PRIMARY PRODUCTIVITY, STANDING CROP AND SEASONAL DYNAMICS OF EPIPHYTIC ALGAE IN A SOUTHERN MANITOBA MARSH POND

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by

Nina Michelle Hooper-Reid

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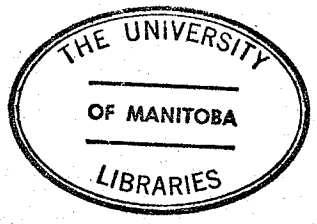

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. Eliot
from Little Gidding

ABSTRACT

The productivity and standing crop of epiphytic algae in a marsh pond and some of the factors controlling the seasonal algal growth were investigated. The use of artificial substrates for the estimation of epiphytic algal production was evaluated. Cellulose-acetate was found to provide a convenient, reliable substrate for epiphytic algal attachment which allowed simple routine application of the C^{14} technique for productivity estimation.

The annual overall primary productivity of epiphytic algae in the marsh pond (Crescent Pond, Delta Marsh, Manitoba) was 1881 kg C yr^{-1} or 26.8 g C m^{-2} pond surface area. Productivity per unit colonizable surface area was greatest on *Scirpus acutus* Muhl. (32.3 g C m^{-2} yr^{-1}) while *Potamogeton pectinatus* L. was the most significant substrate in overall pond productivity (1700 kg C yr^{-1}).

Analysis of photosynthetic C uptake revealed that the photosynthesis to *in situ* light intensity relationship of the epiphytic algae was relatively constant throughout the day. A net loss during the night of 30% of C^{14} assimilated in the previous light period was detected. Apparent uptake of C^{14} by the epiphytic community was cumulative in the light for periods up to 24 hr.

Seasonal growth at two sites in the marsh pond (*Scirpus acutus* and *Potamogeton pectinatus*) was examined in detail. Productivity and standing crop of epiphytic algae were quantified in terms of C^{14}-photosynthetic uptake, cell volume, cell surface area, dry weight and chlorophyll a, protein, carbohydrate and lipid content. Standing crop and productivity increased at both sites in September and October after generally low

summer growth, with the exception of the occurrence of heterocystous blue-green algae at the *Potamogeton* site in July. Factor analysis of inter-relationships among the previously mentioned parameters suggested that cell surface area was more directly related to productivity and various standing crop parameters than was cell volume.

The seasonal growth at the two sites was related to nutrient levels (N, P and Si) and to various physiological indicators of nutrient availability, including chlorophyll content, protein to carbohydrate and lipid ratio, Si debt, storage phosphate levels, alkaline phosphatase activity and nitrogenase activity. Low standing crops in June and early July coincided with indications of deficiency in terms of chlorophyll a content and protein to carbohydrate and lipid ratios at both the *Scirpus* and *Potamogeton* sites. Growth of heterocystous, N_2-fixing blue-green algae and high alkaline phosphatase activity occurred in July at the *Potamogeton* site. Increased standing crop of epiphytic algae in September and October at the *Scirpus* site coincided with higher nutrient levels and absence of deficiency symptoms. A delay in the standing crop increase at the *Potamogeton* site correlated with low nutrient levels and various physiological deficiency symptoms.

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INTRODUCTION

The necessity of a better understanding of the role of epiphytic algae in the littoral zone of freshwater lakes and ponds has become apparent with the increased recognition of the importance of this zone in overall lake productivity and cycling of organic and inorganic compounds. Wetzel (1975), in a discussion of the algae of the littoral zone states "Our lack of knowledge of the complex interactions between the sessile flora and their substrata, and their contributions to the total system productivity represents a major void in contemporary limnology that warrants intensified study."

While the significant contribution to total annual lake production that epiphytic algae may make has been documented in several studies (Allen 1971, Hickman 1971, Kowalczewski 1975), production estimates are scarce. This scarcity may reflect methodological difficulties. Furthermore, little is known about factors controlling epiphytic algal growth. The heterogenous nature of the epiphytic community, with bacterial, fungal, algal and faunal components in close proximity, and the various interactions between this community and its macrophyte substrate and aquatic medium create a complexity of factors which may influence the epiphytic algae in a multitude of ways.

The present study was initiated to quantify epiphytic algal growth in a marsh pond and to examine some factors which may influence seasonal growth. Four areas of investigation can be identified. Initial investigation involved the estimation of primary productivity of epiphytic algae, with necessary evaluation of methodology. In the second area of

investigation, the diurnal pattern of carbon fixation and loss by the epiphytic community was examined. The third area was concerned with the seasonal measurement of various standing crop and productivity parameters and their inter-relationships. The final area of investigation focused on the role of certain nutrients in seasonal growth and community composition of epiphytic algae at two macrophyte sites in the marsh pond. Various physiological indicators of nutrient deficiency were incorporated into the investigation.

CHAPTER 1

LITERATURE REVIEW

LITERATURE REVIEW

Terminology

Terminology pertaining to attached algae is complex and confusing, with numerous synonymous and redefined terms. Since the history of the various terms has been well reviewed (Young 1945; Cooke 1956; Sladeckova 1962; Round 1964; Wetzel 1964; Hutchinson 1967) current terminology will be emphasized in this review.

The term "periphyton" is a collective term commonly used in reference to microfloral growth upon various submerged substrates, especially in studies of algal substrates in lotic environments (e.g. Hansmann and Phinney 1973; Brettum 1974; Dickman 1974). "Aufwuchs" is generally considered to be synonymous with "periphyton" although the term is now used infrequently. However, Woelkerling (1976) followed the classification scheme of Krieger (1933) for desmid populations and restricted the use of "Aufwuchs" to algae associated with a "biological" substrate only.

Moss (1968) and Moore (1974) considered "benthic algae" to be algae associated with interfaces of either sediment and water or attachment surfaces and water, in accord with terminology proposed by Round (1964). Round subdivided the benthic community into "epipelic" (associated with sediments), "epilithic" (with rocks and stones) and "epiphytic" (with macrophytic plants). Moss (1968) recognized three groups within the benthic algae; "epipelic", "attached" (composed of epilithic, episammic and epiphytic) and "mat-forming". Hutchinson (1975) recognized "algal haptobenthos", growing on solid substratum and "algal herpobenthos", growing on or in mud. "Algal herpobenthos" is synonymous with Moss's "epipelic benthic algae", while "algal haptobenthos" corresponds to the "attached benthic algae".

Most recent papers refer to attached algae in terms of the attachment substrata (e.g. epiphytic or epilithic algae). These terms have been used for algae colonizing artificial substrates considered representative of the natural substrate as well as for algae colonizing the actual natural substrate (e.g. Allen 1971). Foerster and Schlichting (1965) used the term "phyco-periphyton" in a sense synonymous with "epiphytic algae".

"Periphyton" or "periphytic algae" seems to be the least confusing collective term for attached and for the most part submerged algae. Specific algal-attachment substrate relationships of periphyton can then be recognized: epiphytic, epilithic, episammic and epipelic. Use of the term "benthic" implies an association with the bottom substrate, which does not occur in the case of epiphytic algae.

Methodology

In the examination of epiphytic algae on natural substrates it has generally been necessary to first remove the algae from the macrophyte surface, although in some cases where leaves were thin and could be cleared by chlorophyll extraction, the epiphytic algae on the leaf surface have been examined directly (Rehbronn 1937) in Sladeckova (1962). Various techniques used for the removal of epiphytic algae from natural substrates have included scraping (Godward 1937; Young 1945; Tai and Hogdkiss 1975), washing with water jets followed by agitation (Hickman 1971), grinding (Douglas 1958) and agitation (Knudson 1957; Foerster and Schlichting 1965; Gough and Woelkerling 1976). Foerster and Schlichting (1965) estimated up to 30% of algal cells were retained on *Myriophyllum* after their agitation procedure, while Gough and Woelkerling (1976) achieved a removal efficiency of 98% from five hosts of widely differing morphology using a method involving agitation and acid hydrolysis.

Once removed from the macrophyte surface and suspended in water, the epiphytic algae have been examined qualitatively and quantitatively by a number of techniques commonly employed for phytoplankton analysis. These have included cell counts, cell volume estimation, dry weight and chlorophyll determinations. Results have generally been expressed in terms of unit area plant surface or total plant surface per unit area of littoral zone, although Gough and Woelkerling (1976) expressed results in terms of cells per unit macrophyte dry weight. The authors stated that results expressed in these terms were of limited value since macrophyte dry weight to surface area ratios were highly variable. Macrophyte surface area has been estimated by mathematical approximation and, in the case of macrophytes with finely dissected leaves, by the use of a surfactant (Harrod and Hall 1962).

Estimation of primary productivity of epiphytic algae attached to natural macrophyte substrates is complicated by interference from the photosynthetic activity of the macrophytes. Assman (1951,1953) in Wetzel (1975), estimated the productivity of *Equisetum* and its associated epiphytic algae by enclosing both in casings and following changes in oxygen concentration. Hickman (1971) and Hickman and Klarer (1973) measured the primary productivity of epiphytic algae from stems of *Equisetum* and *Scirpus* respectively using $C^{14}O_2$ uptake. Prior to incubation, epiphytic algae were scraped from the stem surface and suspended in filtered pond water. Sheldon and Boylen (1975) determined the productivity, on the basis of $C^{14}O_2$ uptake, of *Potamogeton* leaves washed free of epiphytes, the epiphytes alone and the intact macrophyte-epiphyte system.

Many studies of periphytic algae have employed artificial substrates for algal colonization. The artificial substrates provide a uniform attachment substrate which has been submerged for a specific time interval and is readily removable. The historical development of techniques employed for examining periphytic algal growth on artificial substrates has been well reviewed (Cooke 1956; Castenholz 1960; Sladeckova 1962; Wetzel 1964; Hutchinson 1975). While a wide range of substrates have been proposed, glass slides have been used most frequently. Numerous comparisons of periphytic algal growth on natural and artificial substrates have been carried out. Results, especially with regards to epiphytic algae, have been confusing.

Castenholz (1960) used glass plates placed in close proximity to natural rock substrates to study changes in attached algae in a number of freshwater and saline lakes. He concluded that the glass plates were not unduly selective. All periphytic algal species found on natural substrates

were observed on artificial substrates, although some blue-green algae such as *Calothrix* did not occur commonly on the glass slides until 6 weeks of exposure. Peters, Ball and Kevern (1968), using Plexiglas plates, found the same dominant algae occurred on both the natural and artificial substrates. Evans and Stockner (1972) observed that the composition of attached algae on reefs and rock outcrops was similar to that encountered on navigational buoys exposed for 145 days in Lake Winnipeg. These three studies indicate little discrepancy between algal growth on natural inert substrates and artificial substrates. However, studies comparing algae on natural macrophyte and artificial substrates have shown no such agreement in results.

Godward (1937) compared periphytic algal species on stones, macrophytes, mud and horizontally suspended glass slides in Lake Windermere. She concluded that the glass slides were colonized only by algae associated with macrophyte substrates. Certain blue-greens, found only on stones, did not occur on the glass slides.

Foerster and Schlichting (1965) compared algae colonizing glass lantern slides with colonization on *Vallisneria*, *Myriophyllum* and *Sagittaria* as well as on screened *Vallisneria*. Little information was given as to the exposure period or to the placement method for the glass slides within the aquatic vegetation. The authors, in comparing growth on artificial and natural substrates state, "the artificial barren surface gave a false indication of the true productivity trends and indicated only some of the significant genera in the ecosystem". In later discussion, they suggest that the glass slides showed colonization of a barren substrate, comparable to colonization occurring when the wire screen was removed from around the *Vallisneria*. This study has been cited (e.g. Hickman and Klarer

1973) as evidence of discrepancies between natural and artificial substrate algal populations. On the basis of insufficient information on substrate exposure, such interpretation appears unfounded. Rather, results suggest that similar standing crops occurred on recently exposed natural and artificial substrates.

Another frequently cited study of Tippet (1970) supposedly "shows differences between natural epiphytic diatom populations and those found on glass slides" (Hickman and Klarer 1973). Yet, the study involved comparison of glass slides exposed from 2 to 4 weeks with macrophytes exposed for undefined time periods. Part of the apparent discrepancy may have been related to differences in colonization times of the substrates.

Allen (1971) compared productivity of epiphytic algae on artificial and natural macrophyte substrates with similar exposure times using the $C^{14}O_2$ uptake technique. He found similar photosynthetic rates on both substrates.

Mason and Bryant (1975) compared algae on glass rods, exposed in February, with algae on *Typha* stems from the previous year's growth. In the April to December study period, they reported that species of algae on the glass rods were similar to those on stems. The times of peak standing crop coincided for the two substrates, although subsequent changes in standing crop differed. The authors suggested that a better comparison with the glass rods might have been made with *Typha* stems emerging in the spring. Both substrates would then have had equal exposure periods.

Brown (1976) compared colonization of recently submerged *Eleocharis* and glass slides suspended within a bottomless wooden box in the limnetic and littoral zone of a lake. In the limnetic area, the majority of species were chlorophytes with many of the same species appearing on both the slides

and plants. Desmids, filamentous chlorophytes, and cyanophytes formed a larger proportion of the total periphyton on the macrophyte than on the glass slide. In the littoral zone, similar species occurred on both substrates but in different proportions. Genera such as *Coleochaete* and *Protoderma* were more common on artificial than on natural substrates.

These preceding studies illustrate the confusion regarding the reliability of artificial substrates and some of the causes of this confusion. Insufficient attention has been paid to equating exposure periods of substrates and to locating artificial substrates in close proximity to natural substrates. Most studies showing discrepancies between algae on macrophyte and artificial substrates can be criticized for improper artificial substrate exposure. However, it should be recognized that part of the above confusion may be related to real differences in macrophytes and localities.

Algae have generally been scraped from the artificial substrates and methods of quantitative and qualitative analysis similar to those used for algae from natural substrates have generally been employed. Few studies of primary productivity have been carried out. Allen (1971), using $C^{14}O_2$ uptake, incubated Plexiglas slides colonized with epiphytic algae. After incubation, algae were removed by gentle rubbing, redistributed in filtered water and filtered through membrane filters. The filters were dried and radioactivity was determined with a gas-flow Geiger-Mueller counter. Rosemarin (1975), in an examination of periphyton in the Ottawa River, modified this method by placing small pieces of colonized Plexiglas directly into scintillation fluor after incubation with C^{14} and subsequent gentle rinsing.

Standing Crop and Productivity

In any comparison of epiphytic algal standing crop and productivity, the units of expression must be carefully considered. Results may be expressed on the basis of unit area of (1) macrophyte surface, (2) artificial substrate surface, (3) littoral zone colonized by a particular macrophyte, (4) total littoral zone, or (5) total pond or lake area. Total production of epiphytic algae may largely be limited by available colonizable substrate. Results expressed on the basis of either macrophyte surface area or artificial substrate surface area give the best indication of relative standing crop and productivity of the epiphytic algae independent of macrophyte growth.

Studies of seasonal standing crop and productivity of epiphytic algae, summarized in Table 1-1, are reviewed below.

Allen (1971) measured primary productivity and chlorophyll content of epiphytic algae colonizing Plexiglas slides at *Scirpus* and *Najas-Chara* sites in Lawrence Lake, Michigan. Productivity at the *Scirpus* site reached an initial peak in June, decreased in late June, reached maximum levels in July and August, then decreased to winter levels by November. A similar trend was noted at the *Najas-Chara* site, with a third, low peak in productivity in November. Between April and November chlorophyll a levels generally correlated with primary productivity. However, the highest observed level of chlorophyll a, at the *Najas-Chara* site in January, coincided with the lowest observed rate of photosynthesis. According to Allen, the epiphytic algae were responsible for 31.3% of the total annual littoral production, including macrophytes, and 21.4% of the total annual lake production.

Hickman (1971) measured standing crop and primary productivity of

Table 1-1. Standing crop and primary productivity of epiphytic algae on various substrates from several sites.

Lake	Substrate	m^{-2} colonized area	m^{-2} littoral zone
Lawrence L., Michigan (Allen, 1971)	Plexiglas at *Scirpus* site		
	Chlorophyll a		
	maximum	650 mg	383 mg
	Productivity (day^{-1})		
	maximum	2,000 mg C	1,400 mg C
	average	336 mg C	145 mg C
	Plexiglas at *Najas-Chara* site		
	Chlorophyll a		
	maximum	850 mg	7,300 mg
	Productivity (day^{-1})		
	maximum	1,600 mg C	14,000 mg C
	average	258 mg C	1,807 mg C
Priddy Pool, England (Hickman, 1971)	*Equisetum*		
	Chlorophyll a		
	maximum	250 mg	85 mg
	median range	50-100 mg	20-40 mg
	Cell number		
	maximum	48×10^9	12×10^9
	median range	$10\text{-}20 \times 10^9$	$4\text{-}8 \times 10^9$
	Productivity (h^{-1})		
	maximum	200 mg C	140 mg C
	median range	40—80 mg C	30-50 mg C
Langford Spring, England (Hickman, 1974)	*Chiloscyphus*		
	Cell number		
	maximum		20×10^7
	median range		$3\text{-}7 \times 10^7$
	Productivity (h^{-1})		
	maximum		16.3 mg C
	median range		3-7 mg C

Table 1-1. continued

Mikolajskie L., Poland (Kowalezewski, 1975)	*Elodea, Myriophyllum, Potamogeton*		
	Chlorophyll a		
	maximum	33.4 mg	
	average	8 mg	51 mg
	Dry weight		
	average	37 g	
Alderfen Broad, England (Mason and Bryant, 1975)	Glass rods in *Typha* stand		
	Dry weight		
	maximum	19.3 g	
	Dead *Typha* stems		
	Dry weight		
	maximum	18.0 g	
Lake George, New York (Sheldon and Boylen, 1975)	*Potamogeton*		
	Productivity (h^{-1})		
	maximum	.63 mg C	
	median range	0.2-.3 mg C	
	Dry weight		
	maximum	2.2 g	
	median range	0.5—1.5 g	
	Diatom cell number		
	range	3×10^6 - 1.4×10^8	
Plover Cove Reservoir, Hong Kong (Tai and Hodgkiss, 1975)	Wood		
	Chlorophyll a		
	maximum	1,870 mg	
	median range	700-900 mg	
	Diatom cell number		
	maximum	18.6×10^7	
	median range	$4\text{-}6 \times 10^7$	

epiphytic algae attached to *Equisetum* over a two-year period in Priddy Pond, North Somerset. Seasonal productivity rates and standing crop levels, expressed on a macrophyte surface area basis, fluctuated considerably over the two-year period with no consistent seasonal trends, with the exception of highest chlorophyll a levels generally occurring in January and February. Maximum values of chlorophyll a (Table 1-1) were considerably lower than those determined by Allen (1971). Hickman estimated that the yearly standing crop of epiphyton was approximately 27 times that of either phytoplankton or epipelon in Priddy Pond, while primary productivity was approximately 40 times that of the other algal components.

The epiphytic algae attached to *Chiloscyphus*, a liverwort, were examined by Hickman (1973). Productivity and standing crop were closely related, with maxima in both occurring in late April, mid-July and mid-August. A fourth peak occurred in primary productivity in early December and in standing crop in January.

Epiphytic algae on submerged macrophytes were investigated by Kowalczewski (1975). Photosynthetic activity per unit chlorophyll was 5 times higher for phytoplankton than for periphyton. At various sites, the epiphytic algal gross production ranged from 0 - 100% of the total epiphytic and planktonic algal production.

Mason and Bryant (1975) estimated standing crop on the previous year's stems of *Typha*. Standing crop fell from a maximum in April to almost zero by November. Epiphytic algae on glass rods, exposed in spring, reached maximum values in May and declined slightly to a stable level which lasted until the end of the study in December.

Epiphytic algae on *Potamogeton* in an oligotrophic lake were examined

from May to October (Sheldon and Boylen 1975). Maximum photosynthesis and standing crop occurred in July and August. Productivity of epiphytes from lower leaves was ten-fold greater than that from upper leaves. The epiphytic algae assimilated approximately 5% (0.07 - 10.84%) as much inorganic C as the leaves of *Potamogeton amplifolius* from which they were taken.

Tai and Hodgkiss (1975) followed seasonal changes in attached algae on wood in Plover Cove Reservoir, Hong Kong. Chlorophyll a and cell counts followed similar seasonal trends, with maximum growth in winter, moderate spring and autumn growth and low summer growth.

Community Composition

Numerous lists indicating the wide range of algal species which occur in the epiphytic habitat have been published. Among the most comprehensive lists are those of Godward (1937) and Young (1945). Godward recorded a large number of green, blue-green and especially diatom species growing on plants in Lake Windermere. Young (1945) compiled an extensive list of epiphytic species on *Scirpus* culms. As well as the dominant pennate diatoms, members of the Tetrasporales, Ulotrichales, Oedogoniales, Zygnementales, Chlorococcales, Hormogonales, Chroococcales and Centrales were reported. Other organisms present on the culms included invertebrate species, bacteria and fungi.

Hodgkiss and Tai (1976) presented additional evidence to support the generalization of Round (1964) that the epilithic and epiphytic communities are similar in species composition. Hodgkiss and Tai (1976) compared four periphytic communities in Plover Cove Reservoir, Hong Kong. Of a total of 61 species, 54 occurred in the epiphytic community, 46 in the epilithic,

28 in the epipelic and 30 in the episammic. Eighteen of these species occurred on all substrates. The epiphytic community had 45 species in common with the epilithic community, compared to 25 and 27 species in common with the episammic and epipelic communities respectively. Thirteen species shared by the epiphytic and epilithic communities did not occur in the other communities.

The attachment and structure of the epiphytic community on the substrate has been considered by several authors. Round (1964) in a review of periphytic algae, outlined four main attachment mechanisms of epilithic and epiphytic algae. These included forms with mucilage stalks, e.g. *Gomphonema*; direct mucilage attachment, e.g. *Cocconeis;* prostrate disc attachment, e.g. *Coleochaete* and modified basal holdfast cells, e.g. *Oedogonium*. Allen (1971) observed the plant surface beneath epiphytes covered with calcium carbonate crystals and chlorotic diatoms interwoven in a mucilaginous matrix. Allanson (1973), using stereoscan electron microscopy, confirmed the carbonate-mucoid matrix of the epiphytic community.

Seasonal succession of epiphytic algae has been followed by Allen (1971), Hickman (1971, 1974) and Tai and Hodgkiss (1975). During initial colonization of artificial substrates in June in the littoral of Lawrence Lake, Allen (1971) observed some diatoms (*Synedra, Eunotia, Tabellaria*) as well as cyanophytes and bacterial colonies. The community from July until early autumn was dominated by *Gomphonema*, with many smaller diatoms such as *Fragilaria*, and *Cymbella* attached to the *Gomphonema* cells. Other dominant genera included *Oedogonium, Bulbochaete, Zygnema, Chaetophora, Navicula, Cyclotella, Synedra* and *Chlorella*. An understory of *Gloeotrichia* and mucilaginous communities of bacteria occurred occasionally.

Hickman (1971) observed the seasonal cycle of epiphytic algae on *Equisetum* from February 1967 to August 1968 in Priddy Pool. The only consistent trend appeared to be low diatom populations in June, July and August of both years. Hickman (1974) also examined algal growth on *Chiloscyphus*, a leafy liverwort, from February 1967 to February 1968. In February of both years, the community was dominated by *Cocconeis*. A maximum occurred in April, dominated by *Diatoma*, *Meridion*, *Achnanthes* and *Eunotia*. A second population peak in August was dominated by *Achnanthes*. *Nostoc* and *Oscillatoria* appeared in the epiphytic community in August. *Cocconeis* populations began increasing in December and dominated the algal community again by February.

Tai and Hodgkiss (1975) found species of *Fragilaria* and *Nitzschia* were common to sub-dominant throughout the year on wood in Plover Cove Reservoir, with most other species appearing and disappearing at various times. However, all species exhibited optimal growth during the cool winter months and minimum growth during the hot summer months.

While many studies report no clear-cut differences in epiphytic algal composition on various macrophyte species, a few studies have shown some dissimilarities. Prowse (1959) exposed epiphyte-free *Enhydrias*, *Naias* and *Utricularia* in a fishpond. After two weeks, *Gomphonema* occurred in greatest density on *Utricularia*, *Oedogonium* was most common on *Enhydrias* and *Eunotia* most dense on *Naias*. No other reports of such distinct algal preferences have been found.

Foerster and Schlichting (1965) state that the "weed beg" they studied had a "definite ecotype orientation". *Vallisneria*, *Sagittaria* and *Myriophyllum* had an ecotype referred to as the "*Tabellaria-Bulbochaete - Microcystis* synusia" while *Nitella* had a "*Tabellaria - Gomphonema -*

Microcystis synusia". The authors did not indicate the basis for their recognition of such associations. According to their results, *Gomphonema* as well as 17 other genera occurred on all four substrates. Although some genera such as *Bulbochaete* occurred on all substrates except *Nitella*, other genera such as *Meridion* occurred only on *Nitella* and *Vallisneria*.

Woelkerling (1976), in a detailed study of Wisconsin desmids, included an analysis of algae occurring on various macrophyte hosts. Information on total cells of various algal groups per mg dry weight host macrophyte on seven aquatic plant genera in acid and alkaline bogs was presented. Diatoms and blue-green algae had the highest population densities. Significant differences in algal group densities on various hosts within the same lake were reported. However, since algal cell numbers were expressed on a macrophyte dry weight basis, equal surface areas on various macrophytes were not being compared. In contrast to the specificity observed by Prowse (1959), Woelkerling found that *Utricularia* harboured the greatest desmid density as well as representatives of all other algal groups occurring epiphytically.

Factors Affecting Growth

Allen (1971) summarized diagrammatically the metabolic and nutritional interactions between the epiphytic community and the host macrophyte in a typical marl lake. The heterogenous nature of the epiphytic community, with bacterial, fungal, algal and faunal components in close proximity, and the various interactions between this community and the macrophyte substrate create a complexity of factors influencing the growth of epiphytic algae. While various physical, chemical and

biotic factors can be identified, few studies have evaluated the influence of the various factors on seasonal epiphytic algal growth.

Shading by macrophytes may reduce the light availability for epiphytic algae. The extent of light reduction beyond that normally occurring in a water body would depend on the type and growth density of the macrophyte. For example, levels of light penetrating to the water surface in a *Typha domingensis* swamp were found to be less than 10% of light levels above the vegetation (Howard-Williams and Lenton 1975). Dokulil (1973) reported phytoplankton productivity within a *Phragmites* belt to be 35% of that of open water. The difference was attributed mainly to reduced irradiation within the *Phragmites* stand. Straskraba and Pieczynska (1970) demonstrated a fifty-fold increase in phytoplankton productivity and a three-fold increase in attached algal productivity as a consequence of increased light intensity due to removal of *Phragmites* shoots.

Growth of epiphytic algae may be influenced by nutrient competition with macrophytes, as well as by excretion, autolysis and decomposition of macrophytes. The capability of many submerged macrophytes to take up inorganic nutrients from both the sediment via the root system, and the surrounding water, via foliar absorption has been demonstrated (e.g. Denny 1972; Wilson 1972; Nichols and Keeney 1976). Nutrient competition between submerged macrophytes and epiphytic algae was considered by Fitzgerald (1969). Fitzgerald found, in culture studies with a number of aquatic plants, that while phosphorus limitation had no effect on the growth of epiphytes, the limitation of available nitrogen prevented algal growth. The author proposed that the antagonistic effect on algal growth was due to unsuccessful competition for available nitrogen, although the

presence of bacteria-sized organisms which had selective toxicity to certain algae indicated that other causes of aquatic plant-algae antagonism could exist. The results, however, give no direct indication of nutrient competition between the algae and the aquatic plants. There was no evidence of either uptake of nitrogen by the aquatic plants or of growth of the aquatic plants under conditions of nitrogen limitation. Low concentrations of available nitrogen may have been limiting the growth of both the macrophytes and the epiphytes.

Excretion of dissolved organic compounds by macrophytes may be of importance in nutritional interactions within the epiphytic community. Wetzel (1969) demonstrated that excretion by *Najas flexilis* may range from 0.5 to 99% of photosynthetically fixed C, depending on a number of environmental factors. Under most natural conditions, excretion of dissolved organic C is reported to be less than 10%. Hough and Wetzel (1975) reported *in situ* release from *Najas flexilis* in June of 0.5% and in September of up to 2.2% as plants senesced, while *Scirpus subterminalis* excreted 0.3% in October and a maximum of 1.3% in April.

A transfer of C^{14}-labelled compounds from host macrophytes to epiphytic algae has been demonstrated (Allen 1971; Harlin 1973). Radioactive C taken up by *Scirpus* from the atmosphere was observed in the water column and the epiphytic algae within 3 minutes (Allen 1971). In the same study, uptake of C^{14}-labelled extracellular products of *Najas flexilis* by algal and bacterial cultures was demonstrated. Such transfers may have been due to direct organic uptake by the algae, or they may have been indirect transfers, mediated via bacteria. Evidence for the latter was an enhancement, in the presence of bacteria, of the amount of C^{14}-label detected in the algae. Harlin (1973) found transfers

of C^{14}-labelled products between a number of epiphytic marine algae and host substrates. These transfers were bidirectional. In the same study, transfer of P^{32} along the blade of *Phyllospadix* into *Smithora* was demonstrated.

The role of organic compounds in the growth of epiphytic algae in a salt marsh has received considerable attention (Lee, McEnery, Kennedy and Rubin 1975; Saks, Stone and Lee 1976). Results indicate utilization and recycling of organic substrates by various epiphytic algal species. Even though the algae have heterotrophic capabilities, Saks *et al* (1970) suggest that photosynthesis is the major C route for most of the epiphytic algae.

Decomposition of macrophytes would have an obvious influence on nutrient levels in the surrounding water. Higher concentrations of nutrients in littoral water than in open water have frequently been observed (e.g. Howard-Williams and Lenton 1975). These higher nutrient concentrations may enhance the growth of epiphytic algae within the littoral zone.

Young (1945) reported a faunal component of the epiphytic community on *Scirpus* culms comprised of nematodes, rotifers, gastrotrichs, oligochaetes, protozoa, ciliates, copepods, ostracods as well as water mites, mayfly nymphs and larval stages of Trichoptera and Diptera. Possible effects and the extent of grazing on epiphytic algae remain largely unexamined. Allanson (1973), using steroscan microscopy, published photographs of feeding tracks in the epiphytic community on *Chara* made by mayfly nymphs, a copepod, a chydorid and a chironomid larva.

Mason and Bryant (1975) examined grazing by chironomids of periphyton on *Typha* stems. The density of chironomids on dead *Typha* stems

peaked in late May and declined until December, closely paralleling the decline in dry weight of the periphyton. The alimentary canals of 60 chironomid larvae collected from the stems in June were examined. *Glyptotendipes* and *Cricotopus* had ingested algae, while larvae of *Chironomus tetans* contained only rotting *Typha*. The authors suggest that the chironomids, while on the *Typha* stems, almost completely graze the periphyton. However, the control of epiphytic algal standing crop on the stems by factors other than grazing was not considered. No quantitative estimation of grazing was made and evidence that grazing was the main factor reducing standing crop was based on a correlation between larval density and algal standing crop only.

Several studies have attempted to correlate changes in epiphytic algal standing crop with various environmental factors. Knudson (1957) investigated densities of *Tabellaria flocculosa* on shoots of *Phragmites* and *Schoenoplectus* in three English lakes from 1948 to 1952. A generalized account of factors controlling the population density of *Tabellaria* was presented. From November until January, population levels remained constant at high or low levels, indicating that increases due to cell multiplication were balanced by losses due to grazing or wave action. This period coincided with minimum illumination and temperature. A spring increase in population usually occurred during February to April. Following the spring maximum, population levels declined. This decline often correlated with low levels of dissolved silica. As nutrients were renewed, population densities of *Tabellaria* on the shoots reached maximum levels in September or October. Population densities over the winter period in some years remained high and in other years declined rapidly.

Brown (1973) examined littoral periphyton communities on vertically oriented glass slides at four stations in Elk Lake, British Columbia and used regression and correlation techniques to assess environmental factors influencing standing crop and species diversity. Total cell population increases correlated positively with length of slide exposure, pH, temperature, nitrite, nitrate and copper and correlated negatively with oxygen, orthophosphate and hardness. Species diversity generally decreased with increasing duration of slide exposure. Using multiple stepwise regression, 80% of total cell variation at the four stations could be accounted for by length of slide exposure, phosphate and hardness.

Hodgkiss and Tai (1976) investigated four periphytic diatom communities in Plover Cove Reservoir, including the epiphytic community. The major factors governing seasonal variation in standing crop were identified as water level, temperature, nutrients (especially silica, nitrate and phosphate) and summer storm disturbance of attached algae.

CHAPTER 2

PRIMARY PRODUCTIVITY OF EPIPHYTIC ALGAE IN A MARSH POND

INTRODUCTION

In shallow lakes, ponds and marshes, which have dense growths of emergent and submerged macrophytes, an extensive epiphytic community is common on the submerged substrate provided by the aquatic vascular plants. Bacterial, fungal, algal and animal components of such a community have been identified (Young 1945), but there is little information on the metabolism of the community. The purpose of this study has been to provide information on seasonal primary productivity of the algal component.

The attachment of algae to a biotic substrate introduces unique problems to the estimation of productivity, particularly where $C^{14}O_2$ assimilation is to be used as the means. Accordingly, artificial substrates have been utilized. These and other methods of determining periphyton production have been reviewed (Cooke 1956, Sladeckova 1962, Wetzel 1964, 1965). Productivity estimates have involved scraping of the natural substrate prior to incubation with $C^{14}O_2$ (Hickman 1971, 1974) and the rubbing of Plexiglas substrates following incubation (Allen 1971), although scraping of any substrate is both time-consuming and introduces a source of variation. Additionally, the pre-incubation removal of algae from a substrate is inconvenient for '*in situ*' field procedures.

Another purpose of this study was to develop a more convenient method for routine '*in situ*' measurement of periphyton productivity using the $C^{14}O_2$ assimilation technique. Cellulose acetate film, an inert, clear flexible material was found to be readily soluble in dioxane-based scintillation fluid (Bray 1960) without creating problematic quenching. Since the use of this material as an artificial substrate would eliminate

the necessity for sample removal, an evaluation of it was warranted.

MATERIALS AND METHODS

The main experimental site was Crescent Pond (Fig. 2-1), a small isolated body of water in the Delta Marsh (99° 19" W, 50° 7" N) of Manitoba. The spring surface area of the pond is 8.6 ha and the maximum depth 135 cm, although water depth may be reduced by approximately 30 cm during the summer. The maximum length of the pond is 584 m and the maximum width 152 m. Total alkalinity, expressed as $CaCO_3$ (APHA 1971) ranged from 160 mg l^{-1} in May, 1974 to 490 mg l^{-1} in October, 1974, and pH values from 7.6 to 8.6. Crescent Pond has an extensive zone of emergent vegetation in depths up to 40-60 cm (Fig. 2-1). *Typha latifolia* L. is dominant, with narrow distinct zones of *Scirpus acutus* Muhl. and *Phragmites communis* Trin. (Fig. 2-1). The dominant submerged macrophyte is *Potamogeton pectinatus* L. (Fig. 2-1). Primary productivity estimates are for periphyton on these macrophytes in Crescent Pond.

As assessment of the effects of pre-incubation scraping of substrates and a comparison of production of algae on natural, glass and cellulose acetate substrates were conducted. Wooden frames with attached 6.0 cm x 50 cm strips of cellulose acetate film and vertically positioned glass slides were placed among stands of *Typha latifolia*, *Phragmites communis*, *Scirpus acutus* and *Myriophyllum exalbescens* Fern.- *Potamogeton pectinatus* in a blind channel of the Delta Marsh. Each of the four sites was sampled at 2-4 week intervals from July to October, 1973.

At each sampling period random sections of cellulose acetate and natural substrate were cut and returned to the laboratory along with glass

s l ide substrates. Areas of the substrate sections were initially 3 cm, but were later reduced as algal growth became heavy. Material was gently scraped from the substrates and suspended in 25 ml aliquots of filtered marsh water in clear glass incubation bottles. Unscraped cellulose acetate samples were also placed in incubation bottles. Three replicates of unscraped cellulose acetate, scraped cellulose acetate and glass slides, and six replicates of scraped natural substrate were employed for each site at each sampling period. 0.5 ml of NaH $C^{14}O_3$ of known activity (1.4 $\mu Ci\ ml^{-1}$) was injected into each sample bottle and dispersed by shaking. Samples were incubated at 15°C and 260 ft candles for 4 hours. Each sample was then filtered through a 0.45 μ cellulose nitrate filter and the filter rinsed with 10 ml of distilled water. Filters, along with sections of cellulose acetate for unscraped samples, were acidified to remove inorganic carbon and placed in scintillation fluor (Bray 1960). Sample radioactivity was determined in a scintillation counter (Picker Liquimat 220), and efficiency of counting determined by the channels ratio method (Wang and Willis 1965). Inorganic carbon content of sample water was estimated from alkalinity and dissolved CO_2 determinations (APHA 1971) and productivity by the method of Strickland and Parsons (1968).

On the basis of the assessment, cellulose acetate film was used as an artificial substrate for the subsequent estimation of periphyton production in Crescent Pond. The sampling approach involved subsampling with primary units of equal size in the emergent zone and simple random sampling in the submerged zone. In the emergent zone four strata were recognized: *Scirpus*, *Phragmites*, high density *Typha* and low density

Typha (Fig. 2-1). Primary units in each stratum were 0.5 m-wide transects extending from the front edge to the back edge of the emergent vegetation. Two primary units were established as permanent quadrats in each stratum on a random basis. Secondary units were the number of 3.0 cm^2 areas available for epiphytic colonization in each primary unit. Cellulose acetate strips (60 x 5 cm), attached vertically to wooden frames, were placed within the macrophyte stands beside each permanent quadrat, so that the strips extended throughout the water column. Two strata of submerged vegetation were recognized: high density *Potamogeton* and low density *Potamogeton* (Fig. 2-1). Locations of artificial substrates in these strata were determined on a random basis. In all cases placement of the artificial substrate coincided with the spring emergence of the macrophytes.

The submerged surface areas of *Typha*, *Phragmites* and *Scirpus* were measured directly at each sampling period at the permanently established quadrats. Depth was recorded and the percent new area exposed in each sample interval was calculated to allow correction of primary production estimates. For *Potamogeton*, 3 randomly chosen 225 cm^2 quadrats were sampled in each stratum at each sampling interval. All vegetation within each quadrat was removed, washed, dried and weighed. Surface areas were determined from seasonally adjusted surface area/dry weight conversion factors. Light intensity directly above the water surface was estimated at all sites with a Weston Illumination meter.

Production estimates were made at 2-4 week intervals from June-October, 1974 and May-June, 1975. The pond was ice-covered from November to April and the May-June samples represented colonization on submerged surfaces of the previous year's *Typha* and *Scirpus* after overwintering. After June old stems fell to the sediment. *Potamogeton* was mixed into

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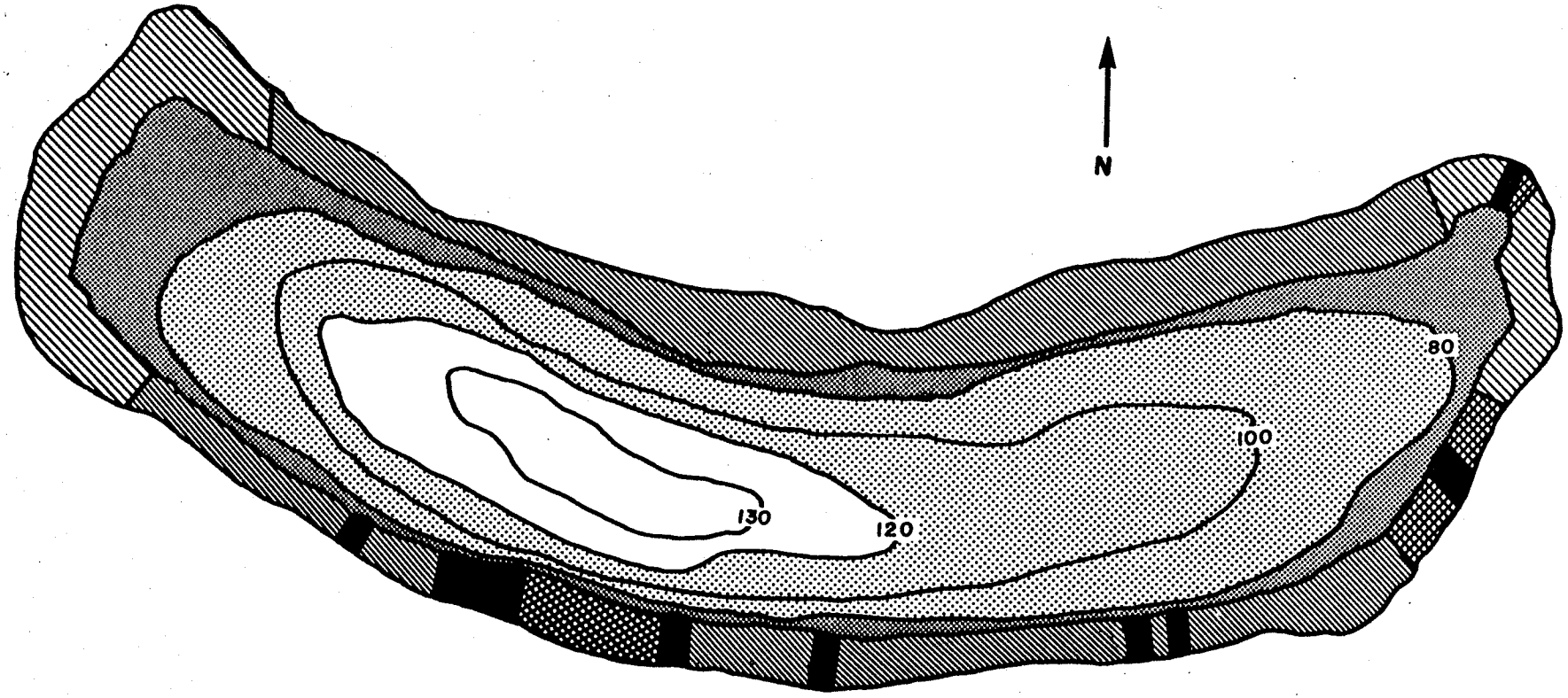
Figure 2-1. Bathymetric map of Crescent Pond, Delta Marsh, Manitoba, illustrating the extent and position of the six sampling strata recognized.



TYPHA - HIGH DENSITY

TYPHA - LOW DENSITY

POTAMOGETON - HIGH DENSITY

POTAMOGETON - LOW DENSITY

SCIRPUS

PHRAGMITES

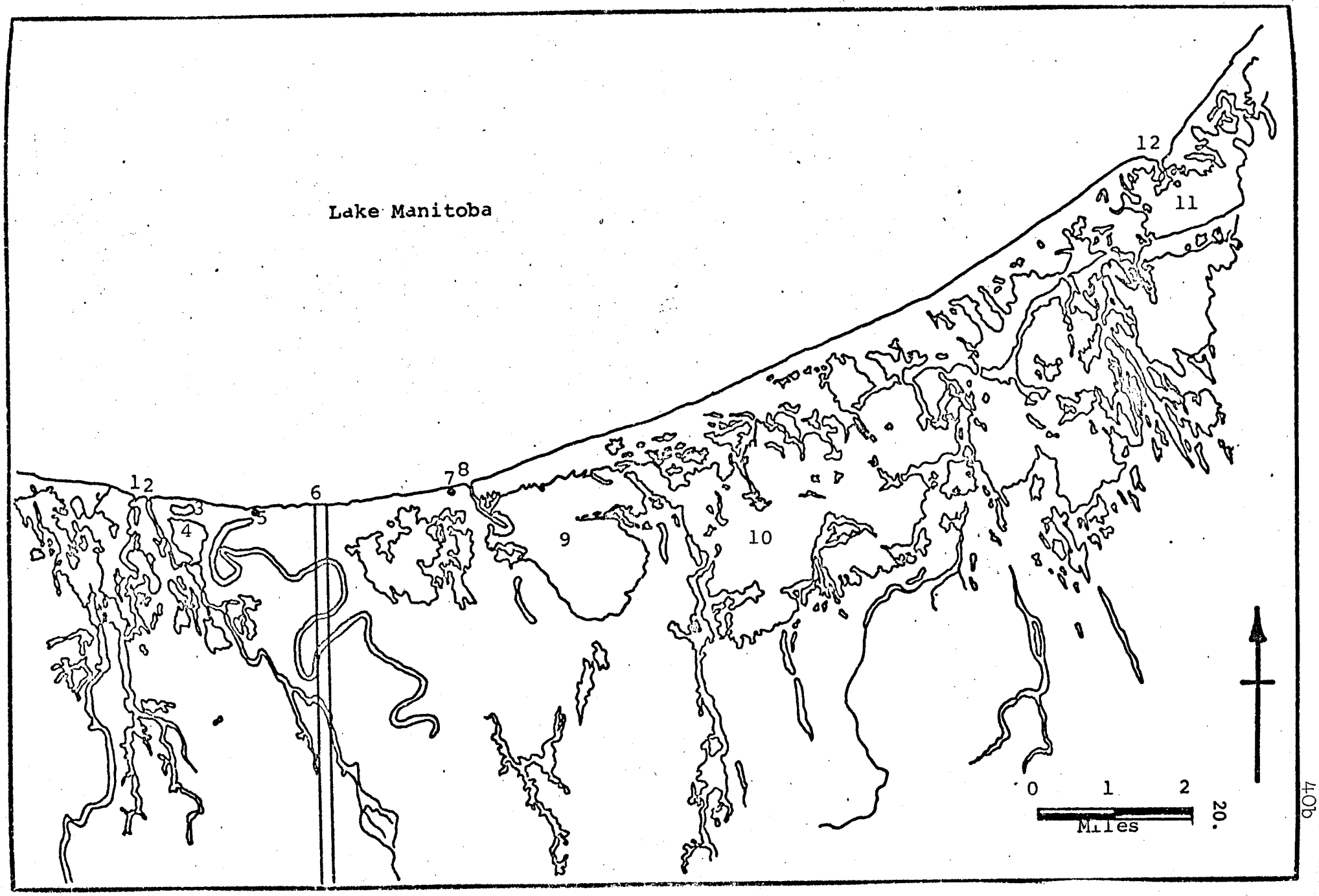
0 50 m

CONTOUR INTERVALS IN CENTIMETRES

LEAF 40 OMITTED IN PAGE NUMBERING.

Figure 2-1b. Location of Crescent Pond in Delta Marsh, Manitoba.

1. Deep Creek
2. Cram Creek
3. Crescent Pond
4. Forsters Bay
5. University of Manitoba Field Station
6. Diversion Channel
7. Delta Beach
8. Delta Channel
9. Cadham Bay
10. Simpson Bay
11. Clandeboye Bay
12. Clandeboye Channel


Lake Manitoba
12
11
12
6
7
8
5
4
9
10
0
1
2
Miles
20.

the sediment by the spring and *Phragmites* was ignored in May-June because of the negligible area submerged in the spring.

Periphyton production was determined as described above for unscraped cellulose acetate with several exceptions. Six determinations of epiphytic algal productivity were made at each of the two permanent quadrats within each macrophyte stratum. The samples of cellulose acetate were chosen on a stratified random basis, placed in light and dark incubation bottles and incubated at their depths of origin for a four hour midday period. That assimilation of $C^{14}O_2$ remained linear for the four hour period was verified. Light and dark control bottles containing only filtered marsh water were also incubated for all samples. Estimates were corrected for dark uptake, incidental phytoplankton activity, and for new surface area available for colonization due to macrophyte growth. Epiphyte production on newly exposed (1 week) cellulose acetate strips was determined at each sample period, as well as the percentage of new macrophyte surface area available for colonization, allowing an appropriate correction of estimates.

Four-hour production values were extrapolated to daily values on the basis of percent total solar radiation, which was continuously monitored with a pyrheliometer (Belfort Instrument Co.). The adequacy of the extrapolation had been tested by overlapping four-hour diurnal incubations, and was found to give values in close agreement. Daily production values were extrapolated to potential maximum values assuming 100% radiation (i.e. cloud-free days), and estimates between sampling periods based on linear interpolations, adjusted for recorded solar radiation.

At each sampling period substrate was collected, scraped, filtered onto pre-combusted glass fiber filters and desiccated for determination

of organic C and N content in an elemental analyzer (Perkin Elmer 240).

RESULTS

No significant differences were found between mean C^{14}-uptake values of epiphytic algae colonizing cellulose acetate and natural substrates for equal periods of time nor between values based on cellulose acetate and glass substrates (Table 2-1). The effect of the removal of epiphytic algae from the substrate prior to incubation was such that when all mean sample pairs are included, there was no significant difference between scraped and unscraped values (Table 2-1). When pairs with unscraped values less than 3.9 $\mu gC\ cm^{-2}$ are compared, the unscraped values are significantly higher, while pairs with unscraped values greater than 3.9 $\mu gC\ cm^{-2}$ are not significantly different (Fig. 2-2).

Analysis of productivity for Crescent Pond indicated a variance to mean relationship indicative of a non-normal distribution. The relationship is described by Taylor's Power Law (Taylor 1961). Following appropriate data transformation (square root), a coefficient of variation ($s/\bar{x}$) was determined for all sample values and averaged. The average coefficient of variation applicable to all productivity values was 31%.

Periphyton productivity at each site is presented in Figure 2-3. Maximum production rates for all substrates occurred in September and October, although high production rates were observed at the *Typha* and *Scirpus* sites immediately after ice-melt (Fig. 2-3). The highest production m^{-2} of macrophyte surface area was at the *Typha* and *Scirpus* sites, although the most significant contribution to the annual overall epiphyte production was attributable to those epiphytes attached to *Typha* and *Potamogeton*

TABLE 2-1

Wilcoxon ranked signs tests on mean pairs of C^{14} uptake values for various substrates and treatments

	Mean Sample Pairs	N[1]	T[2]	a[3]	
A	Cellulose acetate and natural macrophyte substrates	15	64	0.05	n.s
B	Cellulose acetate and glass substrates	15	46	0.05	n.s.
C	Unscraped and scraped cellulose acetate substrates	33	175	0.05	n.s.
D	As C with pairs in which unscraped values <3.9 µgC cm^{-2} included	18	1.5	0.01	s.
E	As C with pairs in which unscraped values >3.9 µgC cm^{-2} included	15	60	0.05	n.s.

[1]N - number of pairs

[2]T - Wilcoxon signed ranks test statistic

[3]a - Level of significance

Figure 2-2. A comparison of epiphytic algal productivity determined from scraped and unscraped cellulose-acetate substrates in Delta Marsh, Manitoba.

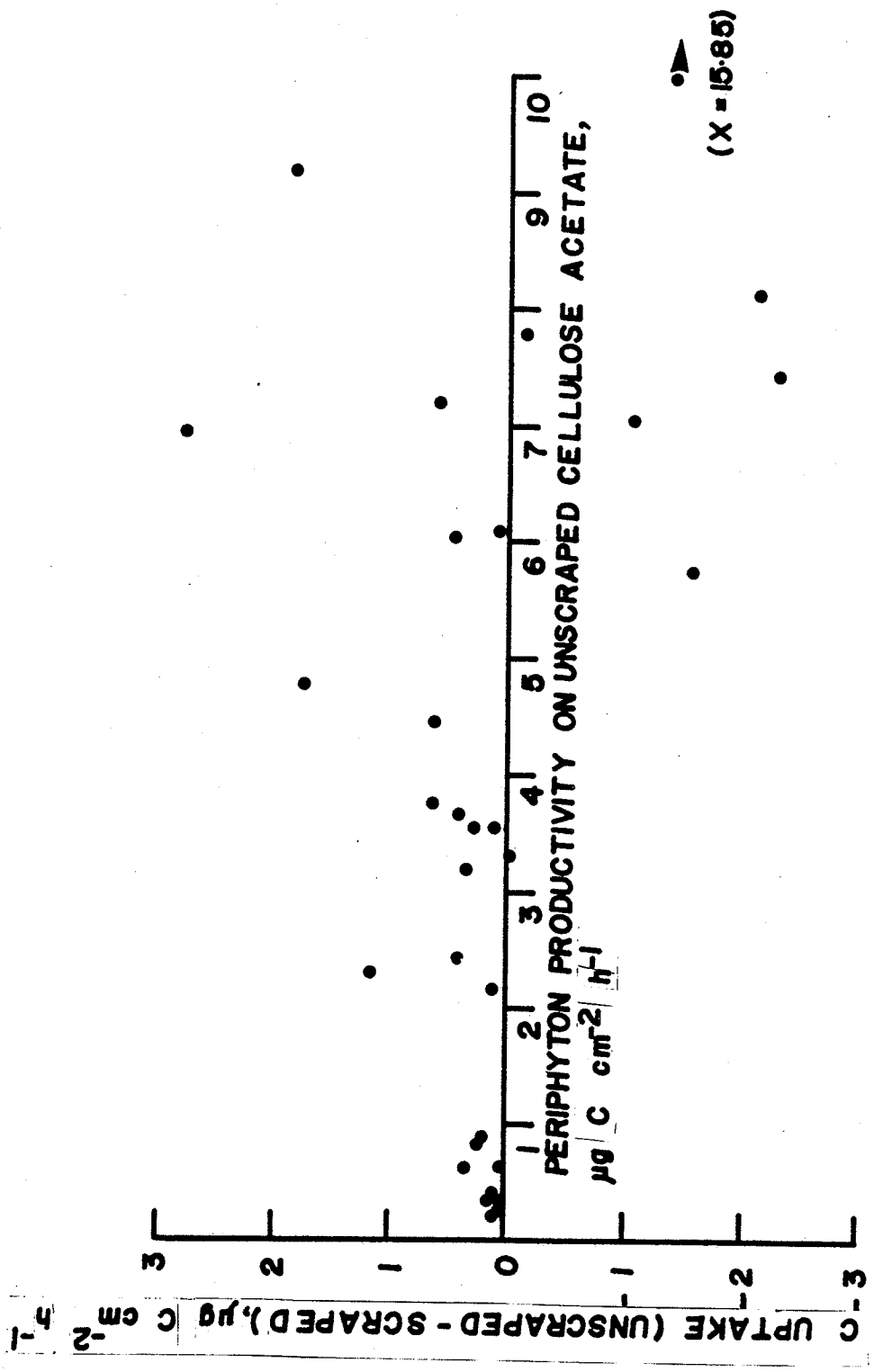
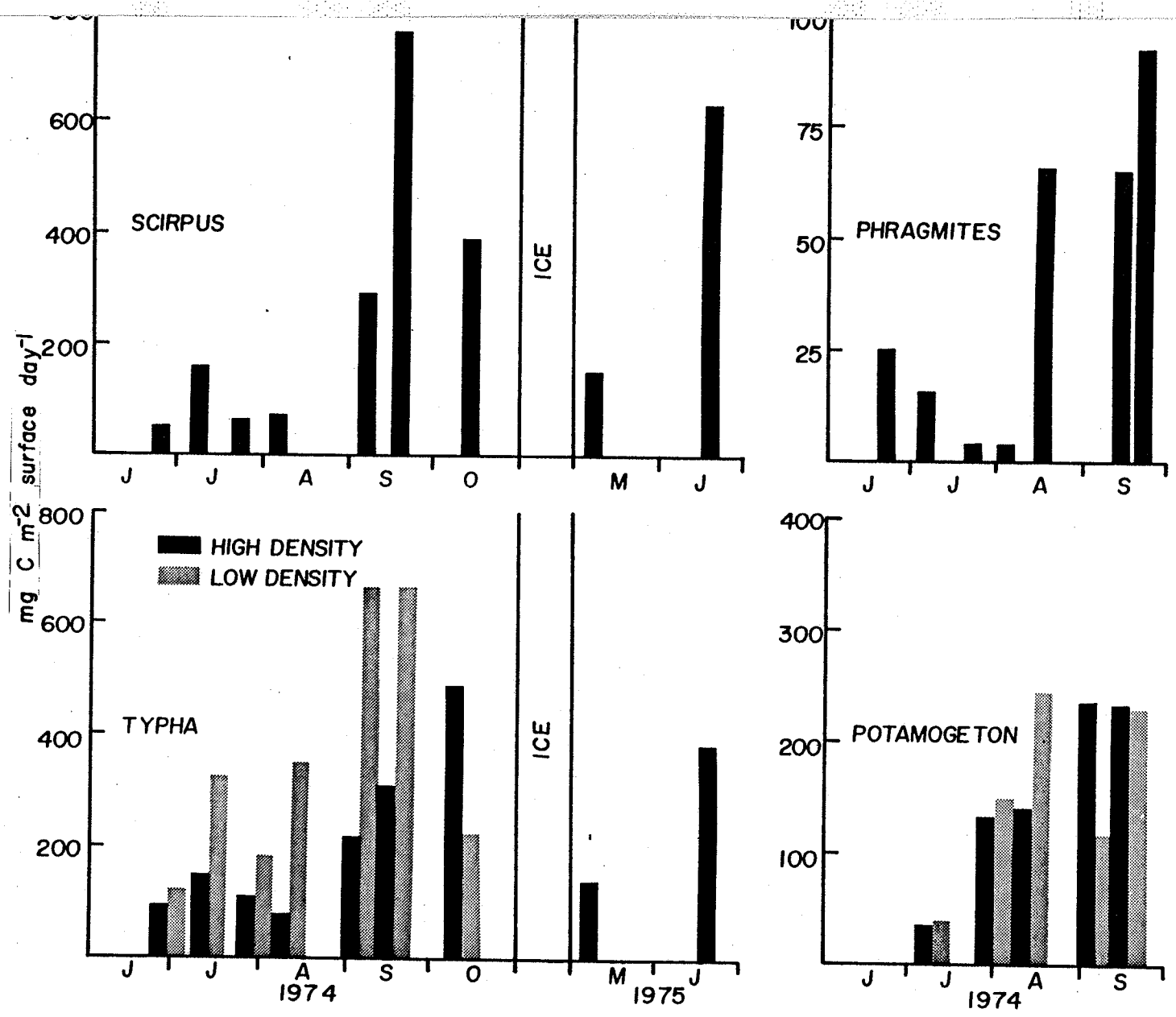

(X = 15·85)
PERIPHYTON PRODUCTIVITY ON UNSCRAPED CELLULOSE ACETATE,
μg C cm⁻² h⁻¹
C UPTAKE (UNSCRAPED - SCRAPED), μg C cm⁻² h⁻¹

Figure 2-3. The seasonal primary productivity of epiphytes on submerged and emergent macrophytes in Crescent Pond, Delta Marsh, Manitoba. Values are maximized assuming 100% radiation.


SCIRPUS
PHRAGMITES
TYPHA
POTAMOGETON
ICE
HIGH DENSITY
LOW DENSITY
mg C m-2 surface day-1
J J A S O M J
1974
1975
J J A S
1974

(Table 2-2). The importance of these substrates is related to the extensive areas of these species available for colonization in Crescent Pond.

The seasonal trend of the organic C content of scraped epiphytic material followed the observed primary production rates (Fig. 2-4). The highest ratios of epiphytic community organic C to productivity were observed in September and October for all but the *Phragmites* site (Table 2-3). The very low productivity and organic C values at the *Phragmites* site resulted in ratios that were more sensitive to minor fluctuations and must therefore be interpreted with caution. The average organic C/organic N ratio was 9.3, with no apparent seasonal trends or substrate differences.

DISCUSSION

The similarity of C^{14}-uptake values for epiphytic algae on artificial and natural macrophyte substrates observed in this study is supported by Allen (1971), who reported similar uptake values for algae on Plexiglas slides and macrophyte surfaces. On the other hand Foerster and Schlichting (1965) and Tippet (1970) reported differences in the algal populations colonizing glass slides and macrophyte substrates. Part of this apparent discrepancy may be related to differences in colonization times of the substrates. For maximum reliability in the use of artificial substrates for the estimation of epiphytic algal production natural colonization times should be ensured by monitoring macrophyte emergence times and growth, and substrates should be placed within macrophyte stands to obtain similar light and chemical environments. Sample scraping should be avoided as it may cause underestimation of uptake values at low productivity levels (Fig. 2-2). Such underestimation may be due to incomplete removal of the algae and/or cell

TABLE 2-2

Primary productivity of epiphytic algae on macrophytes in Crescent Pond; June 1974 - June 1975

Site	Production/m^2 Surface Area g C m^{-2}	Production/m^2 Macrophyte Zone g C m^{-2}	Overall Pond Production Kg C yr^{-1}
Scirpus	32.306	43.490	21.049
Typha High Density	23.047	22.944	142.105
Typha Low Density	28.421	5.679	15.845
Phragmites	2.353	0.642	0.273
Potamogeton High Density	8.643	48.552	1,303.761
Potamogeton Low Density	7.854	11.785	396.138
Total			1,881.
Average per m^2 Total Pond Area			0.027

Figure 2-4. Seasonal particulate organic carbon content of the epiphytic community on submerged emergent macrophytes in Crescent Pond, Delta Marsh, Manitoba during 1974.

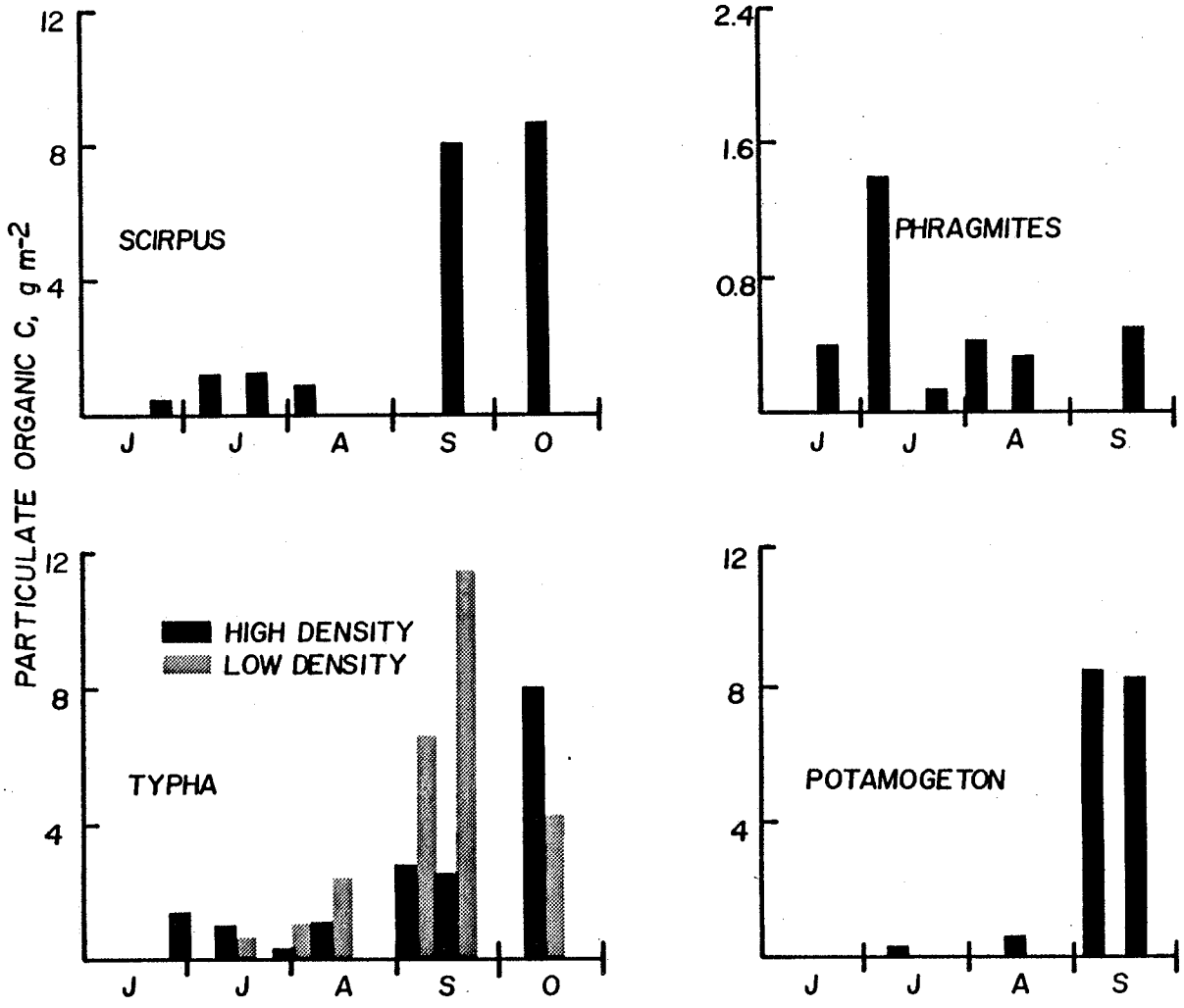

PARTICULATE ORGANIC C, g m-2
12
8
4
SCIRPUS
J
J
A
S
O
2.4
1.6
0.8
PHRAGMITES
J
J
A
S
12
8
4
HIGH DENSITY
LOW DENSITY
TYPHA
J
J
A
S
O
12
8
4
POTAMOGETON
J
J
A
S

TABLE 2-3

Organic C/primary productivity ratios for the epiphytic community in Crescent Pond

Macrophyte Site	Date	Ratio (days)
Typha High Density	1.7.74	13.9
	15.7.74	5.2
	30.7.74	2.9
	12.8.74	11.9
	6.9.74	12.6
	18.9.74	8.0
	13.10.74	16.3
Typha Low Density	15.7.74	1.7
	30.7.74	9.0
	12.8.74	6.3
	6.9.74	9.9
	18.9.74	19.1
	13.10.74	19.0
Scirpus	24.6.74	9.1
	8.7.74	6.3
	22.7.74	16.1
	5.8.74	11.2
	18.9.74	10.6
	13.10.74	22.3
Phragmites	21.6.74	16.2
	5.7.74	56.1
	22.7.74	33.5
	3.8.74	106.0
Potamogeton	10.7.74	8.0
	14.8.74	4.0
	6.9.74	35.4
	18.9.74	35.6

damage.

The fall maximum of periphyton primary productivity observed here was also observed by Allen (1971) in Lawrence Lake. Factors controlling the seasonal growth cycle, including the role of the macrophyte itself, are unknown and require further investigation. Although Crescent Pond was ice-covered for six months, periphyton productivity rates were high after ice-melt. This may be related to the observation of Allen (1971) that a high standing crop under ice conditions may reflect the utilization of storage products or increasing rates of chemo-organotrophy.

The low productivity of epiphytic algae at the *Phragmites* site may be related to the relatively low light intensity at that site (approx. 2%) of open water surface. Productivity values were considerably higher at the low density *Typha* site, which had a higher relative light intensity (approx. 80%) than the high density site (5%). Dokulil (1973) considered reduced light intensity within *Phragmites* stands to be the main factor limiting planktonic production.

The estimated primary productivity of the epiphytic community of 26.8 g C $m^{-2}day^{-1}$ in Crescent Pond compares favourably with the value of 37.9 g C $m^{-2}day^{-1}$ reported for Lawrence Lake (Allen 1971). A direct comparison of the periphyton productivity on *Scirpus acutus* in the two locations is 86.0 mgC m^{-2} macrophyte surface area day^{-1} for Crescent Pond versus 336.0 mgC $m^{-2}day^{-1}$ for Lawrence Lake (Allen 1971). The lower productivity on this substrate in Crescent Pond may be a reflection of the shorter ice-free period in this location.

Biomass/productivity ratios (Table 2-3) agree with values reported by Rosemarin (1975) for a benthic periphyton community, although the ratios are higher than those reported for phytoplankton (Lewis 1974, Rosemarin 1975).

This ratio of the epiphytic community cannot be interpreted as a replacement or turnover time, since the organic C values are for dead cells and non-algal components as well as living algae. The trend towards a higher organic C/productivity ratio in the fall may reflect an accumulation of dead algal cells, an increase in the biomass of non-photosynthetic organisms and/or lowered rates of photosynthesis in response to decreased solar radiation. The average organic C/organic N ratio for the epiphytic community is however in agreement with values reported for phytoplankton populations (Holm-Hansen 1971).

CHAPTER 3

DIURNAL CARBON FIXATION PATTERNS OF EPIPHYTIC ALGAE

INTRODUCTION

In conjunction with the application of the C^{14} uptake technique to the estimation of primary productivity of epiphytic algae (Hooper and Robinson 1976), a series of experiments was carried out on the epiphytic community in Delta Marsh, Manitoba, to determine the relationship between productivity and total solar radiation on a diurnal basis, the effect of incubation time on apparent C^{14} uptake and the extent of losses from the epiphytic community of previously fixed C^{14}.

An artificial substrate, cellulose-acetate, was provided for algal attachment within the macrophyte stands. When properly positioned, the cellulose-acetate substrate provides a reliable attachment surface for the epiphytic algae which may be conveniently used for routine photosynthetic C^{14} uptake determinations (Hooper and Robinson 1976).

METHODS

Diurnal C^{14} uptake experiments were carried out on June 24, 1974, July 1, 1975 and August 24, 1976 for algae colonizing artificial substrates in stands of *Scirpus acutus* Muhl., *Typha latifolia* L. and *Potamogeton pectinatus* L. respectively. Positioning of the artificial substrates and incubation techniques were as described by Hooper and Robinson (1976). Four-hour incubations, with triplicate light bottles, were initiated at sunrise and overlapped by two hours on June 24 and August 24, with no overlap of July 1 samples. Total solar radiation was continuously monitored with a pyrheliometer.

The effect of incubation time on apparent C^{14} uptake was assessed in two ways. Triplicated samples of colonized artificial substrate were collected and C^{14} uptake rates were determined on two occasions under constant incubator conditions (18°C, 400 ft candles) by the method previously described (Hooper and Robinson 1976). Incubation times were 0.5, 1, 2, 3, 4, 6, 12, and 24 hr in one run and 1, 2, 4, 6, 10.5, and 22 hr in a second.

A second method of assessing the effect of incubation time on C^{14} uptake involved the comparison of C uptake of epiphytic algal samples incubated from dawn to dusk with the integrated sum of a series of four-hour incubations, overlapped by two hours, carried out over the same day. The comparisons were carried out on June 24, 1974 and August 24, 1976.

Apparent loss during the night of C^{14} assimilated in the previous day was monitored on June 24, 1974 and August 24, 1976. On June 24, radioactivity in samples, incubated *in situ* for the entire daylight period, was determined for duplicate samples at four-hour intervals during the night. On August 24, radioactivity was determined for triplicate samples after the full dark period only. On June 24, the daylight period was 16 hr and the night period 8 hr, while on August 24 the day-night periods were 14 hr and 10 hr respectively.

Loss in the light and in the dark of C^{14} assimilated in the previous 4 hr incubation period was monitored. On August 24, 9 sample bottles, each containing a square of colonized cellulose-acetate, were incubated *in situ* following previously outlined procedure. At the end of the incubation with C^{14}, the radioactivity of three of the samples was determined, while three other sample squares were quickly put in light bottles containing only filtered marsh water with no isotope and

the final three squares were placed in dark bottles containing only filtered marsh water. Radioactivity was determined after four-hour, *in situ* incubations for both treatments. This series was repeated four times during the day.

Possible excretion of C^{14}-labelled, photosynthetic products by the epiphytic algae was investigated. The acidification and aeration technique of Anderson and Zeutschel (1970) was used. Since results showed consistent low levels of radioactivity, indicating low concentrations of excreted compounds, the possible loss of organic C due to acidification and aeration was examined. Various quantities of C^{14}-labelled glucose were acidified and aerated, with no significant loss of label. Therefore, the absence of detectable excretory products of the epiphytic algae could not be attributed to methodology.

RESULTS

No obvious diurnal shifts in the response to light intensity were observed. The proportion of daily uptake occurring in a four-hour period was highly correlated with the proportion of daily solar radiation occurring within that period (Fig. 3-1) for the entire light period.

There were no significant differences in hourly uptake rates for up to 24 hr (Table 3-1), although in both experiments hourly uptake rates were slightly lower for incubation times greater than 12 hr. The continuous 16 hr *in situ* incubation of June 24, 1974 resulted in an uptake value of 3.26 μg C cm^{-2}, while the integrated uptake value for 7 overlapping four-hour incubations was 3.15 μg C cm^{-2}. On August 24, 1976, the fourteen-hour incubation uptake was 8.11 μg C cm^{-2}, while the integrated uptake was

Figure 3-1 The relationship between the proportion of daily C^{14} uptake occurring in a 4 hr period and the proportion of daily solar radiation occurring within that period.

Note: For computation of regression and correlation of proportional data, values are adjusted by the appropriate variance stabilizing transformation (arcsin $\sqrt{}$).

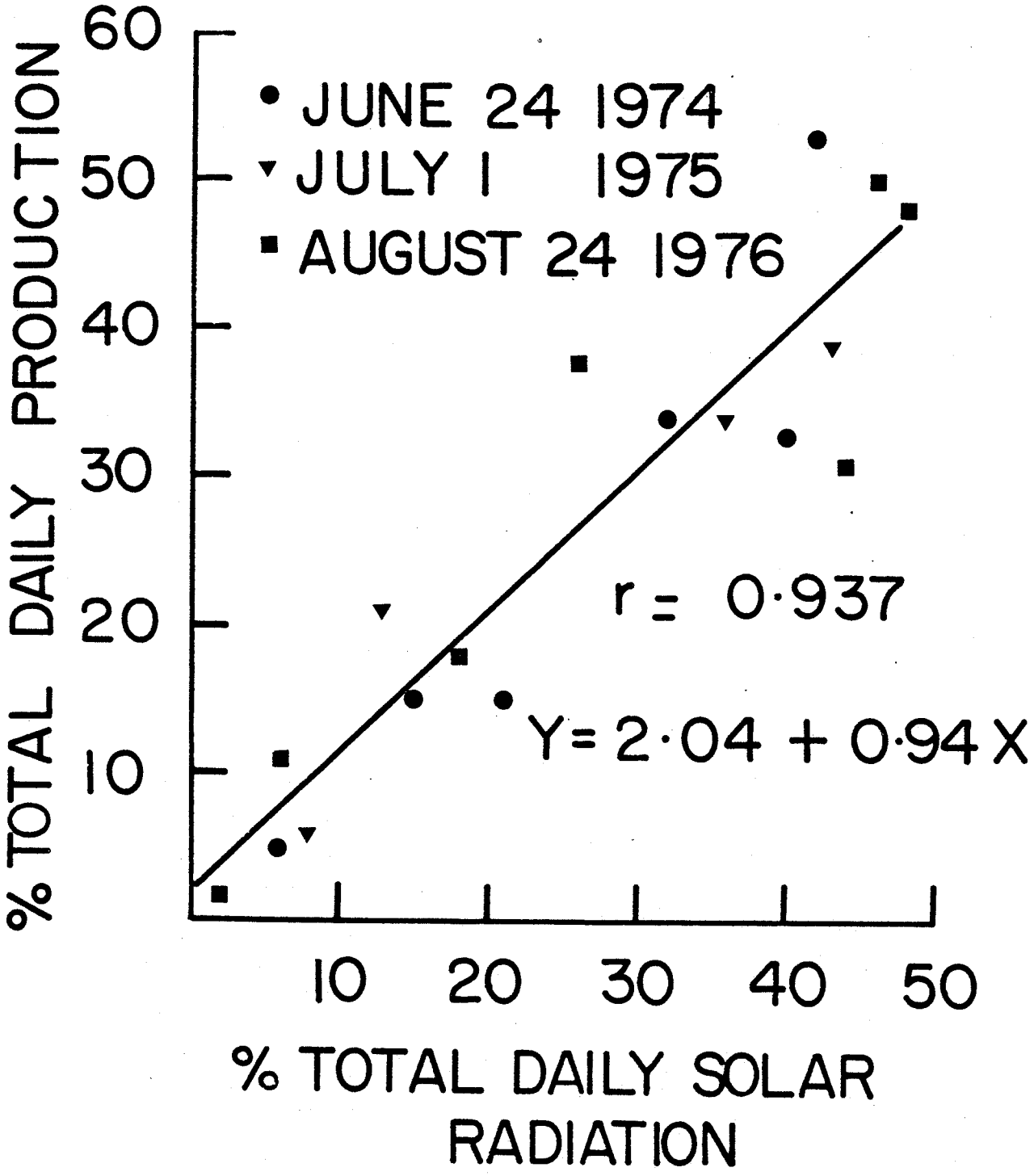

% TOTAL DAILY PRODUCTION
60
50
40
30
20
10
JUNE 24 1974
JULY 1 1975
AUGUST 24 1976
r = 0·937
Y = 2·04 + 0·94 X
10
20
30
40
50
% TOTAL DAILY SOLAR RADIATION

Table 3-1. Effect of increasing incubation time on apparent hourly C^{14} uptake rate under constant incubation conditions.

Date	Incubation Times	Treatments	Replications	F	
Sept. 5/74	(0.5 - 24 hr)	8	3	1.18	ns
Sept. 3/75	(1 - 22 hr)	6	3	1.85	ns

F - F statistic, one-way analysis of variance

ns - no significant treatment differences at 0.05 level

8.23 μg C cm^{-2}.

Loss of label in the night as measured on June 24, 1974 and August 24, 1976 was 28% and 29% respectively. Losses in the light after 4 hrs were negligible and in the dark averaged, on the basis of 4 triplicate determinations, 20% (range 7 - 40%).

DISCUSSION

Numerous studies (e.g. Doty and Oguri 1957; Verduin, 1957; Vollenweider and Nauwerk 1961) have shown diurnal patterns in the photosynthetic response of phytoplankton, generally with a mid-day or afternoon depression attributed to photoinhibition or photorespiration. The lack of afternoon depression in the rate of photosynthetic C uptake by the epiphytic algae observed in this study may be explained by the generally decreased light intensity due to macrophyte shading and to mutual shading by the algae in the epiphytic community. The results support the simplest assumption that can be made in extrapolating part-day to whole-day estimates for epiphytic algae, namely that the photosynthesis to light intensity relationship is relatively constant throughout the day.

The linearity of uptake for up to 16-hr incubations of the epiphytic algae constrasts with decreases in the apparent rate of C^{14} uptake with incubation time reported by workers investigating phytoplankton. Barnett and Hirota (1967) report a ratio of hourly C^{14} uptake after 1 hr of incubation to that after 8 hr to be 1.6 and attribute this to a lag in loss of recently assimilated C^{14}. Eppley and Sharp (1975) report full-day incubations of marine phytoplankton (12-15 hr) have values much less than twice the 6 hr, noon - sunset incubations.

The results of the present study suggest, that in the light, the epiphytic community may be very efficient in net retention of assimilated carbon. This statement is supported by the negligible respiration losses observed in light. It is possible that in the epiphytic community most respired and excreted carbon is rapidly recycled by the algal and bacterial components. Such recycling may be enhanced by the close spatial arrangement of the various community components.

Estimated dark losses of carbon in phytoplankton measured in various ways range from nearly 50% over 12 hr of darkness of newly assimilated C^{14} (Eppley and Sharp 1975) to 20% loss in 24 hr of long term incubation C^{14} label (Ryther 1956). The net loss of 30% in the epiphytic community is within this range. The total loss may have been underestimated due to the possibility of reassimilation of respired C^{14} by heterotrophic and possibly chemosynthetic bacteria (Romanenko 1963).

Eppley and Sharp (1975) suggest 24-hr incubations or application of a correction factor would yield a better estimate of primary production available to grazers. However, correction of primary productivity values obtained for epiphytic algae by 30% would estimate the amount of C assimilated on day 1 that is available at sunrise of day 2. Since C subsequently to be respired during the night was available for grazing at sunset of day 1 and for at least part of the dark period, and since little is known of subsequent respiration losses after sunrise on day 2, the use of light period productivity estimates seems to be most accurate for the estimation of epiphytic algal productivity in Delta Marsh.

CHAPTER 4

SEASONAL DYNAMICS OF EPIPHYTIC ALGAL GROWTH IN A MARSH POND: PRODUCTIVITY, STANDING CROP AND COMMUNITY COMPOSITION

INTRODUCTION

The epiphytic algal community may be an important component of the littoral zone in areas where extensive growth of aquatic vascular plants provides substrates for algal attachment. Yet, relatively few quantitative examinations of epiphytic algae have been carried out. The objectives of this study were to quantify seasonal growth of epiphytic algae in a marsh pond by means of various parameters and to examine any inter-relationships among these parameters.

MATERIALS AND METHODS

Crescent Pond, a small isolated body of water in Delta Marsh, Manitoba, has been previously described (Hooper and Robinson 1976). In early May 1976 this pond was flooded with water from the Assiniboine River diversion channel. Water receded to normal spring levels in late May. Due to reduced seasonal precipitation, the May to October evaporation deficit was 58 cm and the water depth of Crescent Pond decreased correspondingly.

Two sampling sites were established on June 6, 1977 in Crescent Pond, at which cellulose-acetate substrates were positioned (Hooper and Robinson 1976). One site was located within a stand of *Scirpus acutus* Muhl. on the periphery of the pond and the second site in the zone of submerged vegetation (mainly *Potamogeton pectinatus* L. with some *Myriophyllum exalbescens* Fern.). The sampling period extended from late June, 1976, which coincided with the availabilility of new macrophyte substrates for epiphytic colonization to ice formation in mid-October of the same year.

Determinations of various parameters of the epiphytic community attached to the cellulose-acetate were performed on a bi-weekly basis.

Samples were collected from upper and lower depth strata at both sites. Initially, the 'upper depth' was arbitrarily defined as 0 - 35 cm below the water surface and the 'lower depth' from 35 - 70 cm below the water surface. As water levels declined through the summer the ranges were narrowed, until September, when only one range was sampled due to the maximum depth at the sites being less than 35 cm.

Colonized cellulose-acetate strips were collected from the sites, placed in large darkened jars containing marsh water and immediately transported, with minimum agitation, to the laboratory. Arrival at the laboratory was 10 - 20 min after collection time.

The procedure for determining C^{14}-photosynthetic uptake for epiphytic algae has been previously described (Hooper and Robinson 1976). *In situ* uptake for triplicated samples was assessed for 4-hr periods (9 a.m. - 1 p.m.) at both sites from mid-June to mid-September. Mid-day water temperatures and total solar radiation (Belfort pyrheliometer) were reported. In addition, triplicated samples were incubated for the same 4-hr period under constant incubator conditions (20°C, 250 microein m^{-2} sec^{-1}) from mid-June to mid-October.

Cell volumes and cell surface areas were determined by examination of algae scraped from the substrates and resuspended in distilled water. Cells were enumerated using a Sedgewick-Rafter counting cell. Fifteen 258 μ diameter fields were counted on each of three separate slides prepared from a sample. Algae were generally identified to the genus level, with appropriate 'within-genus' size categories to allow computation of cell volume and cell surface area per unit area of substrate. Identifications were based on Prescott (1962) and the U.S. Department of Interior (1966).

For dry weight determinations, a known area (generally 3 cm^2) was

scraped into a pre-dried, pre-weighed aluminum cup containing 1 ml distilled water. The material was dried to constant weight (approximately 12 hr) at 37°C and weighed immediately. Values were corrected for background weight changes of the aluminium cups.

Chlorophylls and total pigments were determined by the common acetone extraction method, with the following modifications for epiphytic algae. Squares of known surface area of cellulose-acetate colonized by algae were placed in 1 ml distilled water with several drops of saturated $MgCO_3$ solution added and algal material was scraped from the acetate into the water. The suspension was then diluted to a volume of 5 mls with undiluted reagent acetone. The solution was transferred to a tissue grinder and ground manually for 30 sec. The final volume was adjusted to 10 mls. Pigments were further extracted by 20 hr in darkness at approximately 4°C. Equations of Parsons and Strickland (1963) for the calculation of chlorophylls a, b, c and carotenoids were used. In addition, samples were acidified and chlorophyll a values were corrected for phaeophytins by the procedure of Lorenzen (1967).

For determination of protein, carbohydrate and lipid levels, squares of cellulose-acetate with epiphytic algae were collected at each sample interval and immediately frozen. Duplicate batch determinations were carried out in October. Protein levels were determined by the Folin-Ciocalteu method of Lowry *et al* (1951). Carbohydrate was determined by the anthrone reaction and lipid by the pinacyanol reaction as outlined by Strickland and Parsons (1972).

Factor analysis on the variables were carried out on log-transformed data to normalize the distribution. A direct factor analysis program (#20

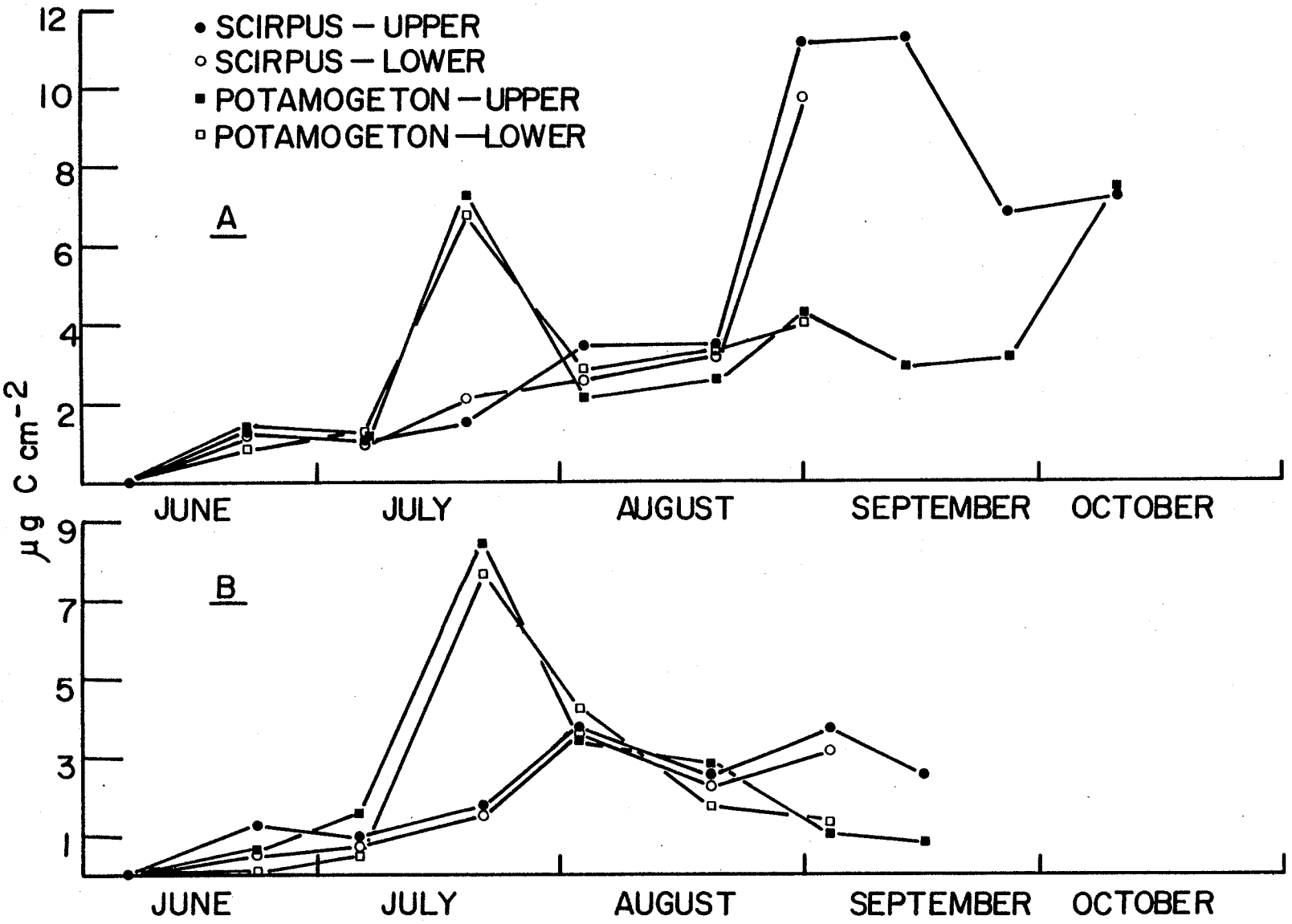
Manitoba Statistical Package, 1976) was used, with application of the Burt-Banks formula (Burt 1952) for significance levels of factor loadings.

RESULTS AND DISCUSSION

Incubator C^{14} uptake results reveal distinct seasonal patterns at the two sites (Fig. 4-1a). At the *Scirpus* site, uptake values increased slightly from June to late August, increased greatly in early September and decreased somewhat in late September. Epiphytic algae at the *Potamogeton* site attained peak uptake values in mid-July, decreased and remained relatively constant until a second increase in uptake in October. An autumnal maximum in epiphytic algal productivity has been observed previously in Crescent Pond (Hooper and Robinson 1976).

Results of 4-hr *in situ* incubations are given in Fig. 4-1b, with no correction for difference in radiation between incubation periods. Uptake estimates based on the *in situ* and incubator incubations were much the same until late August, reflecting the similarity of incubator and *in situ* light and temperature conditions. Differences in light intensity at the upper and lower depths did not seem to significantly affect uptake, perhaps due to the shallowness of the water. There were considerable differences in uptake under the two incubation conditions in September. On the basis of pyrheliometer data, the light did not appear to be the sole factor controlling the decreased '*in situ*' uptake, since light was reduced by only about 30% of mid-summer values. Temperature, however, decreased from mid-day values of 21 - 23°C at all previous incubations, to 14°C and 9°C on September 3 and 12 respectively. These temperature differences may have been primarily responsible for the apparent uptake differences under the two incubation conditions.

Figure 4-1 Seasonal measurement of 4-hr C uptake by epiphytic algae (μg C cm^{-2} substrate surface area) from two marsh sites under; (a) incubator, and (b) *in situ* conditions in Crescent Pond, Delta Marsh, 1976.


• SCIRPUS — UPPER
○ SCIRPUS — LOWER
▪ POTAMOGETON — UPPER
▫ POTAMOGETON — LOWER
A
B
μg C cm⁻²
12
10
8
6
4
2
9
7
5
3
1
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

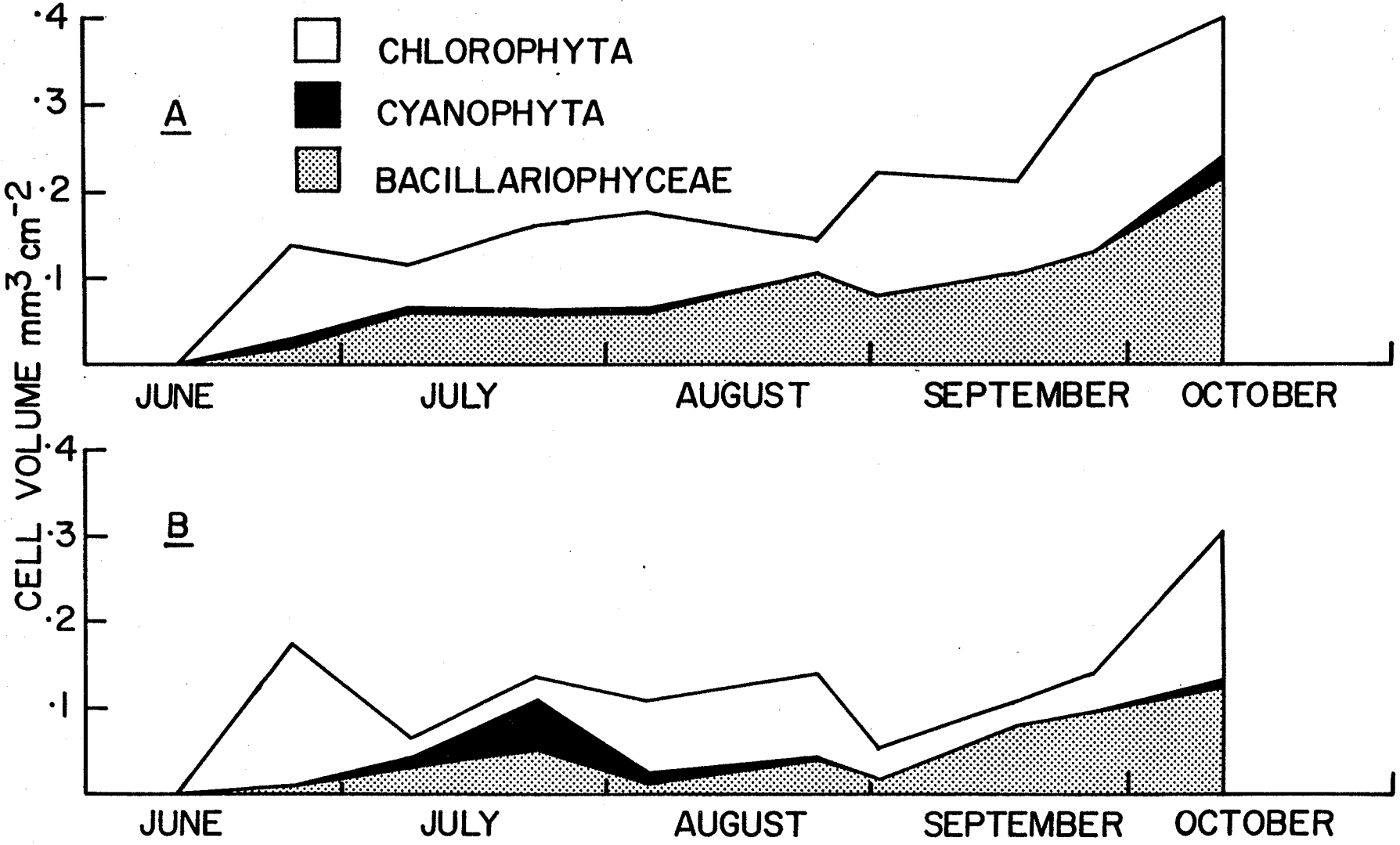
Since there were no obvious differences in cell volumes and compositions between upper and lower depths at both sites, cell volumes are presented for upper depths only (Fig. 4-2). Cell volumes were maximal at both the *Potamogeton* and *Scirpus* sites in October (0.31 mm^3 cm^{-2} and 0.40 mm^3 cm^{-2} respectively). These values are in the lower range of those reported for epilithic algae in Lake Winnipeg (0.32 - 10.00 mm^3 cm^{-2}) by Evans and Stockner (1972).

The epiphytic algae in Crescent Pond belonged almost exclusively to the Chlorophyceae, Cyanophyceae and Bacillariophyceae. The dominant members of the Chlorophyceae were *Coleochaete, Stigeclonium, Oedogonium, Cladophora* and various unidentified filamentous germlings. *Coleochaete* was less prevalent in autumn than in early summer. Cyanophyceae were mainly restricted to the *Potamogeton* site in mid-July, when *Anabaena, Gloeotrichia* and *Calothrix* were observed. An increase in *Oscillatoria* occurred at the *Scirpus* site in October. The dominant diatom species throughout the season at both sites were *Cocconeis placentula* Ehr. and *Cocconeis pediculus* Ehr. Genera which occurred occasionally throughout the season and with greater frequency in October included *Synedra, Fragilaria, Epithemia, Gomphonema, Rhopalodia, Rhoicosphenia, Nitzschia, Navicula, Amphora, Cyclotella, Tabellaria, Cymbella, Eunotia, Achnanthes* and *Diatoma*.

Numerous species lists for epiphytic algae have been compiled and according to the generalizations of Round (1964) and Hutchinson (1975), the community of epiphytic algae in Delta Marsh is typical. Domination of the epiphytic diatoms by *Cocconeis* for at least part of the season has been previously reported (Brown and Austin 1973; Hickman and Klarer 1973).

Chlorophyll <u>a</u> estimates in the epiphytic community reached maximum values in late September at the *Scirpus* site and in October at the

Figure 4-2 Cell volumes of epiphytic algae (mm^3 cm^{-2} substrate surface area) at (a) *Scirpus*, and (b) *Potamogeton* sites in Crescent Pond, Delta Marsh, 1976.


CHLOROPHYTA
CYANOPHYTA
BACILLARIOPHYCEAE
A
B
CELL VOLUME mm3 cm-2
·4
·3
·2
·1
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

Potamogeton site (Fig. 4-3). These maxima, expressed in terms of chlorophyll a corrected for phaeophytin were 4.53 and 6.30 μg cm^{-2} respectively. Chlorophyll a estimates were similar to those reported for epiphytic algae on submerged plants by Kowalczewski (1975) and in the median range of levels reported for algae on *Equisetum* (Hickman 1971).

The proportions of individual chlorophylls (without degradation production correction) and the carotenoids remained relatively constant throughout the season and the average seasonal value for the two depths at the two sites are similar (Table 4-1). The constancy of the chlorophyll proportions may reflect the proportionally consistent growth of green algae and diatoms throughout most of the season (Fig. 4-2). A higher proportion of chlorophyll a to chlorophylls b and c which might have been expected at the *Potamogeton* site in mid-July when blue-green algae made up a significant portion of the cell volume was not evident.

The seasonal average ratio of phaeophytin a to the total of chlorophyll a and phaeophytin at all sites was 0.38, with no apparent seasonal trend. The relationship between trichromatic chlorophyll a estimates and corrected estimates is shown in Fig. 4-4. The trichormatic method consistently overesimated the chlorophyll a, which would be expected. This difference underestimates phaeophytin a by approximately 50%. Since the absorbance of phaeophytin a is 40% less than that of chlorophyll a at 665 mμ (Vernon 1960; Lorenzen 1967) the relationship observed in Fig. 4-4 was very close to that expected due to the absorbance difference.

The carbohydrate, protein and lipid composition of the epiphytic community (Fig. 4-5), averaged, as a percent of the sum of the three components, 43% carbohydrate, 17% lipid and 40% protein, or 18%, 7% and 17% respectively of dry weight.

Figure 4-3 Chlorophyll a (phaeophytin corrected) concentrations ($\mu g\ cm^{-2}$ substrate surface area) of epiphytic algae at (a) *Scirpus* and (b) *Potamogeton* sites in Crescent Pond, Delta Marsh, 1976.

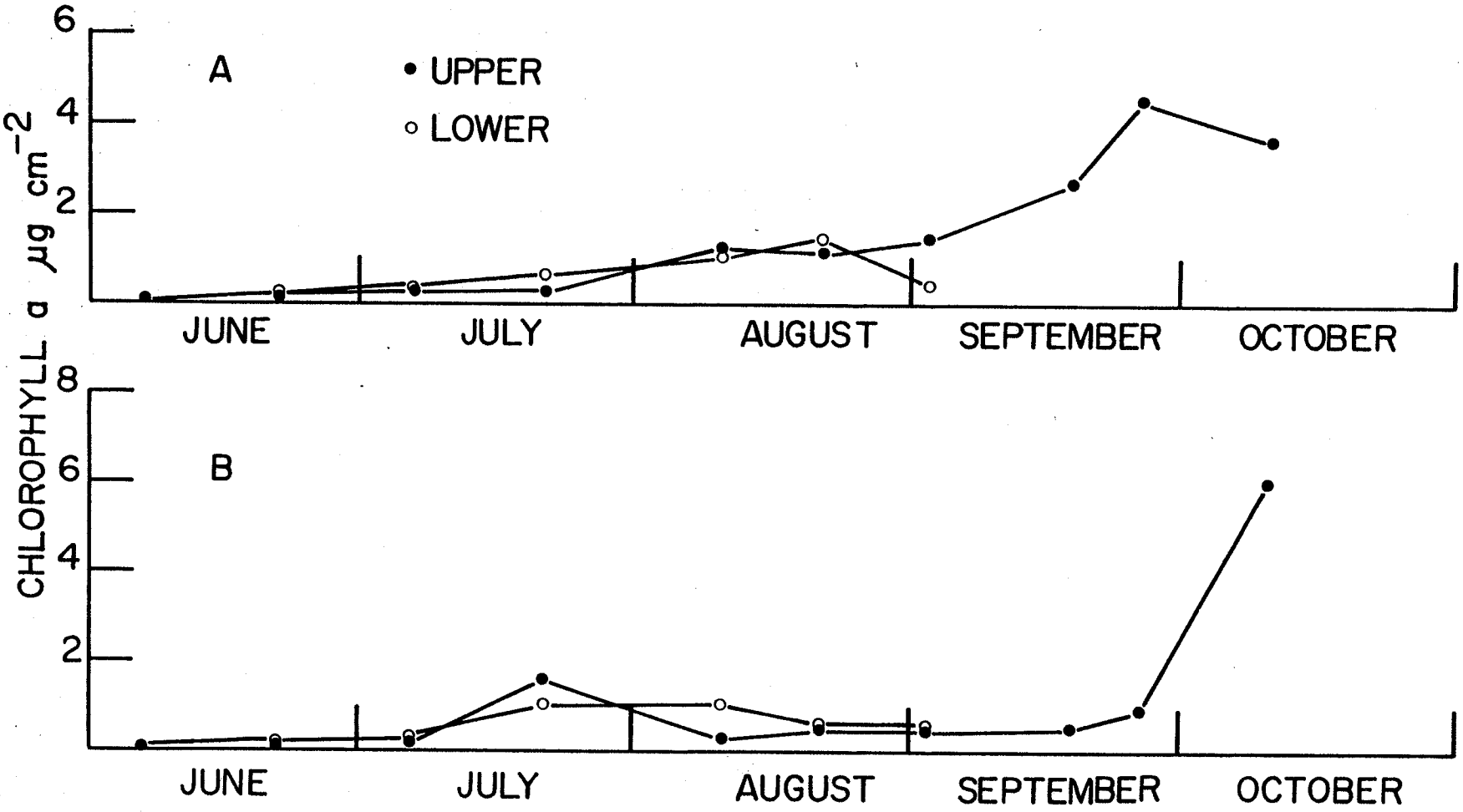

A
• UPPER
○ LOWER
CHLOROPHYLL a μg cm⁻²
6
4
2
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
B
8
6
4
2
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

Table 4-1. Average seasonal proportions of chlorophylls a, b, and c and carotenoids in epiphytic algal pigment at upper and lower depths at the *Scirpus* and *Potamogeton* sites.

Site	Chlorophyll a	Chlorophyll b	Chlorophyll c	Carotenoids
Scirpus upper	.41	.23	.19	.17
Scirpus lower	.41	.19	.21	.19
Potamogeton upper	.41	.22	.18	.19
Potamogeton lower	.44	.23	.11	.22

Figure 4-4 The relationship between the difference of chlorophyll *a* estimates (Parsons and Strickland trichromatic - phaeophytin corrected) and the amount of phaeophytin *a*.

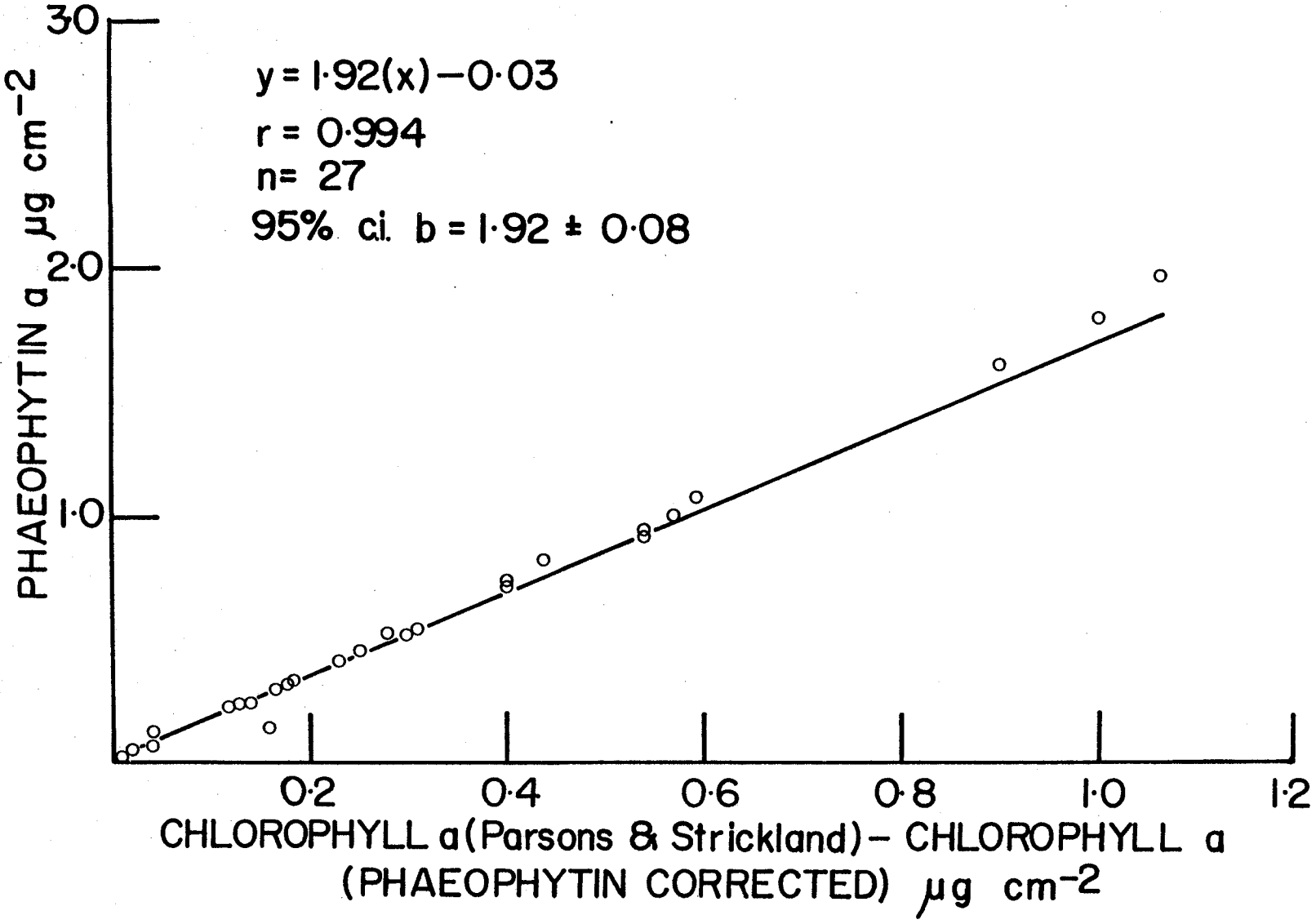
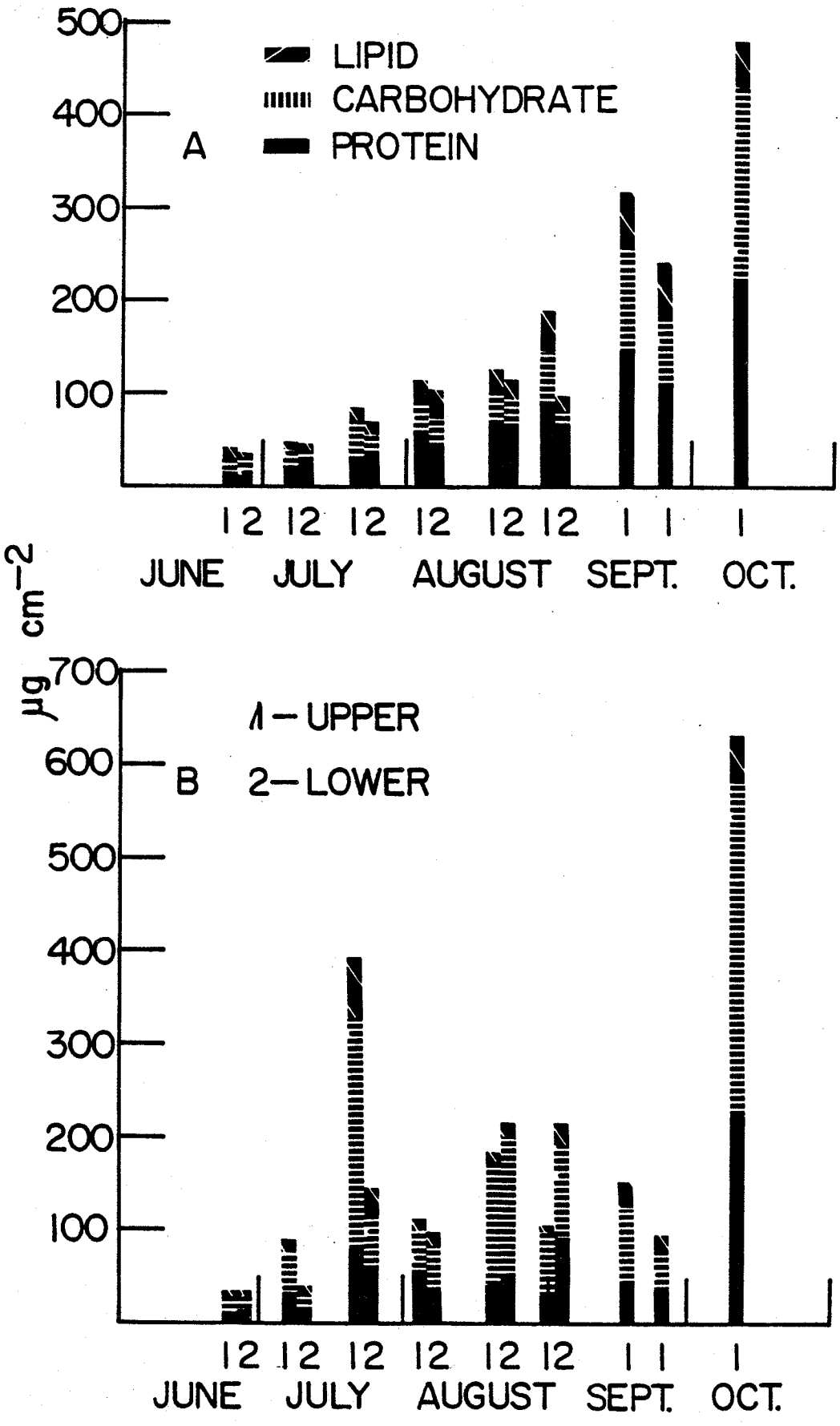

y = 1·92(x) − 0·03
r = 0·994
n = 27
95% ci. b = 1·92 ± 0·08
PHAEOPHYTIN a µg cm⁻²
30
2·0
1·0
0·2
0·4
0·6
0·8
1·0
1·2
CHLOROPHYLL a (Parsons & Strickland) − CHLOROPHYLL a
(PHAEOPHYTIN CORRECTED) µg cm⁻²

Figure 4-5. Protein, carbohydrate and lipid levels of epiphytic algae ($\mu g\ cm^{-2}$) of epiphytic algae on artificial substrates at (a) *Scirpus* and (b) *Potamogeton* sites, Crescent Pond, Delta Marsh, 1976.


500
400
300
200
100
A
LIPID
CARBOHYDRATE
PROTEIN
1 2 1 2 1 2 1 2 1 2 1 2 1 1 1
JUNE JULY AUGUST SEPT. OCT.
μg cm⁻²
700
600
500
400
300
200
100
B
1 – UPPER
2 – LOWER
1 2 1 2 1 2 1 2 1 2 1 2 1 1 1
JUNE JULY AUGUST SEPT. OCT.

For a complete comparison of productivity and standing crop estimates, parameters not yet discussed (dry weight and cell surface area) are presented in Figs. 4-6 and 4-7 . The maximum dry weight reported in this study of 14 g m^{-2} in late September at the *Scirpus* site is similar to maxima reported by Mason and Bryant (1975) of 19.3 and 18.0 g m^{-2} for epiphytic algae on *Typha* stems and glass rods respectively.

The process of factor analysis was used as a device for ordering and simplifying correlations between the various production parameters for 30 observations (Table 4-2). The interpretation of the factor analysis matrix is that while all eight variables are correlated, cell volume has a greater proportion of variance not explained by the common factor (I) than any other of the variables. This is shown by the single significant loading of this variable on factor II. On this basis, it might be said that while 4-hr C^{14} incubations, dry weight, cell surface area, protein, carbohydrate, lipid and chlorophyll a tend to be measuring a common production factor, cell volume represents a somewhat different factor.

Correlation coefficients for log transformed measurements of cell surface area, cell volume, productivity and chlorophyll a from several sources are summarized in Table 4-3. Studies of Paasche (1960) and Smayda (1965) are in accord with the result of this study, namely that algal cell surface area is more highly correlated with productivity than is cell volume. Both authors suggest that cell surface area provides a measure of the assimilative area for nutrient uptake and the area available for chloroplasts, while at the same time minimizing the effect of vacuole volume variation, which makes the interpretation of cell volume difficult. According to Smayda (1965), surface area and plasma volume estimates of standing crop appear to be interchangeable. The greater correlation between

Figure 4-6 Dry weight of epiphytic algae (µg cm^{-2}) on artificial substrates (a) *Scirpus* and (b) *Potamogeton* sites in Crescent Pond, Delta Marsh, 1976.

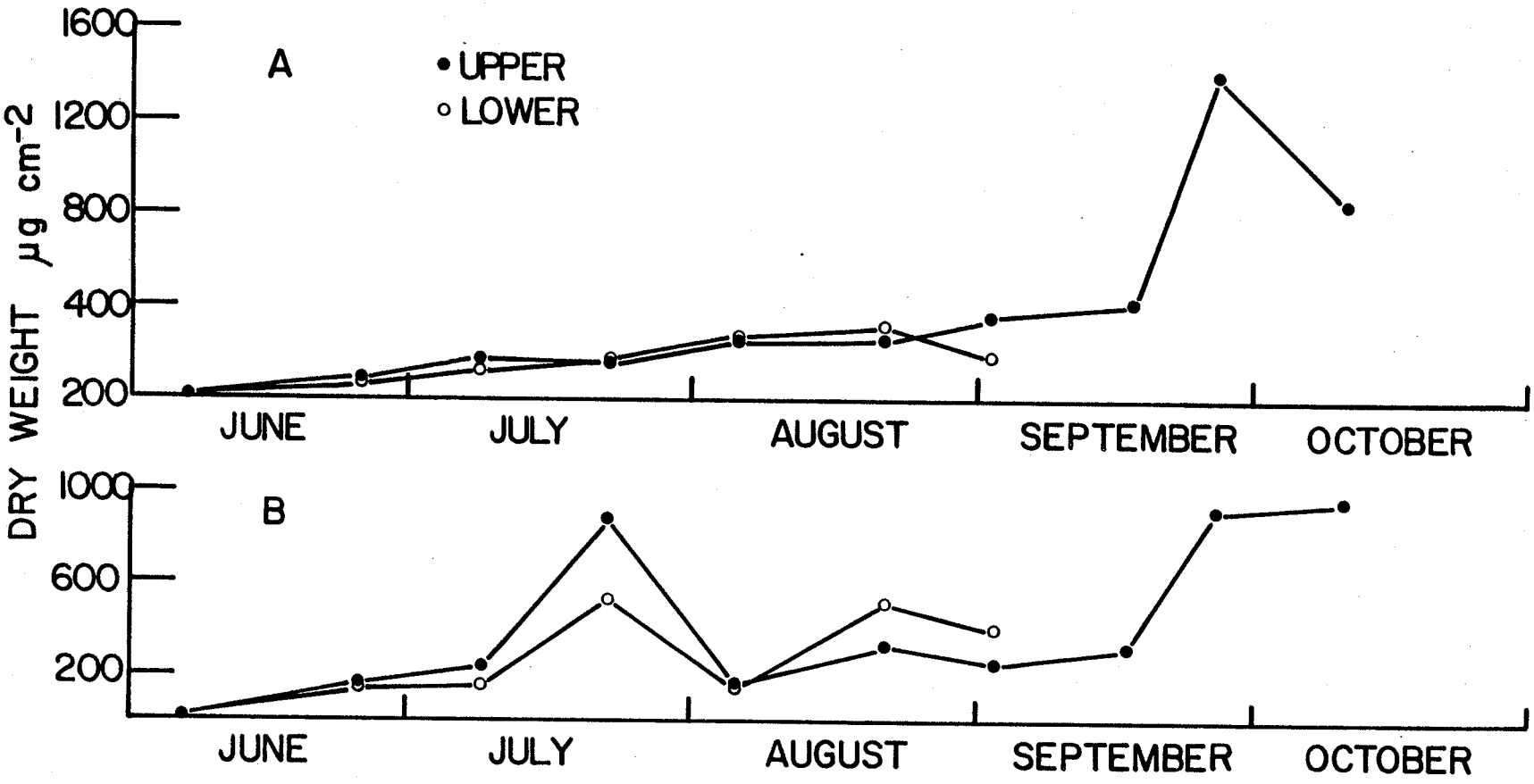

A
• UPPER
○ LOWER
DRY WEIGHT µg cm⁻²
1600
1200
800
400
200
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
B
1000
600
200
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

Figure 4-7 Cell surface area of epiphytic algae (mm^2 cm^{-2} substrate surface area) at (a) *Scirpus* and (b) *Potamogeton* sites in Crescent Pond, Delta Marsh, 1976.

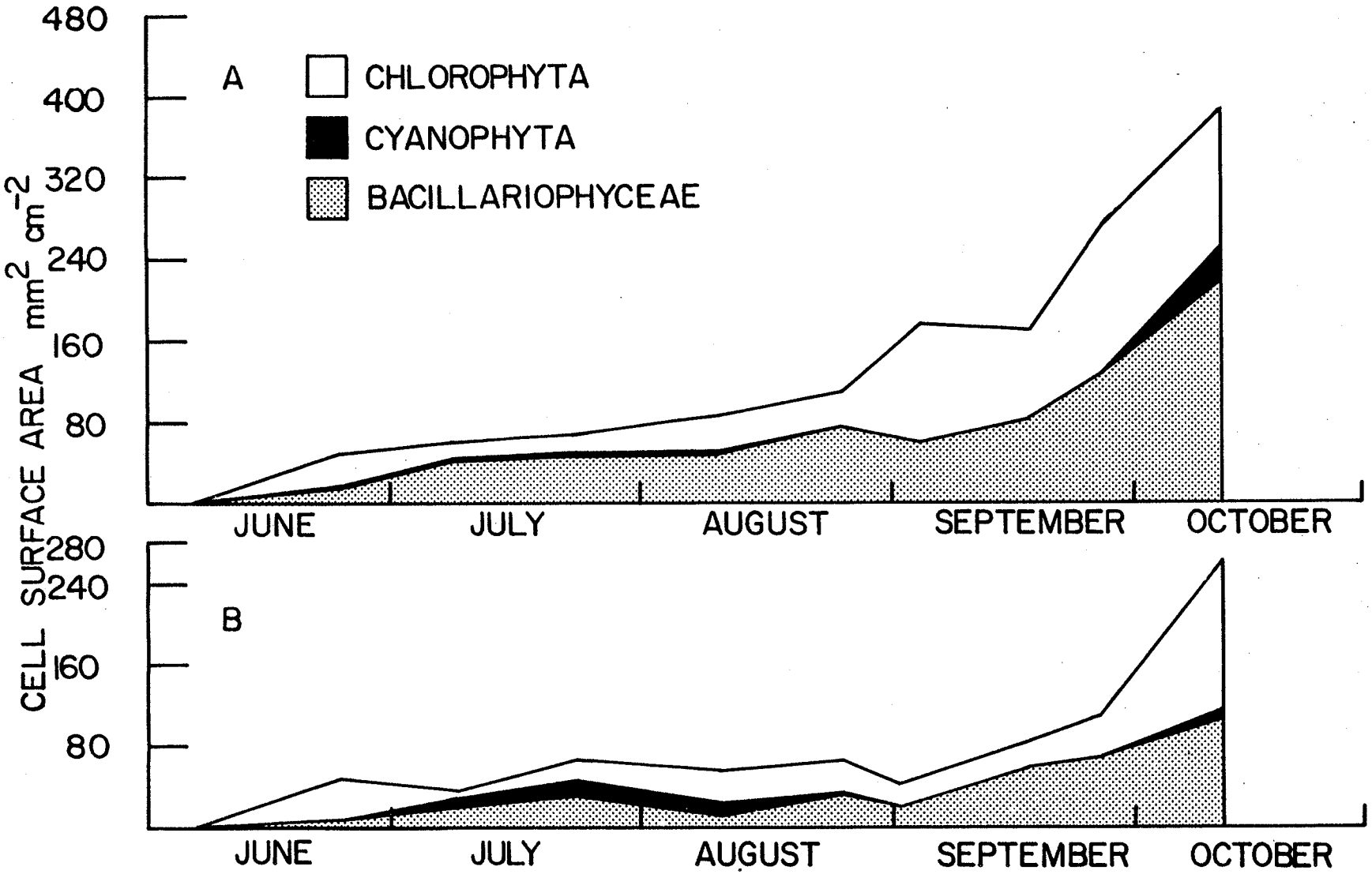

480
400
320
240
160
80
A
CHLOROPHYTA
CYANOPHYTA
BACILLARIOPHYCEAE
CELL SURFACE AREA mm2 cm-2
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
280
240
160
80
B
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

Table 4-2. Principal factor analysis matrix for productivity and standing crop estimates of epiphytic algae.

Measurement	Common Factor Loading I	II	Communality (h^2)
Incubator C^{14} uptake	.86	-.18	.77
Dry weight	.88	-.26	.84
Cell surface area	.94	.25	.95
Cell volume	.70	.60	.86
Protein	.94	-.12	.90
Carbohydrate	.78	-.40	.77
Lipid	.84	.28	.78
Chlorophyll *a*	.93	-.10	.88
Latent Root	5.96	.79	6.75

Table 4-3. Correlation coefficients between log-transformed variables.

Cell surface area and volume	.72	Paasche (1960)
	.82	present study
Productivity and surface area	.74	Paasche (1960)
	.60	Smayda (1965)
	.75	present study
Productivity and cell volume	.62	Paasche (1960)
	.54	Smayda (1965)
	.45	present study
Chlorophyll *a* and surface area	.81	present study
Chlorophyll *a* and cell volume	.45	present study

chlorophyll a and cell surface area than between chlorophyll a and cell volume observed in this study (Table 4-3) supports the suggestion of Paasche (1960) and Smayda (1965) that chlorophyll content is at least a partial function of surface area.

The results of the correlation analysis clearly suggest that surface area is a more meaningful estimator of production capacity and standing crop than is cell volume. Since determination of cell surface area is no more difficult than that of cell volume, microscopically derived estimates of standing crop would be more meaningful when expressed on a cell surface area basis. This is especially important in algal communities such as the epiphytic community where there is a wide range in algal sizes due to the heterogenous composition.

CHAPTER 5

SEASONAL DYNAMICS OF EPIPHYTIC ALGAL GROWTH IN A MARSH POND

COMPOSITION, METABOLISM AND NUTRIENT AVAILABILITY

INTRODUCTION

A previous paper (Hooper and Robinson 1977) discussed seasonal productivity and standing crop of epiphytic algae in Crescent Pond, Delta Marsh, Manitoba from June, 1976 to October, 1976. The purpose of this investigation is to relate measurements of seasonal growth and succession of epiphytic algae to nutrient levels (N, P and Si) in the marsh water and to various physiological indicators of nutrient availability.

Healey (1973, 1975) has reviewed the metabolic effects of nutrient deficiency on algae and discussed the application of these metabolic changes as indicators of nutrient status. Many physiological deficiency studies have been restricted to the use of algal cultures, mainly of planktonic species. Few studies have used these indicators as part of an integrated analysis of seasonal algal succession and production. Since the use of metabolic indicators of nutrient status enable the investigation of seasonal growth to go beyond a strictly correlative approach, several were incorporated into the present study.

Indicators of nutrient stress related to changes in algal composition and metabolism which were followed by chlorophyll content, protein to carbohydrate and lipid ratio, Si debt, storage polyphosphate level and alkaline phosphatase activity. In addition, the possible role of nitrogen fixation in the seasonal growth of epiphytic algae was assessed.

MATERIALS AND METHODS

Crescent Pond, a small isolated body of water in Delta Marsh, Manitoba has been described previously (Hooper and Robinson 1976). Water levels in the pond in the year of this study (1976) have been reported by Hooper and

Robinson (1977), as were the locations of the two sampling sites in *Scirpus acutus* Muhl. and *Potamogeton pectinatus* L. stands and the general biweekly sampling regime.

The establishment of the artificial cellulose-acetate substrates and methods employed for the determination of epiphytic algal cell surface area, dry weight, and chlorophyll, protein, carbohydrate and lipid content were as described in Hooper and Robinson (1977).

Chemical analysis of water from each site was carried out on a monthly basis, with the exception of PO_4-P and Si levels, which were analyzed on a biweekly basis at the *Potamogeton* site. All samples were taken in late afternoon from a depth of 20 cm or maximum depth if the water level had receded to less than 20 cm. Water was immediately filtered (Whatman GF/C glass fiber filter), refrigerated and transported to the laboratory for the analysis of NH_3-N, NO_3-N, total dissolved nitrogen (TDN), soluble reactive silicon (Si), total dissolved phosphorus (TDP) and PO_4-P using procedures described by Stainton *et al* (1974).

Analyses of Si debt, alkaline phosphatase activity and storage polyphosphate involved collection of squares of colonized cellulose-acetate from Crescent Pond on a biweekly basis at 9 a.m. and then immediate return to the laboratory as previously described (Hooper and Robinson 1977). The size of the cellulose-acetate samples varied, depending on the density of epiphytic growth. Each sample was prewashed in the appropriate medium prior to incubation or extraction in order to ensure uniform nutrient conditions. After the assay was completed, the exact size of the cellulose-acetate was measured.

The Si debt was determined on the basis of 24 hr Si uptake. Samples were placed individually in polycarbonate flasks containing 59 ml Rodhe VIII culture media (Rodhe 1948) in Nichols (1973), adjusted to a Si concentration of 3.4

mg l^{-1}. Samples were incubated at 20°C for 24 hr (10 a.m. to 10 a.m.); with a light source of 250 microein m^{-2} sec^{-1} from 10 a.m. to 10 p.m., darkness from 10 a.m. to 6 a.m. and light again from 6 a.m. to 10 a.m. The 24-hr incubation, with both light and dark period, was chosen to eliminate the effect of any periodicity of uptake. Duplicate samples for upper and lower depths at each site along with duplicate control flasks, containing complete medium only, were employed. At the end of the incubation period, concentrations of soluble reactive silicon were determined.

Polyphosphate was extracted from the epiphytic algae by modification of the procedure of Fitzgerald and Nelson (1966). Each sample of cellulose-acetate with attached algae was placed in 40 ml of 5 mg l^{-1} $MgSO_4$ solution and polyphosphate was extracted in a boiling water bath for 90 min. The cellulose-acetate was then removed with minimum agitation and the concentration of PO_4-P in the extracting solution was determined, with appropriate correction for sample blanks. Duplicate extractions were carried out for each depth at each site.

Alkaline phosphatase activity was assessed using p-nitrophenylphosphate as the substrate. The epiphytic algae, attached to cellulose-acetate, were placed in a solution of 23 ml P-deficient Rodhe VIII media (Rodhe 1948) in Nichols (1973) and 3 ml buffer (1 M Tris, 0.02 M $MgCl_2$, pH 8.5). Four ml of p-nitrophenylphosphate (30 mg l^{-1}) was added. The sample was incubated at 35-37°C for 2 hr. Incubation was terminated by removal of the cellulose-acetate samples. The absorbance of the remaining solution was measured immediately at 410 mμ. One unit of alkaline phosphatase is defined as the amount of enzyme liberating 1 μmole of nitrophenol hr^{-1} under the prescribed conditions. A molar absorptivity coefficient for p-nitrophenol of 1.62×10^4 (Berman 1970) was used. A sample blank, without p-nitrophenyl-

phosphate, and a control blank, without epiphytic algae, were run with each duplicate sample.

The acetylene reduction technique of Flett *et al* (1975) with some modifications to permit the use of epiphytic algae colonizing artificial substrates was employed to detect nitrogenase activity. On a bi-weekly basis, at 10:30 a.m., strips of cellulose-acetate were collected from the upper and lower depths (one depth only after September due to receding water levels) at the two Crescent Pond sites and immediately returned to the laboratory in darkened bottles containing marsh water. Sections of acetate, generally 1.0 x 1.5 cm, were placed in 50 ml glass syringes filled with fresh prefiltered marsh water (Whatman GF/C glass fiber filters). The prefiltration, which minimized planktonic interference, is advisable only where water is well-oxygenated and changes in dissolved oxygen concentration would therefore be insignificant. The volume of water in each syringe was reduced to 30 ml. The syringe was inverted and approximately 7 ml of acetylene was allowed to flow into the syringe at low pressure over the water and cellulose-acetate sample. The gas volume was adjusted to 5.0 ml and the syringe was sealed. The sample was shaken for 10 s or until no more acetylene would dissolve. The syringes were incubated at 20°C and 250 microein m^{-2} sec^{-1} for 2 hr. Blanks, syringes containing only prefiltered marsh water and acetylene, were incubated from 0 and 2 hr to correct for possible ethylene contamination, especially from the acetylene source, and for background acetylene reduction in prefiltered marsh water.

The assay was terminated by drawing air into the syringe until the vapor phase volume was 15 ml. The syringe was resealed and shaken for 30 s to equilibrate the vapor and aqueous phases and the final vapor phase volume

was recorded. The gas phase of the syringe was transferred to small (12 ml) serum bottles by displacement of distilled water from the serum bottles. Sealed bottles were stored 2-4 weeks in a freezer prior to determination of ethylene concentration. The ethylene concentration was determined with a hydrogen-flame ionization gas-chromatograph using a phenylisocyanate-Porosil C packed column. The actual quantity of ethylene produced per unit area of substrate was calculated by correcting the concentration of ethylene in the vapor phase for the concentration of ethylene dissolved in the aqueous phase. A 3:1 ratio of moles $C_2 H_4$ produced: moles N_2 fixed was assumed.

RESULTS AND DISCUSSION

Standing Crop and Nutrient Levels

The results of biweekly seasonal measurements of productivity and standing crop of epiphytic algae have been presented in detail elsewhere (Hooper and Robinson 1977). Standing crop, in terms of cell surface area, is summarized in Fig. 5-1. Initial colonization of the substrate in June at the *Scirpus* site by diatoms and green algae was followed by a period of low productivity and standing crop which lasted until late August, when growth measurements showed increases. Maximum values were reached in September and October. At the *Potamogeton* site, the growth of blue-green algae in July increased standing crop. With the exception of the occurrence of blue-green algae, standing crop levels at the *Potamogeton* site were relatively uniform for samples collected from early June until late September. Between late September and mid-October standing crop increased to maximum seasonal levels at this site. A period of relatively constant standing crop is interpretated as a period when losses of algae from the substrate were balanced by algal production. When production was greater than losses, standing crop increased.

Nutrient levels in Crescent Pond showed distinct seasonal trends (Fig. 5-2). Levels of soluble reactive silicate (Si), total dissolved phosphorus (TDP) and PO_4-P were highest in May and June samples and decreased to minimum values in July and mid-August, while levels of total dissolved nitrogen (TDN), NO_3-N and NH_3-N remained low in samples from May to mid-August. Nutrient levels at the two sites were similar during this period. After mid-August concentrations of TDP, TDN, Si and NH_3-N increased at both sites, with generally higher levels at the *Scirpus* site.

Figure 5-1 Cell surface area (mm^2 cell surface area cm^{-2} artificial substrate) of epiphytic algae at the *Scirpus* (o —— o) and *Potamogeton* (● — ●) site in Crescent Pond. Values are means of upper and lower depth samples.

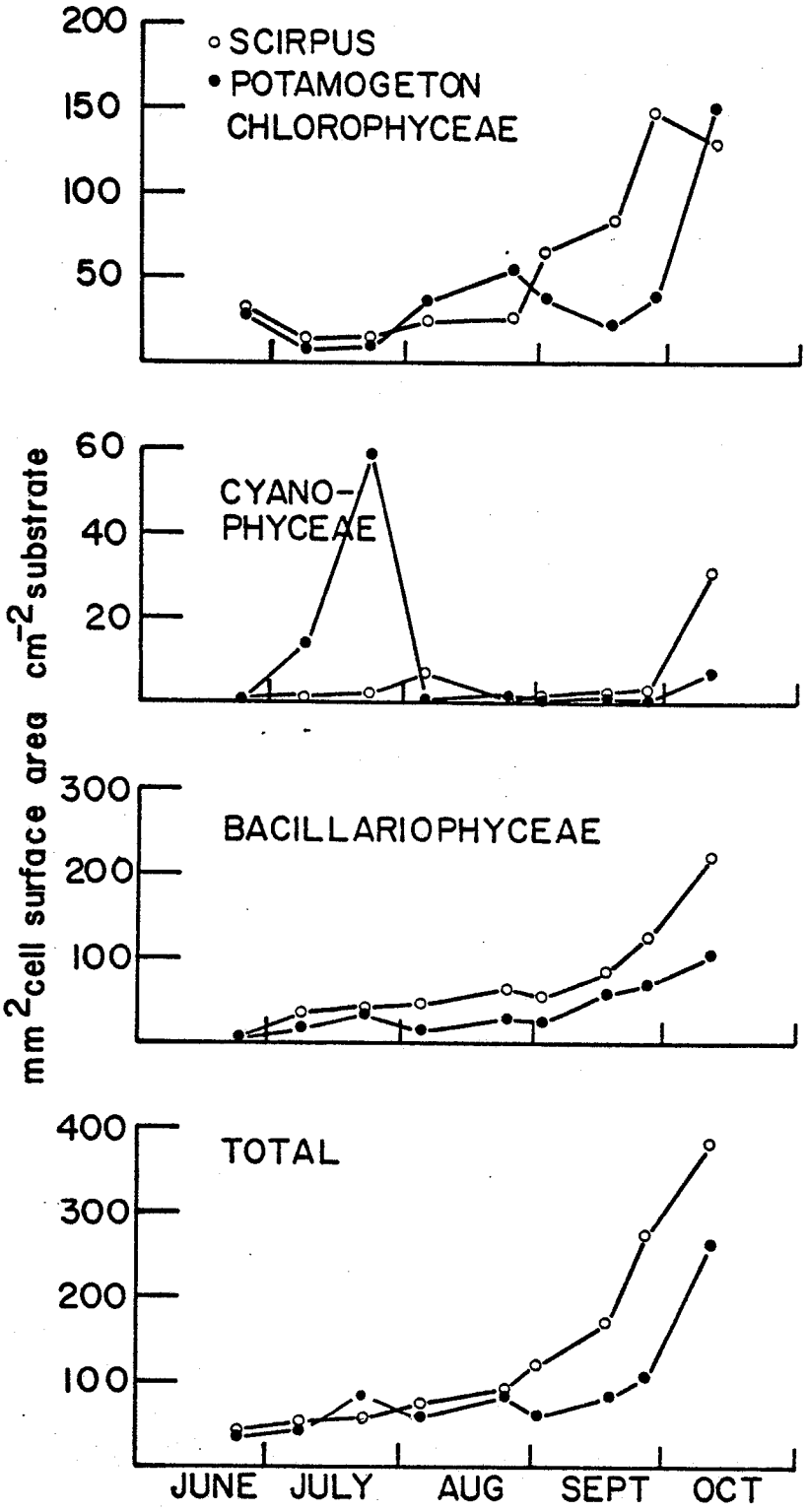
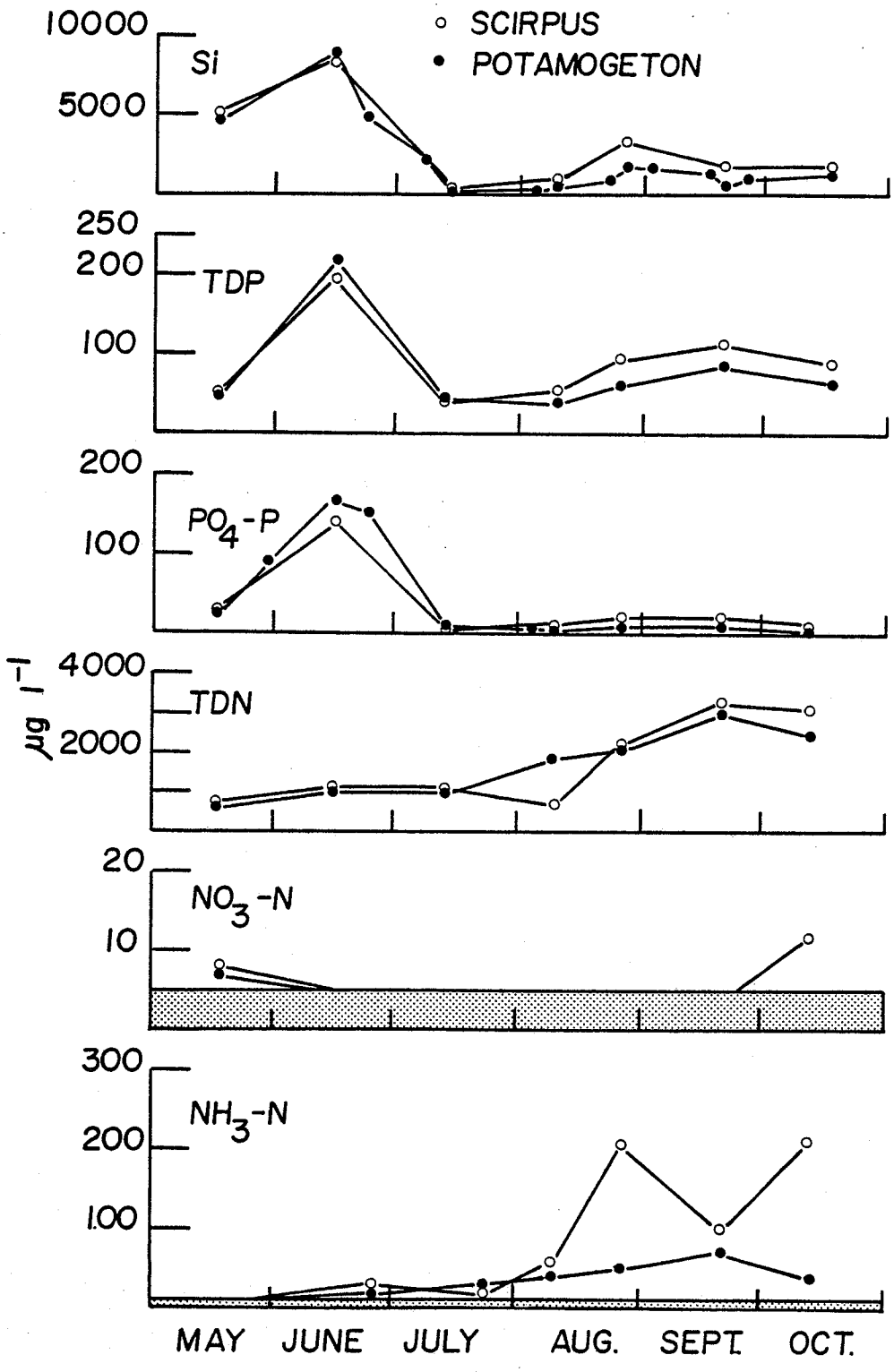

200
150
100
50
○ SCIRPUS
• POTAMOGETON
CHLOROPHYCEAE
60
40
20
CYANO-
PHYCEAE
300
200
100
BACILLARIOPHYCEAE
mm² cell surface area cm⁻² substrate
400
300
200
100
TOTAL
JUNE
JULY
AUG
SEPT
OCT

Figure 5-2 Seasonal changes in Si, TDP, PO_4-P, TDN, NO_3-N, NH_3-N at the *Scirpus* (o —— o) and *Potamogeton* (● — ●) sites in Crescent Pond, Delta Marsh from May to October, 1976.

▒▒▒▒ - limit of detection


SCIRPUS
POTAMOGETON
10000
5000
Si
250
200
100
TDP
200
100
PO4-P
4000
2000
TDN
μg l-1
20
10
NO3-N
300
200
100
NH3-N
MAY
JUNE
JULY
AUG.
SEPT.
OCT.

Concentrations of PO_4-P and NO_3-N were slightly higher at the *Scirpus* site in September and October than in July and August, while levels of these nutrients at the *Potamogeton* site were unchanged.

Total solar radiation (Belfort pyrheliometer) decreased 40% during the June to October study period. Light intensity at both sites was similar, with afternoon mid-September values at the 10 cm depth in each site of approximately 200-300 microein m^{-2} sec^{-1} (Li-Cor Quantum Meter). Water temperatures in Crescent Pond at a depth of 15 cm at 10 a.m. were in the 20-24°C range from late June to early September, then decreased to the 6-12°C range in late September and October.

The seasonal fluctuation in cell surface area of the epiphytic diatoms (Fig. 5-1) generally correlated with apparent changes in the Si concentration. Initial colonization of the substrates by diatoms occurred when the Si concentration was high and such growth may have partially accounted for the decrease in Si concentration. The surface area of the epiphytic diatoms, dominated by *Cocconeis*, remained approximately constant at both sites when Si was low in mid-summer. Diatom cell surface area increased to maximum levels in mid-October, which coincided with increased Si levels. The higher standing crop of diatoms at the *Scirpus* site in autumn than at the *Potamogeton* site correlated with both higher Si and NH_3-N levels at the *Scirpus* site.

Many studies have reported a general coincidence of decreases in diatom populations with a drop in dissolved silica concentration for both phytoplankton (e.g. Pearsall 1932; Lund 1950, 1963) and periphyton (Tai and Hodgkiss 1975). Pearsall (1932) and Lund (1950) suggest natural levels below 0.5 mg l^{-1} silicate may be limiting to diatom growth. July levels of Si at the Crescent Pond sites were within this limitation range.

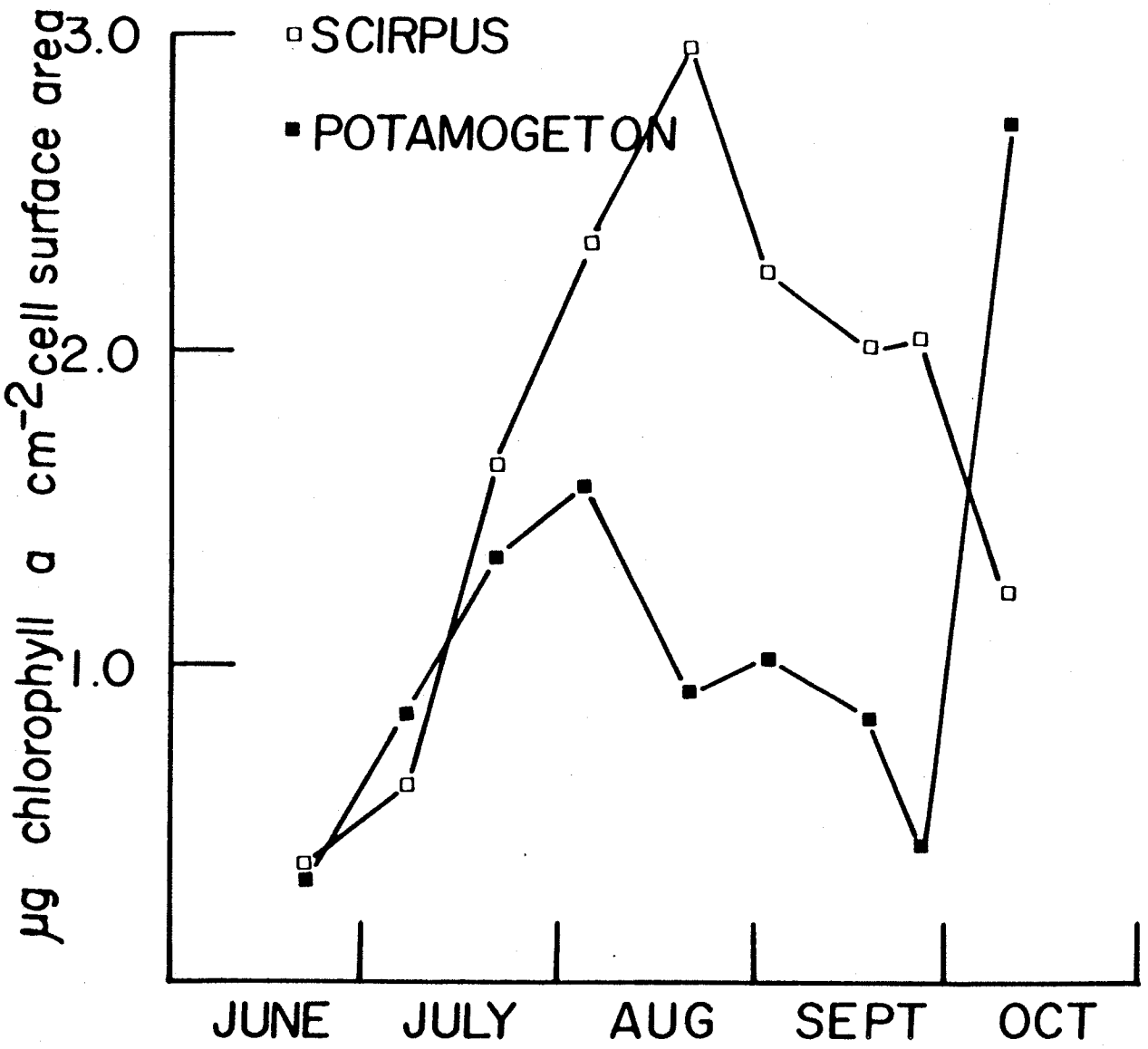
The occurrence of heterocystous blue-green algae at the *Potamogeton* site coincided with low nutrient levels. Such a growth maximum at low nutrient concentrations, especially of combined N, has been generally reported (Fogg, Steward, Fay and Walsby 1973). Since the drop in PO_4-P generally coincided with the increase in blue-green algal standing crop, the growth of the blue-green algae might have been at the expense of stored cellular phosphate (Stewart and Alexander 1971) or as observed by Heath and Cooke (1975), due to synthesis of alkaline phosphatase.

The green algae followed the general seasonal trends of low summer and high autumn standing crops on the artificial substrates after initial colonization in June. While standing crop increased at the *Scirpus* site in early September, an increase at the *Potamogeton* site did not occur until late October. During this period, levels of NO_3-N and NH_3-N were higher at the *Scirpus* site and may in part account for the growth difference at the two sites. The correlation is not direct however, for levels of NO_3 and NH_3 were not higher at the *Potamogeton* site in October than in September. It may be that any combined N available at this site in early October was taken up rapidly for algal growth resulting in no apparent increase in NH_3 or NO_3 concentration at the *Potamogeton* site.

Chlorophyll a Content

The seasonal variation in chlorophyll a content of epiphytic algae at the two sites is presented in Fig. 5-3 on a cell surface area basis. Generally, chlorophyll a content increased similarly at both sites from the time of initial colonization in June until early August, when values at the *Scirpus* site continued to increase while those at the *Potamogeton* site

Figure 5-3. Seasonal chlorophyll a content of epiphytic algae at Crescent Pond *Scirpus* (o —— o) and *Potamogeton* (●——●) sites. Values, averages of samples from upper and lower depths, are expressed in terms of μg chlorophyll a cm^{-2} cell surface area.


SCIRPUS
POTAMOGETON
3.0
2.0
1.0
μg chlorophyll a cm⁻² cell surface area
JUNE
JULY
AUG
SEPT
OCT

declined. Between early August and late September, chlorophyll a content was consistently higher at the *Scirpus* site. In October, chlorophyll a content of the epiphytic algae at the *Potamogeton* site showed a large increase with values greater than those at the *Scirpus* site.

Lowered chlorophyll a content has been reported in a variety of algae as a result of numerous deficiencies. Healey (1975) summarizes the results of chlorophyll a content analysis of cultured algae under various light, temperature and nutrient conditions from a number of sources and suggests that levels less than 5 μg chlorophyll a mg^{-1} dry weight are confined to situations of extreme N and P deficiency. The expression of results on a dry weight basis for natural planktonic and periphytic communities is of limited value, due to the varying proportion of the total dry weight that may actually be algal. In this study, cell surface area has been utilized as a more direct estimate of algal crop. Critical levels expressed on a dry weight basis may however be converted to the appropriate cell surface area basis to give approximate indication of nutrient status if a minor non-algal dry weight component is assumed.

Since the mean dry weight to surface area ratio was significantly higher at the *Potamogeton* site than at the *Scirpus* site (Wilcoxon two-sample test, n=13, =.01) the critical level of 5 μg chlorophyll a mg^{-1} dry weight converts to 1.5 μg chlorophyll a cm^{-2} cell surface area at the *Scirpus* site, while at the *Potamogeton* site it is equivalent to 2.4 μg chlorophyll a cm^{-2}. At the *Scirpus* site, a larger proportion of the total dry weight was algal, since the dry weight to cell surface area ratio was smaller at this site even although algal composition was similar to that at the *Potamogeton* site. As a result of this, the conversion of critical levels based on dry weight to a cell surface area basis may be more reliable when based on the *Scirpus*

site dry weight to cell surface area ratio, because the non-algal dry weight component was smaller at this site.

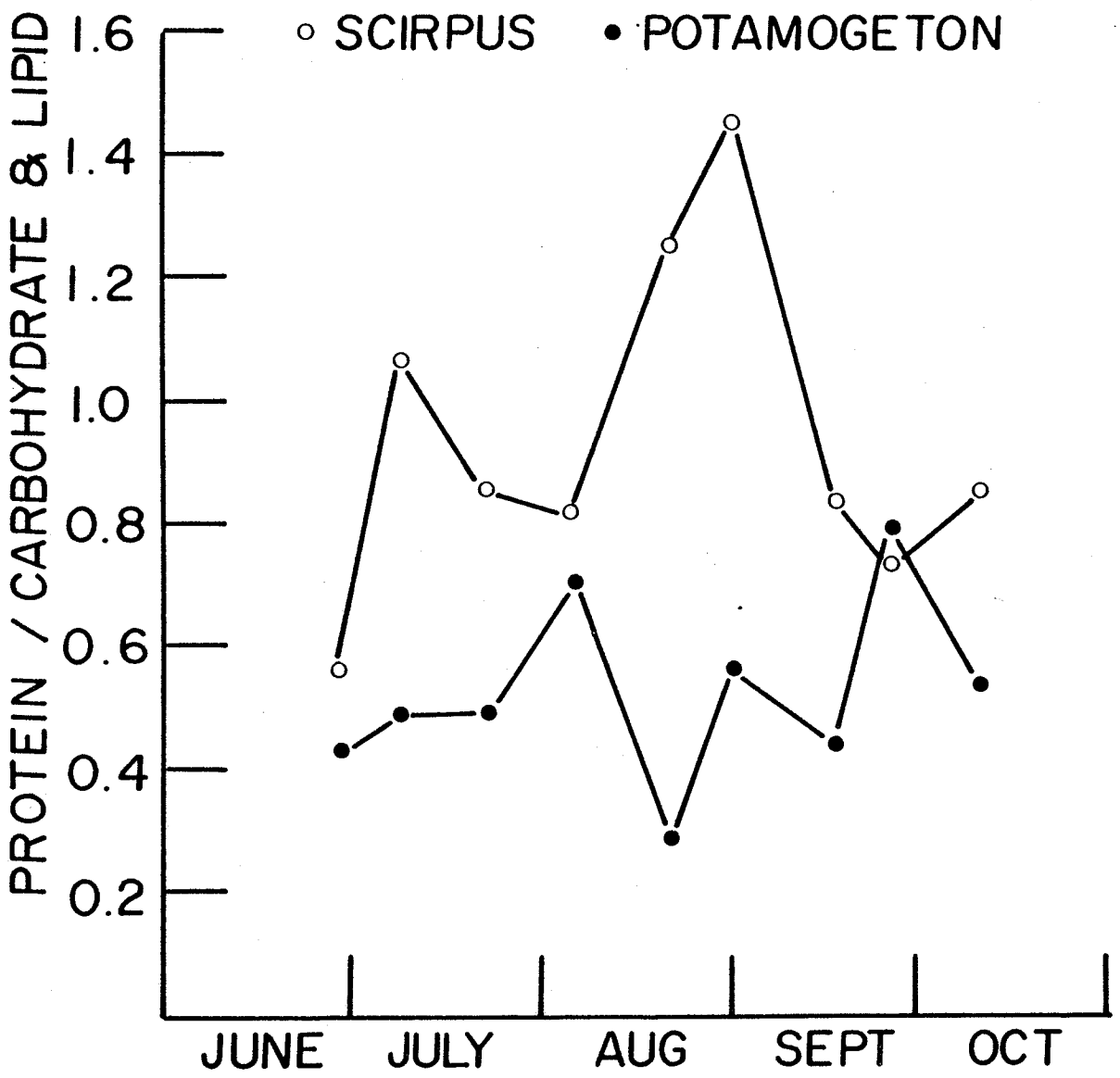
If the value of 1.5 µg chlorophyll a cm^{-2} cell surface area is applied to the results (Fig. 5-3) there is indication of deficiency at both sites in June and July. The epiphytic algae at the *Scirpus* site were generally above the level of deficiency from August until October, while at the *Potamogeton* site, chlorophyll a values were below the 1.5 µg level until October, when the chlorophyll a content increased.

Protein, Carbohydrate and Lipid Content

The protein to carbohydrate and lipid ratios are presented on a seasonal basis for the epiphytic community (Fig. 5-4). Seasonal ratios were higher at the *Scirpus* site than at the *Potamogeton* site in all samples with the exception of those collected in late September, when ratios at the two sites were approximately equal. The ratio at the *Scirpus* site was lowest in June (0.56) and highest in early September (1.45). At the *Potamogeton* site, the range of values was less, with a minimum of 0.29 in late August and a maximum of 0.79 in late September.

Healey (1975) compiled protein to carbohydrate and lipid ratios of cultured algae from a number of sources and suggested that values below 0.5 were indicative of N or P deficiency, while those above 0.7 indicated nutrient sufficiency. Ratios for epiphytic community at the *Scirpus* site were consistently above the level indicative of nutrient sufficiency, with the exception of the initial June sample when the ratio indicated moderate deficiency. The epiphytic community at the *Potamogeton* site however, had ratios indicative of moderate or extreme deficiency at all sample times, except late September, when the ratio indicated nutrient sufficiency.

Figure 5-4. Seasonal ratios of protein/carbohydrate and lipid for epiphytic algae at the *Scirpus* (o —— o) and *Potamogeton* (● — ●) sites at Crescent Pond. Values are means of upper and lower depth samples.


SCIRPUS
POTAMOGETON
PROTEIN / CARBOHYDRATE & LIPID
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
JUNE
JULY
AUG
SEPT
OCT

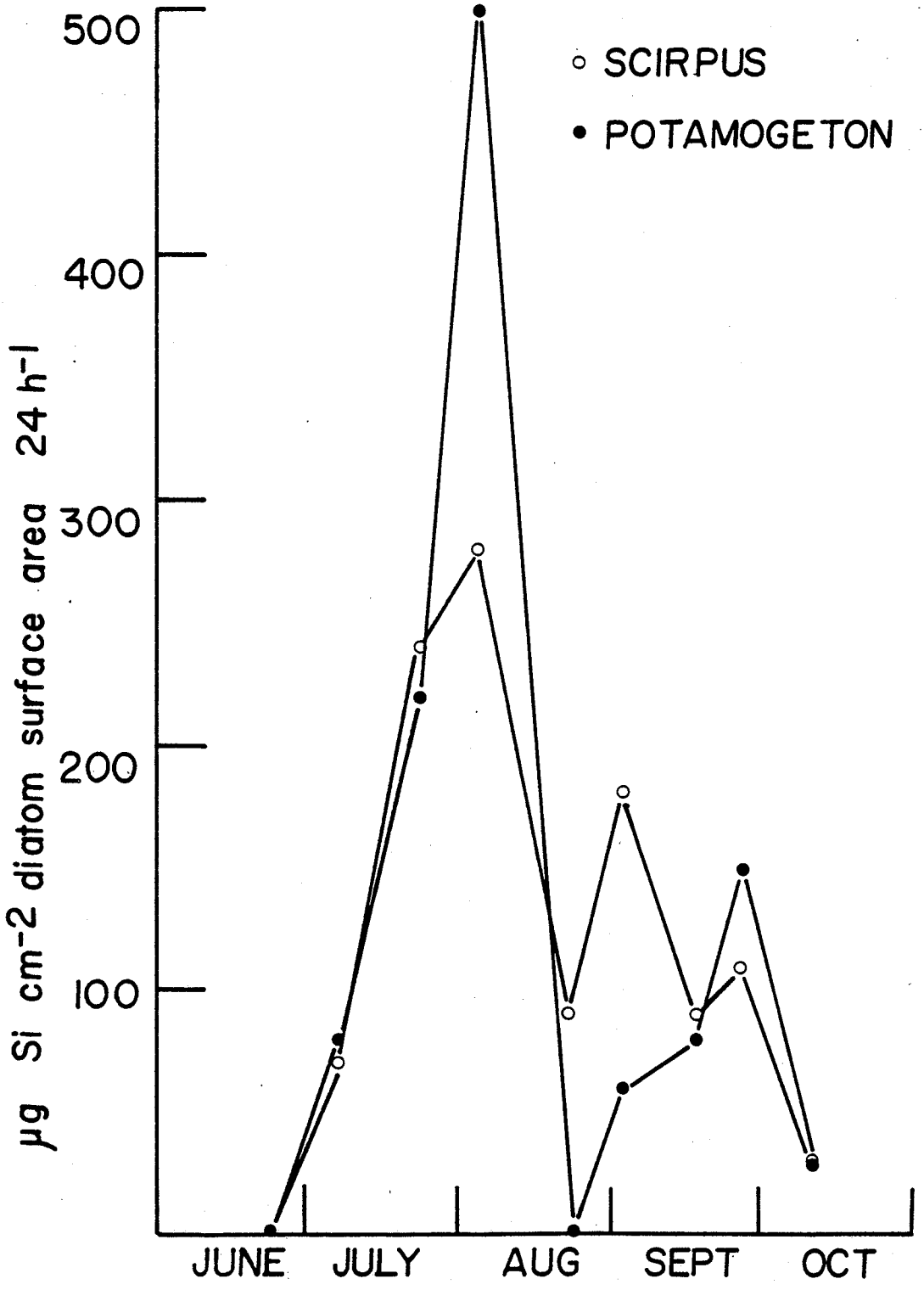
Si Debt

A generally observed metabolic response to nutrient deficiency is the increased ability of algal cells to take up the deficient nutrient. A surge in Si uptake following Si readdition to Si starved diatoms has been demonstrated (Conway, Harrison and David 1976; Busby and Lewin 1967). Lewin (1955) presented evidence that total Si uptake increases as cellular content decreases in *Navicula pelliculosa*. Such increased Si uptake might then be expected to be a useful indicator of '*in situ*' Si deficiency, a similar approach to that used by Healey (1975) to assess N or P deficiency by measurement of nutrient debt.

The uptake of Si in a 24-hr period increased from indetectable levels at both the *Scirpus* and *Potamogeton* sites in late June to maxima of 280 and 500 μg Si cm^{-2} diatom surface area 24 hr^{-1} at the two sites respectively in early August (Fig. 5-5). After early August, Si debt values were below 18 at both sites, with some fluctuation. Final values at both sites in October decreased to 30 μg Si cm^{-2} diatom surface area 24 hr^{-1}.

The period of maximum Si debt (mid-July to mid-August) coincided with the time of apparent low diatom growth (Fig. 5-1) and low Si levels (Fig. 5-2). This increased uptake occurring under apparent deficiency conditions is consistent with culture studies and suggests, on a tentative basis, that Si debt reflects *in situ* Si status. Obviously, there is a need for further evaluation of Si debt under various conditions with a wide range of diatom species before critical levels indicative of nutrient deficiency could be established. Low Si debt may not necessarily be attributable to Si sufficiency, since prolonged starvation may eventually produce senescent cells incapable of Si uptake.

Figure 5-5. Si debt (μg Si mm^{-2} diatom surface area 24 h^{-1}) of epiphytic diatoms at the *Scirpus* (o —— o) and *Potamogeton* (● —— ●) site in Crescent Pond. Values are means of upper and lower depth samples.


500
400
300
200
100
μg Si cm⁻² diatom surface area 24 h⁻¹
SCIRPUS
POTAMOGETON
JUNE
JULY
AUG
SEPT
OCT

Extractable Phosphate and Alkaline Phosphatase

Levels of extractable phosphate (Fig. 5-6) from the epiphytic community decreased between initial June and July samples at both the *Potamogeton* and *Scirpus* sites. Levels at the *Potamogeton* site continued to decline while those at the *Scirpus* site increased. Extractable phosphate at the *Scirpus* site was generally greater than at the *Potamogeton* site from mid-July until late September with the exception of the early September sample when levels at the *Potamogeton* site had increased. In mid-October, extractable phosphate levels at both sites were approximately the same.

Fitzgerald and Nelson (1966) suggest that concentrations of extractable phosphate below 80 mg P 100 mg^{-1} dry weight indicate a likelihood of the growth of the algae being limited by available phosphate. This level corresponds to approximately 0.24 µg P cm^{-2} cell surface area, based on the dry weight to cell surface area ratio for epiphytic algae at the *Scirpus* site. Using the 0.24 µg level, the *Scirpus* epiphytic algae were apparently only P-limited in July, while the algae at the *Potamogeton* site were limited in late July, early August and in September.

The highest alkaline phosphatase activity (Fig. 5-7) occurred at both sites in mid-July, with maximum activity of 1155 mµmole P cm^{-2} cell surface area h^{-1} at the *Potamogeton* site. Following this July peak, activity at both sites generally decreased to low October levels, with some fluctuations. Healey (1975) suggests, on the basis of p-nitrophenylphosphate substrate, that rates of alkaline phosphatase activity above 2 µmole mg^{-1} dry weight h^{-1} are likely to be associated with P deficiency. This value converts to 590 and 943 mµmole cm^{-2} h^{-1} for epiphytic algae at the *Scirpus* and *Potamogeton* sites respectively. The only activity greater than either of these levels occurred in mid-July at the *Potamogeton* site.

Figure 5-6. Extractable phosphate from epiphytic algae (μg PO_4-P cm^{-2} cell surface area) at two sites, *Scirpus* (o —— o) and *Potamogeton* (● --- ●) in Crescent Pond. Values are means of upper and lower depth samples.

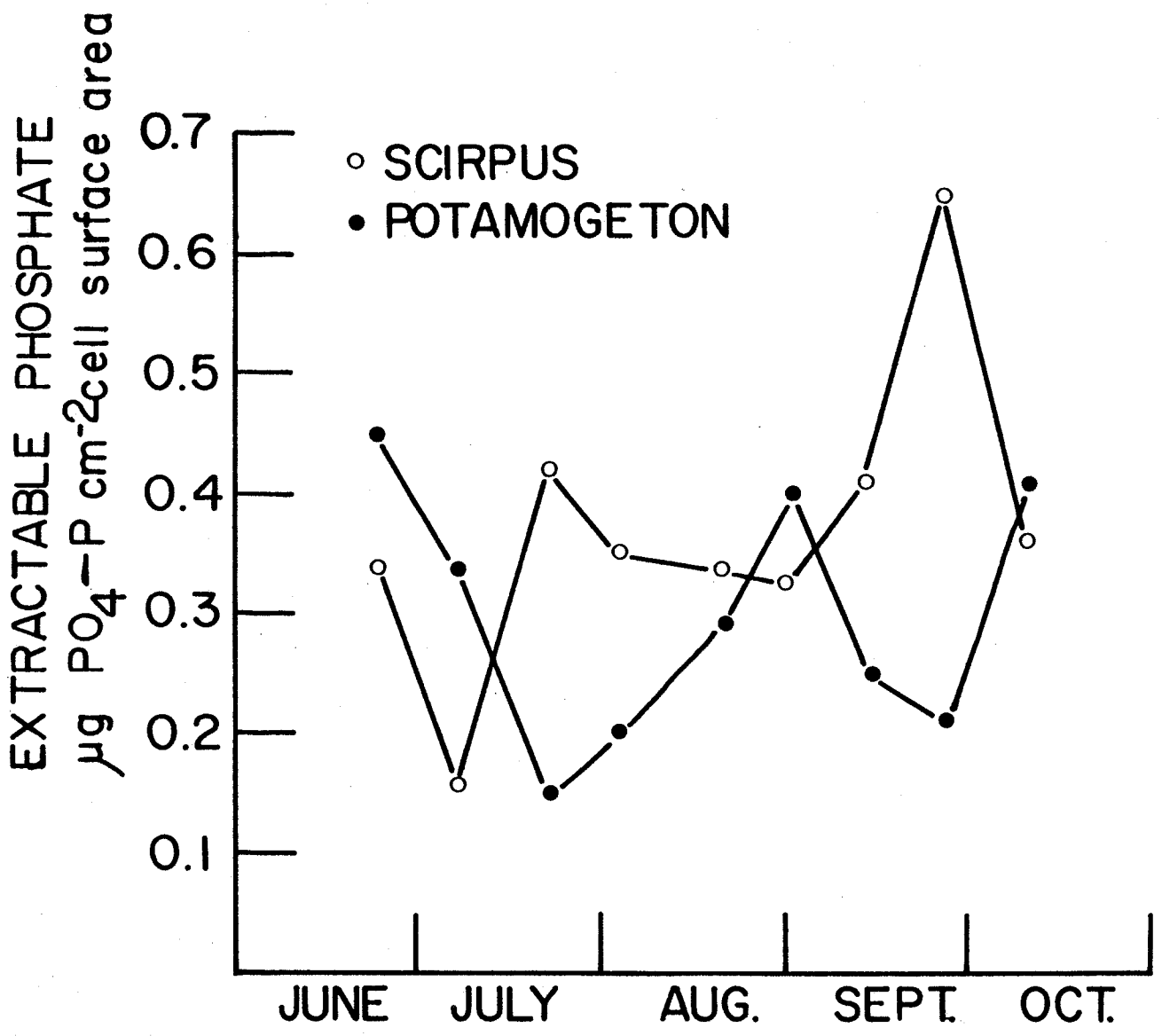
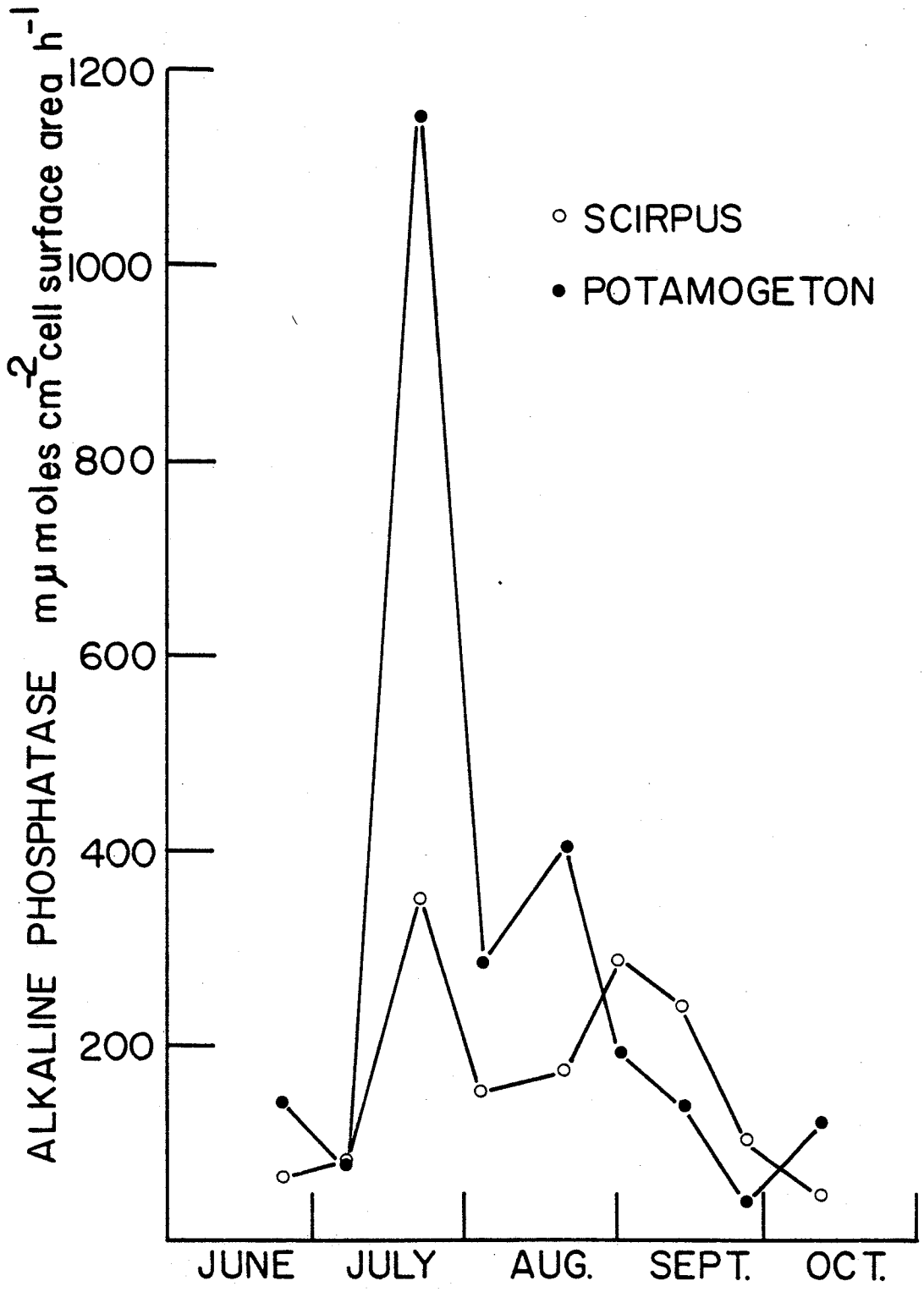

EXTRACTABLE PHOSPHATE
µg PO4–P cm-2cell surface area
0.7
0.6
0.5
0.4
0.3
0.2
0.1
SCIRPUS
POTAMOGETON
JUNE
JULY
AUG.
SEPT.
OCT.

Figure 5-7 Alkaline phosphatase activity of epiphytic algae (mμmole P cm^{-2} cell surface area hr^{-1}) at the *Scirpus* (o —— o) and *Potamogeton* (● —— ●) sites in Crescent Pond. Values are mean of upper and lower depth samples.


ALKALINE PHOSPHATASE mμmoles cm⁻² cell surface area h⁻¹
1200
1000
800
600
400
200
SCIRPUS
POTAMOGETON
JUNE
JULY
AUG.
SEPT.
OCT.

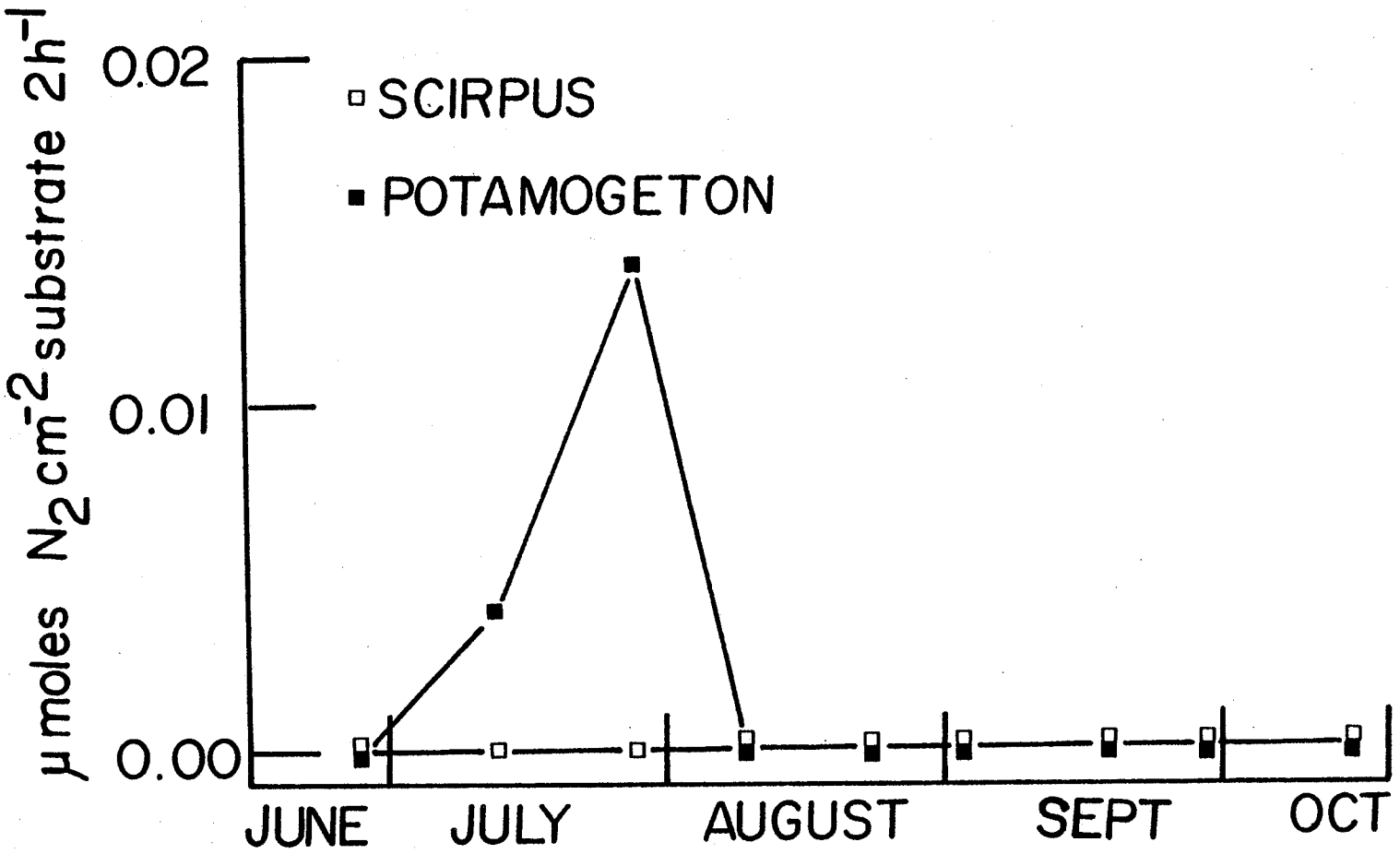
The peak in alkaline phosphatase activity at the *Potamogeton* site in late July coincided with low PO_4-P levels, low extractable phosphate levels (Fig. 5-6) and with a peak in heterocystous blue-green algal growth at the *Potamogeton* site (Fig. 5-1). Higher levels of alkaline phosphatase in cultured blue-green algae relative to other algal groups have been reported (Fitzgerald and Nelson 1966). Heath and Cooke (1975) report high alkaline phosphatase levels in an Ohio kettle lake associated with *Aphanizomenon* blooms. It appears that at the *Potamogeton* site, the blue-green algae, which appeared when PO_4-P levels were low, depended on alkaline phosphatase to obtain phosphate, as well as utilization of accumulated polyphosphates.

There should generally be an inverse relationship between extractable phosphate (Fig. 5-6) and alkaline phosphatase activity (Fig. 5-7). When storage phosphate levels at the *Potamogeton* site were lower in July and August than those at the *Scirpus* site, alkaline phosphatase levels were higher at the *Potamogeton* site than at the *Scirpus* site. In September, there was indication of P-limitation at the *Potamogeton* on the basis of extractable phosphate levels, but the epiphytic algae at the site were not producing alkaline phosphatase.

Nitrogenase Activity

Nitrogenase activity in Crescent Pond (Fig. 5-8) was highest at the *Potamogeton* site in July, with an assayed maximum rate of 178×10^{-4} moles N_2 fixed cm^{-2} per 2 h incubation. No significant activity by algae colonizing the artificial substrate in the *Scirpus* zone was detected. The peak in N_2 fixation at the *Potamogeton* site corresponded with an increase in cyanophyte cell volume (Fig. 5-1) essentially confirming that fixation was a function of the blue-green algae, particularly since the surrounding water was probably O_2-saturated throughout the experimental period. The hetero-

Figure 5-8 Nitrogenase activity of epiphytic algae (μmoles N_2 cm^{-2} substrate 2 h^{-1}) at the *Scirpus* (o —— o) and *Potamogeton* (● —— ●) site in Crescent Pond. Values are mean of upper and lower depth sample.


μ moles N_2 cm^{-2} substrate $2h^{-1}$
0.02
0.01
0.00
SCIRPUS
POTAMOGETON
JUNE
JULY
AUGUST
SEPT
OCT

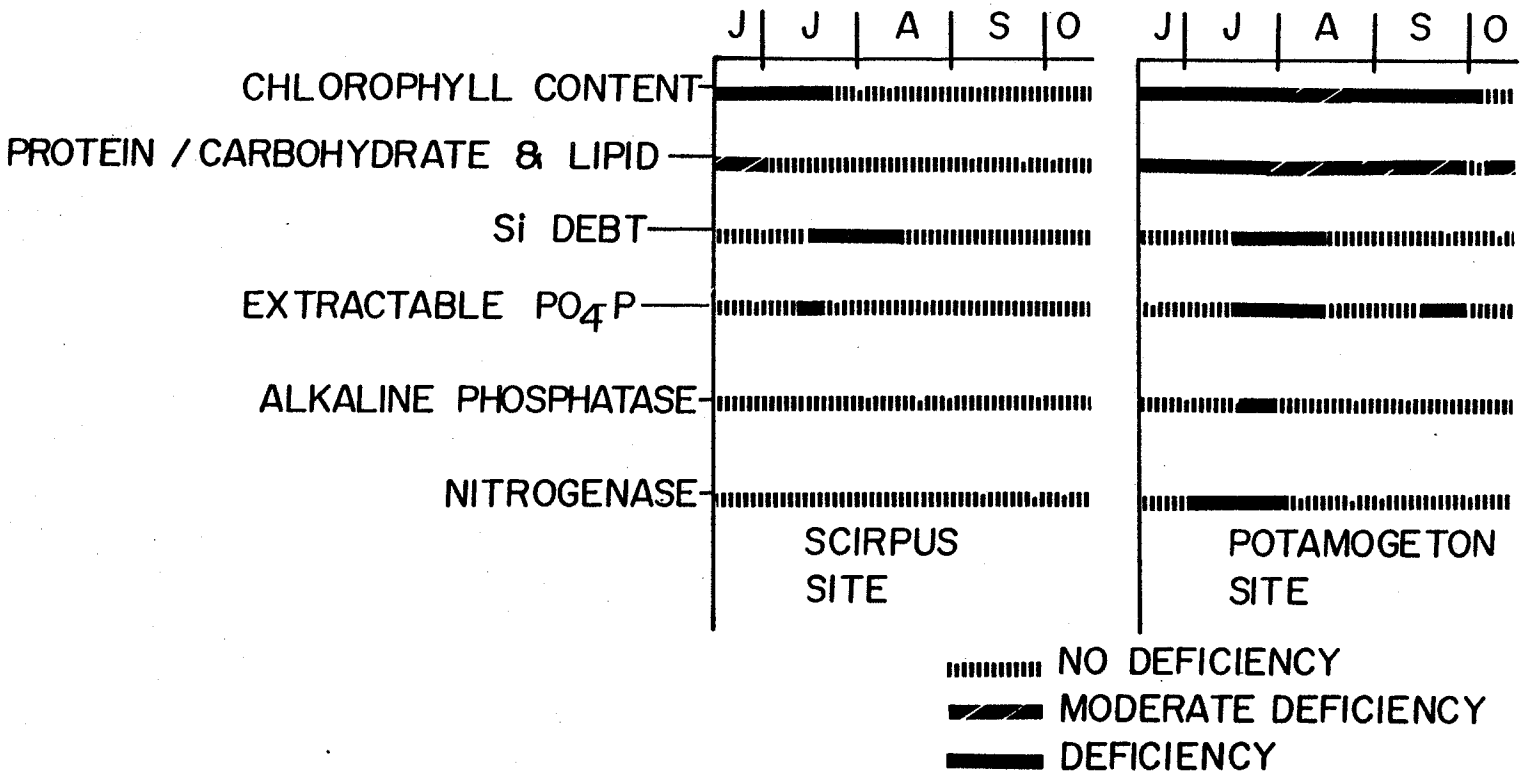
cystous algae occurring on the substrate were *Anabaena* spp., *Gloeotrichia* spp. and *Calothrix* spp.

General Discussion

The results of various physiological indicators used in this study of epiphytic algae are summarized in Fig. 5-9. In June and early July when the artificial substrates were initially colonized and standing crops at the two sites were generally low, there was indication of deficiency on the basis of chlorophyll a content and protein to carbohydrate and lipid ratio, especially at the *Potamogeton* site. Concentrations of available P and Si were high and there was no physiological indication of limitation by either of these nutrients. Obviously, there are numerous factors which could have been limiting epiphytic algal growth, including the low NO_3-N and NH_3-N concentrations. In the July to August period, when nutrients were apparently low, there was evidence of Si limitation of diatom growth at both sites and growth of N_2-fixing heterocystous blue-green algae coincident with high alkaline phosphatase activity at the *Potamogeton* site. There was indication of nutrient deficiency at the *Potamogeton* site in terms of all physiological indicators, while only extractable phosphate levels indicated P deficiency at the *Scirpus* site. Standing crops at both sites were similar and generally low.

The lack of indication of nutrient deficiency at the *Scirpus* site from September to October corresponded to increased standing crop of epiphytic algae and higher levels of N, P and Si at this site. At the *Potamogeton* site, where standing crop, especially of green algae, did not increase until October, there was indication of deficiency in terms of

Figure 9 Summary of physiological indicators of nutrient deficiency for epiphytic algae at (a) *Scirpus* and (b) *Potamogeton* sites in Crescent Pond, Delta Marsh. (▦ - no deficiency, ▨ - moderate deficiency, ■ - deficiency).


J
J
A
S
O
CHLOROPHYLL CONTENT
PROTEIN / CARBOHYDRATE & LIPID
Si DEBT
EXTRACTABLE PO_4P
ALKALINE PHOSPHATASE
NITROGENASE
SCIRPUS SITE
POTAMOGETON SITE
NO DEFICIENCY
MODERATE DEFICIENCY
DEFICIENCY

chlorophyll a content, protein to carbohydrate and lipid ratios and extractable phosphate levels. When standing crop did increase at this site in October, there was no indication of deficiency, with the exception of a protein to carbohydrate and lipid ratio suggestive of moderate deficiency. The relationship between standing crop and nutrient levels during this period has been previously discussed. In general, higher nutrient levels at the *Scirpus* site in September and October corresponded to increased standing crop of epiphytic algae and absence of indication of deficiency, while the lower standing crop at the *Potamogeton* site coincided with low nutrient levels and indicators of deficiency. It is not apparent which nutrient or other factor triggered the growth of epiphytic algae in October at the *Potamogeton* site.

The physiological indicators used in this study provided additional information on the seasonal growth of epiphytic algae. These indicators generally verified observed correlations between changes in standing crop and fluctuations in nutrient levels. There is a need for further study on the effects of deficiencies on various algal populations in order to establish more rigorous critical levels indicative of deficiency, especially on a cell surface area basis.

CONCLUDING DISCUSSION

The objectives of this study were to investigate productivity and standing crop of epiphytic algae in a marsh pond and to analyze the role of nutrients in the regulation of seasonal epiphytic growth. Results revealed that the epiphytic algae were an important component of the Crescent Pond ecosystem. The annual estimated productivity of 1881 kg C yr^{-1} (Chapter 2) for the epiphytes in the pond was as high as approximately 39% of that of the aquatic macrophytes (Appendix 1). Such a significant contribution to pond production indicates active metabolic activity which undoubtedly influences the cycling of organic and inorganic compounds through the littoral zone.

The retention of assimilated C during the light period, with negligible apparent respiration or excretion losses in the light (Chapter 3) suggests that the epiphytic community may be efficient in C utilization. Preferential use of CO_2 produced by respiration might be an advantage to photosynthetically-active epiphytic algae in the alkaline marsh water, with its predominate bicarbonate source. Furthermore, because of the close spatial proximity of the algal, bacterial and fungal components of the epiphytic community, excreted products may be rapidly reassimilated. The high productivity of the epiphytic community may be partially due to rapid recycling of various compounds and accompanying metabolic efficiency.

A comparative study of productivity and various standing crop estimates of epiphytic algae revealed general correlation between measurements of ^{14}C-photosynthetic uptake under constant incubation conditions, cell surface area, dry weight and chlorophyll *a*, protein, carbohydrate and lipid content (Chapter 4). Cell volume showed lower correlation with the various para-

meters. This lower correlation is of significance, since cell volume is a commonly employed measure of standing crop. It would appear that cell surface area may be a more meaningful expression of epiphytic algal standing crop than cell number.

Peak productivity and standing crop of epiphytic algae in Crescent Pond generally occurred in the autumn. Analysis of seasonal growth with respect to nutrient levels in the pond and physiological indicators of nutrient deficiency suggested the concentrations of P, Si and N may be important controlling factors at the *Scirpus* and *Potamogeton* sites studied in the pond (Chapter 5). Summer growth appears to have been limited by low nutrient levels. The physiological indicators generally verified observed correlations between changes in standing crop of epiphytic algae and fluctuations in nutrient levels. Although the epiphytic algae at the *Potamogeton* and *Scirpus* sites were separated by less than 12 m and seasonal algal growth was generally similar, some significant differences did occur in epiphytic algal growth. For example, heterocystous, N_2-fixing blue-green algae occurred in mid-July at the *Potamogeton* site and not at the *Scirpus* site.

Although this study of epiphytic algae satisfies the initial objectives, it leaves unresolved many significant gaps in the overall understanding of the epiphytic community. Some results in this study have implications beyond the study's scope and suggest areas which warrant further investigation. The observation of carbon retention during the day in the epiphytic community suggests rapid recycling may be occurring. The detection of N_2 fixing blue-green algae at the *Potamogeton* site suggests a potential beneficial effect of epiphytic algae on submerged macrophytes. General areas in which further investigation is required include metabolic exchanges occurring within the

epiphytic community, interactions between the epiphytic community and host macrophytes, faunal activity associated with the epiphytic community and the role of bacteria and fungi in the community.

SUMMARY

A study was carried out to quantify epiphytic algal growth in a marsh pond (Crescent Pond, Delta Marsh, Manitoba) and to examine some factors controlling seasonal growth.

Initial investigation involved the estimation of primary productivity, with necessary evaluation of methodology. Cellulose-acetate substrates were found to provide a reliable attachment surface which could be used for routine photosynthetic C^{14} uptake determinations of the epiphytic algae. No significant differences were found between mean C^{14} uptake values of epiphytic algae colonizing cellulose-acetate and natural substrates for equal periods of time or between values based on cellulose-acetate and glass substrates.

Annual productivity of epiphytic algae in Crescent Pond was estimated to be 1880 kg C yr^{-1} or 26.8 g C m^{-2} pond surface area with a coefficient of variation of 31%. The highest productivity per unit macrophyte surface area was at a *Scirpus acutus* site, while *Potamogeton pectinatus* was the most significant substrate in overall productivity. The importance of *Potamogeton* was related to the extensive area of the submerged plant available for epiphytic algal colonization. Organic C to primary productivity ratios were higher in the autumn than in the summer. The average organic C to organic N ratio was 9.3, with no apparent seasonal trends or substrate differences.

Diurnal C fixation patterns of the epiphytic community were investigated. The photosynthesis to *in situ* light intensity relationship was relatively constant throughout the day. Apparent uptake of C^{14} by the epiphytic community was cumulative in the light for periods up to 24 hr. A net loss

during the night of 30% of C^{14} assimilated in the previous light period was detected, while negligible losses were observed in the light. Results suggested that, in the light, the epiphytic community may be efficient in net retention of assimilated carbon.

Seasonal growth of epiphytic algae colonizing artificial substrates positioned in a stand of *Scirpus acutus* and in a zone of *Potamogeton pectinatus* was analyzed in detail. Productivity, in terms of C^{14}-photosynthetic uptake and standing crop in terms of cell volume, cell surface area, dry weight and chlorophyll a, protein, carbohydrate and lipid content were followed from June until October 1976. Standing crop and productivity increased at both sites in September and October, after generally low summer growth with the exception of the occurrence of heterocystous blue-green algae at the *Potamogeton* site in July. Common genera occurring in the epiphytic community included *Coleochaete, Stigeoclonium, Oedogonium, Cladophora, Cocconeis, Synedra, Fragilaria, Gomphonema* and other pennate diatoms. *Anabaena, Gloeotrichia* and *Calothrix* occurred in July at the *Potamogeton* site.

Maximum values for epiphytic algae at the *Scirpus* and *Potamogeton* sites were observed to be 0.40 mm^3 cm^{-2} and 0.30 mm^3 cm^{-2} cell volume; 383 mm^2 cm^{-2} and 266 mm^2 cm^{-2} cell surface area; 4.53 µg cm^{-2} and 6.30 µg cm^{-2} chlorophyll a and 1408 µg cm^{-2} and 963 µg cm^{-2} dry weight, respectively. The seasonal average ratio of phaeophytin a to the total of chlorophyll a and phaeophytin a at all sites was 0.38 with no apparent seasonal trend. The carbohydrate, protein and lipid composition of the epiphytic community averaged, as a percent of the sum of the three components, 43% carbohydrate, 17% lipid and 40% protein or 18%, 7% and 17% respectively of dry weight.

The process of factor analysis was used as a device for ordering and simplifying correlations between various production parameters. Cell volume had a greater proportion of variance not explained by the common factor than any other of the variables. Cell surface area was more closely correlated with productivity and chlorophyll a than was cell volume. Results suggested that cell surface area was a more meaningful estimator of production capacity and standing crop than was cell volume.

The seasonal growth of epiphytic algae at the two sites in Crescent Pond was related to nutrient levels (N, P and Si) and to various physiological indicators of nutrient availability, including chlorophyll content, protein to carbohydrate and lipid ratios, Si debt, storage phosphate levels, alkaline phosphatase activity and nitrogenase activity. In June and early July, when the artificial substrates were initially colonized and standing crops at the two sites were generally low, there was indication of deficiency on the basis of chlorophyll a content and protein to carbohydrate and lipid ratio, especially at the *Potamogeton* site. Concentrations of available P and Si were high and there was no physiological indication of limitation by either of these nutrients. In the July to August period, when nutrients were apparently low, there was evidence of Si limitation of diatom growth at both sites and growth of N_2-fixing heterocystous blue-green algae coincident with high alkaline phosphatase activity at the *Potamogeton* site. There was indication of nutrient deficiency at the *Potamogeton* site in terms of all physiological indicators, while only extractable phosphate levels indicated P deficiency at the *Scirpus* site.

The lack of indication of nutrient deficiency at the *Scirpus* site from September to October corresponded to increased standing crop of epiphytic algae and higher levels of N, P and Si at this site. At the *Potamogeton* site,

where standing crop, especially of green algae, did not increase until October, there was indication of deficiency in terms of chlorophyll *a* content, protein to carbohydrate and lipid ratios and extractable phosphate levels.

APPENDIX I

Macrophyte Production in Crescent Pond

Above-ground macrophytic standing crop in Crescent Pond (1974) was estimated on a dry weight basis and converted to an approximate C basis using a conversion factor from dry weight to C of 0.44 (Table 6-1). [The inaccuracy of this commonly used conversion factor has been discussed by Westlake (1965) and the fact that the macrophyte production in Crescent Pond is crudely estimated is stressed.] The total macrophyte production was 4817.9 kg C or 688 kg C ha^{-1} pond surface area.

Macrophyte production may be compared with epiphytic algal productivity in Crescent Pond of 1881 kg C or 268 kg C ha^{-1} lake surface yr^{-1} (Table 2-2). The epiphytic production was 39% that of the macrophytes. Wetzel, Rich, Miller and Allen (1972) reported littoral algal production and macrophyte production in Lawrence Lake, Michigan to be 399 and 879 kg C ha^{-1} lake surface yr^{-1} respectively. Littoral algal production was 45% that of the macrophytes. The results of Wetzel *et al* (1972) were similar to those reported for Crescent Pond.

Table 6-1. Macrophyte production in Crescent Pond based on 1974 shoot densities.

Macrophyte	Shoots mg^{-2}	Dry wt g $shoot^{-1}$	Area m^{-2}	Total dry wt kg
Phragmites communis	50	9.8	308	150.9
Scirpus acutus	109	5.3	484	279.6
Typha latifolia				
low density	5	42.0	2736	574.6
high density	30	42.0	6192	7801.9
Potamogeton pectinatus				
low density	19 g m^{-2}		33628	638.9
high density	56 g m^{-2}		26854	1503.8
				10949.7

Conversion to C = 4817.9 kg C

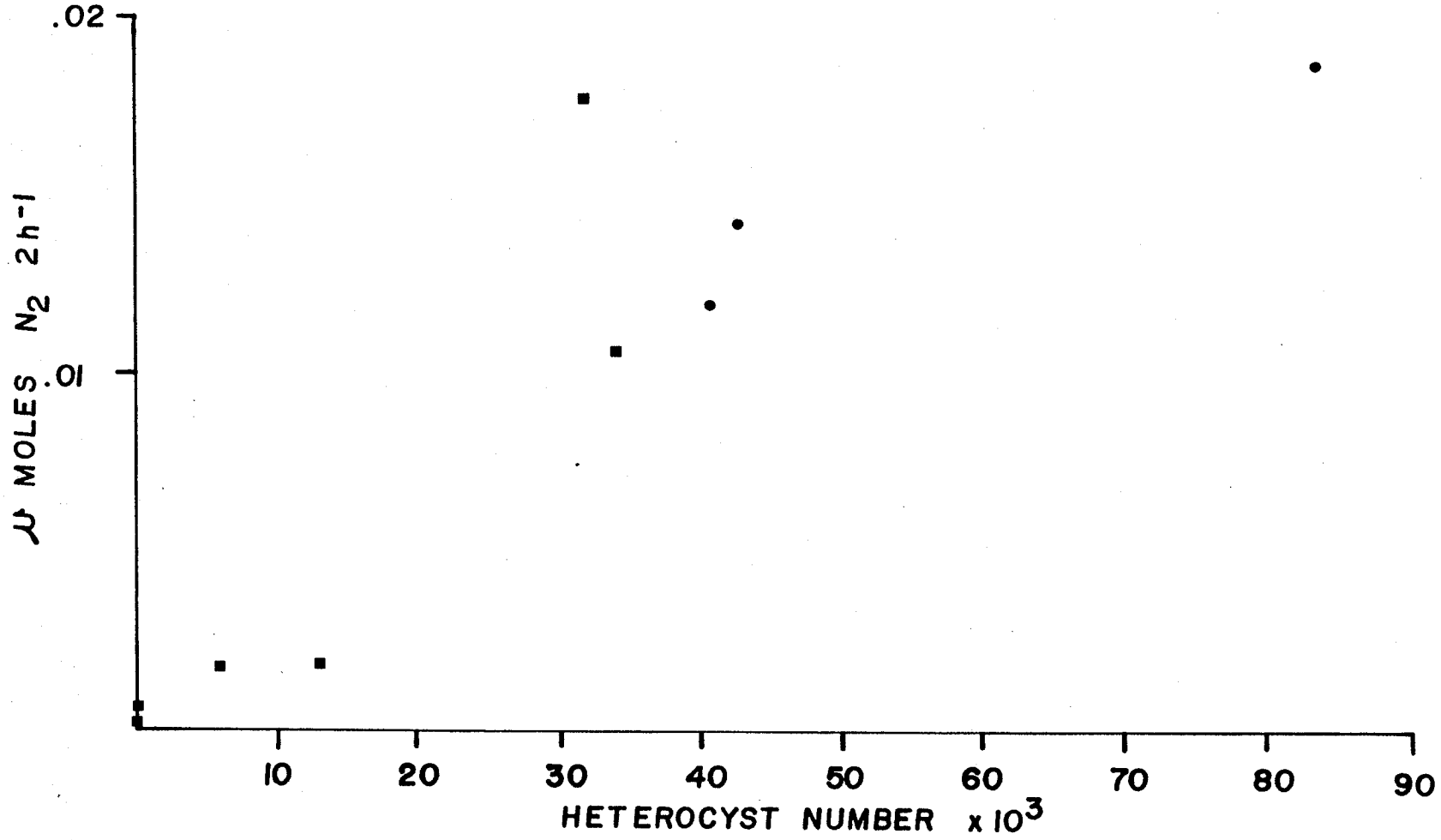
APPENDIX II

Nitrogenase Activity and Heterocysts in the Epiphytic Community

The relationship between nitrogenase activity (Chapter 5) and the number of heterocysts in the epiphytic algae is presented in Figure 6-1. This relationship confirms the well-documented role of the blue-green algal heterocysts in N_2 fixation under oxic conditions (e.g. Horne and Goldman 1972). At Crescent Pond, the mean heterocyst to blue-green cell ratio was 0.07, with values ranging from 0.06 to 0.11. These values are higher than the range reported by Horne and Goldman (1972) of 0.009 - 0.017 for planktonic algae, but within the range of ratios of 0.006 to 0.6 reported by Horne and Carmiggelt (1975) for stream benthic algae.

Figure 6-1 The relationship between nitrogenase activity and the number of heterocysts in the epiphytic algal community.

■ mixed epiphytic algae ● *Gloeotrichia natans*


.02
.01
μ MOLES N_2 $2h^{-1}$
10
20
30
40
50
60
70
80
90
HETEROCYST NUMBER x 10^3

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