in which diatoms assumed overwhelming dominance on substrata throughout the remainder of the 6-week experiment. The increasing incidence of green algae by week 6 in the 1.0mg·L^{-1} enclosure was confirmed by samples collected sporadically over a subsequent 4-week period.

Figure 11: Total algal biovolume over the 6-week period of the experiment in the control enclosure (●---●) and in enclosures treated with 0.01 (o---o), 0.1 (o-·-o) and 1.0 (o···o) $mg \cdot L^{-1}$ terbutryn. Error bars are the SD of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.

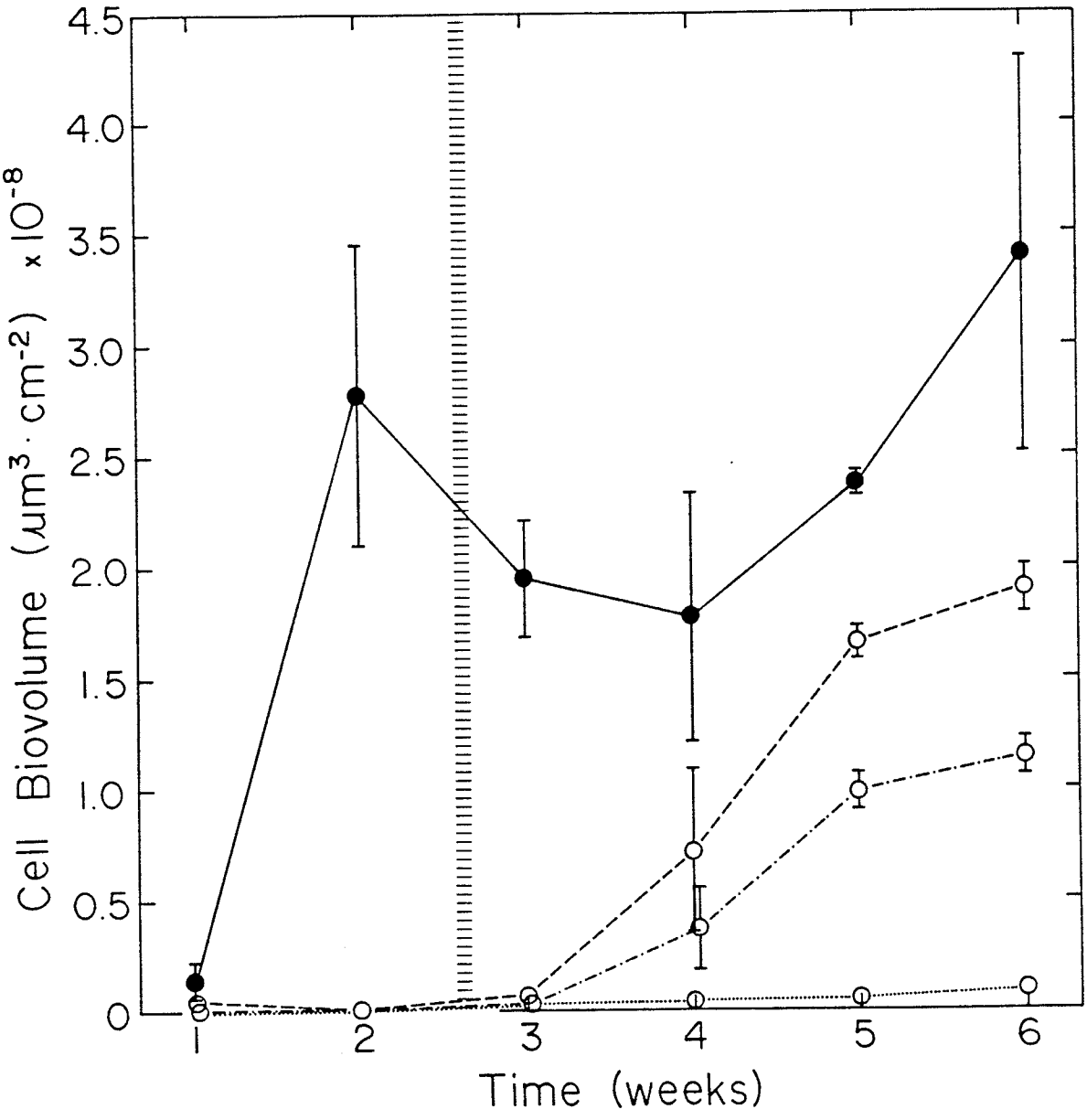
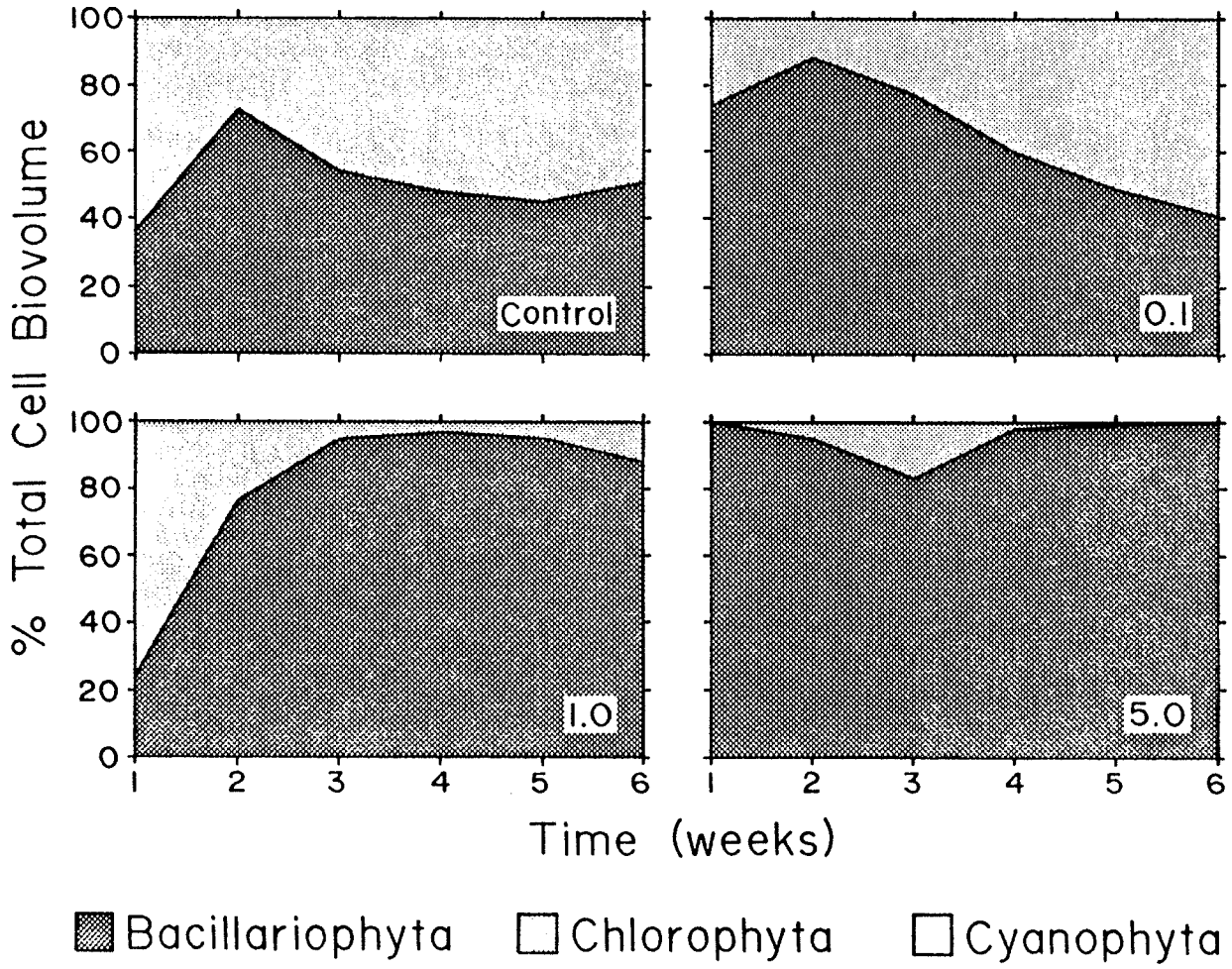

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Cell Biovolume (μm³ · cm⁻²) x 10⁻⁸
1
2
3
4
5
6
Time (weeks)

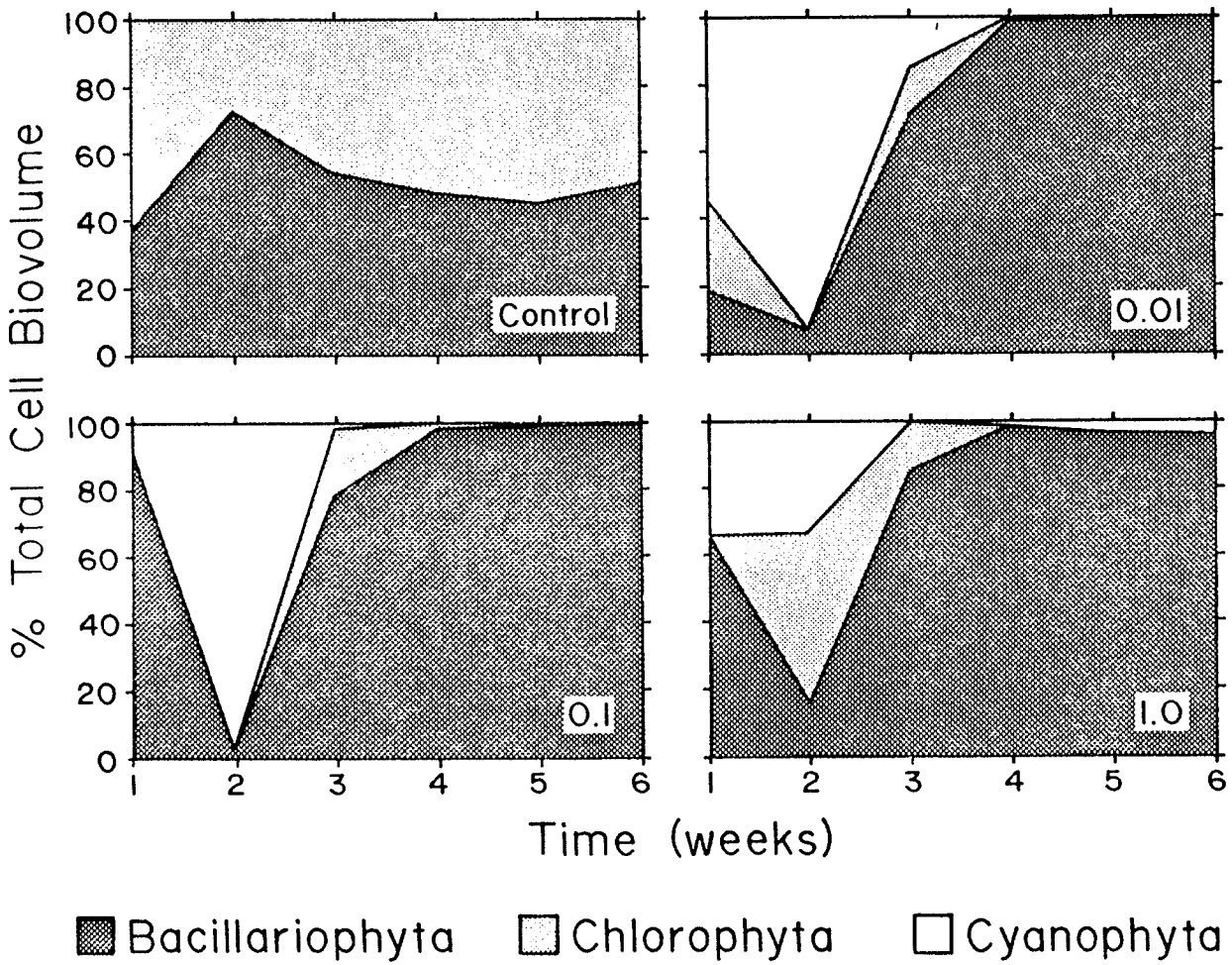
Figure 12: Change in proportions of the three major algal divisions over the 6-week period of the experiment in the control and simazine-treated enclosures (0.1, 1.0 and 5.0mg·L^{-1}).


% Total Cell Biovolume
100
80
60
40
20
0
Control
0.1
1.0
5.0
1
2
3
4
5
6
Time (weeks)
Bacillariophyta
Chlorophyta
Cyanophyta

Responses of algal community structure to initial terbutryn exposure differed markedly from those for simazine (Figure 13). Although algal density was very low in all treatments, a large proportion of total biovolume was assumed by a small (< 5 um length) cyanophyte of the order Chamaesiphonales, which, despite careful examination of more dense communities, was not found in control and simazine enclosure samples. Relative increases in diatom abundance following flooding were concomitant with the disappearance of this taxon. Increases in green algal density were not apparent in subsequent sampling of substrata from terbutryn-treated enclosures.

Seventy-three periphytic algal taxa were observed during this experiment, with the largest number recorded in control and 0.1mg·L^{-1} simazine enclosures. Species richness increased from the pre- to post-flood periods in all enclosures (Table 10) although the difference was most pronounced in enclosures in which pre-flood algal inhibition was high. Changes in the specific taxonomic composition of periphyton in experimentally manipulated enclosures are best illustrated by the two most numerically dense taxa. Cocconeis placentula (varieties lineata and euglypta) was the most abundant (on both numbers and biovolume bases) diatom taxon. In the control and 0.1mg·L^{-1} simazine enclosure (Figure 14), Cocconeis accounted for ca. 25% of total algal biovolume throughout the experiment. Absolute numbers of this diatom

Figure 13: Change in proportions of the three major algal divisions over the 6-week period of the experiment in the control and terbutryn-treated enclosures (0.01, 0.1 and 1.0mg·L^{-1}).


% Total Cell Biovolume
100
80
60
40
20
0
Control
0.01
0.1
1.0
1
2
3
4
5
6
Time (weeks)
Bacillariophyta
Chlorophyta
Cyanophyta

increased rapidly in the first 2 weeks of sampling and then reached a stable plateau population of about 2.8×10^5 cells$\cdot$cm^{-2}. Differences between the two enclosures were not significant at the 5% level. Prior to enclosure flooding, Cocconeis was present in periphyton exposed to high treatment levels of simazine in approximately the same proportions as the control although absolute density was reduced by approximately 99% in both cases. Following flooding, cell densities in 1.0 and 5.0mg$\cdot$L^{-1} enclosures increased at rates ($4.5 \pm 0.3 \times 10^4$ and $3.6 \pm 1.5 \times 10^4$ cells$\cdot$cm$^{-2}\cdot$d^{-1} respectively) similar to rates observed in initial samples of control and 0.1mg$\cdot$L^{-1} enclosures substrata ($4.0 \pm 1.2 \times 10^4$ and $2.8 \pm 1.5 \times 10^4$ cells$\cdot$cm$^{-2}\cdot$d^{-1} respectively). Stable plateaus were attained at levels greater than in the control (ca. 4.2×10^5 cells$\cdot$cm^{-2}) with the exception of the last sample date in which densities were not significantly different. In addition, the proportion of total biovolume attributable to Cocconeis was considerably greater than in the 1.0 and 5.0mg$\cdot$L^{-1} enclosures, reaching greater than 90% by week 4.

Strikingly similar results were found in terbutryn-treated enclosures in the post-flood period (Figure 15). Following the herbicide exposure period, in which the abundance of Cocconeis was very low (< 0.1% of control) in all enclosures, densities increased rapidly in the 0.01 and 0.1mg$\cdot$L^{-1} enclosures ($5.5 \pm 3.1 \times 10^4$ and $3.3 \pm 1.9 \times 10^4$

Figure 14: Density of Cocconeis placentula (Inset: proportion of total biovolume) over the 6-week period of the experiment in the control enclosure (•---•) and in enclosures treated with 0.1 (o---o), 1.0 (o-·-o) and 5.0 (o···o) $mg \cdot L^{-1}$ simazine. Error bars are the SD of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.

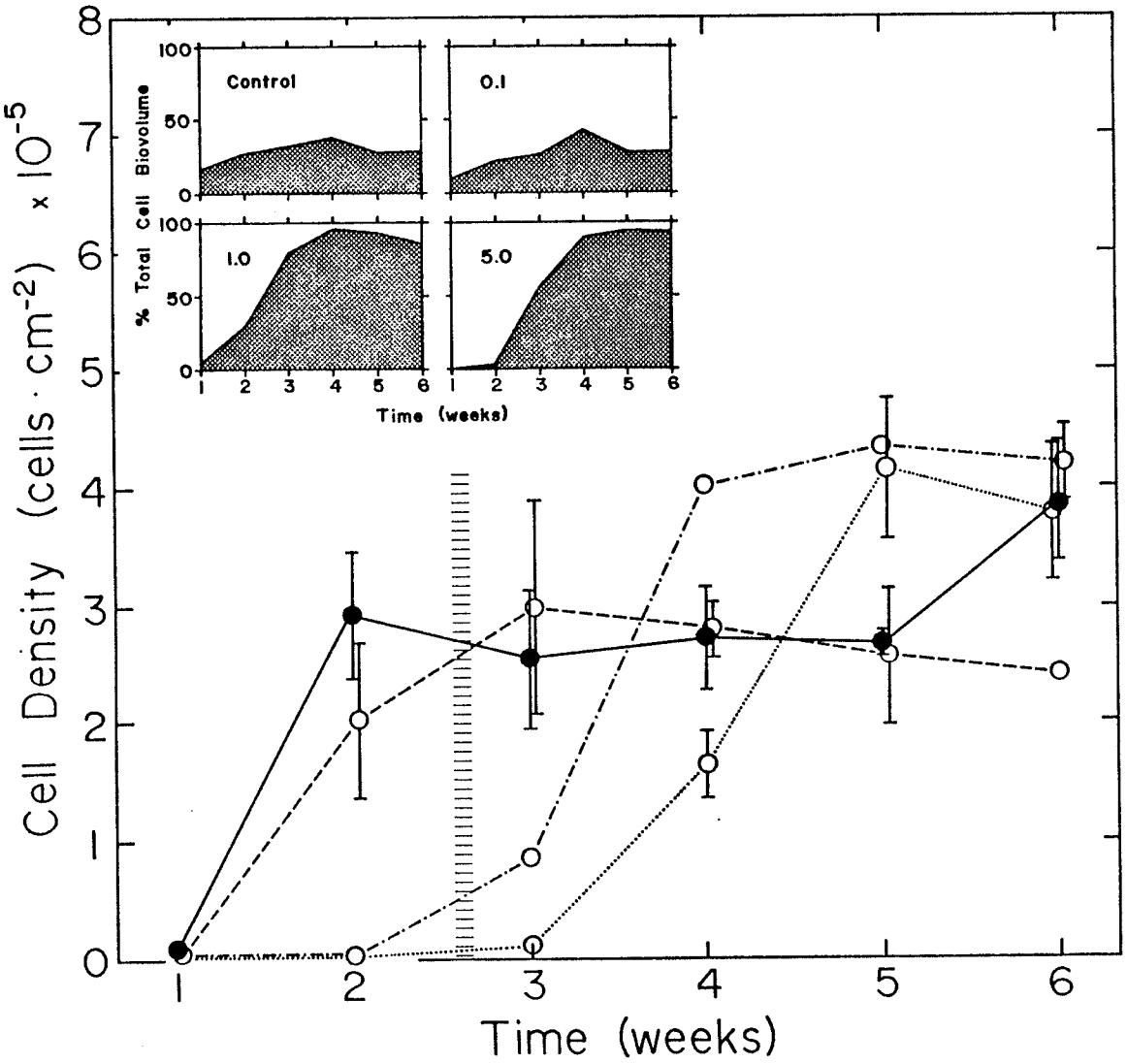

Cell Density (cells · cm⁻²) x 10⁻⁵
Time (weeks)
% Total Cell Biovolume
Control
0.1
1.0
5.0
Time (weeks)

TABLE 10

Species list of periphytic algal taxa

collected from artificial substrata positioned within experimental enclosures. 1 = control, 2-4 = simazine (0.1, 1.0 and 5.0 $mg \cdot L^{-1}$ respectively), 5-7 = terbutryn (0.01, 0.1 and 1.0 $mg \cdot L^{-1}$ respectively), A = pre-flood, B = post-flood, P = < 5% total density, C = 5 - 25% total density, A = > 25% total density.

Species	1 A	1 B	2 A	2 B	3 A	3 B	4 A	4 B	5 A	5 B	6 A	6 B	7 A	7 B
Bacillariophyta														
Achnanthes lanceolata Breb.ex.Kutz.	P	P	P	P				P				P		P
A. minutissima Kutz.				P		P								
Amphora ovalis (Kutz.)Kutz.	P	P	P	P		P	C					P		
A. perpusilla (Grun.)Grun.		P		P										
Anomoeoneis sphaerophora (Ehr.)Pfitzer										P				
Bacillaria paxillifer (Mull.)Hendey		P		P		P				P		P	P	P
Caloneis amphisbaena (Bory.)Cleve		P				P					P			
C. bacillum (Grun.)Cleve	P	P	P	P		P								
Cocconeis placentula Ehr.	A	A	A	A	A	A	C	A	P	A	P	A	P	A
Cyclotella comta (Ehr.)Kutz.	P													
C. glomerata Bachmann			P	P		P	C	P				P		P
C. meneghiniana Kutz.		P				P				P			P	
Cymbella cistula (Hemp.)Grun.		P												
C. muelleri Hust.		P				P								
Denticula sp.	P	P		P										
Diatoma vulgare Bory.			P				C							
Epithemia adnata (Kutz.)Breb.	P	P	P	P		P	C	P		P		P		P
E. turgida (Ehr.)Kutz.		P	P	P				P		P		P		P
Fragilaria brevistriata Grun.								P						
F. capucina Rabh.	P	P	P	P	P		A		P	P	C	C	P	P
Gomphonema affine Kutz.	P	P	P	P	P			P		P				
G. parvulum Kutz.	C	P	C	C	P	P		P		P		P		
G. subclavatum Grun.	P	P	P	P		P		P		P		P		P
G. truncatum Ehr.				P						P				P
Gyrosigma spencerii (Quek.)Griff.&Henfr		P					C							
Navicula capitata Ehr.		P	P	P		P	C	P		P	C		P	P
N. circumtexta Meist.ex Hust.				P										
N. cryptocephala Kutz.	P	P	P	P	P	P	C	P	P	P	C	P		
N. cuspidata (Kutz.)Kutz.										P				
N. elmorei Patr.	P													
N. halophila (Grun.)Cl.		P	P											
N. menisculus (Grun.)Grun.		P	P	P						P				
N. minuscula Grun.	P	P	P	P										
N. oblonga (Kutz.)Kutz.				P										
N. pelliculosa (Breb.ex Kutz.)Hilse		P	P	P								P		

Table 10 continued

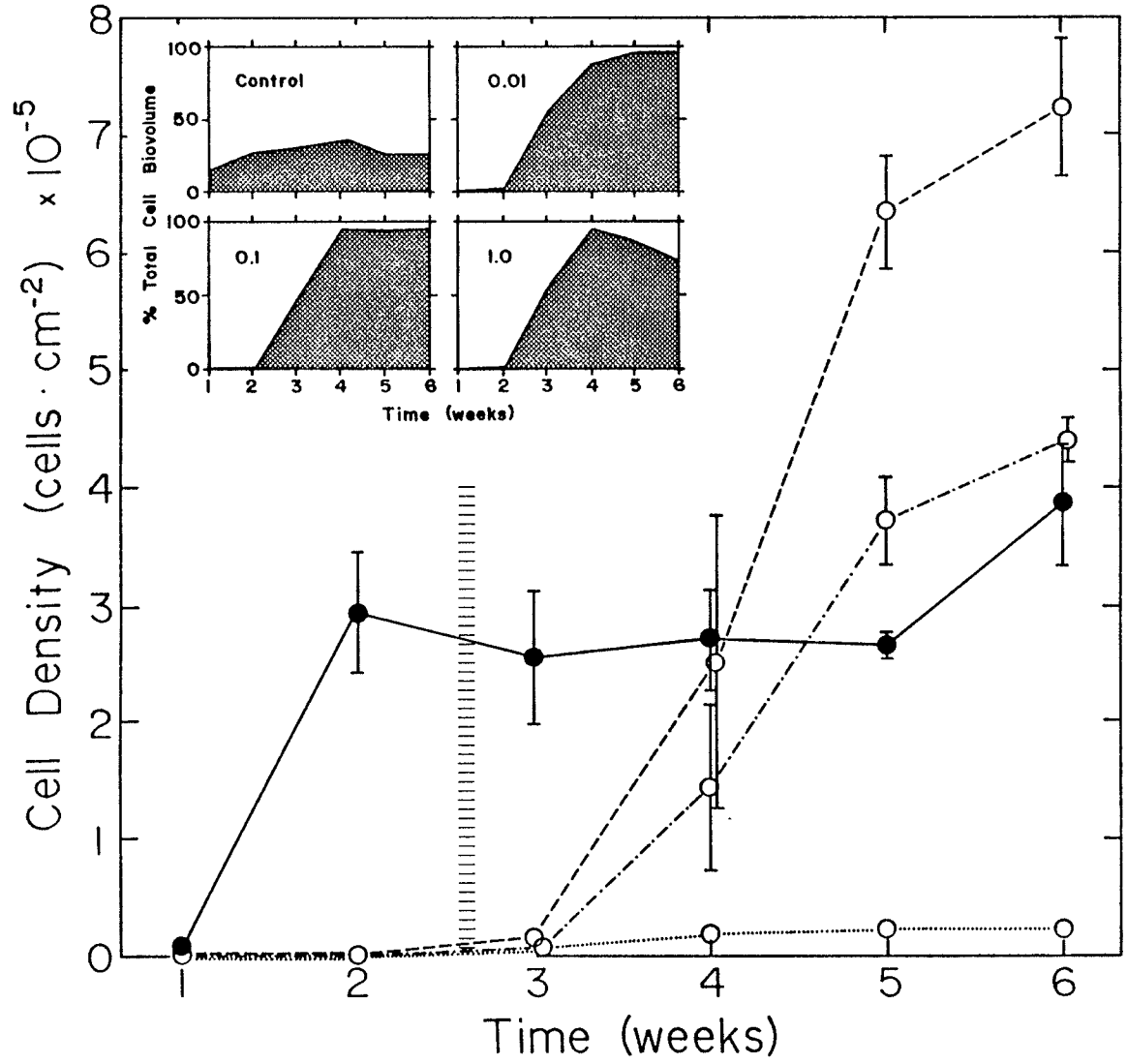
N. peregrina (Ehr.)Kutz.				P			C							
N. pupula Kutz.	P	P	P	P			C			P			P	
N. pygmaea Kutz.					P			P				P		
N. radiosa Kutz.		P												
N. rhyncocephala Kutz.		P		P			C	P		P		P		P
N. secura Patr.			P											
N. seminulum Patr.						P								
N. tenera Hust.	P	P	P											
Nitzschia acicularis W.Sm.			P	P				P				P		
N. amphibia Grun.	P	P	P	P		P				P		P		P
N. denticula Grun.	P									P				
N. dissipata (Kutz.)Grun.	P	P		P						P				
N. hungarica Grun.				P								P		
N. inconspicua Grun.	P	P	P	P		P	C			P		P	P	P
N. linearis W.Sm.			P	P										
N. longissima (Breb.)Ralfs.		P								P				
N. palea (Kutz.)W.Sm.	C	P	C	C	C	P	C	P		P	C	P	P	P
N. pusilla Kutz.	P													
N. sigmoidea (Ehr.)W.Sm.				P										
Opephora martyi Herib.		P			P			P						
Rhoicosphenia curvata (Kutz.)Grun.ex Ra		P	P	P				P		P		P		
Rhopalodia gibba (Ehr.)O.Mull.							C							
Synedra delicatissima W.Sm.	P	P	P	P						P				
S. fasciculata (Ag.)Kutz.	P	P		P				P		P		P		
S. rumpens Kutz.			P	P	P	P								P
S. ulna (Nitz.)Ehr.	P	P	P	P	P	P				P				
Unknown diatom sp.													P	P
Chlorophyta														
Bulbochaete sp.	C	C	C	C	A	P		P	P	P		P	P	P
Coleochaete irregularis Pring.						P								
C. orbicularis Pring.			P	P										
C. scutata Breb.	C	P	C	C		P		P		P		P		
Pediastrum boryanum (Turp.)Meneghini	P	P	P							P				
Scenedesmus quadricauda Breb.				P										
Stigeoclonium sp.	A	A	A	A	A	C		P	P	P		P		P
Cyanophyta														
Anabaena sp.			P											
Chamaesiphonales									A	A	A	P	A	C
Gloeotrichia sp.		P	P	P	P					P		P		
Species Richness	29	43	36	45	13	24	15	21	6	33	6	27	11	19

cells·cm^{-2}·d^{-1}) to attain densities greater than the control (7.2 x 10^5 and 4.4 x 10^5 cells·cm^{-2} for the 0.01 and 0.1mg·L^{-1} treatments respectively). Only a slight increase in Cocconeis density was seen at 1.0mg·L^{-1} (relative maxima of 500 and 22,600 cells·cm^{-2} in the pre- and post-flood periods respectively) although this taxon had nevertheless assumed dominance of the periphyton community by week 3.

The filamentous green alga Stigeoclonium sp. was the numerically dominant Chlorophyte taxon in all enclosures. In control and 0.1mg·L^{-1} simazine enclosure (Figure 16), density of this genus increased throughout the experimental period, particularly in the last 3 weeks. Differences between the two treatments were not significant (5% level). On the other hand, this taxon occurred only rarely in the 1.0 and 5.0mg·L^{-1} enclosures prior to and following enclosure flooding, and contributed only a small proportion of total biovolume (< 4%) up to week 6. This was also true for all terbutryn-treated enclosures, in which the genus never contributed more than 1% of total biovolume (Figure 17) in any of the three treatment levels.

Ordination of algal periphyton community structure data was based on both specific density and biovolume. Respective multivariate models fitted to both simazine and terbutryn datasets resulted in stress values (measures of the poorness-of-fit of the model to experimental data - Kruskall & Wish 1978) of less than 0.13 (13%) in two dimensions, and

Figure 15: Density of Cocconeis placentula (Inset: proportion of total biovolume) over the 6-week period of the experiment in the control enclosure (•---•) and in enclosures treated with 0.01 (o---o), 0.1 (o-·-o) and 1.0 (o···o) $mg \cdot L^{-1}$ terbutryn. Error bars are the SD of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.

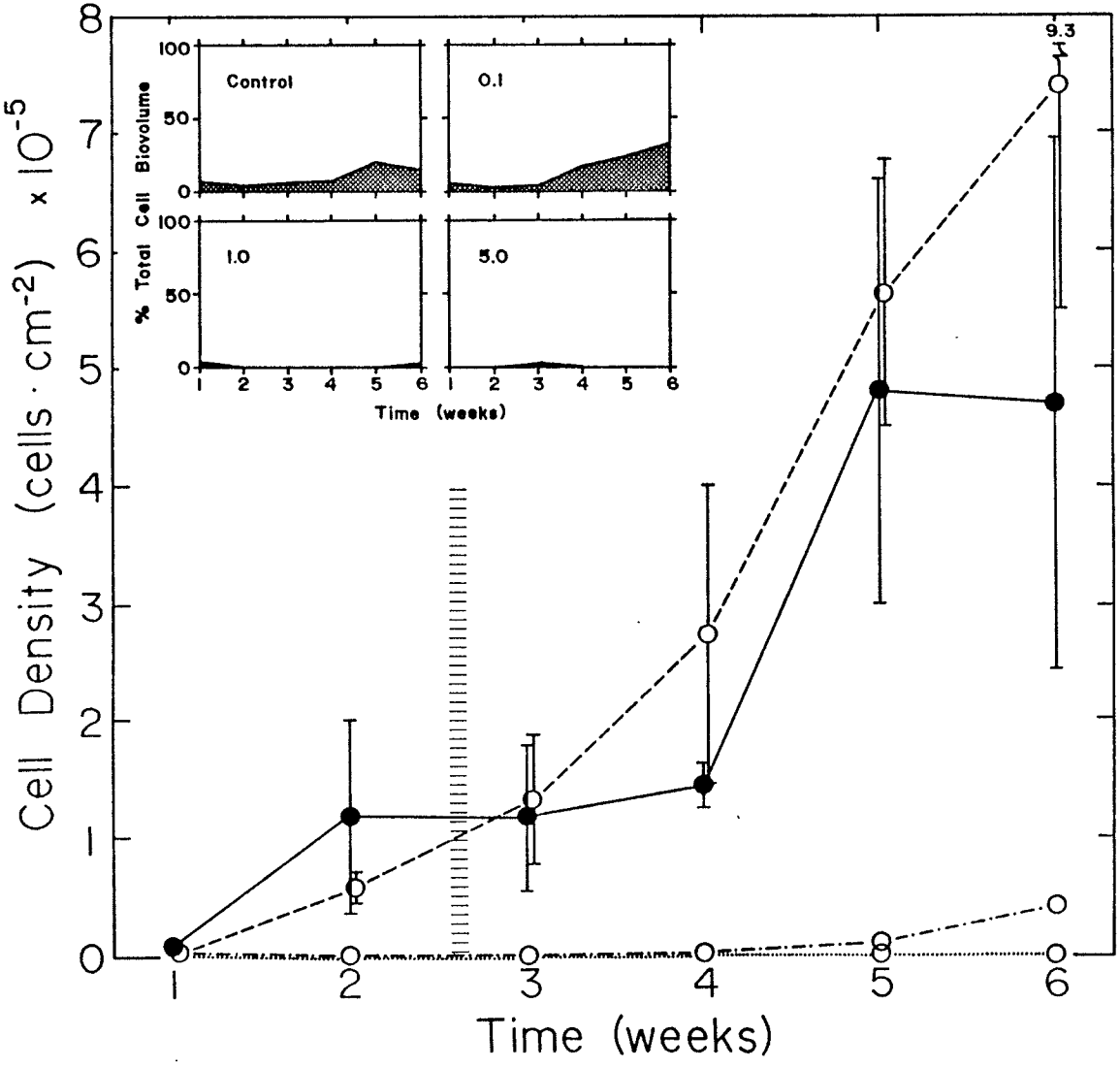

Cell Density (cells · cm^{-2}) x 10^{-5}
Time (weeks)
% Total Cell Biovolume
Control
0.01
0.1
1.0
Time (weeks)

explained greater than 92% of variance in scaled experimental data. In the case of both herbicides, use of either abundance measure resulted in similar spatial representations. Analyses of data matrices based on density, however, yielded lower overall stress values (< 6%) and provided more readily interpretable results. Consequently, only these data are presented.

Data from week 1 samples from the control and all simazine enclosures are represented as points in the lower right quadrant of the 2-dimensional ordination diagram (Figure 18). Since the Euclidean measure of dissimilarity is based on total abundance as well as specific composition, the apparent similarity of these samples, in which densities were consistently low (Figure 10), is not surprising.

Temporal sequences in community composition in each enclosure are represented by lines connecting successive samples in each case (Figure 18). The sequences in control and 0.1mg·L^{-1} simazine enclosures initially are oriented diagonally to the two dimensions and following week 3-4, nearly perpendicular to initial samples. Slight divergence between the enclosures is seen in week 6 samples. By contrast, samples from the 1.0 and 5.0mg·L^{-1} samples progress only in the former direction and at an apparently different angle to the control and 0.1mg·L^{-1} treatments (Figure 18).

Figure 16: Density of Stigeoclonium sp. (Inset: proportion of total biovolume) over the 6-week period of the experiment in the control enclosure (•---•) and in enclosures treated with 0.1 (o---o), 1.0 (o-·-o) and 5.0 (o···o) $mg \cdot L^{-1}$ simazine. Error bars are the SD of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.


Cell Density (cells · cm-2) x 10-5
Time (weeks)
9.3
Control
0.1
1.0
5.0
% Total Cell Biovolume
Time (weeks)

The bidirectional orientation of the temporal sequence in control samples is also illustrated in comparison with terbutryn-treated enclosures (Figure 19), whereas samples from 0.01 and 0.1mg·L^{-1} treatments exhibit a linear sequence. Owing to their consistently low total abundance, none of the samples from the 1.0mg·L^{-1} treatment are discriminated from week 1 samples in the lower, right quadrant of the plot.

Figure 17: Density of Stigeoclonium sp. (Inset: proportion of total biovolume) over the 6-week period of the experiment in the control enclosure (●---●) and in enclosures treated with 0.01 (o---o), 0.1 (o-·-o) and 1.0 (o···o) $mg \cdot L^{-1}$ terbutryn. Error bars are the SD of replicates (n = 3). The approximate period of enclosure flooding is indicated by the vertical bar.

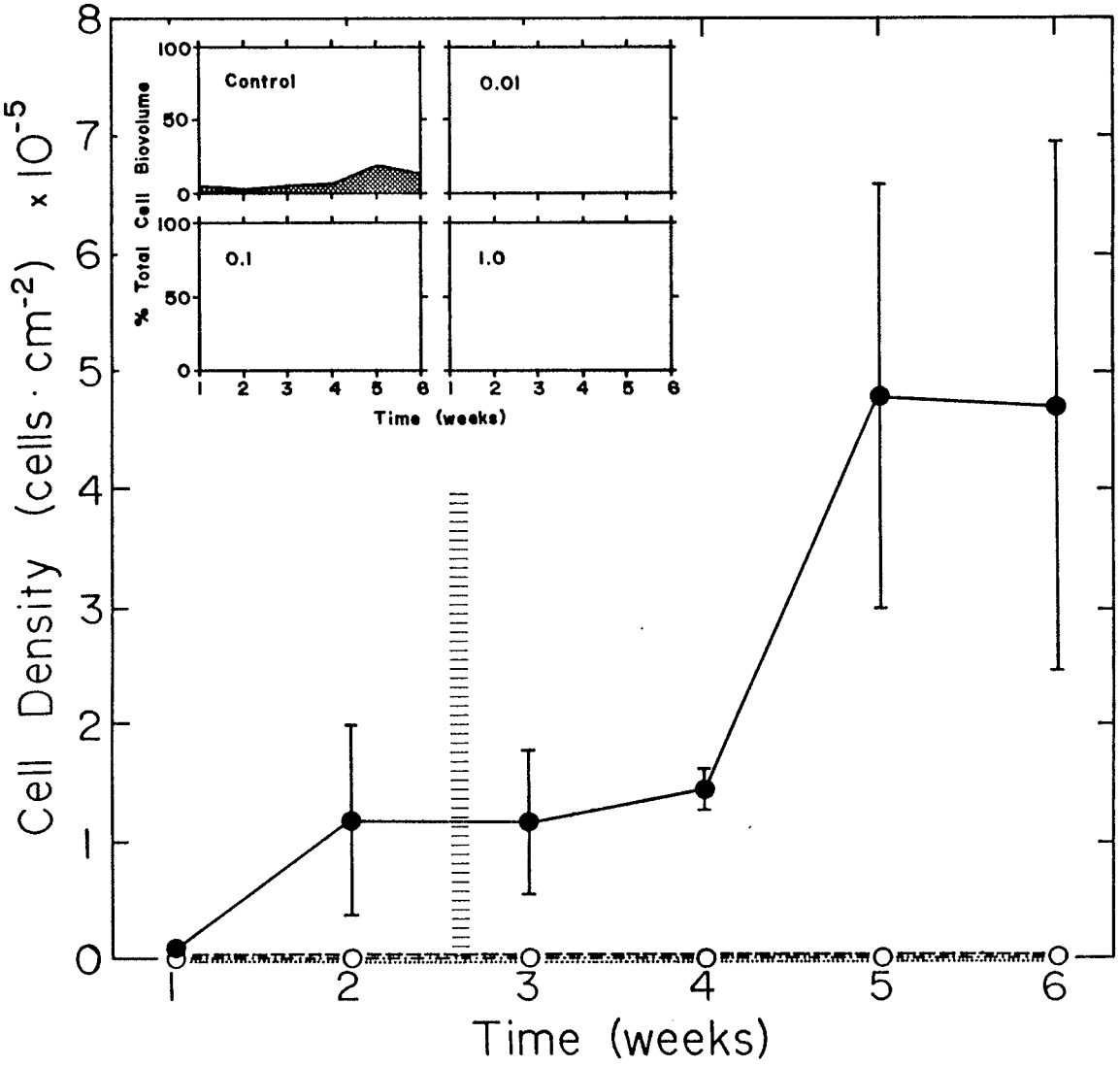

Cell Density (cells · cm^{-2}) x 10^{-5}
Time (weeks)
% Total Cell Biovolume
Control
0.01
0.1
1.0
Time (weeks)

Figure 18: Nonmetric multidimensional scaling of Euclidean distances between algal communities in control and simazine-treated enclosures. Week 1 samples are situated in the lower right quadrant of the plot and temporal sequences are indicated by lines connecting successive samples from the control (•---•), 0.1 (o---o), 1.0 (o-·-o) and 5.0 (o···o)$mg \cdot L^{-1}$ enclosures. Axes are dimensionless.

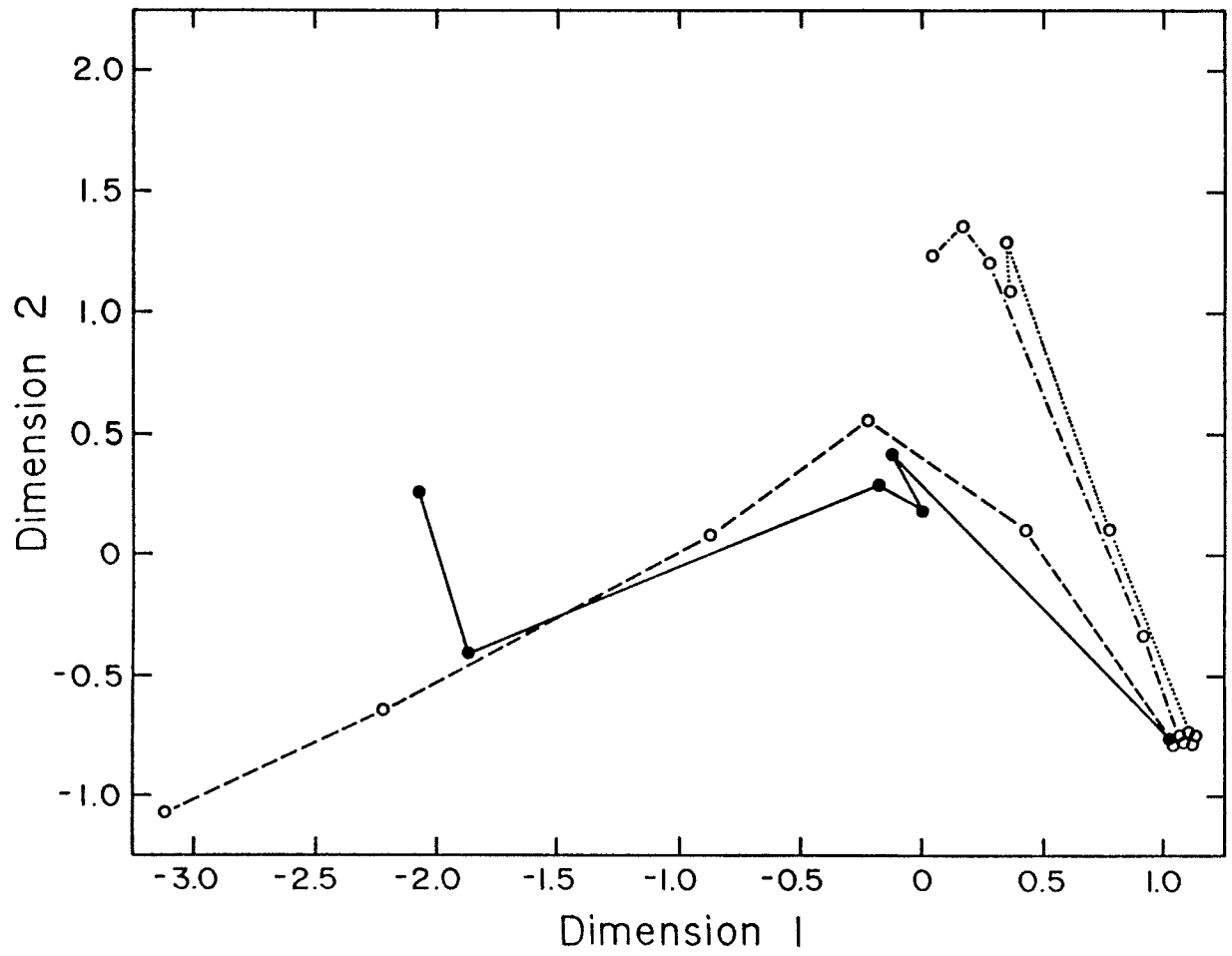
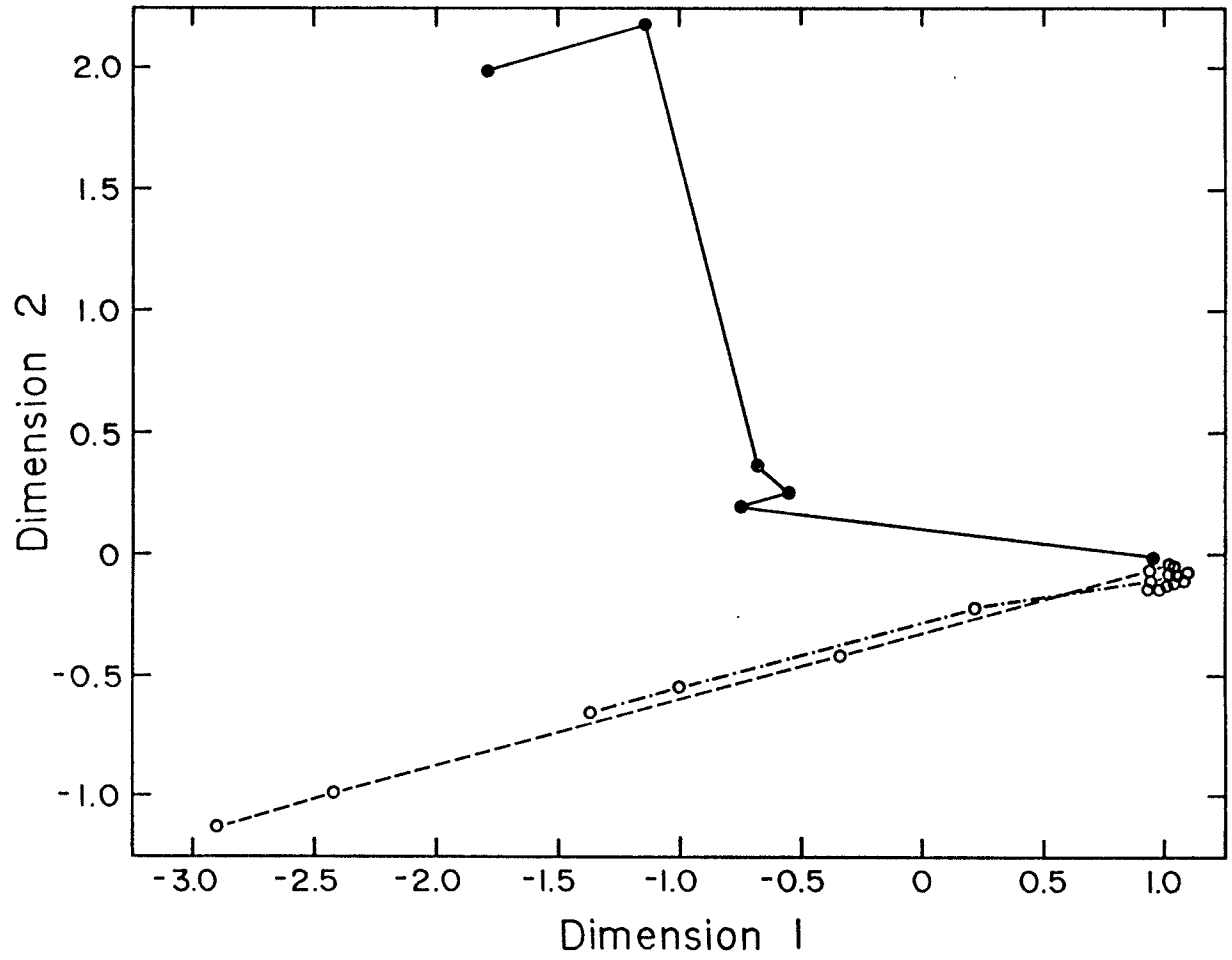

2.0
1.5
1.0
0.5
0
-0.5
-1.0
Dimension 2
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
Dimension 1

Figure 19: Nonmetric multidimensional scaling of Euclidean distances between algal communities in control and terbutryn-treated enclosures. Week 1 samples are situated in the lower right quadrant of the plot and temporal sequences are indicated by lines connecting successive samples from the control (•---•), 0.01 (o---o), 0.1 (o-·-o) and 1.0 (o···o)mg·L^{-1} enclosures. Axes are dimensionless.


2.0
1.5
1.0
0.5
0
-0.5
-1.0
Dimension 2
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
Dimension 1

3.4 DISCUSSION

Evaluation of the degree of toxicity of the two herbicides based on total periphyton biovolume is consistent with those made previously (Chapter 2), and indicate that terbutryn was much more toxic than simazine at similar levels. Linear regression analyses of biovolume data with corresponding chlorophyll and photosynthesis data resulted in correlation coefficients of 0.96 and 0.62 respectively (both $p < 0.0005$).

The apparent correspondence of chlorophyll, photosynthetic rates and the algal biovolume measurements presented here should not be taken as evidence of redundancy. Although each parameter responded to herbicide toxicity in a similar manner in these experiments, differing results have been obtained in other enclosure experiments under specific circumstances (Goldsborough unpubl.). For example, photosynthetic measurements based on ^{14}C-assimilation rates do not differentiate oxygenic from anoxygenic sources in the presence of photosynthetic bacteria, which would be insensitive to photosystem II-inhibiting triazine herbicides (Moreland 1980). Under favorable conditions of anaerobiosis and high concentrations of reduced compounds (both are secondary effects of herbicide addition - Chapter 5), such bacteria may flourish (eg. Appendix B). Furthermore, while chlorophyll a measurements are sensitive to vegetative biomass, current spectrophotometric methods are unable to account for the spectral

overlap of this pigment with those of bacteriochlorophylls c and d (Stanier & Smith 1960) when these latter pigments are present in periphyton extracts (Appendix B). Of the three measures, biovolume calculations most directly reflect algal success. Any direct extrapolation of biovolume measurements to productivity would, however, assume that all production is photolithotrophic, so that the potential contribution of heterotrophy to maintenance of algal populations is ignored. While these interferences are not necessarily implicit in all data, and may occur only rarely, the use of multiple measures of total algal production guards against spurious conclusions, and can provide additional insight into toxicological effects.

With the increasing availability of computer facilities, the ability to describe community structure and dynamics with multivariate models is becoming more widespread. Some foresight as to the salient features important to the determination of 'ecological distance' may, however, yet be necessary. It is our belief that two parameters are of major significance in describing the toxicological effects of a compound to a natural community. These include both the absolute density of the community (function) and its structure. It is insufficient to conclude on the basis of total algal biomass that a compound has no 'long-term effects' if that compound had in fact eliminated a major species. At the same time, if all species were inhibited to similar ex-

tents, comparison of control and treatments merely on the basis of relative species composition overlooks secondary effects of reduced biomass on energy flow between trophic levels. The Euclidean function used here incorporates both of these aspects, although the extent to which other distance indices do so varies. Similarly, the use of nonmetric ordination minimizes model stress resulting from an inappropriate assumption regarding the correspondence of the distance function chosen and true ecological distance (Kruskal & Wish 1978). Eigenvector ordination techniques (which do not require the input of calculated distances) may or may not provide a satisfactory representation of the above two factors. For example, while we have used detrended correspondence analysis (DCA) to successfully discriminate phytoplanktonic, haptobenthic, and herpobenthic algal communities in shallow eutrophic ponds (Shamess et al. 1985), that analysis based comparisons between communities on the relative proportions of constituent taxa (Hill 1979). Thus, similar ordination of the present simazine data matrix, in which reduction in total biomass was arguably as important as change in specific composition, resulted in a poor separation of samples along one axis with little information drawn from a second, and did not allow the differentiation of temporal successional patterns.

By contrast, the sequence of colonization of newly available substratum in the control and $0.1 mg \cdot L^{-1}$ simazine enclo-

sure is summarized by the bidirectional orientation of samples by the present ordination (Figures 18-19). The two directions may in effect summarize the two major components involved in succession, namely expansion in the horizontal and vertical planes. The divergence of succession as a result of herbicide exposure is also well described and may reflect the loss of community 3-dimensionality.

The sequences of periphyton succession on substrata from the control and 0.1mg·L^{-1} simazine treatments were qualitatively similar to that described by Hoagland et al. (1982). Within a few days of substratum placement, a visible bacterial coating had developed over the entire surface of each. Within 9 days of substratum exposure (week 1), several periphytic algal taxa had become established (Table 10), including the majority of those which would become abundant over the subsequent 5-week period. Cocconeis increased in abundance between weeks 1 and 2, while stalked diatoms (Gomphonema parvulum, G. subclavatum and G. affine) and rosette-shaped clusters of Nitzschia palea were most abundant between weeks 2 and 4. Finally, the density of filamentous green algae increased significantly between weeks 4 and 6 (cf. Figures 16-17). The maintenance of a stable population of C. placentula between weeks 2 and 6 is consistent with the 'tolerance' model of succession, which allows for the persistence of healthy, intact early colonists in a developing community. While aspects of 'facilitation' by early

colonists (particularly bacteria) cannot be ruled out, the presence of later colonists (green algae) in week 1 samples implies that there are no algal prerequisites to their colonization and that a period of delay prior to their proliferation (weeks 4-6) may be a reflection of slow rates of growth.

No major taxon (> 5% of total density) was present in simazine-treated enclosures which was not also present on control substrata. Moreover, there was no evidence that a clearly herbicide resistant/tolerant community had developed in the 2.5 week period prior to enclosure flooding, although the lower relative abundance of filamentous green algae at 5.0mg·L^{-1} suggested that these taxa were selectively inhibited to a greater extent than others. Kosinski (1984) has drawn a similar conclusion in studies of the impact of atrazine on stream periphyton. Due to the extremely low algal density in all terbutryn-treated enclosures, little can be said of the response of community structure to herbicide addition. The exceptionally high abundance of a periphytic blue-green alga in these enclosures, however, suggests that this taxon possesses some means of herbicide resistance/tolerance. At the same time, its disappearance from 0.01 and 0.1mg·L^{-1} treatments, and reduced abundance in 1.0mg·L^{-1} following flooding indicates that it is a poor competitor for resources with less tolerant species. Analogous conclusions have been reached in experiments dealing with triaz-

ine-resistant terrestrial weed biotypes (Conard & Radosevich 1979), and suggests that herbicide tolerance in general may be achieved at the expense of ecological fitness.

The post-flood successional sequence in herbicide-treated enclosures more closely resembled Connell & Slatyer's (1977) 'inhibition' model in which an early colonist (Cocconeis placentula) effectively excludes other colonists. From an ecological standpoint, the dominance of Cocconeis in herbicide-perturbed periphyton communities is of considerable interest due to its reported ubiquity in lotic and lentic environments over a wide salinity range (McIntire & Moore 1978; Patrick & Reimer 1966), including a variety of substratum types in the Delta Marsh (Hooper-Reid & Robinson 1978; G.Kruszynski unpubl.). Despite its widespread distribution and relative abundance, however, definition of its niche has proven elusive. A survey of the literature has indicated a preference for slow moving water in lotic habitats (Jones 1978), horizontal positioning (Godward 1934), moderate specific conductance and alkalinity (Patrick & Reimer 1966; Higashi et al. 1981; Tuchmann & Blinn 1979), moderate water temperature (Hickman & Klarer 1974; Tuchmann & Blinn 1979; Hickman 1982), high nitrate concentration (Patrick 1978) and low light intensity (Hickman & Klarer 1974; Hickman 1982; Jenkerson & Hickman 1983). Other factors which may be related to high Cocconeis density include reduced susceptibility to grazing pressure (Patrick 1970;

Kesler 1981), extended colonization period (Kesler 1981), increased substratum age (Siver 1978; Rogers & Breen 1981) and mid-late summer season (Brown & Austin 1973; Kesler 1981; Hickman 1982). Some of these conclusions have undoubtedly arisen from subjective interpretations of assumed cause and effect relationships between observed periodicity and environmental factors. Reconciliation of some apparently contradictory factors may be possible, although there is a clear need for autecological studies of common periphytic algal species such as Cocconeis, and of the possible existence of distinguishable ecotypes.

Interspecific competition between Cocconeis and other periphytic diatoms has been documented. Brown & Austin (1973) found that the density of C. placentula on glass slides was correlated with the density of Achnanthes minutissima such that increases in the proportion of one corresponded to decreases in the other. Patrick (1978) described a similar relationship between C. placentula and A. lanceolata while Tuchmann & Blinn (1979) have reported a relationship between the relative proportions of C. placentula and Amphora coffeiformis (Ag.)Kutz. in the periphyton colonizing submerged macrophytes. Outcome of competition in the latter study was apparently influenced by temperature, with Cocconeis prevailing at temperatures less than 26°C. In these examples, it is presumed that both competitors share a common niche such that occupation by one reduces space available for the other (Patrick 1978).

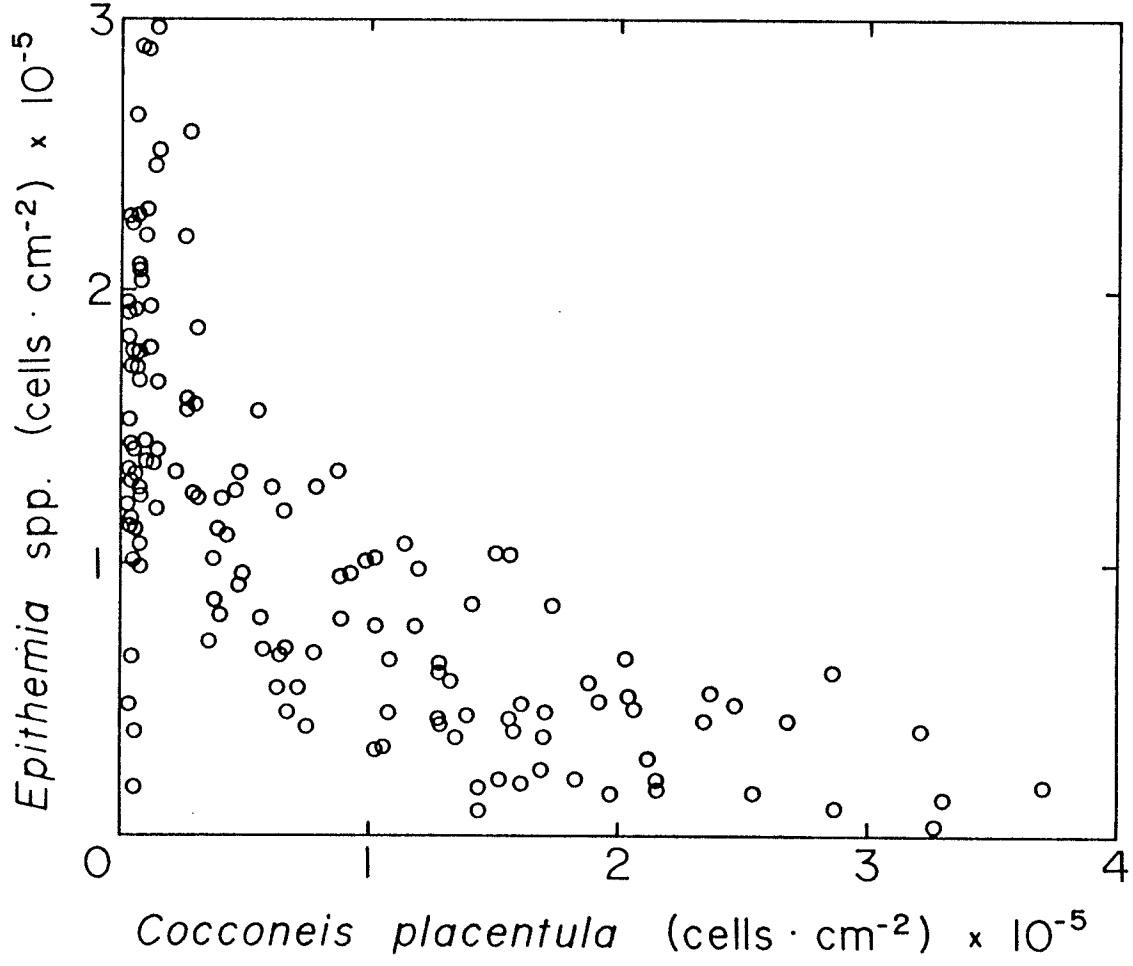
Additional evidence that Cocconeis actively competes for space on substrata is provided by data of the periphyton community structure colonizing vertically-positioned acrylic rods in the Delta Marsh (Robinson & Goldsborough unpubl.). In these samples, periphyton communities were dominated by C. placentula, Epithemia adnata and E. turgida, and evidence of an inverse relationship between the absolute density of Epithemia spp. and Cocconeis was found (Figure 20). Moreover, samples collected 12 days following substratum placement (29 July) were characteristically dominated by Epithemia, while those collected 4 weeks later (18 August) were predominately composed of Cocconeis. It appeared that Cocconeis was the superior competitor under the the prevailing environmental conditions by week 4, although the basis upon which predominance was achieved could not be determined. Tuchmann & Blinn (1979) report a similar relationship between Epithemia and Cocconeis which they attributed to low ($< 18^{o}C$) temperature preference by Epithemia. In the present data, the densities of Epithemia spp. were consistently low (Table 10).

The suggestion that development of large populations of Cocconeis is favored by low light intensity is particularly attractive, as it could explain why Cocconeis may be outcompeted in a spatially dense community by individuals of increasing physical stature. Several lines of evidence, however, suggest that this explanation may be too superficial.

Firstly, the correlation of Cocconeis density with low ambient light intensity is based on the observed environmental distribution of Cocconeis rather than on determination of its photosynthetic saturation characteristics and adaptability. Secondly, the presence of low light conditions in an environment where Cocconeis is reported does not a priori assume dominance by Cocconeis; although this taxon is abundant on many habitats in the Delta Marsh, it is notably rare on the undersides of dense mats of Lemna minor (Goldsborough & Robinson 1985) where ambient light intensities are low. Thirdly, Cocconeis achieved dominance in the experimental enclosures used here despite high water clarity (n = 0.0302 cm^{-1}), and light intensity (up to 650 $uE \cdot m^{-2} \cdot s^{-1}$)(Chapter 2). These observations imply that the occurrence of Cocconeis under low light conditions is not based on physiological requirements.

The ability of Cocconeis to proliferate under conditions of low light may be reconciled with the present observations by presuming that the role of light is in determination of total periphytic biomass and hence density of resource competitors. If it is then assumed that Cocconeis is an opportunistic competitor (irrespective of light intensity), its dominance in mid-late summer (when shading by macrophytes reduces competition) and at greater depth (Godward 1937) and in winter (Hickman 1982) are consistent.

Figure 20: Apparent relationship between the density of Cocconeis placentula and Epithemia spp. (E. adnata and E. turgida).


Epithemia spp. (cells · cm-2) x 10-5
Cocconeis placentula (cells · cm-2) x 10-5
0
1
2
3
4

Data concerning the role of grazers in determination of Cocconeis populations are contradictory. Dickman & Gochnauer (1978) concluded that intensive grazing pressure by benthic invertebrates prevented C. placentula from out-competing other species for space on tiles placed in a small stream. Conversely, Kesler (1981) found that selective grazing of other algae by the snail Amnicola resulted in the proliferation of Cocconeis. Moreover, Higashi et al. (1981) determined that the proportion of Cocconeis in the faeces of the snail Sinotaia historica was less than its proportion of the periphyton community, while those of other species were the same or greater. In the present investigation, intermittent observations of colonized acrylic rods which had been grazed (predominantly by snails) indicated that little periphytic material escapes grazing. By virtue of its tightly appressed growth habit, in which a large portion of the raphidinate valve is effectively cemented to the substratum by a mucilage pad (Round 1981), it is not unreasonable to presume, however, that some Cocconeis cells would escape grazing. Medlin (1980) has invoked this explanation for the increased abundance of Cocconeis scutellum as a result of limpet grazing. Given the rapid rate at which Cocconeis populations increased on substrata in post-flood herbicide enclosures (maximum = 5.5×10^4 cells$\cdot$cm$^{-2}\cdot$d^{-1}), which is significantly greater than maxima reported for total diatom increments in other habitats (eg. 2.5×10^3 cells$\cdot$cm$^{-2}\cdot$d^{-1} - Stevenson 1983), cells which escape grazing

would be provided with a substratum virtually free of competitors and may be able to disseminate over the substratum very quickly to form nearly mono-specific stands.

Without experimental verification of assertions drawn here, there can be no definitive explanation for the shift in periphyton community structure resulting from herbicide exposures. A hypothesis which is offered, however, states that the temporal delay in the onset of succession brought about by herbicide exposure had two significant effects. One effect was a change in abiotic environmental factors to conditions which favored colonization by Cocconeis. It has been noted that Cocconeis achieves peak biomass in mid-late summer in the Delta Marsh (G.Kruszynski unpubl.), although this distribution may have bases more complex than mere seasonal change, and may include effects of nutrient supply, light and temperature on periphyton growth. Moreover, the incidence of maximum cover by aquatic macrophytes in the Delta Marsh appears to coincide with the incidence of Cocconeis and may relate to possible light effects discussed earlier. This explanation cannot, however, be applied in a macrophyte-free enclosure. Differences in mean ambient water temperature with time were minimal ($< 2^{o}C$) are unlikely to have a bearing on the present results.

The chemical environment in our experimentally manipulated enclosures did not remain constant. Soon after herbicide addition, substantial increases in some dissolved nutrients

(ammonia, phosphorus, nitrate, silicon) and decreases in dissolved oxygen were detected (Chapters 2 and 5). Such effects have been widely documented although the cause is yet unclear. Many attribute these changes to release of labile compounds during plant decay (eg. Anderson 1981), while experiments using pairs of bottomed and unbottomed enclosures indicate that stimulated sediment efflux may be involved (Chapter 5).

As a result of enclosure flooding, nutrient levels in all enclosures decreased. Within 3-4 days, nutrient concentrations in all terbutryn-treated enclosures, and the 5.0mg·L^{-1} simazine enclosure increased. Sixteen days after flooding, decreases in concentration of ammonia and silicon did occur in the 0.01mg·L^{-1} terbutryn treatment. This latter observation may be significant, as the largest Cocconeis population was established in this enclosure.

A second significant effect of the herbicide treatment which may have favored Cocconeis was the reduction of total periphyton density (and hence resource competitors) at the time of substratum recolonization by resident species. It is plausible that Cocconeis predominates because it is best able to colonize at the earliest possible time, grows quickly and is able to deplete a limiting resource before potential competitors can become established. Circumstantial evidence suggests that Cocconeis is at least partially tolerant of the herbicides used here and so might be expect-

ed to be an early colonist in enclosures in which low (but still phytotoxic to many species) herbicide concentrations existed. It was abundant in the post-flood 5.0mg·L^{-1} simazine enclosure, and it maintained an apparently viable population (increase in density with time of ca. 457 ± 157 cells·cm^{-2}·d^{-1}) in the 1.0 mg·L^{-1} post-flood terbutryn enclosure. Moreover, intact frustules of C. placentula (containing cytoplasm and plastids) were observed in all experimental enclosures prior to flooding. The implication is that C. placentula is either able to photosynthesize in the presence of sufficient quantities of the herbicide to reduce total biovolume (detectable levels of carbon assimilation were observed in all pre-flood herbicide-treated enclosures, although the contribution by Cocconeis is unknown) or can maintain itself in the face of photosynthetic inhibition through heterotrophy. This later assertion is not unreasonable given the demonstration of glucose uptake by this species (Pip & Robinson 1982) and the anticipated high organic levels in the marsh and the expectation that levels of organic compounds also increase as a secondary consequence of herbicide addition. Tuchmann & Blinn (1979) have cited the rarity of C. placentula on chemically inert artificial substrata and its abundance on adjacent macrophytes as evidence of a heterotrophic requirement of this species, although this observation is not corroborated here.

In conclusion, the responses upon which recovery of a community following stress is determined must be re-evaluated. Conclusions based on numbers alone provide some indication of the return of community function but not of structure. The present study points out the importance of considering both function and structure in toxicological assessment, for even short herbicide exposures had profound effects on periphyton community structure. Long-term monitoring will be necessary to establish whether these changes in structure are irreversible and whether they do have a bearing on the productivity of subsequent trophic levels.

Chapter IV

STRUCTURAL AND FUNCTIONAL RESPONSES TO SHORT HERBICIDE EXPOSURES

4.1 INTRODUCTION

A common supposition of herbicide usage in aquatic environments is that selective pressure may favor the rapid establishment of resistant algal biotypes given the short generation time of algal cells (Hurlbert 1975). Induction of varying degrees of herbicide resistance through mutation has been achieved in unispecific laboratory cultures using ultraviolet light and/or mutagenic chemicals (McBride et al. 1977; Galloway & Mets 1982), or simply through repeated exposure to the herbicide (Cho et al. 1972; Bednarz 1981). Observations of marked increases in periphyton biomass succeeding herbicide (diquat, endothall, copper) treatment of a small lake (Hodgson & Carter 1982) suggest proliferation of resistant populations. Attempts to experimentally elicit resistance in natural algal communities have, however, provided varied results. Based on *in vivo* chlorophyll fluorescence measurements, deNoyelles et al. (1982) concluded that a physiologically resistant community of phytoplankton had developed in ponds treated with 0.02 and 0.5 $mg \cdot L^{-1}$ atrazine. Shifts in community structure were also detected. deNoyelles & Kettle (1983) reported similar findings for si-

mazine-treated ponds. On the other hand, the use of a chronic dose (0.01 $mg \cdot L^{-1}$) of atrazine to induce resistance in a lotic periphyton community (Kosinski & Merckle 1984; Kosinski 1984) was unsuccessful.

Assessment of the physiological impact of herbicides on algae has largely centered on the calculation of dose-response curves for cultures of individual clones (eg. Walsh & Alexander 1980; Blanck et al. 1984; Stratton 1984). What this approach neglects in extrapolation to potential effects on natural periphytic algal communities is the complex series of inter- and intraspecific interactions which can exist in a spatially complex algal mat. Evidence for the establishment of resistant populations in natural phytoplankton assemblages based on observed changes in specific proportions (eg. Schwartz et al. 1981; Scott et al. 1985) following herbicide treatment may be misleading in the context of a periphytic community, since it might be unclear whether any such changes were the result of direct toxic effects or secondary effects of the herbicide on niche availability (attachment space, nutrient and light supply etc.). Nonetheless, the impact of a herbicide exposure is at least partially a function of specific resistance to the herbicide concentration in the adjacent medium, which in turn may be mediated by such factors as sorption/desorption processes, chemical and biological degradation etc. Evidence that the architecture of a periphyton community may influence the

levels of toxicity of a herbicide derives from studies in which mass transport limitation of organic and inorganic materials (including the herbicide 2,4-D) in dense periphyton mats (Neal et al. 1967; Bruno et al. 1982; Lewis et al. 1983) was demonstrated. Furthermore, O'Neal & Lembi (1983) suggested that self-shading in thick metaphytic algal mats could limit the effect of photosynthetic inhibitors. By extrapolation, the observation that increases in periphyton community stature with time through the growth of taxa possessing erect and pedunculate growth habits (Hoagland et al. 1982) may imply that increasingly shaded underlying vegetation could escape herbicidal effects. Such effects would be most pronounced in quiescent environments in which the period of exposure to the toxicant was short. While the widespread applicability of any interaction of community structure and chemical toxicity is yet unknown, it could in part explain Blanck's (1985) observation that the EC_{50} (concentration of a compound causing 50% inhibition of a metabolic process) to aliphatic amines of periphyton sampled from several rivers in Sweden varied with algal biomass.

To address the question whether herbicide resistance in natural marsh periphyton communities can be induced through short exposures, and to determine the extent to which community structure and development may bear on inherent sensitivity, two experiments were conducted (experiments C and F, Appendix A) in which colonization of artificial substrata

situated within littoral enclosures by periphyton was monitored prior to and subsequent to the addition of simazine. Temporal changes in algal biomass, community composition, and static sensitivity to herbicide (EC_{50}) of periphyton within a herbicide treated enclosure were compared to those of an unperturbed control.

'Resistance' is used conditionally throughout in the absence of specific physiological mechanisms and may include varying aspects of tolerance.

4.2 MATERIALS AND METHODS

In each experiment, two cylindrical enclosures were constructed (Chapter 2) and positioned in channels of the Delta Marsh, on the southern end of Lake Manitoba, Canada (99° 19'W, 50° 7'N) in water of ca. 60 cm depth. Submerged macrophytes contained within the enclosures were harvested 6 days prior to the beginning of the experiments.

Pre-scored artificial substrata (Goldsborough et al. 1986) were positioned vertically within each enclosure and a colonization period of 14 days was allowed prior to experimental manipulations. Technical grade simazine (>97.7% a.i.) was dispensed in a gauze sac in a quantity sufficient to give approximately 1.0 $mg \cdot L^{-1}$ in the enclosure water volume (ca. 300L) and was added to the water of one enclosure (designated 'H'). Regular squeezing of the sac in the first 2 days following addition ensured complete and rapid solu-

tion of the herbicide. The other enclosure (designated 'C') was maintained as an untreated control.

The concentration of simazine in both the treated and untreated enclosures was assayed at weekly intervals using ultraviolet spectrophotometry (Mattson et al. 1970). Surface water was concurrently sampled from within both enclosures and from the adjacent marsh and the concentration of ammonia, silicon and total reactive phosphorus (TRP) analyzed after the methods of Stainton et al. (1977). Dissolved oxygen concentration was measured using a YSI model 51B meter.

As a result of brief (ca. 14 hours) incidental enclosure flooding, the herbicide treatment of enclosure H in experiment 1 consisted of a 7-day exposure prior to flooding, and an 11-day exposure following readdition of herbicide 9 days after the flood. Biweekly sampling of algal substrata was timed so that the first collection preceded the initial herbicide addition by 1 day (week 1), the second followed the flooding by 6 days (week 3), and the last was made 11 days after the second herbicide treatment (week 5). Initial sampling of substrata from the two enclosures of experiment 2 was undertaken 1 day prior to herbicide addition (week 1) and subsequently at biweekly intervals (weeks 3 and 5). No enclosure flooding occurred during the experiment, although heavy wave action against enclosure H walls 3 days after the week 3 sampling may have resulted in limited water exchange with the surrounding marsh.

During each sampling, six substrata were randomly selected from each enclosure. Three 2 cm pieces were subsampled from a position on each corresponding to 30-36 cm above the sediment/water interface and were placed in tubes containing 20 mL triple-filtered water from the adjacent marsh (twice through Whatman GF/C, once through 0.45 um Sartorius cellulose nitrate filters). A 6 cm segment from the 36-42cm rod position was sampled into filtered marshwater and preserved with acid Lugol's for algal species enumeration. The priphyton on the remainders of each rod (50 cm) was detached with a stiff-bristled paintbrush and filtered onto Whatman GF/C filters for chlorophyll a analyses (Chapter 2). In the second experiment (weeks 3 and 5), these samples were subdivided into two even portions and one half analyzed for particulate phosphorus content (Stainton et al. 1977).

Tubes containing 2 cm rod segments received one of five simazine concentrations (0.1, 0.5, 1.0, 2.5, 5.0 $mg \cdot L^{-1}$ using 0.5 mL methanol as a carrier) via a randomized incomplete block design. Each treatment was triplicated. One half milliliter of methanol alone was added to three additional control samples. One mL standardized $NaH^{14}CO_3$ (ca. 0.5 $uCi \cdot mL^{-1}$) was added to each tube, which were then incubated in a growth chamber at 20-23°C for 4 hours at 50 $uE \cdot m^{-2}s^{-1}$. Samples were filtered through 0.45 um filters to collect dislodged cells and the activity of the rod segment and filter determined through liquid scintillation counting

(Chapter 2). Concurrent determination of available inorganic carbon in the incubation medium (APHA 1980) permitted the calculation of carbon fixation rates (ug $C \cdot cm^{-2} \cdot h^{-1}$) of periphyton.

Specific productivity (carbon fixed per unit chlorophyll) was utilized in EC_{50} calculations to standardize intra-substratum variability. Relativization of these values to the mean of the three control samples (100%) yielded the level of inhibition at each simazine concentration. EC_{50} (and corresponding 95% fiducial limits) of data for each enclosure on each sampling date was calculated by probit analysis (SAS 1982).

Determinations of the taxonomic composition of periphyton from preserved rod segments, and the correction of diatom counts for the inclusion of dead, intact frustules followed the methods previously described (Chapter 3). Total algal biovolume was calculated by multiplying mean cell volume of each taxon (determined from a sample of at least 20 cells of each taxon, with calculation based on the nearest geometric solid) by its cell abundance and summing the result for all taxa.

4.3 RESULTS

4.3.1 Experiment 1

No simazine was detected in water of enclosure C throughout the experiment. Following addition of herbicide to enclosure H immediately after the first substratum sampling, the concentration in the water remained at ca. 0.9 $mg \cdot L^{-1}$ for 8 days prior to flooding, after which a residual concentration of 0.26 $mg \cdot L^{-1}$ was found (Figure 21A). The presence of herbicide following enclosure flooding likely reflects incomplete replacement of enclosed water due to short flood duration. Readdition of herbicide subsequently increased the concentration to 1.26 $mg \cdot L^{-1}$, the highest level observed during the experiment (Figure 21A).

Pretreatment levels of dissolved oxygen and ammonia within the two enclosures and in the surrounding marshwater were similar (Table 11). By the second week of the experiment (following simazine addition to enclosure H), the oxygen level in enclosure H was 4.6 $mg \cdot L^{-1}$ lower than in the control, while ammonia and silicon levels were markedly higher. This trend continued throughout the experiment, with ammonia and silicon concentrations as much as 70 and 11 times greater than corresponding control levels respectively (Table 11).

Total cell biovolume was low at the first sampling date (Figure 22B). Rapid colonization occurred in the period between the first and second sampling in both enclosures,

Figure 21: Simazine concentration in enclosures treated with 1.0 $mg \cdot L^{-1}$.
A - experiment 1, B - experiment 2.

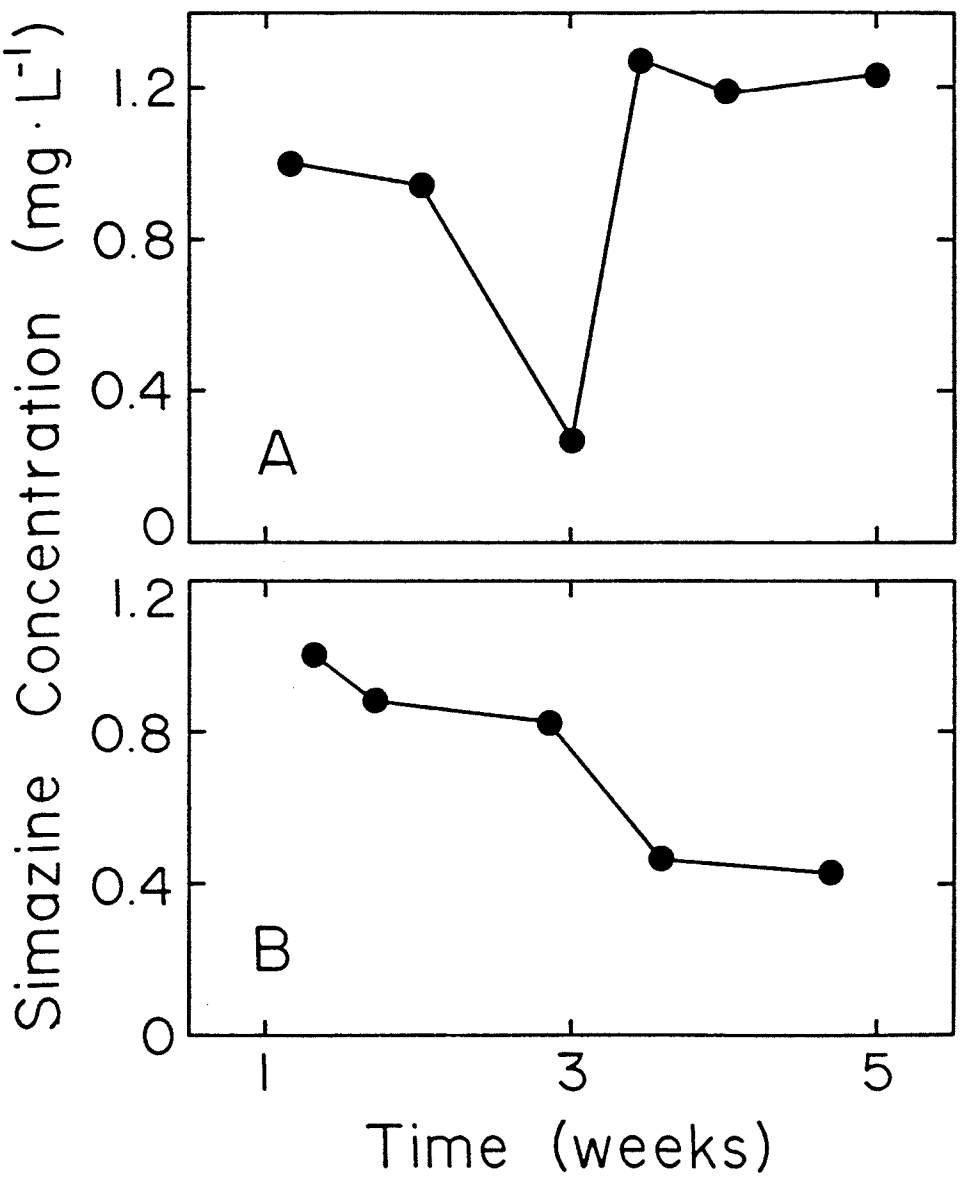

Simazine Concentration (mg·L⁻¹)
1.2
0.8
0.4
0
A
1.2
0.8
0.4
0
B
1
3
5
Time (weeks)

TABLE 11

Nutrient analyses results - experiment 1

Concentration (in $mg \cdot L^{-1}$) of dissolved oxygen, ammonia (NH_3-N), and silicon in water from a treated (H) and untreated (C) enclosures and in the adjacent marsh (M).

Week	Oxygen			Ammonia			Silicon		
	H	C	M	H	C	M	H	C	M
1	5.90	6.00	6.45	0.09	0.13	0.01	-	-	-
2	7.65	12.25	11.00	0.28	<0.01	<0.01	1.92	0.75	0.14
3	8.80	9.85	11.80	0.29	0.02	<0.01	1.18	0.34	0.14
4	10.20	13.90	14.80	0.60	0.02	<0.01	1.87	0.17	0.54
5	9.00	13.40	12.40	0.70	0.01	<0.01	3.42	0.16	0.83

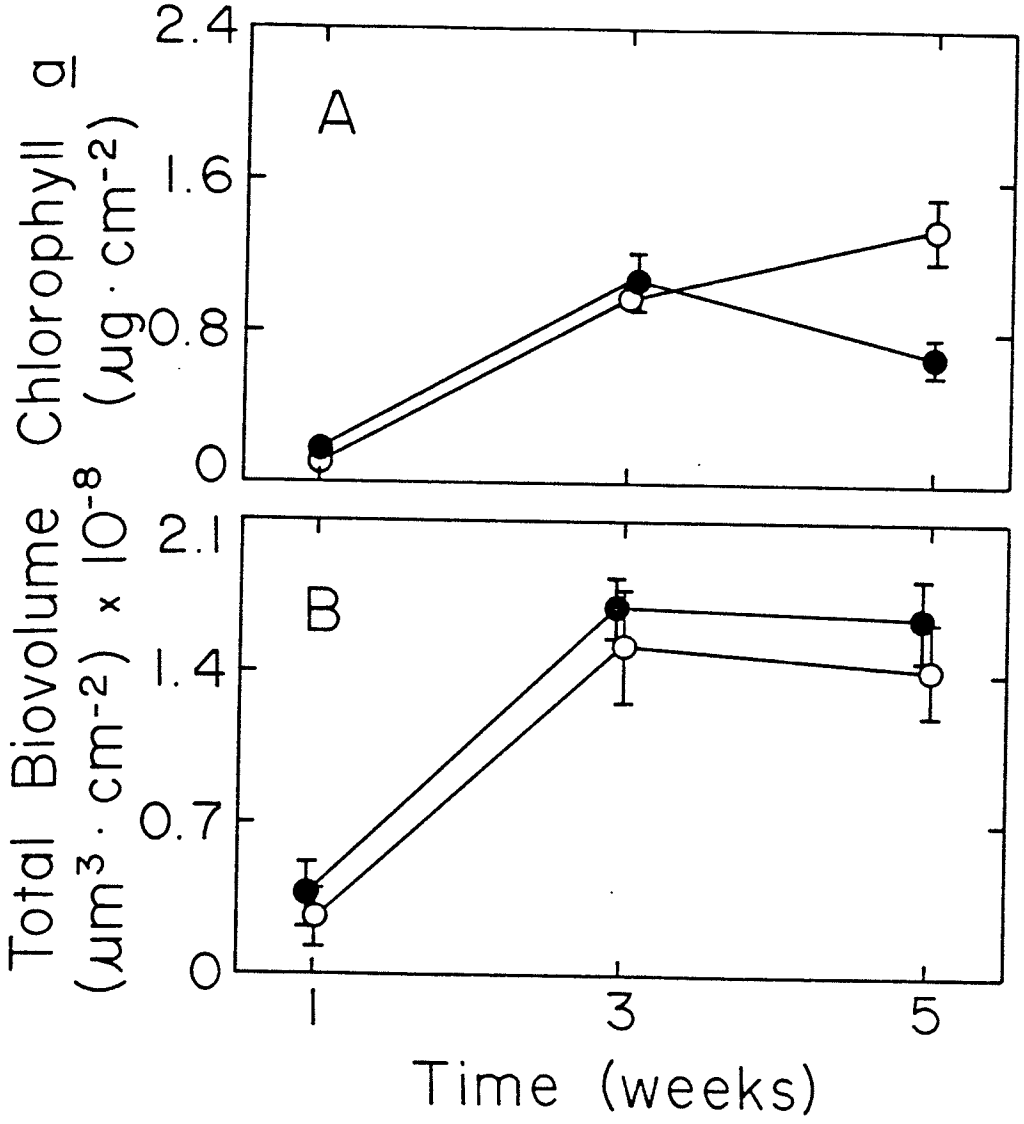
- no data

while a slight net decrease in biovolume had occurred by the final sampling. Overall mean biovolume on enclosure H substrata was greater than on control substrata (1.2×10^6 versus 1.0×10^6 $um^3 \cdot cm^{-2}$) although the difference was not statistically significant ($p=0.0723$).

Low pigment concentrations on the first week of sampling (Figure 22A) were coincident with low algal biovolume. No significant differences were observed between enclosures, although chlorophyll a levels were slightly higher in enclosure H. After the initial herbicide application, levels in H were only slightly less than in the control (week 3). Control substratum levels of chlorophyll a increased between the second and third sampling, while those in enclosure H decreased (Figure 22A) concomitant with an increase in pheophytin a (data not shown). Analyses of variance confirmed that the changes in pigment concentrations with sampling date were significantly different between the two enclosures ($p<0.001$) as a result of the large difference between the enclosures on the third sampling date.

Differences in C-fixation rate of periphyton on substrata from each enclosure (in marsh water containing no herbicide) were insignificant on week 1, but rates for enclosure H samples were substantially higher than those of corresponding control samples on week 3 (Figure 23). By week 5, enclosure H samples had increased slightly, while C samples decreased by ca. 20%. Overall mean rates in the H enclosure were sig-

Figure 22: Periphytic algal biomass (experiment 1) on artificial substrata positioned in the control enclosure (o) and the herbicide-treated enclosure (•).
A - chlorophyll *a* ± SD (n=6). B - total cell biovolume ± SD (n=3).


Total Biovolume Chlorophyll a
(μm³ · cm⁻²) x 10⁻⁸ (μg · cm⁻²)
2.4
1.6
0.8
0
A
2.1
1.4
0.7
0
B
1
3
5
Time (weeks)

nificantly higher ($p=0.0034$) than in the C enclosure. Calculations of specific productivity (carbon fixed per unit of chlorophyll a) suggested that levels of photosynthetic efficiency between the enclosures on week 1 were similar (2.2 and 1.5 ug C·ug chl $a^{-1}\cdot h^{-1}$ for C and H respectively). Control values decreased consistently over the subsequent two samplings (0.4 and 0.3) while those of enclosure H did not (1.2 and 1.9 for weeks 3 and 5 respectively).

The EC_{50} values of periphyton in the two enclosures prior to addition of herbicide were similar (Table 12). On subsequent sampling dates, the sensitivity of the enclosure C periphyton to simazine did not change significantly. Following the first herbicide addition to H, however, the EC_{50} for the periphyton in that enclosure increased markedly, although overlap of the 95% fiducial limits with those of the control suggests the difference was not significant. The week 5 EC_{50} (after the second herbicide application) was significantly greater than the control by two times (Table 12).

Periphyton colonizing the artificial substrata in the control enclosure was composed primarily of diatoms (>90% of total algal biovolume in all samples - Figure 24). The remaining biovolume was attributable to multicellular green algae. In general, no major taxon (>5% total biovolume) was observed in H which was not also present in the control, although significant enclosure differences in the densities of predominant taxa were observed.

Figure 23: Carbon fixation rate of periphyton (experiment 1) on artificial substrata sampled from the control enclosure (open bars) and the herbicide treated enclosure (shaded bars) at 6 levels of added simazine (± SD, n = 3).

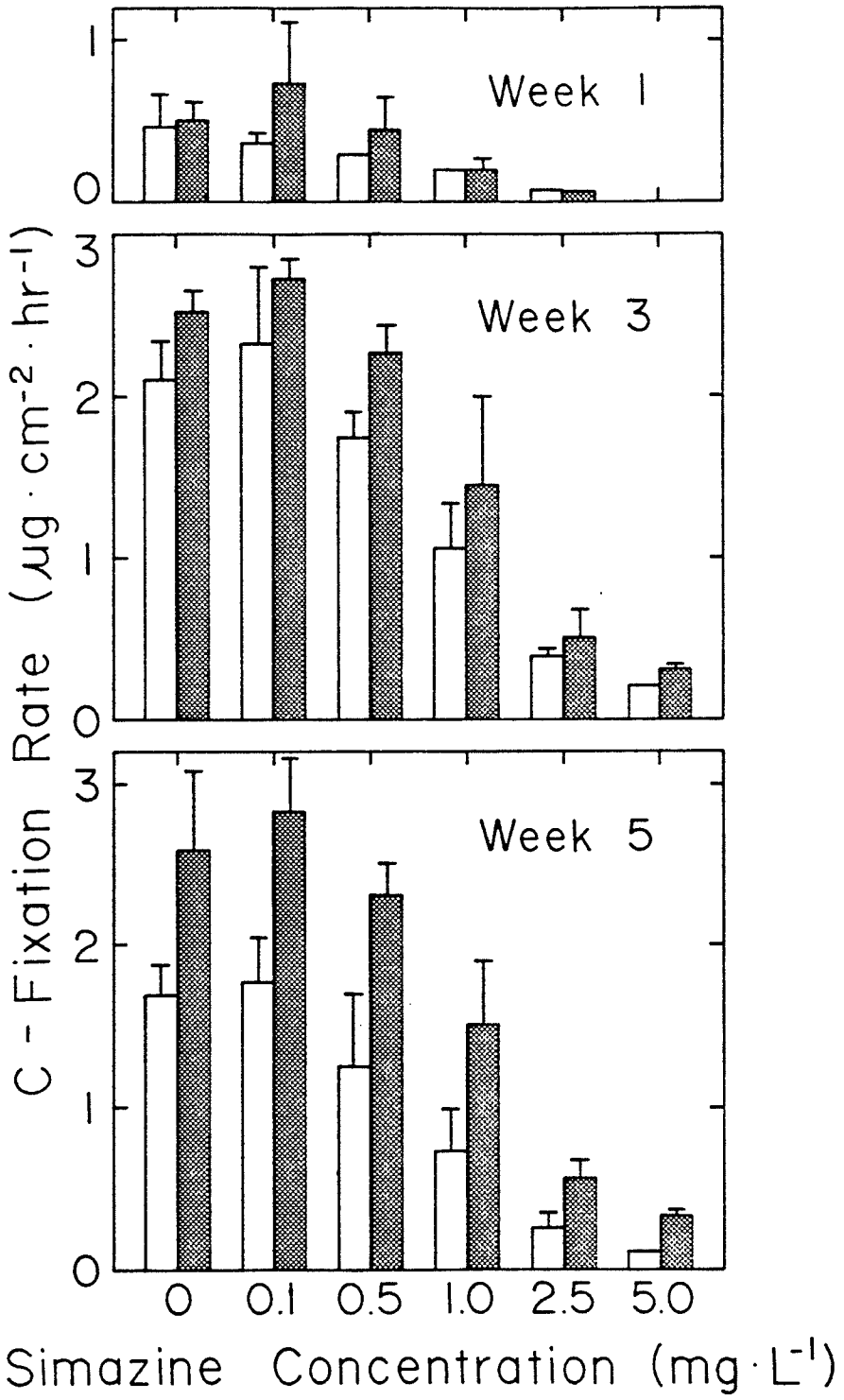

Week 1
Week 3
Week 5
C - Fixation Rate (µg · cm⁻² · hr⁻¹)
0
1
2
3
0
0.1
0.5
1.0
2.5
5.0
Simazine Concentration (mg · L⁻¹)

TABLE 12

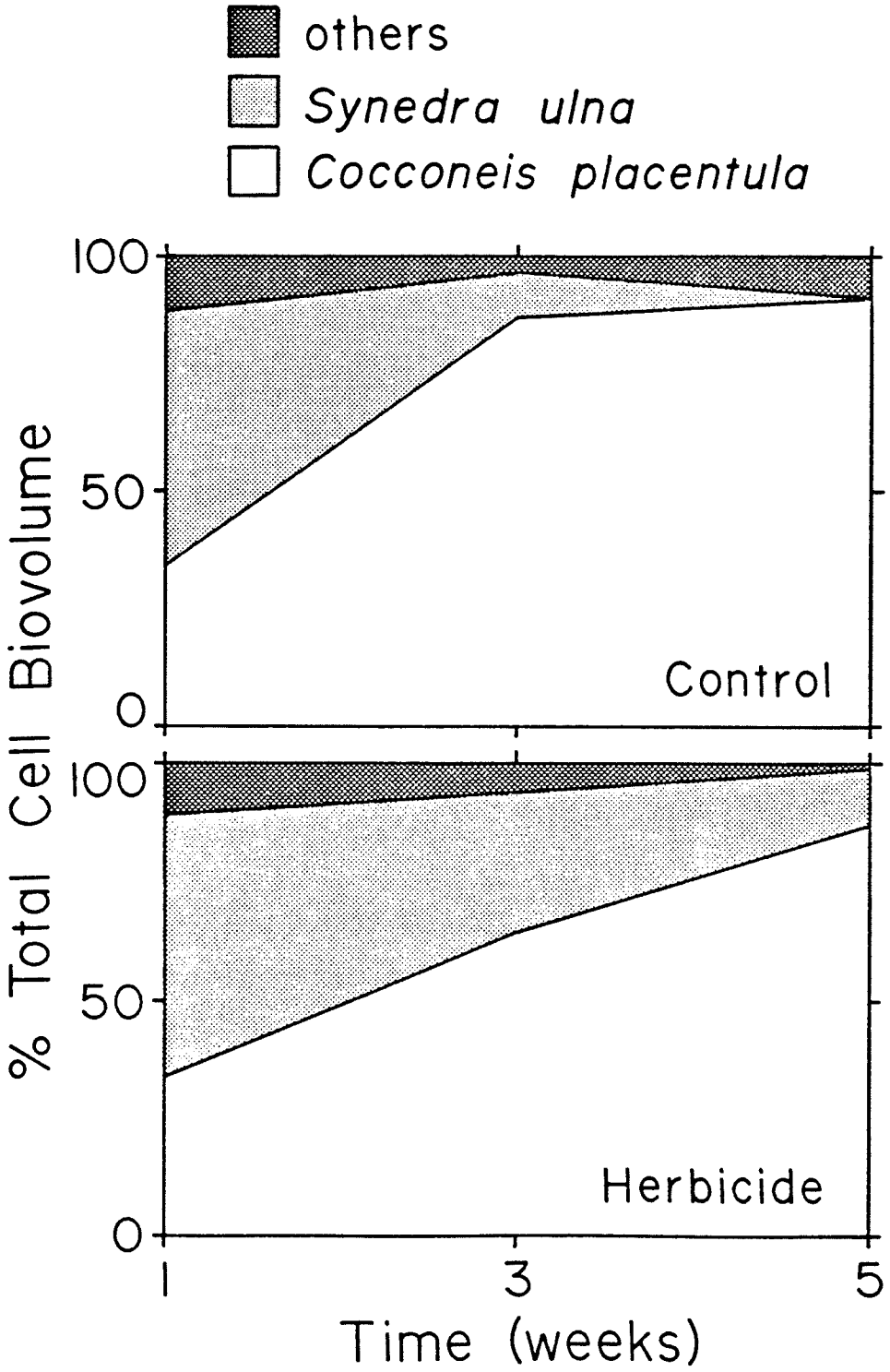
Estimated EC_{50} (+ 95% fiducial limits)

of marsh periphyton in two enclosures to simazine (experiment 1)

Week	Control	Herbicide
1	0.71 (-)	0.79 (-)
3	0.86 (0.71 , 1.04)	1.13 (0.81 , 1.55)
5	0.66 (0.52 , 0.83)	1.32 (1.00 , 1.74)

- data not available

Figure 24: Proportions of total cell biovolume (experiment 1) contributed by the dominant algal taxa on artificial substrata positioned in the control enclosure and the herbicide-treated enclosure. Data represent the means of 3 replicates.


others
Synedra ulna
Cocconeis placentula
100
50
0
Control
100
50
0
Herbicide
% Total Cell Biovolume
1
3
5
Time (weeks)

Cocconeis placentula Ehr. (including the varieties lineata and euglypta) was the most abundant taxon encountered in all samples and it comprised an increasing percentage of total biovolume with time (Figure 24). Its density in enclosure H increased to 5.7 ± 0.4 x 10^5 cells·cm^{-2} by week 5. Maximum density in the control was attained on week 3 (5.3 ± 0.5 x 10^5 cells·cm^{-2}). Differences between the two enclosures were not significant ($p>0.5$) although a significant interaction with time was due to the lower density of Cocconeis in enclosure H on week 3, and higher density on week 5 than in corresponding control samples. Two other common diatom taxa, Synedra ulna (Nitz.) Ehr. and Gomphonema parvulum Kutz. were slightly more abundant in H on week 1 than in control samples but were significantly more dense than on control substrata in the subsequent two samples ($p<0.001$).

Filamentous green algae made up the remainder of total algal biovolume in control samples (Figure 24). The three most abundant taxa (Stigeoclonium sp., Coleochaete scutata (deBreb.)Pring. and Bulbochaete sp.) exhibited similar changes in density with time; initial densities in the control enclosure were low (<1.5 x 10^3 cells·cm^{-2}) and reached relative maxima on week 5 (4.1 ± 1.3 x 10^4, 1.0 ± 0.5 x 10^4 and 0.2 ± 0.2 x 10^4 cells·cm^{-2} respectively). Corresponding densities in the treated enclosure were less than 2 x 10^3 cells·cm^{-2} on all sampling dates.

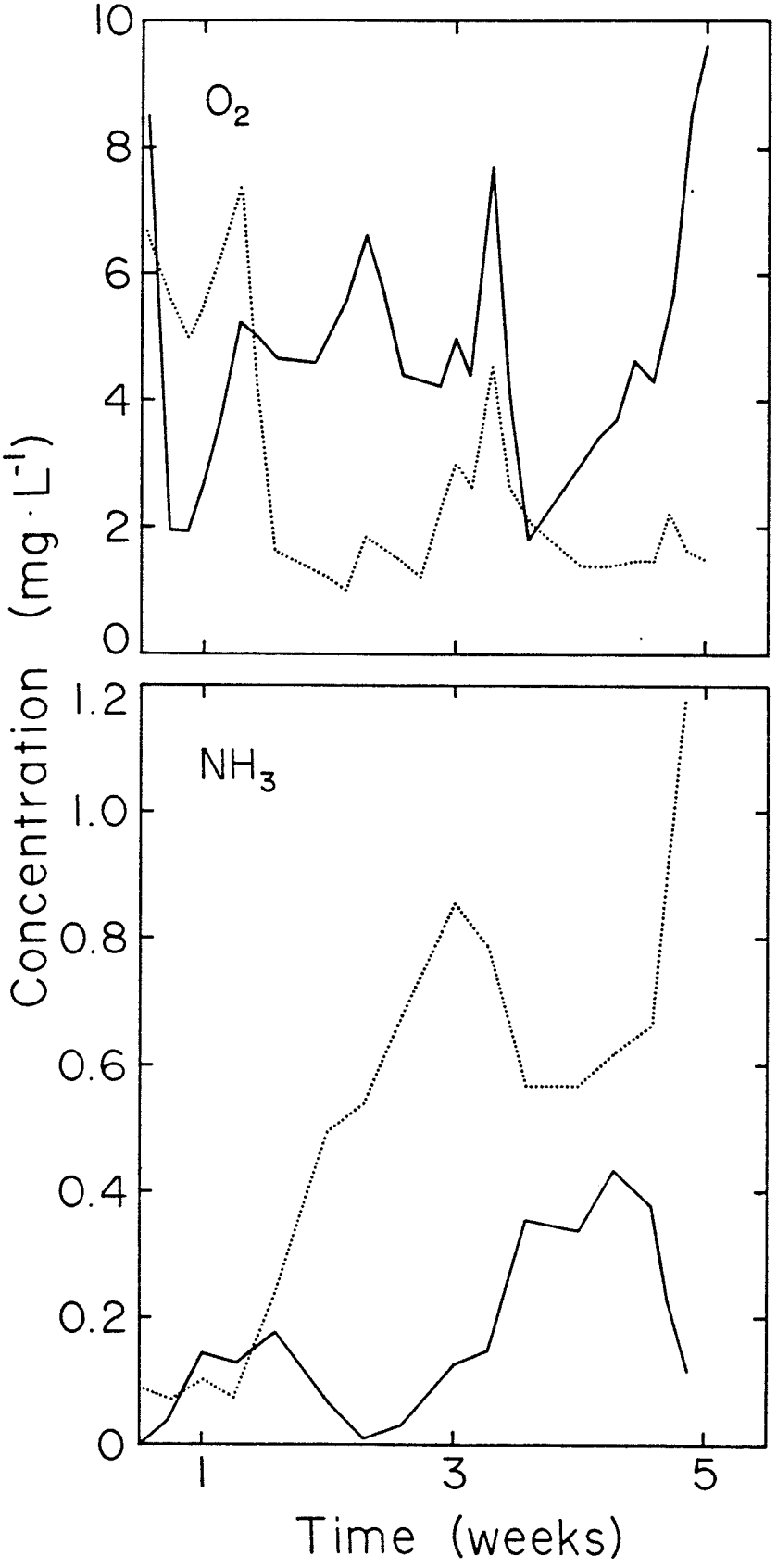
4.3.2 Experiment 2

No simazine was detected in water of the control enclosure throughout the 5 week duration of the experiment. The initial concentration in enclosure H decreased with time from ca. 1.0 $mg \cdot L^{-1}$ to 0.83 $mg \cdot L^{-1}$ by week 3 (Figure 21B). A sudden decrease a week later (to 0.46 $mg \cdot L^{-1}$) may be related to wave action against enclosure walls noted 6 days after the week 3 sampling, since the observed level on week 5 was not substantially different from the value of the preceding week.

Dissolved oxygen levels in both enclosures (Figure 25) decreased immediately prior to the first experimental manipulation. The level in enclosure C, however, increased to about 5 $mg \cdot L^{-1}$ for the following 2 weeks. An increase noted in enclosure H was followed by the marked decrease to ca. 1 $mg \cdot L^{-1}$ soon after herbicide addition. Aside from a short-lived increase around week 3, oxygen level in H remained low and was consistently lower (particularly by week 5) than those in the control enclosure.

Ammonia levels in enclosure C varied inversely with ambient oxygen levels (Figure 25B). Ammonia levels in the simazine treated enclosure were significantly greater than in the control, and increased more or less throughout the experiment. Dissolved silicon levels were not routinely determined in this experiment but were found to be insignificantly different between enclosures at the 5% level (data

Figure 25: Changes in concentration of dissolved oxygen and ammonia in the control enclosure (---) and the herbicide treated enclosure (···) over the 4 week duration of experiment 2.


10
8
6
4
2
0
O_2
1.2
1.0
0.8
0.6
0.4
0.2
0
NH_3
Concentration (mg·L^{-1})
1
3
5
Time (weeks)

not shown). TRP levels were measured on 2 dates (week 3 and 5) and were considerably higher in enclosure H (9 and 37 $ug \cdot L^{-1}$ respectively on week 3; 31 and 161 $ug \cdot L^{-1}$ on week 5).

Algal biomass (expressed in terms of total cell biovolume and chlorophyll a content) was higher on substrata collected from enclosure H than enclosure C on week 1 prior to addition of simazine (Figure 26). Reasons for this difference are unclear, but may relate to slight differences in water chemistry (ammonia, phosphate) noted between enclosures soon after their placement. Chlorophyll a levels increased consistently with time over the 5 week period in the control while in enclosure H they decreased slightly between weeks 1 and 3 but increased between weeks 3 and 5. Biovolume determinations were variable on week 3 (CV = 64%) but indicated that a slight increase occurred between each sampling period of the experiment.

Particulate P levels of periphyton from substrata in the control enclosure were consistently higher than in enclosure H (Table 13) but were not significantly different between sampling periods (week 3 and 5). Phosphorus levels on enclosure H substrata had increased by 50% over the same period.

Rates of carbon assimilation by periphyton within each enclosure (in the absence of herbicide) were significantly higher in enclosure H on weeks 1 and 3 than in the control,

Figure 26: Periphytic algal biomass (experiment 2) on artificial substrata positioned in the control enclosure (o) and the herbicide-treated enclosure (•).
A - chlorophyll *a* ± SD (n=6). B - total cell biovolume ± SD (n=3).

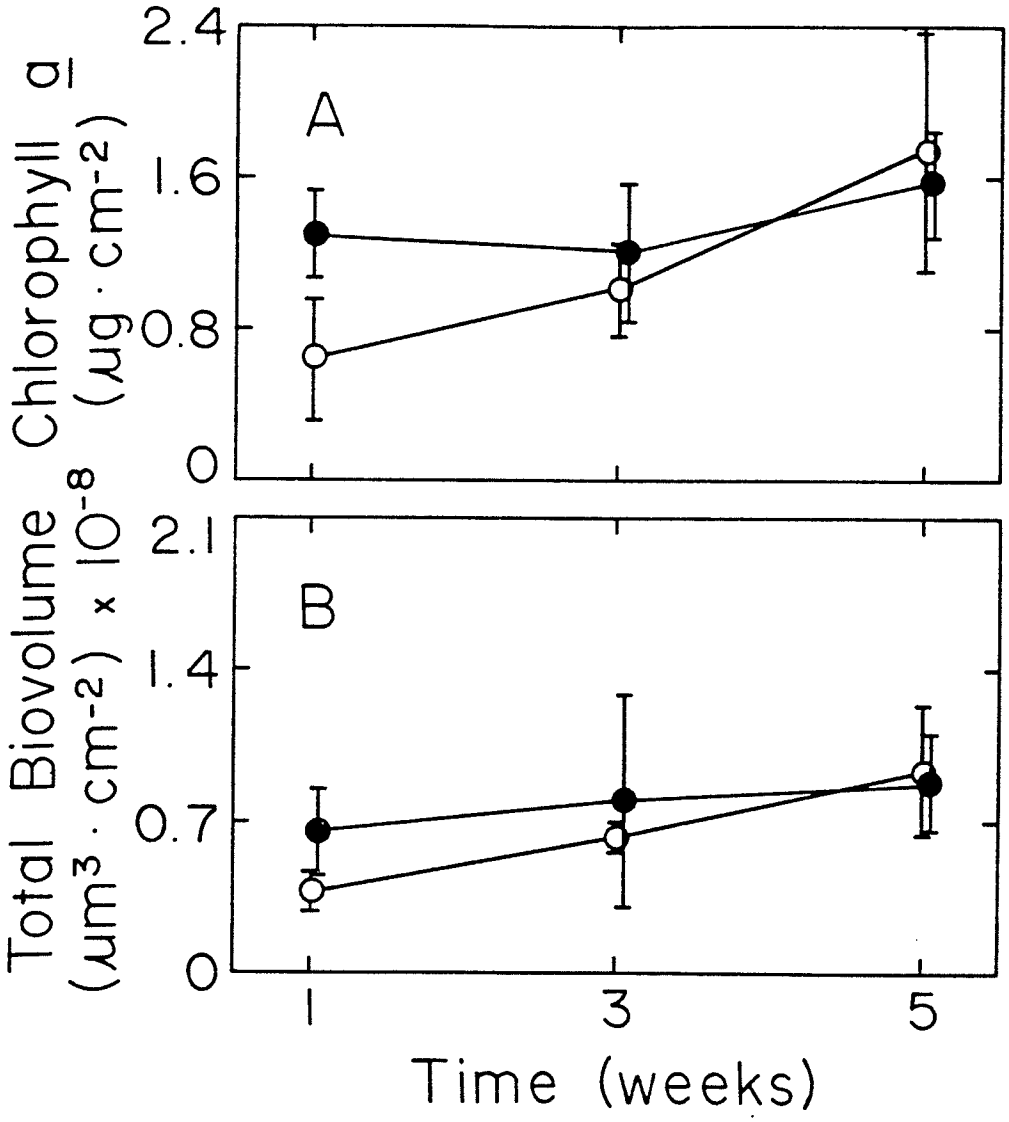

Total Biovolume ($\mu m^3 \cdot cm^{-2}$) x 10^{-8}
Chlorophyll a ($\mu g \cdot cm^{-2}$)
A
B
2.4
1.6
0.8
0
2.1
1.4
0.7
0
1
3
5
Time (weeks)

TABLE 13

Particulate phosphorus ($ug \cdot cm^{-2}$) of periphyton on substrata from control and simazine treated enclosures (experiment 2) ± SD (n=6)

Week	Control	Herbicide
3	0.87 (± 0.16)	0.41 (± 20.12)
5	0.87 (± 0.10)	0.66 (± 0.07)

but decreased to considerably less than control samples on week 5 (Figure 27). Rates of the control samples generally increased with time, although specific photosynthesis decreased consistently (2.4, 2.0 and 1.6 ug C·ug chl *a* $^{-1}\cdot h^{-1}$ for weeks 1,3 and 5 respectively). Photosynthetic rates of enclosure H samples on week 1 and 3 were similar but decreased on week 5. Specific photosynthesis rates exhibited a commensurate trend (1.9, 2.1 and 1.2 ug C·ug chl *a* $^{-1}\cdot h^{-1}$ for the 3 weeks respectively).

EC_{50} values of the control and herbicide-treated enclosure were not significantly different on week 1 (Table 14) and did not change over the duration of the experiment in enclosure C. Over the same period, EC_{50} of enclosure H periphyton increased (to a value significantly greater than that of the control) on week 3 and decreased on week 5.

The periphyton community in enclosure H was qualitatively dissimilar to that in enclosure C (Figure 28). *Gomphonema parvulum* and *G. subclavatum* (Grun.) Grun. were abundant on the former substrata but were notably rare (<1% total biovolume) in the control. This difference was maintained throughout the experiment, including substrata collected prior to addition of herbicide. *Rhoicosphenia curvata* (Kutz.) Grun.ex.Rabh. was slightly more abundant in enclosure H samples as well. Small individuals from the Order Chamaesiphonales were common only in the control samples on week 1.

Figure 27: Carbon fixation rate of periphyton (experiment 2) on artificial substrata sampled from the control enclosure (open bars) and the herbicide treated enclosure (shaded bars) at 6 levels of added simazine (± SD, n = 3).

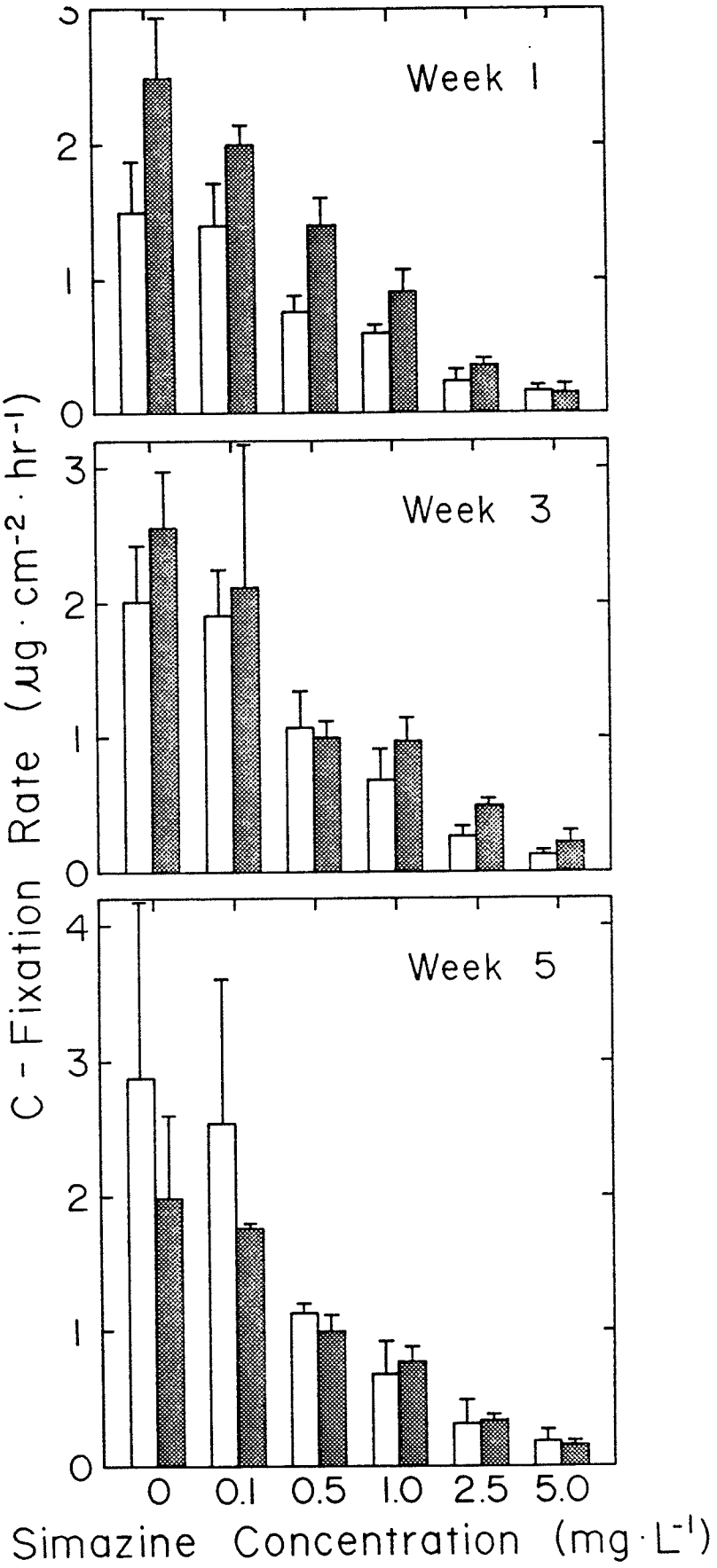

Week 1
Week 3
Week 5
C - Fixation Rate (µg · cm-2 · hr-1)
0
1
2
3
4
0
0.1
0.5
1.0
2.5
5.0
Simazine Concentration (mg · L-1)

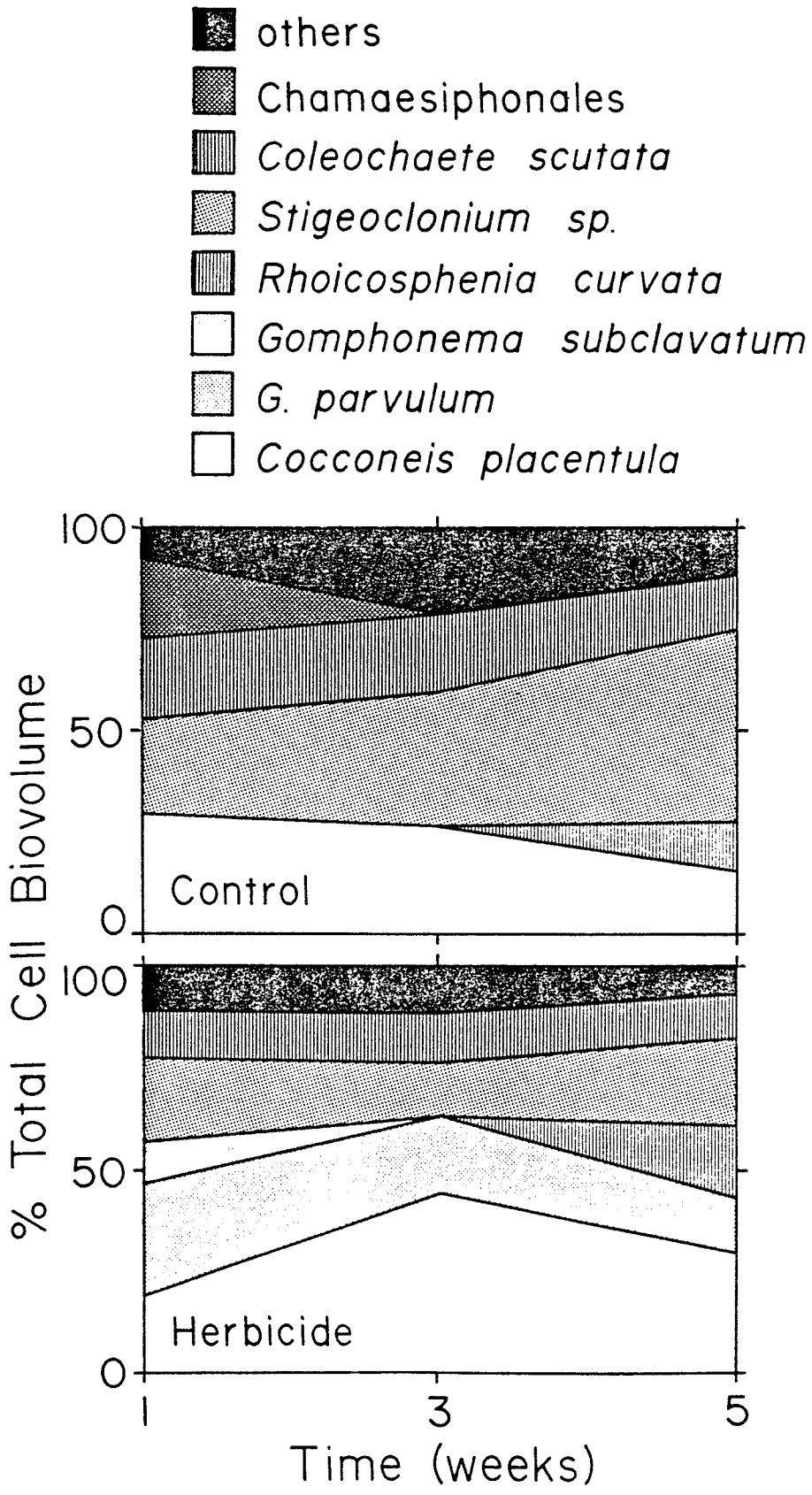
TABLE 14

Estimated EC_{50} (+ 95% fiducial limits)

of marsh periphyton in two enclosures to simazine (experiment 2)

Week	Control	Herbicide
1	0.55 (0.19 , 1.14)	0.66 (0.43 , 0.96)
3	0.51 (0.46 , 0.57)	0.85 (0.60 , 1.17)
5	0.61 (0.40 , 0.85)	0.65 (0.58 , 0.72)

Figure 28: Proportions of total cell biovolume (experiment 2) contributed by the dominant algal taxa on artificial substrata positioned in the control enclosure and the herbicide-treated enclosure. Data represent the means of 3 replicates.


others
Chamaesiphonales
Coleochaete scutata
Stigeoclonium sp.
Rhoicosphenia curvata
Gomphonema subclavatum
G. parvulum
Cocconeis placentula
100
50
0
Control
100
50
0
Herbicide
% Total Cell Biovolume
1
3
5
Time (weeks)

The density of Cocconeis placentula in the control community did not increase after week 1 and maintained a stable population of approximately 6 x 10^4 cells·cm^{-2}. Densities in enclosure H, where it generally formed a larger proportion of total biovolume, did not initially differ from the control, but increased on week 3 (1.4 ± 0.2 x 10^5 cells·cm^{-2}) and decreased slightly on week 5 (1.1 ± 0.2 x 10^5 cells·cm^{-2}). The densities of filamentous green algal taxa, which constituted the remainder of cell biovolume in both enclosures, were reduced in abundance in enclosure H on week 3 by as much as 50% from the preceding sample. At the same time, the density of Stigeoclonium sp., the major taxon of this group, increased with time in the control to reach a relative maximum of 4.5 ± 2.3 x 10^5 cells·cm^{-2} on week 5. Following the decline on week 3, its density increased on week 5 (from 1.0 ± 0.7 x 10^5 to 1.8 ± 0.3 x 10^5 cells·cm^{-2}) in the herbicide-treated enclosure. The density of Coleochaete scutata was similar in each enclosure on all dates, with the exception of week 3 samples (4.3 ± 1.3 x 10^4 and 1.0 ± 0.8 x 10^4 cells·cm^{-2} in enclosures C and H respectively).

4.4 DISCUSSION

While increases in nutrient levels and decreases in dissolved oxygen commonly follow herbicide treatment of enclosures (Chapters 2 and 5), the lack of substantial reductions in *in situ* algal biomass (as indicated by total cell biovolume and chlorophyll) with 1.0 $mg \cdot L^{-1}$ simazine additions found here (Figures 22 and 26) contradicts the relatively high level of inhibition noted at this concentration in an earlier study (Chapters 2 and 3). One explanation for this discrepancy may relate to the nature of the periphyton community which initially developed on experimental substrata. Whereas substrata used in the previous experiment were colonized by periphyton in the presence of herbicide (thereby 'selecting' for a community of more herbicide-resistant taxa), the precolonization period used here ensured that the algal community sampled on week 1 consisted of individuals of varying sensitivity. Thus, any inability to differentiate cells living at the time of collection from recently deceased cells containing cytoplasm in the latter case could result in erroneous estimates of total viable cell biovolume. This source of error would be most prevalent in the period immediately following herbicide addition as cells, which had colonized the substrata during the preceding 14-day period, inevitably accumulated as identifiably dead individuals. Although some evidence suggests that empty diatom frustules may persist on substrata for several months (Owen et al. 1979), no increase in the proportions of dead

cells were detected with time in samples from either enclosure (data not shown). Moreover, while the dead-cell estimates for individual taxa varied over a small range in all samples, differences between taxa were substantial and appeared to vary with the relative sizes of the organisms (Table 15). These ranges also overlap corresponding species estimates from the previous experiment (C.placentula 2.5-22.5%, G.parvulum 5.9-26.1%, S.ulna 32.9-46.9%). This leads us to believe that the 'dead' cells were largely artifacts of the relative susceptibility of individual taxa to breakage and loss of cytoplasmic stain during sample preparation (due to differences in cell morphology and frustule thickness). We therefore conclude that most algal cells enumerated in substratum samples were probably living at the time of collection and thus that undetected effects of simazine on cell biovolume estimates were small. Similar effects on chlorophyll-based estimates of algal biomass are also ruled out on the basis that reported chlorophyll a levels on substrata were corrected for the presence of pheophytin a (which would accumulate as the native pigment degraded in senescing cells).

A corollary to the differences in substratum colonization between experiments is that the structural development of the enclosure H community at the time of herbicide addition may have contributed to the apparent lack of effect of short exposures on algal biomass (on bases described in the Intro-

TABLE 15

Estimates of dead-cell proportions

of major diatom taxa in samples from both enclosures (experiments 1 & 2) and corresponding mean cell biovolume and length.

Taxon	Range % Dead	Biovolume ($um^3 \cdot cell^{-1}$)	Length (um)
Cocconeis placentula	9.5 - 22.5	250	9 - 35
Gomphonema parvulum	5.8 - 9.3	475	15 - 28
G.subclavatum	19.7	460	31 - 43
Rhoicosphenia curvata	1.9 - 3.5	475	13 - 32
Synedra ulna	34.8 - 49.4	10840	231 - 327

duction). This hypothesis can be disputed, however, on two grounds. Firstly, the prevailing communities in both enclosures of experiment 1 were dominated by sessile diatoms (chiefly Cocconeis) and rosette-like clusters of diatoms, and was therefore structurally simple (see, for example, the SEM photographs in Dickman & Gochnauer 1978 and Korte & Blinn 1983 in which C.placentula forms a two-dimensional 'pavement' upon substrata). Aside from the green algae, which were reduced in abundance in enclosure H and the slightly greater occurrence of erect and stalked diatoms, differences in architectural complexity between each enclosure on each of the three dates were slight and so likely affected neither the proximity of cells to phytotoxic levels of simazine nor the amount of light penetrating to the substratum surface. Secondly, in examining the EC_{50} of the control enclosure in both experiments, no significant change occurred between each of three sampling dates despite increases in biomass and (at least in experiment 2 where overlying green algae increased in density with time) structural complexity. Moreover, the community which developed in enclosure C during experiment 2 was arguably more complex (fewer sessile diatoms, more pedunculate and heterotrichous algae) than on corresponding dates in experiment 1. Yet, without exception, the EC_{50} values were less in experiment 2 than in experiment 1 (only significantly so on week 3); the reverse should have been true had the structure of the community ameliorated herbicidal impact in the former case.

The results of the current experiments can be reconciled with preceding results by the fact that, in each case, the herbicide concentration was not constant over the experimental duration. During experiment 1, for example, the first exposure lasted for only 7 days with the result that the community in enclosure H was exposed to a herbicide concentration approximately one quarter of the added amount for 1 week prior to the week 3 sampling. Since the level of inhibition of photosynthesis at this concentration was less than 10% (Figure 23), considerable growth could occur in this period. Estimates of the potential rate of accumulation of populations of C.placentula (Chapter 3) are fully consistent with the increase in density of 3.8×10^5 cells$\cdot$cm^{-2} of this taxon between weeks 1 and 3. The shorter period of time available for population growth in this enclosure (1 week versus 2 weeks in the control) may explain its slightly lower density as compared to the control on week 3. Subsequent herbicide levels were high (week 5) and substantial reduction in algal chlorophyll content was detected. Increases in the density of C.placentula were not as marked as in the preceding interval but nevertheless indicate that populations of this diatom can continue to grow in the presence of a photosynthetic inhibitor (see later).

While herbicidal effects on total algal biomass were small, changes in community structure were observed following herbicide addition on week 3, and more evidently on week

5 of experiment 1. Green algae were reduced in abundance while intermediate stages of succession (erect and pedunculate diatoms - after Hoagland et al. 1982) were more abundant. This is in agreement with previous findings and reinforces our contention that normal successional processes can be redirected by simazine, even though effects on total productivity may be less evident (Chapter 3).

Experiment 2 results are consistent with the hypothesis that observed community growth occurred in the period during which herbicide concentration was low enough to permit resumption of growth. The herbicide concentration was greater than 0.8 $mg \cdot L^{-1}$ for the first 2 weeks of the experiment and resulted in a slight decrease in algal biomass (Figure 26). Moreover, there was a significant effect of herbicide exposure in experiment 2 on particulate phosphorus levels on substrata, although the extent to which particulate phosphorus is correlated with algal biomass and exogenous supply is unknown. The residual concentration in the latter 2 weeks (0.46 $mg \cdot L^{-1}$) could have resulted in no more than ca. 60% inhibition of photosynthesis (Figure 27) so continued growth and the reestablishment of green algae were possible by week 5.

Interpolation of the results of a previous investigation suggested that the LC_{50} (herbicide concentration resulting in 50% reduction in biomass) of simazine for growth of periphyton lay between 0.1 and 1.0 $mg \cdot L^{-1}$ (Chapter 2). Biomass

(as indicated by chlorophyll *a* level, photosynthetic rate and total algal biovolume) was reduced to ca. 10% of an unperturbed control by 1.0 $mg \cdot L^{-1}$ simazine (Chapters 2 and 3). It is therefore clear from the level of inhibition of photosynthesis at 1.0 $mg \cdot L^{-1}$ in the control enclosures (23-46% of untreated samples, mean = 39%) that the EC_{50} values calculated here are overestimates of LC_{50} for periphyton growth. Since all sample tubes were mixed thoroughly and regularly during incubation, it is unlikely that the herbicide concentration to which periphyton was exposed was appreciably decreased by mass transport limitation within the mat in the incubation medium. Rather, the most probable explanation is that 4-hour herbicide exposures (the length of the incubation) do not adequately assess all effects of simazine on algal metabolism. Although the mechanism of action of simazine is in inhibition of photosynthetic electron transport (Moreland et al. 1959), secondary effects due to accumulation of toxic levels of nitrite (Ries et al. 1967), loss of RNA (Hawxby & Mehta 1977; Mehta & Hawxby 1979) and cellular and organelle membrane disruption (Mehta & Hawxby 1979; Dabydeen & Leavitt 1981) might also occur with increasing exposure time. The value of EC_{50} levels, then, lies in the ability to compare the effects of compounds on the potential autotrophic growth of samples collected on a spatial and/or temporal basis.

An implication of the discrepancy between EC_{50} and LC_{50} is that laboratory-generated dose-response data should be used cautiously in extrapolations to effects on growth of natural populations where the relative contributions of autotrophic carbon assimilation (the impact on which EC_{50} is a measure) and heterotrophy (either obligate or facultative) are unknown. It is not necessarily implicit that observed algal growth is the result of autotrophy alone; Pip & Robinson (1982), for example, demonstrated that many common epiphytic algal taxa which grow primarily through the incorporation of inorganic carbon may also be able to utilize organic carbon substrates. The employment of facultative heterotrophy by algal populations as a means of maintaining viability during short periods of photosynthetic inhibition (eg. Ashton et al. 1966) would be a worthwhile ecological strategy. This possibility cannot be discounted by the high rates of C-fixation of periphyton from enclosure H (0 $mg \cdot L^{-1}$ added simazine) because these samples were incubated in water from the surrounding marsh rather from the herbicide-treated enclosure (ie. under conditions conducive to autotrophic metabolism). While previous results have suggested that Cocconeis placentula (and perhaps other less abundant taxa) may possess heterotrophic capabilities (Chapter 3) in light of its growth in enclosures treated with of simazine and terbutryn, the fact that EC_{50} for simazine is greater than LC_{50} (assuming that any secondary physiological effects of the herbicide can be accounted for by the difference be-

tween EC_{50} and LC_{50}) suggests that the importance of heterotrophy in the community studied here, if present, lies in short-term cellular maintenance and not as a significant contributor to algal growth. The static measurement of EC_{50} also does not take into account any interaction of increased nutrient supply (Table 11, Figure 25) with herbicide toxicity on observed algal growth. Tubea et al. (1981) showed that increased nitrogen concentration could significantly increase effects of herbicides on growth of algal cultures, while our unpublished observations suggest that simazine toxicity to periphyton is less in the presence of high nutrient concentrations.

Rates of specific photosynthesis of periphyton samples from the simazine-treated enclosures were generally equal to or greater than corresponding rates of samples from unmanipulated enclosures. This suggests that EC_{50} estimates in the former case were not the result of an inhibited community responding to further additions (in laboratory inoculations) to a lesser extent than an unperturbed community. On this basis, the present findings indicate that herbicide resistance can develop in lentic periphyton communities after short (7 days) exposures. Such development, however, can only apparently occur at relatively high herbicide concentrations since comparisons of enclosure H EC_{50} (Tables 12 and 13) with ambient herbicide concentration (Figure 21) shows that significant increases in EC_{50} only occurred when

simazine concentration was greater than ca. 0.8 $mg \cdot L^{-1}$. If of widespread applicability, these data and the lack of inhibition at 0.1 $mg \cdot L^{-1}$ (Figures 23 and 27) may explain why Kosinski (1984) was unable to detect the development of resistance by lotic periphyton exposed to 0.01 $mg \cdot L^{-1}$ atrazine (chemically similar to simazine). Moreover, this level (0.8 $mg \cdot L^{-1}$) is generally higher than that found in streamwater following terrestrial runoff (Frank et al. 1982; Anderson et al. 1978; Smith et al. 1975) or used to control nuisance aquatic vegetation (eg. Schwartz et al. 1981) so would counterindicate the widespread development of resistance by native periphytic algal communities in response to short-term contamination.

A second implication of these data is that resistance, once developed, does not persist at herbicide levels less than 0.8 $mg \cdot L^{-1}$. The increase in EC_{50} noted in experiment 2 on week 3 was short-lived and was not significantly greater than for the control community on week 5, when herbicide levels had decreased. This suggests that resistance in general is conferred at the expense of ecological fitness (Conard & Radosevich 1979; Ahrens & Stoller 1983) and that resistant populations would only thrive under continued exposure. The 'threshold' simazine concentration noted above may reflect the point at which the disadvantage of poor competitive ability for resources is offset by the ability to grow in the presence of a herbicide.

Individuals of the Order Chamaesiphonales, which were previously found in abundance in herbicide-treated enclosures (Chapter 3), were common only in the control enclosure (experiment 2), indicating that their distribution is related to factors other than simple resistance to herbicide. There was little consistent taxonomic evidence to substantiate the development of a resistant algal population in simazine-treated enclosures, aside from the slightly higher densities assumed by Gomphonema parvulum and Synedra ulna in enclosure H (experiment 1). Whether these changes were the result of true resistance or redirection of succession as a consequence of the previous herbicide exposure is unknown, but changes in their densities did not correlate systematically with changes in the EC_{50} of the whole community. Similar attempts to relate the density of Cocconeis placentula with EC_{50} showed that if data from enclosure H (both experiments pooled) were used in correlation, a good relationship was evident ($r=0.96$, $n=6$) whereas the correlation was much poorer ($r=0.69$, $n=12$) if all data (irrespective of enclosure) were combined. This is due to the abundance of Cocconeis on substrata from both enclosures of experiment 1 despite marked differences in EC_{50} on week 5. The import is that if the relationship is valid, then the density of C.placentula is uncorrelated with level of resistance except under specific conditions (such as exposure to herbicide) which would further imply that an indistinguishable biotype had developed. Microscopic examination of individuals from

the populations in each enclosure revealed no significant ($p>0.5$) differences in cell size (frustule length, width, depth) or morphology, nor did the proportions of the two constituent varieties appear to change with treatment despite contentions that they have distinct ecological requirements (Kingston et al. 1983). Considerably more information on the autecology of C.placentula is necessary before the basis of this finding can be evaluated.

An interesting finding of the present experiments is that herbicide treatment did not lead to the development of monospecific stands of Cocconeis placentula, which was the result of a previous study (Chapter 3). The hypothesis invoked to explain those findings, that C. placentula is an opportunistic competitor able to gain community dominance on previously barren substrata in the absence of resource competitors, is, however, consistent with present data. In experiment 1, which was conducted in late fall (11 September - 9 October), C. placentula was abundant on substrata from both enclosures prior to herbicide additions. Records of seasonal patterns of abundance of major diatom taxa in the Delta Marsh (G.Kruszynski, unpubl.data) indicate that Cocconeis populations flourish in late summer. The substrata of experiment 1 were thus colonized at a time conducive to the development of Cocconeis, as were those in enclosures colonizing after simazine and terbutryn treatments (Chapter 3) in a similar seasonal period (18 August - 31 August). Of

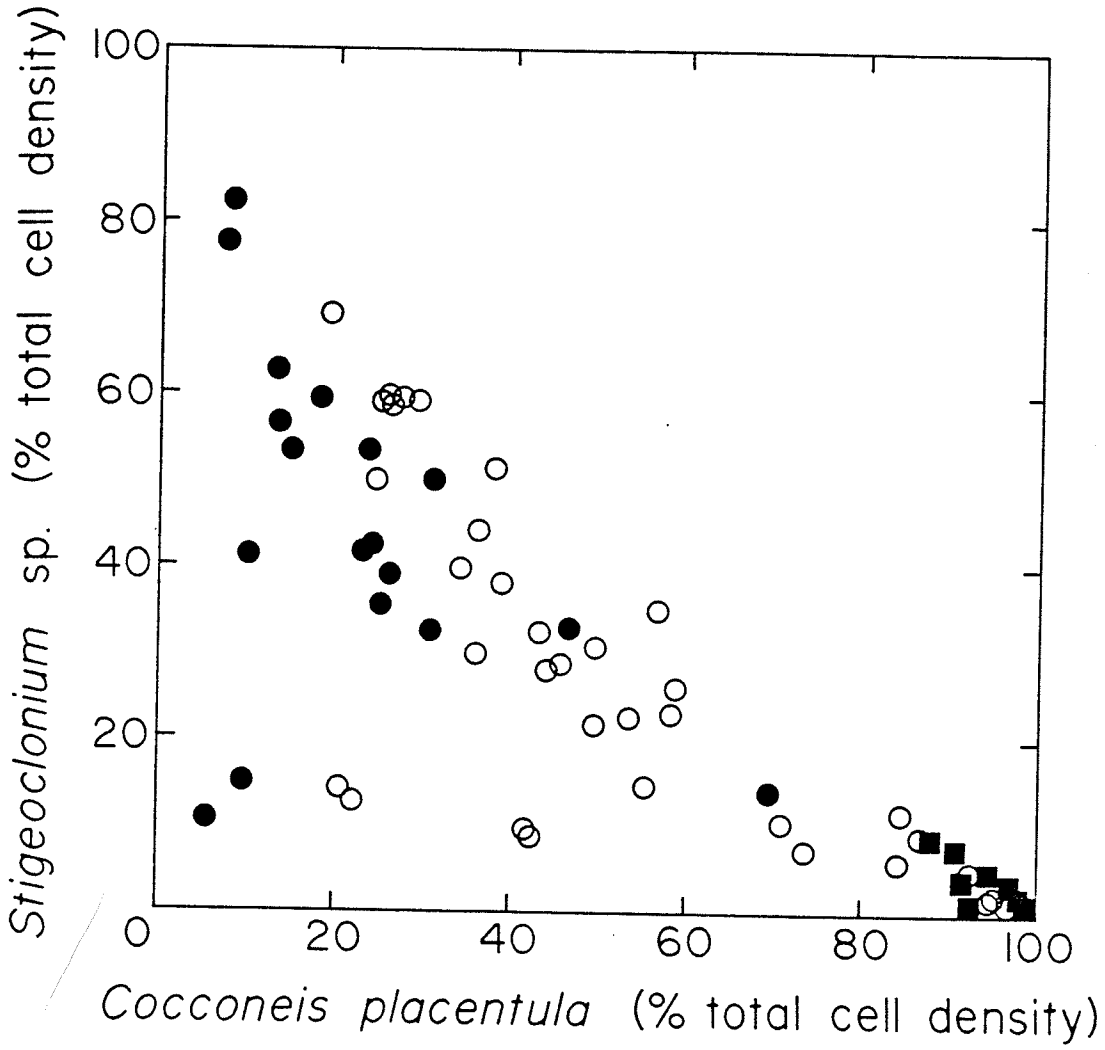
note, however, is the higher plateau population achieved by Cocconeis in experiment 1 (both in the C and H enclosures) than in the prior control (2.8×10^5 cells·cm^{-2}) while the proportion of green algae (particularly Stigeoclonium) was less. Experiment 2, on the other hand, was conducted in mid-summer (18 July - 15 August), when Cocconeis generally forms a smaller proportion of periphytic algal biomass. This is consistent with the much reduced population of Cocconeis seen in the control, while green algae (and to a lesser extent, pedunculate diatoms) were considerably more abundant. Taken together, the three experiments indicate that an inverse relationship may exist between the maximum density that Cocconeis populations can achieve on newly available substrata and the abundance of competitors (primarily multicellular green algae)(Figure 29). The apparent separation of the experiments along a temporal axis (Figure 29) indicates that the outcome of resource competition may have bases rooted in seasonally mediated factors and, significantly, that the specific outcome of a herbicide exposure may depend on seasonal timing of herbicide treatment. At the same time, the observation that the Cocconeis population increased in enclosure H (experiment 2) during the period in which green algal populations waned (week 3) and subsequently decreased when conditions (likely low herbicide concentration) favored green algal reestablishment indicates that Cocconeis is not favored by temporal change alone. The data of Fairchild et al. (1985) show that C.placentula and

Stigeoclonium tenue are favored by additions of nitrogen and phosphorus. If this is interpreted as evidence that both taxa are poor relative competitors at low nutrient levels but effectively compete at high relative nutrient status, our data indicate that C. placentula can surplant Stigeoclonium if given a further competitive advantage by selective inhibition of the green algae by herbicides (and perhaps secondarily by the increase in nutrient supply with herbicide treatment). In the original experiment, a substantial population of Stigeoclonium did not subsequently develop in the 3-week period following removal of herbicide, perhaps because Cocconeis was able to deplete nutrient supplies during a short period of competitive advantage to a level unsuitable for the green algae. Where Stigeoclonium becomes established, its heterotrichous growth habit renders it better able to maximize light and nutrient receipt over a wider absorptive area away from the substratum (Fairchild et al. 1985), while Cocconeis may be increasingly shaded and nutrient starved near the substratum.

In conclusion, the present experiments demonstrate that herbicide resistance can develop in periphyton communities following short exposures without necessarily resulting in gross taxonomic shifts. Changes in specific proportions must be interpreted in terms of resource competition as well as toxicological impacts on individual taxa. Finally, prolonged studies using relatively high simazine levels (> 1.0

Figure 29: Experimental summary comparing the relative proportions of total cell density contributed by Cocconeis placentula and Stigeoclonium sp. on artificial substrata following achievement of stable population size by C.placentula. (Experiment 1 - ■, experiment 2 - •, previous experiment - o).

mg·L^{-1}) will be needed to determine the feasibility of long-


Stigeoclonium sp. (% total cell density)
100
80
60
40
20
0
20
40
60
80
100
Cocconeis placentula (% total cell density)

term maintenance of community productivity through the development of resistant biotypes.

Chapter V

EFFECT OF SIMAZINE ON SEDIMENT NUTRIENT FLUX

5.1 INTRODUCTION

A commonly observed secondary effect of herbicide usage in freshwater is a pronounced and often sustained increase in dissolved nutrients (e.g. Michaud et al. 1979; Anderson 1981; Murphy et al 1981; Tucker & Busch 1982). Various mechanisms have been proposed to explain this phenomenon. One is that death of aquatic macrophytes following herbicide treatment allows release of sedimentary nutrient pools which would otherwise be assimilated by the plants (Fish 1966). Alternately, decreases in oxygen production and concomitant increases in bacterial activity (Fry et al. 1973; Ramsay & Fry 1976) may be indicative of nutrient release from labile inorganic and organic pools within decaying macrophytes (e.g. Michaud et al. 1979; Peverly & Johnson 1979; Anderson 1981). This is based largely on laboratory experiments such as those of Nichols & Keeney (1973) in which marked increases in nitrogenous and phosphorus compounds occurred following herbicide kill of macrophytes.

A third possibility is that the decline in dissolved oxygen which often occurs after treatment (e.g. Ashton et al. 1980; Boyle 1980; Murphy et al. 1981) is the important fac-

tor, since it has been observed that where deoxygenation does not occur, no increase in nutrients is observed (Brooker & Edwards 1975). This may be the result of increased rates of release of reduced compounds from the sediment as redox potential decreases under conditions of anaerobosis (Mortimer 1941; 1942). However, while Simsiman et al. (1972) and Wingfield & Johnson (1981) have demonstrated a decline in redox potential following herbicide addition to microcosms, data of Boyle (1980) suggest that redox changes in situ cannot adequately account for changes in nutrient concentration in all situations.

At the same time, several lines of evidence suggest that macrophyte decay cannot account for all of the observed changes in dissolved nutrients. Peverly & Johnson (1979) observed that the increase in the amount of nitrogenous compounds following use of diquat exceeded the amount available in the vegetation of the treated ponds. Moreover, in comparative studies using covered experimental enclosures with and without sediment contact, and with and without macrophytes, Kistritz (1978) showed that the amount of ammonia released by sediments during normal (in the absence of herbicide treatment) decomposition was approximately five times that released by macrophytes, whereas macrophyte decay yielded twice as much orthophosphate as sediment. Further, previous experiments (Chapter 2) have documented a substantial increase in ammonia and silicon coinciding with addi-

tion of simazine and terbutryn to enclosures containing no macrophytes and that the magnitude of the increase was related to the concentration of the herbicide. These observations can be explained by the work of Jansson (1980), which showed that inhibition of epipelon (that community which inhabits sediment surfaces) in sediment cores using mercuric chloride increased the supply of ammonia to the water column. Thus, some or all of the increase in certain inorganic nutrients may be a reflection of the amount which is normally intercepted by epipelic autotrophs.

To examine the possible role of sediment nutrient flux in response to herbicide treatment, an experiment was designed using littoral enclosures with and without sediment contact (none of which contained macrophytes) situated in the Delta Marsh (experiment G, Appendix A). Changes in concentrations of total ammonia, silicon, and total reactive phosphorus following addition of three concentrations of simazine were monitored; the difference in rate of change between enclosures was assumed to be due to the contribution of the sediments. Since the maximum increase in these nutrients following herbicide treatment occurs within 2 weeks (Chapter 2), the results of the first 23 days following addition of simazine are reported here.

5.2 MATERIALS AND METHODS

Eight PVC littoral enclosures (120cm high, 78cm diameter) were constructed as previously described (Chapter 2). Four enclosures were modified to include a sealed floor 90cm from the top of the enclosure (Figure 3B). These floors were constructed by attaching a 78cm diameter hoop of 1.2 x 0.6cm PVC to the inner surface of the enclosure with solvent cement. A PVC disc of appropriate size was glued onto the hoop and the joint between the floor and enclosure walls sealed with silicone rubber. Eight holes approximately 2cm in diameter were drilled in the enclosure walls just beneath the floor to allow water and air to escape from the space beneath the floor during placement of enclosures. Two smaller holes were drilled in the floor on opposite sides of the enclosure and hard plastic tubing was passed through the holes and glued so that the tubes extended from just beneath the floors to about 10cm above the enclosure. These tubes served as vents to release into the atmosphere any gases which accumulated under the floors during the experiment.

Experimental enclosures were positioned in Crescent Pond, a small, shallow pond (maximum depth=123cm) within the Delta Marsh on the southern end of Lake Manitoba in water of approximately 60cm depth. Macrophytes in the area were harvested 10 days prior to enclosure placement. Unmodified open-bottomed (OB) enclosures were pushed into the sediments to a depth of 35cm. Enclosures with sealed floors (SB) were

floated into position. As pond water was slowly added to the top, they settled into the sediments so that the floors rested on the sediment interface. Water addition continued until SB enclosures contained about the same volumes of water as OB enclosures (ca. 300L). Experimental manipulation of enclosures was delayed for 12 days following placement to allow disturbed sediments in OB enclosures to settle.

Unformulated technical grade simazine (98% a.i.) was dispensed into gauze sacs to yield final enclosure concentrations of 0.1, 1.0, and 5.0mg·L^{-1}. Herbicide treatments were assigned so that one SB and one OB enclosure received each herbicide concentration. Sacs were suspended in the water of each enclosure and were squeezed at regular intervals in the first 2 days following addition to ensure complete and rapid solution of herbicide. Two remaining enclosures (one SB and one OB) were maintained as untreated controls.

Water was sampled from each enclosure from a depth of 10cm several times prior to herbicide addition, and at daily intervals following treatment. Samples were analyzed for dissolved silicon, total ammonia (ammonia plus ammonium ion), and total reactive phosphorus after the methods of Stainton et al. (1977). Dissolved oxygen concentration was measured on a daily basis using a YSI model 51B oxygen meter. The concentration of simazine in water of treated enclosures was determined weekly using ultraviolet spectrophotometry Mattson et al. (1970).

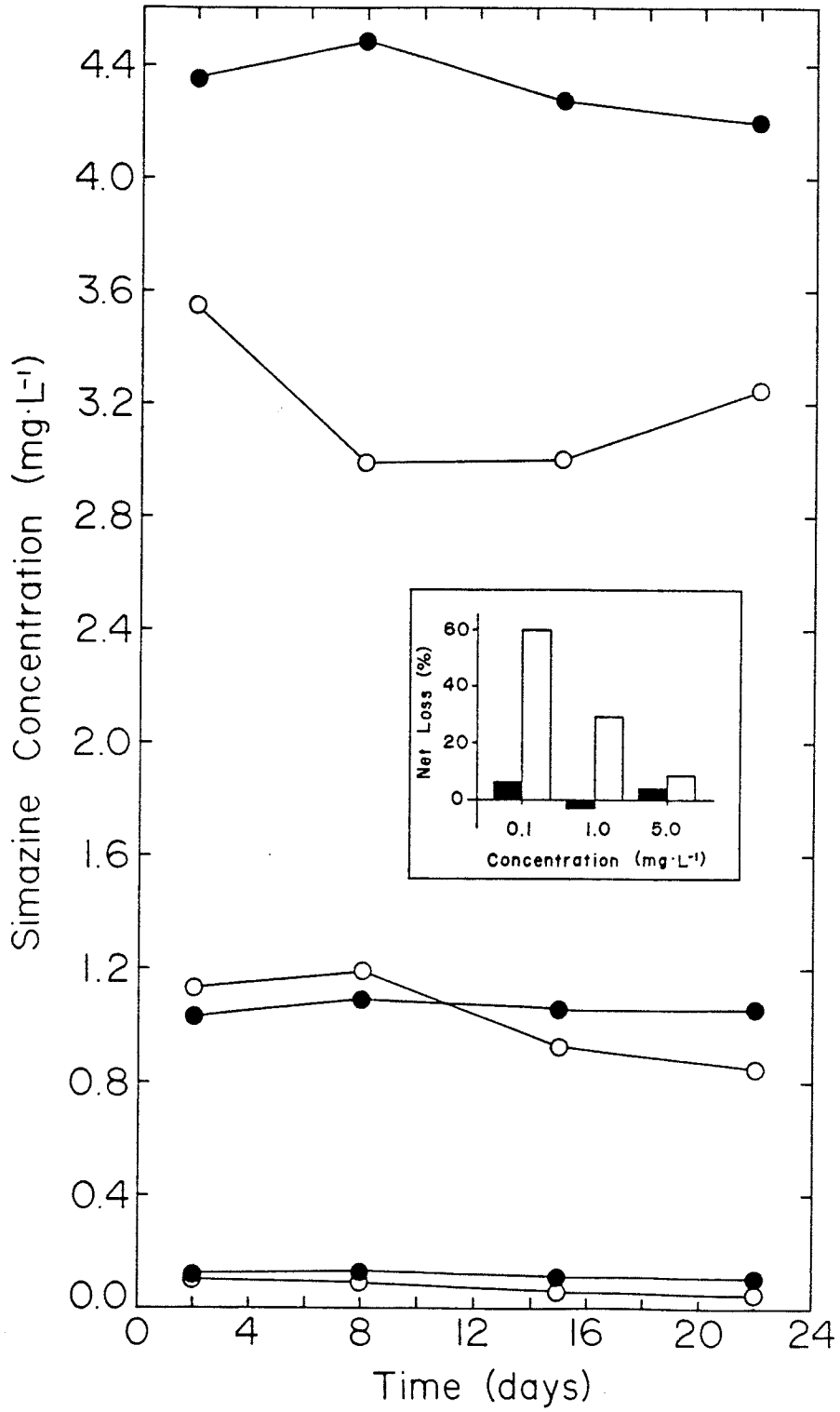
5.3 RESULTS

No simazine was detected in water from control enclosures. The concentrations of herbicide in each pair of treatments were initially similar (2 days post-addition) with the exception of the 5.0mg·L^{-1} treatment level, where the OB enclosure reached the maximum water solubility for simazine at 20°C of 3.5mg·L^{-1} (WSSA 1983). The SB enclosure exhibited a much higher initial concentration 4.3mg·L^{-1}), and decreased by only 4-5% over the experimental period (Figure 30). Concentrations in OB enclosures consistently decreased from initial levels by 8%, 30%, and 60% in the 5.0, 1.0, and 0.1mg·L^{-1} treatments respectively.

Within a few hours of enclosure placement, dissolved oxygen concentration in SB enclosures had decreased by ca. 2mg·L^{-1} whereas in OB enclosures, they had decreased by ca. 4mg·L^{-1}. Seven days later (5 days prior to herbicide addition), dissolved oxygen in SB enclosures was greater than or equal to outside waters, whereas in OB enclosures concentrations were ca. 2mg·L^{-1} lower than outside and were more variable than SB enclosures.

No change in oxygen was observed in the first 12 hours following herbicide treatment in any enclosure (Figure 31); however, deoxygenation began within 24 hours at higher treatment levels (1.0 and 5.0mg·L^{-1}). In the controls, very little change was observed over the 23 day period (Figure 31). A slight initial decrease occurred in the 0.1mg·L^{-1} OB

Figure 30: Assayed herbicide concentration in water of open-bottom (o) and sealed-bottom (•) enclosures treated with three concentrations of simazine. Inset: Amount of herbicide lost from the water of each enclosure during the 23 day period of the experiment expressed as a percentage of the initial concentration.


Simazine Concentration (mg·L⁻¹)
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
4
8
12
16
20
24
Time (days)
Net Loss (%)
60
40
20
0
0.1
1.0
5.0
Concentration (mg·L⁻¹)

enclosure during the first 8 days. In all pairs, oxygen levels were higher in SB enclosures, but progressively declined in relation to controls with increasing simazine concentration (Figure 31).

Significant differences in total ammonia concentration were observed in SB and OB enclosures prior to treatment, and were particularly evident in the enclosures assigned to the 0.1mg·L^{-1} OB treatment (Figure 32). These differences were first observed 5 days before treatment and changed little until addition of the herbicide. No changes in concentration occurred following addition of 0.1mg·L^{-1} simazine (Figure 32), but at 1.0 and 5.0mg·L^{-1} treatment levels, both OB enclosures showed detectably higher ammonia levels within 24 hours and increased consistently over the first 1-3 days. The corresponding SB enclosures showed no initial change (Figure 32).

Net sediment ammonia flux was calculated by determining the rate of increase in concentration in the first 15 days for all treatments (the slope of a least-squares linear regression), subtracting the SB value from the corresponding OB value and converting the resultant flux to mass per unit sediment area over time using the enclosure volumes (calculated from water depth) and enclosed sediment area (ca. 0.47m^2). These data (Table 16) indicate a small net release of ammonia by the control and 0.1mg·L^{-1} treatments over the experimental period, with a progressively larger rate of release at 1.0 and 5.0mg·L^{-1} application levels.

Figure 31: Dissolved oxygen concentrations in water of open-bottom (o) and sealed-bottom (•) enclosures treated with three concentrations of simazine.

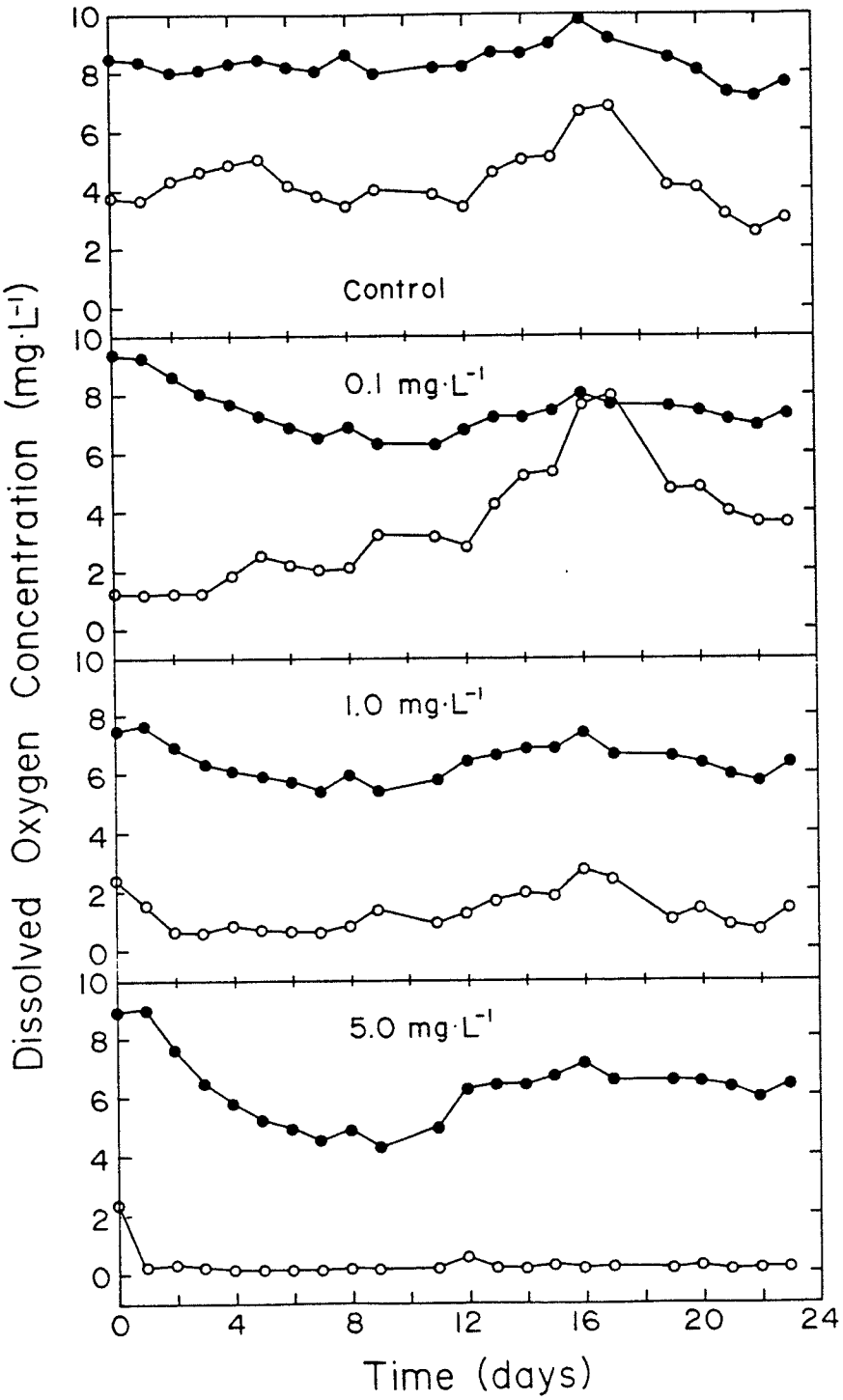

Dissolved Oxygen Concentration (mg·L⁻¹)
Control
0.1 mg·L⁻¹
1.0 mg·L⁻¹
5.0 mg·L⁻¹
0
2
4
6
8
10
0
4
8
12
16
20
24
Time (days)

Figure 32: Ammonia concentration in water of open-bottom (o) and sealed-bottom (•) enclosures treated with three concentrations of simazine.

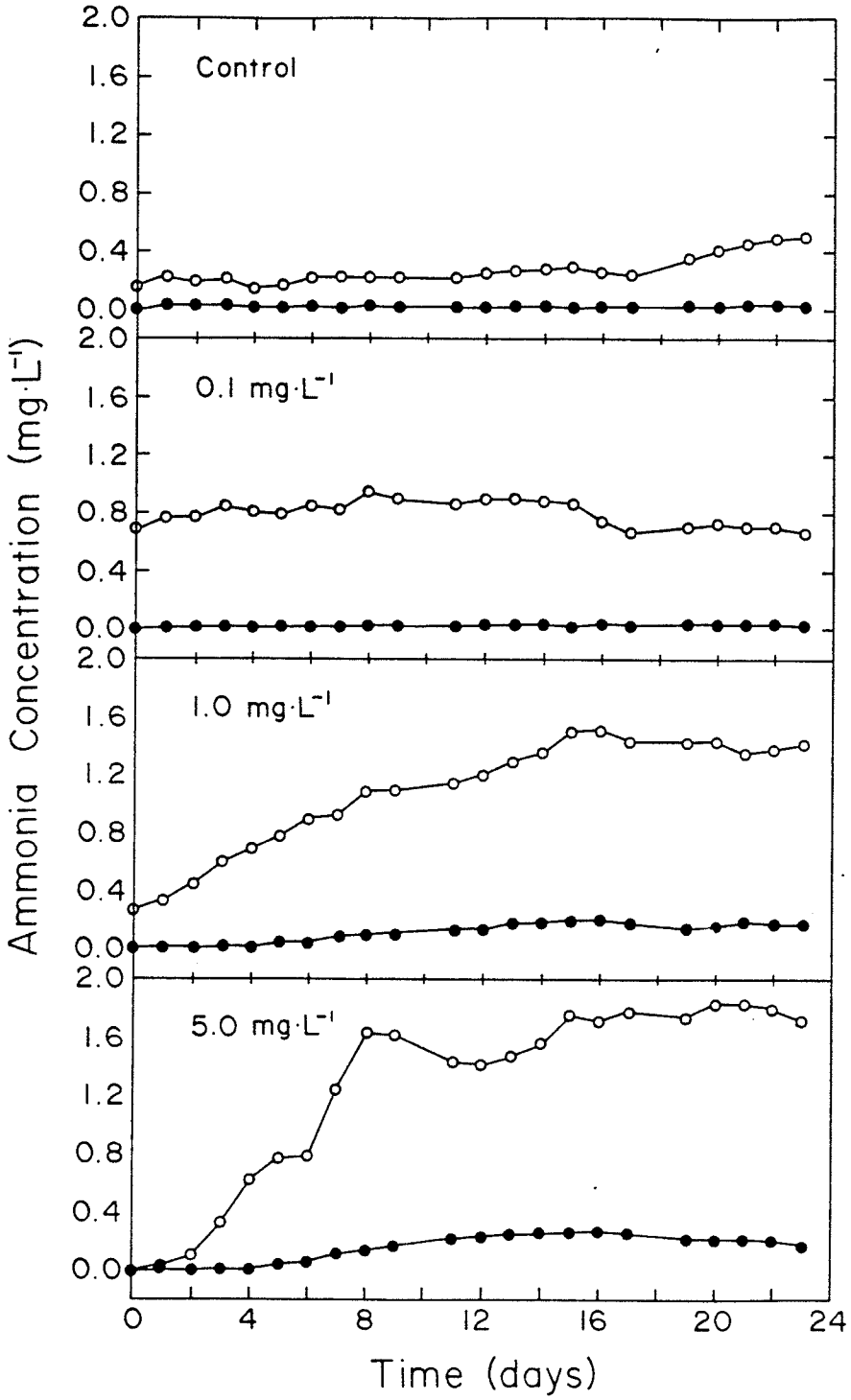

Control
0.1 mg·L⁻¹
1.0 mg·L⁻¹
5.0 mg·L⁻¹
Ammonia Concentration (mg·L⁻¹)
Time (days)
2.0
1.6
1.2
0.8
0.4
0.0
0
4
8
12
16
20
24

TABLE 16

Net sediment nutrient flux

of total ammonia, total reactive phosphorus (TRP) and silicon in response to three levels of simazine.

	Sediment flux ($mg \cdot m^{-2} \cdot day^{-1}$)		
Treatment	Ammonia	TRP	Silicon
control	4.63	0.49	5.25
0.1$mg \cdot L^{-1}$	4.68	-0.90	-92.95
1.0$mg \cdot L^{-1}$	35.79	5.34	207.59
5.0$mg \cdot L^{-1}$	64.58	19.65	247.95

Total reactive phosphorus (TRP) levels in the enclosures exhibited trends very similar to those of ammonia (Figure 33), with increases in OB herbicide-treated enclosures detected within 48 hours of addition. SB enclosure levels at 1.0 and 5.0mg·L^{-1} increased only slightly (Figure 33). Calculations of sediment flux using all data (Table 16) confirmed the increase in flux with increasing simazine concentration. Slight net sediment adsorption (negative flux) was observed at 0.1mg·L^{-1}.

Initial silicon level determinations were erratic due to analytical difficulties. No significant change occurred in the control and 0.1mg·L^{-1} enclosures following treatment, although levels in SB enclosures were near the limit of detection (Figure 34). Initial differences between SB and OB enclosures treated with 1.0 and 5.0mg·L^{-1} simazine widened following treatment, with marked increases in OB enclosures. Net sediment flux for silicon calculated using all data increased in proportion to increasing herbicide concentration (Table 16). As found for TRP, net negative flux was observed at 0.1mg·L^{-1} simazine.

Figure 33: Total reactive phosphorus concentration of open-bottom (o) and sealed-bottom (•) enclosures treated with three concentrations of simazine.

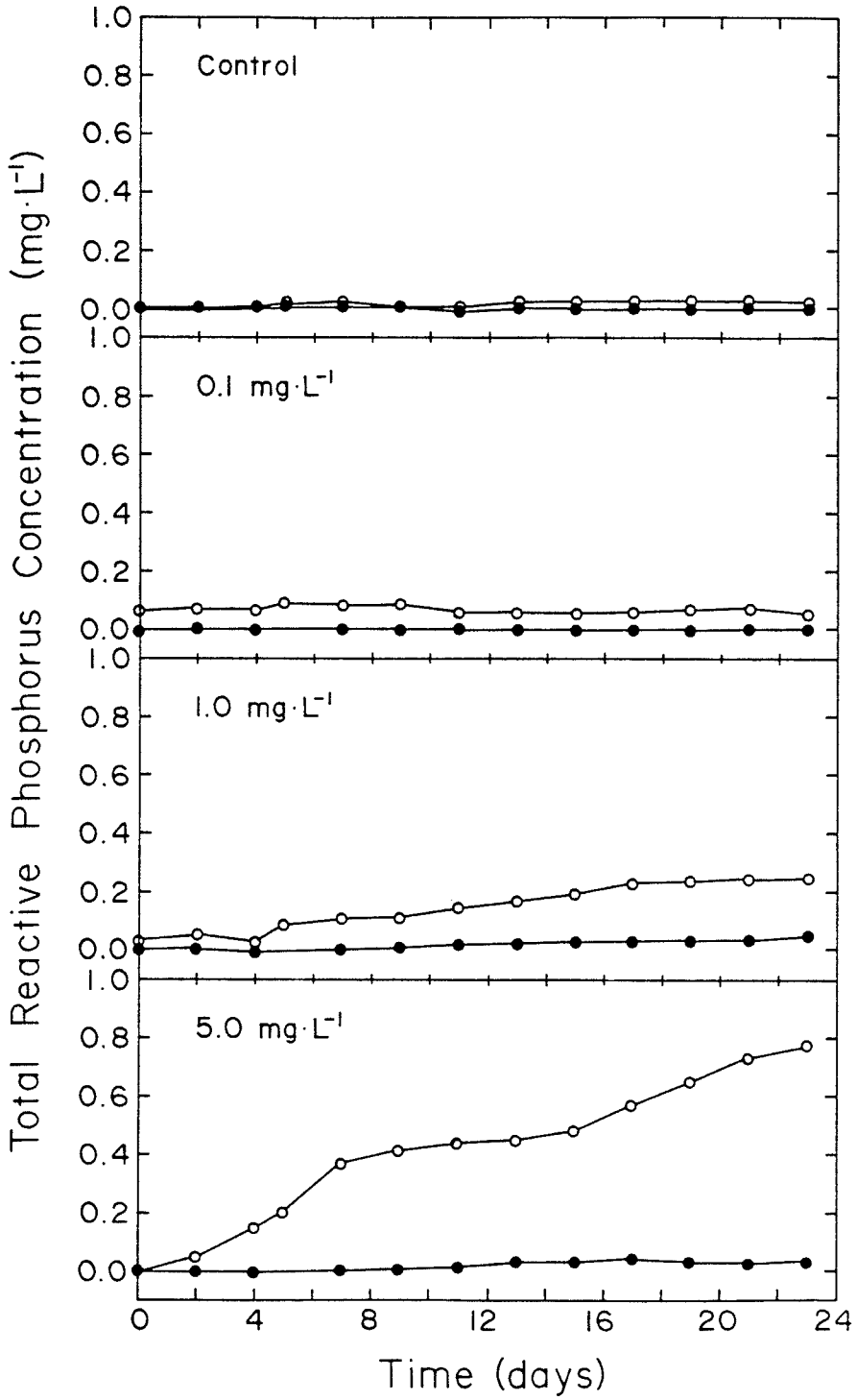
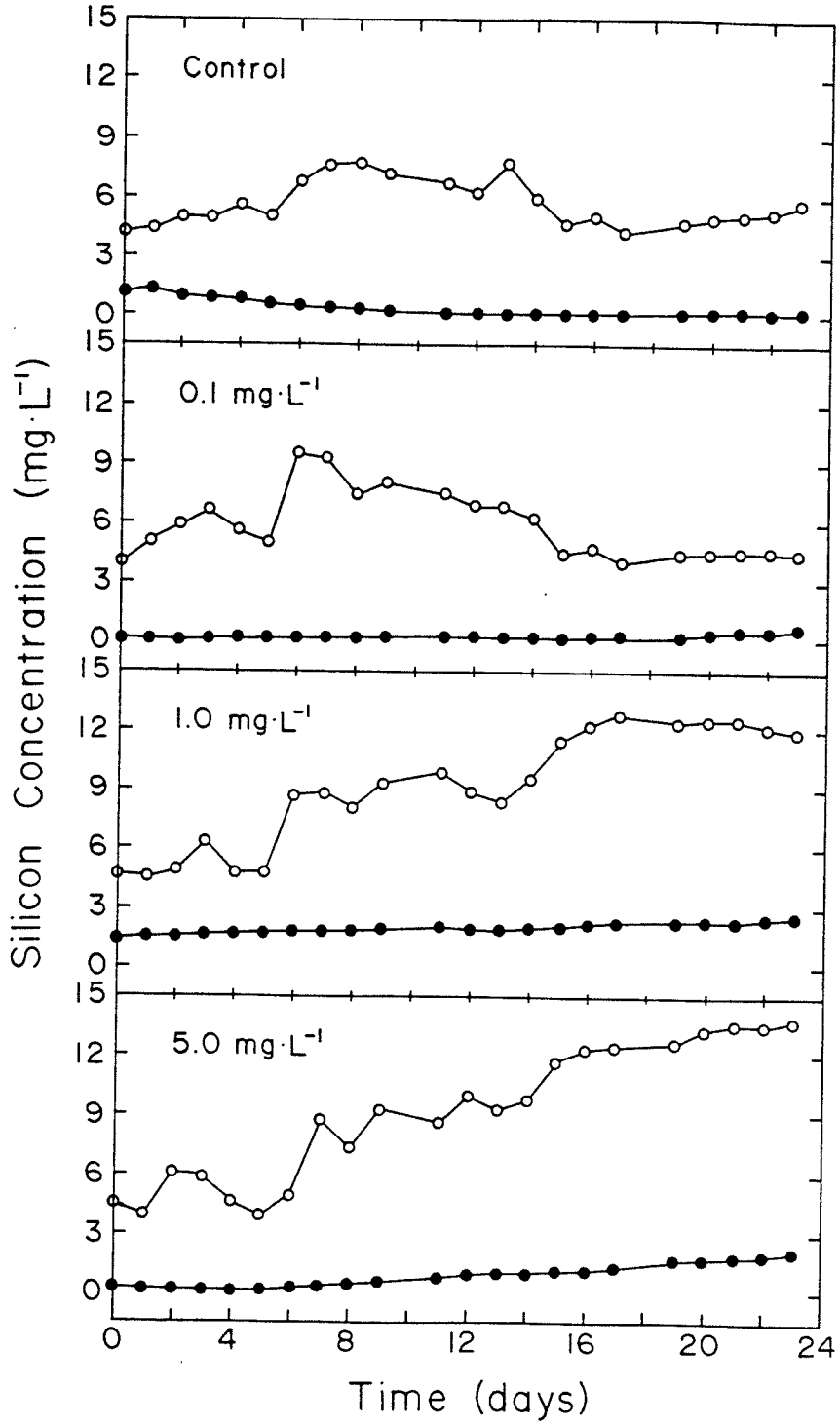

Control
0.1 mg·L⁻¹
1.0 mg·L⁻¹
5.0 mg·L⁻¹
Total Reactive Phosphorus Concentration (mg·L⁻¹)
Time (days)
0
4
8
12
16
20
24
0.0
0.2
0.4
0.6
0.8
1.0

Figure 34: Silicon concentration of open-bottom (o) and sealed-bottom (•) enclosures treated with three concentrations of simazine.


Control
0.1 mg·L⁻¹
1.0 mg·L⁻¹
5.0 mg·L⁻¹
Silicon Concentration (mg·L⁻¹)
Time (days)
0
3
6
9
12
15
0
4
8
12
16
20
24

5.4 DISCUSSION

During this experiment, two phases in the change in dissolved oxygen status of OB versus SB enclosures were identified. The first occurred soon after enclosure placement, and was characterized by lower oxygen concentrations in OB enclosures. This trend continued over the pretreatment period of 12 days suggesting that the decrease was a reflection of normal BOD and/or COD of marsh sediments. At the same time, lower oxygen levels appeared to be correlated with higher nutrient levels (possibly through an influence on sediment redox), explaining the initial (day 0) nutrient differences between enclosures (Figures 32-34).

The second change in dissolved oxygen levels of the enclosures occurred following herbicide treatment, specific levels being a function of herbicide concentration. This is indicative of a change in photosynthetic oxygen evolution and corresponding increases in BOD due to decay processes. Since macrophytes were not present in the enclosures, the observed deoxygenation may reflect inhibition of sediment epipelon, with some lesser influence upon planktonic and haptobenthic organisms. In light of the fact that simazine concentrations in OB enclosures decreased significantly over the experimental period (Figure 30), sediment adsorption of herbicide is probable, thereby increasing the phytotoxic concentration at the sediment/water interface. This is particularly true for the $5.0 mg \cdot L^{-1}$ OB treatment, since the

amount of added herbicide in excess of the maximum observed water concentration (ca. 3.5mg·L^{-1}) was not accounted for. Furthermore, significantly higher concentrations noted in the SB enclosures suggests strongly that sediment adsorption occurred in the corresponding OB enclosures.

The change in oxygen concentration in relation to the herbicide level coincided with substantial changes in the concentrations of total ammonia, TRP, and silicon, pointing to a connection between inhibition of sediment biota and increase in nutrients. Given that the primary effect of triazine herbicides is on photosynthesis (Moreland 1980) and that the results found here were similar to those of the light-exluding enclosure experiment of Kistritz (1978), in that significant sediment release of ammonia and phosphorus occurred under conditions of low dissolved oxygen in both cases, it is likely that autotrophic biota are important determinants of the ultimate rate of nutrient flux from sediments. The present data do not indicate whether this relationship is direct, involving loss of the nutrient-trapping epipelic layer (Jansson 1980), or indirect, through lower photosynthetically-evolved dissolved oxygen levels which would either stimulate sediment release (through lower redox potential) or sustain high levels of reduced or sparingly soluble species generated by other means. Further elucidation of the correlation between epipelon and sediment nutrient flux is clearly needed.

A major assumption of this work is that the observed differences in nutrient levels between SB and OB enclosures following herbicide treatment reflects all and only sediment effects (i.e. that all inputs and outputs for nutrients to the pairs of enclosures other than sediment contact were the same). However, concurrent observations on the development of periphyton in the enclosures suggests that this may not be true, since algal colonization was generally much higher in OB as compared to SB enclosures. Rapid colonization occurred in all enclosures during the pretreatment period and autolysis of this material probably explains the slight increase in nutrients in 1.0 and 5.0mg·L^{-1} SB treatment enclosures over time.

Preliminary data indicate that the difference in algal colonization between SB and OB enclosures following herbicide treatment was greatest in control and 0.1mg·L^{-1} pairs, and decreased in relation to herbicide concentration at 1.0 and 5.0mg·L^{-1} simazine. This is in agreement with our previous findings where levels of periphytic chlorophyll were observed to be approximately 75%, 55%, and 5% of a control at 0.1, 1.0 and 5.0mg·L^{-1} simazine respectively (Chapter 2). It is likely, then, that the fluxes reported for the control treatment are somewhat underestimated, and the discrepancy between observed and actual sediment flux would decrease with decreasing periphyton growth (i.e. at higher herbicide levels). These differences may in part explain net negative

fluxes calculated for TRP and silicon in the $0.1 mg \cdot L^{-1}$ treatment. On the basis that differences in levels of periphyton colonization between SB and OB enclosures were of lesser magnitude that would be expected from the differences in nutrient concentrations, we do not believe that underestimation is of sufficient magnitude to obscure the observed effect of herbicide concentration on sediment flux rates (Table 16). Moreover, reduction in dissolved oxygen levels with herbicide treatment (Figure 31) would be expected to reduce the contribution of bioturbation by sediment invertebrates on sediment nutrient flux (Rutgers van der Loeff et al. 1984) with the result that calculated fluxes at higher simazine treatment levels were also underestimates.

Because some investigators indicate an interaction between herbicide addition and nutrient status (e.g. Murphy et al. 1981; Tucker & Busch 1982) while other do not (e.g. Brooker & Edwards 1973; Strange 1976), there may be several interactive factors determining the ultimate ambient nutrient concentration. The present work points out the potential importance of sediment effects, even though the controls of the processes involved are yet not clear. One should not discount, however, the potential importance of such factors as the size and species composition of macrophyte communities, which would determine the magnitude of biotically stored nutrient pools and the relative susceptibility to subsequent release and/or mineralization. Fur-

thermore, the potential for development of resistant plant biotypes (including periphytic and planktonic algae) will determine the extent of immediate reuse of released nutrients. In this regard, several authors who found no increase in nutrients do report the occurrence of new macrophyte species (Fish 1966; Brooker & Edwards 1973) and/or phytoplankton blooms (Walsh et al. 1971) following herbicide treatment. The composition of suspended and sediment bacterial populations may also have a bearing on ambient nutrient levels. Their resistance levels to the specific herbicides, ability to degrade organic nutrients and release inorganic compounds, proximity to plant detrital particles falling to the sediments, and their capacity in nitrification may all be important. Sediment characteristics are also likely to be important (e.g. organic content, texture, porosity, and sorptive ability for nutrients). For example, many sediments have high capacity for ammonia and orthophosphate (Lerman 1978) so that if interstitial pools are small, adsorption may exceed release.

Clearly, more intensive examination of some of these factors will be necessary before a complete understanding of secondary effects of herbicides in aquatic systems is gained.

Finally, the high nutrient fluxes observed at $5.0 mg \cdot L^{-1}$ simazine relative to the control points to an interesting tangent of the present work. This rate may reflect poten-

tial sediment contribution of nutrients to the overlying marsh and therefore the impact of the development of epipelon on marsh nutrient dynamics. Preliminary data for interstitial sediment water concentrations of ammonia and silicon at the study site (up to 6.7 and 7.2mg·L^{-1} respectively) suggest that flux based simply on molecular diffusion should be large, since overlying water concentrations of these nutrients (and particularly ammonia) are comparatively much lower. The importance of sediment flora, then, should be considered in examinations of nutrient cycling in shallow water bodies where development of an extensive epipelic community may occur.

Chapter VI

SUMMARY

In one experiment, concentrations of the aquatic herbicides simazine and terbutryn were added to *in situ* enclosures of marsh water. Colonization of acrylic substrata by periphyton was monitored through measurements of chlorophyll *a* accumulation, carbon assimilation rate and total algal cell biovolume. No change in these parameters, relative to an untreated control, was observed at 0.1 $mg \cdot L^{-1}$ simazine over a 3-week period, with increasing inhibition (to approximately 98%) at 1.0 and 5.0 $mg \cdot L^{-1}$. Algal biomass was reduced more than 90% with terbutryn concentrations of 0.01 $mg \cdot L^{-1}$ and higher. Upon reduction of herbicide concentrations in manipulated enclosures, increases in algal growth were detected within one week, with growth rate (rate of increase in algal chlorophyll, total biovolume or carbon fixation rate) equal to or greater than that of samples from the control enclosure.

Pre-flood community structure of periphyton in simazine-treated enclosures was qualitatively similar to that of control samples, while a small blue-green alga was abundant only in terbutryn-treated enclosures. After enclosure flooding, substrata from most experimental enclosures were

colonized predominantly by Cocconeis placentula, while this taxon accounted for about 25% of total biovolume on substrata from the control and 0.1 $mg \cdot L^{-1}$ simazine enclosures. Recovery of algal communities following herbicide stress must therefore be evaluated carefully to include effects on both community structure and function.

In a second series of experiments, the static sensitivity of periphyton carbon fixation rate of samples from control and simazine-treated enclosures to five concentrations of added simazine was determined. Increased herbicide resistance / tolerance, as indicated by increases in EC_{50}, resulted from short (ca. 7 days) exposures, although EC_{50} decreased when herbicide concentration was less than 0.8 $mg \cdot L^{-1}$. Levels of inhibition detected at 1.0 $mg \cdot L^{-1}$ were less than those observed for growth at this concentration, implying that EC_{50} is an overestimate of LC_{50}. There was no evidence that a resistant taxon developed in response to simazine addition, although the density of C.placentula correlated well with EC_{50} in treated enclosures.

Collation of community structural analyses of samples collected during the preceding experiments showed that a relationship may exist between the maximum density that C.placentula populations achieve on artificial substrata and the density of the heterotrichous green alga Stigeoclonium sp. The prevalence of C.placentula in some enclosures following decreases in herbicide concentration and not in oth-

ers suggests that the specific outcome of herbicide treatments on algal community structure may depend on the seasonal timing of the addition and the autecological and synecological responses of constituent taxa.

Secondary effects of herbicide addition on enclosed water chemistry were examined by adding one of three concentrations of simazine to macrophyte-free enclosures with and without sediment contact. Changes in the concentrations of total ammonia, total reactive phosphorus and silicon were monitored, and net sediment flux was calculated from the difference in nutrient concentration between pairs of bottomed and unbottomed enclosures. Rates of sediment release for all three nutrients were unaltered by 0.1 $mg \cdot L^{-1}$ simazine in relation to a control, whereas rates increased proportionally at 1.0 and 5.0 $mg \cdot L^{-1}$. Increases in dissolved nutrients which commonly follow herbicide treatment of shallow waters may therefore not be attributable solely to macrophyte decay, but may also involve a complex interaction of biotic and abiotic sediment nutrient exchange processes. Further work is needed to determine to what extent primary (herbicide toxicity) and secondary (increased nutrient supply) effects influence periphyton growth in herbicide-treated enclosures.

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Appendix A

SUMMARY OF ENCLOSURE EXPERIMENTS

Summary of enclosure experiments performed at two sites in the Delta Marsh, 1982-1984.

Experiment	Start[1]	End[2]	Year	Site
A	13 May	9 July	1982	BC
B	18 July	2 October	1982	BC
C	22 August	10 October	1982	BC
D	16 May	23 June	1983	CP
E	20 June	5 August	1983	CP
F	20 June	15 August	1983	CP
G	27 July	16 September	1983	CP
H/I	9 August	23 September	1984	CP
J	27 June	23 August	1983	CP

[1] date of enclosure placement
[2] date of final sample collection
[3] BC = Blind Channel, CP = Crescent Pond

Appendix B

DETECTION OF BACTERIOCHLOROPHYLL IN PERIPHYTON EXTRACTS

The trichromatic method of Strickland & Parsons (1972) was used in this investigation for determination of chlorophyll concentrations in crude 90% acetone extracts. This method involves measurement of the specific absorbance of extracts at 630, 645 and 665nm with substitution into the following formulae:

(1) Chl $\underline{a}$ = 10 x $(11.6A_{665} - 1.31A_{645} - 0.14A_{630}) / V$

(2) Chl $\underline{b}$ = 10 x $(20.7A_{645} - 4.34A_{665} - 4.42A_{630}) / V$

(3) Chl $\underline{c}$ = 10 x $(55.0A_{630} - 4.64A_{665} - 16.3A_{645}) / V$

where $\underline{A_{630}}$, $\underline{A_{645}}$ and $\underline{A_{665}}$ are absorbances measured in a 1cm pathlength cuvette, $\underline{V}$ is the volume of water extracted (L) and concentrations are in units of $ug \cdot L^{-1}$. Pigment concentrations in samples of periphyton are derived by replacing the denominator of the above formulae by the substratum surface area (cm^2).

This method corrects for potential error due to absorbance of particulate material in solution (arising primarily from macerated glass fiber filters) by measuring specific absorbance of the solution at 750nm (A_{750}). The basis for the correction is that particulate materials will absorb

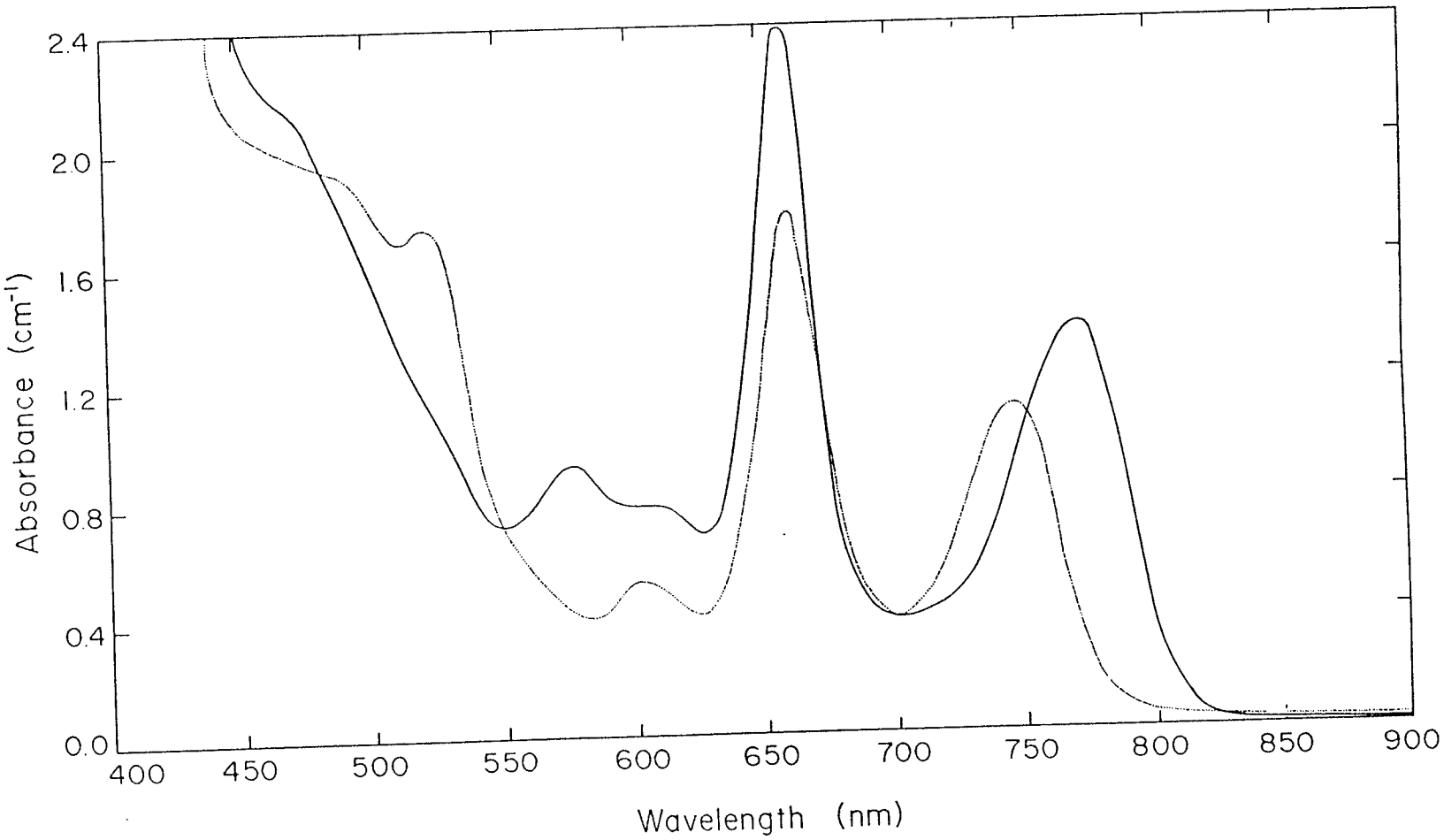
light at 750nm, but that constituent plant pigments will not (Strickland & Parsons 1972). It is further assumed that A_{750} is roughly equivalent to the specific absorbance due to particulates at 630, 645 and 665nm, so that subtraction of A_{750} from A_{630}, A_{645} and A_{665} provides a more accurate measure of net chlorophyll absorbance. The monochromatic estimation method of Lorenzen (1967) also employs A_{750} as a correction factor for A_{665}.

During two experiments performed in this investigation, extracts of periphytic material were found to have exceptionally high A_{750} values which were not visually attributable to particulate material. These extracts were generally brownish green in colour (as opposed to a usual green colour) and were characteristic of specific experimental enclosures. An absorption spectrum of a representative sample in 90% acetone (Figure 35 - solid line) showed that these solutions exhibited a near-infrared absorption maximum in the range of 770-773nm, which, upon acidification (ca. 10^{-2}N HCl), shifted to 748-750nm (Figure 35 - dashed line). These findings suggested that a pigment(s) not present in other samples was a major component of these extracts. Attempts to isolate these pigments by 2-dimensional ascending paper chromatography using the method of Jeffrey (1961) (developed in the dark - dimension 1: 4% propanol in petroleum ether; dimension 2: 30% chloroform in petroleum ether) were unsuccessful, presumably due to degradation of the pigment(s)

during concentration of the extract under an air stream (see later discussion) and the poor resolution of chromatograms. However, on the basis of the observed absorption maximum, and the assumption that photosynthetic plant pigments absorb light primarily in the range of 400-700nm, it was concluded that the solutions contained bacteriochlorophyll a (after the terminology of Jensen et al. 1964). This pigment exhibits its maximum absorbance in the range of 769-773nm in a variety of organic solvents (including acetone)(Wassink et al. 1939; Katz & Wassink 1939; Jensen et al. 1964; Sauer et al. 1966) and its degradation product, bacteriopheophytin a, absorbs maximally at 745-750nm. Bacteriochlorophyll a is the primary pigment of anoxygenic photosynthetic bacteria in the families Chromatiaceae and Rhodospirilliaceae (Pfennig 1978).

Several methods were encountered in the literature for quantitative measurement of bacteriochlorophyll a. All were based on monochromatic formulae, since bacteriochlorophyll a absorbs in a clearly distinct absorbance range from other photosynthetic bacterial pigments (b,c and d)(Jensen et al. 1964). The oldest method, described by van Niel & Arnold (1938), involved the quantitative conversion of the apparently light-instable pigment to its more stable pheophytin using HCl. The absorbance of this solution was measured at 667.8 nm and the concentration of bacteriochlorophyll a calculated in comparison with the extinction coefficient of a

Figure 35: Absorption spectrum of periphyton extract (1cm pathlength) from enclosure I, experiment G. Solid line - whole extract (90% acetone), dashed line - acidified extract.


2.4
2.0
1.6
1.2
0.8
0.4
0.0
Absorbance (cm⁻¹)
400
450
500
550
600
650
700
750
800
850
900
Wavelength (nm)

standard solution. It was assumed that the amount of pheophytin produced was equivalent to the amount of bacteriochlorphyll a in the original sample. Lascelles (1956) updated this method by measuring absorbance of the pheophytin solution at 750nm (its absorption maximum). Recent evidence suggests that concentrated solutions of bacteriochlorophyll a in pure, clean solvents are actually rather stable (Mauzerall 1978), and several authors have based estimation of concentration on absorbance of the native pigment (Takahashi & Ishimura 1968; Cohen-Bazire et al. 1957; Guerrero et al. 1985). A calculation formula used by the former authors for solutions of 90% acetone was as follows:

(4) Bact a ($ug \cdot L^{-1}$) = ($25.2A_{772}$ x v) / (V x L)

where 25.2 is an extinction coefficient ($mL \cdot mg^{-1} \cdot cm^{-1}$) for the pigment, A_{772} is the specific absorbance of the acetone solution at 772nm, v is the volume of the extract (mL), V is the water volume sampled (L) and L is the light pathlength (cm). Similar methods have also been used for estimation of bacteriochlorophyll d residues (Caldwell & Tiedje 1975).

In the present investigation, the latter method was chosen over the more laborious acid-conversion method since crude solutions were found to be relatively stable in the 30-60 minutes period from extraction of pigments to measurement of absorbance; light absorbance of specific wavelengths by solutions kept in the dark for up to 4 hours decreased only slightly.

The formula adopted for direct estimation of bacteriochlorophyll *a* in unacidified extracts was as follows:

(5) Bact *a* ($ug \cdot cm^{-2}$) = ($911.5A_{770}$ x v) / (69 x SA)

where 911.5 is the molecular weight of bacteriochlorophyll ($ug \cdot umole^{-1}$), A_{770} is specific absorbance of a 1 cm pathlength solution at 770nm, v is the volume extracted (10 mL), 69 is a molar extinction coefficient ($mL \cdot umole^{-1} \cdot cm^{-1}$) of bacteriochlorophyll *a* in acetone at 770nm (Mauzerall 1978) and SA is the surface area (cm^2) of the substratum.

To simplify this calculation for routine measurements made at 750nm, a relationship between A_{750} and A_{770} values of solutions containing bacteriochlorophyll *a* was established (Figure 36):

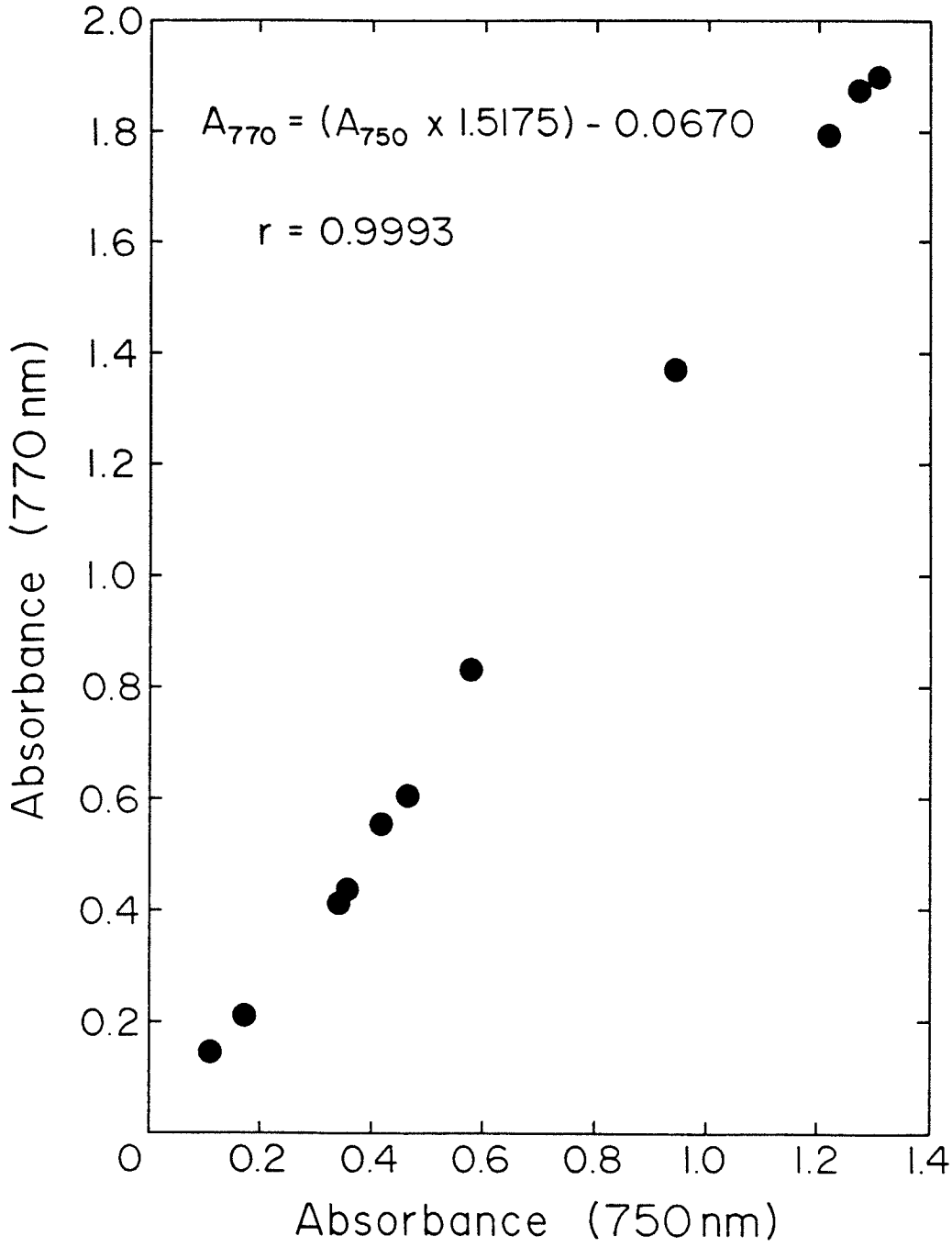
(6) $A_{770} = 1.5175A_{750} - 0.0670$ (r=0.9993, n=11)

This regression was substituted for the A_{770} term in formula (5) to yield the following:

(7) Bact *a* = ((911.5 x v)($1.5175A_{750}$ - 0.670)) / (69 x SA)

The algorithm for calculation of bacteriochlorophyll *a* in periphyton samples was as follows. Prior to particulate correction of A_{630}, A_{645} and A_{665} values for a given sample, the corresponding A_{750} value was inserted into the bacteriochlorophyll formula (7). Since A_{750} values $\leq 0.044cm^{-1}$

Figure 36: Relationship between A_{750} and A_{770} of samples containing bacteriochlorophyll <u>a</u>.


$A_{770} = (A_{750} \times 1.5175) - 0.0670$
r = 0.9993
Absorbance (770 nm)
Absorbance (750 nm)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

give negative results from formula (6), samples containing little or no bacteriochlorophyll were effectively discriminated, while those yielding a positive value were assumed to contain bacteriochlorophyll. Strickland & Parsons (1972) noted that solutions of chlorophyll with $A_{750} > 0.02$ (irrespective of pathlength) should not be used to provide reliable estimates, so it is unlikely that bacteriochlorophyll-free samples would be encountered with $A_{750} > 0.044 cm^{-1}$. To avoid using A_{750} as an indicator of "particulate" absorbance of these samples, the subsequent correction step was skipped at the expense of a small degree of error. Concentrations of chlorophylls a, b and c and correction for pheophytin a could then be calculated using formulae (1 to 3) (Strickland & Parsons 1972) and bacteriochlorophyll a by formula 7.

Sample data from experiment D (1983) illustrate the advantages and disadvantages of the present calculation formula (Table 17). In that experiment, substratum samples were collected at weekly intervals for 4 weeks from an enclosure treated with $1.0 mg \cdot L^{-1}$ simazine, and 60cm segments were used for chlorophyll analyses. On the first week, epiphytic biomass was low, although disintegration upon maceration of the filters used in collecting epiphyton resulted in a moderately turbid solution which could not be satisfactorily clarified either by centrifugation (15 minutes @ 1000 g) or filtration (Whatman GF/C). After correction using A_{750}, the Strickland & Parsons formula (1) confirmed undetectable

chlorophyll a levels. Because of the absorbance limitation noted earlier, the modified formulae (1 + 7) erroneously resulted in positive bacteriochlorophyll (due to high A_{750}) and chlorophyll a (due to lack of correction of A_{665} by A_{750}) levels. These data emphasized that the particulate content of samples to be screened for bacteriochlorophyll by this method must be low ($\leq 0.044 cm^{-1}$).

The incidence of sample turbidity observed on week 1 did not recur during subsequent sampling, with the result that chlorophyll a concentration estimates from the two methods on week 2 were the same. In general, the two methods will always yield equivalent results when $A_{750} \leq 0.044 cm^{-1}$ (when bacteriochlorophyll is absent).

On the third and fourth weeks of sampling, an apparent bacterial bloom was observed in the enclosure as periphytic material on sampled substrata increased markedly. An absorption spectrum similar to Figure 35 was recorded for each of 3 samples on each date. The standard Strickland & Parsons correction factor resulted in significant reduction in A_{665}, so estimates of chlorophyll a were much lower than those provided by the modified formula (Table 17). This suggests that the use of A_{750} in correction of A_{665} may result in serious underestimation of chlorophyll a concentrations when bacteriochlorophyll a is present. This assertion is qualified by the assumption that the observed A_{665} value represents chlorophyll a absorbance, although it is noted

that *in vitro* absorption spectra of bacteriochlorophylls *c* and *d* are very similar to those of chlorophyll *a* (Table 18). Eloranta (1985) has attempted to differentiate the two on the basis of absorption spectra and paper chromatographic separation. Takahashi & Ichimura (1968) reported individual concentrations of chlorophyll *a*, and bacteriochlorophylls *a*, *c* and *d* in depth profiles of several Japanese lakes based on analytical techniques and calculation formulae similar to those used here. The basis by which separation of a mixture of these pigments was achieved was unfortunately not described.

Speculatively, the present samples may have contained members of the bacterial family Chlorobiaceae, which contain bacteriochlorophylls *c* and *d*, and smaller (5-10% of total) quantities of *a* (Sybesma 1970) within a mixed community of green and purple bacteria. This does not, however, preclude the presence of algal chlorophyll *a* in these samples; corresponding paper chromatograms contained spots with Rf values and absorption spectra similar to chlorophyll *a*. Wet and ashed microscopic preparations of samples from experiment G (enclosure I), which exhibited significant bacteriochlorophyll *a* concentrations, were found to contain large populations of blue-green, euglenid and diatom taxa (data not shown). It is therefore suggested that while chlorophyll *a* estimates provided by the present formulae counteract inherent underestimation by the standard formulae, caution should

TABLE 17

Comparison of calulated concentration using two methods

Calculated chlorophyll concentrations from data from experiment D (enclosure #3, 1983) using the Strickland & Parsons formula and the modified formula for addition detection of bacteriochlorophyll *a* (± SE, n=3).

Time (weeks)	Concentration ($ug \cdot cm^{-2}$) S & P formula	Modified S & P Chl. *a*	Modified S & P Bact. *a*
1	0	0.012 (0.012)	0.012 (0.012)
2	0.007 (0.003)	0.007 (0.003)	0
3	0.062 (0.018)	0.237 (0.026)	0.142 (0.010)
4	1.063 (0.278)	0.944 (0.224)	0.080 (0.040)

TABLE 18

Absorption maxima of some chlorophylls and bacteriochlorophylls*

Pigment	Maxima (nm)					
Chlorophyll *a*	410	430	534	578	615	662
b	430	455	-	549	595	644
Bacteriochlorophyll *c*	412	432	-	-	622	660
d	406	425	530	575	612	650

*source: Stanier & Smith 1960.

be exercised in interpretation of absolute pigment levels. Furthermore, this limitation should be noted for applications which presume to measure bacteriochlorophyll concentrations in natural samples containing algal chlorophylls (eg. Caldwell & Tiedje 1975; Guerrero et al. 1985).

A restriction of the present model is that it does not allow for correction of bacteriochlorophyll a estimates for any pheophytin a present in the original sample. Such a correction should be possible if an independent estimate of bacteriochlorophyll, derived using the A_{750} value of the sample after acidification (noting the potential errors for this latter method), is compared to that of the present method in a manner similar to that used by Lorenzen (1967) to estimate the pheophytin content in samples of chlorophyll a.

In conclusion, the present modification attempts to alleviate limitations of the Strickland & Parsons formula used for estimation of chlorophyll a concentrations in extracts of periphyton containing significant concentrations of bacteriochlorophyll a. While it does not allow for accurate differentiation of algal and green bacterial pigments (if both are present), it does provide additional useful information from periphyton samples containing purple photosynthetic bacteria.

Appendix C

COMPUTER SIMULATION OF THE PERSISTENCE OF SIMAZINE IN ENCLOSURES

C.1 INTRODUCTION

As the use of herbicides in modern agricultural and forestry practise becomes widespread, knowledge of the persistence of these compounds in all aspects of the environment is of increasing importance. This information can be used in conjunction with data characterizing the relative toxicity of the compound to biota to predict the impact of inputs to the environment. Unfortunately, generation of persistence data under natural conditions for all possible environmental contaminants is neither practical nor possible. As a result, the use of simulation models, which provide gross estimations of the environmental partitioning of a compound from minimal data of its chemical and physical properties (Rao & Davidson 1980), is becoming increasingly common. Examples are models which predict degradation rates (eg. Walker et al. 1983) and overall fate (Jury et al. 1984) of pesticides in soil, extent of loss of terrestrially-applied compounds via runoff (Wauchope & Leonard 1980) and movement of these compounds between various components of the aquatic ecosystem (Mackay et al. 1981; Roberts et al. 1981). The latter models are of particular use in aquatic toxicology, as they

may provide an initial direction in determining the orientation of detailed monitoring programs, and some expectations of the duration of persistence of the modelled compound.

In this investigation, gross approximations of herbicide residue levels in experimentally manipulated enclosures were performed in order to monitor ambient levels of treatments, and to detect the effects of enclosure flooding on these levels. No explicit data regarding partitioning of the herbicide between the water (where it was initially added) and other components of the enclosed littoral marsh can be offered. To address some aspects of the potential environmental persistence and fate of the herbicides used here, a simulation was conducted using a model proposed by the National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality (Roberts et al. 1981). This model was chosen because it allowed the definition of a specific aquatic 'system' (the enclosure) within limits defined by the accuracy of crude estimations.

While the NRC model required a reduced amount of specific input by the user, it was inevitably limited by the availability of elementary data. Therefore, the present simulation was restricted to simazine, for which much of the necessary data existed. The simulation was conducted using the computer program described by Roberts et al. (1981). The original program was written in Tektronix BASIC and was subsequently translated into Microsoft BASIC by Dr. D.C.G.

Muir. The present program is a derivation of the latter version, revised by the author to include disk storage of input data and compatability with both the Apple DOS and Apple CP/M operating systems. Copies of the source code are available on request.

C.2 THE MODEL ECOSYSTEM

The model defines four compartments of the aquatic environment. Compartment 1 is water and includes all constituent dissolved organic and inorganic compounds. Compartment 2, the catchall, includes all particulates suspended in the water column (suspended sediments, phytoplankton, bacteria etc.) except fauna. In the present context, an attempt was made to incorporate periphyton into this component as well. Compartment 3 is the sediment, specifically defined as that layer in active chemical contact with the overlying water column. Compartment 4 is defined as biota by Roberts et al. (1981) but should be more closely defined as fauna (the most important component being fish). Since fish were absent from the enclosure system, data inputs for compartment 4 specifically pertaining to fish were neglected (see later for explanation).

The data entered into the model describe physical and chemical properties of the enclosed aquatic environment (Table 19) and simazine (Table 20), as well as compound-specific degradation rates (Table 21) and inter-compartmental

transfer rates and partition coefficients (Table 22). Values were drawn from the literature where possible, or were estimates based on model defaults and/or empirical formulae. The specific origins of these data are given in the appropriate table or are discussed later.

TABLE 19

System-specific data (Delta Marsh, Crescent Pond)

System-specific data input to the NRC model for prediction of the persistence of simazine in in experimental enclosures.

Parameter	Input data
latitude (^{o}N)	50
water depth (cm)	63
water volume (L)	300
concentration of catchall ($mg \cdot L^{-1}$)	25
sediment organic content (%)	15
sediment weight (gm)	7650
month	July
water temperature (^{o}C)	20
light attenuation with depth	low
average fish weight (gm)	0.1
number of fish	0
simazine concentration in input volume ($mg \cdot L^{-1}$)	300
volume of solution input (L)	1
critical percentage	10

TABLE 20

Compound-specific physical properties

input to NRC model for the prediction of the persistence of simazine in experimental enclosures. See text for source of data not given in table.

Parameter	Input data	Source
compound name	simazine	
melting point (°C)	226	WSSA 1983
molecular weight	201.7	WSSA 1983
vapour pressure (mm Hg)	6.1 x 10-9	WSSA 1983
water solubility ($mg \cdot L^{-1}$)	3.5	WSSA 1983
octanol-water part. coeff.	97	
organic matter part. coeff.	175	
Henry's constant ($Pa \cdot m^{-3} \cdot mol^{-1}$)	4.6735×10^{-5}	
extinction coeff. ($M^{-1} \cdot cm^{-1}$) (290 - 400nm)	all 0	
quantum yield	1	
fraction biodegradable	0	

TABLE 21

Compound-specific degradation rates

Compound-specific degradation rates input to NRC model for prediction of the persistence of simazine in experimental enclosures. See text for sources. First order or pseudo-first order kinetics are assumed. All units in day^{-1}

Compartment	Rate
water	
volatilization	6.113×10^{-6}
hydrolysis	8.299×10^{-7}
photolysis	5.702×10^{-6}
catchall	0.018
sediment	0.028
biota	0

TABLE 22

Transfer rates and partition coefficients

used as input to NRC model for prediction of the persistence of simazine in experimental enclosures. See text for sources.

Parameter	Input data
Partition coefficients	
sediment / water	12.5
catchall / water	37.5
fish / water (bioconcentration)	1.0
Transfer rates	
water -> sediment ($mg^{-1} \cdot day^{-1}$)	10
sediment -> water ($mg^{-1} \cdot day^{-1}$)	1.78
water -> catchall ($mg^{-1} \cdot day^{-1}$)	67
catchall -> water ($mg^{-1} \cdot day^{-1}$)	1.78
water -> fish (day^{-1})	1.1779
fish -> water (day^{-1})	1.1779

C.3 MODEL INPUT

C.3.1 Enclosure system parameters

The model simulates the addition of 1 L of a 300 $mg \cdot L^{-1}$ simazine solution (initial concentration = 1.0 $mg \cdot L^{-1}$) to an enclosure located in the Crescent Pond (99° 19'W, 50° 7'N). Enclosure volume varied seasonally with pond level, although an assumed mean (ca. 300 L) is representative of most enclosure experiments. Based on enclosure diameter (78 cm), this would represent an enclosed water depth of 63 cm (Table 19). Actual observations of water depth in the pond indicated that the level varies temporally within a range of about 50-90 cm (data not shown). The enclosed sediment area within a typical enclosure was 4780cm^2. Based on assumed values for sediment bulk density and 'active' depth (1.6$gm \cdot cm^{-3}$ and 1.0cm respectively - Muir, pers.comm.), an estimate of sediment weight within the enclosure was 7650gm. Sediment samples were collected from within enclosures in the Crescent Pond in 1984 for determination of organic matter content. These samples were dried overnight to constant weight at 105°C, and organic percentage estimated by loss-on-ignition from 2gm samples incinerated for 16 hours at 375°C (McKeague 1978). The mean of 4 replicates was 15.05 ± 0.30.

Most enclosure experiments were conducted in mid-summer (Appendix A) so the model based the simulation in the month of July. Water temperature in this period fluctuates widely as a result of winds and the shallow water column, but a es-

timate of 20°C is probably representative. The light attenuation with water depth (set as either 'high' or 'low') was assumed to be low due to the relative clarity of pond water in this period (mean $n=0.0302\ cm^{-1}$).

No data exist for the concentration of suspended material (catchall) in Crescent Pond water, although qualitative observations derived from water filtrations suggest that the value is low. Since the largest apparent biotic component within enclosures (the periphyton) was not quantified by the model, an estimate of total periphyton biomass was incorporated into the model as catchall. Total surface area for periphyton colonization was estimated as 20,000 cm^2 (15348 and 4562 cm^2 on enclosure walls and on 36-60 cm long acrylic rods respectively). Assuming an average periphyton biomass (expressed as chlorophyll a) in July of 1.0 $ug \cdot cm^2$ (Hooper-Reid & Robinson 1978), this represents a total chlorophyll a content of 20mg. This in turn was converted to organic matter using a 1% chlorophyll/organic matter ratio (F.P.Healey, pers.comm.). The resulting weight (2000mg) was expressed in units of enclosure volume (300L) as ca. $7mg \cdot L^{-1}$. An estimate of total catchall concentration was taken as 25 $mg \cdot L^{-1}$ to account for the uncertainty inherent in the above calculation and other unquantified (and potentially significant) catchall components.

Although the enclosure system included no fish (except on rare occasions when one was accidentally trapped during en-

closure placement), the input value for average fish weight could not be set to zero without causing the simulation to terminate prematurely. To circumvent this problem, a low value (0.1 gm·fish^{-1}) was entered and the number of fish per enclosure was set to zero.

The critical percentage of the modelled system (which may be used in calculation of the efficiency of various degradative pathways in influencing loss of the compound from the system) was set at 10% (the model default).

C.3.2 Simazine properties

Some basic chemical properties of simazine were readily available for model input (WSSA 1983), while others were estimated from data reported in chemical literature (Table 20). The latter include the octanol-water partition coefficient (Kow - a crude estimator of biological membrane partitioning), which was estimated as the arithmetic mean of 88 (Rao & Davidson 1980) and 155 (Kenaga & Goring 1980). The organic matter partition coefficient (Koc) was based on the geometric mean (due to the higher variability of estimates) of 138 (Rao & Davidson 1980), 135 (Kenaga & Goring 1980) and 284 (Glotfelty et al. 1984). This estimate assumed that the effect of colloidal material in solution on Koc is minimal, although Means & Wijayaratne (1982) have found that Koc of atrazine (chemically similar to simazine) may increase to 13,000 in the presence of colloids. Henry's constant (used

in the calculation of volatilization rate) was estimated as the ratio of vapour pressure (in Pa) to water solubility (in mole$\cdot m^{-3}$).

The model default for quantum yield of simazine (ratio of the number of molecule quantities undergoing photolytic reactions to the number of light quanta absorbed by the molecules) of 1.0 was used (ie. maximum potential photolysis when energy is absorbed) because no data were available to allow more accurate input. The default value for the fraction of the molecule which was biodegradable (ratio of biotic degradation rate to export rate) was used (Roberts et al. 1981). Changes in these parameters had marginal effects on the resulting simulation.

The rate of volatilization has been assumed to be very low in simulations of the persistence of simazine in soil (Walker 1976a;b) and a calculated first-order rate substantiates this conclusion (Table 21). This rate was calculated from the following formula, which is based on 2-film (air,water) theory:

$$1/Kol = 1/Kw + RT/HKg$$

where Kol is the mass transfer rate between liquid and gas phases ($m\cdot hour^{-1}$), Kw and Kg are mass transfer coefficients for water and gas respectively ($m\cdot hour^{-1}$), R is the universal gas constant (8.314 $Pa\cdot m^3\cdot mol^{-1}\cdot K^{-1}$), T is the environmental temperature (^0K) and H is Henry's constant. The val-

ues of Kw and Kq for simazine (7.17×10^{-3} and 8.36 respectively) were estimated from similar values offered by Liss & Slater (1974) for oxygen and water in oceans (which may be overestimates of actual rates in a sheltered pond) with correction for the molecular weight of simazine (Table 20). The resultant Kol value (1.6047×10^{-7}) was converted to a first-order rate constant by division by pond depth (63 cm).

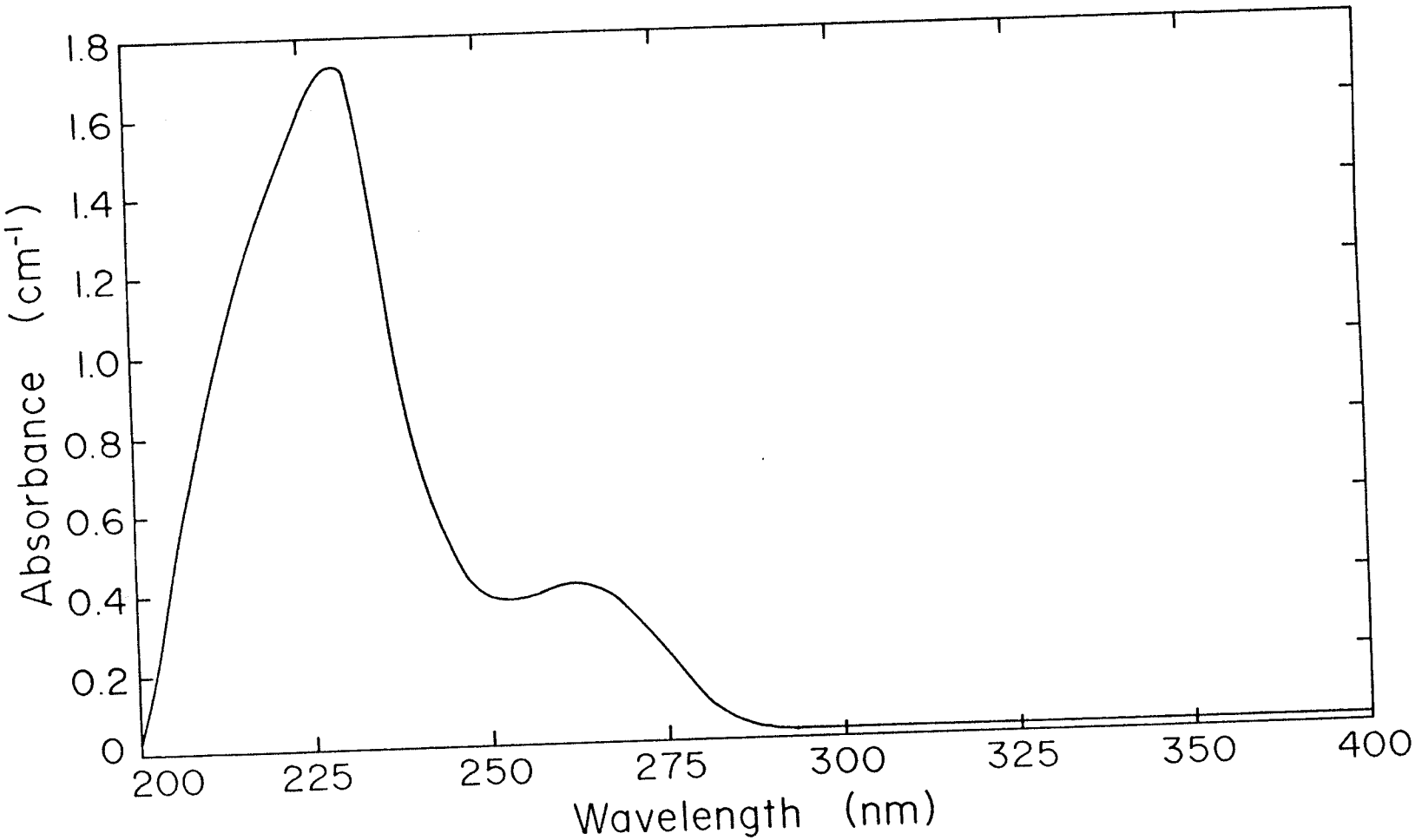
The ability of simazine to undergo photolysis is based on the extent to which the herbicide molecule absorbs ultraviolet light in the range of 290-400nm (wavelengths <290nm are absorbed by the upper atmosphere). An option in the model allowed the input of extinction coefficients of the herbicide in water at light wavelengths in the above range (measured in 10nm increments). However, while reports in the literature suggest that such absorption is low (Pape & Zabik 1970), no explicit ultraviolet absorption spectra could be found. A spectrum was, however, generated (Figure 37) for a 1×10^{-4} M ($20.17 mg \cdot L^{-1}$) methanolic simazine solution in 1cm quartz cuvettes using a Unicam SP-800 scanning spectrophotometer. Although these data are not directly comparable to the extinction in water (the technique is limited by the low water solubility of simazine), the differences are not likely to be important because no appreciable light absorption occurred at light wavelengths above 290nm (Figure 37). At the same time, while these data would imply

that photolysis of simazine is negligible, the presence of sensitizing agents in natural waters (many types of organic molecules which absorb light in the near ultraviolet and transfer shorter wavelength energy directly to the herbicide molecule) may result in appreciable photolytic losses (Harris 1978).

Actual rate of aqueous photolysis of simazine was derived from Ruzo et al. (1973), in which photolytic losses of simazine were determined in pure water at 300nm in the absence of a photosensitizing agent. While no substantive data for simazine exist, Burkhard & Guth (1976) have determined that the photolysis rate of atrazine increases 5 times upon addition of a 1% acetone solution. Thus, the rate may vary in Delta Marsh water with the availability of sensitizers and incident light intensity.

Few data for simazine hydrolysis rate are available in the literature in which rates were measured independantly of total 'degradation' in soil samples. Burkhard & Guth (1981) reported a first-order half-life at pH=7.0 and temperature of 25°C of >200 days (rate constant = 0.0035 day^{-1}). As the half-life was not definitively measured, however, this constant may represent a serious overestimate. It was, therefore, assumed that a rate measured for atrazine would yield a more accurate estimate. In solutions of circumneutral pH values and temperature in the range of 20-25°C, rate constants of 1.0543×10^{-6} (Glotfelty et al. 1984), 8.64×10^{-7}

Figure 37: Simazine ultraviolet absorption spectrum in methanol (1×10^{-4}M, 1cm pathlength).


Absorbance (cm⁻¹)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
200
225
250
275
300
325
350
400
Wavelength (nm)

(Plust et al. 1981) and 5.714 x 10^{-7} day^{-1} (Gamble et al. 1983) were found, and an arithmetic mean (8.299 x 10^{-7} day^{-1}) was used in simulation calculations. Restrictions on this estimate relate to effects of environmental pH (relatively constant in the range 7.5-8.5), temperature and dissolved organic matter content on in situ rates of hydrolysis. In the last respect, Khan (1978) found hydrolysis rate of atrazine to increase several orders of magnitude upon addition of 5% fulvic acid.

Overall degradation rate of simazine in catchall was estimated from rates in terrestrial soil. Although Jones et al (1982) noted that the degradation rate of atrazine was approximately ten times greater in estuarine sediment than in terrestrial soil, the degree of correspondence may vary with water content (Walker 1974), the size of the 'degrader' population (Scow 1978) and other site-specific factors. The chosen results therefore are probably accurate to within an order of magnitude. The final mean was based on 8 individual estimates (Table 23).

A mean of several degradation rates reported by Tucker & Boyd (1981) for the rate of loss of simazine from in vitro sediment samples was used for the sediment compartment (0.028 day^{-1}). Muir & Yarechewski (1982) have shown, however, that degradation rate of terbutryn decreases significantly under anaerobic conditions, and Jones et al. (1982) reported similar findings for atrazine degradation in anae-

TABLE 23

Degradation rates of simazine in soil

Estimate (day^{-1})	Source
0.009	Walker 1976a
0.022	Walker & Thompson 1977
0.040	Walker 1978
0.014	Rao & Davidson 1980
0.022	"
0.029	Walker & Brown 1983
0.012	Walker et al. 1983
0.012	Pestemer et al. 1984
mean 0.018	

robic estuarine systems. As a result, the rate used here (which was determined in mixed and presumably aerobic flasks) may be an overestimate of the rate of simazine degradation in presumably anaerobic marsh sediments.

A bioconcentration factor (BCF) of 1.0 (Table 22) was used in simulation calculations (Kenaga & Goring 1980), which indicates that simazine is neither actively excluded nor concentrated by biota. Based on the rapid clearance rate of simazine by fish (Rodgers 1970) and the low BCF, degradation in biota was assumed to be negligible.

The calculation of chemical partitioning into sediment (Ks) can be estimated from the relation (Roberts et al. 1981):

$$Ks = Koc \times \%OM/100$$

where %OM is the organic matter content of the sediment. The calculated value of Ks was 20.6, although the accuracy of this value may depend on the possible restrictions on the accuracy of Koc in the presence of colloids (see earlier). This compares favorably with an experimentally determined value of 12.5 (Glotfelty et al. 1984) used in the present simulation. The data of Glotfelty et al. (1984) suggest that partitioning of atrazine into catchall is 3 times Ks, so a Kca value for simazine of 37.5 was used here. Evidence that this rate is of the correct order of magnitude for suspended organic material can be drawn from the data of Dabydeen & Leavitt (1981), which showed that an equilibrium con-

centration of simazine in the tissue of Elodea canadensis in a $3mg \cdot L^{-1}$ solution was approximately $22mg \cdot kg^{-1}$. Assuming a plant density of 1.0, this corresponds to a Koc (concentration in plant / concentration in solution) of 7.3.

Calculation of mass transfer rates of simazine between the four model compartments was based primarily on model defaults because very little information exist regarding the magnitude of intercompartmental fluxes of pesticides. The value for water/sediment transfer rate was taken as 10 $mg^{-1} \cdot day^{-1}$ (Roberts et al. 1981), from which the water/catchall rate (67 $mg^{-1} \cdot day^{-1}$) was calculated according to the formula (Roberts et al. 1981):

water/catchall = water/sediment x 100 / %OM

Catchall/water transfer (water/catchall divided by Kca) and sediment/water transfer (water/sediment / (Kca x %OM)/100) rates were each 1.78 $mg^{-1} \cdot day^{-1}$

Rates of clearance of simazine by fish were estimated from the data of Rodgers (1970) in which the simazine concentration of green sunfish tissue, determined after 24 hour oral exposures, was used to calculate a half-life and a first-order rate constant (1.1779 day^{-1}). The fish clearance rate constant is equal to the fish/water transfer rate because degradation in fish was earlier assumed to be negligible. Since the BCF (1.0) is then the ratio of rate of entry into the biota to its rate of exit, the former rate is also 1.1779 day^{-1}.

C.4 MODEL OUTPUT

The Roberts et al. (1981) model provides 'fixed' and 'dynamic' simulations of chemical persistence. The former estimates the retentive capacity of the modelled compound in an equilibrated system. The levels of the compound in each compartment of this system are assumed to be stable and proportional to partition coefficients and transfer rates between the respective compartments (after a period of continuous input of the compound sufficiently long to allow equilibration). The fixed solution provides the relative fractions of the equilibrium compound concentration present in each compartment, and the relative contributions of various loss mechanisms (degradation rates) to the overall rate of loss of compound.

Since this investigation involved single herbicide applications to enclosures over short time periods, equilibration of simazine between model compartments likely did not occur. The results of the fixed solution do, however, permit comparisons on a common basis of simazine persistence with other environmental pollutants.

The simazine retentive half-life of the modelled enclosure system was 147.5 days. When compared with values provided by Roberts et al. (1981) for several benchmark compounds, including chloroform (<1 day), methyl parathion (12 days), the dioxin TCDD (7.8 years) and DDT (3335 years), one may conclude that simazine is a moderately persistent chemi-

cal. By contrast, similar calculations performed for atrazine using a default 'pond system' of the Roberts et al. (1981) model (Goldsborough, unpubl.data), provided an estimate of retentive capacity of 18 days. The discrepancy of the two results for similar s-triazines may in part be resolved by quantitative differences between the two modelled systems, but more importantly may reflect the effects of errors inherent in estimations of reaction rates. The former simulation drew on more recently reported rates of hyrolysis, photolysis and volatilization of s-triazines, and was less reliant on estimated data.

Summaries of fractional retention and degradation are given in Table 24 and show that the majority (75%) of added simazine is predicted to remain in the water compartment. The next largest sink for herbicide is the sediment (24%) with only marginal quantities in catchall and biota. The largest degradative pathway occurs in the sediment (99.7% of total degradation).

The prevalence of simazine in the water compartment suggests that the use of crude, whole water extracts in the detection of simazine residues within enclosures (Chapters 1,2,3), while limited in scope, did provide meaningful information on the relative persistence of simazine between various treatments. The above data indicate that a more intensive investigation should be directed at quantification of residue levels in sediments, and to a less extent, degradative losses in water and sediment.

TABLE 24

Results of fixed solution

indicating fractional retention of simazine in the four model compartments and the proportions of total degradative losses attributed to various mechanisms.

	Percent of Total
Fractional Retention	
Water	75.78
Catchall	0.07
Sediment	24.15
Biota	negligible
Fractional Degradation	
Water	
Volatilization	negligible
Photolysis	0.06
Hydrolysis	negligible
Catchall	0.19
Sediment	99.74
Biota	negligible

The 'dynamic' simulation of the NRC model provides a time sequence plot showing the change in concentration of the compound in each model compartment as the system approaches equilibrium. These data are of particular use in examining the fate of compounds which equilibrate between the compartments slowly, and for the modelling of single input events. Therefore, of three 'pollution event' options available in the model under the dynamic solution (single input / continuous input to equilibrium / continuous input to equilibrium with subsequent cessation), the single input model was most appropriate to the present enclosure system.

The temporal scale of the dynamic solution calculations was extended over a period of 2552 days (ca. 7 years) by the computer model. At the end of this period, simazine concentrations in each of the four compartments were 1.2×10^{-4}, 4.5×10^{-3}, 6.7×10^{-4} and 1.2×10^{-4} $mg \cdot kg^{-1}$ in water, catchall, sediment and biota respectively. For the sake of brevity, only data representing the first 1000 days after input are plotted (Figures 38 and 39).

Apparent differences in the concentrations of simazine in the four compartments (Figure 38 - water , Figure 39 - catchall, sediment, biota) are due to respective weight differences between the four compartments. By performing mass balance calculations, it may be seen that the largest proportion of herbicide is contained in the water compartment (as found by the fixed solution).

Figure 38: Results of dynamic solution indicating predicted change in simazine concentration over a 1000-day period in the water compartment of a simulated enclosure system after a single input of $1.0 mg \cdot L^{-1}$ (day 0).
Inset: enlarged view showing apparent linear phase which occurred between days 3 and 45.

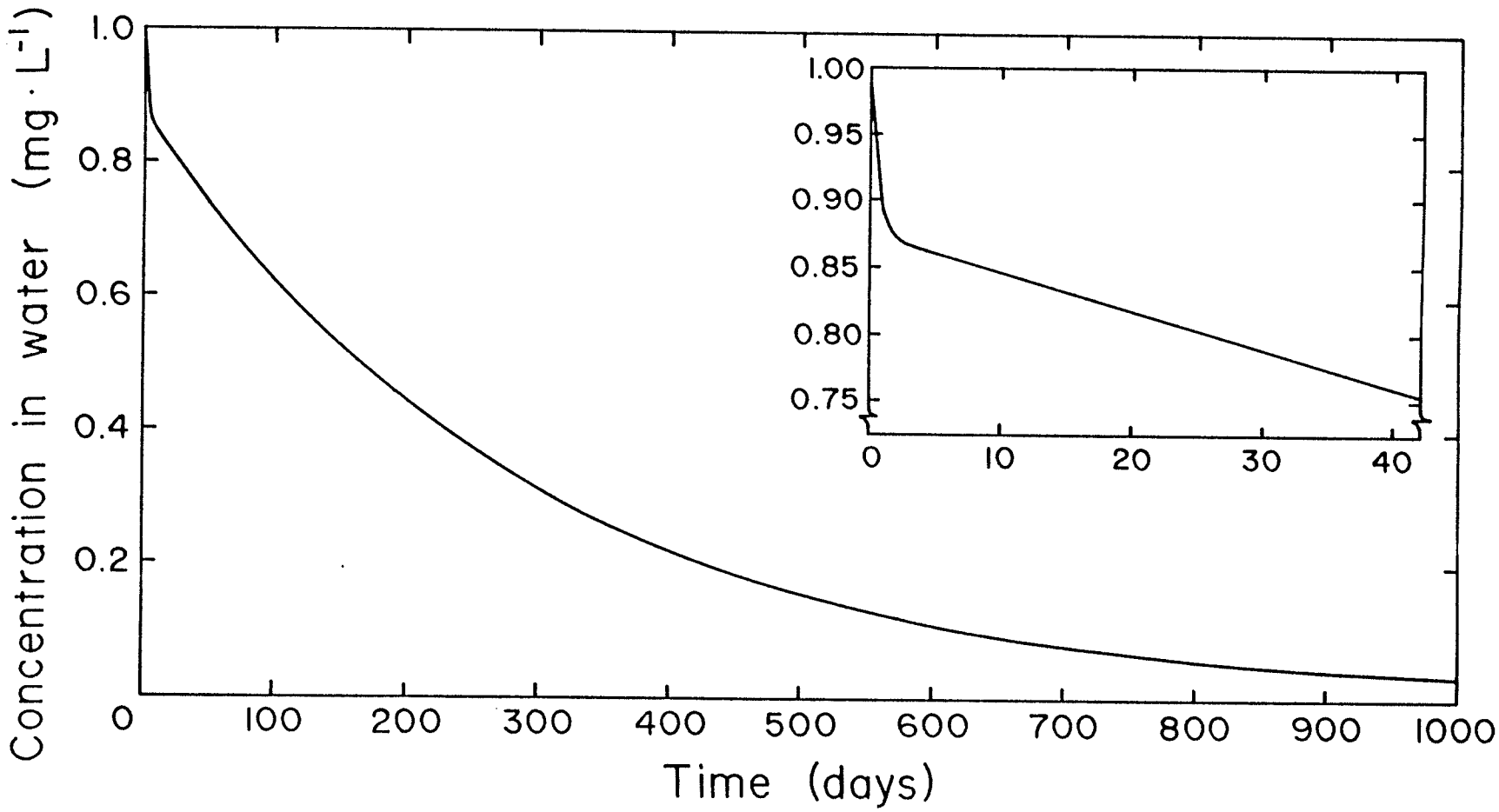
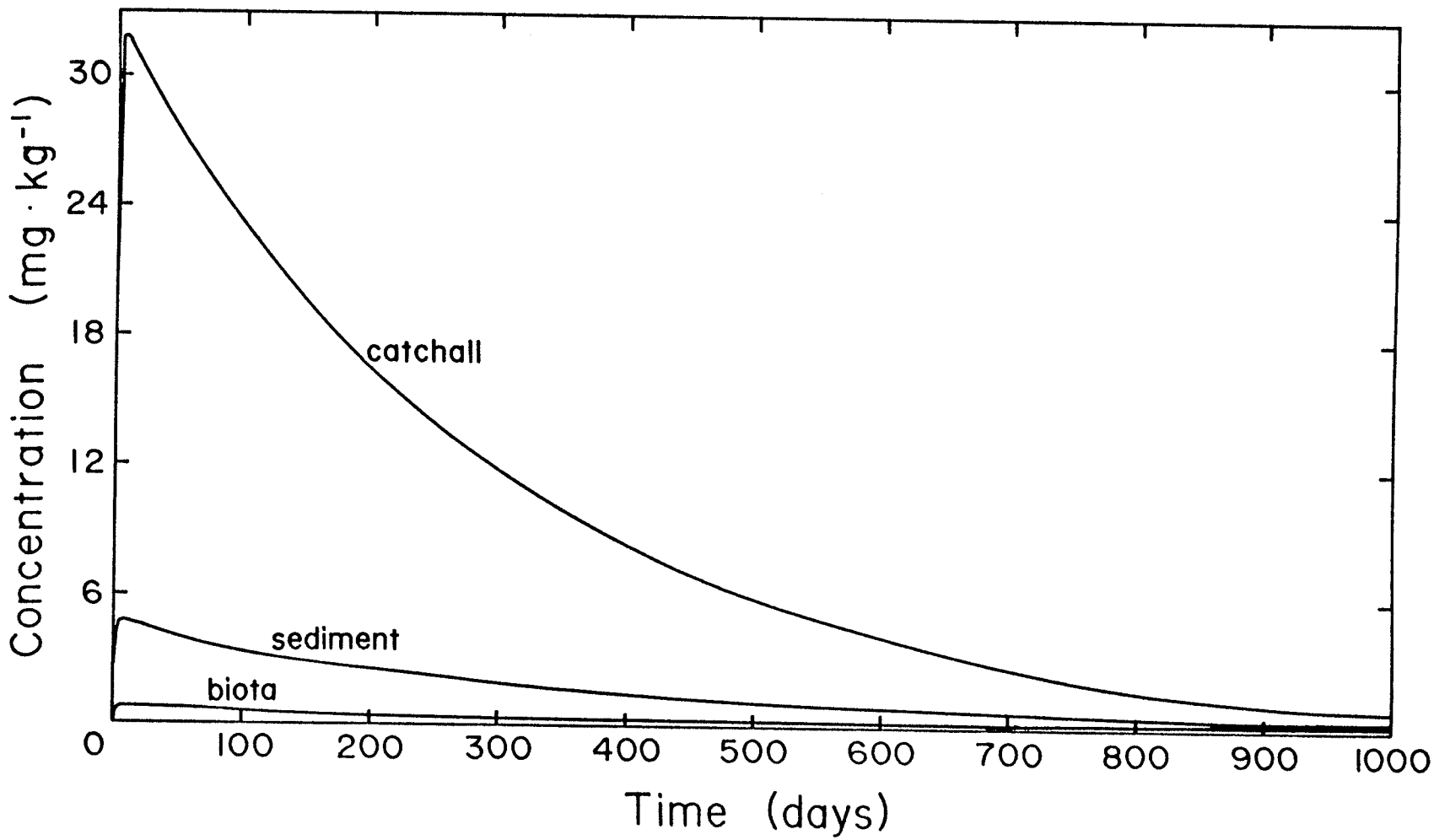

Concentration in water (mg·L⁻¹)
1.0
0.8
0.6
0.4
0.2
0
100
200
300
400
500
600
700
800
900
1000
Time (days)
1.00
0.95
0.90
0.85
0.80
0.75
0
10
20
30
40

Figure 39: Results of dynamic solution indicating predicted changes in simazine concentration over a 1000-day period in the catchall, sediment and biota compartments of a simulated enclosure system after a single input of 1.0mg·L^{-1} (day 0).


Concentration (mg · kg⁻¹)
30
24
18
12
6
0
catchall
sediment
biota
100
200
300
400
500
600
700
800
900
1000
Time (days)

The maximum observed simazine concentration in all compartments occurred in the first 0-3 days, and decreased significantly thereafter. The water concentration decreased rapidly from the initial input concentration of 1.0mg·L^{-1} within the first 2 days (Figure 38 inset), probably as a result of adsorption into the catchall, biota and sediment compartments (in which concentration increased in the same period). Subsequently, the concentration in the water compartment decreased at a nearly linear rate (2.8ug·day^{-1}, r=-0.9998) between days 3 and 42 (a period corresponding approximately to the duration of most enclosure experiments - Appendix A).

Owing to incidental enclosure flooding during many experiments, quantitative comparisons of simulated with observed residue levels in water of the present studies are limited. Data collected from OB enclosures (experiment G) are most suited to such comparison, as they covered a 37-day period during which no flooding occurred. Univariate linear regression of estimated simazine concentration in 0.1 and 1.0mg·L^{-1} treatments resulted in correlation coefficients of -0.93 and -0.99 respectively and slopes (rates of change in concentration) of -2.2 and -16.6ug·day^{-1} respectively. Data from a 5.0mg·L^{-1} treatment were not considered, because the observed residue levels (maximum = 3.62mg·L^{-1}) were in excess of maximum water solubility in pure water (Table 20). Under such conditions, the present simulation and its inher-

ent assumptions of chemical behavior are inappropriate (Roberts et al. 1981).

The above two rates of decrease in concentration are similar when expressed on a relative basis (2.2% and 1.7% day^{-1}) and compare favorably with data collected within a similar time period in other enclosure experiments (S.E.Gurney, pers.comm.). These rates are, however, considerably greater than would be predicted from the linear period of the simulation (0.3% day^{-1}). This difference likely is a reflection of the accumulated inaccuracy of estimation in the various inputs to the model, particularly in regards to mass transfer rates (Table 22) and water degradation rates (Table 21) under ambient environmental conditions. As a result, it is suggested that the present simulation overestimates the relative persistence of simazine in the Delta Marsh.

C.5 EVALUATION OF THE MODEL

The simulation model of Roberts et al. (1981) provides several advantages for prediction of chemical persistence. These include relative simplicitiy of the input data and the provision for a user-defined model ecosystem and input event. The value of defining persistence in terms of these specific inputs can be seen in the substantial variability in results of studies of changes in concentration of simazine in different water bodies. Mauck et al. (1976) found

that the simazine concentration in the water of small, artificial ponds treated initially with 1.0mg·L^{-1} decreased from 0.45 to 0.22mg·L^{-1} within 45 days. Similar relative losses occurred in ponds treated with 0.3 and 0.1mg·L^{-1} and residual concentrations of <0.01, 0.03 and 0.09mg·L^{-1} were noted in the 0.1, 0.3 and 1.0mg·L^{-1} treatments respectively after 346 days. Rapid loss of simazine from treated aquarium water (to ca. 10% within 14 days) was reported by Hawxby & Mehta (1979), while the amount in enclosed sediment increased to ca. 20% of the added quantity. Hydamaka et al. (1977), on the other hand, provided data which show that no significant change in simazine concentration occurred within 63 days of a treatment (input concentration not specified) of farm ponds in southern Manitoba. Klassen & Kadoum (1979) reported rates of loss of atrazine from treated ponds ranging from 0.003-0.4% per days of the added amount. The data of Mauck et al. (1976) show that the decline in concentration following a second addition of simazine to ponds (1 year after the first addition) was less than seen in the initial treatment; a residual concentration of 0.42mg·L^{-1} was detected 456 days after a second 1.0mg·L^{-1} treatment.

On the above basis, the model provides data which may be used in the elucidation of likely areas of environmental concern in specific instances (for example, indicating where in an aquatic ecosystem a compound may be expected to accumulate). The accuracy with which such predictions are made,

however, is contingent on the accuracy of input data. For many compounds estimates of physical and chemical properties necessary to the model are unavailable. This is particularly tru for older compounds which were introduced prior to registration requirements for such information (Muir, pers.comm.) and is amply evident from the paucity of much of these data for simazine, which has seen widespread commercial use since the late 1950's.

Furthermore, many physical and chemical properties of the modelled compound are under complex environmental influence. In the present context, the effects of such variable factors as photosensitizer concentration and water turbidity on photolysis rate, surface water movement on volatilization potential, and pH and organic material effects on hydrolysis may well be important. The ability of microorganisms to degrade the compound may depend on the ability of ambient populations to adapt to the presence of the compound, their affinity to it, and interactions and competition between individual taxa for the energy source. Changes in the proportions of bacterial species of various nutritive strategies following introduction of herbicides have been documented (Fry et al. 1973; Ramsay & Fry 1976). Moreover, Lewis et al. (1983) have shown that a relationship exists between degradation rate of 2,4-D ester by periphyton and the flow rate of medium across the substratum, suggesting that transport limitation may occur on a spatially and temporally variable scale.

A temporal basis for variability in most input parameters is not incorporated in the present model. The potential for change in rate of an inputted photolysis rate with season, for example, may be a serious omission, although the model does account for variation in light intensity with season. Other considerations include effects of temperature on rates of chemical and biological reactions. Such effects would be particularly evident in north temperate climates with extreme annual temperature cycles with corresponding effects on rate of hydrolysis, catchall mass etc.

Unlike some other aquatic environental fate models (eg. Mackay et al. 1981), the NRC model is limited by its lack of definition of hydrodynamic factors in the transport of the chemical between compartments, including effects of inflow and outflow of water (with and without constituent pollutants), sedimentation of suspended solids (with adsorbed pollutants) and variable sediment resuspension. In shallow littoral ponds, these factors may be particularly important in determining the distribution of pollutants. Moreover, the importance of the air as an individual compartment in the fate of volatile compounds is not realized by this model (Muir, pers.comm.).

Finally, the lack of differentiation of such important determinants of environmental fate of chemicals as aquatic macrophytes, periphytic algae and bacteria, zoobenthos, phytoplankton and zooplankton from "catchall" is a serious

omission limiting the applicability of the model in pond environments.

In conclusion, while valuable preliminary information may be gained from the use of screening persistence models such as that of Roberts et al. (1981), there is yet no acceptable alternative to actual environmental monitoring for the examination of persistence and fate of environmental pollutants.

Appendix D

EFFECT OF LOW TERBUTRYN LEVELS ON ALGAL PRODUCTIVITY

The results of experiment B, which examined the effects of simazine and terbutryn on periphytic algal community structure and function (Chapters 2 and 3) did not allow the resolution of a 'no-effect' level for terbutryn; levels of inhibition of chlorophyll *a*, photosynthetic rate and algal biovolume were greater than 95% at all concentrations tested (0.01, 0.1 and 1.0 $mg \cdot L^{-1}$). An experiment conducted in 1983 (experiment D - Appendix A) attempted to examine this question using a 0.001 $mg \cdot L^{-1}$ terbutryn treatment in addition to 0.01 and 0.1 $mg \cdot L^{-1}$ treatments. Samples of artificial substrata from control and herbicide-treated enclosures were collected at weekly intervals over a 5-week period, although results from week 5 were discarded because enclosure flooding between weeks 4 and 5 resulted in the addition of large floating mats of filamentous green algae (which were abundant in the surrounding marsh) to all enclosures.

Methods used for the determination of terbutryn residues, photosynthetic rates and chlorophyll *a* content were described in Chapter 2.

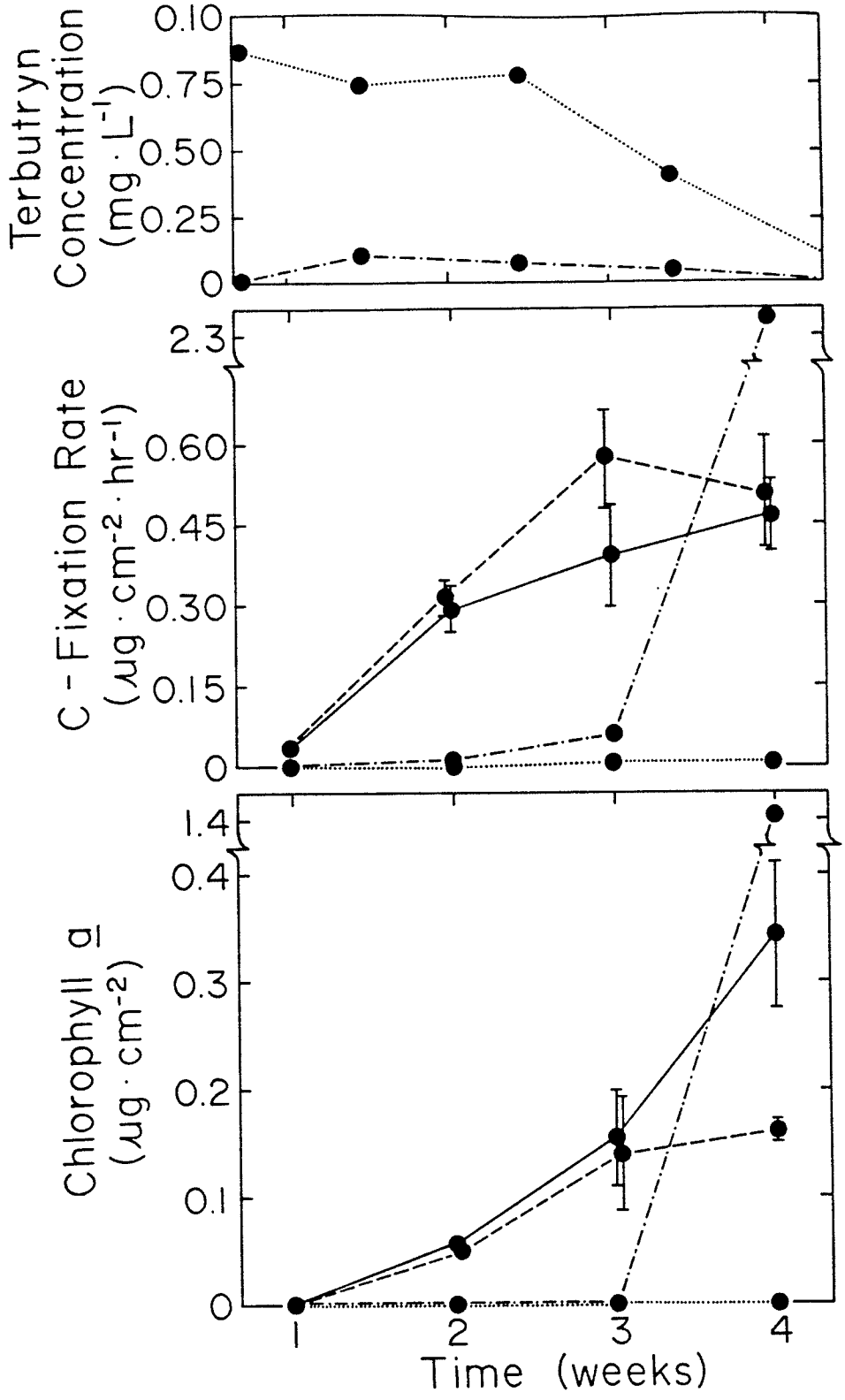
Owing to a 0.01 $mg \cdot L^{-1}$ detection limit of the method used for terbutryn residue analyses, terbutryn was not detected in the 0.001 $mg \cdot L^{-1}$ enclosure during the experiment. Levels in the two higher treatment level enclosures were slightly less than the initial added concentration (Figure 40) and likely reflects losses due to sediment adsorption. Similar results were reported in experiment B. Decreases in herbicide concentration between weeks 3 and 4 were due to partial enclosure flooding as a result of intense wave action against all enclosures, while terbutryn was not detectable in any enclosure following complete enclosure flooding between weeks 4 and 5.

In the first 3 weeks of sampling, there were no apparent effects of 0.001 $mg \cdot L^{-1}$ terbutryn treatment on algal photosynthetic rate or chlorophyll a content (Figure 40). Levels of inhibition of photosynthesis at 0.01 and 0.1 $mg \cdot L^{-1}$ in the same period were approximately 88 and 96% respectively, while inhibition levels of chlorophyll content were 98 and 100% respectively. On week 4, chlorophyll and photosynthetic rate were significantly higher on substrata from the 0.01 $mg \cdot L^{-1}$ treatment, while chlorophyll concentrations in the 0.001 $mg \cdot L^{-1}$ enclosure were less than on substrata from the control enclosure. No explanation can be offered for the former observation, although it may be related to a 50% reduction in terbutryn concentration in this enclosure observed between weeks 3 and 4. Furthermore, photosynthesis

and chlorophyll content of samples collected on week 5, despite contamination by intertwined green algae, were ca. 20% of week 4 values.

On the basis on the present data, it is concluded that a community LC_{50} (concentration giving 50% inhibition of community productivity) of marsh periphyton lies between 0.001 and 0.01 $mg \cdot L^{-1}$ terbutryn.

Figure 40: Effect of low terbutryn levels. Top: Assayed terbutryn concentration over a 4-week period in enclosures treated with 0.01 (•-·-•) and 0.1 (•···•) $mg \cdot L^{-1}$ terbutryn. No herbicide was detected in the 0.001 $mg \cdot L^{-1}$ treatment enclosure.
Photosynthetic rate (middle) and chlorophyll a level (bottom) of periphyton on artificial substrata over a 4-week period in the control enclosure (•---•) and in enclosures treated with 0.001 (•---•), 0.01 (•-·-•) and 0.1 (•···•) $mg \cdot L^{-1}$ terbutryn.
Error bars are the SE of replicates (n = 3).


Terbutryn Concentration (mg·L⁻¹)
0.10
0.75
0.50
0.25
0
C - Fixation Rate (µg·cm⁻²·hr⁻¹)
2.3
0.60
0.45
0.30
0.15
0
Chlorophyll a (µg·cm⁻²)
1.4
0.4
0.3
0.2
0.1
0
1
2
3
4
Time (weeks)

Appendix E

A SIMPLE BIOASSAY FOR PHOTOSYSTEM II INHIBITORS IN WATER

USING IN VIVO CHLOROPHYLL FLUORESCENCE*

E.1 INTRODUCTION

There has been increasing emphasis on the development of bioassays for environmental toxicants. Those for detection of herbicide toxicity have ranged from variable phototaxis of Aedes larvae (Simonet et al. 1976) to the growth of Lemna fronds in solutions containing herbicide (Muir et al. 1980; 1981). The use of algae as assay organisms is particularly desirable, since effects on a photosynthetic organism may be observed directly at a unicellular level. Previous use of algae include variable effects of herbicides on algal chlorophyll production (Kratky & Warren 1971; Lefebvre-Drouet & Calvet 1978), packed cell volume (Ashton et al. 1966), cell density (Castelfranco & Bisalputra 1965) and oxygen evolution (Hollister & Walsh 1973). At a simpler level, subjective assessments of herbicide concentration effects have been based on degree of trichome migration by blue-green algae (Noll & Bauer 1973), and relative degree of growth of algal cells in aqueous media (Vance & Smith 1969; Cooper et al. 1978), or on agar plates impregnated with herbicide

(Thomas et al. 1973; Wright 1975). While each method has inherent advantages, many are limited by high cost, lengthy procedure, qualitative assessment of effect and/or low sensitivity.

In vivo chlorophyll fluorescence has been used extensively to examine effect of herbicides on photosynthetic electron transport (Prezelin 1981). It has been shown that upon addition of an electron transport inhibitor, light energy is diverted into fluorescence, and as a result, many authors have used DCMU-induced fluorescence as an indicator of maximum cellular photosynthetic capability (Cullen & Renger 1979, Roy & Legendre 1979; Prezelin & Ley 1980; Vincent 1980; 1981). Variable response of chlorophyll fluorescence to specific herbicide levels has also been used to detect triazine-resistant terrestrial weed biotypes (Ali & Souza Machado 1981) and phytoplankton communities (deNoyelles et al. 1982). It has further been shown that the degree of increase in fluorescence as a result of photosynthetic inhibition is directly related to the concentration of the inhibitor (Zweig et al. 1963; Lien et al. 1977), an observation which probably relates to the number of reaction sites of photosystem II inhibited by a given herbicide concentration.

On the basis of this latter observation, we propose a bioassay using unialgal cultures in which the response of fluorescence yield to concentration of inhibitor is known, so that a change in in vivo chlorophyll fluorescence brought

about by addition of a water sample containing herbicides to the cultures can be interpolated to an estimate of concentration. This paper reports the use of this bioassay to detect changes in concentration of the herbicide terbutryn, a potent photosystem II inhibitor (WSSA 1983), in a laboratory microcosm over a period of time.

E.2 MATERIALS AND METHODS

Clones of the diatom Nitzschia acicularis W.Smith and the green alga Scenedesmus dimorphus (Turp.) Kuetzing were respectively isolated from the periphyton and plankton of the Blind Channel of the Delta Marsh, Manitoba, Canada (99° 19'W, 50° 7'N). Both were cultured in modified MBL medium (Nichols 1973), with added boron (0.16 uM) and silicon increased to 800 uM. Under aeration in a growth chamber, both cultures were grown in uniform suspension under continuous light (60-100 $uE \cdot m^{-2} \cdot s^{-1}$) at a temperature of 16°C. A continuous culture apparatus using a peristaltic pump (Buchler 'Dekastaltic') to add fresh medium and remove algal culture from the reaction flask (ca. 0.69 dilutions day^{-1}) allowed maintenance of physiological steady state, thereby fixing maximum chlorophyll fluorescence in response to photosynthetic inhibitors at a more or less constant level.

Chlorophyll fluorescence was measured using a Turner 111 fluorometer, equipped with a standard cuvette door, blue light source (Turner 110-853), and blue excitation (Kodak

Wratten 47B) and red emission (Corning 2-64) filters. Aperture sizes were chosen such that readings for algal cultures were at least 20% of full scale. This allowed sufficient sensitivity to resolve small differences influorescence upon addition of herbicide, particularly at lower inhibitor concentrations.

For fluorometric determinations, a known volume of cell culture was centrifuged for 10 minutes at approximately 2000 rpm. While maintaining low light conditions (<5 $uE \cdot m^{-2} \cdot s^{-1}$), the supernatant was decanted and replaced by either fresh medium (for purposes of obtaining calibration responses) or water samples of unknown terbutryn concentration (for purposes of herbicide assay). Five milliliter aliquots of the resulting suspensions were dispensed into several matched fluorometer cuvettes. To cuvettes containing medium, 0.1mL of 100% methanol containing increasing amounts of terbutryn were added to yield final herbicide concentrations of 0.5 to 1000 $ug \cdot L^{-1}$ (approximately 2×10^{-9} to 4×10^{-6} M). A blank consisted of algal culture plus 0.1mL methanol containing no herbicide. For the sake of consistency, methanol was also added to cuvettes containing unknowns. Preliminary experiments showed that addition of methanol lead to less than a 7% increase in fluorescent response over control samples.

Fluorescence of each cuvette was measured twice within 30 minutes of addition of the herbicide stock (or addition of

the water sample). The cuvettes were kept at a low light intensity (<1 $uE \cdot m^{-2} \cdot s^{-1}$) between measurements. Maximum response time for most samples was less than 10 minutes. Fluorometer readings (F) were used to calculate a fluorescence response index (FRI) for each sample using the formula:

$$FRI = 1 - F(blank)/F(sample)$$

Samples for terbutryn analyses were generated by spiking an aquarium containing 2.4kg Delta Marsh sediment and 60L distilled water with unformulated technical grade terbutryn (>98% a.i.) in 100% methanol to give an initial concentration of about 10 $ug \cdot L^{-1}$. Regular additions of water counteracted evaporative losses. Over a 31-day period, unfiltered water samples were collected from the aquarium at 2-4 day intervals and assayed for terbutryn via chlorophyll fluorescence. Aquarium water was also tested for 'dead' fluorescence. Neither these samples nor solutions of terbutryn alone showed appreciable fluorescence.

On the same sampling dates, water was also collected for terbutryn determination by gas chromatography (Muir 1980). For GC analysis, 1.0L of water (unfiltered) was extracted into 400mL methylene chloride, dried over anhydrous sodium sulfate, and evaporated to near dryness in a rotary evaporator. The residue was redissolved in 5mL ethyl acetate and the concentration of terbutryn determined with a Varian 2100 gas chromatograph equipped with a nitrogen-phosphorus detec-

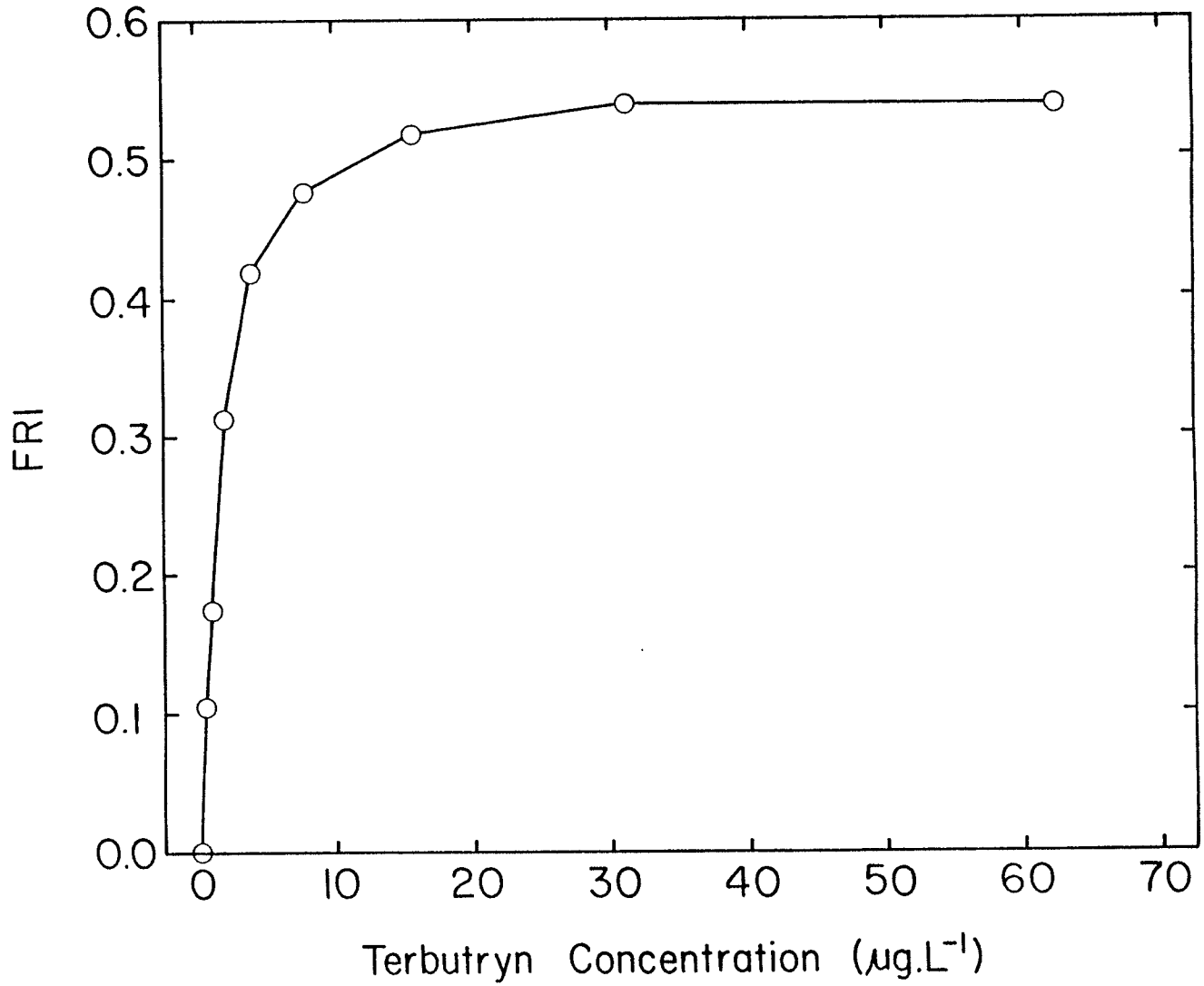
tor [1.8m x 2.0mm glass column 3% OV-17, detector temperature 220°C, gas flows as in Muir(1980)] against a standard solution in ethyl acetate.

E.3 RESULTS AND DISCUSSION

A typical example of the response of FRI to terbutryn concentration is given in Figure 41 for N.acicularis. The data appear to follow a hyperbolic function rather than the sigmoid curve typical of dose-response functions. This may be due in part to insufficient data at concentrations less than 0.5 $ug \cdot L^{-1}$, although Lien et al. (1977) also reported a hyperbolic response of fluorescence to increasing concentrations of DCMU and simazine in Chlamydomonas reinhardi. Replication of single concentrations during all experiments showed a mean coefficient of variation of 5%. It is important to note that a constant value for FRI (FRImax) was achieved at about 30 $ug \cdot L^{-1}$ (1.24×10^{-7} M), indicating that the index used here is only equivalent to fluorescence-based photochemical indices of other authors [FRI, (Cullen & Renger 1979), CFC and CPC (Vincent 1980; 1981)] at concentrations greater than this threshold.

Since the magnitude of the maximum fluorescence response is apparently related to photosynthetic capacity (Samuelsson & Oquist 1977), the curve in Figure 41 would only be constant as long as physiological steady state is maintained. For the sake of a bioassay in which many unknown samples are

Figure 41: Relationship between fluorescence response index (FRI) of N.acicularis grown in continuous culture and terbutryn concentration.

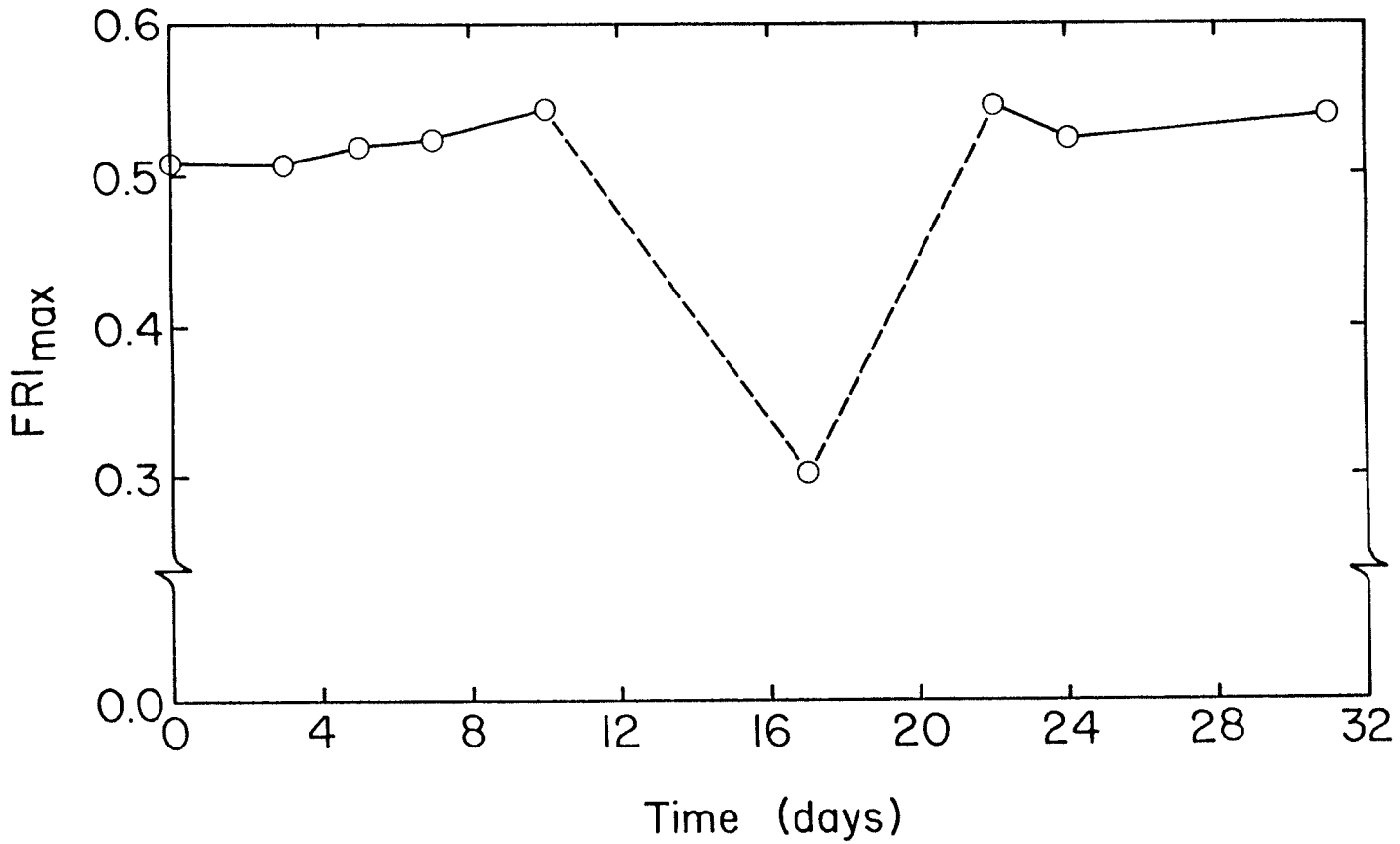

0.6
0.5
0.4
0.3
0.2
0.1
0.0
FRI
0
10
20
30
40
50
60
70
Terbutryn Concentration (µg.L⁻¹)

examined over a period of time, the stability of the response with concentration is of considerable importance. For this reason, the use of continuous culture technique for supply of algal cells is highly desirable. In the present experiment, FRImax of the Nitzschia culture, which was determined throughout the experiment, varied slightly (Figure 42) except for an intervening period in which defective culture medium caused the culture to wash out (days 12 to 17). Nevertheless, it was possible to define a standard curve of FRI versus concentration on day 17, even though it was considerably different from that shown in Figure 41.

Once the FRI function is known, the FRI of samples containing aquarium water could be interpolated directly into units of terbutryn. Alternatively, it is possible to linearize the hyperbola via a double reciprocal transformation, from which a predictive equation can be derived. The double reciprocal plot is preferable to other means of transformation, since it tends to emphasize data at lower concentrations, which is particularly beneficial for a bioassay in which low limits of detection are stressed.

A compilation of data for dates on which FRImax of Nitzschia was constant (Figure 42) is shown in transformed format (Figure 43). The level of fit to a straight line is very good ($r = 0.97$) although higher regression correlations were achieved by regressing individual dates separately (maximum $r = 0.99$). This is evident from the higher degree

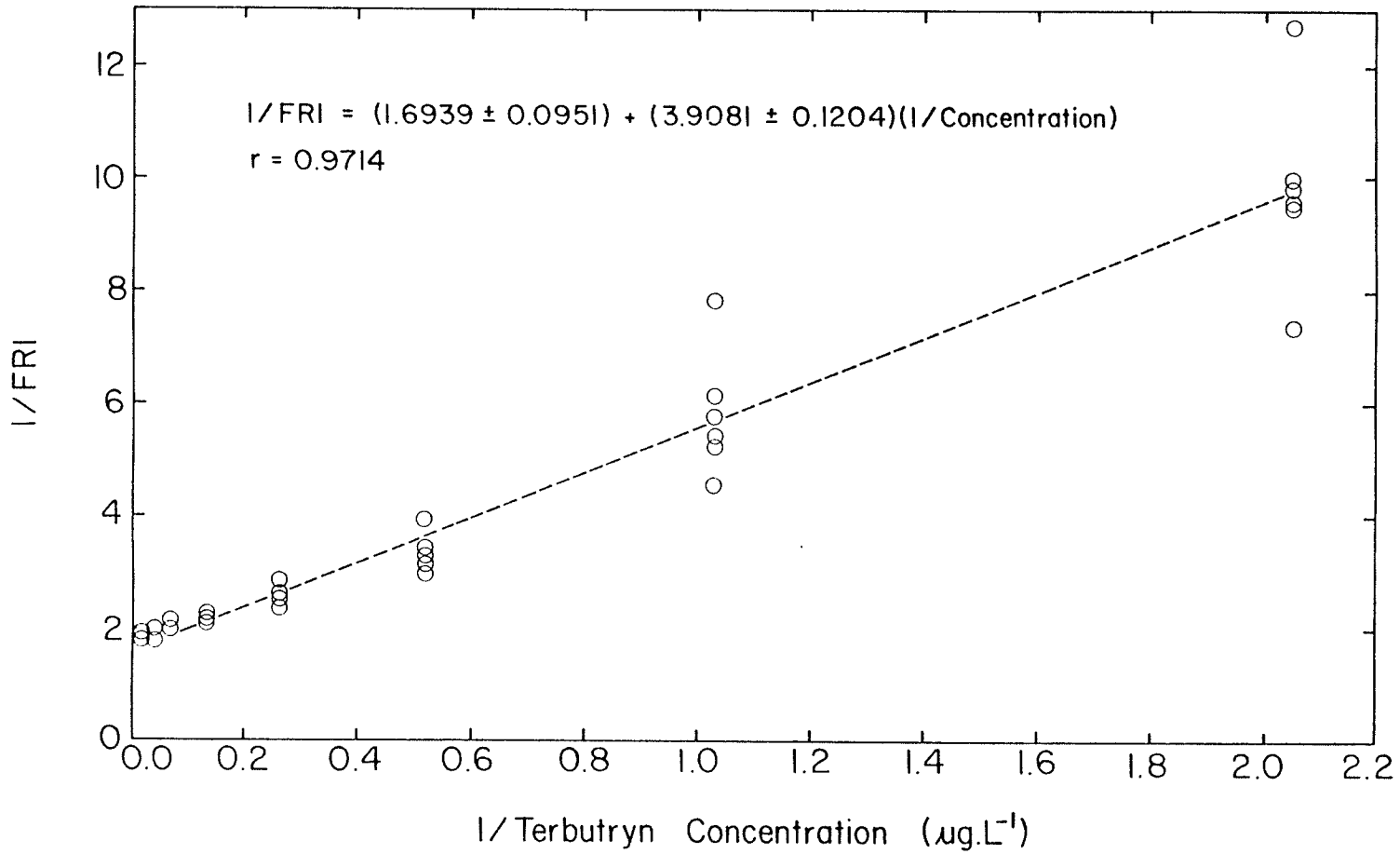
Figure 42: Maximum fluorescence response index (FRImax) of N.acicularis a teach date on which terbutryn bioassay was performed. Dashed line indicates the period over which the culture washed out due to defective medium.


0.6
0.5
0.4
0.3
0.0
FRImax
0
4
8
12
16
20
24
28
32
Time (days)

of scatter at lower concentrations (Figure 43), and probably relates to the fact that FRImax could not be held perfectly constant due to equipment limitations. As a result, for optimum accuracy, standard curves should perhaps be generated on a daily basis.

Using daily prediction equations, FRI values of water from the aquarium were converted to units of terbutryn (Figure 44). Results from the bioassay agreed closely with those by gas chromatography, with estimates from both methods typically within $2ug \cdot L^{-1}$. In each case, initial estimates were near the theoretical applied concentration of 10 $ug \cdot L^{-1}$, with declining concentrations in following days, presumably a result of sediment adsorption (Muir et al. 1981). In general, estimates by bioassay were higher than those by GC from day 10 onwards. This was particularly evident on day 22 when estimates differed by ca.7 $ug \cdot L^{-1}$. A possible explanation for these discrepancies may lie in the presence of phytotoxic degradative products such as N-deethylated terbutryn (Muir et al. 1981) which would tend to increase the estimate given by the bioassay. However, this possibility seems unlikely, since chromatographs did not consistently exhibit identifiable peaks other than that of terbutryn, although this avenue was not rigourously investigated. Alternatively, there may have been a variable effect of incubation medium composition on fluorescent yield. FRI standards were determined in defined medium whereas unknowns

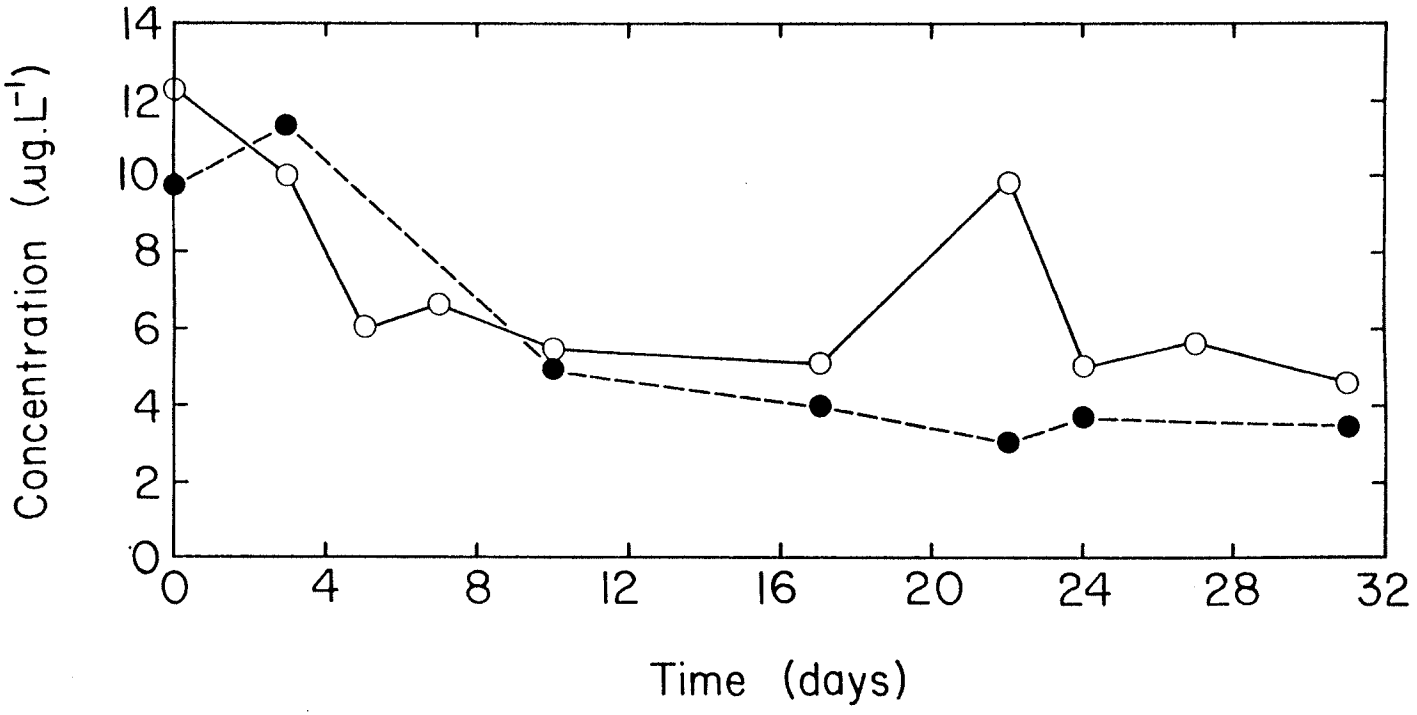
Figure 43: Standard curve of the relationship between FRI of N.acicularis and terbutryn concentration following double reciprocal transformation. Standard errors for the regression coefficients are based on eight replications (see text). Overlapping points have been deleted.


1/FRI = (1.6939 ± 0.0951) + (3.9081 ± 0.1204)(1/Concentration)
r = 0.9714
1/FRI
0
2
4
6
8
10
12
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
1/Terbutryn Concentration (µg.L⁻¹)

were composed of aquarium water of varying composition. Kiefer (1973) showed that nitrogen limitation in Thalassiosira pseudonana and phosphorus limitation in Monochrysis lutheri could increase fluorescent response in the absence of an inhibitor. Conversely, Vincent (1980) found the DCMU-induced fluorescence of natural phytoplankton communities to decrease upon addition of nitrogen. Even the type of nitrogen supplied (either as ammonia or nitrate) has been shown to significantly alter fluorescent response to DCMU by Oscillatoria chalybea (Bader & Schmid 1980).

Potential effects of nutrient composition on the bioassay were investigated in the latter part of the experiment. This was accomplished by diluting aquarium water with known amounts of culture medium prior to addition to the algal pellet. Since fresh medium was of high overall nutrient status, increasing dilutions enriched aquarium water while at the same time diluting terbutryn in the sample by a known amount. Estimates of herbicide concentration given by each dilution could be corrected for the dilution to derive an estimate of concentration in the original sample. Results showed that, with the possible exception of data on day 31, the estimate of concentration decreased with increasing enrichment; estimates at 1/4 dilution were consistently lower than at all others (Figure 45). Significantly, these latter estimates were nearer those from GC than those of undiluted samples on each date. This suggests that herbicide determi-

Figure 44: Concentration of terbutryn in the water of a laboratory microcosm over a 31-day period determined by the *in vivo* chlorophyll fluorescence bioassay, using *N.acicularis* (o--o) and by gas chromatography (•--•).

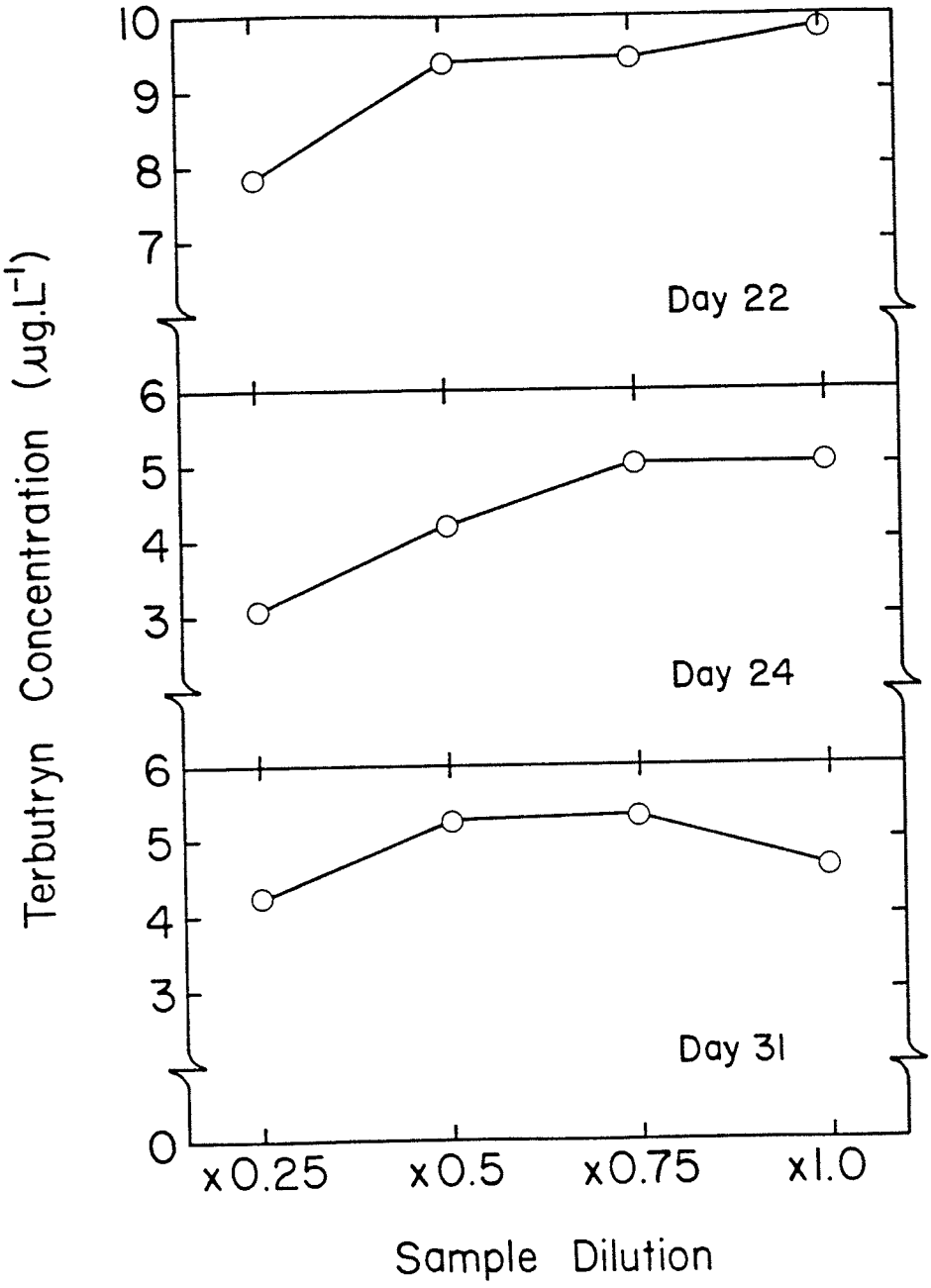

Concentration (µg.L⁻¹)
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
28
32
Time (days)

nation via bioassay may be improved by correction for sample nutrient composition using various dilutions in instances where this proves to be a significant factor.

Some advantages of the in vivo chlorophyll fluorescence bioassay over conventional means of herbicide residue analysis are its rapid and simple operation and relative cheapness. Moreover, it requires very small sample water volumes (ca. 5mL) while maintaining high sensitivity to small changes in concentration. If concentration of water samples can be achieved without degrading herbicide residues (perhaps through freeze drying), much lower detection limits for certain herbicides may be possible than currently available. Finally, results may be more biologically meaningful than estimates of absolute herbicide residue by GC. A limitation of the method is, however, that it is sensitive only to those chemicals which interfere with electron transport through photosystem II of photosynthesis (Moreland 1980).

In these experiments, Nitzschia acicularis proved to be an excellent assay organism at low concentrations, although in general terms, the limits of detection would be set by the range of concentrations over which FRI of a culture varied, which in turn would be determined by individual species sensitivity. Ideally, a multispecies approach incorporating clones of varying sensitivity could increase the utilizable range of the method. For example, data for the FRI function with terbutryn concentration for Scenedesmus dimorphus grown

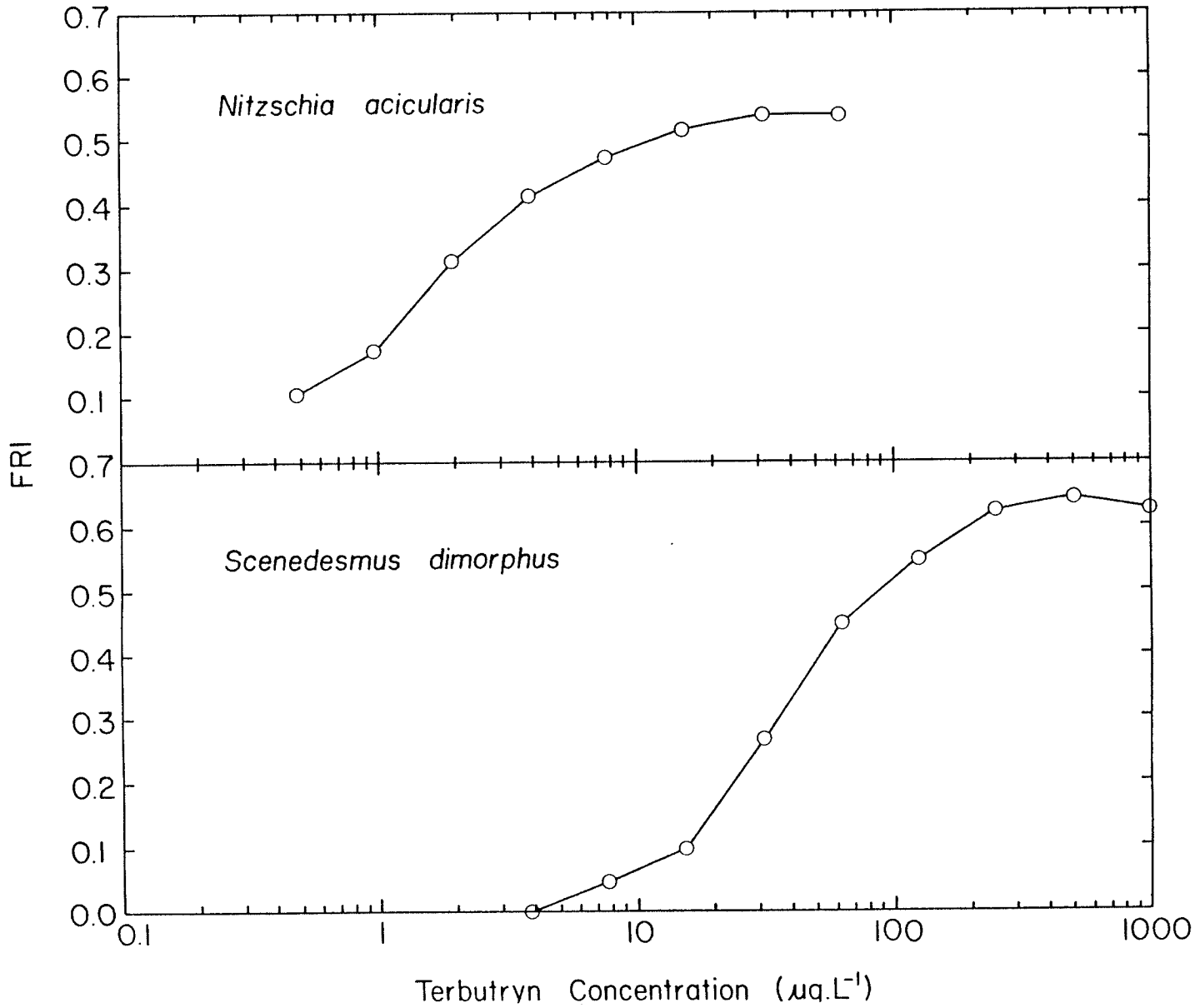
Figure 45: Effect of dilution of water samples with culture medium on the estimate of terbutryn concentration in the original sample derived by the *in vivo* chlorophyll fluorescence bioassay.


Terbutryn Concentration (µg.L⁻¹)
10
9
8
7
Day 22
6
5
4
3
Day 24
6
5
4
3
Day 31
0
x0.25
x0.5
x0.75
x1.0
Sample Dilution

at similar dilution rate to Nitzschia acicularis (Figure 46) showed that this clone was much more tolerant of high herbicide levels than the Nitzschia clone yet still responded in a similar manner within a defined, albeit higher, range of concentrations, inferring that this species would be well suited to detection of higher herbicide levels.

In conclusion, in vivo chlorophyll fluorescence represents a potentially useful bioassay tool which is not only directly related to the primary effect of electron transport inhibitors on photosynthesis, but is also more sensitive than bioassay methods previously proposed. As a result, it should have potential applications for herbicide residue analyses in natural waters.

Figure 46: Comparison of the response of FRI of N.acicularis and S.dimorphus clones (grown in continuous culture at similar dilution rates) to terbutryn concentration.


Nitzschia acicularis
Scenedesmus dimorphus
FRI
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.1
1
10
100
1000
Terbutryn Concentration (µg.L⁻¹)