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ECOLOGY OF GRACILARIA TIKVAHIAE  
MCLACHLAN (GIGARTINALES,  
RHODOPHYTA) IN THE GREAT BAY  
ESTUARY, NEW HAMPSHIRE

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**ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN (GIGARTINALES,  
RHODOPHYTA) IN THE GREAT BAY ESTUARY, NEW HAMPSHIRE**

*University of New Hampshire*

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ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN  
(GIGARTINALES, RHODOPHYTA) IN THE  
GREAT BAY ESTUARY, NEW HAMPSHIRE.

BY

CLAYTON ARTHUR PENNIMAN

B.S. (Biology), University of Maine at Orono, 1974

A DISSERTATION

Submitted to the University of New Hampshire  
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the Requirements for the Degree of

Doctor of Philosophy

in

Botany

September 1983

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ABSTRACT

ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN  
(GIGARTINALES, RHODOPHYTA) IN THE  
GREAT BAY ESTUARY, NEW HAMPSHIRE.

By

Clayton Arthur Penniman

University of New Hampshire, September, 1983

The reproductive phenology, growth and variation of chemical composition of Gracilaria tikvahiae from the Great Bay Estuary, N.H. were evaluated. A major objective was an analysis of the chemical composition, particularly agar content and properties, of plants separated into reproductive categories. The net photosynthetic responses of G. tikvahiae to several irradiance, temperature and salinity regimes were determined.

Gracilaria tikvahiae plants from the Great Bay Estuary were vegetative throughout most of the year. However, discrete maxima of tetrasporic and spermatangial plants occurred during June-July and for cystocarpic plants during July-August. The in situ growth of Gracilaria tikvahiae was highest during June-September, with maximum rates of 11%/day. The growth cycle of G. tikvahiae plants was most

strongly correlated with water temperature. Seasonal variations of surface irradiance and dissolved inorganic nitrogen were not related to the growth cycle of G. tikvahiae.

Gracilaria tikvahiae had annual cycles of ash, dry weight, carbohydrate, agar, carbon, nitrogen and phycoerythrin contents. In contrast, little variation in protein, phosphorus or chlorophyll occurred. The changes in tissue carbon, nitrogen, carbohydrate and agar had summer minima and winter maxima. However, the ash content was maximal in summer and lowest during winter. The total tissue nitrogen of G. tikvahiae did not decrease below 2% of dry weight. No significant differences in chemical composition were noted between reproductive stages. The agar content of Gracilaria tikvahiae varied between 7% (summer) and 23% (winter). The gel strengths and 3,6-anhydrogalactose content of G. tikvahiae agar were highest in the summer. There were no significant differences in 3,6-anhydrogalactose, sulfate, ash content, gel strength or viscosity between agar, extracted with hydroxide pretreatment, from cystocarpic or tetrasporic plants.

The net photosynthesis of Gracilaria tikvahiae was light-saturated at 200-600  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , but it was not inhibited at 1440  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . G. tikvahiae had increasing net photosynthetic rates from 5° to 25°C. Maximum net photosynthesis occurred between 25° and 35°C, while rates decreased at 37.5°C. The net photosynthetic responses at 25° and 30°C were stable after acclimation times of one to four days, but declined after three days at 35°C. G. tikvahiae has a euryhaline net photosynthetic response between 5 g/kg and 40 g/kg.

ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN  
(GIGARTINALES, RHODOPHYTA) IN THE  
GREAT BAY ESTUARY, NEW HAMPSHIRE.

PART 1.

REPRODUCTIVE PHENOLOGY AND GROWTH.

## 1.1 INTRODUCTION

The cosmopolitan genus Gracilaria is widely used as a source of the phycocolloid agar (Michanek 1975, Mathieson 1982). In North America several projects have been conducted to determine the aquaculture potential of various Gracilaria species (Edelstein et al. 1976, 1981, Edelstein 1977, C. Bird et al. 1977a, Ryther et al. 1979, Lindsay and Saunders 1979, 1980, Saunders and Lindsay 1979). The latter investigations have demonstrated rapid growth rates of some Gracilaria species under various artificial conditions. However, less is known of G. tikvahiae's growth, as well as its reproductive phenology, in situ (Taylor 1975, C. Bird et al. 1977b).

Growth studies of several Gracilaria species indicate that maximum growth or standing crop coincides with seasonal temperature and/or irradiance maxima, at least in temperate habitats (Conover 1958, Edwards and Kapraun 1973, C. Bird et al. 1977a, 1977b, Rosenberg and Ramus 1981, 1982). In particular, in situ growth rates of G. tikvahiae compare favorably with those determined in aquaculture (see Table 1-V). The seasonality of reproduction coincides with growth/standing crop maxima for G. tikvahiae (C. Bird et al. 1977b) and G. verrucosa (Jones 1959a, 1959b). In

contrast, no similar coincidence was shown for G. bursapastoris and G. coronopifolia (Hoyle 1978).

Gracilaria tikvahiae (N. Bird et al. 1977), G. verrucosa (Ogata et al. 1972, C. Bird et al. 1982) and G. foliifera (McLachlan and Edelstein 1977) have isomorphic triphasic life histories of the Polysiphonia-type (Dixon 1973). However, seasonal field collections of these seaweeds have generally found substantial deviations from the life histories in culture (N. Bird 1975, 1976, C. Bird et al. 1977a). Jones (1959a) noted that spermatangial plants of G. verrucosa were much less common than either tetrasporic or cystocarpic plants in Great Britain. In contrast, Gracilaria (verrucosa type) in British Columbia (Whyte et al. 1981) and some populations of G. tikvahiae in the Canadian Maritimes (C. Bird et al. 1977b) had a preponderance of tetrasporic plants. However, one attached population of G. tikvahiae from Barrachois Harbour (Nova Scotia) apparently conforms to the life history demonstrated in culture (N. Bird 1976).

The present report represents one section of a project concerning the ecology of Gracilaria tikvahiae within the Great Bay Estuary (N.H.). The current paper describes the in situ growth and reproductive phenology of G. tikvahiae within the Great Bay Estuary. A portion of this research was summarized previously (Penniman 1977) with the plant referred to as Gracilaria foliifera (Forsskal) Boergesen.



However, the alga has subsequently been designated Gracilaria tikvahiae (Chapman et al. 1977, McLachlan et al. 1977, Edelstein et al. 1978, McLachlan 1979).

## 1.2 METHODS

Attached plants of Gracilaria tikvahiae were collected randomly by SCUBA divers between 2 m to 4 m below mean low water at Cedar Point ( $43^{\circ} 7.68' N$ ,  $70^{\circ} 51.67' W$ ), Thomas Point ( $43^{\circ} 4.93' N$ ,  $70^{\circ} 51.92' W$ ) and Nannie Island ( $43^{\circ} 4.13' N$ ,  $70^{\circ} 51.83' W$ ) within the Great Bay Estuary (Figure 1-1). Monthly collections were made from May 1976 to October 1977. Ice cover during January and February 1977 prevented collection at Nannie Island. Individual plants from each collection were sorted by reproductive status (i.e. vegetative, cystocarpic, spermatangial or tetrasporic). The categories indicated only the presence of the reproductive structures but did not necessarily indicate reproductive potential. Each sample was rinsed briefly in tap water, drained, blotted dry and its fresh weight determined. The samples were dried at room temperature in moving air; then dried for 48 hr at  $60^{\circ}C$  in vacuo; and reweighed. The proportion of each reproductive category was expressed as the percent of the total dry weight per individual collection. The dried plant material was chemically analyzed (Parts 2 and 3).

The growth of Gracilaria tikvahiae plants collected at Thomas Point was measured at Adams Point (43° 5.48' N, 70° 51.93' W) with three methods. First, twenty apical fragments (1-2 g fresh wt) were placed in a 0.125 inch nylon mesh bag subdivided into twenty separate compartments. The bag was suspended horizontally on a 0.75 inch PVC pipe frame (1 m x 1 m) maintained by flotation at a constant depth of 1 m in approximately 5 m deep water. In the other two enclosure methods, the plants were tethered to cement blocks placed at -1.0 m, to maximize irradiance and ensure that emersion did not occur. Two sets of twenty plants were attached to the blocks; one set was enclosed in individual net bags similar to those described previously, while the remaining set of twenty plants was tied to the blocks by monofilament lines. All of the growth experiments were conducted on the eastern shore of Adams Point (Figure 1-1).

Monthly growth rates were measured as increases in fresh weight and calculated in terms of percent growth/day by the following formula:

$$G = [(W_t/W_o)^{1/t} - 1] \times 100$$

where G=percent increase in fresh weight/day,  $W_o$ =initial weight, and  $W_t$ =weight after t days (Hoyle 1978). The plants were cleaned of epiphytes weekly, pruned to minimize shelf-shading, or replaced when necessary during each monthly weighing. Growth in the floating net bags was measured from April 1978 to August 1979. The two other

treatments, also initiated in April, were terminated after October 1978 due to plant fragmentation and loss as a result of wave action during autumn storms.

The values for hydrographic and water nutrient chemistry data used in this study were obtained from a baseline hydrographic survey of the Great Bay Estuary (Emerich Penniman et al. 1983). The water samples for the latter study were taken during ebb tide from 0.0, -1.2 and -4.0 m at locations adjacent to the study sites used in the Gracilaria investigation (Figure 1-1). Dissolved inorganic nutrients (i.e.  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and  $\text{PO}_4^{3-}$ -P) were analyzed using Technicon Autoanalyzer methods (Glibert and Loder 1977). Surface irradiance values, measured with an Eppley model PSP pyranometer, were obtained from data collected by the N.H. State Climatologist located at Durham, N.H. (G. Pregent, personal communication). A factor of 0.5 was used to approximate PAR (i.e. photosynthetically active radiation) from the total daily solar irradiance values (Szeicz 1974).

The average seasonal curves for the hydrographic and water chemistry data (May 1976 to October 1977) were analyzed by periodic regression techniques (Hackney and Hackney 1977, 1978). The periodic regression model contained a harmonic term:

$$Y_i = a_0 + a_1 \cos(2\pi/12x_i) + a_2 \sin(2\pi/12x_i) + e_i$$

where  $y_i$  is the dependent variable;  $a_0, a_1, a_2$  are constants;  $x_i$  is the independent variable (i.e. a time series), and  $e_i$  is the residual term. Statistical analyses of growth rates were performed using a similar periodic regression model. The periodic regression analyses were conducted with a routine (BMDO4R) from the BMD package (Dixon 1977). Correlations between growth rates and environmental parameters were calculated with MINITAB (Ryan et al. 1976) and SPSS (Nie et al. 1975). The plant tissue chemistry data used in correlations with growth rates are from 1976-1977 (Part 2).

### 1.3 SITE DESCRIPTION

The Great Bay Estuary (New Hampshire-Maine) extends from the mouth of the Piscataqua River in Portsmouth, to Little Bay, then past Furber Straits into Great Bay. The Estuary also includes the tidewater portions of the seven rivers which drain into the basin (Figure 1-1). Tides are equal-semidiurnal, with a vertical range of 2.0-3.0 m (National Ocean Survey 1982). The Estuary is well-mixed by tidal currents that may exceed 100 cm/s in certain areas (Swenson et al. 1977).

The distribution of Gracilaria tikvahiae within the Estuary is primarily limited to Little and Great Bays (Hehre and Mathieson 1970, Mathieson et al. 1981). Perennial, subtidal populations of G. tikvahiae occur at each of the four study sites (Figure 1-1). Most of the plants are attached individuals between -1 to -4 m. Although the bottom at these sites is generally composed of mud/silt, other substrata, such as bivalve shells (particularly Crassostrea virginica), rocks and sunken logs, are present. The algal flora associated with Gracilaria at these sites has been described by Mathieson et al. (1981).

The water temperatures at Cedar Point, Thomas Point and Nannie Island (collection sites) from May 1976 to October 1977 and at Adams Point (growth study site) from April 1978 to August 1979 varied from a winter low of  $-1.9^{\circ}\text{C}$  to a summer high of  $25^{\circ}\text{C}$  (Figures 1-2 and 1-4). Salinities varied from 8 g/kg during spring runoff to maximum values of 32 g/kg (Figures 1-3 and 1-5). The temperature and salinity regimes at Cedar Point, Thomas Point and Nannie Island were similar during May 1976 to October 1977 (Figures 1-2a and 1-3a). A regression model (Table 1-I) calculated for the average seasonal cycle of temperature at Cedar Point, Thomas Point and Nannie Island (Figure 1-2b) had a significant periodic component ( $R^2=91.7$ ). However, the average annual cycle of salinity (Figure 1-3b) did not conform as closely to the periodic model ( $R^2=44.0$ ) due to episodic spring runoff.

The monthly values of  $\text{NH}_4^+$ -N and  $(\text{NO}_3^- + \text{NO}_2^-)$ -N at Cedar Point, Thomas Point and Nannie Island are variable, although distinct seasonal fall-winter maxima and summer minima are apparent (Figure 1-6). Minor summer increases in ammonium levels were evident during 1976 and 1977. A comparable cycle of dissolved inorganic nitrogen occurred during April 1978 to August 1979 at Adams Point (Figure 1-8). The seasonal cycles of  $\text{NH}_4^+$ -N,  $(\text{NO}_3^- + \text{NO}_2^-)$ -N and total dissolved inorganic nitrogen (i.e. the sum of the two former components) at Cedar Point, Thomas Point and Nannie Island have significant periodic components,  $R^2=56.8$ , 73.3 and

77.2, respectively (Table 1-I). Dissolved phosphate concentrations at Cedar Point, Thomas Point and Nannie Island varied between a June low near 0  $\mu\text{g-at P/L}$  to a January high of 2  $\mu\text{g-at P/L}$  (Figure 1-9). The low seasonal variation of dissolved phosphate (Figure 1-9b) was reflected in the low  $R^2$  (34.3) of the periodic regression model (Table 1-I). Similar reduced seasonal variation in phosphate was present at Adams Point during April 1978 to August 1979. Total surface irradiance at Durham (N.H.), approximately 7.5 km from Adams Point, for the period April 1978 to July 1979, varied from summer highs of  $600 \text{ cal}\cdot\text{cm}^{-2}\cdot\text{day}^{-1}$  to winter lows of  $150 \text{ cal}\cdot\text{cm}^{-2}\cdot\text{day}^{-1}$  (Figure 1-11).



## 1.4 RESULTS

### REPRODUCTIVE PHENOLOGY

Vegetative plants dominated the populations at Cedar Point, Thomas Point and Nannie Island from September to May, while the maximum abundance of reproductive plants occurred during June to August for 1976 and 1977 (Figures 1-12 to 1-15). Tetrasporic plants had a discrete reproductive periodicity with maxima in June-July, decreasing to negligible amounts throughout the remainder of the year (Figures 1-12 to 1-15). Cystocarpic plants had maximum abundance during June-August, while lesser amounts occurred during other times (Figures 1-12 to 1-15). Cystocarps observed during the winter to early spring probably represented residual structures that had released their carpospores. The refractory nature of the cystocarps themselves, relative to the tetrasporangial or spermatangial sori, explains their persistence. During 1976 the maximal abundance of both cystocarpic and tetrasporic fronds occurred progressively later at Nannie Island, Thomas Point and, finally, Cedar Point; however, this temporal difference was not observed in 1977.

Plants with spermatangial sori (i.e. of the textorii-type sensu Yamamoto 1975) were not identified until May 1977. However, during the period they were observed, spermatangial plants had a distinct reproductive periodicity similar to both the tetrasporic and cystocarpic thalli. Spermatangial fronds were most abundant during June-July (of 1977) at all three sites (Figures 1-12 to 1-14). Cystocarpic plants occurred in greater amounts than spermatangial plants during the summer of 1977 when both phases were collected (Figures 1-12 to 1-15). In general the proportion of each reproductive phase was similar for corresponding collections at the three sites (Figure 1-15).

#### GROWTH

The growth rates of Gracilaria tikvahiae at Adams Point were maximal during June to August (Figure 1-16). There was a significant periodic component of the seasonal growth cycle and there were significant differences between the enclosure treatments (Table 1-II). The growth of plants tethered to cement blocks at -1.0 m was significantly greater than those held in mesh bags (SNK,  $p < 0.05$ ). One exception in July was due to an anomalous decrease in growth of the tethered plants, perhaps due to storm/wave-induced fragmentation.

The differences in growth rates between the three methods suggest some differential shading due to the net enclosures. Also the net bags may have protected crustacean grazers and therefore increased their numbers in the bags relative to the tethered plants. However, little evidence of grazing was observed for any plant in the three treatments. While the bags probably shaded the enclosed plants to some degree, loss due to fragmentation was decreased by enclosure. The tethered plants, however, were subject to considerable fragmentation which may have contributed to the July depression of growth. Plants in mesh bags held 1.0 m below the water surface had zero growth rates from December 1978 to April 1979. Growth increased during May to July in 1979 as in 1978. The maximum growth rates coincided with the period of maximum reproduction.

Growth (as arcsine transformed % fresh weight/day in -1.0 m mesh bags) was highly correlated ( $r^2=0.914$ ) with temperature (Table 1-III). Surface irradiance was substantially less correlated with growth as a single factor ( $r^2=0.580$ ) or thru partial correlation with temperature held constant ( $r_{GL.T}=-0.460$ ;  $r^2_{GL.T}=0.212$ ,  $p=0.057$ ). Inorganic nitrogen (as  $NH_4^+-N$ ,  $(NO_3^-+NO_2^-)-N$  or total dissolved inorganic nitrogen) was negatively correlated ( $r=-0.455$ ,  $-0.607$ , and  $-0.589$ , respectively) with growth. Correlations with dissolved inorganic nutrients did not increase when a one month lag was introduced to the nutrient data (i.e. growth versus the previous month's dissolved nutrient

concentrations). Plant tissue carbon, nitrogen, and phosphorus were all negatively correlated with growth, while ash content was positively correlated with growth rate (Table 1-III). Relationships between plant tissue chemistry and dissolved nutrients will be addressed in Part 2.

A multiple correlation model (Table 1-IV) was constructed for growth using the factors listed in Table 1-III. Factors were added to the model such that each partial F-value was significant at  $p < 0.05$ . Thus, temperature and dissolved  $\text{PO}_4^{3-}$ -P accounted for 96.3% of the variance in the seasonal growth data with no significant contribution from any other factor in Table 1-III or respective interaction terms.

## 1.5 DISCUSSION

In contrast to other Gracilaria populations that are dominated by loose or entangled individuals (Taylor 1975, N. Bird 1976, C. Bird et al. 1977b, Goldstein 1981), G. tikvahiae within the Great Bay Estuary (New Hampshire) occurs primarily as attached plants. The rapid currents in the Estuary (Swenson et al. 1977) may not allow development of extensive, unattached G. tikvahiae populations. Similarly, although certain G. tikvahiae populations in the Canadian Maritimes are entangled in Mytilus edulis byssi, no similar association occurs in the Great Bay Estuary.

While vegetative Gracilaria tikvahiae plants predominated for most of the year in the Great Bay Estuary, there were distinct reproductive maxima. Tetrasporophytes were dominant in June-July of both years. Similarly, cystocarpic plants were at a maximum in June-August. Although not observed until the second year of the study, spermatangial plants were most common during June-July (1977). Cystocarpic and tetrasporic plants had approximately equal biomass, while lesser amounts of male plants were observed. Such information provides putative evidence that G. tikvahiae within the Great Bay Estuary has a classical Polysiphonia-type life history, as described in

vitro (N. Bird et al. 1977). However, the reduced amounts of spermatangial versus cystocarpic plants in the present investigation suggest a deviation from a 1:1 female:male ratio.

Reproductive patterns similar to those in the Great Bay Estuary have been observed in an attached population of Gracilaria tikvahiae from Barrachois Harbour, Nova Scotia (N. Bird 1975, 1976). In contrast, several unattached populations of the same species in the Canadian Maritimes have a predominance of tetrasporophytes, with reduced levels of gametophytes (N. Bird 1976, C. Bird et al. 1977b). The dominance of tetrasporophytes has been attributed to a greater longevity of diploid than gametophytic plants in the detached state (C. Bird et al. 1977b). Gracilaria verrucosa in the Menai Straits (U.K.) has a reproductive cycle (Jones 1959a) comparable to G. tikvahiae in the Great Bay Estuary. In Ceylon G. verrucosa has a similar reproductive cycle to G. verrucosa in the Menai Straits (Durairatnam 1965). Reproductive plants of G. verrucosa occur throughout the year in Manila Bay, with reduced numbers of spermatangial relative to cystocarpic and tetrasporic plants (Trono and Azanza-Corrales 1981). Isaac (1956) found populations of G. verrucosa (as G. confervoides) in South Africa that were reproductive throughout the year, with more cystocarpic than tetrasporic plants, and no male plants recorded. In contrast G. edulis, G. foliifera and G. corticata from India had a predominance of tetrasporic relative to gametophytic

plants (Umamaheswara Rao 1973, 1975).

As with G. tikvahiae, both G. verrucosa and G. foliifera follow a Polysiphonia-type life history in culture (Ogata et al. 1972, McLachlan and Edelman 1977, C. Bird et al. 1982). Hoyle (1978) observed that G. coronopifolia and G. bursapastoris were reproductive year-round in Hawaii, the former having significantly more male than female plants and both species having greater numbers of tetrasporophytes than gametophytes. Several populations of Gracilaria in British Columbia (Saunders and Lindsay 1979, Bunting et al. 1980, Whyte et al. 1981) had variable reproductive phenologies. Specifically, attached populations of Gracilaria (verrucosa type) produced all three reproductive phases but tetrasporic plants were most abundant (Saunders and Lindsay 1979, Whyte et al. 1981). In contrast, intertidal beds of Gracilaria sp., without holdfasts and entangled in Mytilus edulis byssi, lacked gametophytes and had a spring maxima of tetrasporophytes (Saunders and Lindsay 1979). The taxon formerly designated as G. verrucosa in British Columbia differs in chromosome number from plants of this species collected at the type locality in Great Britain (C. Bird et al. 1982). Thus, references to G. verrucosa in British Columbia should not be equated with the taxon in Great Britain.

As outlined above, Gracilaria may have a wide range of in situ life history strategies, depending upon the taxon and specific habitat characteristics. The variations from in vitro theoretical life histories (Ogata et al. 1972, N. Bird et al. 1977, C. Bird et al. 1982, McLachlan and Edelstein 1977) are similar to those of other red algae throughout their ranges (Dixon 1973). Reports of the predominance of tetrasporophytes or the limited occurrence of spermatangial plants may reflect very specific microhabitat requirements of each reproductive phase (Mathieson and Burns 1975, Norall et al. 1981), differences in the longevity of the observable reproductive structure (Kapraun 1978), or the difficulty of identifying male plants (Ngan and Price 1980). In general unattached populations of Gracilaria, including G. tikvahiae, have reproductive patterns characterized by the absence of a particular phase(s) or at least a pronounced inequality of phases (Causey et al. 1946, Stokke 1957, Edwards and Kapraun 1973, C. Bird et al. 1977b, Saunders and Lindsay 1979). In contrast, attached populations such as those in the Great Bay Estuary seem to conform more closely to a Polysiphonia-type life history in situ.

The seasonal growth of Gracilaria tikvahiae in the Great Bay Estuary is limited to May-September. In contrast, the plant's growth is restricted to three months in Barrachois Harbour, Nova Scotia, with senescence in late August (N. Bird 1976). The maximum growth rate of tethered



plants at -1.0 m in the present study was 11%/day in June-July (avg. 7.5%). Although comparisons between single plant measurements and mass culture growth rates are difficult, due to differences in plant density, it is apparent (Table 1-V) that the in situ growth rates of G. tikvahiae in the Great Bay Estuary are comparable to those recorded under various aquaculture regimes.

Gracilaria tikvahiae in New England and the Canadian Maritimes appears to be restricted to relatively shallow embayments where summer temperatures are sufficiently high to support growth (i.e. greater than 15°C). Such a distribution leads to a coincidence of maximum growth/standing crop with seasonal maxima of temperature and irradiance (Conover 1958, N. Bird 1975, C. Bird et al. 1977a). Stokke (1957) reported that G. verrucosa populations in Norway were absent from open coastal locations and restricted to protected warm embayments. Kim and Humm (1965) and Causey et al. (1946) reported that the growth of G. foliifera and G. verrucosa was limited to warm water periods. Similarly Rosenberg and Ramus (1981, 1982) measured maximum growth of G. foliifera in North Carolina during periods of maximum temperature and irradiance.

The growth of Gracilaria tikvahiae in New Hampshire was highly correlated with water temperature. In culture the growth of G. tikvahiae is limited to temperatures greater than 12°C (Edelstein et al. 1976, N. Bird et al. 1979).

However, results from the present study indicate that growth can occur at slightly lower temperatures (i.e.  $10^{\circ}\text{C}$ ), at least within the Great Bay Estuary. Growth was less correlated with surface irradiance than water temperature. However, the use of surface irradiance may not be representative of in situ values for a turbid estuary. Lindsay and Saunders (1980) and Whyte et al. (1981) have shown that the growth/standing crop of Gracilaria in British Columbia is correlated with irradiance rather than seawater temperature. Similarly, Hansen (1977) found a positive correlation between growth and irradiance for Iridaea cordata, but no relation with ambient temperature, dissolved nitrogen nor phosphate. LaPointe et al. (1976) found no correlation between either temperature or irradiance and growth with Gracilaria in a flow-through aquaculture system. LaPointe (1981) also found no correlation between dissolved inorganic nitrogen and growth of cultured G. foliifera, but there was a strong correlation with irradiance. However, as the cultures were maintained at  $25^{\circ}\text{C}$  in these experiments, the temperature may have been suboptimal.

As would be expected for a euryhaline plant such as Gracilaria tikvahiae, little correlation was apparent between salinity and growth (Table 1-III). Because G. tikvahiae populations in the Great Bay Estuary are perennial, the plants must tolerate annual salinity variations from 8 g/kg to 32 g/kg. N. Bird et al. (1979) found the growth of G. tikvahiae in culture was greatest at

20 g/kg to 40 g/kg. Although G. tikvahiae in the Great Bay Estuary tolerates low salinities, most growth occurs (primarily due to temperature limitations) during periods of higher salinities (i.e. 25 g/kg to 32 g/kg). In contrast G. verrucosa tends to be less tolerant of low salinities, while G. foliifera is more euryhaline (Kim and Humm 1965). Although LaPointe (1981) found a relatively strong negative correlation between growth and ash content of G. foliifera (i.e.  $r=-0.85$ ), a positive correlation of similar magnitude was found in the present study between in situ growth of G. tikvahiae and ash content (Table 1-III).

In the present study, the seasonal growth cycles of Gracilaria tikvahiae appeared to be unrelated to the cycle of dissolved inorganic nitrogen and plant tissue nitrogen, as indicated by the negative correlations of these factors (Table 1-III). The growth of G. tikvahiae (as G. foliifera) in flow-through cultures was saturated at 1.0-1.5  $\mu\text{M}$  dissolved inorganic nitrogen (DeBoer et al. 1978). In the present growth study, ambient total dissolved inorganic nitrogen declined below this concentration only during July, August and November 1978. As temperature and light may limit growth in November, the only period when nitrogen may have been limiting was during July-August 1978. Consideration of nitrogen limitation strictly in terms of ambient concentrations is simplistic, as water motion can act to enhance nutrient availability at suboptimal concentrations (Conover 1968, Gerard and Mann 1979, Parker

1981, 1982, Gerard 1982c). Given the rapid tidal currents in the Great Bay Estuary (Swenson et al. 1977), nitrogen depletion probably would not occur within the Gracilaria beds below concentrations detected in the water column. As demonstrated by Ryther et al. (1981), Gracilaria has the capacity to rapidly assimilate and store quantities of nitrogen sufficient to support growth for two weeks in nutrient-depleted water. The latter phenomenon may contribute to the lack of correlation between growth and water nitrogen concentrations (Gerard 1982b, LaPointe and Tenore 1981).

LaPointe and Ryther (1979) have shown that Gracilaria tikvahiae tissue carbon/nitrogen (weight) >10 indicated nitrogen-limited growth. As C/N ratios of natural populations of G. tikvahiae show substantial seasonal variation (see Part 2), the specific values given by LaPointe and Ryther (1979) for cultured material probably have little direct application to in situ plants. However, in the present growth study, C/N increased to >10 only during July-August, and C/N did not correlate strongly with growth. LaPointe and Tenore (1981) stated that tissue C/N values will be related to growth only when nitrogen contents are growth-limiting. Finally, the lack of correlation with water nitrogen and growth may be a reflection of the inadequacy of a monthly measurement of dissolved nitrogen reflecting the dynamic nutrient conditions found in an estuary. However, this sampling strategy would have no

bearing on tissue nitrogen versus growth relationships.

Hanisak (1982) found that 2% was the critical internal nitrogen concentration for Floridian Gracilaria tikvahiae (i.e. the growth of plants with less than 2% nitrogen would be nitrogen-limited). At no time did the nitrogen content of G. tikvahiae plants from the Great Bay Estuary decline below 2%. Thus, it would appear that G. tikvahiae within the Great Bay Estuary is rarely limited by ambient nitrogen availability. Rosenberg and Ramus (1981, 1982) reported growth rates of G. foliifera in a flow-through culture system were correlated with dissolved inorganic nitrogen. However, the plants used were from the intertidal zone but examined in a continuously submerged state. In addition "ambient" nitrogen concentrations in their culture tanks were substantially higher than corresponding in situ levels, due to ammonium enrichment from animals colonizing the seawater pipes. The maximum total plant nitrogen measured by Rosenberg and Ramus (1982) was only one-half of that found in the present study (i.e. 1.4% vs. 2.8%-4.2%).

Single factor (i.e. simple) correlations inferred a strong positive relationship between temperature and growth. Further development of this bivariate correlation gave a multiple correlation model including the factors temperature and dissolved reactive phosphate (Table 1-IV). No other factor (of those listed in Table 1-III) added significantly to the multiple correlation model. The model therefore

reflects a high dependency of in situ growth on temperature, as well as the temporal uncoupling of growth from other factors such as surface irradiance and dissolved inorganic nitrogen. The inclusion of dissolved phosphate in this model is of interest. Limitation of marine algal growth is generally attributed to nitrogen rather than phosphorus, particularly in open ocean and coastal areas (Ryther and Dunstan 1971, Topinka and Robbins 1976, Chapman and Craigie 1977, Hanisak 1979a, 1979b, 1982, Chapman and Lindley 1980, Gagne and Mann 1981, DeBoer 1981, Rosenberg and Ramus 1982, Gerard 1982a, 1982b). However inner estuarine sites may have phosphorus or nitrogen limitation depending upon season or individual runoff events (Wallentinus 1979, Anderson et al. 1981). Absolute amounts of dissolved nitrogen and phosphorus as well as ambient N/P must be known (Waite and Mitchell 1972, Kautsky 1982).

While dissolved inorganic nitrogen concentrations within the Great Bay Estuary were higher than in many other coastal areas where macroalgal nutrient limitation has been studied (Chapman and Craigie 1977, Asare 1979, Chapman and Lindley 1980, Gagne and Mann 1981, Gerard 1982a, 1982b), large fluctuations occurred during the study period. Phosphate concentration was relatively stable, and changes in available N/P (water) were primarily due to differences in nitrogen concentration. The inclusion of dissolved phosphate in the multiple correlation model suggests a need for further study of growth-limiting nutrients in estuarine

areas. Harlin and Thorne-Miller (1981) working with Gracilaria tikvahiae in Ninigret Pond (Rhode Island) found the greatest increase in standing crop after in situ phosphate enrichment, relative to nitrate or ammonium additions. However, none of their standing crop changes were statistically greater than in the control area. Similarly, Cladophora glomerata in the Baltic Sea was phosphorus-limited in some seasons (Wallentinus 1979).

While phosphorus may limit macroalgal growth in some estuarine/brackish habitats, comparisons are restricted due to site-specific nutrient variations and species-specific responses to nutrient concentrations (Prince 1974, Harlin and Thorne-Miller 1981, Kornfeldt 1982). Furthermore, it is difficult to relate growth limitation in the present study to dissolved inorganic phosphate concentrations because of the lack of strong seasonal variation in dissolved phosphate, little seasonal variation in plant phosphorus content, and a low correlation between dissolved phosphate and plant phosphorus. Although most emphasis has been placed on nitrogen limitation of marine macroalgal growth, it would seem valuable for a further evaluation of the interactions between ambient dissolved nitrogen and phosphorus, growth, and plant tissue chemistry, particularly of estuarine species.

## 1.6 SUMMARY

- 1). Gracilaria tikvahiae in the Great Bay Estuary displayed discrete summer reproductive maxima for cystocarpic, spermatangial and tetrasporic stages.
- 2). Cystocarpic plants occurred in slightly greater amounts than did spermatangial plants during the reproductive period.
- 3). Growth of Gracilaria tikvahiae was limited to warm water periods in the Great Bay Estuary (i.e. May - September).
- 4). Growth rates of Gracilaria tikvahiae in the Great Bay Estuary correlated most highly with water temperature and dissolved inorganic phosphate via a multiple correlation model.
- 5). Growth of Gracilaria tikvahiae was unrelated to seasonal variations in dissolved inorganic nitrogen. Ambient nitrogen concentrations rarely limit growth of G. tikvahiae in the Great Bay Estuary.



Table 1-I. Regression (periodic model) analyses of variance tables for average annual cycles of hydrographic and water nutrient parameters in the Great Bay Estuary.

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a) Temperature

source	df	MS	F
periodic regression	2	12795.68	2369.6 ***
residual	429	5.40	

$R^2=91.7$

b) Salinity

source	df	MS	F
periodic regression	2	4119.88	165.3 ***
residual	421	24.93	

$R^2=44.0$

c)  $\text{NH}_4^+-\text{N}$

source	df	MS	F
periodic regression	2	317.88	93.9 ***
residual	143	3.39	

$R^2=56.8$

Table 1-I. (continued.)

d)  $(\text{NO}_3^- + \text{NO}_2^-) - \text{N}$ 

source	df	MS	F
periodic regression	2	894.90	198.2 ***
residual	144	4.52	
$R^2 = 73.3$			

e) Total dissolved inorganic nitrogen

source	df	MS	F
periodic regression	2	2202.16	247.9 ***
residual	146	8.90	
$R^2 = 77.2$			

f)  $\text{PO}_4^{3-} - \text{P}$ 

source	df	MS	F
periodic regression	2	6.89	38.2 ***
residual	146	0.18	
$R^2 = 34.3$			

\*\*\*  $p \leq 0.001$

Table 1-II. Regression (periodic model) analysis of variance table of growth rates (arcsine transformed) at Adams Point, April 1978 to October 1978.

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source	df	MS	F
periodic	2	1809.1	373.5 ***
treatment	2	396.7	81.9 ***
interaction	4	10.0	2.1 ns
residual	312	4.84	

$R^2=74.7$

\*\*\*  $p < 0.001$   
 ns  $p > 0.05$

Table 1-III. Correlations of growth (arcsine transformed) with environmental parameters and corresponding plant tissue chemistry.

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Factor	r	r <sup>2</sup> (x100)	p
Temperature	0.956	91.4	0.000
Salinity	0.584	34.0	0.009
Irradiance	0.761	58.0	0.000
(NH <sub>4</sub> <sup>+</sup> )-N	-0.455	20.7	0.044
(NO <sub>3</sub> <sup>-</sup> +NO <sub>2</sub> <sup>-</sup> )-N	-0.607	36.8	0.008
DIN	-0.589	34.6	0.010
PO <sub>4</sub> <sup>3-</sup> -P	0.315	9.9	0.126
Plant-C	-0.854	72.9	0.000
Plant-N	-0.840	70.6	0.000
Plant-P	-0.733	53.7	0.001
Plant-C/N	0.729	53.1	0.001
Plant-Ash	0.910	82.9	0.000

(Factors are: temperature and salinity - water; irradiance - surface irradiance; NH<sub>4</sub><sup>+</sup>-N, (NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>)-N, PO<sub>4</sub><sup>3-</sup>-P - water; DIN - total dissolved inorganic nitrogen; plant-C, plant-N, plant-P, plant-ash - % composition of C, N, P and ash as dry weight; plant-C/N - weight ratio.)

Table 1-IV. Multiple correlation model of growth (arcsine transformed) with environmental parameters and corresponding plant tissue chemistry.

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Step	Factors	R	$R^2$ (x100)
1	Temperature	0.956	91.4
2	Temperature $PO_4^{3--P}$	0.981	96.3

(Factors were added to the model to satisfy  $p < 0.05$  of F of partial SS for each added factor. No other factors listed in Table 1-III, as well as all interactions, increased the model significantly with respect to this criterion. Both models were significant at  $p < 0.001$ .)

Table 1-V. Comparative growth rates for Gracilaria species both in situ and in culture.

Species	Location	%/day	Source
<u>G. tikvahiae</u>	Hill River, P.E.I.	5	Taylor 1975
<u>G. tikvahiae</u>	Pomquet Harbour, N.S.	7.1	C. Bird <u>et al.</u> 1977a
<u>G. tikvahiae</u>	Ninigret Pond, R.I.	2.9	Asare 1979
<u>G. tikvahiae</u>	culture, N.S.	16.5	N. Bird <u>et al.</u> 1979
<u>G. tikvahiae</u>	culture, N.S.	14	Edelstein <u>et al.</u> 1976
<u>G. tikvahiae</u>	culture, N.S.	24	N. Bird 1975
<u>G. "chorda"</u>	culture, B.C.	3.0	Bunting <u>et al.</u> 1980
<u>G. foliifera</u>	culture, N.C.	5	Rosenberg & Ramus 1982
<u>G. foliifera</u>	culture, Ireland	13.7	Guiry & Ottway 1981
<u>G. "foliifera"</u>	culture, W.H.O.I.	14	DeBoer <u>et al.</u> 1978
<u>G. "verrucosa"</u>	culture, Israel	7.5	Friedlander & Lipkin 1982
<u>G. "verrucosa"</u>	culture, B.C.	5.8	Saunders & Lindsay 1979
<u>G. verrucosa</u>	Menai Straits, U.K.	10	Jones 1959b
<u>G. bursapastoris</u>	culture, Hi.	2.7	Hoyle 1978
<u>G. coronopifolia</u>	culture, Hi.	1.5	Hoyle 1978
<u>G. bursapastoris</u>	culture, Hi.	8.3	Hunt <u>et al.</u> 1982
<u>G. coronopifolia</u>	culture, Hi.	7.8	Hunt <u>et al.</u> 1982

Figure 1-1. Map of the Great Bay Estuary (New Hampshire-Maine) showing location of collection and growth study sites. Cedar Point (CP), Adams Point (AP), Thomas Point (TP) and Nannie Island (NI).

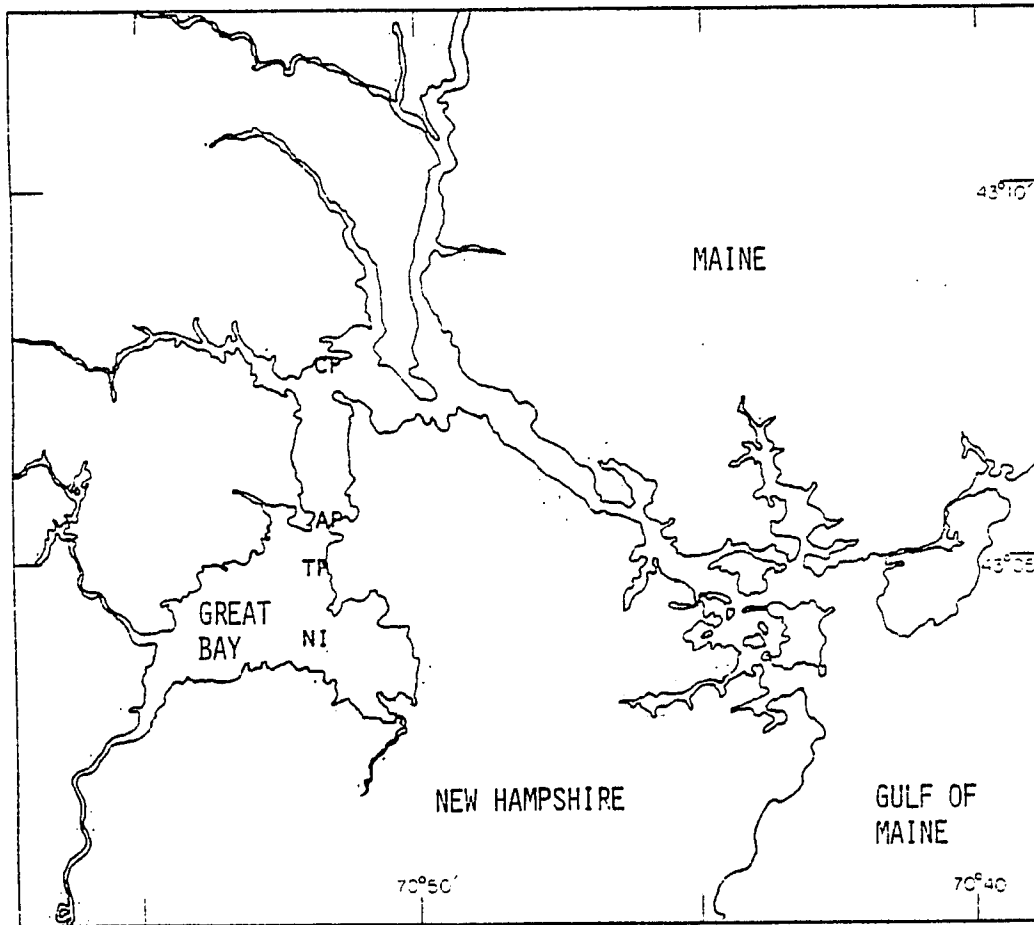


FIGURE 1-1



Figure 1-2. Water temperatures ( $^{\circ}\text{C}$ ) at collection sites from May 1976 to October 1977. a) Cedar Point (octagons), Thomas Point (triangles) and Nannie Island (squares). b) Average (+2 SE) of the three sites.

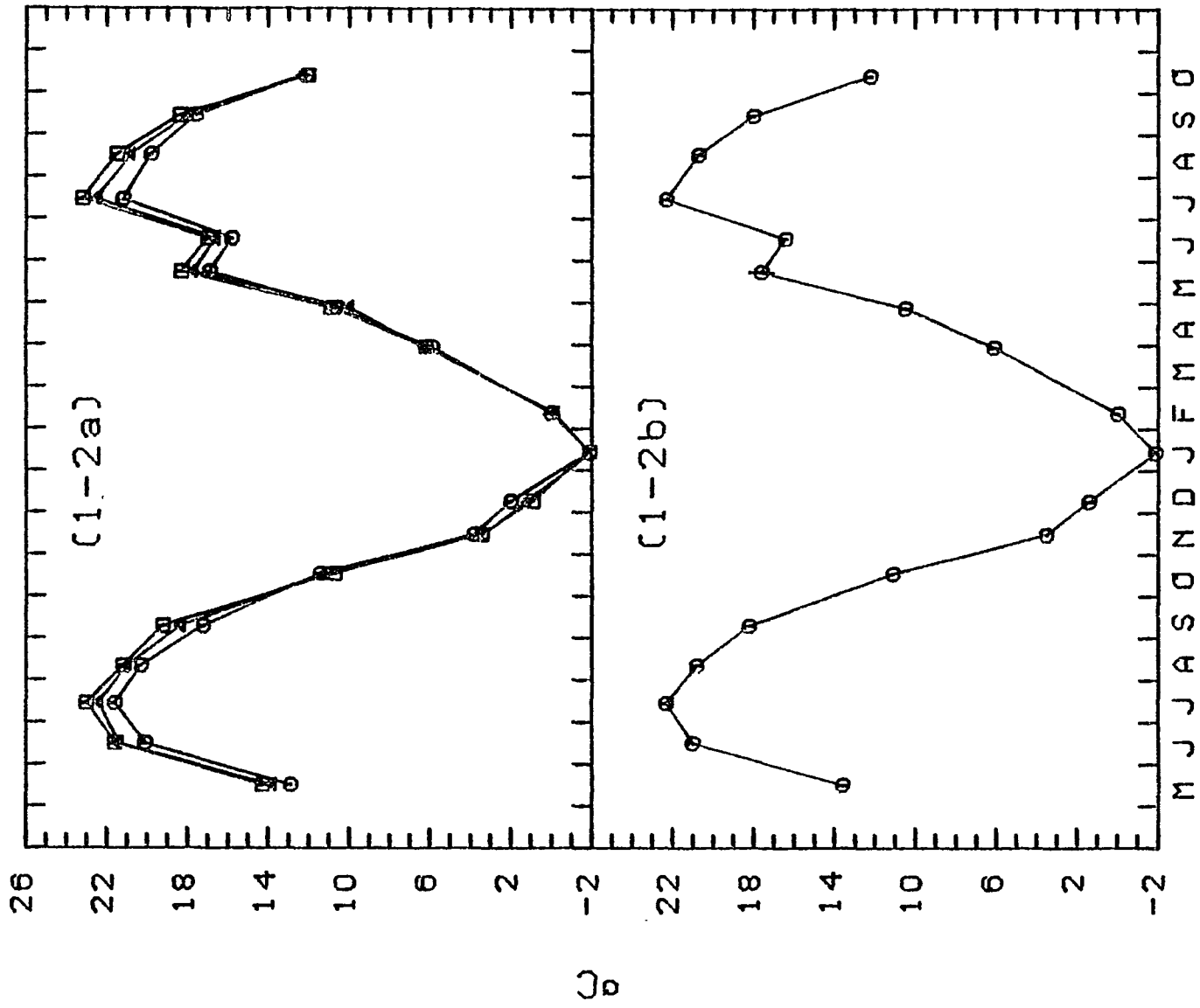


Figure 1-2

Figure 1-3. Salinities (g/kg) at collection sites from May 1976 to October 1977. a) Cedar Point (octagons), Thomas Point (triangles), and Nannie Island (squares). b) Average (+2 SE) of the three sites.

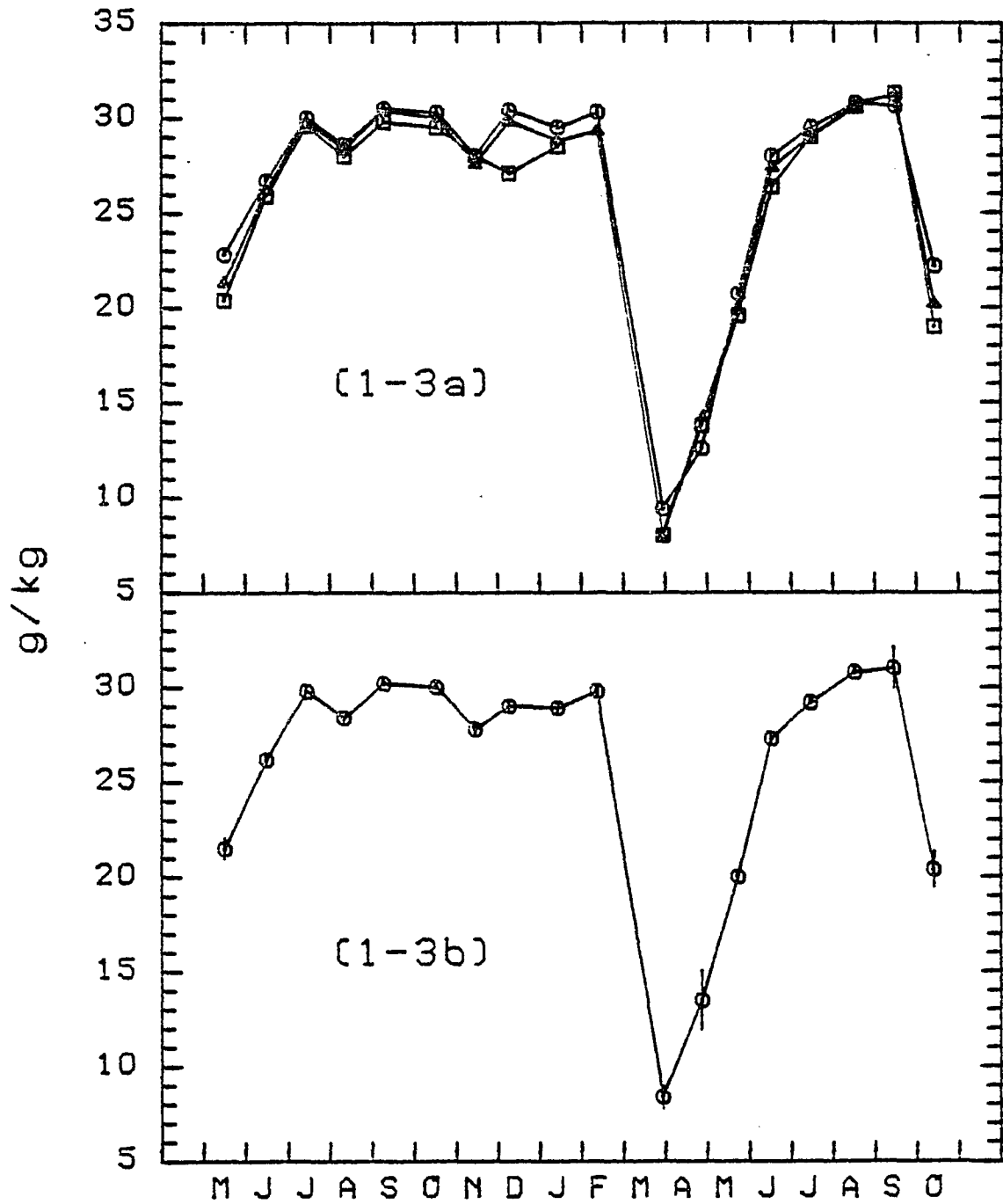


Figure 1-3

Figure 1-4. Water temperatures ( $^{\circ}\text{C}$ ) at Adams Point from April 1978 to August 1979. Error bars indicate +2 SE.

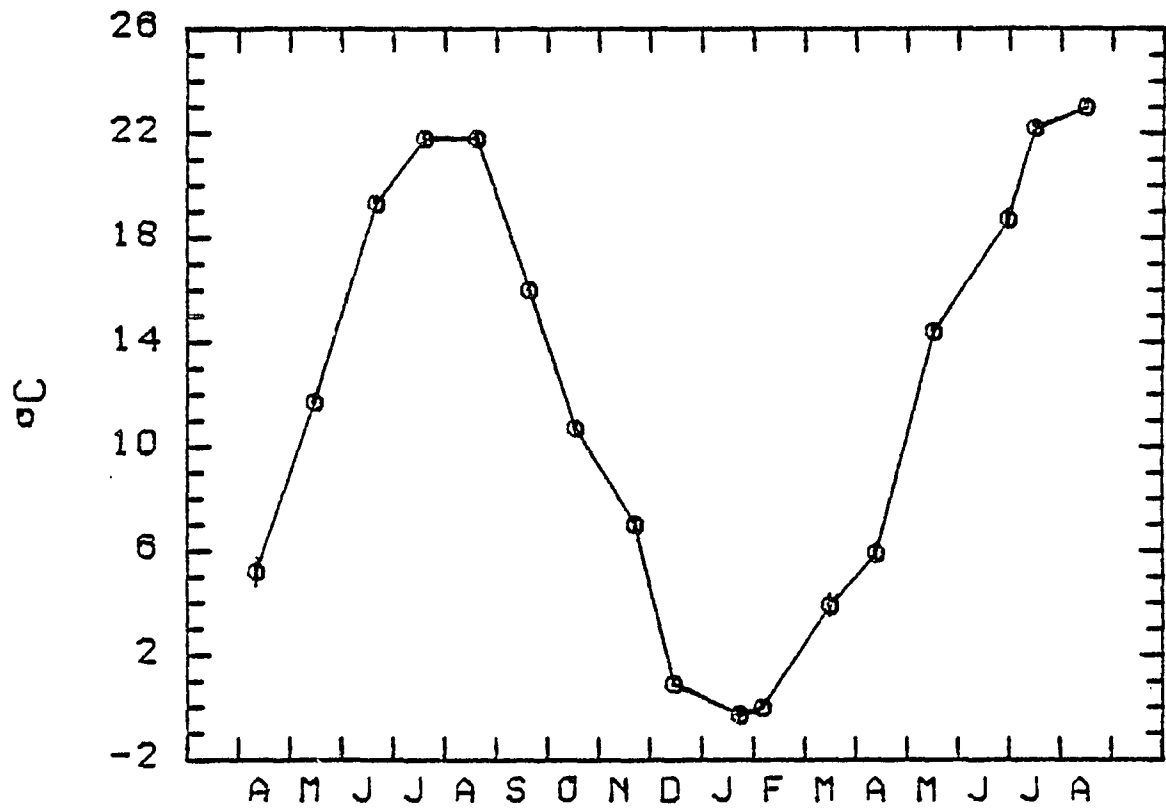


Figure 1-4

Figure 1-5. Salinities (g/kg) at Adams Point from April 1978 to August 1979. Error bars indicate  $\pm 2$  SE.

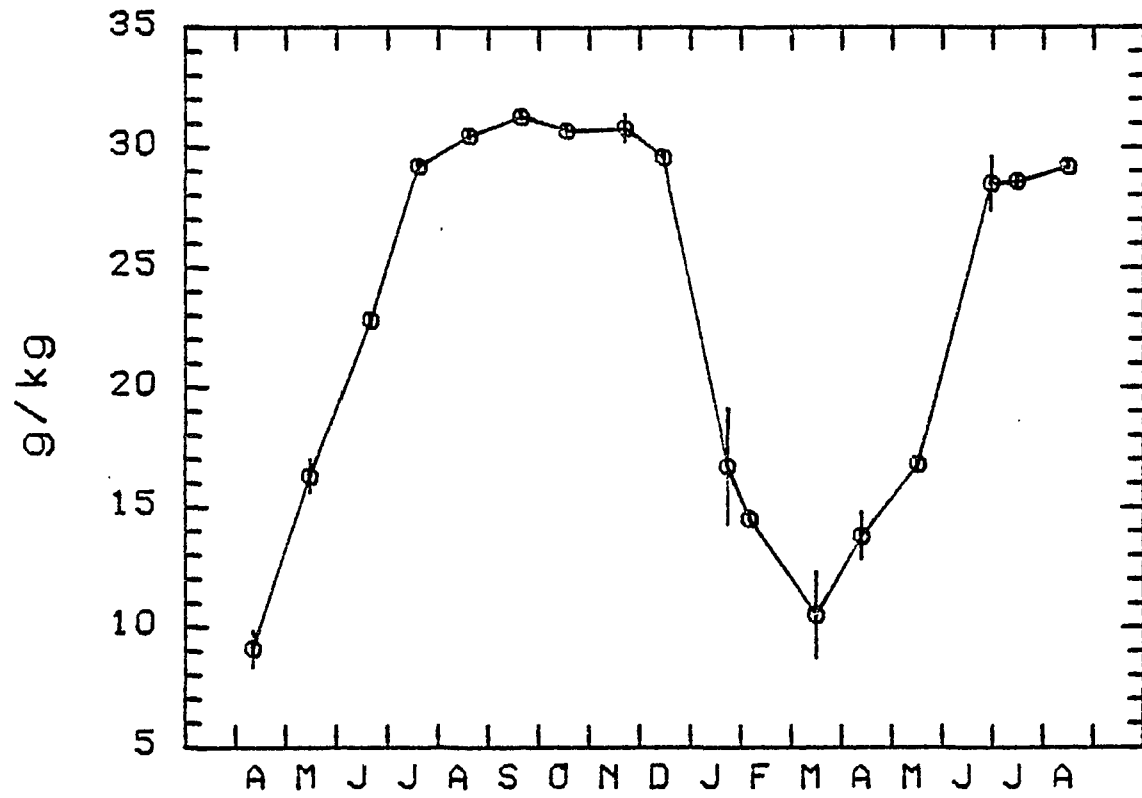


Figure 1-5



Figure 1-6. Dissolved inorganic nitrogen concentrations ( $\mu\text{g-at N/L}$ ) at collection sites from May 1976 to October 1977. a)  $\text{NH}_4^+$ -N and b)  $(\text{NO}_3^- + \text{NO}_2^-)$ -N at Cedar Point (octagons), Thomas Point (triangles) and Nannie Island (squares).

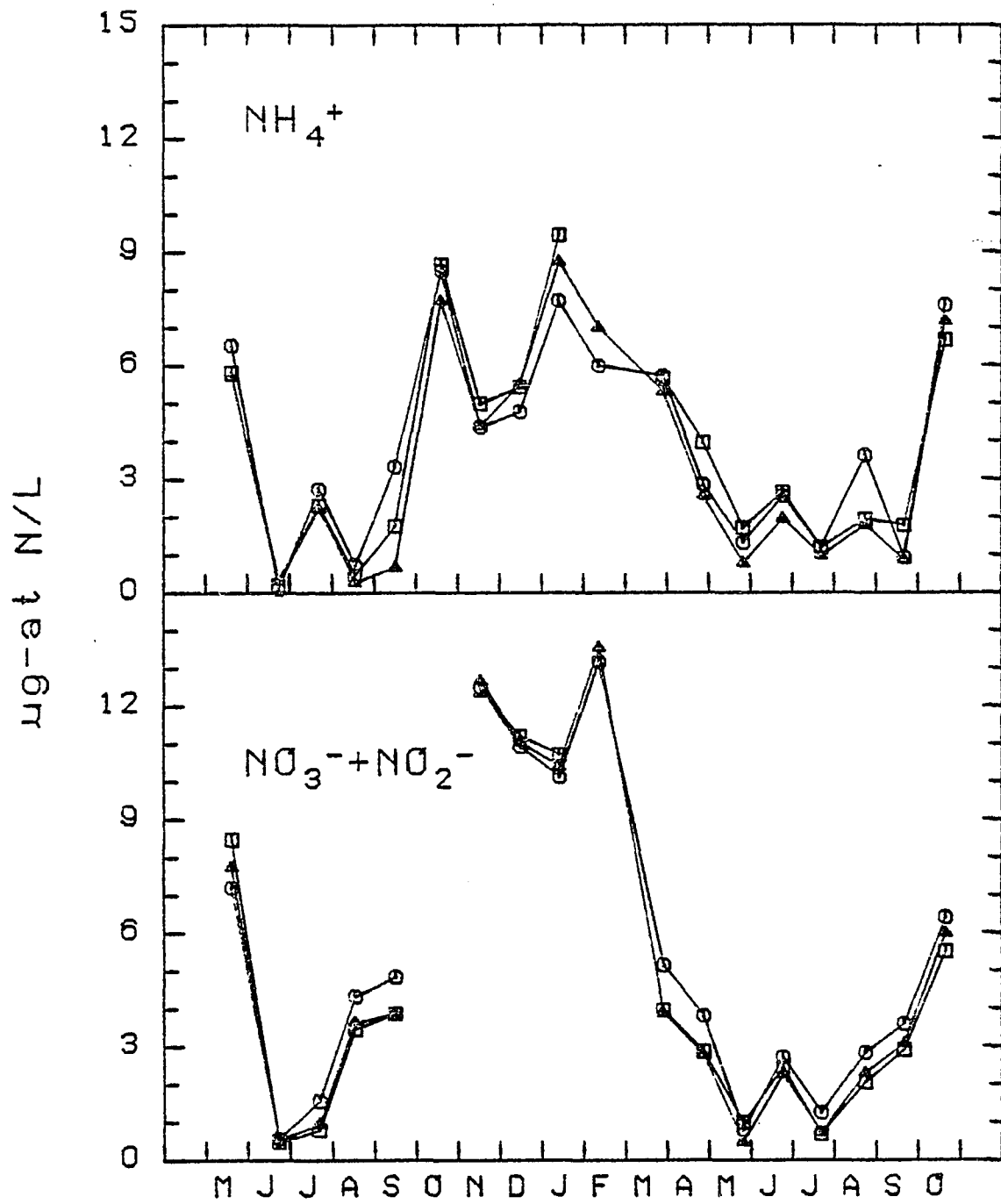


Figure 1-6

Figure 1-7. Average dissolved inorganic nitrogen concentrations ( $\mu\text{g-at N/L}$ ) of the three collection sites from May 1976 to October 1977.  $\text{NH}_4^+\text{-N}$  (octagons),  $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$  (triangles), and total inorganic nitrogen (squares). Error bars indicate  $\pm 2$  SE.

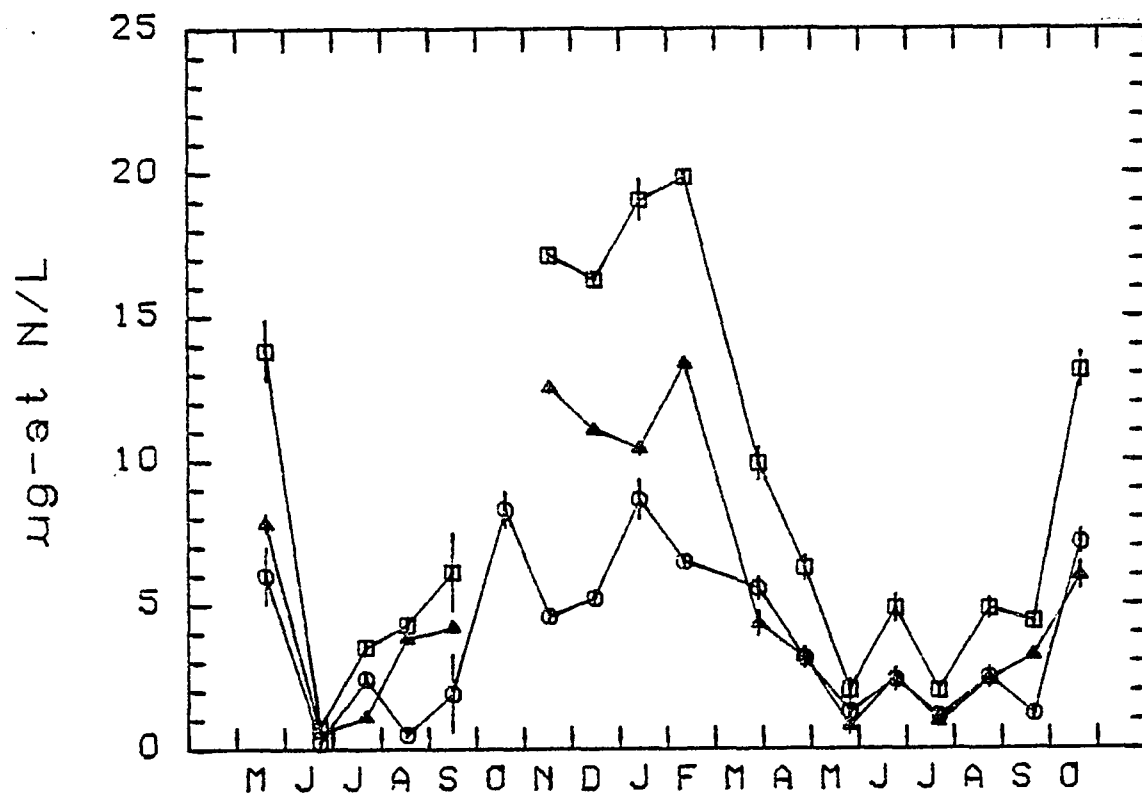


Figure 1-7

Figure 1-8. Dissolved inorganic nitrogen concentrations ( $\mu\text{g-at N/L}$ ) at Adams Point from April 1978 to August 1979.  $\text{NH}_4^+$ -N (octagons),  $(\text{NO}_3^- + \text{NO}_2^-)$ -N (triangles) and total inorganic nitrogen (squares). Error bars indicate +2 SE.

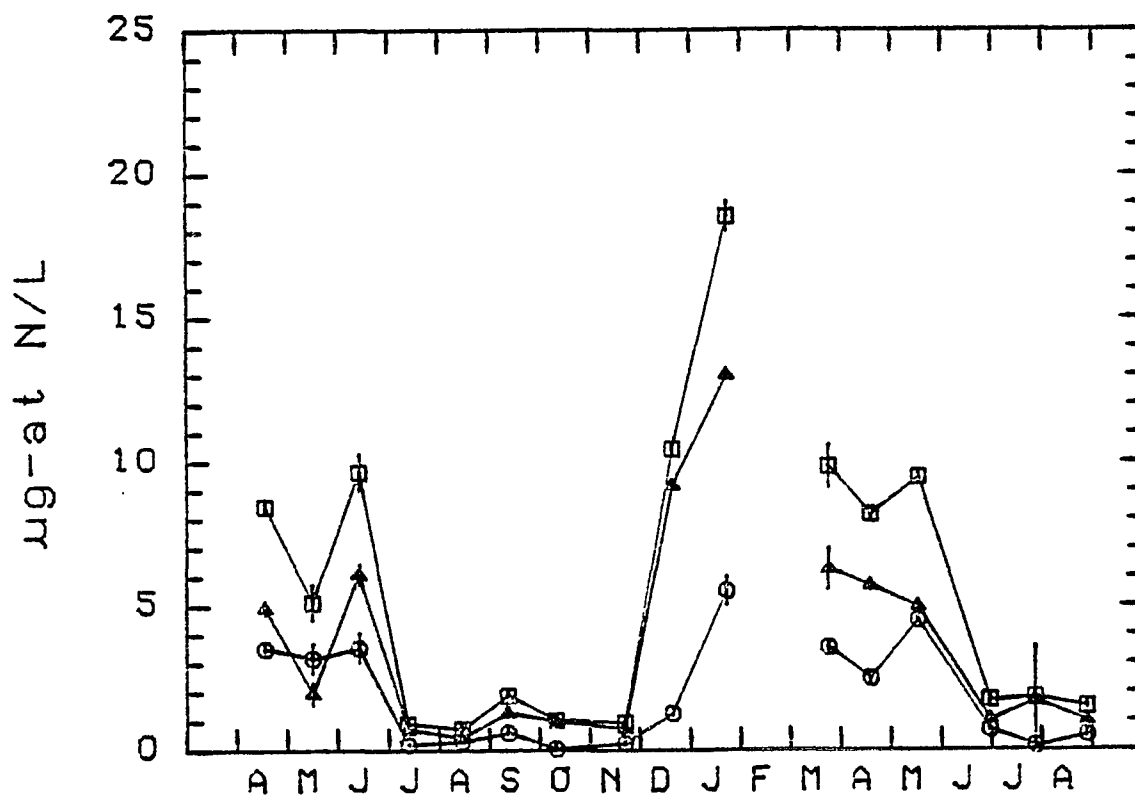


Figure 1-8

Figure 1-9. Dissolved  $\text{PO}_4^{3-}$ -P concentrations ( $\mu\text{g-at P/L}$ ) at collection sites from May 1976 to October 1977. a) Cedar Point (octagons), Thomas Point (triangles) and Nannie Island (squares). b) Average (+2 SE) of the three sites.

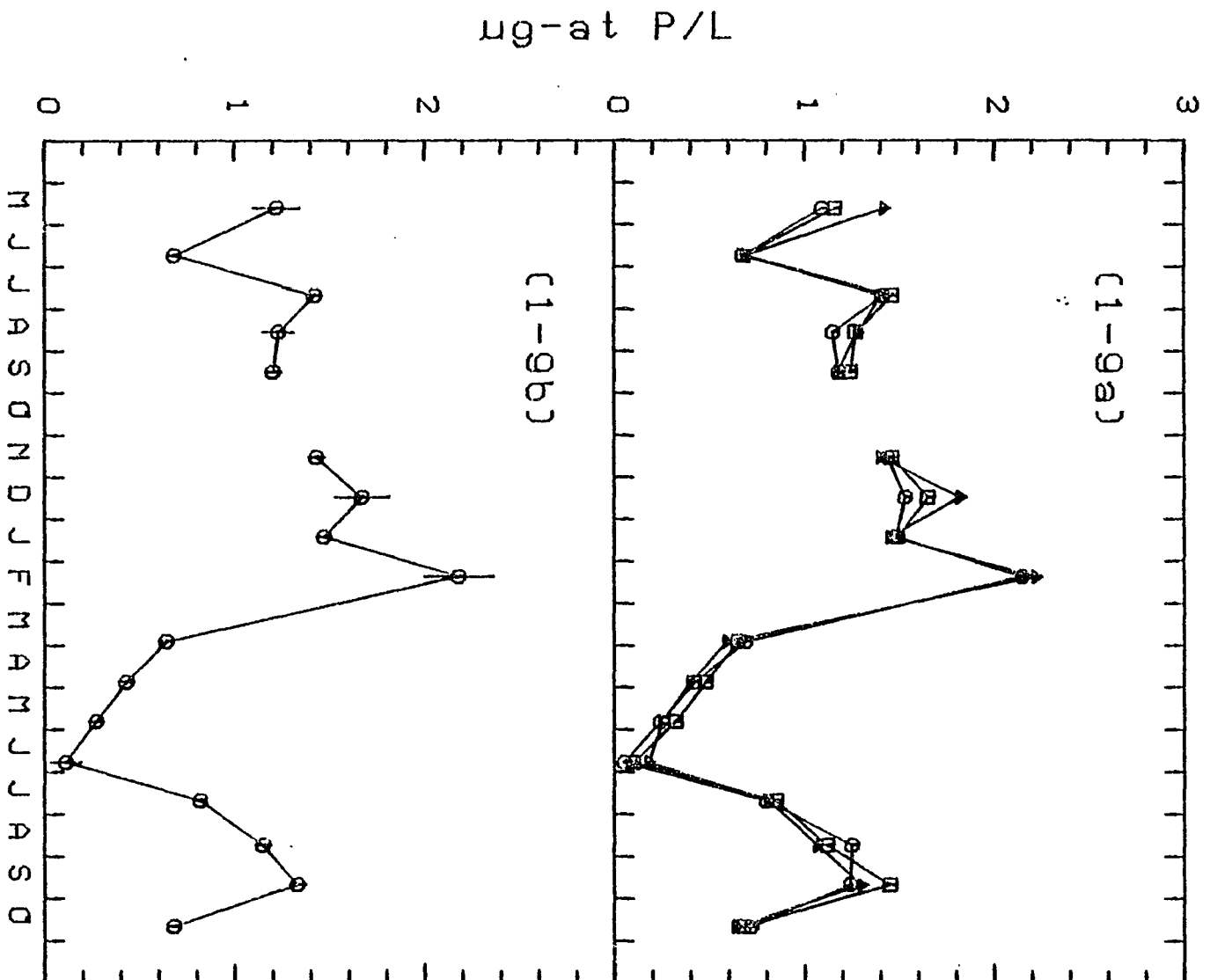


Figure 1-9



Figure 1-10. Dissolved  $\text{PO}_4^{3-}$ -P concentrations ( $\mu\text{g-at P/L}$ ) at Adams Point from April 1978 to August 1979. Error bars indicate +2 SE.

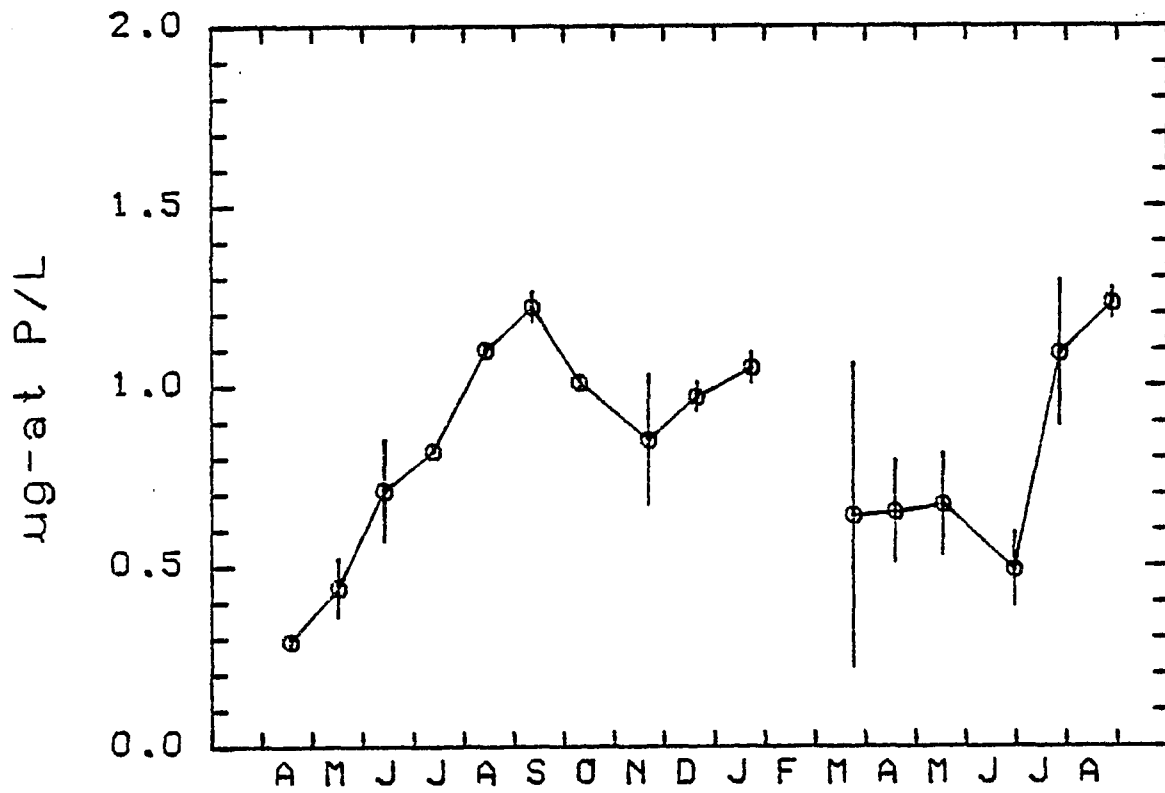


Figure 1-10

Figure 1-11. Total surface irradiance ( $\text{cal}\cdot\text{cm}^{-2}\cdot\text{day}^{-1}$ ) (octagons) and PAR (triangles) at Durham, N.H. from April 1978 to July 1979.

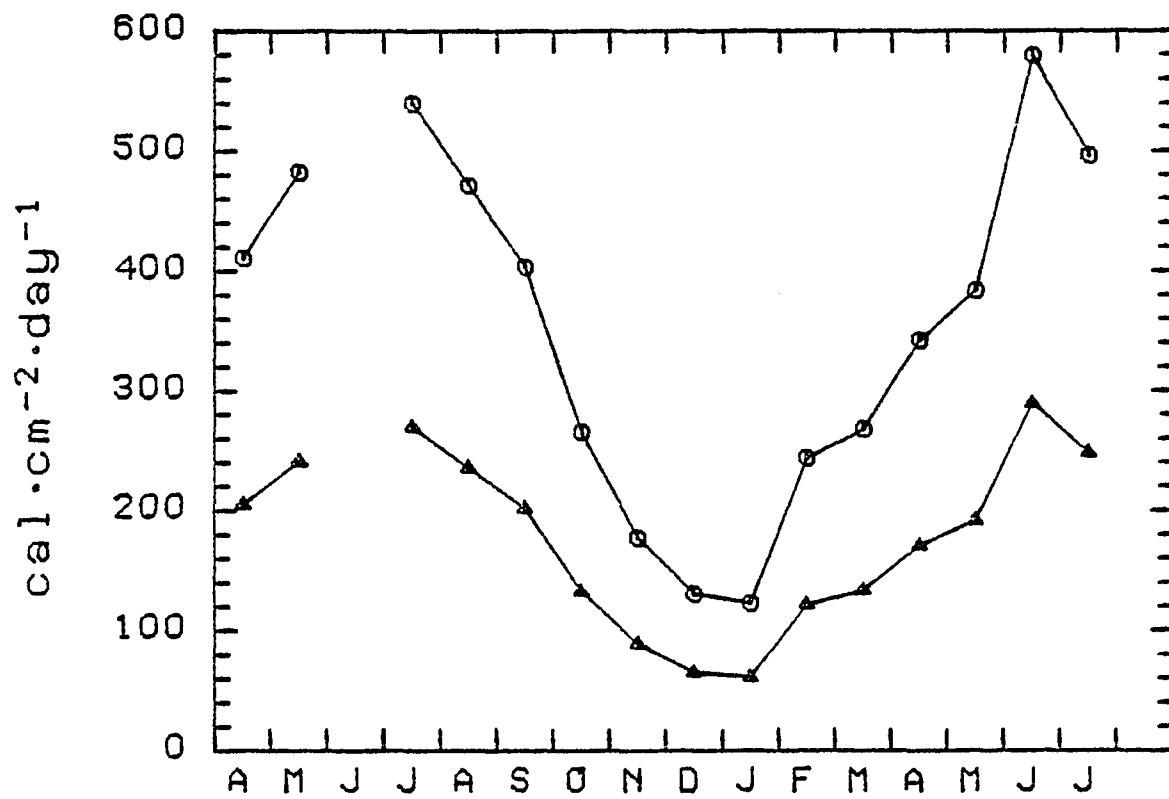


Figure 1-11

Figure 1-12. Reproductive phenology of Gracilaria tikvahiae at Cedar Point from May 1976 October 1977. Percent composition of each stage as percent of total individual collection biomass; vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds) and tetrasporic (squares) plants.

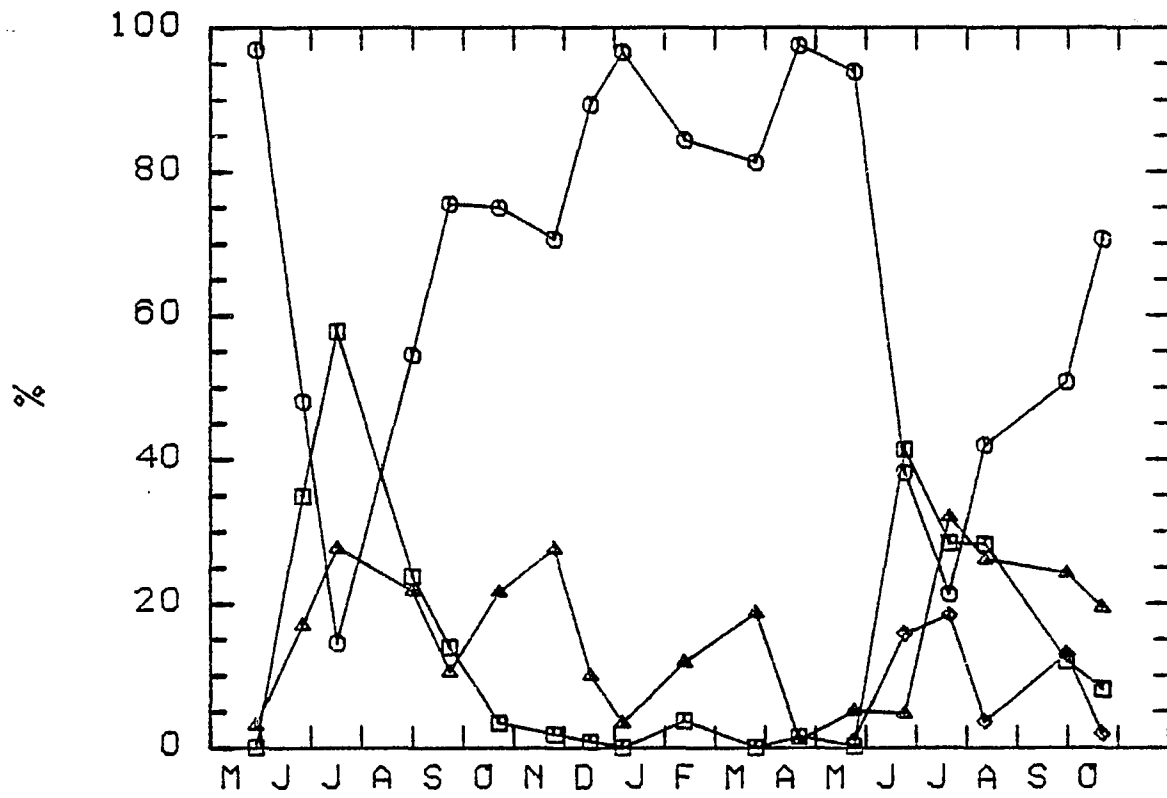


Figure 1-12

Figure 1-13. Reproductive phenology of Gracilaria tikvahiae at Thomas Point from May 1976 to October 1977. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds) and tetrasporic (squares) plants. Percent composition as described for Figure 1-12.

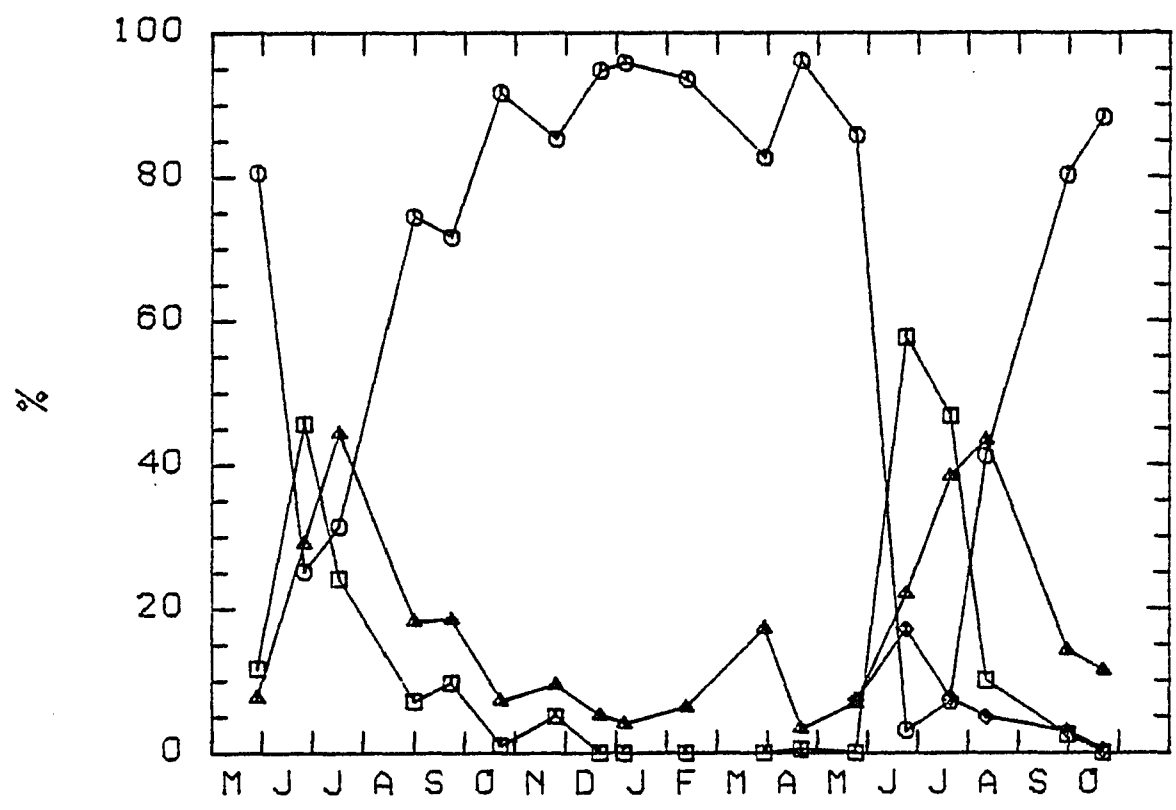


Figure 1-13



Figure 1-14. Reproductive phenology of Gracilaria tikvahiae at Nannie Island from May 1976 to October 1977. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds) and tetrasporic (squares) plants. Percent composition as described for Figure 1-12.

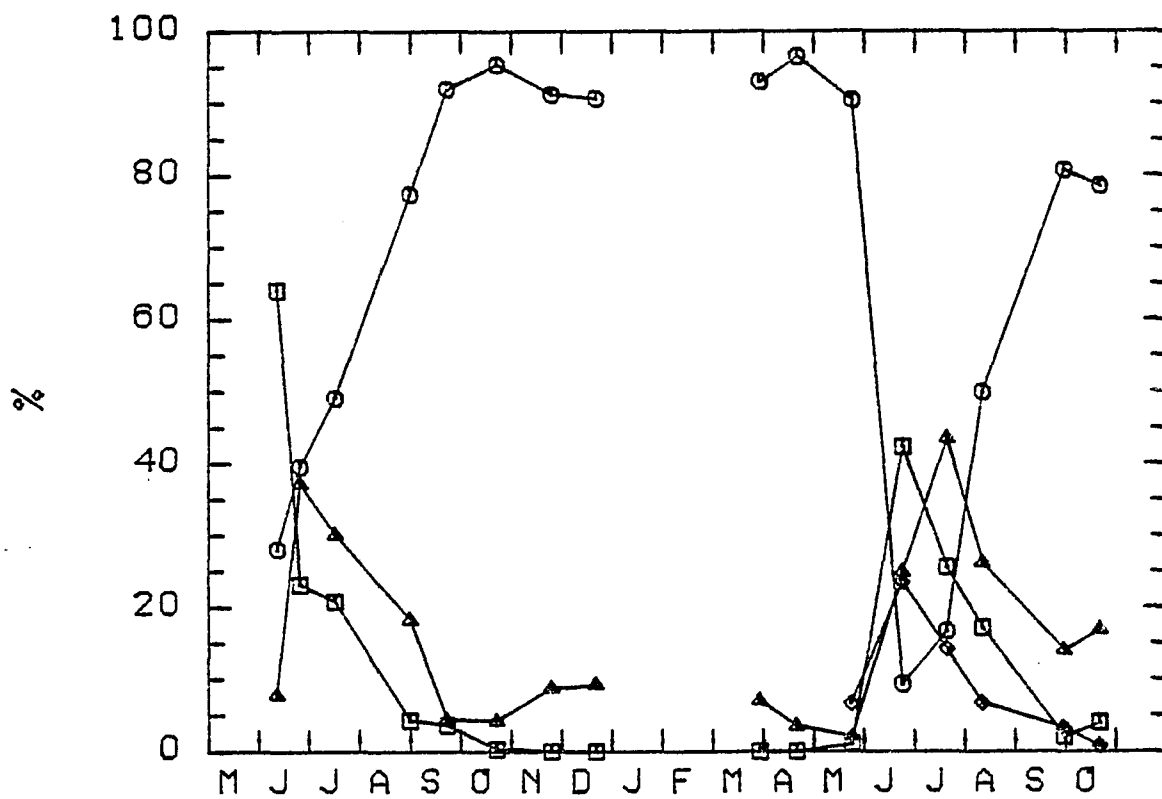


Figure 1-14

Figure 1-15. Average ( $\pm 1$  SE) reproductive phenology of Gracilaria tikvahiae for the three collections sites from May 1976 to October 1977. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds) and tetrasporic (squares) plants. Percent composition as described for Figure 1-12.

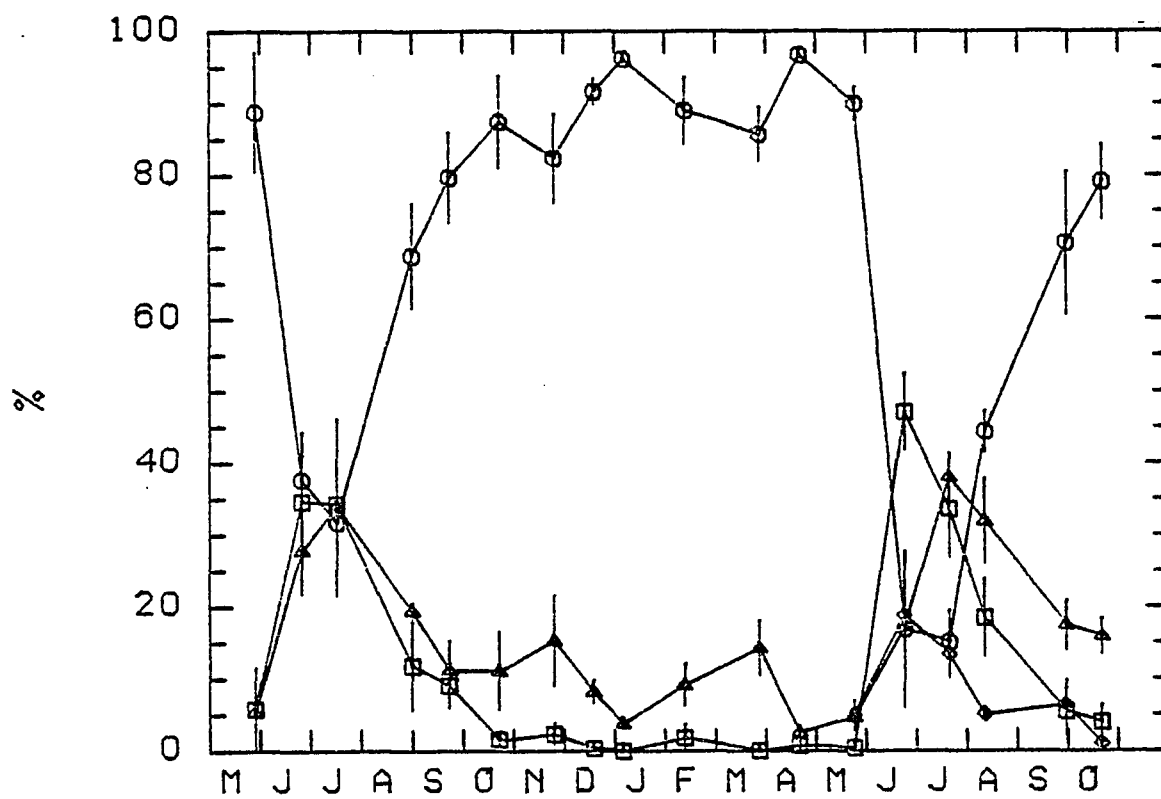


Figure 1-15

Figure 1-16. Growth rates of Gracilaria tikvahiae at Adams Point from April 1978 to August 1979. Tethered plants (squares), plants in net bags tied to blocks (triangles), plants in net bags at -1.0 m (octagons). Growth measured as percent increase in fresh weight per day (mean  $\pm$  1 SE).

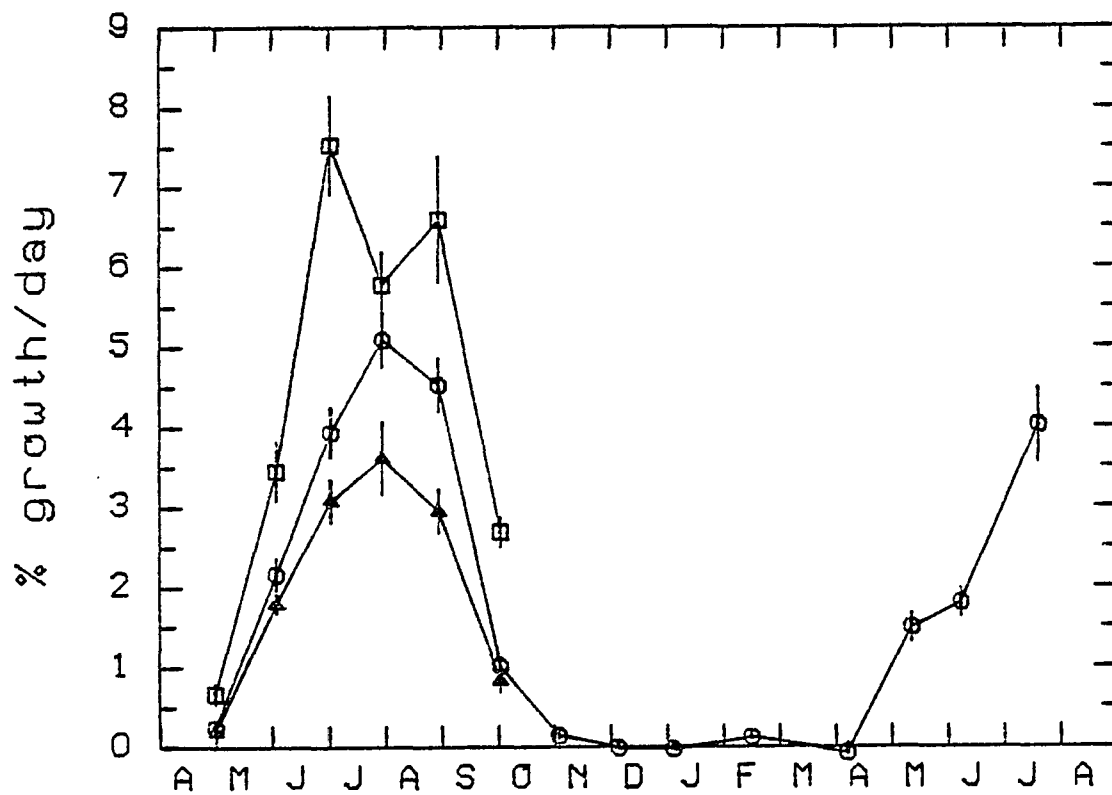


Figure 1-16

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ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN  
(GIGARTINALES, RHODOPHYTA) IN THE  
GREAT BAY ESTUARY, NEW HAMPSHIRE.

PART 2.

VARIATION IN CHEMICAL COMPOSITION.



## 2.1 INTRODUCTION

Recently the mariculture of the red alga Gracilaria tikvahiae McLachlan, and closely related species, have been extensively studied (LaPointe et al. 1976, Whyte and Englar 1976, 1979c, DeBoer and Ryther 1977, Ryther et al. 1979, LaPointe and Ryther 1978, Lindsay and Saunders 1979, 1980, Mathieson 1982), to assess their potential as sources of the phycocolloid, agar, and for methane production (Ryther et al. 1979, Mathieson 1982). In conjunction with these studies, analyses of the chemical composition of the aquacultured populations have been conducted (LaPointe and Ryther 1978, 1979, DeBoer 1979, K. Bird et al. 1981, 1982, LaPointe 1981).

The relations between several chemical components and various environmental factors have been investigated in several aquacultured and in situ populations of Gracilaria (C. Bird et al. 1977, Penniman 1977, Hoyle 1978a, 1978b, DeBoer 1979, K. Bird et al. 1981, Rosenberg and Ramus 1981, 1982a, 1982b). By manipulating temperature, irradiance, or dissolved nutrients, a seaweed aquaculturalist may control the composition (and perhaps the specific properties of individual components) of the seaweed crop (LaPointe and Ryther 1979, LaPointe 1981). There is an inverse

relationship between tissue nitrogen (or protein) and carbon (or carbohydrate) in several aquacultured and natural populations of seaweeds (Butler 1931, 1936, Neish and Shacklock 1971, Dawes et al. 1974, Mathieson and Tveter 1975, 1976, Durako and Dawes 1980, LaPointe 1981). However, exceptions to this correlation have been reported (C. Bird et al. 1977, Penniman 1977).

Recent studies have also demonstrated differences in growth rates (Edelstein 1977, Edelstein et al. 1976) and chemical composition (McCandless et al. 1973, 1975, Hosford and McCandless 1975, Pickmere et al. 1975, Waaland 1975, Doty and Santos 1978, Kim and Henriquez 1979) between haploid and diploid plants of an individual seaweed species. For example, a segregation of carrageenan fractions occurs between cystocarpic and tetrasporic stages of several gigartinalean algae (i.e. kappa-carrageenan in gametophytes and lambda-carrageenan in tetrasporophytes) (McCandless et al. 1973, 1975, Hosford and McCandless 1975, Pickmere et al. 1975, Waaland 1975). Proposals have been advanced suggesting the aquaculture of isolated reproductive stages of seaweeds to produce specific fractions of carrageenan (Shacklock et al. 1973, Mathieson 1982), heretofore only available via costly fractionation procedures. Some Gracilaria species may exhibit differences in the absolute content of agar between the reproductive phases (Kim and Henriquez 1979, Whyte and Englar 1979b, Whyte et al. 1981). However, Penniman (1977) presented preliminary data

suggesting that Gracilaria tikvahiae (as G. foliifera) populations within the Great Bay Estuary (New Hampshire), did not show any differences in agar yield between reproductive phases. Similar results have been published for G. coronopifolia, G. bursapastoris (Hoyle 1978a) and Eucheuma spp. (Dawes et al. 1977). The current paper expands preliminary conclusions (Penniman 1977) regarding the content and seasonal cycles of several major chemical components of G. tikvahiae. Analyses of the physical and chemical properties of the agar from G. tikvahiae are presented in Part 3.

It should be emphasized that there is considerable confusion regarding the taxonomy of Gracilaria, both worldwide, and along the East Coast of North America (Chapman et al. 1977, McLachlan et al. 1977, McLachlan 1979, Hoek 1982). Thus, McLachlan (1979) combined all of the previously recognized taxa of Gracilaria in the geographic range of New Brunswick-Nova Scotia to New Jersey under the name Gracilaria tikvahiae. Between New Jersey and the Caribbean, confusion still remains as to the identities of closely related Gracilaria taxa (the source of much of the algal biomass used in several aquaculture projects).

## 2.2 METHODS

Gracilaria tikvahiae plants were collected monthly by SCUBA divers from May 1976 to October 1977 at three sites (i.e. Cedar Point, Thomas Point, and Nannie Island) in the Great Bay Estuary. See Part 1 for a description of these sites.

Immediately after collection, apical tips were excised from the plants for pigment analyses. Algal samples from each station were sorted into vegetative, cystocarpic, tetrasporic and spermatangial material. All phases were not present in each monthly collection (see Part 1). Each sample was prepared as follows; (1) it was rinsed briefly in tap water, (2) a fresh weight was determined after blotting excess moisture with Kimwipes, (3) the sample was dried at room temperature in moving air, (4) the sample was dried further in a vacuum oven at 60°C for 48 hr, (5) and a dry weight was determined. The dry samples were ground to pass through a 40-mesh screen using a Wiley mill, dried again in a vacuum oven, and stored in dessicators. The samples were subsequently analyzed as described below. With the exception of the pigment analyses, all the following procedures were conducted on dry, ground samples sorted as to reproductive status.

## DRY WEIGHT

A dry/fresh weight ratio, expressed as percent dry weight, was calculated for each Gracilaria tikvahiae sample. The fresh and dry weights were determined as described above.

## ASH

The ash content of portions of each Gracilaria sample was determined. Triplicate portions (0.10-0.25 g) were combusted in porcelain crucibles for 48 hr at 500°C in a muffle furnace.

## AGAR

The agar extraction techniques employed generally followed the method described by Kim (1970). The amount of algal material used for each extraction did not exceed 30 g. Replication of the agar extractions was limited by the amount of plant material available from each collection (for each reproductive stage). Gracilaria samples were pretreated with 5% sodium hydroxide (3:1, vol of NaOH:dry wt Gracilaria) and heated for 3 hr at 80°-90°C. The samples were then rinsed with distilled water (24:1, vol water:dry wt Gracilaria) at 10°C to remove excess NaOH, and then drained. A second equal volume of water was added to the samples and the pH was then adjusted to 6.0 with 1.0N hydrochloric acid. The mixture was heated at 90°-100°C and

stirred for 1.5 hr to extract the agar. Diatomaceous earth and calcium chloride dihydrate (to aid filtration) were added to the extraction (1:1, wt filter aid:dry wt Gracilaria; 0.02:1, wt  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ :dry wt Gracilaria). The agar solution was filtered in a pressure bomb through Whatman #2 filter paper. The solid residue (filter cake) was re-extracted at  $90^\circ$ - $100^\circ\text{C}$  for 30 min and then filtered. The two filtrates were combined, allowed to cool and gel, and then frozen. After 8-10 hr, the frozen agar filtrates were thawed and excess water was separated from the insoluble agar by squeezing in a nylon cloth. The agar was washed twice in 85% isopropanol (24:1, vol isopropanol:dry wt Gracilaria), then dried in vacuo at  $60^\circ\text{C}$  for 48 hr, and ground in a Wiley mill to pass through a 40-mesh screen. Subsequent analyses of these agar samples are reported in Part 3.

#### CARBOHYDRATE

Soluble carbohydrates were extracted from triplicate portions (5-10 mg) of dry, ground Gracilaria samples in 15 ml of hot 5% trichloroacetic acid for 2 hr. After centrifugation, aliquots of the solutions were analyzed for carbohydrate content by the phenol-sulfuric acid method of Dubois et al. (1956), using D-glucose as a standard. Absorbances were measured at 490 nm in 1.0 cm pyrex cuvettes with a Beckman DBG spectrophotometer.

## PROTEIN

Triplicate 10-25 mg portions of each Gracilaria sample were analyzed for protein content. Each replicate was homogenized in 10 ml of 1.0N NaOH using a Ten Broeck grinder and then allowed to extract for 8-10 hr. Aliquots (0.5 ml) of the homogenates were neutralized with 1.0N HCl and subsequently analyzed for protein content by the Folin phenol method (Lowry et al. 1951) as described by Umbreit et al. (1972). Absorbances were measured at 750 nm in 1.0 cm pyrex cuvettes, using a Beckman DBG spectrophotometer. Bovine serum albumin (Fraction V, Sigma Chemical Co.) was used as a protein standard.

## CARBON AND NITROGEN

Total carbon and nitrogen contents of dry, ground Gracilaria samples were measured in duplicate on a Hewlett-Packard model 185 elemental analyzer. Cystine was used as a standard. Carbon and nitrogen analyses were performed on all eighteen monthly collections from Thomas Point, but only for three-month intervals on samples from Cedar Point and Nannie Island due to financial limitations.

## PHOSPHORUS

The phosphorus content of triplicate ashed portions (50-200 mg) of Gracilaria samples was determined. The ashed material was dissolved in 25 ml of 10% HCl by heating at

90°-100°C for 1.5 hr. Aliquots of digests were analyzed for ortho-phosphate with the molybdate-ascorbic acid method (Golterman 1970). Absorbances were read at 665 nm in 4.0 cm pyrex cuvettes with a Beckman DBG spectrophotometer.

#### CHLOROPHYLL a AND PHYCOERYTHRIN

The chlorophyll a content of ten, fresh, apical tips (25-75 mg fresh wt) of Gracilaria tikvahiae was determined on each monthly collection, prior to sorting into reproductive groups. The fresh weights of the plant tips were measured and the chlorophyll was extracted by grinding in 12 ml of 90% acetone, using a Ten Broeck homogenizer, over ice, for 1.5 min. The samples were kept for 2 hr at 4°C in the dark to allow further chlorophyll extraction, and then centrifuged. Absorbances of the supernatants were measured at 750 and 665 nm in 4.0 pyrex cuvettes on a Beckman DBG spectrophotometer. The chlorophyll a content was calculated, after correction for the 750 nm turbidity reading, with the extinction coefficient,  $E=87.67$  (Jeffrey and Humphrey 1975).

The phycoerythrin content of six, replicate, fresh, apical tips of Gracilaria tikvahiae was determined on each monthly collection from November 1976 to October 1977. The plant tips were homogenized as described for chlorophyll; however, the solvent used was 10 ml of 0.1M (pH 6.5) phosphate buffer (Moon and Dawes 1976). The extracts were



centrifuged and the pellet re-extracted as described above. The absorbances of each supernatant (i.e. first and second extraction) were measured at 750 and 565 nm in 4.0 cm cuvettes on a Beckman DBG spectrophotometer. Phycoerythrin content was calculated, after correction for the 750 nm turbidity reading, with the absorption coefficient,  $A=81.0$  (O'hEocha 1971). The phycoerythrin content for each plant tip was determined by adding the measurements of the two extractions.

Concurrent with the pigment analyses, ten, fresh, apical tips from each collection were weighed (fresh wt), dried in vacuo at  $60^{\circ}\text{C}$  for 48 hr, and reweighed (dry wt). The percent dry weights were then used to express pigment concentration as mg chlorophyll (or phycoerythrin) per gram dry weight algal material.

#### STATISTICAL ANALYSES

The chemical components of Gracilaria tikvahiae were plotted for the eighteen month period of the investigation. Missing points indicate either the lack of a specific reproductive phase for that month or the inability to sample due to ice cover (i.e. January-February 1977 at Nannie Island). The seasonal curves were analyzed by fitting periodic regression equations to the data. The model for the periodic regression analyses contained a harmonic term, as follows:

$$y_i = a_0 + a_1 \cos(2\pi/12x_i) + a_2 \sin(2\pi/12x_i) + e_i$$

where  $y_i$  is the dependent variable (i.e. a chemical component),  $a_0$ ,  $a_1$ ,  $a_2$  are constants,  $x_i$  is the independent variable (i.e. a time series), and  $e_i$  is the residual term (Hackney and Hackney 1977, 1978). The model is appropriate for analyses of data that vary periodically through time. It has the advantage of being simpler mathematically than techniques such as time-series autocorrelation analysis (Williams et al. 1981) and is conceptually more straightforward than equations of higher degree (i.e. greater than third) polynomials (Hackney and Hackney 1977).

A multiple regression model was developed that included the harmonic function and a term to represent the reproductive phase of the algal samples (i.e. cystocarpic or tetrasporic) as main effects and the respective interaction terms. The multiple regression analysis of variance allowed tests of significance for a seasonal periodic component as well as those differences in chemical composition due to reproductive phase. Only the differences between cystocarpic and tetrasporic plants were examined because vegetative plants could be either haploid or diploid. Although the data for male plants were included in the seasonal graphs, no statistical comparisons were made by regression methods since male plants were collected infrequently. The regression model was used to analyze the dry weight, ash, agar, protein, carbohydrate, carbon,

nitrogen, and phosphorus data. The samples of Gracilaria tikvahiae from the three collection sites (i.e. Cedar Point, Thomas Point, and Nannie Island) represented replicates. The full regression model was not used to analyze data on pigment content as the samples were not separated into distinct reproductive groups. All regressions and ANOVA were performed with MINITAB (Ryan et al. 1976), SPSS (Nie et al. 1975) and/or the BMD04R routine from the BMD statistical package (Dixon 1977). Correlations between plant tissue chemistry and environmental data were calculated with the SPSS statistical package (Nie et al. 1975). All regression and correlation analyses were performed on arcsine transformed data (Sokal and Rohlf 1981).

## 2.3 RESULTS

### DRY WEIGHT

Similar cycles of low dry weight values of Gracilaria tikvahiae in the summer increasing to maximum values in the winter were apparent at all three sites (Figure 2-1). There were no consistent differences between vegetative, cystocarpic, tetrasporic and spermatangial phases during the eighteen month period (Figure 2-2). Analysis of variance (Table 2-I) showed a significant interaction between the main effects (i.e. the periodic effect and the effect of reproductive phase). The regression model had an  $R^2=72.4$  ( $p<0.001$ ).

### ASH

High values of ash content in Gracilaria tikvahiae (40% - 50%) occurred during both summers, while lower percent ash (24% - 32%) occurred during the winter (Figure 2-3). Average values (i.e. reproductive phases averaged over the three sites) illustrated in Figure 2-4 show the seasonal trends analyzed in Table 2-I. The regression model explained 69.2% of the variation in ash content. The effect of reproductive phase (i.e. cystocarpic or tetrasporic) was

not significant, but the main effect for the periodic term was highly significant (Table 2-I).

#### AGAR

The agar contents of vegetative, cystocarpic, tetrasporic and spermatangial Gracilaria tikvahiae plants at the three sites were low during the summer (Figure 2-5). The values for the summer of 1976 (7% - 18%) were lower than during 1977 (12% - 20%). Similar cycles were apparent at all three stations. Regression analysis of variance of the average agar content (Figure 2-6) showed a significant interaction term (Table 2-I). There were no absolute differences in agar content between cystocarpic and tetrasporic plants (Figure 2-6), but rather a seasonal shift with tetrasporic plants attaining maximum agar content earlier than cystocarpic plants.

#### CARBOHYDRATE

The seasonal variation of total soluble carbohydrate content in vegetative, cystocarpic, tetrasporic and spermatangial plants of Gracilaria tikvahiae showed similar trends at the three sites (Figure 2-7). Low values of 24% - 30% were present during both summers, while winter levels generally exceeded 40%. Each phase exhibited a similar cycle of soluble carbohydrate content (Figure 2-8). Analysis of variance showed no significant difference in

carbohydrate content between cystocarpic and tetrasporic plants (Table 2-I). The regression model ( $R^2=59.6$ ) gave a highly significant seasonal component ( $p<0.001$ ).

#### PROTEIN

The protein content of Gracilaria tikvahiae was relatively constant throughout the year with the exception of slightly lower protein levels in June-July of both 1976 and 1977 (Figure 2-9). The trend was apparent at all three stations. With few exceptions, the protein levels varied from 10% to 13% of dry weight. The average protein values had no strong seasonal cycle nor any differences between reproductive groups (Figure 2-10). The trend is emphasized in the regression model by the low  $R^2$  (14.1) and by the lack of any significant difference between cystocarpic and tetrasporic plants.

#### CARBON

The total carbon levels in Gracilaria tikvahiae plants from Thomas Point (Figure 2-11) had a seasonal cycle of low summer (25% - 30%) and high winter values (32% - 37%). No consistent differences were evident between the four reproductive phases. The data for Cedar Point and Nannie Island are not illustrated; however they are included in the regression analysis of variance (Table 2-I). Regression ANOVA showed no significant differences between cystocarpic

and tetrasporic phases (Table 2-I). There was a highly significant ( $p < 0.001$ ) periodic component to the regression model ( $R^2 = 61.9$ ).

#### NITROGEN

Summer values of total tissue nitrogen of Thomas Point Gracilaria tikvahiae plants were 2.0% - 2.5%, while winter levels were 3.5% - 4.0% (Figure 2-12). Nitrogen measurements were made on plants from Cedar Point and Nannie Island at three-month intervals (as with total carbon measurements). While these data are not illustrated, they were used in the regression analysis of variance. No consistent differences in nitrogen content due to reproductive phase were apparent (Figure 2-12). Comparison by regression ANOVA of cystocarpic and tetrasporic plants for nitrogen content, indicated no significant differences between these stages (Table 2-I). The regression model ( $R^2 = 66.8$ ) indicated a highly significant seasonal component.

#### PHOSPHORUS

The seasonal trends of tissue phosphorus content for Gracilaria tikvahiae within the Great Bay Estuary varied between 0.2% and 0.4% of dry weight (Figure 2-13). Regression ANOVA showed no significant differences in phosphorus content (Figure 2-14) between cystocarpic and tetrasporic plants (Table 2-I). There was a low  $R^2$  (22.8)

for the overall regression model, indicative of the lack of a strong seasonal cycle in phosphorus content.

#### COMPONENT RATIOS

The C/N, C/P and N/P ratios (on both weight and atomic bases) for Thomas Point plants are illustrated in Figure 2-15. The C/N ratios were low during the fall to spring (8-10, by weight) and higher in the summer (12-14, by weight) at Thomas Point (Figure 2-15a). The C/P ratios were quite variable, with no consistent seasonal trends (Figure 2-15b). The values primarily reflect variation in carbon content since phosphorus was rather stable. Figure 2-15c illustrates the N/P ratios for the four reproductive groups at Thomas Point. Lower (9-12, by weight) and higher (12-15, by weight) values of N/P were seen in summer and winter, respectively. Carbohydrate/protein ratios (Figure 2-16) varied from low summer (1.5-3.5) to higher winter values (3.5-4.2).

#### CHLOROPHYLL a AND PHYCOERYTHRIN

Chlorophyll content (mg/g dry wt) had little seasonality (Figure 2-17) and no consistent differences between stations. The lack of a strong seasonal variation was supported by the low  $R^2$  of the periodic regression models fitted to the chlorophyll data for the three stations (Table 2-II).



Phycoerythrin content was analyzed for twelve months (November 1976 - October 1977) at Cedar Point, Thomas Point and Nannie Island (Figure 2-18). Low summer and high winter values of phycoerythrin were evident (Table 2-II).

#### CORRELATION ANALYSES

Correlations between Gracilaria tikvahiae tissue composition and various hydrographic and nutrient parameters were calculated (Tables 2-III and 2-IV). Ash content was negatively correlated with all tissue components (Table 2-III). However, ash content was positively correlated with temperature and salinity (Table 2-IV). All other chemical components were negatively correlated with both temperature and salinity. Total tissue nitrogen and protein were positively correlated with dissolved inorganic nitrogen (Table 2-IV). In contrast, tissue phosphorus was not significantly correlated with ambient dissolved phosphate. Tissue nitrogen and carbon were more strongly correlated than protein and carbohydrate (Table 2-III). Chlorophyll and phycoerythrin content were strongly positively correlated.

## 2.4 DISCUSSION

Although much information is available on the chemical composition of Gracilaria tikvahiae and related species in culture (DeBoer and Ryther 1977, DeBoer et al. 1978, D'Elia and DeBoer 1978, LaPointe and Ryther 1978, 1979, K. Bird et al. 1981, 1982, LaPointe 1981, Ryther et al. 1981, Rosenberg and Ramus 1982a, 1982b), relatively little is known concerning the composition of the same plants from in situ populations (DeLoach et al. 1946, Kim and Humm 1965, C. Bird et al. 1977, Asare 1979). The results of the present study indicate substantial seasonal changes in the chemical composition of G. tikvahiae populations within the Great Bay Estuary. In addition they point to relationships between various components and environmental parameters, that are not necessarily the same as those seen in culture (LaPointe and Ryther 1979, LaPointe 1981).

There was a marked seasonal variation in the percent dry weight of Gracilaria tikvahiae plants. The annual cycle of percent dry weight of G. tikvahiae in the Great Bay Estuary was comparable to that described for the same species in Pomquet Harbour, Nova Scotia (C. Bird et al. 1977). The changes in dry weight were negatively correlated with the seasonal growth cycle of G. tikvahiae.

While there were no significant differences in percent dry weight between cystocarpic and tetrasporic plants in the present study, Whyte and Englar (1979b) and Whyte et al. (1981) found large differences between these stages in Gracilaria from British Columbia. The latter two studies gave absolute values of percent dry weight (7% - 20%) similar to those for G. tikvahiae in the Great Bay Estuary. In contrast, Lindsay and Saunders (1980) found lower dry weight values (5% - 9%) in aquacultured G. verrucosa from British Columbia, with a cycle of high winter and low summer values. The lower values probably were due to the removal of surface salts, since the latter samples were rinsed in freshwater after partial drying, rather than while still fresh. Gracilaria chorda from British Columbia had percent dry weight values (8% - 11%) with the seasonal trend of low winter and higher summer values the opposite of that for G. verrucosa (Lindsay and Saunders 1980). Similar dry weight values as those in the present study were reported by Hoffman (1978) for natural populations of G. verrucosa in Florida; as well as by LaPointe and Ryther (1979) with aquacultured G. foliifera populations in Florida. The values of percent dry weight for G. tikvahiae in the present study are comparable to those for other gigartinean species; Hypnea musciformis (Durako and Dawes 1980), Eucheuma nudum (Dawes et al. 1974, 1977, Dawes 1982), and E. isiforme (Dawes et al. 1974).

The ash content of Gracilaria tikvahiae had a seasonal cycle of winter lows (25% - 30%) increasing to 35% - 50% in the summer. C. Bird et al. (1977) showed a similar seasonal trend for G. tikvahiae in Pomquet Harbour, Nova Scotia, although the seasonal amplitude was less than in Great Bay plants. There were no significant differences between cystocarpic and tetrasporic plants with respect to ash content. The ash content of G. tikvahiae in the present study was comparable to that of other Gracilaria species (Whyte and Englar 1976, 1979c, 1980b, Hoffman 1978, LaPointe and Ryther 1979, LaPointe 1981). Yang (1982) reported values of 6.2% - 13% ash in G. verrucosa from Taiwan which are substantially lower than those for G. tikvahiae in the present study. Gracilaria corticata from India showed little seasonal variation of ash content (Sumitra-Vijayaraghavan et al. 1980). Several species of Eucheuma had ash contents generally less than 25%, with little seasonal variation (Dawes et al. 1977, Dawes 1982). Hypnea musciformis (Durako and Dawes 1980) also had a seasonally stable ash content of approximately 45%. Although Munda and Kremer (1977) and Munda and Garrasi (1978) have demonstrated a strong relationship between media salinity and ash content for several fucoids, there was a relatively low positive correlation between ash and in situ salinity for Great Bay G. tikvahiae. Ash content is only a variable approximation of the inorganic content of algal material due to agar liberating sulfuric acid in amounts dependent upon the

associated cations, as well as volatilization of chloride (Larsen 1978).

Under aquaculture conditions, Gracilaria foliifera had an ash content of 35% - 60% (LaPointe and Ryther 1979, LaPointe 1981). The ash content of natural populations of G. tikvahiae in the Great Bay Estuary was positively correlated with growth ( $r=0.91$ , see Part 1), but LaPointe (1981) found a negative correlation of similar magnitude ( $r=-0.85$ ) between ash and growth for G. foliifera in aquaculture. The same contrast was true for ash and C/N content in the present study ( $r=0.70$ ) versus G. foliifera in aquaculture ( $r=-0.99$ ) (LaPointe and Ryther 1979). Morgan and Simpson (1981) discuss the relationship of increasing ash contents with low growth rates in aquacultured Palmaria palmata, the opposite of the trend with G. tikvahiae. The nitrogen and ash contents for P. palmata were negatively correlated.

The protein content in Gracilaria tikvahiae showed little seasonal variation. Similarly, the protein contents of Sargassum pteropleuron (Prince and Daly 1981) and Eucheuma spp. (Dawes et al. 1974) showed reduced annual variation. Insoluble nitrogen in Laminaria longicruris from the St. Lawrence Estuary also exhibited minimal annual changes (Anderson et al. 1981), probably due to high dissolved nitrogen concentrations. The protein content in New Hampshire populations of G. tikvahiae was higher than

similar populations of Chondrus crispus (Mathieson and Tveter 1975) and Gigartina stellata (Mathieson and Tveter 1976). The comparatively low protein content in C. crispus (Mathieson and Tveter 1975) may be a reflection of storage of organic nitrogen as L-citrullinyl-L-arginine (which would not be measured by Lowry protein analyses) rather than as protein (Laycock and Craigie 1977). In contrast to the present study, C. Bird et al. (1977) found a large seasonal cycle of protein content (i.e. as Kjeldahl-nitrogen x 6.25) in G. tikvahiae from Pomquet Harbour, Nova Scotia. Since many seaweeds have the ability to accumulate intracellular pools of inorganic nitrogen (Chapman and Craigie 1977, Asare 1979, Rosenberg and Ramus 1982a, Gagne et al. 1982, K. Bird et al. 1982), low molecular-weight nitrogen compounds (Laycock and Craigie 1977, Laycock et al. 1981, Rosenberg and Ramus 1982a), as well as high molecular-weight nitrogen compounds (e.g. protein), the use of total tissue nitrogen (or Kjeldahl-nitrogen) times a factor (6.25) to estimate protein content is probably erroneous (Harrison and Mann 1975, Gaines 1977, Rice 1982). In view of this confusion, the protein estimates for G. tikvahiae by C. Bird et al. (1977) may be higher, due to the inclusion of amino acids, etc., than if the values had been determined by standard Lowry protein methodology.

In contrast to the lack of an annual cycle in protein content, total tissue nitrogen in Gracilaria tikvahiae had a large seasonal cycle, with low summer and high winter

values. The internal nitrogen content paralleled ambient dissolved inorganic nitrogen in the Great Bay Estuary ( $r=0.74$ ), similar to the relationship for several other macroalgae (Asare 1979, Wheeler and North 1981). In Great Bay G. tikvahiae populations a combination of factors may allow the plants to maintain internal nitrogen at levels greater than 2% of dry weight: (1) relatively high dissolved inorganic nitrogen concentrations in the Great Bay Estuary (see Part 1), (2) rapid currents (Swenson et al. 1977) that enhance diffusive transport, (3) storage of internal nitrogen reserves (Ryther et al. 1981), and (4) a high affinity for absorption of dissolved nutrients (DeBoer et al. 1978, D'Elia and DeBoer 1978). Asare (1979) found a seasonal cycle of total tissue nitrogen for G. tikvahiae and Neogardhiella baileyi in Ninigret Pond, Rhode Island, similar in phase to Great Bay G. tikvahiae. However, his nitrogen values were substantially lower than those for G. tikvahiae in the present study. The differences may be due to: (1) dissolved inorganic nitrogen concentrations in the Great Bay Estuary are generally greater than those in Ninigret Pond (Asare 1979) and (2) the rapid currents in the Great Bay Estuary ( $>20$  cm/s during flood and ebb tides in the Great Bay, Swenson et al. 1977) would enhance algal nutrient uptake (Conover 1968, Parker 1981, 1982, Gerard 1982c). Although Asare (1979) does not describe current regimes in Ninigret Pond it is unlikely that they would be comparable to those in the Great Bay Estuary (see Harlin and

Thorne-Miller 1981). Also, Asare studied unattached G. tikvahiae and therefore the prior hydrographic and nutrient conditions to which they had been exposed were unknown. Hoyle (1978b) measured the seasonal cycles of total nitrogen content of Hawaiian plants of G. coronopifolia and G. bursapastoris which, although similar to those of G. tikvahiae, had absolute values one-half those in the present study. Ambient dissolved nitrate was much reduced (Hoyle 1978b) compared to the Great Bay Estuary. Rosenberg and Ramus (1982a) cultured G. foliifera in tanks with flowing-seawater over a fourteen month period; their values of total tissue nitrogen were much lower than Asare's (1979) or the present study. The dissolved inorganic nitrogen concentrations in the tanks used by Rosenberg and Ramus (1982a) were lower than in the Great Bay Estuary or Ninigret Pond (Asare 1979).

Comparison of the tissue chemistry of natural populations versus aquacultured plants may not be advisable as the latter regimes may introduce conditions which would not occur in situ (e.g. simultaneous elevated temperature and dissolved inorganic nitrogen). The apparent luxury consumption of nitrogen in conjunction with relatively high dissolved inorganic nitrogen concentrations may decrease the correlation of total tissue nitrogen with dissolved inorganic nitrogen and of tissue nitrogen with growth rate. Thus, it would appear to be necessary to examine the response of an alga both in situ and in culture



(e.g. aquaculture tanks, etc.) to adequately describe its potential physiological responses.

Tissue nitrogen in Gracilaria tikvahiae was inversely related to ambient salinity in the Great Bay Estuary. The latter relationship corresponds with that demonstrated for several brown algae (Munda 1977, Munda and Kremer 1977, Munda and Garrasi 1978).

The seasonal pattern of total tissue nitrogen for Gracilaria tikvahiae paralleled changes in total carbon and soluble carbohydrate. The correspondence was reflected by the high correlation of nitrogen with carbon and carbohydrate (i.e.  $r=0.80$  and  $r=0.65$ , respectively). Several studies have indicated the opposite pattern (i.e. low winter and high summer values of carbohydrate content) in Hypnea musciformis (Durako and Dawes 1980), Chondrus crispus (Butler 1936), and Eucheuma nudum (Dawes et al. 1977). Carbohydrate content was seasonally stable in New Hampshire Gigartina stellata (Mathieson and Tveter 1976) but had an erratic cycle in Chondrus crispus (Mathieson and Tveter 1975). Carbohydrate and total nitrogen were inversely related in aquacultured Palmaria palmata (Morgan and Simpson 1981). The pattern exhibited by Gracilaria tikvahiae for carbon and carbohydrate may be a reflection of the Great Bay Estuary being relatively nitrogen-rich as compared with conditions in many of the aforementioned studies (Dawes et al. 1974, Mathieson and Tveter 1975,

1976). C. Bird et al. (1977) discuss a pattern of carbohydrate and protein variation in G. tikvahiae from Pomquet Harbour, Nova Scotia similar to that in Great Bay populations. In Nova Scotian plants both carbohydrate and protein contents exhibited summer lows and winter highs.

Neither protein, carbohydrate, carbon, nor nitrogen showed significant differences between cystocarpic and tetrasporic plants in Gracilaria tikvahiae. Similarly, Dawes et al. (1977) found no differences in protein nor carbohydrate contents between cystocarpic and tetrasporic samples of several Florida Eucheuma species.

The C/N (by weight) in actively growing Gracilaria verrucosa (Niell 1976) was similar to that in G. tikvahiae. In Great Bay G. tikvahiae, C/N (by atoms) frequently rose above ten, which was determined to indicate nitrogen-limited growth for G. foliifera in aquaculture (D'Elia and DeBoer 1978). However, the tissue nitrogen content never decreased below the 2% value determined by Hanisak (1982) as indicating growth-limiting conditions in G. tikvahiae. Gordon et al. (1981) found a similar critical nitrogen content of 2.1% for the estuarine green alga Cladophora albida. Hanisak (1979b) has shown that plant tissue nitrogen analyses are generally better indications of growth-limiting internal nitrogen concentrations than are C/N data, as the latter values are dependent on both carbon and nitrogen metabolism (Gordon et al. 1981). Hanisak

(1979b) found that the critical tissue nitrogen content of Codium fragile was 1.9% of dry weight. In Rhode Island populations of the same species, an annual cycle of 0.75% nitrogen (summer) to 3.72% (winter) indicated changes from nitrogen-limitation to luxury nitrogen-storage (Hanisak 1979b).

Further support for the fact that Great Bay populations of G. tikvahiae were not nitrogen-limited, arises from a comparison of the high summer dissolved inorganic nitrogen concentrations in the Great Bay Estuary (Part 1) relative to other coastal (Ryther and Dunstan 1971, Wheeler and North 1981) or estuarine (Hanisak 1979a, Asare 1979) sites where seasonal plant nitrogen and carbon cycles have been examined. Cultured G. verrucosa from British Columbia (Lindsay and Saunders 1980) had an average nitrogen content of 3.5% - 4.0% (as well as C/N = 8-11) and did not appear to be nitrogen-limited at this internal nitrogen concentration. Parker (1982) indicated that nitrogen uptake in nitrogen-limited G. tikvahiae was saturated at a current velocity of 7.5 cm/s. As shown by (Swenson et al. 1977) current velocities in the Great Bay Estuary are generally much greater than this value. Gerard (1982a, 1982b) demonstrated that nitrogen starvation was probably rare for southern California populations of Macrocystis pyrifera due to the plants' nitrogen-storage abilities and to periodic upwelling and terrestrial runoff of nitrogen-rich water.

Morgan and Simpson (1981) found an inverse relationship between growth rate and tissue nitrogen content with Palmaria palmata grown under conditions where nitrogen was not limiting. Similar relationships between growth and nitrogen content were present for Great Bay Gracilaria tikvahiae as well as similar populations from Pomquet Harbour, Nova Scotia (C. Bird et al. 1977). Yoder (1979) discussed differences between nutrient-limited and light-limited growth and stated that high tissue nitrogen indicated either adequate nitrogen and fast growth, or low growth and nitrogen conservation.

The annual cycle of agar content in Great Bay Gracilaria tikvahiae corresponded with total carbon and soluble carbohydrate, with summer minima and winter maxima. Similar patterns were seen in aquacultured Gracilaria from British Columbia (Lindsay and Saunders 1979). In contrast, Hoyle (1978b) found a winter minimum of agar content for G. coronopifolia. Asare (1979), working in Rhode Island, found no overall annual cycle for agar in G. tikvahiae nor carrageenan in Neogardhiella baileyi. His findings may have resulted from the use of drift plant material (Asare 1979). New Hampshire populations of Chondrus crispus showed a seasonal cycle of carrageenan content with high summer and low winter-spring values (Fuller and Mathieson 1972, Mathieson and Tveter 1975). Several tropical carrageenophytes, Hypnea cervicornis, H. chordacea, H. nidifica (Mshigeni 1979) and an agarophyte Gelidiella

acerosa (Thomas et al. 1975, 1978) showed cycles of phycocolloid content similar to Great Bay G. tikvahiae. The agar yields for N.H. G. tikvahiae were similar to those of several other Gracilaria species (Young 1974, Hoyle 1978a, 1978b, Lindsay and Saunders 1979, 1980, Whyte and Englar 1979a, 1981, Abbott 1980, K. Bird et al. 1981, Yang 1982). Agar content of G. verrucosa (as G. confervoides) from Beaufort, North Carolina varied between 20% - 35% with the highest yield occurring during the summer (DeLoach et al. 1946). Kim and Humm (1965) found the agar content of North Carolina G. foliifera to be twice that of N.H. G. tikvahiae.

A comparison of agar yields between different species (e.g. Rama Rao 1977, Durairatnam 1980) is limited by differences in extraction techniques, seasonal variation in agar content (if only single measurements are made), possible seasonal variation in agar extractability (DeLoach et al. 1946), as well as possible confusion of Gracilaria species. It should be noted that agar extraction using an alkaline modification procedure (as in the present study) may cause somewhat reduced agar yields (versus no alkaline step) with G. verrucosa, G. sjoestedtii (Durairatnam and Santos 1981), and G. tikvahiae (Young 1974) but not for G. foliifera (Matsushashi and Hayashi 1972).

No differences in magnitude of agar content were noted between reproductive stages of Great Bay Gracilaria tikvahiae. In contrast, Kim and Henriquez (1979), Whyte and Englar (1979b) and Whyte et al. (1981) have observed differences in agar yield between various reproductive stages of G. verrucosa. However, none of these differences were confirmed statistically. Hoyle's (1978a) study with Hawaiian G. coronopifolia and G. bursapastoris supports the lack of agar yield differences between reproductive stages, as observed in the present study for G. tikvahiae. Further comparisons of the agar composition from G. tikvahiae are presented in Part 3.

The seasonal variations of agar yield for Great Bay Gracilaria tikvahiae were not inversely related to either nitrogen or protein content, as compared to the carrageenan or agar and protein levels in natural populations of Gigartina stellata (Mathieson and Tveter 1976), Chondrus crispus (Mathieson and Tveter 1975), Hypnea musciformis (Durako and Dawes 1980), G. coronopifolia or G. bursapastoris (Hoyle 1978b) or of aquacultured C. crispus (Neish and Shacklock 1971), Neoagardhiella baileyi (DeBoer 1979), or G. foliifera (DeBoer 1979, K. Bird et al. 1981). Liu et al. (1981) and Yang (1982) found a negative correlation between, temperature or light, and agar content in pond-cultured G. verrucosa in Taiwan. Similar relationships occurred in the present study. The yield of agar from Ghanaian G. dentata (John and Asare 1975) was

greatest during periods of maximum growth, as well as for carrageenan in Hypnea musciformis and H. valentiae (Rama Rao and Krishnamurthy 1978). The opposite relationship was evident for Great Bay G. tikvahiae. Carrageenan content of aquacultured Hypnea musciformis was inversely related to growth (Guist et al. 1982). Thus, there would appear to be contrasts between species with a positive relationship between growth and phycocolloid (or carbohydrate) content and those species where the opposite relationship holds (as in the present study). Whether the differences result from various factors limiting growth (e.g. nutrients, irradiance, temperature, etc.) remains to be studied.

There was little seasonal variation in Great Bay Gracilaria tikvahiae tissue phosphorus content. Few other studies of annual variation in macroalgal tissue phosphorus are available for comparison (Wort 1955, Young and Langille 1958, Wallentinus 1975, Whyte and Englar 1980a). The seasonal variation of phosphorus content in Macrocystis integrifolia and Nereocystis leutkeana ranged from 0.3% to 1.3% (Wort 1955), which was higher than in Great Bay G. tikvahiae. In contrast, the percent phosphorus content of Chondrus crispus in Nova Scotia ranged from 0.22% to 0.34% (Young and Langille 1958), similar to the data in the present study. Gordon et al. (1981) determined that the internal phosphorus content limiting growth in estuarine Cladophora albida was 0.33%. In the present study summer phosphorus levels in G. tikvahiae fell below this value.

However, comparisons between species with respect to physiological parameters such as critical internal nutrient concentrations are tenuous. Supplemental phosphate additions in a Rhode Island coastal lagoon (Harlin and Thorne-Miller 1981) resulted in greater increases in standing crop with G. tikvahiae, than either additional nitrate or ammonium. Further research of in situ phosphorus requirements of G. tikvahiae and other estuarine seaweeds is desirable.

The N/P atomic ratios of Great Bay Gracilaria tikvahiae were always higher than 16, the value representative of oceanic phytoplankton (Redfield 1958). Wallentinus (1975) found that N/P atomic ratios were generally greater than 15 for Cladophora glomerata in the Baltic Sea. However due to the wide variation of N/P in macroalgae (Imbamba 1972, Kornfeldt 1982, Kautsky 1982), little information regarding nutrient limitation can be derived from this ratio alone.

The chlorophyll a content of Great Bay Gracilaria tikvahiae showed relatively little seasonal variation. The values were generally higher than those measured by Rosenberg and Ramus (1982b) for G. foliifera populations from Beaufort, North Carolina. The phycoerythrin content of Great Bay G. tikvahiae had low summer and high winter values. Gracilaria verrucosa from the Adriatic Sea had a similar annual cycle of phycoerythrin content (Kosovel and Talarico 1979) as found in the present study; the latter



authors also found a negative correlation between phycoerythrin and chlorophyll content. In contrast, phycoerythrin and chlorophyll content in N.H. populations of G. tikvahiae were positively correlated ( $r=0.86$ ). The chlorophyll content in Floridian G. verrucosa (Hoffman 1978) was similar in magnitude to values in the present study. Chlorophyll content was more variable in Florida populations of Eucheuma isiforme (Moon and Dawes 1976) than the Great Bay populations of G. tikvahiae. Moon and Dawes (1976) also found an annual cycle with summer maxima for both chlorophyll and phycoerythrin content in E. isiforme, opposite to the present results. Although Rosenberg and Ramus (1982b) found a cycle of phycoerythrin content for G. foliifera similar to the present study, their absolute values were lower. The range of pigment values in Great Bay G. tikvahiae was similar to those for phycoerythrin and chlorophyll contents in several Porphyra species (Oohusa et al. 1977, Amano and Noda 1978).

Wallentinus (1975) found significant correlations between chlorophyll content and both tissue nitrogen and phosphorus in Baltic Sea populations of Cladophora glomerata. No similar correlations were apparent in G. tikvahiae. The phycoerythrin, but not chlorophyll content, of Great Bay G. tikvahiae was significantly correlated with total tissue nitrogen ( $r=0.70$ ). High pigment content of Great Bay G. tikvahiae was paralleled by high tissue nitrogen. Similar patterns were noted in

G. foliifera from North Carolina (Rosenberg and Ramus 1982b). LaPointe (1981), K. Bird et al. (1982) and Ryther et al. (1981) have hypothesized that the phycobilins in G. tikvahiae may act as storage sites for nitrogen as well as being accessory pigments. Elevated pigment levels (i.e. darker thalli) have been observed in aquacultured plants of Palmaria palmata under low temperature and low light as well as high temperature and high light regimes (Morgan and Simpson 1981).

As noted earlier there have been few parallel comparisons of aquacultured and natural populations of seaweeds and few conclusions can be drawn regarding varying chemical composition(s) under these contrasting regimes. Whyte and Englar (1976, 1979c) compared the composition of Gracilaria populations from natural and aquaculture conditions; even so, their study did not include adequate controls, as only pre- and post-aquaculture samples were examined and no concomitant changes in the natural population were followed during the experiment. Guist et al. (1982) examined in situ and aquacultured Hypnea musciformis in Florida and found parallel seasonal changes of carrageenan content. While there are inherent differences between studies of native and cultured algae, the contrasting results from the present study and those of aquacultured G. foliifera (LaPointe and Ryther 1979, LaPointe 1981) suggest the need for more parallel investigations. Furthermore, comparisons of natural and

cultured populations may be inappropriate as combinations of conditions produced in culture (e.g. high temperature and elevated dissolved inorganic nitrogen) may never occur simultaneously in the field. Discrepancies may also arise due to physiological or biochemical differences between coastal and estuarine populations of similar or closely related macroalgae.

In summary, Great Bay populations of Gracilaria tikvahiae had marked seasonal cycles of several chemical components. Most of these cycles included summer minima and winter maxima (i.e. agar, carbohydrate, carbon, nitrogen, phycoerythrin and dry weight), and were the opposite of the plant's growth cycle. In contrast, ash content, showed an annual cycle with summer maxima and winter minima (i.e. similar to growth). The contrasts between these opposite cycles could cause attenuation or depression of relationships between individual components since the ash content included such a large proportion of the dry weight of the alga. There were no significant differences in magnitude between cystocarpic and tetrasporic plants in all components studied. In addition there were no consistent differences in composition between reproductive and vegetative plants.

## 2.5 SUMMARY

- 1). Cystocarpic and tetrasporic plants of Gracilaria tikvahiae from Great Bay showed no differences in the various chemical components (i.e. dry weight, ash, agar, carbohydrate, protein, carbon, nitrogen, and phosphorus).
- 2). A strong seasonal cycle was apparent for dry weight, ash, agar, carbohydrate, carbon, nitrogen and phycoerythrin contents for Great Bay Gracilaria tikvahiae, but not for protein, phosphorus and chlorophyll contents.
- 3). All of the aforementioned components of Great Bay Gracilaria tikvahiae, except for ash and chlorophyll, were significantly, positively correlated with ambient water temperature.
- 4). The total tissue nitrogen content of Great Bay Gracilaria tikvahiae did not decrease below 2% determined to be the growth-limiting concentration for this species (Hanisak 1982).

5). The carbon and nitrogen (and agar and nitrogen) contents of Great Bay Gracilaria tikvahiae were positively correlated.

Table 2-I. Regression (periodic model) analyses of variance tables for individual chemical components of Gracilaria tikvahiae with comparisons between cystocarpic and tetrasporic stages.

a) Dry weight

source	df	MS	F	
periodic	2	175.9	104.08	***
stage	1	2.00	1.19	ns
interaction	2	11.94	7.07	**
residual	85	1.69		
$R^2=72.4$ ***				

b) Ash

source	df	MS	F	
periodic	2	506.2	92.37	***
stage	1	6.52	1.19	ns
interaction	2	13.23	2.41	ns
residual	85	5.48		
$R^2=69.2$ ***				

c) Agar

source	df	MS	F	
periodic	2	173.8	23.78	***
stage	1	3.52	0.48	ns
interaction	2	25.18	3.44	*
residual	74	7.31		
$R^2=42.6$ ***				

Table 2-I. (continued.)

## d) Carbohydrate

source	df	MS	F	
periodic	2	344.2	59.76	***
stage	1	11.85	2.06	ns
interaction	2	6.65	1.15	ns
residual	84	5.76		
$R^2=59.6$ ***				

## e) Protein

source	df	MS	F	
periodic	2	7.26	6.28	***
stage	1	0.71	0.61	ns
interaction	2	0.34	0.29	ns
residual	84	1.16		
$R^2=14.1$ *				

## f) Carbon

source	df	MS	F	
periodic	2	36.48	34.09	***
stage	1	1.88	1.76	ns
interaction	2	0.13	0.12	ns
residual	43	1.07		
$R^2=61.9$ ***				

Table 2-I. (continued.)

## g) Nitrogen

source	df	MS	F	
periodic	2	223.4	42.88	***
stage	1	2.52	0.48	ns
interaction	2	1.39	0.27	ns
residual	43	5.21		

$$R^2=66.8 \quad ***$$

## h) Phosphorus

source	df	MS	F	
periodic	2	87.60	11.70	***
stage	1	1.60	0.21	ns
interaction	2	4.26	0.57	ns
residual	84	7.49		

$$R^2=22.8 \quad ***$$

\*\*\*  $p < 0.001$   
 \*\*  $0.001 < p < 0.01$   
 \*  $0.05 < p < 0.1$   
 ns  $p > 0.05$



Table 2-II. Regression (periodic model) analyses of variance tables for pigment content of Gracilaria tikvahiae.

a) Chlorophyll

source	df	MS	F
periodic regression	2	52.71	4.04 *
residual	45	13.03	

$R^2=15.2$

b) Phycoerythrin

source	df	MS	F
periodic regression	2	55.66	8.46 **
residual	32	6.58	

$R^2=34.6$

\*\* 0.001 < p < 0.01

\* 0.05 < p < 0.1

Table 2-III. Correlation analyses between chemical components of *Gracilaria tikvahiae*.

	dry wt	ash	agar	carboh.	protein	C	N	P	chloro.	phycoe.
ash	-0.881 77.6 ***	$\begin{bmatrix} r \\ r^2 \times 100 \\ p \end{bmatrix}$								
agar	0.515 26.5 ***		-0.593 35.2 ***							
carboh.	0.806 65.0 ***	-0.844 71.2 ***	0.599 35.9 ***							
protein	0.400 16.0 ***	-0.545 29.7 ***	0.334 11.2 ***	0.272 7.4 ***						
C	0.757 57.3 ***	-0.899 80.8 ***	0.574 32.9 ***	0.833 69.4 ***	0.564 31.8 ***					
N	0.835 69.7 ***	-0.877 76.9 ***	0.513 26.3 ***	0.649 42.1 ***	0.766 58.7 ***	0.797 63.5 ***				
P	0.592 35.0 ***	-0.610 37.2 ***	0.311 9.7 ***	0.309 9.5 ***	0.612 37.5 ***	0.611 37.3 ***	0.821 67.4 ***			
chloro.	-0.047 0.2 ns	-0.115 1.3 ns	0.152 2.3 ns	0.002 0.0 ns	0.078 0.6 ns	0.289 8.4 *	0.158 2.5 ns	0.145 2.1 ns		
phycoe.	0.491 24.1 ***	-0.555 30.8 ***	0.388 15.1 *	0.373 13.9 *	0.360 13.0 *	0.600 36.0 **	0.704 49.6 ***	0.509 25.9 ***	0.858 73.6 ***	
C/N	-0.693 48.0 ***	0.702 49.4 ***	-0.406 16.5 ***	-0.406 16.5 ***	-0.748 56.0 ***	-0.516 26.6 ***	-0.912 83.2 ***	-0.770 59.2 ***	-0.066 0.4 ns	-0.574 32.9 **

Table 2-IV. Correlation analyses between chemical components of *Gracilaria tikvahiae* and corresponding hydrographic and water nutrient chemistry data.

	temp.	salin.	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	DIN	PO <sub>4</sub> <sup>3-</sup>
dry wt	-0.836 69.8 ***	-0.287 8.2 ***	0.567 32.1 ***	0.687 47.1 ***	0.696 48.5 ***	0.210 4.4 **
ash	0.802 64.4 ***	0.419 17.5 ***	-0.549 30.1 ***	-0.591 35.0 ***	-0.623 38.8 ***	-0.128 1.6 ns
agar	-0.405 16.4 ***	-0.098 1.0 ns	0.284 8.1 ***	0.295 8.7 ***	0.300 9.0 ***	0.062 0.4 ns
carboh.	-0.715 51.1 ***	-0.314 9.8 ***	0.428 18.3 ***	0.557 31.1 ***	0.550 30.2 ***	0.228 5.2 **
protein	-0.352 12.4 ***	-0.163 2.7 ***	0.420 17.6 ***	0.279 7.8 ***	0.334 11.1 ***	-0.076 0.6 ns
C	-0.753 56.7 ***	-0.353 12.4 ***	0.546 29.8 ***	0.585 34.2 ***	0.599 35.9 ***	0.124 1.5 ns
N	-0.825 68.1 ***	-0.456 20.8 ***	0.682 46.5 ***	0.683 46.6 ***	0.737 54.3 ***	-0.008 0.0 ns
P	-0.448 20.0 ***	-0.331 11.0 ***	0.366 13.4 ***	0.293 8.6 ***	0.353 12.5 ***	-0.025 0.1 ns
chloro.	-0.084 0.7 ns	-0.025 0.1 ns	0.159 2.5 ns	-0.071 0.5 ns	0.005 0.0 ns	0.094 0.9 ns
phycoe.	-0.462 21.4 **	-0.127 1.6 ns	0.544 29.6 ***	0.500 25.0 **	0.590 34.8 ***	0.344 11.9 *
C/N	0.689 47.4 ***	0.413 17.1 ***	-0.603 36.4 ***	-0.584 34.1 ***	-0.639 40.9 ***	0.087 0.8 ns

Figure 2-1. Seasonal variation of percent dry weight of Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

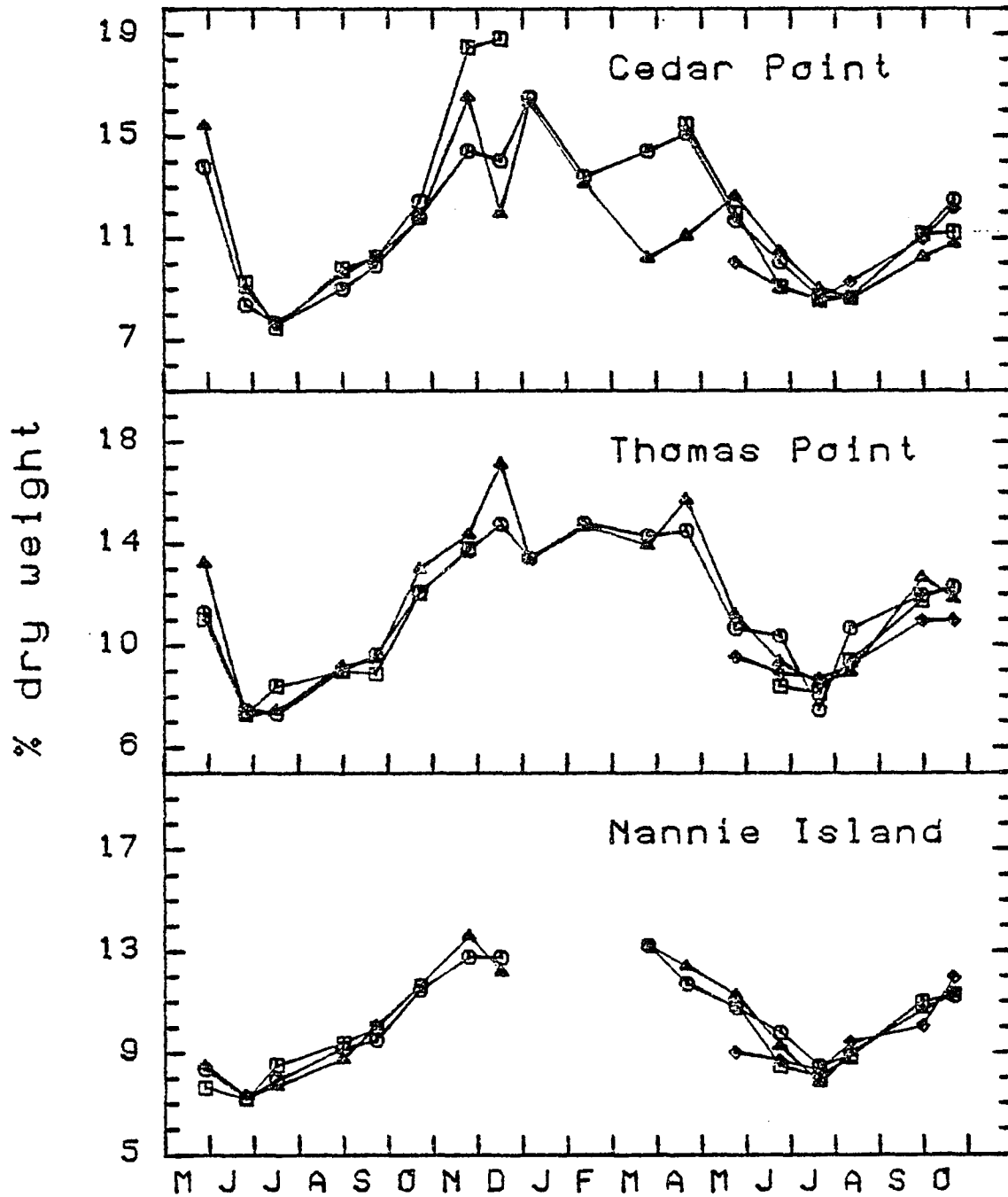


Figure 2-1

Figure 2-2. Average seasonal variation ( $\pm 1$  SE) of percent dry weight of Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds) and tetrasporic (squares) plants.

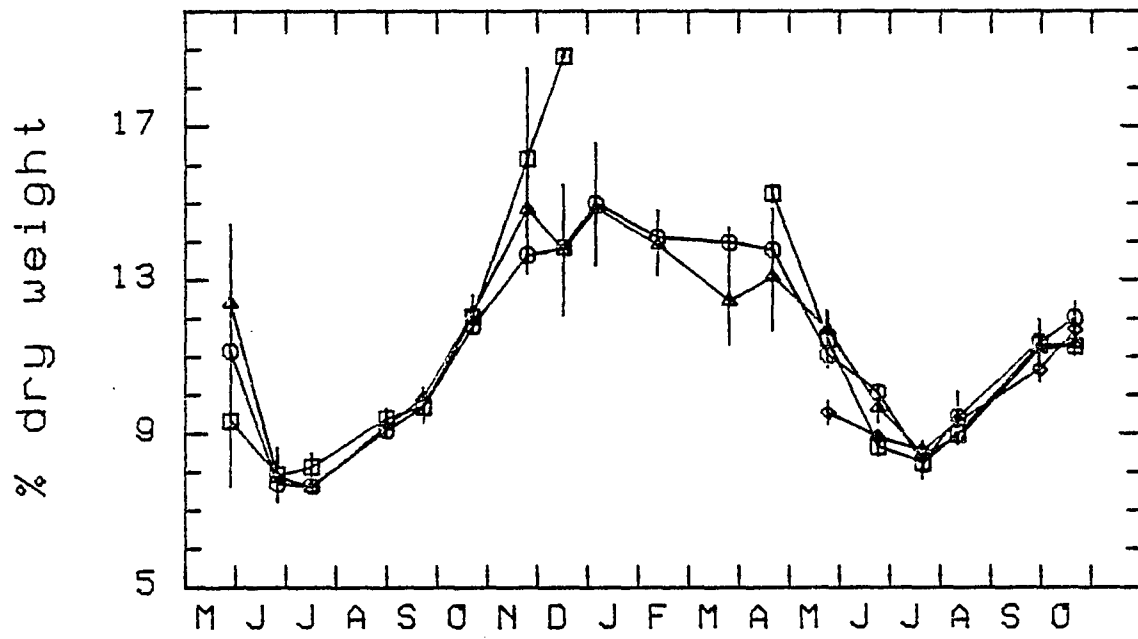


Figure 2-2

Figure 2-3. Seasonal variation of percent ash of Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate +1 SE for analytical errors.



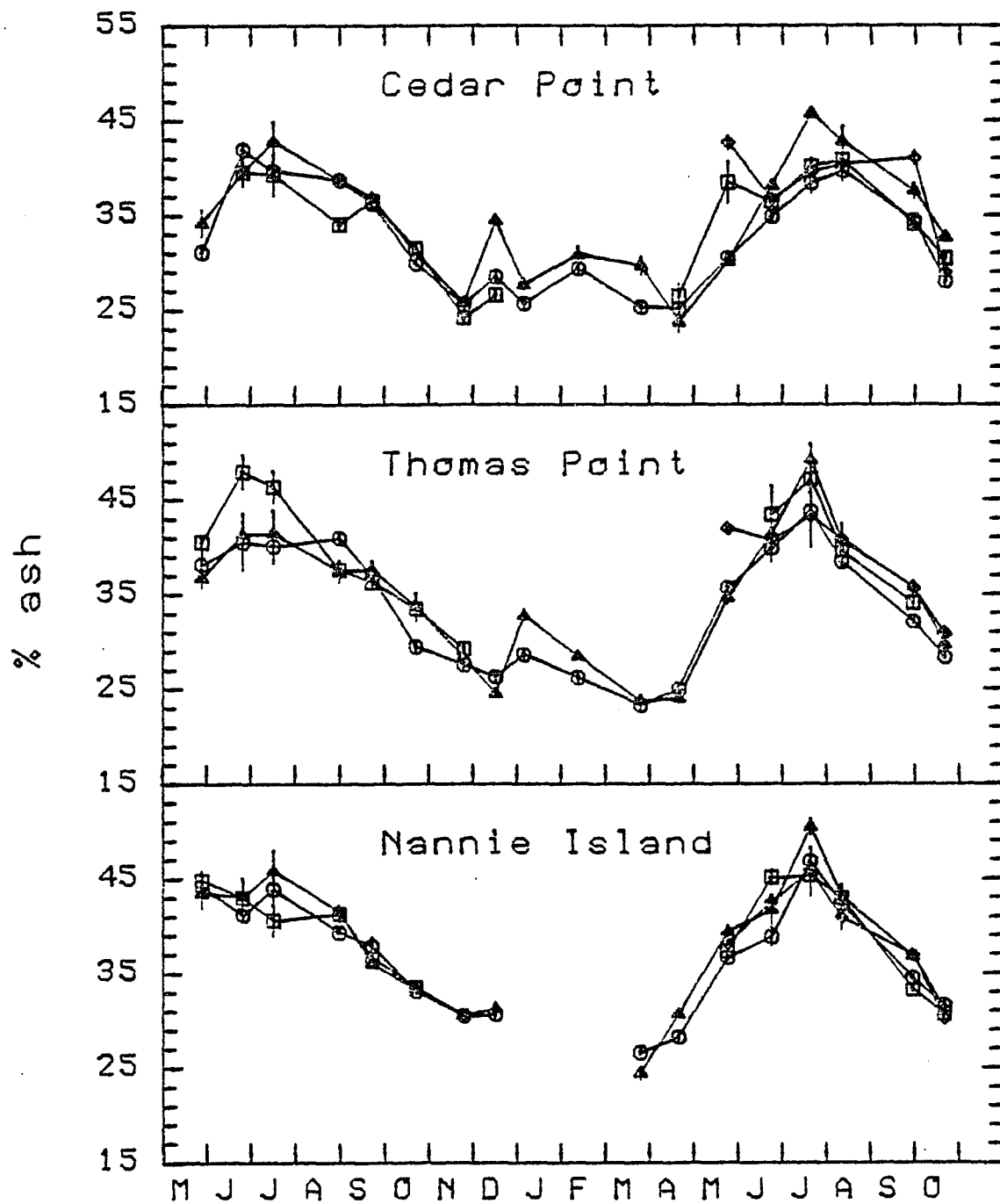


Figure 2-3

Figure 2-4. Average seasonal variation ( $\pm 1$  SE) of percent ash of Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

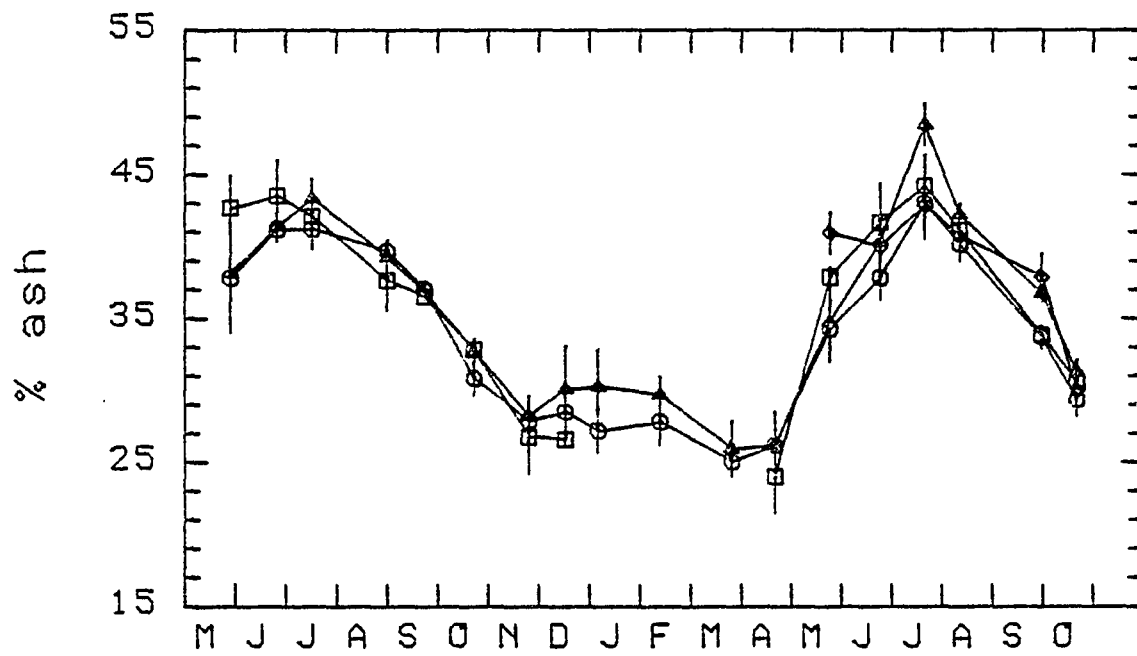


Figure 2-4

Figure 2-5. Seasonal variation of percent agar of Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.

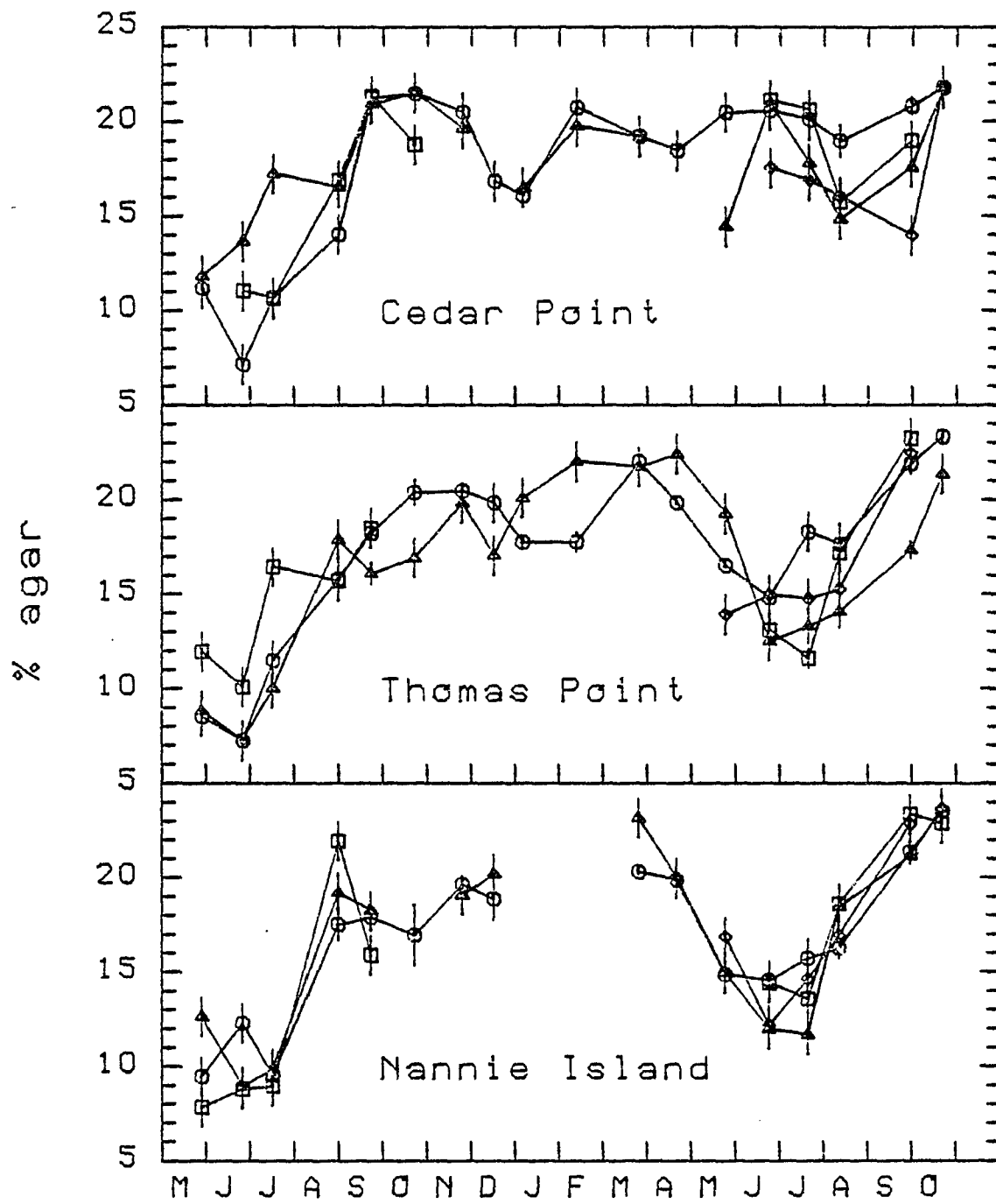


Figure 2-5

Figure 2-6. Average seasonal variation ( $\pm 1$  SE) of percent agar of Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

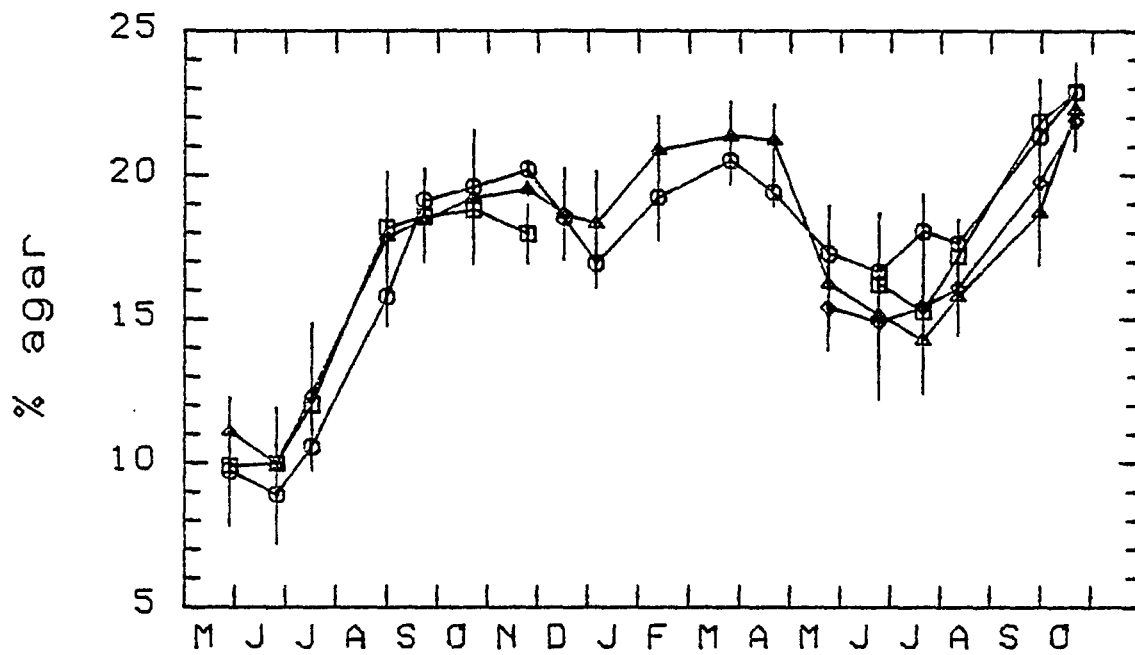


Figure 2-6

Figure 2-7. Seasonal variation of percent carbohydrate of Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.



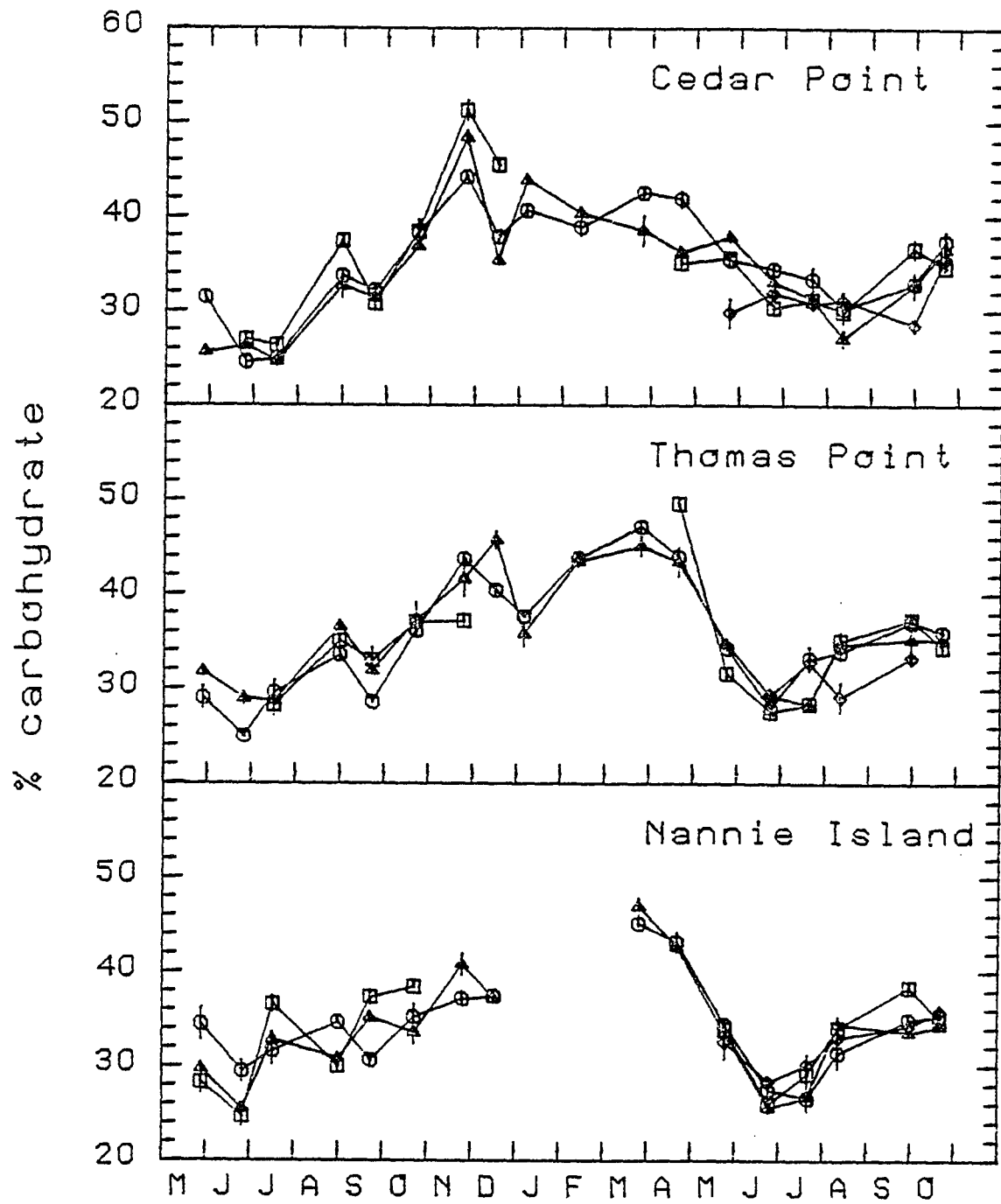


Figure 2-7

Figure 2-8. Average seasonal variation ( $\pm 1$  SE) of percent carbohydrate of Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

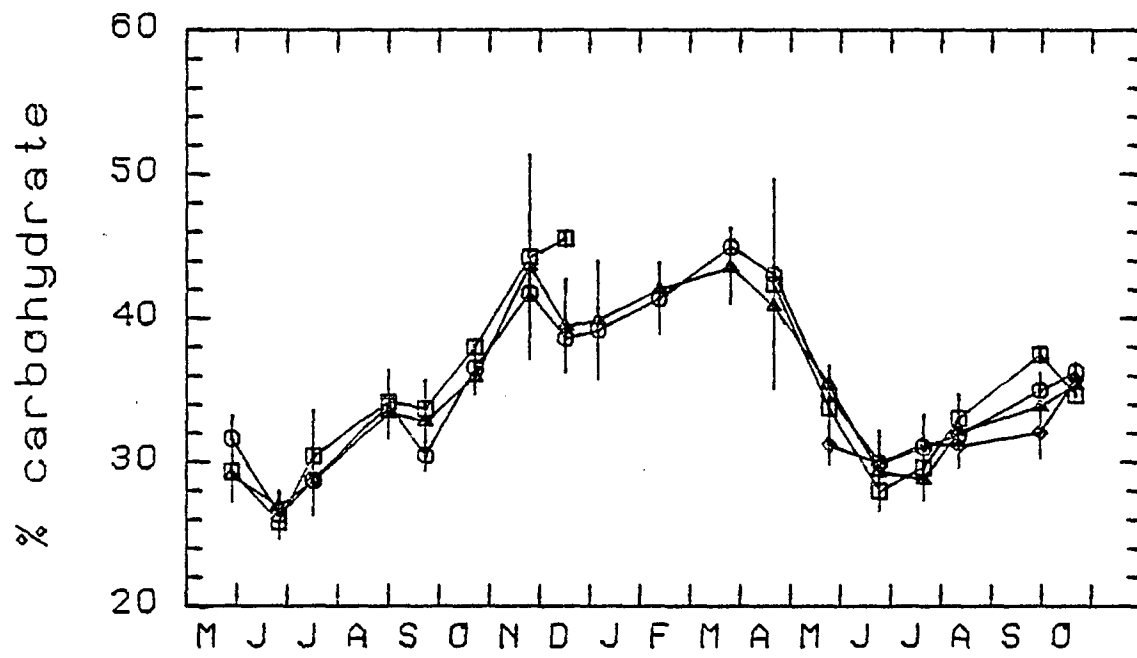


Figure 2-8

Figure 2-9. Seasonal variation of percent protein of Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.

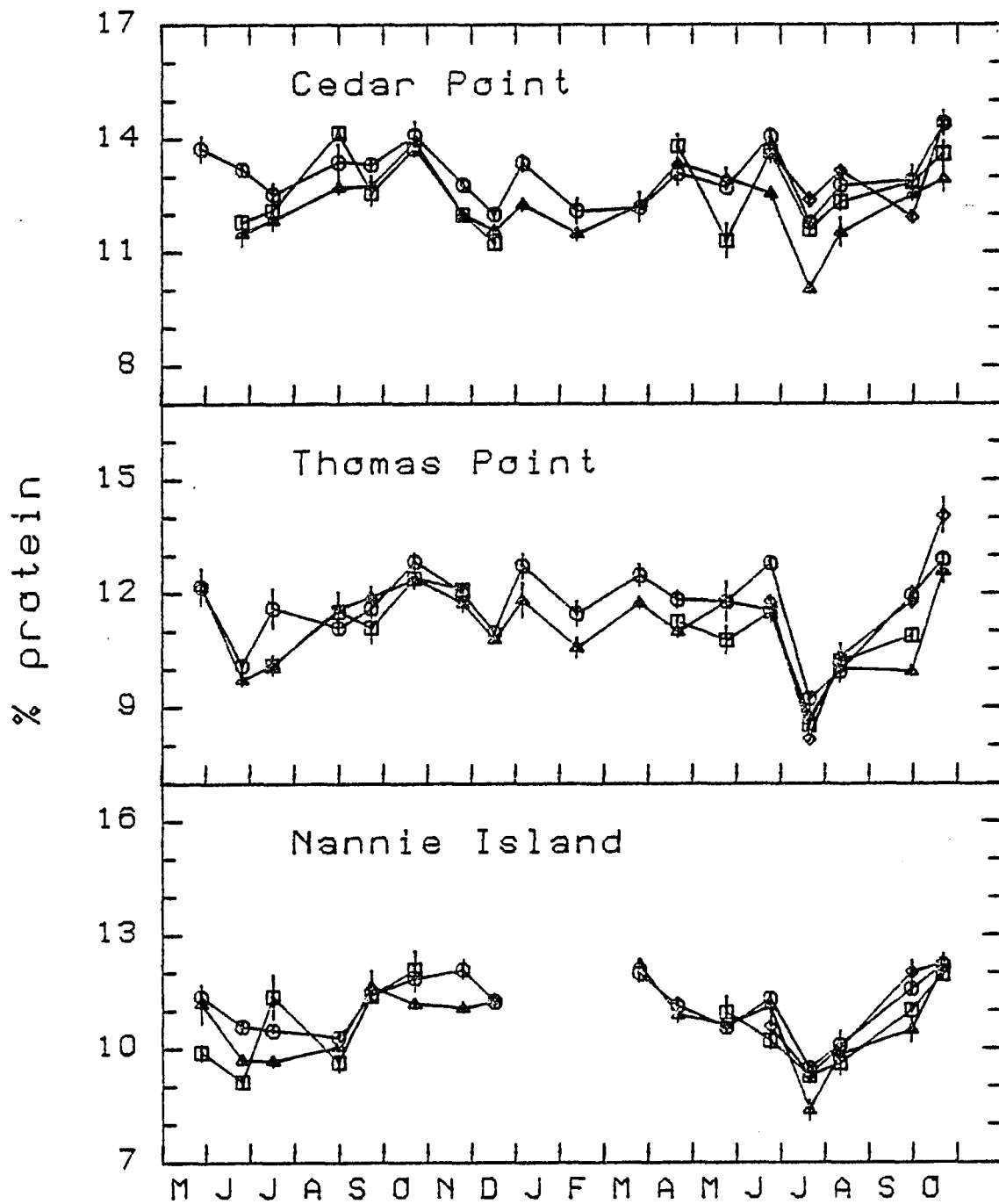


Figure 2-9

Figure 2-10. Average seasonal variation ( $\pm 1$  SE) of percent protein of Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

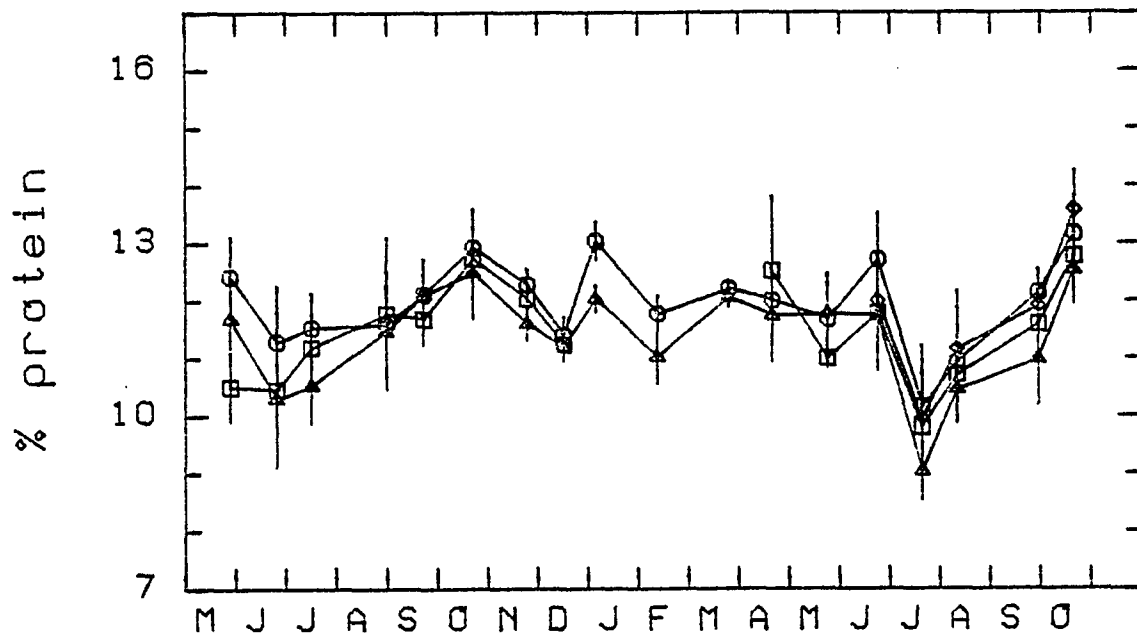


Figure 2-10

Figure 2-11. Seasonal variation of percent carbon of Gracilaria tikvahiae plants from Thomas Point. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.



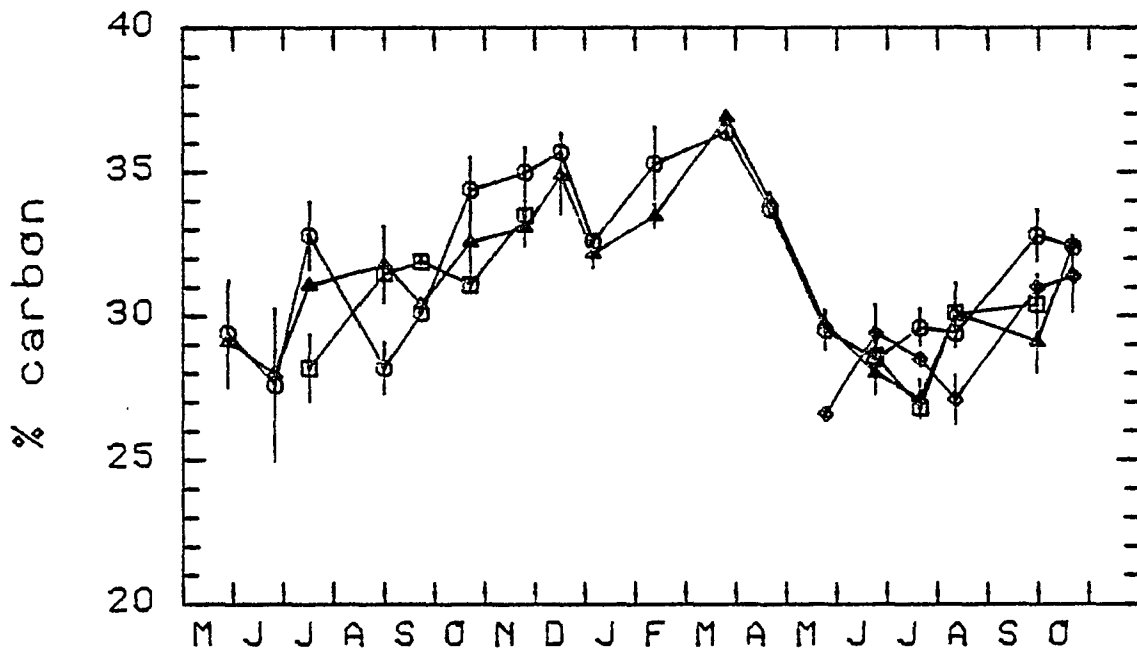


Figure 2-11

Figure 2-12. Seasonal variation of percent nitrogen of Gracilaria tikvahiae plants from Thomas Point. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate +1 SE for analytical errors.

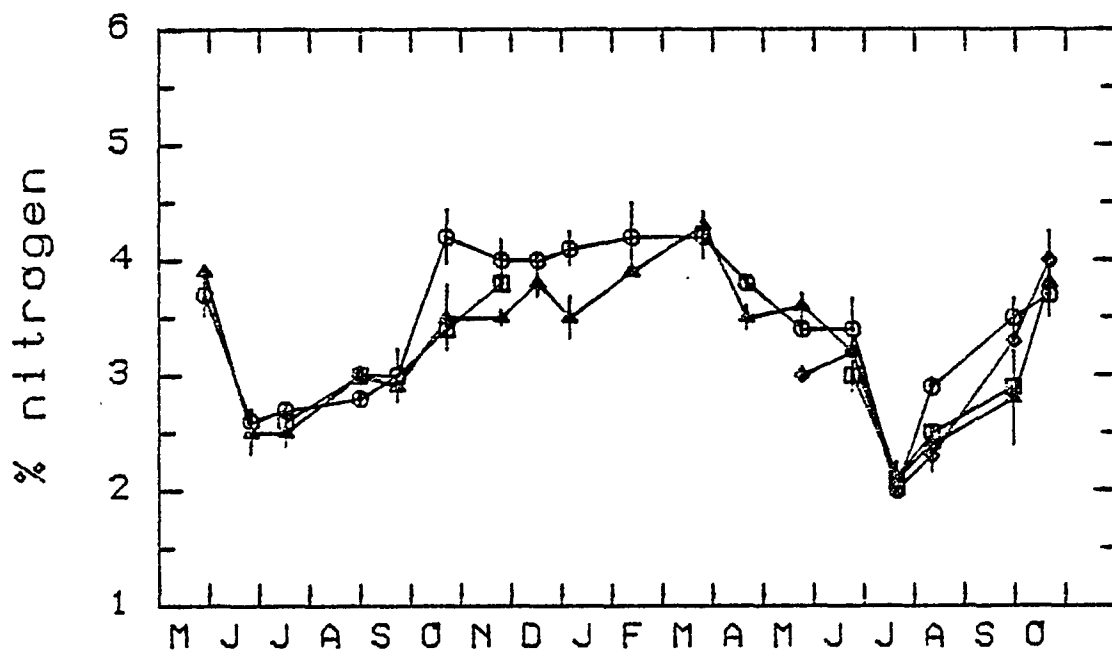


Figure 2-12

Figure 2-13. Seasonal variation of percent phosphorus of Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.

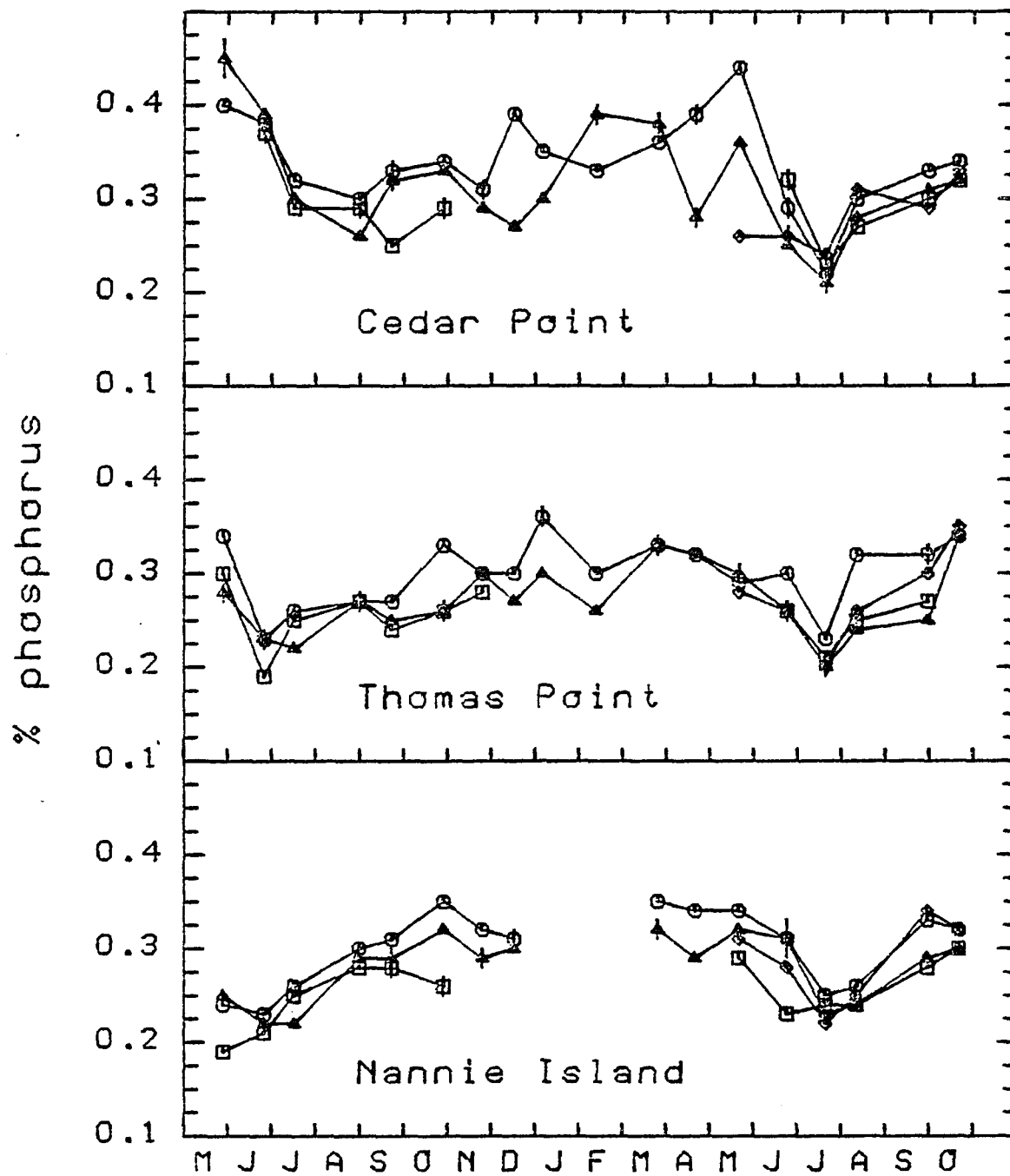


Figure 2-13

Figure 2-14. Average seasonal variation ( $\pm 1$  SE) of percent phosphorus of Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

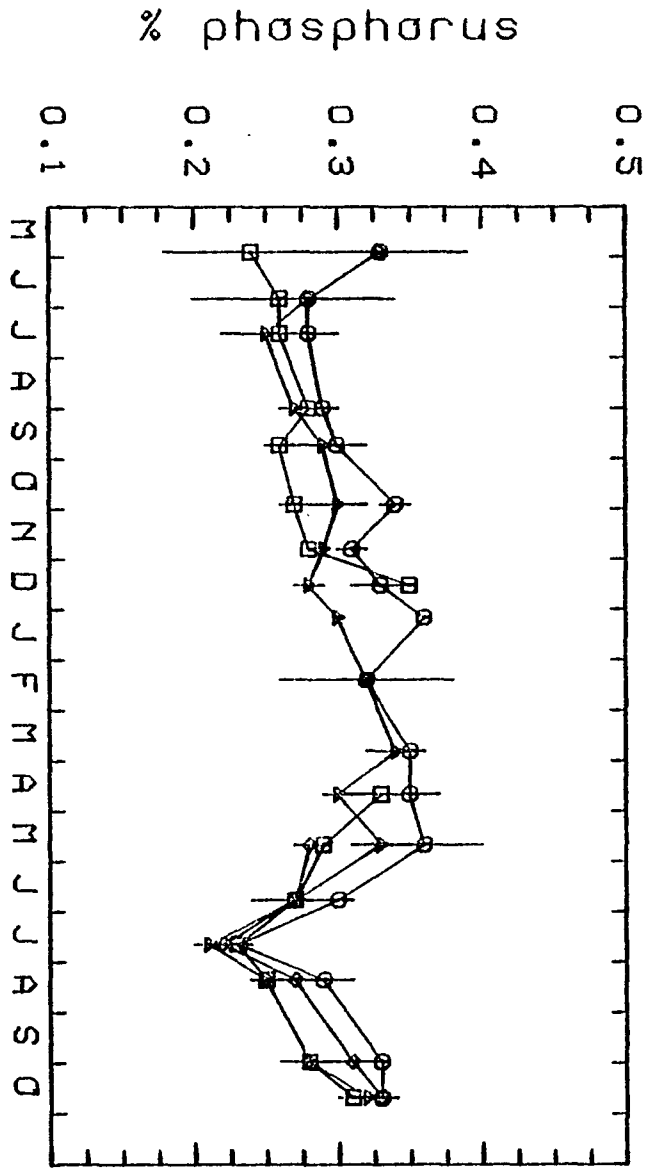


Figure 2-14

Figure 2-15. Seasonal variation of elemental ratios (by atoms and by weight) of Gracilaria tikvahiae plants from Thomas Point. a) Carbon/nitrogen, b) carbon/phosphorus, and c) nitrogen/phosphorus. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.



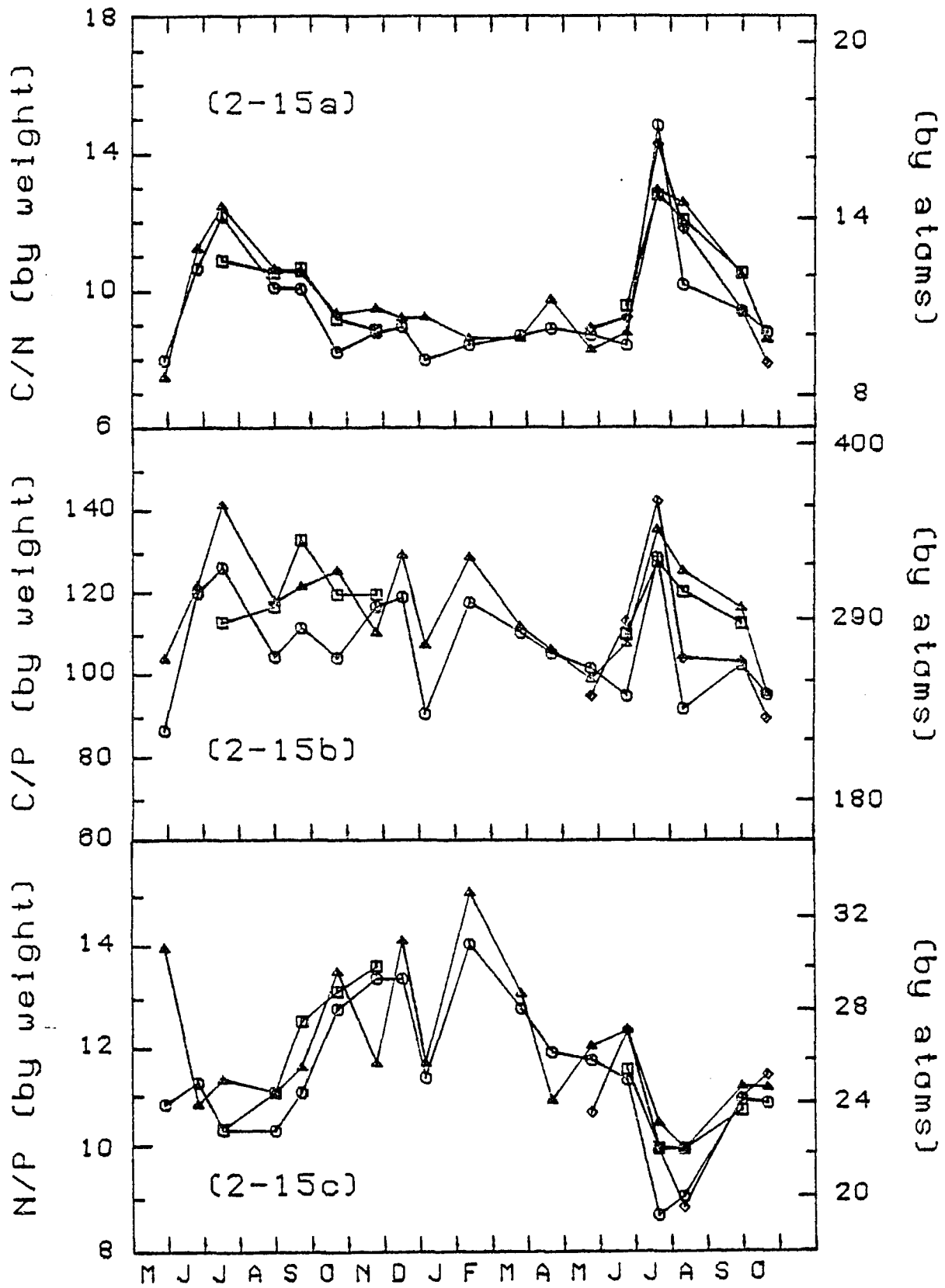


Figure 2-15

Figure 2-16. Seasonal variation of carbohydrate/protein ratios of Gracilaria tikvahiae plants from Thomas Point. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

carbohydrate/protein

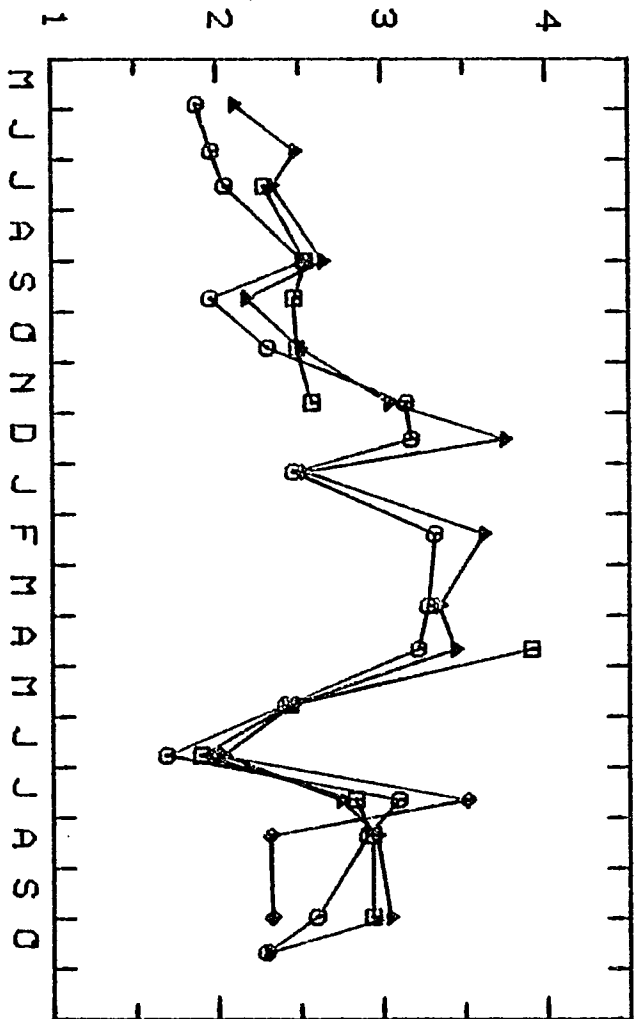


Figure 2-16

Figure 2-17. Seasonal variation of chlorophyll content (mg/g dry wt) of Gracilaria tikvahiae plants from Cedar Point (octagons), Thomas Point (triangles), and Nannie Island (squares). Error bars indicate  $\pm 1$  SE.

chlorophyll (mg/g dry wt)

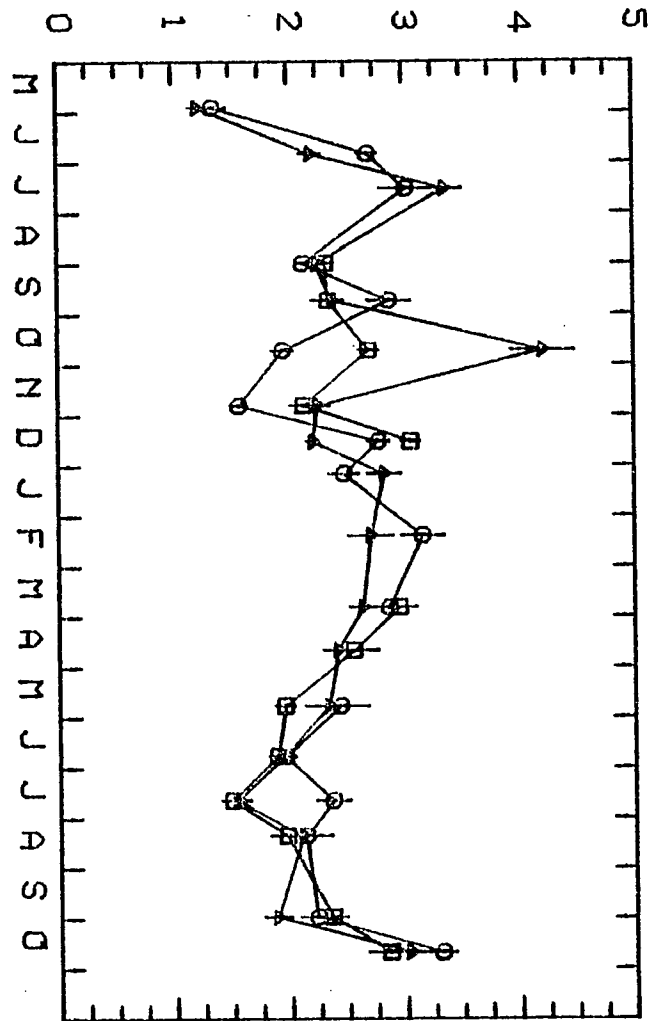


Figure 2-17

Figure 2-18. Seasonal variation of phycoerythrin content (mg/g dry wt) of Gracilaria tikvahiae plants from Cedar Point (octagons), Thomas Point (triangles), and Nannie Island (squares). Error bars indicate +1 SE.

phycoerythrin (mg/g dry wt)

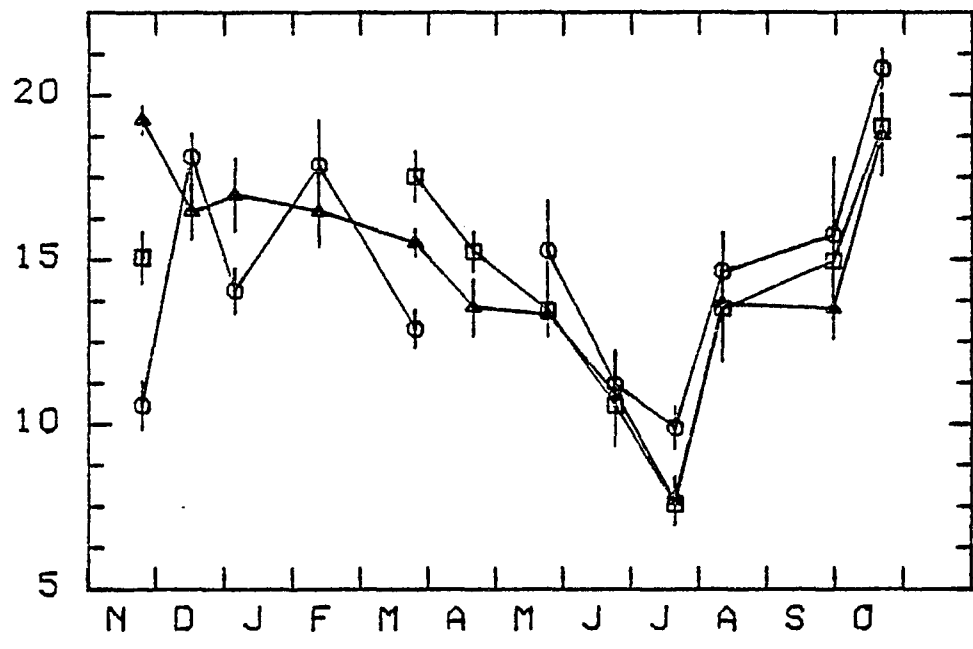


Figure 2-18

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ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN  
(GIGARTINALES, RHODOPHYTA) IN THE  
GREAT BAY ESTUARY, NEW HAMPSHIRE.

PART 3.

VARIATION IN AGAR PROPERTIES.

### 3.1 INTRODUCTION

Agar is a heterogeneous, polydisperse phycocolloid with a backbone composed of alternating 1,3-linked B-D-galactopyranose and 1,4-linked 3,6-anhydro-a-L-galactopyranose residues (Guiseley 1968, Yaphe and Duckworth 1972, McCandless 1981). The repeating, neutral disaccharide typifies the limit polysaccharide, agarose (Percival and McDowell 1967, Arnott et al. 1974, Guiseley and Renn 1977). However, the regular structure may be masked by a variety of side groups, including ester sulfate, methoxyl, pyruvic acid, and carboxyl residues (Hong et al. 1969, Duckworth et al. 1971, Duckworth and Yaphe 1971a, 1971b, Young et al. 1971, Izumi 1972, Yaphe and Duckworth 1972, Guiseley and Renn 1977, McCandless 1981). Variations from the limit agarose molecule result in a wide range of agar polysaccharides with varied composition and properties, dependent upon the algal source (i.e. within the Rhodophyceae) of the agar and the specific extraction techniques employed (Yaphe and Duckworth 1972, Guiseley and Renn 1977). In general, three extreme structural types can be described for agar: (1) neutral agarose, (2) pyruvated agarose with little ester sulfate, and (3) galactans with much sulfate but no 3,6-anhydrogalactose or pyruvate

(Duckworth and Yaphe 1971a, Yaphe and Duckworth 1972, McCandless 1981). In addition, Gracilaria agars, in particular, may contain large amounts of methoxyl residues as 6-O-methyl-D-galactose (Izumi 1972, Duckworth and Yaphe 1971a).

Carrageenans are sulphated polysaccharides similar to agar in structure (Percival and McDowell 1967, Guiseley 1968, McCandless 1981). In a variety of carrageenophytes, specific fractions of carrageenan have been shown to be restricted, within a given species, to individual haploid or diploid plants (McCandless et al. 1973, 1975, Hosford and McCandless 1975, Pickmere et al. 1975, Waaland 1975, Parsons et al. 1977, McCandless 1981). For example, with Chondrus crispus, lambda-carrageenan is restricted to the tetrasporic stage, while kappa-carrageenan is limited to gametophytes (McCandless et al. 1973). In contrast, species of Eucheuma, which contain either iota or kappa-carrageenan, do not show such ploidy level polysaccharide distinctions (Dawes et al. 1977, DiNinno and McCandless 1978, Doty and Santos 1978, Dawes 1979).

Interest has arisen in chemical and/or physical property differences between the agar from haploid and diploid plants of Gracilaria species. Thus, while Kim and Henriquez (1979), Whyte and Englar (1979a), and Whyte et al. (1981) have found differences in agar yield and gel strength between cystocarpic and tetrasporic plants of

G. verrucosa, Hoyle (1978a) did not find such differences in G. coronopifolia and G. bursapastoris. Penniman (1977) suggested that G. tikvahiae from the Great Bay Estuary, New Hampshire, did not show differences in agar yield between haploid and diploid plants. In the present paper, further data is presented concerning chemical and physical properties of agar from G. tikvahiae. Further, while information is available regarding the composition of agar from aquacultured G. tikvahiae (K. Bird et al. 1981), much less is known of agar from natural populations of the same macroalga (Asare 1979, 1980).

### 3.2 METHODS

The methods used for collecting Gracilaria tikvahiae samples from the Great Bay Estuary and the subsequent agar extractions were described previously in Parts 1 and 2, respectively. In the present section, the methods used to analyze several physical and chemical properties of agar are outlined. Although most of the agar extractions employed an alkaline pretreatment stage (see Part 2), several extractions were performed without this step. A comparison of corresponding agar properties of samples extracted with and without alkaline pretreatment is described. All of the analyses were performed on G. tikvahiae agar samples as well as on a sample of Difco Bacto-agar used as an internal standard.

#### 3,6-ANHYDROGALACTOSE

Triplicate portions (5-12 mg) of agar samples were dissolved by boiling in 100 ml distilled water. Aliquots of these solutions were then analyzed for 3,6-anhydrogalactose content with the methods of Yaphe and Arsenault (1965) as described by Craigie and Leigh (1978). D-fructose was used as a standard. Absorbances (555 nm) were measured in 1.0 cm pyrex cuvettes with a Beckman model 35 spectrophotometer.



## SULFATE

The ester sulfate content of the agar samples was measured with the procedures of Jackson and McCandless (1978). Triplicate portions (10-20 mg) of agar samples were hydrolyzed in 1.0N HCl in sealed tubes at 100°-105°C for 3 hr. Aliquots of these digests were analyzed for sulfate content by measuring the turbidity produced following addition of barium chloride (Jackson and McCandless 1978). Ammonium sulfate was used as a standard. Turbidity was measured in 1.0 cm pyrex cuvettes at 500 nm with a Beckman model 35 spectrophotometer. Several parallel sulfate analyses using a gravimetric technique, following precipitation of the hydrolyzed sulfate with barium chloride, were conducted to confirm the results of the turbidimetric analyses (Guiseley and Renn 1977). Sulfate analyses were performed on all eighteen monthly samples from Thomas Point but only on every third monthly sample from Cedar Point and Nannie Island (as described in Part 2, Carbon and Nitrogen Methods).

## PYRUVATE

An attempt was made to analyze the pyruvate content, 4,6-O-(1-carboxyethylidene)-D-galactose, of agar samples using the lactate dehydrogenase method (Duckworth and Yaphe 1970). However, no pyruvate was detected in Gracilaria tikvahiae agar samples. Young (1974) did not detect

pyruvate by this method in Nova Scotian G. tikvahiae agar. However, Duckworth et al. (1971) and Young et al. (1971) found 0.10% - 0.13% pyruvate in agar from this species. Craigie and Leigh (1978) mention difficulty obtaining stable absorbance values with the lactate dehydrogenase method.

#### ASH

The ash content was measured in triplicate (25-50 mg) portions of agar samples. Each replicate was combusted in a porcelain crucible for 48 hr at 500°C in a muffle furnace.

#### GEL STRENGTH

The gel strengths ( $\text{g}/\text{cm}^2$ ) of 1% gels of agar samples were measured with a 1.0  $\text{cm}^2$  plunger on a Marine Colloids gel tester (Marine Colloids Division, FMC Corporation, Rockland, Maine). Gel strength is defined as the force required to fracture a 1% agar gel (Guiseley and Renn 1977). The gels were prepared in 70 mm x 50 mm pyrex crystallizing dishes, then allowed to stand for 2 hr at 10°C. After inverting the gel in the crystallizing dish, gel strength was measured as described above. As each analysis required a rather large amount of agar (1.8 g), gel strengths were not determined on all agar samples.

## VISCOSITY

The viscosities of 1% solutions of triplicate portions of agar samples were determined. Measurements were made at 65°C using a Brookfield model LVT with cone plate microviscometer (Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts). Viscosities were measured on selected samples as described previously under the sulfate methods.

## STATISTICAL ANALYSES

The chemical and physical properties of agar from Gracilaria tikvahiae were plotted for the eighteen month period of study. Missing points indicate either the lack of analysis on a specific sample or the lack of collection due to ice cover (see Part 1).

The seasonal curves for the properties of agar from cystocarpic and tetrasporic plants were analyzed by a periodic multiple regression model (Hackney and Hackney 1977, 1978) described in Part 2. As the periodic model for ash and viscosity data was not significant ( $p > 0.05$ ), the data were analyzed by two-way ANOVA via multiple regression. All regressions and ANOVA were performed using MINITAB (Ryan et al. 1976), SPSS (Nie et al. 1975) and/or the BMD04R routine from the BMD statistical package (Dixon 1977). Correlations between agar properties, plant tissue chemistry and environmental variables were calculated with the SPSS

statistical package (Nie et al. 1975). All regression and correlation analyses were calculated with arcsine transformed data (Sokal and Rohlf 1981).

### 3.3 RESULTS

#### 3,6-ANHYDROGALACTOSE

Variations in 3,6-anhydrogalactose content (Figures 3-1 and 3-2) had a significant periodic component (Table 3-I), indicating a strong seasonal cycle, with winter-spring minima (34%) and summer maxima (48%). There were no significant differences in 3,6-anhydrogalactose content between cystocarpic and tetrasporic plants (Table 3-I). The 3,6-anhydrogalactose content of a Difco Bacto-agar sample, used as an internal standard, was 45.02% ( $\pm 0.02$ , SE).

#### SULFATE

Ester sulfate content of agar from Gracilaria tikvahiae varied from 3.5% to 5.8% (Figure 3-3). There was a significant annual cycle (Table 3-I) with high summer and low winter values, although some exceptions were apparent. No significant differences in sulfate content were evident between cystocarpic and tetrasporic plants (Table 3-I). The Difco Bacto-agar internal standard had 2.86% sulfate ( $\pm 0.06$ , SE).

## ASH

Although there were monthly differences in ash content (Figures 3-4 and 3-5), the variations did not follow a periodic cycle (Table 3-I). Ash values generally varied from 4% - 9% (Figure 3-5). There were no differences in ash content of agar from cystocarpic and tetrasporic Gracilaria tikvahiae plants. The ash content of the Difco Bacto-agar sample was 4.93% (+0.04, SE).

## GEL STRENGTH

Gel strengths (Figure 3-6) of Gracilaria tikvahiae agar samples were generally low from fall through spring (<160 g/cm<sup>2</sup>) and higher during the summer (>200 g/cm<sup>2</sup>). There was a significant periodic component to the seasonal variation (Table 3-I). No significant differences in the annual cycle were noted between agar from cystocarpic and tetrasporic plants (Table 3-I). The gel strength of the Difco Bacto-agar sample was 159 g/cm<sup>2</sup> (+4.2, SE).

## VISCOSITY

The viscosities of 1% Gracilaria tikvahiae agar solutions (at 65°C) were between 5 and 20 centipoise. There was no regular periodic component to the annual variation (Table 3-I). However, differences between months were significant (Table 3-I). No significant differences in viscosities of agar from cystocarpic or tetrasporic plants

were noted (Table 3-I). The viscosity of the Difco Bacto-agar sample was 3.2 cp ( $\pm 0.05$ , SE).

#### CORRELATION ANALYSES

Simple correlations between Gracilaria tikvahiae agar properties and several parameters describing plant chemistry, growth, and environmental conditions are listed in Table 3-II. There was a significant negative correlation between 3,6-anhydrogalactose and ester sulfate content ( $r=-0.38$ ) and between gel strength and ester sulfate ( $r=-0.25$ ). A positive correlation was apparent between gel strength and 3,6-anhydrogalactose content ( $r=0.54$ ). Agar yields were negatively correlated with 3,6-anhydrogalactose content ( $r=-0.48$ ) and gel strength ( $r=-0.75$ ), but positively correlated with sulfate ( $r=0.37$ ). Total plant tissue carbon, nitrogen and phosphorus were all negatively correlated with 3,6-anhydrogalactose and gel strength (Table 3-II). Plant ash content and growth rate were positively correlated with both 3,6-anhydrogalactose and gel strength of Gracilaria tikvahiae agar (Table 3-II). Ambient water temperature was positively correlated with 3,6-anhydrogalactose ( $r=0.45$ ), gel strength ( $r=0.41$ ), and agar ash contents ( $r=0.27$ ). The opposite relationship was apparent for these parameters and ambient total dissolved inorganic nitrogen (Table 3-II).

COMPARISONS OF AGAR EXTRACTED WITH  
AND WITHOUT HYDROXIDE PRETREATMENT

Agar yields between parallel extractions with and without hydroxide pretreatment were generally comparable (Table 3-III). However, one sample (i.e. vegetative plants from Thomas Point, September 1976) had lower agar content when extracted with alkaline pretreatment. The 3,6-anhydrogalactose content of vegetative, cystocarpic and tetrasporic Gracilaria tikvahiae plants was greater in agar samples extracted with hydroxide pretreatment (Table 3-III). Agar ash and ester sulfate contents were consistently reduced after extraction with hydroxide pretreatment (than without this step). In contrast, gel strength was greater in hydroxide treated agar samples from vegetative and cystocarpic plants, but less in agar from tetrasporic plants (Table 3-III). With one exception (i.e. agar from vegetative plants collected at Thomas Point, September 1976), viscosities were always lower in agar extracted with hydroxide pretreatment.



### 3.4 DISCUSSION

The 3,6-anhydrogalactose and ester sulfate contents of agar from Great Bay Gracilaria tikvahiae varied seasonally. Asare (1979, 1980) found similar cycles for Rhode Island G. tikvahiae agar. However, as his agar extraction techniques did not include a hydroxide pretreatment (as in the present study), comparisons between the two studies are inappropriate. It is interesting to note, however, that the sulfate values for Rhode Island G. tikvahiae (Asare 1979, 1980) were generally lower than those from the present study. The opposite would have been expected as hydroxide pretreatment (used in the present study) can remove 6-O-sulfate from L-galactose, producing 3,6-anhydrogalactose (McCandless 1981). The increase in 3,6-anhydrogalactose and concomitant decrease in ester sulfate content from agar extracted with hydroxide pretreatment has been demonstrated with a variety of Gracilaria species (Hong et al. 1969, Duckworth et al. 1971, Tagawa and Kojima 1972, Young 1974, Whyte and Englar, 1976, 1979b, 1980).

No significant differences were apparent in the seasonal cycles of 3,6-anhydrogalactose or ester sulfate contents cystocarpic or tetrasporic plants of Great Bay Gracilaria tikvahiae. Whyte and Englar (1979a) and Whyte et

al. (1981) describe similar variations of these agar components in vegetative, tetrasporic and gametophytic plants of a Gracilaria species from British Columbia.

As mentioned by Asare (1979), it is apparent from the large seasonal variation in agar components, such as 3,6-anhydrogalactose or sulfate, that to adequately describe the composition of agar from an individual algal species, the annual cycle must be considered. The ranges of Gracilaria tikvahiae agar components are greater than differences between some Gracilaria species for these same components (Duckworth et al. 1971). Thus, the values for 3,6-anhydrogalactose and ester sulfate content in Great Bay G. tikvahiae are comparable to those for several other Gracilaria species (Hong et al. 1969, Duckworth et al. 1971, Whyte and Englar 1976, 1979a, 1979b, 1980, Whyte et al. 1981).

The ash content of agar in Gracilaria tikvahiae from the Great Bay Estuary varied between 4% and 9%. As would be expected there was a positive correlation between agar ash and sulfate contents. Accordingly, the ash content was lower in agar extracted with a hydroxide pretreatment step. A similar observation was noted for several other Gracilaria species (Hong et al. 1969).

The gel strengths of Great Bay Gracilaria tikvahiae agar were greatest during the summer. Seasonal variation in gel strength has been noted for other Gracilaria species

(Kim and Humm 1965, Oza 1978, Lindsay and Saunders 1980, Yang et al. 1981, Yang 1982). The range of gel strength values in the present study corresponded to other reports for G. tikvahiae (Duckworth et al. 1971). There were no differences in the seasonal cycles of gel strength between cystocarpic and tetrasporic plants of Great Bay G. tikvahiae. Such results agree with those of Hoyle (1978a) for Hawaiian G. bursapastoris and G. coronopifolia, but they contrast with the differences in gel strength between gametophytes and tetrasporophytes of Gracilaria sp. (Whyte and Englar 1979a, Whyte et al. 1981), and G. verrucosa (Kim and Henriquez 1979). Comparisons between gels strength values of agar from various algal species are difficult due to the variety of extraction and gel strength techniques used (Yaphe and Duckworth 1972). In general, the gel strengths of Gracilaria tikvahiae agar are somewhat lower than for either G. verrucosa or G. sjoestedtii (Abbott 1980, Durairatnam and Santos 1981). The agar of G. tikvahiae will form gels of comparable strength to those of the closely related species (C. Bird and McLachlan 1982), G. bursapastoris (Hoyle 1978a, 1978b).

The annual cycle of gel strength for Great Bay Gracilaria tikvahiae was correlated with its 3,6-anhydrogalactose content. In contrast, K. Bird et al. (1981) found two conflicting correlations between 3,6-anhydrogalactose and gel strength (i.e.  $r=-0.94$  and  $r=0.23$ ) in agars from two series of aquaculture experiments with G. tikvahiae. In

accord with the results of Matsushashi (1977) and K. Bird et al. (1981), there was a significant negative correlation between gel strength and agar yield in Great Bay G. tikvahiae. However, contrasting relationships between gel strength and total plant tissue nitrogen were evident between the present study ( $r=-0.55$ ) and that of K. Bird et al. (1981). Furthermore, while sulfate was negatively correlated with gel strength in the present study, the opposite relationship was reported by K. Bird et al. (1981). As discussed in Part 2, there appeared to be contrasting relationships between the responses of in situ New Hampshire and aquacultured Florida populations of Gracilaria tikvahiae.

In the present study, hydroxide pretreatment during agar extractions increased the gel strength of agar from vegetative and cystocarpic plants, but decreased it in agar from tetrasporic plants. The differences between extractions with and without hydroxide pretreatment were generally within experimental error. Whyte and Englar (1980) observed that contrasting changes in gel strength of agars extracted with and without hydroxide pretreatment differed among the various morphotypes of British Columbia Gracilaria. Further research into these differences in Gracilaria tikvahiae agar is warranted, however. The increase in gel strength due to alkaline treatment during agar extraction is attributable to an increase in the agarobiose subunits in the agar molecules, thus enhancing

the relative proportion of agarose (i.e. gelling) molecules (Yaphe and Duckworth 1972, McCandless 1981). The phenomenon is apparent with agar from a variety of Gracilaria species (Hong et al. 1969, Matsushashi and Hayashi 1972, Tagawa and Kojima 1972, Durairatnam and Santos 1981). As mentioned above, the opposite response (i.e. lower gel strength as a result of alkali modification) may occur, perhaps due to polymer degradation (Whyte and Englar 1980).

Agar viscosities varied substantially (i.e. 5 - 20 cp). Viscosities did not differ significantly between agar from cystocarpic or tetrasporic plants of Great Bay Gracilaria tikvahiae. Whyte and Englar (1979a) and Whyte et al. (1981) observed differences between the viscosities of gametophytic and tetrasporic British Columbia Gracilaria. The viscosity values in the present study are comparable to those of agar from other Gracilaria species (Young 1974, Whyte and Englar 1976, 1979b). In general, viscosities were lower in agar extracted with hydroxide pretreatment. Similar changes in viscosities were observed for agar from British Columbia Gracilaria by Whyte and Englar (1976, 1979b). However, Young (1974) found the opposite trend with agar from Nova Scotian G. tikvahiae.

In conclusion, Gracilaria tikvahiae agar (extracted with hydroxide pretreatment) had seasonal variations of 3,6-anhydrogalactose, ester sulfate, ash and gel strength. There were no significant differences in the annual cycles

of these properties dependent upon the reproductive stage of the alga. The latter observation is in contrast to Kim and Henriquez (1979), Whyte and Englar (1979a) and Whyte et al. (1981), but it is in agreement with Hoyle (1978a). Therefore, ploidy level agar differences within Gracilaria appear to be restricted to individual species. In the closely related species G. tikvahiae and G. bursapastoris, no differences in agar composition are apparent between haploid and diploid individuals (present study, Hoyle 1978a). However, in G. verrucosa (Kim and Henriquez 1979) and in other related species (Whyte and Englar 1979a, Whyte et al. 1981) such differences in agar composition may be present.

### 3.5 SUMMARY

- 1). There were no significant differences in 3,6-anhydrogalactose, ester sulfate, ash, gel strength or viscosity between agar extracted (with hydroxide pretreatment) from cystocarpic and tetrasporic plants of Gracilaria tikvahiae from the Great Bay Estuary.
- 2). Gel strength and 3,6-anhydrogalactose were greatest during the summer, while sulfate content was greatest during the winter.
- 3). Sulfate content was inversely related to gel strength, while the opposite relationship held for gel strength and 3,6-anhydrogalactose.
- 4). Agar yield was negatively correlated with both gel strength and 3,6-anhydrogalactose content.

Table 3-I. Regression (periodic model) and two-way analyses of variance tables for chemical and physical properties of Gracilaria tikvahiae agar with comparisons between cystocarpic and tetrasporic stages.

a) 3,6-anhydrogalactose

source	df	MS	F	
periodic	2	32.05	20.96	***
stage	1	2.63	1.72	ns
interaction	2	2.12	1.38	ns
residual	72	1.53		
$R^2=39.2$ ***				

b) Sulfate

source	df	MS	F	
periodic	2	38.42	5.20	*
stage	1	20.50	2.77	ns
interaction	2	0.05	0.01	ns
residual	36	7.39		
$R^2=26.8$ *				

c) Ash

source	df	MS	F	
month	17	10.70	3.76	***
stage	1	1.18	0.42	ns
interaction	10	2.93	1.03	ns
residual	46	2.84		
$R^2=53.4$ ***				



Table 3-I. (continued).

## d) Gel strength

source	df	MS	F	
periodic	2	82.65	8.31	**
stage	1	17.62	1.77	ns
interaction	2	4.63	0.47	ns
residual	36	9.94		
$R^2=34.9$ **				

## e) Viscosity

source	df	MS	F	
month	17	18.63	3.44	**
stage	1	8.08	1.49	ns
interaction	7	2.83	2.83	ns
residual	14	5.42		
$R^2=76.8$ ***				

\*\*\*  $p < 0.001$   
 \*\*  $0.001 < p < 0.01$   
 \*  $0.05 < p < 0.1$   
 ns  $p > 0.05$

(Note: ash and viscosity analyzed via two-way ANOVA multiple regression model, all other components analyzed via multiple regression ANOVA with periodic component.)

Table 3-II. Correlation analyses between Gracilaria tikvahiae agar properties, plant tissue chemistry and growth, and selected water hydrographic and nutrient parameters.

	3,6-AG	SO <sub>4</sub>	gel st.	visc.	ash <sub>a</sub>	agar
SO <sub>4</sub>	-0.385 14.8 ***	$\left[ \begin{array}{l} r \\ r^2 \times 100 \\ p \end{array} \right]$				
gel st.	0.544 29.5 ***			-0.254 6.5 *		
visc.	-0.027 0.1 ns	0.272 7.4 **	0.213 4.5 ns			
ash <sub>a</sub>	-0.121 1.5 ns	0.354 12.5 ***	0.032 0.1 ns	-0.133 1.8 ns		
agar	-0.476 22.7 ***	0.374 14.0 ***	-0.746 55.7 ***	0.148 2.2 ns	-0.266 7.1 ***	
C <sub>p</sub>	-0.629 39.6 ***	0.044 0.2 ns	-0.464 21.5 ***	-0.085 0.7 ns	-0.065 0.4 ns	0.574 32.9 ***
N <sub>p</sub>	-0.477 22.7 ***	-0.125 1.6 ns	-0.553 30.6 ***	-0.050 0.3 ns	-0.167 2.8 ns	0.513 26.3 ***
P <sub>p</sub>	-0.350 12.3 ***	-0.032 0.1 ns	-0.470 22.1 ***	-0.052 0.3 ns	0.056 0.3 ns	0.311 9.7 ***
ash <sub>p</sub>	0.518 26.9 ***	0.036 0.1 ns	0.528 27.8 ***	0.058 0.3 ns	0.190 3.6 **	-0.593 35.2 ***
growth	0.590 34.8 ***	0.129 1.7 ns	0.515 26.5 ***	0.148 2.2 ns	0.278 7.7 **	-0.476 22.6 ***

Table 3-II. (continued.)

	3,6-AG	SO <sub>4</sub>	gel st.	visc.	ash <sub>a</sub>	agar
temp.	0.451	0.099	0.411	0.016	0.267	-0.405
	20.4	1.0	16.9	0.0	7.1	16.4
	***	ns	***	ns	***	***
DIN	-0.422	-0.010	-0.398	-0.022	-0.270	0.300
	17.8	0.0	15.9	0.0	7.3	9.0
	***	ns	***	ns	**	***
PO <sub>4</sub> <sup>3-</sup>	-0.253	0.154	0.089	0.140	0.094	0.062
	6.4	2.4	0.8	2.0	0.9	0.4
	**	ns	ns	ns	ns	ns

Factors are: agar components, 3,6-AG - % 3,6-anhydro-galactose, SO<sub>4</sub> - % ester sulfate, ash<sub>a</sub> - agar % ash; agar properties, gel st. - gel strength (g/cm<sup>2</sup>), visc. - viscosity (cp); plant tissue chemistry, agar - % agar, C<sub>p</sub> - total % carbon, N<sub>p</sub> - total % nitrogen, P<sub>p</sub> - total % phosphorus, ash<sub>p</sub> - % plant ash; growth - growth rate (%/day); ambient hydrographic and water nutrient parameters, temp. - water temperature (°C), DIN - total dissolved inorganic nitrogen (ug-at N/L), PO<sub>4</sub><sup>3-</sup> - dissolved phosphate (ug-at P/L). For significance levels see Table 3-I.

Table 3-III. Comparison of agar yields from Gracilaria tikvahiae plants from Thomas Point and associated composition and properties of corresponding extractions with and without hydroxide pretreatment.

	vegetative		cystocarpic		tetrasporic	
	with OH <sup>-</sup>	no OH <sup>-</sup>	with OH <sup>-</sup>	no OH <sup>-</sup>	with OH <sup>-</sup>	no OH <sup>-</sup>
yield (%)	18.24 <	26.93 <sup>(1)</sup>	12.52 <	13.02 <sup>(3)</sup>	13.09 >	11.57 <sup>(3)</sup>
	21.92 <	24.27 <sup>(2)</sup>	13.31 >	9.50 <sup>(4)</sup>	11.61 >	11.06 <sup>(4)</sup>
3,6-AG (%)	42.93 >	38.80	43.62 >	40.70	-	-
	41.16 >	36.41	43.93 >	41.36	44.33 >	43.44
SO <sub>4</sub> (%)	4.50 <	6.63	4.14 <	6.68	4.48 <	6.15
	4.37 <	5.99	4.70 <	7.03	4.23 <	5.34
ash (%)	5.62 <	8.00	4.59 <	7.03	-	-
	4.24 <	6.82	4.36 <	8.42	4.41 <	5.96
gel st. (g/cm <sup>2</sup> )	117 >	139	164 >	138	191 <	214
	114 >	83	-	-	214 <	259
visc. (cp)	19.4 >	13.6	5.2 <	42.9	14.5 <	28.7
	9.8 <	32.7	11.3 <	39.6	10.6 <	27.1

- (1) September 1976 collection  
 (2) September 1977  
 (3) June 1977  
 (4) July 1977

Figure 3-1. Seasonal variation of percent 3,6-anhydrogalactose content of agar from Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.

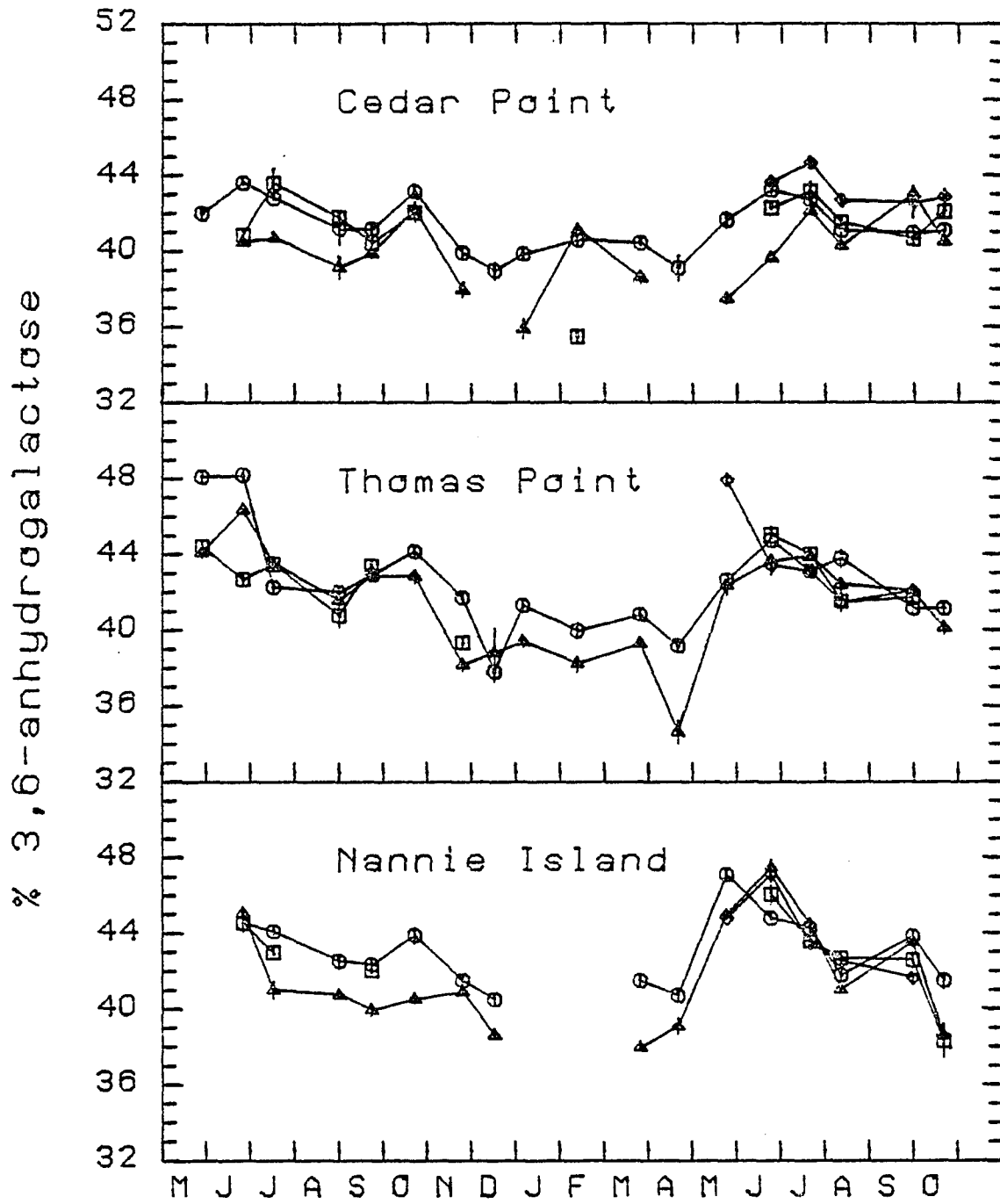


Figure 3-1

Figure 3-2. Average seasonal variation ( $\pm 1$  SE) of percent 3,6-anhydrogalactose content of agar from Gracilaria tikvahiae plants in the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

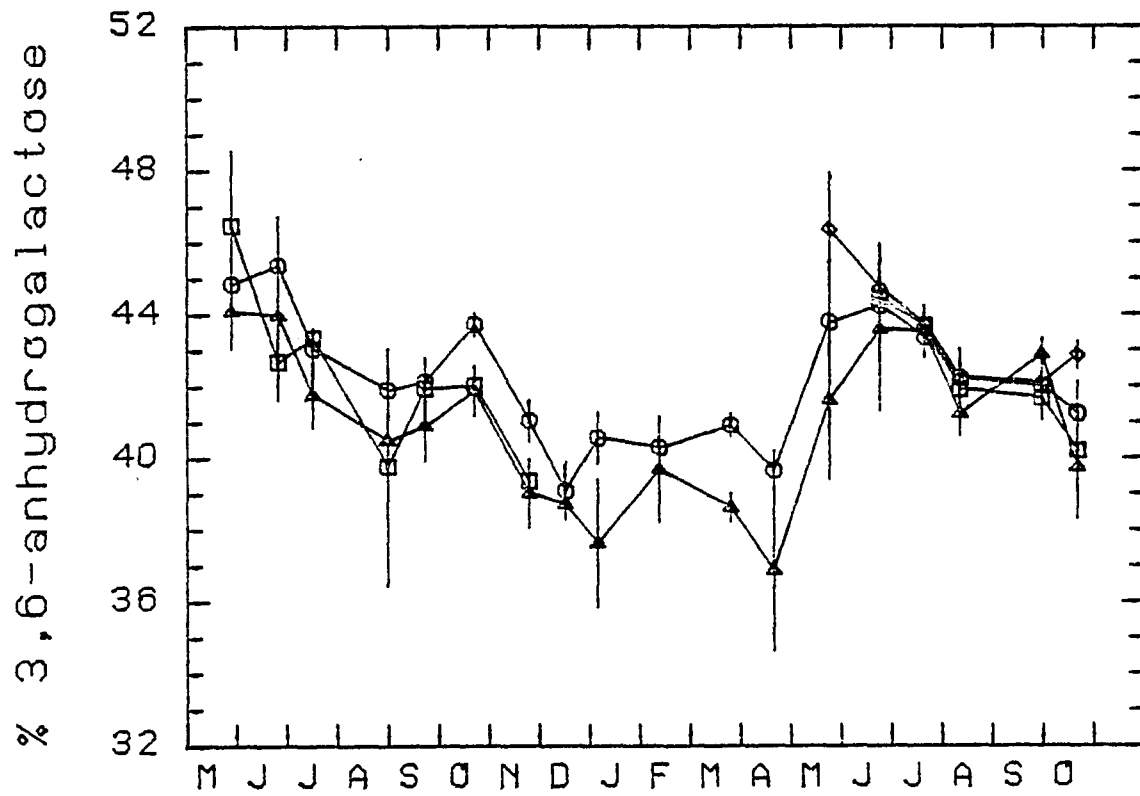


Figure 3-2



Figure 3-3. Seasonal variation of percent ester sulfate content of agar from Gracilaria tikvahiae plants from Thomas Point. Vegetative (octagons), cystocarpic (triangles), and tetrasporic (squares) plants. Error bars indicate +1 SE for analytical errors.

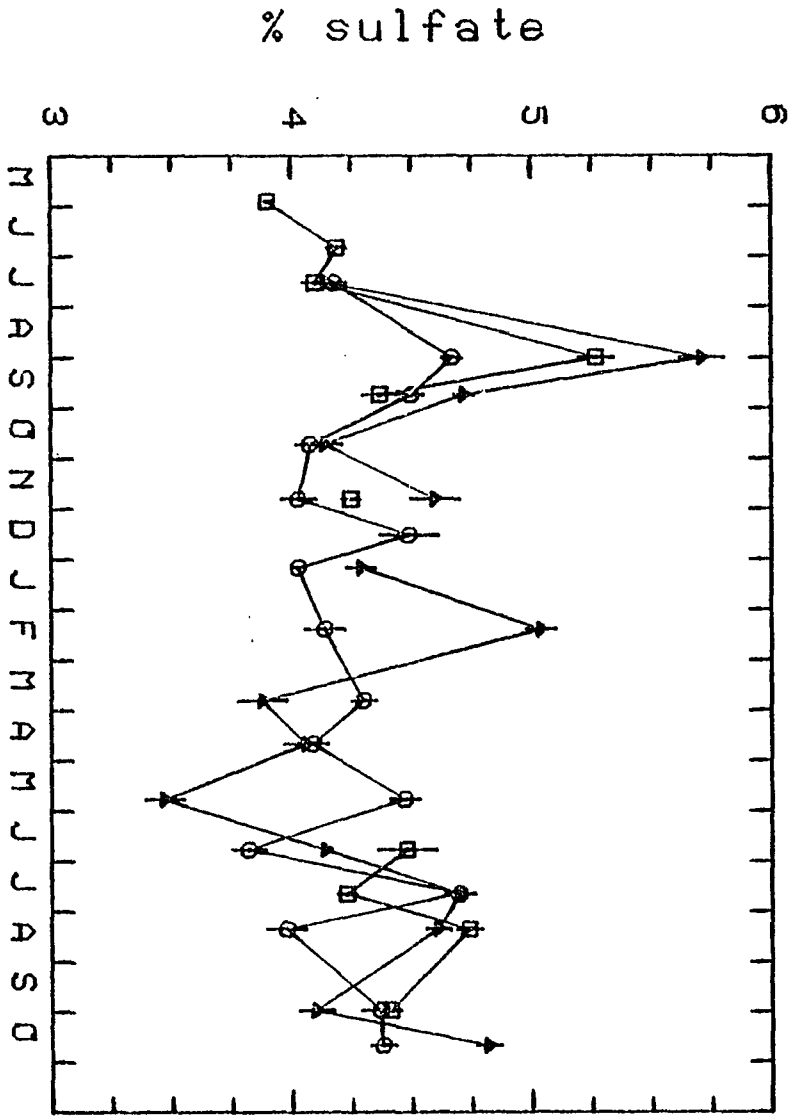


Figure 3-3

Figure 3-4. Seasonal variation of percent ash content of agar from Gracilaria tikvahiae plants from Cedar Point, Thomas Point and Nannie Island. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.

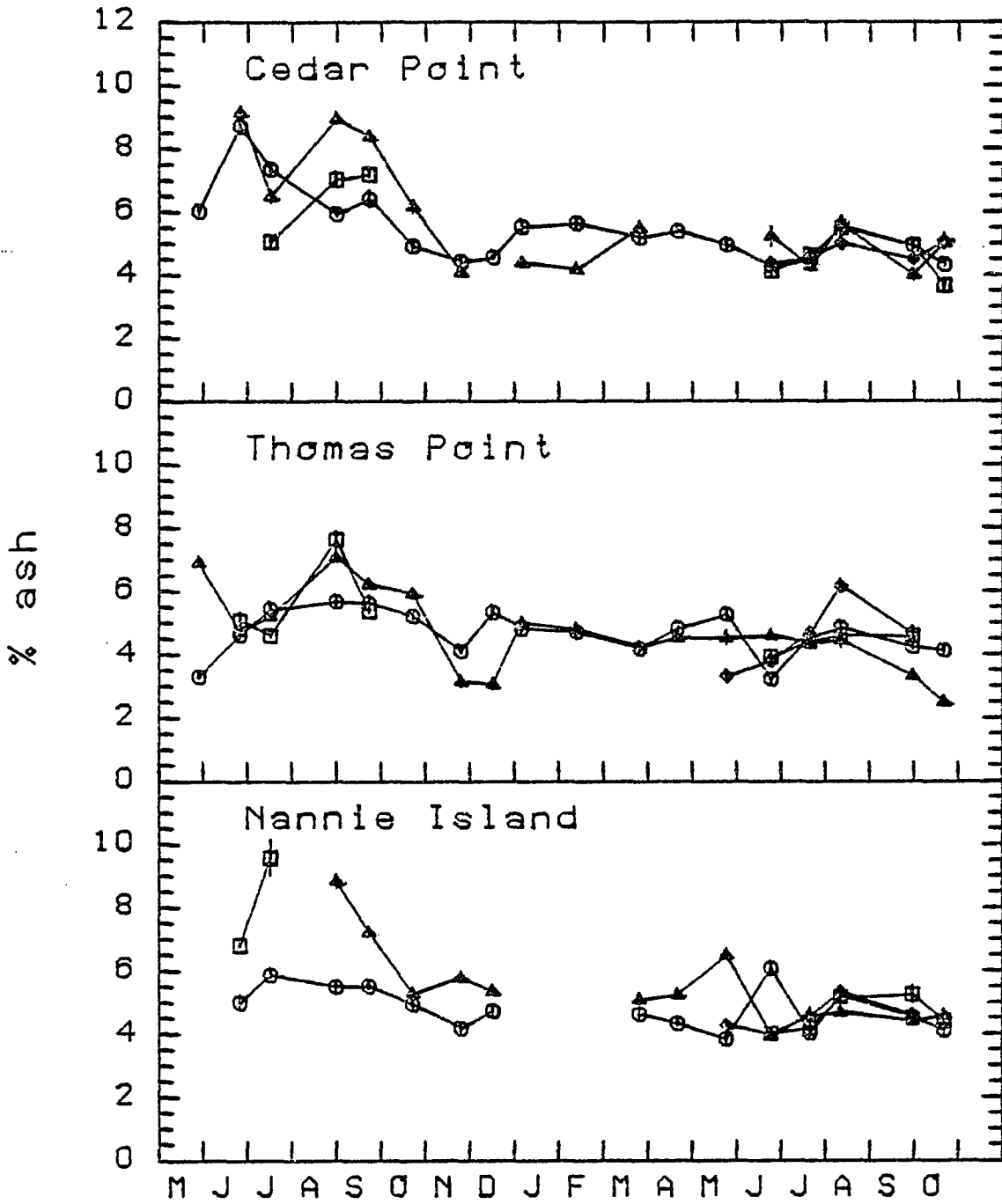


Figure 3-4

Figure 3-5. Average seasonal variation ( $\pm 1$  SE) of percent ash content of agar from Gracilaria tikvahiae plants from the Great Bay Estuary. Vegetative (octagons), cystocarpic (triangles), spermatangial (diamonds), and tetrasporic (squares) plants.

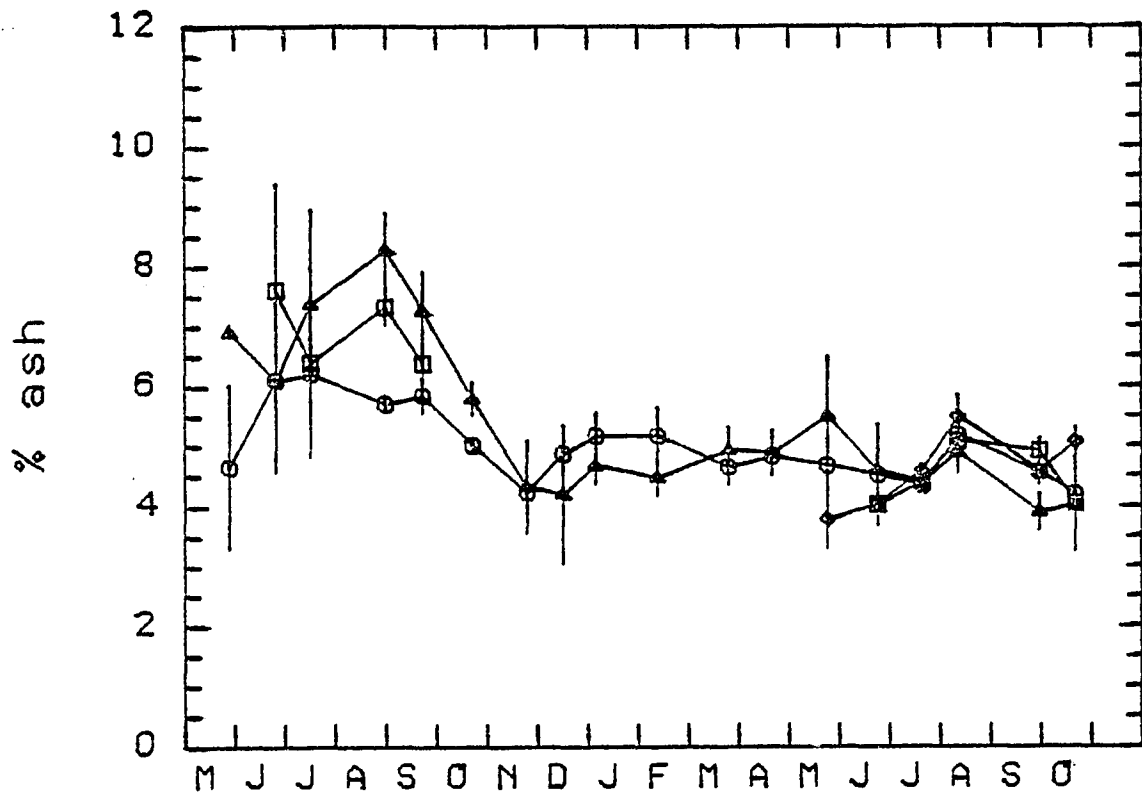


Figure 3-5

Figure 3-6. Seasonal variation of gel strength ( $\text{g}/\text{cm}^2$ ) of agar from Gracilaria tikvahiae plants from Thomas Point. Vegetative (octagons), cystocarpic (triangles), and tetrasporic (squares) plants. Error bars indicate  $\pm 1$  SE for analytical errors.

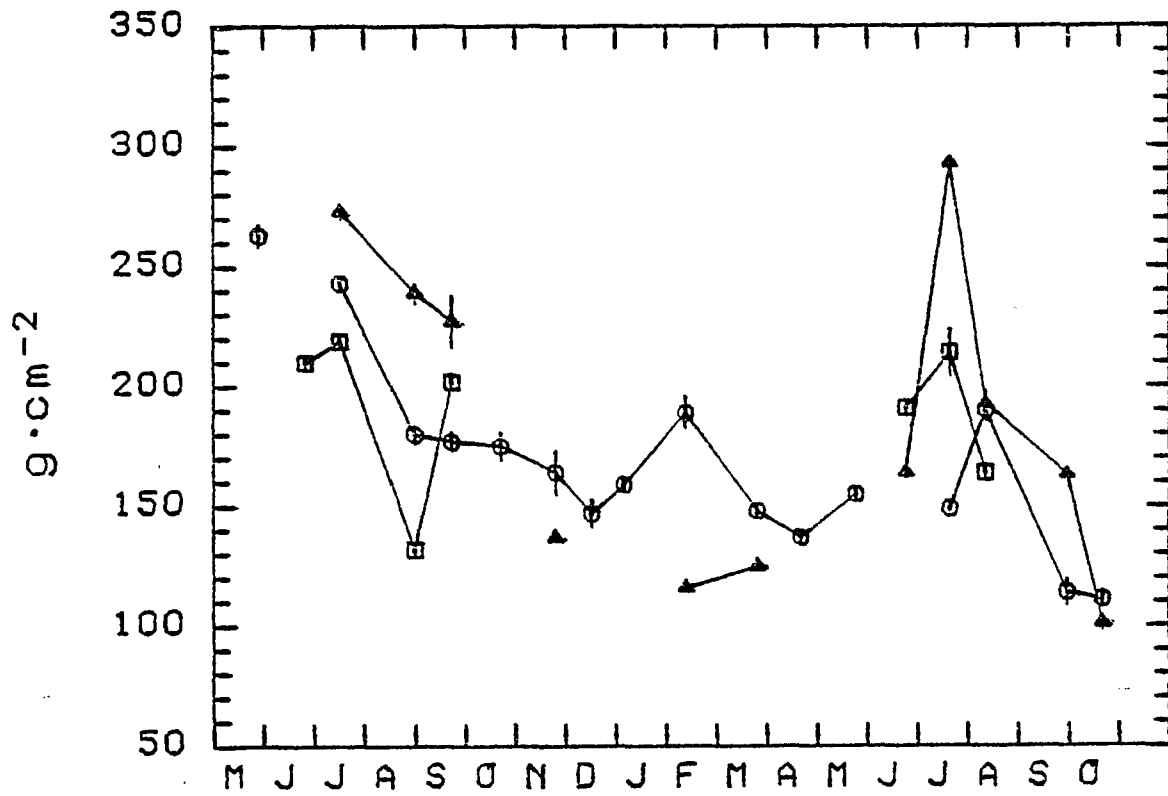


Figure 3-6



Figure 3-7. Seasonal variation of viscosity (cp) of agar from Gracilaria tikvahiae plants from Thomas Point. Vegetative (octagons), cystocarpic (triangles), and tetrasporic (squares) plants. Error bars indicate +1 SE for analytical errors.

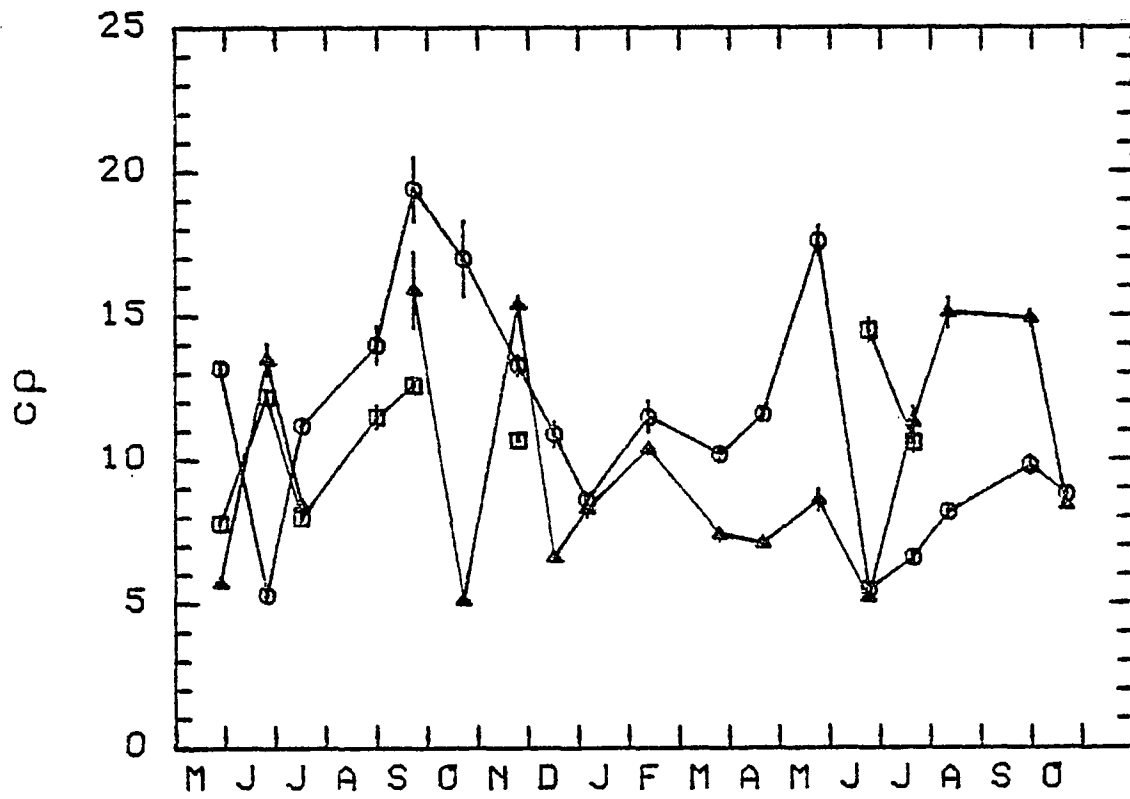


Figure 3-7

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ECOLOGY OF GRACILARIA TIKVAHIAE MCLACHLAN  
(GIGARTINALES, RHODOPHYTA) IN THE  
GREAT BAY ESTUARY, NEW HAMPSHIRE.

PART 4.

PHOTOSYNTHESIS.



#### 4.1 INTRODUCTION

The cosmopolitan red algal genus Gracilaria is an important source of the phycocolloid agar (Michanek 1975, Jensen 1979). Although it is currently not commercially exploited, Gracilaria tikvahiae McLachlan is a major component of the estuarine flora of the northwest Atlantic (Conover 1958, Edelstein et al. 1967, Hehre and Mathieson 1970, C. Bird et al. 1976, 1977b, Mathieson et al. 1981). Several investigators have studied the growth of G. tikvahiae in culture (N. Bird 1975, Edelstein et al. 1976, 1981, Edelstein 1977, N. Bird et al. 1979, Ramus and van der Meer 1983) and in natural conditions (Taylor 1975, C. Bird et al. 1977a, Edelstein et al. 1981), but few reports are available concerning its photosynthetic responses to environmental variables (Fralick and DeBoer 1977, Ramus and van der Meer 1983). At present the documented distribution of G. tikvahiae extends from Nova Scotia to New Jersey (Chapman et al. 1977, McLachlan 1979), with its occurrence farther south being somewhat ambiguous (Hoek 1982).

Several photosynthetic studies of other Gracilaria species have been conducted (Rosenberg and Ramus 1982). For example, Dawes et al. (1978) and Hoffman and Dawes (1980)

found that Florida plants of G. verrucosa were tolerant of a broad range of irradiances, salinities, and temperatures in accordance with the plants' estuarine distribution.

Similarly, Adriatic G. verrucosa (as G. confervoides) showed broad photosynthetic tolerance to temperature and salinity conditions (Simonetti et al. 1970). The present research was initiated in order to describe the effects of quantum irradiance, temperature and salinity on the net photosynthesis of G. tikvahiae. The paper represents a portion of a study dealing with the seasonal growth, reproductive phenology, physiological ecology and chemical composition of G. tikvahiae from a northern New England estuary (see Parts 1-3).

In the Great Bay Estuary of New Hampshire-Maine, Gracilaria tikvahiae occurs predominantly at inner estuarine sites, attached in subtidal beds where sufficient substrata (e.g. rocks, sunken logs, bivalve shells) are available (see Part 1). In these habitats, the plants are exposed to a wide range of salinity (5-30 g/kg) and temperature (-1.9<sup>o</sup>-27<sup>o</sup>C) regimes, while available light may be limited by extreme turbidity changes (Emerich Penniman et al. 1983). As these fluctuations may occur over a relatively short time (i.e. hours-days) similar acclimation periods were employed in this study.

## 4.2 METHODS

Plants of Gracilaria tikvahiae were collected by dredging between -2 to -4 m in a predominantly attached population at Thomas Point, Great Bay, N.H. ( $43^{\circ} 4.93' N$ ,  $70^{\circ} 51.92' W$ ), described in Part 1. The plants were held for 1-10 days under indirect, natural light at ambient temperature and salinity conditions in flow-thru seawater trays at the Jackson Estuarine Laboratory. Plants acclimated to winter and summer conditions were collected during January-March 1980 and July-September 1980, respectively. Plants used in Winkler dissolved oxygen experiments were collected in October-November 1980. In all experiments vegetative apical tips approximately 3 cm in length were excised with a razor blade and quickly wiped to remove contaminating epiphytes. The tips were preconditioned in 0.45  $\mu$ -filtered seawater in the dark at a temperature and salinity corresponding to the experimental run. All plant apices were preconditioned for 24 hr except those used in the temperature-acclimation experiments.

The photosynthetic responses of Gracilaria tikvahiae were measured manometrically with a Gilson differential respirometer (Model GRP-14) or with Winkler determinations (Strickland and Parsons 1972) of dissolved oxygen changes in

300 ml B.O.D. bottles. In the first instance, plant tips (avg. dry weight, 5.4 mg) were placed in manometric flasks with 5.0 ml artificial seawater (Instant Ocean) containing bicarbonate-carbonate buffer (Chapman 1962). A "carbon-dioxide buffer" solution (Umbreit et al. 1972) was added to the center well (0.30 ml) and sidearm (0.25 ml) of each flask to maintain a 2% carbon dioxide atmosphere.

Manometric flasks (with plant apices) were attached to the respirometer, allowed to equilibrate with shaking (100 cycles/min) at the experimental temperature for 60 min in the dark, and then exposed to light for 30 min. The manometric system was then closed and readings taken every 15 min for one hour. At the end of the photosynthetic run, the plant tips were removed from the flasks, blotted dry, and their fresh weights determined. Each tip was then halved. One portion was used to determine dry weight (80°C in vacuo for 24 hr), while the other piece was extracted with 90% acetone by grinding in a 25 ml Ten Broek homogenizer. After centrifugation, the chlorophyll content of this extract was determined with a Beckman DBG or Model 34 double beam spectrophotometer. The chlorophyll a concentrations were calculated with the extinction coefficient ( $E=87.67$ ) of Jeffrey and Humphrey (1975). All photosynthetic rates are net photosynthesis except when stated otherwise. Rates of net photosynthesis were expressed both per dry weight and per chlorophyll content.

Illumination in the respirometer water bath was provided by fourteen 50 watt General Electric flood lamps, run at 140 volts with a Stafco transformer (model 2PF1010) set at its maximum (1.4 kva). The setting allowed a somewhat higher maximum light intensity than 120 v. Irradiance was adjusted with variable numbers of nylon screens to approximate neutral density filters. Light was measured at the bottom of the manometric flasks with a Li-Cor model LI-185 quantum meter and a LI-1925 submersible quantum sensor and with a Weston foot-candle meter.

The net photosynthetic rates of winter- and summer-acclimated tips of Gracilaria tikvahiae were measured under a variety of light, temperature and salinity conditions. Two light experiments were conducted with winter and summer plants over a quantum irradiance range of 6-1440  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (20-5050 ft-c) at 15°C and 25°C. Similarly two temperature experiments were run with salinities of 10 and 25 g/kg. Two photosynthesis-salinity experiments were conducted at 10°C and 25°C. All of the temperature and salinity experiments were performed with a quantum irradiance of 870  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Individual plant apices were subjected to a single experimental combination of light, temperature and salinity conditions. In each manometric experiment 9-12 flasks were used.

To test the interaction of pretreatment time and temperature on photosynthetic response, summer-acclimated apices were conditioned at 25<sup>o</sup>, 30<sup>o</sup> or 35<sup>o</sup>C for 0, 1, 2, 3 or 4 days. The plants were conditioned in the dark in filtered 25 g/kg seawater, which was changed every 24 hr. Net photosynthesis was then measured manometrically at temperatures corresponding to the pretreatment conditions and at 870  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

A set of manometric experiments was conducted to detect any possible diel variation in photosynthetic rate. Three sets of plant apices, which had been held for 5 days in the flow-thru seawater trays, were conditioned in 25 g/kg filtered seawater at 25<sup>o</sup>C for 24 hr in the dark. The net photosynthetic rates at 870  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  were measured during the time periods: 0930-1030, 1230-1330, and 1530-1630.

A series of net photosynthetic measurements was made using Winkler dissolved oxygen techniques. Autumn-acclimated plants were conditioned as described previously, and then placed in 300 ml glass-stoppered bottles filled with 0.45  $\mu$ -filtered seawater. The bottles were strapped to the manometric flask supports in the respirometer water bath. The intent of this design was to approximate the manometric conditions, with respect to agitation, quantum irradiance and temperature. Net photosynthetic rates relative to quantum irradiance (at 25<sup>o</sup>C with 25 g/kg seawater) and temperature (at 870  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with 25 g/kg

seawater) were evaluated. Five to six experimental bottles, each containing one plant tip, and two control bottles were used. Dissolved oxygen concentrations were measured on an aliquot of the seawater used to initially fill each bottle and on the contents of each bottle after four hours, when the plant tips were removed. The dry weights and chlorophyll contents of the apices were determined as described above. The average dry weight of the apices was 0.013 g, a plant weight to bottle volume ratio of 0.04 g/L (Littler, 1979).

The effects of the initial oxygen concentration of the incubation medium on photosynthetic rates were determined. Four sets of eight bottles were used for this experiment, which was conducted in full natural sunlight (1750  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) at  $25^{\circ}\pm 1^{\circ}\text{C}$ . Initial oxygen concentrations of 0.5, 7.9 and 14.6 ppm (corresponding to 6, 110 and 200% saturation) were obtained by bubbling seawater with nitrogen, air and oxygen, respectively. Non-aerated seawater, 5.3 ppm oxygen (74% saturation), was also used. The bottles were vigorously agitated by hand every 10 min. Oxygen concentrations before and after the photosynthetic run were determined as described above.

Analyses of variance, Student-Newman-Keuls multiple comparison of means and Student's t-tests were performed on arcsine transformed data (Sokal and Rohlf 1969, 1981) with the computer statistical packages SPSS (Nie et al. 1975) and

MINITAB (Ryan et al 1976). Tests of significance were performed at the  $p=0.05$  level. The raw net photosynthesis-irradiance data were used to estimate values for the net photosynthetic rates at light saturation and the saturating quantum irradiances in the equation of Steele (1962), as modified by Jassby and Platt (1976). The formula is as follows:

$$P = \underline{a}Ie^{(-\underline{a}I/eP_m)} \quad \text{for } 0 \leq I \leq P_m e / \underline{a}$$

$$P = P_m \quad \text{for } I > P_m e / \underline{a}$$

(where  $P$ =gross photosynthetic rate,  $P_m$ =light saturated gross photosynthetic rate,  $I$ =quantum irradiance, and  $\alpha$ =slope, i.e.  $\underline{a}$ , of initial portion of light curve). The formula was chosen from the eight photosynthesis-light relationships described in Lederman and Tett (1981) as the one that best suited the present data. The model-fitting involved the simultaneous and independent estimation of the parameters  $P_m$  and  $\alpha$  by using the Marquardt-Levenberg algorithm to minimize sums-of-squares (Knott 1979). The advantages of this model-fitting procedure are described by Lederman and Tett (1981). As Jassby and Platt's equation applies to gross photosynthesis, the net photosynthetic rates measured in the present study were converted by adding a respiration value determined as the zero-light  $y$ -intercept of the linear regression of points representing the lowest three quantum irradiance values. Similarly compensation quantum irradiances were calculated as the intercept at  $P=0$ .



Subsequent curve-fitting procedures used the adjusted data. However, final tabular and graphic displays were corrected to give original net photosynthetic rates.

### 4.3 RESULTS

#### QUANTUM IRRADIANCE

The net photosynthesis-irradiance responses of winter- and summer-acclimated Gracilaria tikvahiae at 15° and 25°C were measured by manometric techniques (Figure 4-1). An analagous photosynthesis-irradiance relationship of autumn-acclimated plants at 25°C was measured by Winkler methods (Figure 4-2). The respiration rates (Table 4-I) were calculated as the zero-light y-intercept of each photosynthesis-irradiance curve. The initial slope (alpha) and maximum net photosynthetic rate ( $P_m$ ), simultaneously estimated from each of the raw data sets using the photosynthesis-irradiance equation of Jassby and Platt (1976), are given in Table 4-I. The latter parameters were used to plot best-fitting curves (Figures 4-1 and 4-2). Light-saturated photosynthetic rates (Figure 4-1 and Table 4-I) were significantly higher for summer than winter plants at 15° and 25°C, with the differences being greater at 25°C. No significant light-inhibition was apparent at the highest quantum irradiance ( $1440 \mu E \cdot m^{-2} \cdot s^{-1}$ ). The initial slopes of the four curves in Figure 4-1a were similar. The responses were comparable whether the rates were expressed on a chlorophyll content or dry weight basis. Absolute

chlorophyll content was significantly lower in winter ( $2.23 \pm 0.05$  mg chlorophyll/g dry wt, mean  $\pm 2$  SE) than in summer plants ( $2.42 \pm 0.06$ ).

Using the net photosynthesis-irradiance data, expressed in terms of chlorophyll content (Figures 4-1a and 4-2), saturation ( $I_s$ ) and compensation ( $I_c$ ) quantum irradiances were calculated (Table 4-II). The saturation quantum irradiances of winter and summer plants at  $15^\circ\text{C}$ , 298 and  $216 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively, were not significantly different. However, at  $25^\circ\text{C}$  the saturation quantum irradiance for winter plants ( $383 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) was significantly less than for summer plants ( $583 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Summer plants had a significantly greater  $I_s$  at  $25^\circ$  than  $15^\circ\text{C}$ ; winter plants did not show such a difference. The compensation quantum irradiances for winter and summer plants, calculated by regression of the initial segment of each light curve, were 8 to  $10 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Table 4-II). The photosynthesis-irradiance curve determined by the dissolved oxygen method (Figure 4-2) was similar to those determined manometrically. The  $P_m$  of the former was approximately one-half of the latter. The saturation quantum irradiance in the October plants measured with the Winkler techniques was intermediate to those of winter and summer plants. The value for  $I_c$  calculated in the October plants was  $5 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Table 4-II).

## TEMPERATURE

The net photosynthesis-temperature responses for winter- and summer-acclimated plants at 10 g/kg and 25 g/kg were similar regardless of whether the rates were expressed on a chlorophyll or dry weight basis (Figure 4-3). The average chlorophyll content of winter apices ( $2.22 \pm 0.06$  mg chlorophyll/g dry wt) was significantly lower than the value in summer tips ( $2.62 \pm 0.09$ ). Winter and summer plants exhibited increasing photosynthetic rates from  $5^{\circ}$  to  $25^{\circ}\text{C}$ . With the exception of winter plants at 10 g/kg, the photosynthetic rates expressed on a chlorophyll basis were comparable from  $25^{\circ}$  to  $35^{\circ}\text{C}$ , as demonstrated by Student-Newman-Keuls multiple comparisons of means (Table 4-IIIa). On a dry weight basis the responses were similar with maximum photosynthesis at  $25^{\circ}$  to  $35^{\circ}\text{C}$ , except in summer plants at 25 g/kg (Table 4-IIIb). The photosynthetic rates decreased dramatically at  $37.5^{\circ}\text{C}$  (Figure 4-3).

As shown in Figure 4-3, there were few seasonal differences in the net photosynthesis-temperature responses. Significant differences in rates (chlorophyll) were evident between winter and summer plants at  $5^{\circ}$ ,  $30^{\circ}$ ,  $35^{\circ}$  and  $37.5^{\circ}\text{C}$  in 10 g/kg and at  $5^{\circ}$ ,  $10^{\circ}$  and  $37.5^{\circ}\text{C}$  in 25 g/kg. With rates expressed in terms of dry weight, there were no differences between winter and summer plants in 10 g/kg at any temperature. The only seasonal differences in 25 g/kg seawater were at  $5^{\circ}$  and  $37.5^{\circ}\text{C}$  (dry weight). Overall, there

were no consistent differences in photosynthetic rates between winter and summer-acclimated plants.

The net photosynthetic rates (chlorophyll content) of winter-acclimated plants were significantly higher in 25 g/kg than 10 g/kg seawater at all temperatures, except 5°, 20° and 35°C (Figure 4-3a). In contrast, the rates (chlorophyll) of summer plants in 25 g/kg were significantly greater from 20° to 37.5°C than in 10 g/kg. The differences were not as pronounced when photosynthetic rates were expressed per unit dry weight. Specifically, in winter plants the rates were higher in 25 g/kg at 30° and 37.5°C than in 10 g/kg and for summer plants they were higher in 25 g/kg at 25° and 30°C.

The net photosynthesis-temperature response of Gracilaria tikvahiae measured by dissolved oxygen techniques was similar to that measured manometrically (Figure 4-4), although absolute rates were approximately one-half those shown in Figure 4-3. Overall the plant had a broad photosynthetic tolerance to temperature.

#### TEMPERATURE ACCLIMATION

The net photosynthesis of summer plants was not significantly different for acclimation times of zero to four days at 25° or 30°C (Figure 4-5). However, at 35°C the photosynthetic rates decreased significantly (SNK) after three days.

## SALINITY

The net photosynthesis-salinity responses of winter- and summer-acclimated Gracilaria tikvahiae plants at 10° and 25°C were similar whether photosynthesis was expressed in terms of chlorophyll (Figure 4-6a) or dry weight (Figure 4-6b). The chlorophyll content of winter tips ( $2.26 \pm 0.07$  mg chlorophyll/g dry wt) was significantly less than of summer tips ( $2.49 \pm 0.08$ ). There was a slight tendency for higher photosynthetic rates at salinities between 20 and 35 g/kg, particularly at 25°C. However a SNK comparison of means showed no consistent significant trend (Table 4-IV). Overall, G. tikvahiae has an extremely euryhaline photosynthetic response.

The net photosynthesis-salinity responses of Gracilaria tikvahiae were significantly greater at 25° than at 10°C in both winter and summer plants (Figure 4-6). When expressed on a chlorophyll basis, photosynthesis at 25°C was significantly greater for summer than winter plants at all salinities above 5 g/kg. Summer photosynthetic rates per unit dry weight at 25°C were greater than corresponding winter rates at all salinities. There were no significant differences between photosynthetic rates of winter and summer plants at 10°C for 5 g/kg to 25 g/kg. At 30 g/kg to 40 g/kg winter plants at 10°C had higher rates than summer plants.

## TIME OF DAY

The net photosynthesis of Gracilaria ( $25^{\circ}\text{C}$ , 25 g/kg,  $870 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) measured during 0930-1030, 1230-1330 and 1530-1630 hr is shown in Figure 4-7. Analysis of variance indicated no significant differences between the means for the three time intervals.

## INITIAL OXYGEN CONCENTRATION

The net photosynthesis of Gracilaria tikvahiae as measured by Winkler techniques decreased with increasing initial dissolved oxygen concentration (Figure 4-8). Analysis of variance showed that the differences were significant. However, multiple comparison of means by the SNK test (Table 4-V) showed that the rates of air-purged samples were not significantly less than those in  $\text{N}_2$ -purged bottles. Thus, during the other experiments using dissolved oxygen bottles, increases in oxygen concentration should not have appreciably lowered photosynthetic rates. As this particular experiment was performed in natural sunlight ( $1750 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), with manual, intermittent agitation, it should be noted that absolute rates are similar to those measured under incandescent light-saturated conditions with continuous, mechanical agitation.

#### 4.4 DISCUSSION

Gracilaria tikvahiae has a net photosynthetic tolerance to light intensities up to at least  $1440 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . In contrast other sublittoral seaweeds show some degree of photoinhibition of net photosynthesis at light intensities approaching ambient sunlight (Mathieson and Dawes 1974, Brinkhuis and Jones 1974, Mathieson and Norall 1975a, 1975b, Arnold and Murray 1980). Intertidal species, however, generally exhibit greater tolerance to full sunlight (Mathieson and Burns 1971, Brinkhuis et al. 1976, King and Schramm 1976, Niemeck and Mathieson 1978, Chock and Mathieson 1979, Tseng et al. 1981). Fralick and DeBoer (1977) showed that G. tikvahiae (as G. foliifera) could withstand light intensities up to 2500 ft-c, approximately one-half the maximum used in the present study. A related species, G. verrucosa from a Florida mangrove habitat showed no photoinhibition at 2000 ft-c (Dawes et al. 1978). Similarly, Hoffman (1978) found no significant depression of net photosynthesis in G. verrucosa from two northern Gulf of Mexico populations at light intensities up to 1800 ft-c. Ramus and Rosenberg (1980) and Ramus (1981) inferred that light inhibition occurred in G. foliifera at ambient light conditions, while Rosenberg and Ramus (1982) stated that



light inhibition occurred at  $600 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for shade-adapted G. foliifera from Beaufort, North Carolina.

The photosynthesis-light equation of Jassby and Platt (1976) was used in the present study to calculate more reliable values for compensation and saturation quantum irradiances and for maximum photosynthetic rates than could be visually estimated. Net photosynthesis in Gracilaria tikvahiae was light-saturated at  $200\text{--}600 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (800-2000 ft-c), depending on the season and temperature. The latter values corresponded to those determined for G. tikvahiae by Ramus and van der Meer (1983) and for G. foliifera (Rosenberg and Ramus 1982). The  $I_s$  data from G. tikvahiae in the present study were similar to records for other subtidal red algae. For example, Chondrus crispus is light saturated at c1000 ft-c (Mathieson and Burns 1971), while the  $I_s$  values for Ptilota serrata, Phyllophora truncata, and Callophyllis cristata are  $100\text{--}300 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Mathieson and Norall 1975a), versus 800-1000 ft-c for Gracilaria verrucosa (Hoffman 1978) and 1000-1500 ft-c for Hypnea musciformis (Dawes et al. 1976). Intertidal species exhibit saturation at comparable or slightly higher irradiances. Gigartina stellata saturates at 2100 ft-c (Mathieson and Burns 1971), G. exasperata at  $300 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Merrill and Waaland 1979) and Iridaea cordata at  $150\text{--}250 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Hansen 1977).

The saturating quantum irradiances calculated for Gracilaria tikvahiae with the equation of Jassby and Platt (1976) corresponded more closely to visually estimated values of Arnold and Murray (1980) with several green seaweeds than those estimated by graphic interpolation of the linear initial slope of the light curve and the horizontal line through  $P_m$  (i.e.  $I_k$ ). As many of the species described above are relatively optically opaque (sensu Ramus 1978) their photosynthesis-irradiance curves tend to approach saturation more gradually than those of thinner, more translucent plants.

Among others, King and Schramm (1976), Dawes et al. (1976) and Durako and Dawes (1980) have found seasonal changes in  $I_s$  values for several seaweeds. In the present study, Gracilaria tikvahiae exhibited significantly greater  $I_s$  values for summer versus winter plants at 25°C; in addition the values for  $P_m$  also changed seasonally. Similarly King and Schramm (1976), Dawes et al. (1976), Moon and Dawes (1976), and Durako and Dawes (1980) found that  $P_m$  was dependent upon season. With G. tikvahiae no significant differences in  $I_c$  values were found seasonally nor at different temperatures. In contrast, King and Schramm (1976) found reduced compensation intensities for winter plants. The compensation quantum irradiances measured in G. tikvahiae are similar to those listed from several green seaweeds (Arnold and Murray 1980).

The optimal temperatures of net photosynthesis in Gracilaria tikvahiae were between 25° to 35°C, with a marked decrease at 37.5°C. The latter response was similar for acclimation times up to three days; by four days at 35°C, photosynthesis declined. The temperature optimum shown here is higher than in several other New England estuarine algae. For example, Polysiphonia subtilissima and P. nigrescens have photosynthetic optima at 25° to 30°C (Fralick and Mathieson 1975), while Ascophyllum nodosum and its detached ecad scorpioides have thermal optima of 15° to 25°C (Chock and Mathieson 1979). Summer plants of Fucus vesiculosus var. spiralis (Niemeck and Mathieson 1978) had a temperature optimum comparable to G. tikvahiae. The photosynthesis-temperature response exhibited by G. tikvahiae in the present study is equivalent to that found by Fralick and DeBoer (1977) in southern New England populations. The cosmopolitan agarophyte G. verrucosa has a similar eurythermal photosynthetic response (Dawes et al. 1978, Mizusawa et al. 1978). In contrast the tropical genus Eucheuma has a more restricted thermal optimum of approximately 20° to 25°C for Caribbean (Mathieson and Dawes 1974) and 30°C in Hawaiian species (Glenn and Doty 1981), with photosynthesis declining at higher or lower temperatures.

No seasonal differences in temperature optima of net photosynthesis were observed in the present study of Gracilaria tikvahiae. In contrast summer-acclimated plants

of Fucus spiralis, F. vesiculosus and F. vesiculosus var. spiralis can tolerate higher temperatures than winter plants (Niemeck and Mathieson 1978). Chondrus crispus (Mathieson and Norall 1975b), Hypnea musciformis (Durako and Dawes 1980), Phycodrys rubens, Callophyllis cristata and Phyllophora truncata (Mathieson and Norall 1975a) all have similar patterns. Higher absolute rates of net photosynthesis have been described in summer than in winter plants of Ascophyllum nodosum and its detached ecad scorpioides (Chock and Mathieson 1979).

The effects of salinity on photosynthesis in macroalgae are complicated by both the ionic composition of the media and the length of the acclimation time (Gessner and Schramm 1971, Yarish et al. 1979, Dawes and McIntosh 1981). After a one day acclimation period in artificial seawater, Gracilaria tikvahiae had a broad euryhaline net photosynthetic response. A slight trend of higher photosynthetic rates at 25 g/kg to 35 g/kg was evident. Fralick and DeBoer (1977) found optimal photosynthesis for G. tikvahiae at salinities less than 30 g/kg. Several other estuarine and intertidal algae have a similar euryhaline tolerance (Mathieson and Burns 1971, Fralick and Mathieson 1975, Dawes et al. 1976, 1978, Niemeck and Mathieson 1978, Chock and Mathieson 1979, Yarish et al. 1979, Dawes and McIntosh 1981). In contrast subtidal coastal species are more stenohaline (Kjeldsen and Phinney 1972, Mathieson and Dawes 1974, Zavodnik 1975, Ohno 1976, Dawes et al. 1977).

In the present study no seasonal differences were observed in relative salinity tolerance, although the absolute photosynthetic rates were greater in summer than in winter plants (at 25°C). In contrast Hypnea musciformis had lower salinity optima and higher absolute rates in winter versus summer plants (Dawes et al. 1976). Bostrychia binderi had highest photosynthetic rates, at several salinities, in August-October (Davis and Dawes 1981).

As suggested previously there are difficulties in interpreting photosynthesis-salinity results with media prepared from diluted seawater (natural or artificial). Differences in carbon dioxide-bicarbonate concentrations (Hammer 1969, Ohno 1976, Dromgoole 1978a) or cationic composition, particularly  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  (Yarish et al. 1980, Dawes and McIntosh 1981), may modify the effects of salinity alone on net photosynthesis. In view of these limitations it is best to interpret such results in a relative rather than an absolute sense. In general Gracilaria tikvahiae has a net photosynthesis salinity tolerance over the range of salinity variations within the Great Bay Estuary (Emerich Penniman et al. 1983). It also appears tolerant, at least briefly, of salinities approaching those of coastal water.

No diel variations in the photosynthetic rate of Gracilaria tikvahiae were detected in the present experiment. In contrast diel photosynthetic rhythms have been measured under constant conditions in several

macroalgae (Terborgh and McLeod 1967, Britz and Briggs 1976, Mishkind et al. 1979, Kageyama et al. 1979, Hoffman 1978, Hoffman and Dawes 1980, Ramus 1981) and microalgae (Humphrey 1979, Harding et al. 1981a, 1981b). Field determinations have also demonstrated photosynthetic rhythms in seaweeds that were not coincident with irradiance cycles (Ramus and Rosenberg 1980, Hoffman and Dawes 1980). However, Blinks and Givan (1961) observed no daily photosynthetic rhythms in several intertidal algae. Sweeney (1963) attributes the latter results to measurements being made at suboptimal light levels. However, Harding et al. (1981b) have demonstrated diel differences in the light-limited portion of the photosynthesis-irradiance curve of Ditylum brightwellii. Harding et al. (1981b) also observed a strong relationship between growth rate or growth phase in D. brightwellii cultures and the amplitude of diel oscillations in light-saturated photosynthesis. Pronounced diel photosynthesis differences were present in rapidly dividing cells, whereas cells in the stationary phase showed little evidence of such a rhythm. Thus, differences in growth rate or age of algae may influence the amplitude of diel photosynthetic cycles. The apparent lack of a diel net photosynthetic rhythm in Gracilaria tikvahiae in the present study may be explained according to the findings of Harding et al. (1981b). That is, since the G. tikvahiae plants had been held in dim natural light and thus would have exhibited depressed growth rates, a minimal amplitude of a

photosynthetic rhythm would be expected (sensu Harding et al. 1981b).

Net photosynthesis in Gracilaria tikvahiae decreased linearly with increasing initial media oxygen concentrations. A similar response was demonstrated for the brown alga Carpophyllum maschalocarpum (Dromgoole 1978b). Sensitivity of photosynthesis to dissolved oxygen levels has been demonstrated in other seaweeds. Inhibition occurred whether photosynthesis was measured as oxygen evolution (Downton et al. 1976, Littler 1979) or carbon uptake (Black et al. 1976, Hatcher et al. 1977, Burris 1977, Gluck and Dawes 1980). Some microalgae also have reduced photosynthetic rates at high oxygen levels (Beardall et al. 1976, Burris 1977). Although Chaetomorpha showed the opposite trend of increasing photosynthetic rates with higher oxygen levels, this may have been an artifact of inconsistent chlorophyll extraction between treatments (Burris 1977). Gluck and Dawes (1980) found no increase in oxygen production in G. verrucosa under enhanced or lowered oxygen concentrations with various photorespiratory inhibitors.

The depression of photosynthesis with elevated oxygen levels may be due to a combination of oxygen enhanced respiration (Hough 1976, Downton et al. 1976, Dromgoole 1978b) at least up to oxygen levels that saturate dark respiration (Dromgoole 1978b), photorespiration (Tolbert 1974, Burris 1977, Dromgoole 1978b), or a direct oxygen

inhibition of gross photosynthesis (Dromgoole 1978b). Ambient seawater oxygen concentrations may result in lowered in situ photosynthetic rates for Gracilaria tikvahiae in contrast to potential rates under artificially reduced dissolved oxygen levels. However, only small differences in photosynthetic rates would be apparent with respect to seasonal or diel dissolved oxygen changes (6-10 ppm) in a well-mixed estuary such as the Great Bay Estuary (Emerich Penniman et al. 1983).



#### 4.5 SUMMARY

- 1). The net photosynthesis of Gracilaria tikvahiae was light saturated at  $200-600 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  but it was not inhibited at quantum irradiances up to  $1440 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The values for  $I_s$  were dependent upon season and temperature.
- 2). Net photosynthesis of Gracilaria tikvahiae increased with increasing temperatures from  $5^\circ$  to  $25^\circ\text{C}$ . The rates at  $25^\circ$  to  $35^\circ\text{C}$  were equivalent. Net photosynthesis decreased markedly at  $37.5^\circ\text{C}$ .
- 3). Net photosynthesis of Gracilaria tikvahiae at  $25^\circ$  and  $30^\circ\text{C}$  was relatively constant up to four days acclimation. However, by four days at  $35^\circ\text{C}$ , the net photosynthetic rate had declined.
- 4). Winter and summer plants of Gracilaria tikvahiae had a broad euryhaline net photosynthetic response (from 5 g/kg to 40 g/kg) at  $10^\circ$  and  $25^\circ\text{C}$ .

- 5). No diel variation in net photosynthesis of Gracilaria tikvahiae was observed with measurements made in the morning, early afternoon and late afternoon.
  
- 6). The rate of net photosynthesis in Gracilaria tikvahiae decreased linearly with increased dissolved oxygen levels.

Table 4-I. Parameters calculated by data-generated model of Jassby and Platt's (1967) photosynthesis-irradiance equation ( $P_m$  and  $\alpha$ ) and by linear regression ( $R$ ). Net photosynthesis is expressed as a)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$ , b)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$  and c)  $\text{mg O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$ ; respiration values are in terms of  $\text{g dry wt}^{-1}$ . Values are means with 95% confidence intervals in parentheses.

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Experimental conditions	R	$\alpha$	$P_m$
a) winter 15°C	4.2	0.37 (0.32-0.42)	40.6 (38.5-42.7)
25°C	8.4	0.56 (0.47-0.65)	78.9 (73.5-84.3)
summer 15°C	7.7	0.63 (0.54-0.72)	50.1 (47.4-52.8)
25°C	7.7	0.53 (0.46-0.60)	113.6 (107.0-120.2)
b) winter 15°C	10.1	0.86 (0.76-0.96)	84.6 (81.0-88.2)
25°C	17.4	1.24 (1.05-1.43)	176.7 (165.2-188.2)
summer 15°C	17.5	1.67 (1.44-1.90)	117.9 (112.2-123.6)
25°C	17.6	1.03 (0.93-1.13)	287.5 (273.2-301.8)
c) autumn 25°C	1.5	0.33 (0.28-0.38)	51.2 (48.0-54.4)

Table 4-II. Compensation and saturation quantum irradiances ( $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for net photosynthesis-irradiance curves in Figures 4-1a and 4-2. Values are means with 95% confidence interval for  $I_s$ .

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Experimental conditions	$I_c$	$I_s$
winter 15°C	9.9	298 (251-345)
25°C	7.7	383 (310-456)
summer 15°C	7.9	216 (179-253)
25°C	9.1	583 (494-672)
autumn 25°C (Winkler)	4.8	422 (345-499)

Table 4-III. Student-Newman-Keuls comparisons of means from net photosynthesis-temperature experiments (Figures 4-3 and 4-4). Temperatures are in order of increasing magnitude of corresponding mean photosynthetic rates (expressed as a)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$ , b)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$  and c)  $\text{mg O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$ ). Values underlined indicate means for those conditions are not significantly different at  $p < 0.05$ .

Experimental conditions		Temperature ( $^{\circ}\text{C}$ )						
a)	winter 10 g/kg	5	10	15	20	25	<u>30</u>	35
	25 g/kg	5	10	15	20	25	<u>35</u>	30
	summer 10 g/kg	5	10	15	20	25	<u>30</u>	35
	25 g/kg	5	10	15	20	25	<u>30</u>	35
b)	winter 10 g/kg	5	10	15	20	25	<u>30</u>	35
	25 g/kg	5	10	15	20	35	<u>25</u>	30
	summer 10 g/kg	5	10	15	20	30	<u>35</u>	25
	25 g/kg	5	10	15	20	35	<u>25</u>	30
c)	autumn 25 g/kg	5	10	<u>15</u>	20	35	<u>25</u>	30

Table 4-IV. Student-Newman-Keuls comparisons of means from net photosynthesis-salinity experiments (Figure 4-6). Salinities are in order of increasing magnitude of corresponding mean photosynthetic rates (expressed as a)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$  and b)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$ ). Values underlined indicate means for those conditions are not significantly different at  $p \leq 0.05$ .

Experimental conditions		Salinity (g/kg)							
a) winter	10°C	<u>15</u>	5	<u>35</u>	10	20	25	40	30
	25°C	5	<u>10</u>	15	20	40	30	25	35
summer	10°C	40	5	35	10	30	15	25	20
	25°C	5	10	40	35	15	20	25	30
b) winter	10°C	15	10	5	35	20	30	25	40
	25°C	40	35	30	5	15	10	20	25
summer	10°C	40	30	10	35	5	15	20	25
	25°C	15	10	40	30	5	20	25	35

Table 4-V. Student-Newman-Keuls comparison of means from net photosynthesis-oxygen concentration experiment (Figure 4-8). Oxygen concentrations are in order of increasing magnitude of corresponding mean photosynthetic rates (expressed as  $\text{mg O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$ ). Values underlined indicate means for those conditions are not significantly different at  $p \leq 0.05$ .

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Initial oxygen concentration (ppm)			
(O <sub>2</sub> )	(air)	(ambient)	(N <sub>2</sub> )
<u>15.1</u>	7.89	5.29	0.46
<hr/>		<hr/>	

Figure 4-1. Net photosynthesis (measured manometrically) versus quantum irradiance. Each point is the mean of 9-12 replicates. Error bars indicate 95% confidence intervals. Best-fitting curve is drawn for each set of conditions using parameters in Table 4-I. Photosynthesis is expressed as a)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$  and b)  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$ . Summer plants at  $15^\circ\text{C}$  (triangles) and  $25^\circ\text{C}$  (diamonds), winter plants at  $15^\circ\text{C}$  (octagons) and  $25^\circ\text{C}$  (squares).



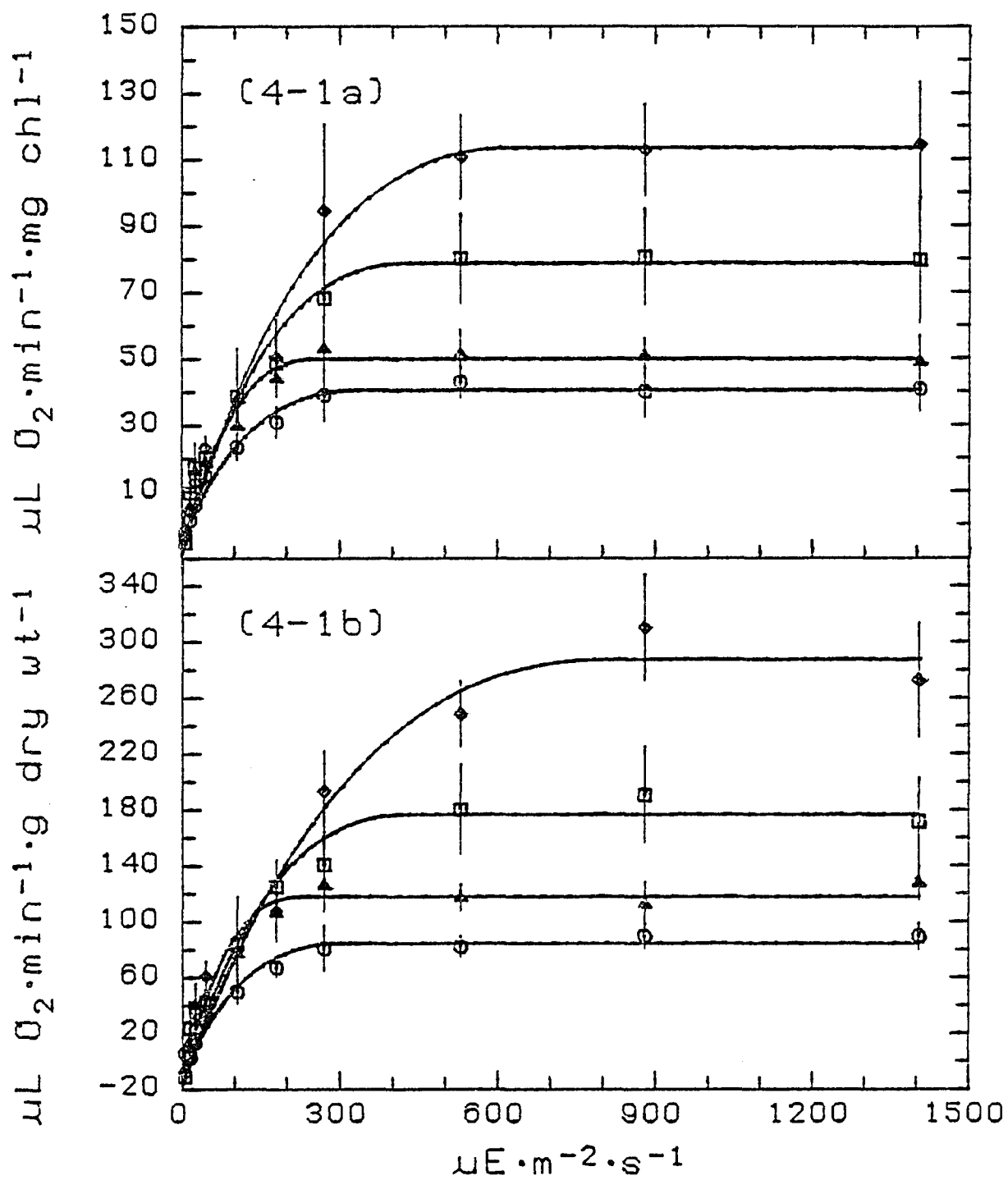


Figure 4-1

Figure 4-2. Net photosynthesis (measured by Winkler method) versus quantum irradiance at 25°C. Each point is the mean of 5-6 replicates. Error bars indicate 95% confidence intervals. Best-fitting curve is drawn from parameters in Table 4-1c. Photosynthesis is expressed as  $\text{mg O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$  and as  $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{mg chlorophyll}^{-1}$ .

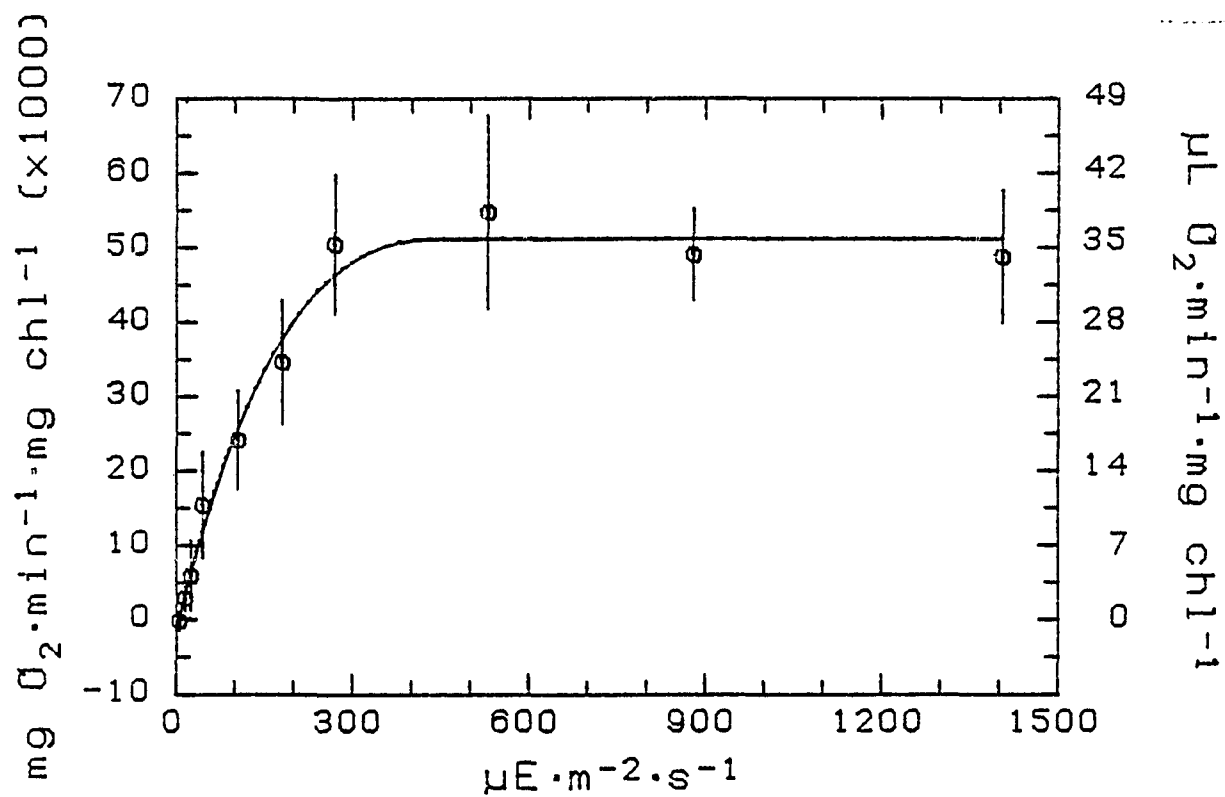


Figure 4-2

Figure 4-3. Net photosynthesis (measured manometrically) versus temperature (at  $870 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 25 g/kg). Each point is the mean of 9-12 replicates. Error bars indicate 95% confidence intervals. Photosynthesis is expressed as a)  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$  and b)  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$ . Summer plants in 10 g/kg (squares) and 25 g/kg (diamonds), winter plants in 10 g/kg (octagons) and 25 g/kg (triangles).

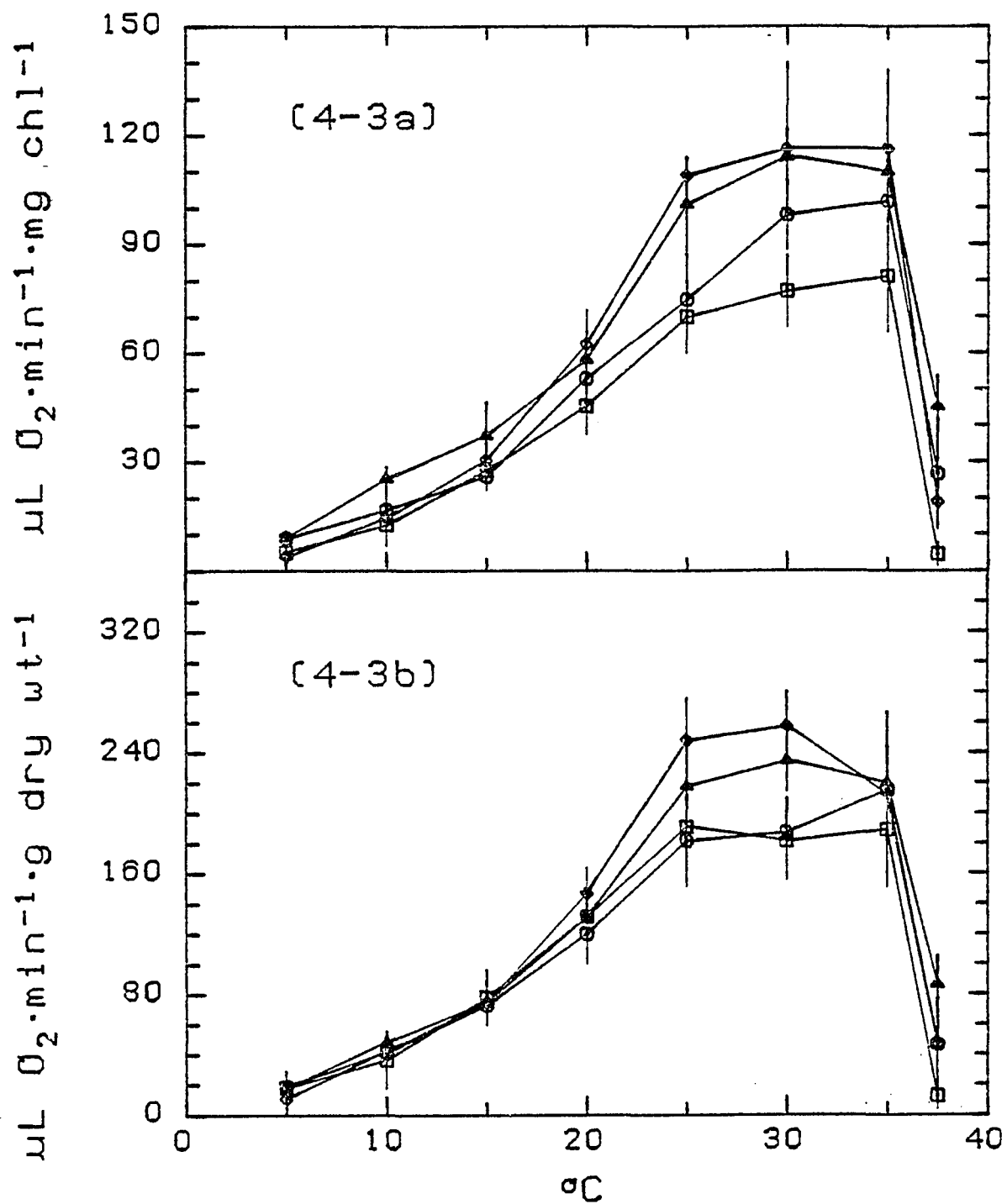


Figure 4-3

Figure 4-4. Net photosynthesis (measured by Winkler method) versus temperature (at  $870 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 25 g/kg). Each point is the mean of 5-6 replicates. Error bars indicate 95% confidence intervals. Photosynthesis is expressed as  $\text{mg O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$  and as  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$ .

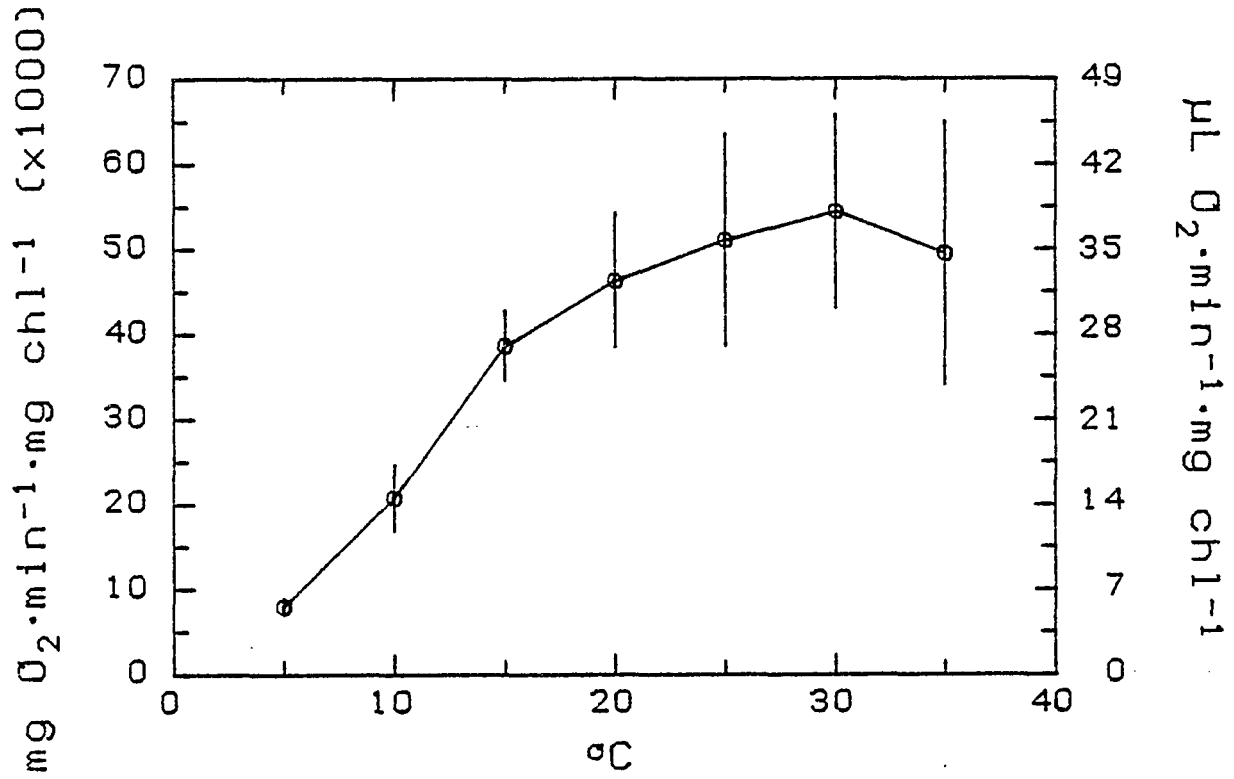


Figure 4-4

Figure 4-5. Net photosynthesis (measured manometrically) for summer-acclimated plants in relation to acclimation time at 25° (octagons), 30° (triangles) and 35° C (squares) (at 870  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 25 g/kg). Each point is the mean of 9-12 replicates. Error bars indicate 95% confidence intervals. Photosynthesis is expressed as  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$ .



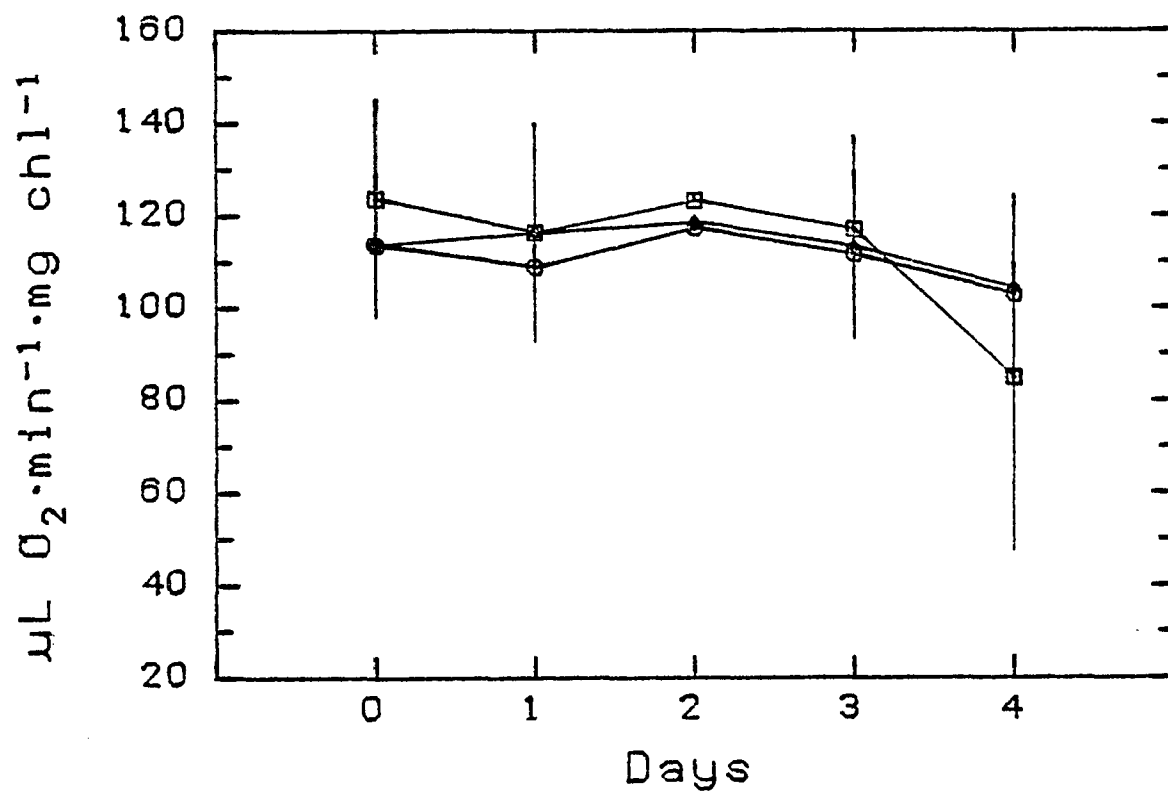


Figure 4-5

Figure 4-6. Net photosynthesis (measured manometrically) versus salinity (at  $870 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Each point is the mean of 9-12 replicates. Error bars indicate 95% confidence intervals. Photosynthesis is expressed as a)  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$  and b)  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1}$ . Summer plants at  $10^\circ\text{C}$  (squares) and  $25^\circ\text{C}$  (diamonds), winter plants at  $10^\circ\text{C}$  (octagons) and  $25^\circ\text{C}$  (triangles).

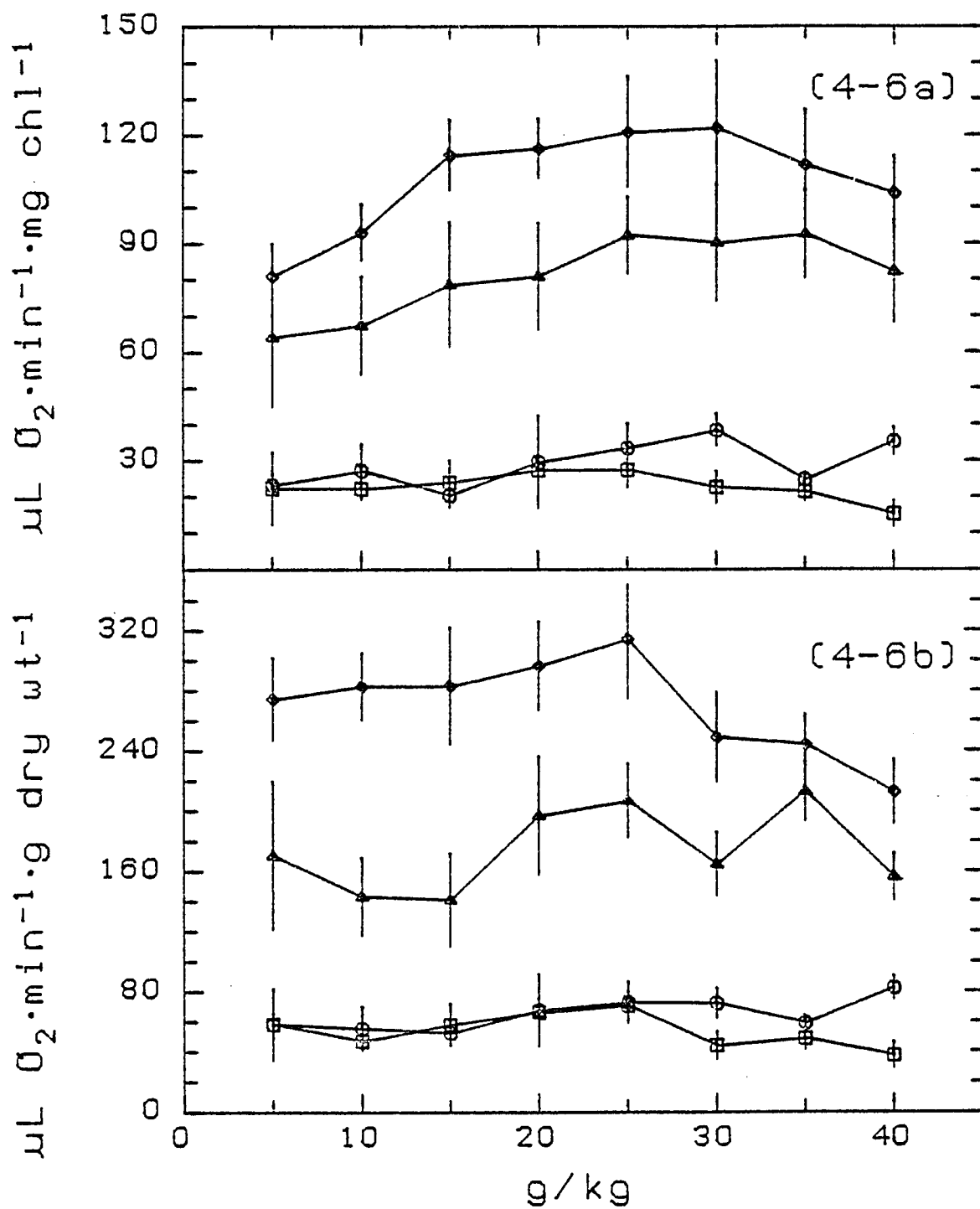


Figure 4-6

Figure 4-7. Net photosynthesis (measured manometrically) in relation to time of day (at  $870 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $25^{\circ}\text{C}$ , 25 g/kg). Each point is the mean of 9-12 replicates. Error bars indicate 95% confidence intervals. Photosynthesis is expressed as  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$ . Time periods 1) 0930-1030, 2) 1230-1330, and 3) 1530-1630.

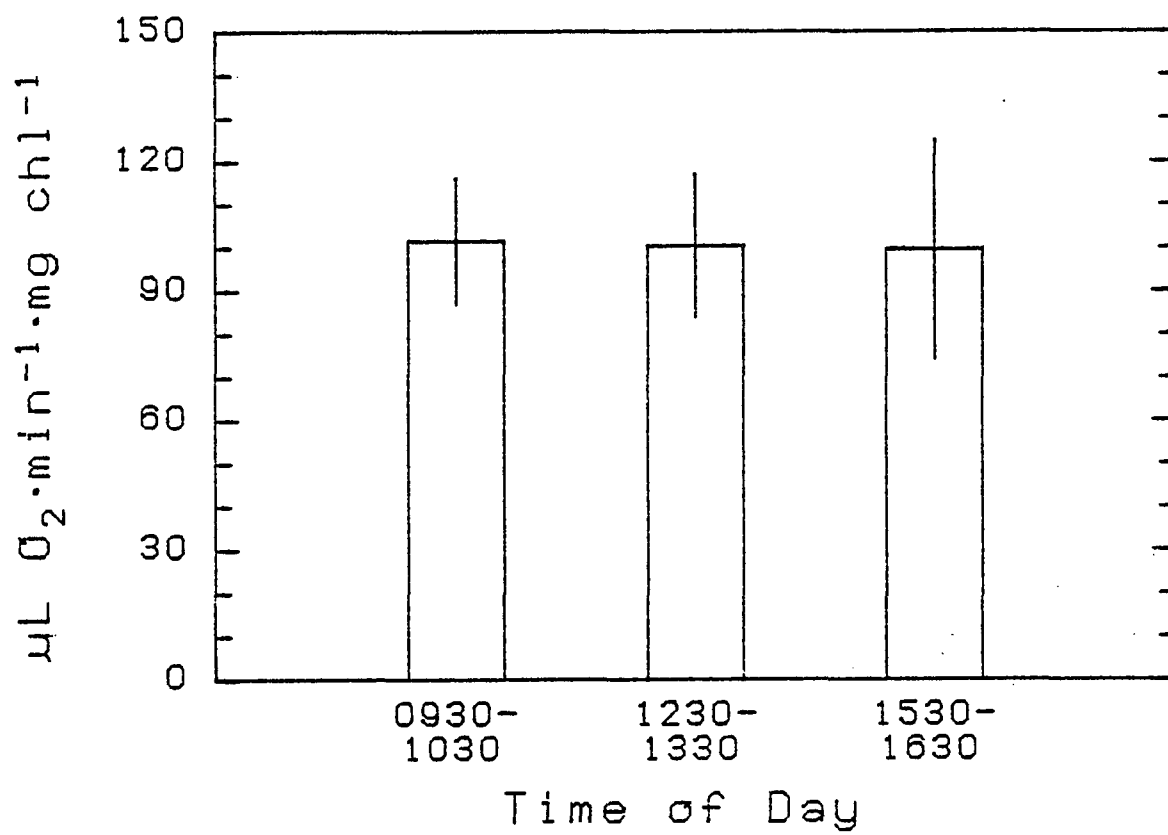


Figure 4-7

Figure 4-8. Net photosynthesis (measured by Winkler method) in relation to initial dissolved oxygen concentration (at  $1750 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $25^\circ\text{C}$ ,  $25 \text{ g/kg}$ ). Each point is the mean of 6 replicates. Error bars indicate 95% confidence intervals. Photosynthesis is expressed as  $\text{mg O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$  and as  $\mu\text{l O}_2\cdot\text{min}^{-1}\cdot\text{mg chlorophyll}^{-1}$ .

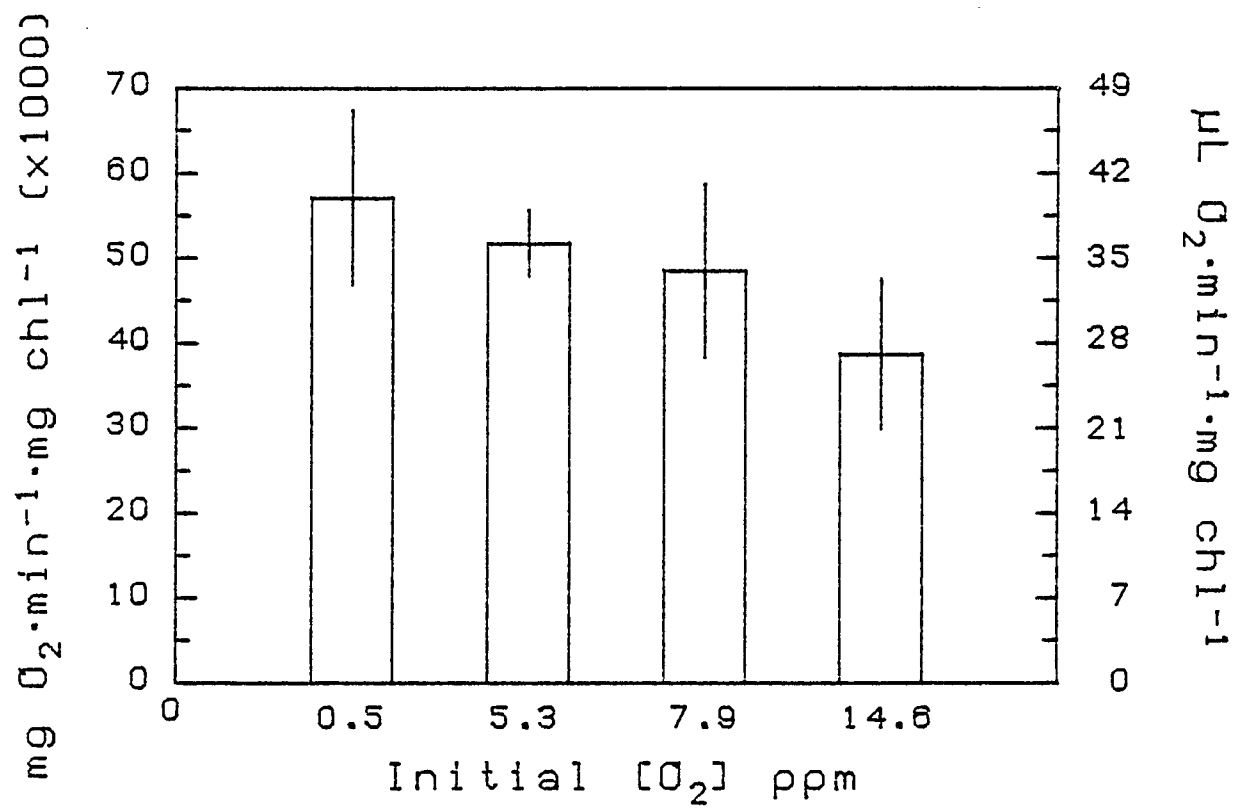


Figure 4-8

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