AN ABSTRACT OF THE THESIS OF

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Title: Nitrogen and Phosphorus Uptake by Enteromorpha prolifera (Mull.) J. Ag.

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The influence of tissue composition and desiccation on uptake of NH_4^+, NO_3^- and PO_4^{3-} by Enteromorpha prolifera (Mull.) J. Ag. was examined. Tissue composition and uptake rates of field-collected plants suggested that E. prolifera in Yaquina Bay, Oregon was not likely to have been nitrogen-limited, but may have been phosphorus-limited during 1985. Kinetic parameters for NH_4^+ and NO_3^- uptake were determined. For NH_4^+ uptake, V_{max} was 188 µmol NH_4^+ g dry wt^{-1} h^{-1} and K_s ranged from 9.3 to 13.4 µM and there was no difference between kinetic parameters of plants with low and high tissue nitrogen content. The K_s and V_{max} for NO_3^- uptake were higher for plants with low tissue nitrogen than for plants with high tissue nitrogen. V_{max} was 169 and 75.4 µmol NO_3^- g dry wt^{-1} h^{-1} and K_s, 13.3 and 2.31 µM for low and high tissue nitrogen plants, respectively. Estimates of field uptake rates from kinetic curves and ambient nitrogen concentrations agreed well with in situ measurements and with those calculated from tissue content and growth

rates. Estimates of uptake in the field suggested that NO_3^- uptake comprised up to 30% of total nitrogen uptake during the summer.

Nutrient uptake after desiccation was variable and variation tended to increase with increasing desiccation. Phosphate was released after desiccation during all experiments and release during the first 15 minutes comprised up to 12.2% of total tissue phosphorus. Nitrate was not consistently released but at times released NO_3^- comprised up to 2.4% of total tissue nitrogen. Ammonium uptake after desiccation decreased slightly but inconsistently.

Rates of NH_4^+ and NO_3^- uptake were determined by ^{15}N accumulation in plant tissue and by disappearance of nutrient from the medium. Overall, the agreement between rates calculated by the two methods was good, averaging 82.7% (SD = 15.8%) and 91.2% (SD = 13.7%) for NH_4^+ and NO_3^- uptake, respectively. An average of 93.4 and 96.0% of added $^{15}NH_4^+$ and $^{15}NO_3^-$ was recovered from the medium and/or plant tissue at the end of the incubations. Indirect evidence suggested that both bacterial uptake and regeneration of NH_4^+ may contribute to discrepancies between NH_4^+ uptake rates calculated by ^{15}N accumulation and disappearance of NH_4^+ from the medium.

Nitrogen and Phosphorus Uptake by *Enteromorpha prolifera* (Mull.) J. Ag.

by

Margaret C. O'Brien

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CHAPTER I

GENERAL INTRODUCTION

Macroalgal mats are important contributors to nearshore primary production (Shellem and Josselyn 1982, Pregnall and Rudy 1985) and their tendency to proliferate in eutrophic areas makes them important to nutrient cycling (Kautsky 1982). There have been relatively few studies on nutrient physiology of macroalgae compared with the abundance of such studies for phytoplankton. Uptake of nutrients is the first step in the processes that lead eventually to incorporation of the nutrients into cellular components. This thesis investigates the uptake of nitrogen and phosphorus by Enteromorpha prolifera (Mull.) J. Ag. growing on a mudflat in Yaquina Bay, Oregon.

Yaquina Bay is a well-mixed estuary during the summer and fall and partly-mixed during the winter and spring (Kulm 1965). Variations in hydrography and chemical characteristics are seasonal due to high rainfall during the winter. Mudflats comprise about 35% of the estuarine surface (Cox 1973), and are covered with dense mats of Ulva spp. and Enteromorpha spp. from late spring through mid-fall. The standing crop of either genus may reach 500 g dry wt m^{-2} during July and August (Davis 1981). Chemical characteristics of the water column from 1983 to 1985 have been described by Collins (1987) and Garber et al. (1984). In the lower bay, salinity ranges from 27 to

$34^{o}/oo$ and temperature from 10 to $18^{o}C$. Concentrations of NO_3^- reach 25 - 30 μM in the fall and winter and are correlated with high runoff. During the summer, NO_3^- is also advected into the bay during upwelling events and concentrations up to 14 μM have been observed. Ammonium ranges between 1 and 4 μM and is supplied mainly from regeneration from the sediments (Collins 1987). Phosphate is supplied from the sediments, the Yaquina River and from the ocean during upwelling events. Concentrations range from 0.7 to 1.4 μM.

Primary production of Enteromorpha and Ulva in temperate estuaries ranges from 3 to 47 g C m^{-2} day^{-1} and growth rates up to 0.27 day^{-1} have been measured (Davis 1981, Shellem and Josselyn 1982, Pregnall and Rudy 1985). Rates of primary production by macroalgae in Yaquina Bay are among the highest reported in the literature and the algal mats have been estimated to contribute about 50% to total estuarine primary production during the summer (Collins 1987).

The factors controlling the growth of macroalgae on the Yaquina Bay mudflats are not known. It is possible that light limits growth in the fall. Davis (1981) found that light reaching the sediments was saturating for photosynthesis in July but not in September of 1980. Conversely, Collins (1987) found that light levels at the sediment surface were not likely to be limiting to growth during the fall and winter of 1984-85. Growth rates of Enteromorpha and related genera increase with additions of nutrients suggesting that some algal mats may be nitrogen- (Waite and Mitchell 1972, Kautsky 1982) or phosphorus-limited (Birch et al. 1981, Schramm and Booth 1981).

Collins (1987) found that the ratios of *in situ* nitrogen:phosphorus uptake were lower than predicted by demand and suggested that growth of macroalgae in Yaquina Bay may be nitrogen-limited.

Mats of *Enteromorpha* and related genera control nutrient cycling in some estuaries. Welsh (1980) found that *Ulva lactuca* mats in a Long Island estuary regulated the seasonal flux of nutrients between the adjacent channel and salt marsh. During the summer, the mats intercepted pulses of nutrients regenerated by the salt marsh sediments before they reached the channel. In the fall, the mats exported NH_4^+, NO_2^- and PO_4^{3-} to the channel as the algae became senescent. In a Scotland estuary, Owens et al. (1979) and Owens and Stewart (1983) found that algal mats trapped nitrogen in the sediments. The nitrogen accumulated by *Enteromorpha* spp. accounted for 98% of the total nitrogen taken up by primary producers. An estimated 88% of macroalgal production was subsequently buried by sediments. Only 14.4% of this nitrogen was remineralized and released to the overlying water as NH_4^+ after burial for 28 days. Owens and Stewart (1983) estimated that the input of macroalgae to the sediments was sufficient to support all of the secondary production on the mudflat. Collins (1987) found that the macroalgal mats in Yaquina Bay were able to remove more than ten times the NO_3^- and twice the PO_4^{3-} that was supplied to the estuary by the Yaquina river during the summer.

Although existing studies indicate the importance of macroalgal mats to estuarine ecology, they have provided only broad estimates of

macroalgal nutrient utilization and uptake capabilities. Nutrient uptake rates change in response to environmental and biological conditions that cannot always be controlled or even adequately defined in the field (reviewed by Harrison and Druehl 1982, Hanisak 1983). In situ incubations require controls to account for the contributions of phytoplankton, macroalgae, bacteria and sediments to the observed changes in nutrient concentration. To examine physiological processes in detail, thalli must be brought into the laboratory.

The relationship of uptake to nutrient concentration is generally described by a rectangular hyperbola using the Michaelis-Menten equation (Harrison and Druehl 1982). This equation yields two values that describe uptake: the maximum uptake rate, V_{max}, and the concentration at which uptake is one-half the maximum rate, K_s. Comparisons of these values indicate the relative efficiency with which algae acquire nutrients (Healey 1980, Hanisak 1983). The relative utilization of NO_3^- and NH_4^+ in field populations can be predicted if the kinetics of uptake of these two forms of nitrogen are known (Topinka 1978).

The use of stable nitrogen isotopes has become common in studies of phytoplankton nutrition (reviewed by Harrison 1983). Isotopes have been particularly useful for elucidating the importance of recycled forms of nitrogen, mainly NH_4^+, since uptake can be measured where there is no net change in the concentration of dissolved nutrient. ^{15}N has been used with macroalgae in only a few studies.

Owens and Stewart (1983) and Owens et al. (1979) used $^{15}NH_4^+$ in *in situ* incubations to study *Enteromorpha* spp. production and decomposition. Williams and Fisher (1985) used ^{15}N to measure NH_4^+ uptake kinetics of *Caulerpa cupressoides*. These studies begin to address the potential uses and associated problems of ^{15}N in studies of macroalgae. Among these problems is the incomplete recovery of isotope during experiments and methods must be devised in which the isotope is conserved. *E. prolifera* has a simple, undifferentiated thallus for which current ^{15}N methodology is easily adapted.

Phytoplankton respond to nitrogen starvation by exhibiting extremely high, transient rates of NH_4^+ uptake (e.g. McCarthy and Goldman 1979). Recently, several studies have examined macroalgal uptake during nutrient limitation and starvation and a similar response is often observed. This response may be more extreme for ephemeral algae with high growth rates when compared to algae with slow growth rates. Rosenberg et al. (1984) found that transient uptake of NH_4^+ by *Chordaria flagelliformis*, a fast-growing summer ephemeral, exceeded the V_{max} by a factor of 3.4 - 4.8 and the degree of enhancement was inversely related to thallus nitrogen content. The enhancement of uptake by a slower growing perennial, *Fucus distichus*, was lower, but still up to 3 times the V_{max}.

Tolerance to exposure at low tide is a major factor in controlling distribution of marine algae (Schonbeck and Norton 1980). However, the effects of exposure on algal physiology are not well known. The responses of photosynthesis and respiration to emergence

have been described for several species (Johnson et al. 1974, Quadir et al. 1979). Net photosynthetic rates of high intertidal fucoids increased at approximately 20% desiccation, presumably due to increased CO_2 flux to the plant surface (Brinkhuis et al. 1976, Quadir et al. 1979). The gross photosynthetic rates of thin or sheet-like forms were reduced by desiccation, although net photosynthetic rates sometimes remained positive (ibid). Gross photosynthesis by Enteromorpha prolifera in an Oregon estuary decreased by 75% at 10 - 30% water loss (Pregnall 1983). Beers and Eshel (1983a, b) found that net photosynthetic rates of a tropical Ulva species were much lower when emerged than when submerged. They attributed this partly to the alga's preference for HCO_3^-, a form of inorganic carbon available only in water, rather than entirely to the stress of dehydration. Wiltens et al. (1978) classified Ulva scagelii and Enteromorpha linza as "desiccation sensitive" based on fluorescence induction curves of thalli undergoing desiccation and rehydration. They found that tolerant forms recovered upon rehydration when as much as 98% of the internal water had been lost whereas sensitive forms must retain much more than 25% to recover. Under some circumstances, fronds of Ulva have been shown to become resistant to desiccation by limiting water loss (Jenik and Lawson 1967).

The continuation of photosynthesis while the plant is emerged may create a short term deficit in nutrients relative to carbon (Thomas and Turpin 1980). Thomas and Turpin (ibid) investigated nutrient uptake of Fucus distichus when thalli had been resubmerged

after desiccation. They found that uptake of both nitrogen forms was enhanced at about 30% water loss in thalli that were likely to have been nitrogen-limited in the field. Enhancement of PO_4^{3-} uptake was also observed after maintenence in PO_4^{3-} free water for 24 h. Thomas (1983) observed an enhancement of NO_3^- uptake by Enteromorpha spp. after slight desiccation and NO_3^- release at higher desiccation levels. Since net photosynthesis may continue when desiccation-sensitive species are emerged, these plants also may develop a nutrient deficit. Alternatively, a large proportion of carbon fixed by Enteromorpha prolifera while emerged may be lost after resubmergence (Pregnall 1983) and a deficit may not occur.

This thesis examines the influence of some environmental factors on short term uptake of nitrogen and phosphorus of Enteromorpha prolifera from the Idaho Point tidal flat in Yaquina Bay, Oregon. Seasonal changes in the nitrogen and phosphorus content of algae are examined with reference to potential nutrient limitation in Chapter II. The kinetics of nitrogen uptake by nitrogen-limited and nitrogen-replete thalli of E. prolifera are presented in Chapter III. In addition, uptake measured by nutrient disappearance from the medium and accumulation of ^{15}N in the plant tissue have been compared. The influence of desiccation on the uptake of nitrogen and phosphorus by E. prolifera is covered in Chapter IV.

CHAPTER II

CARBON, NITROGEN AND PHOSPHORUS CONTENT OF *ENTEROMORPHA PROFIFERA*

The measurement of carbon, nitrogen and phosphorus concentrations in plant tissue is a relatively simple method of estimating the degree of nutrient limitation in field populations since the composition of algal tissue reflects the nutrient regime in which the alga is growing (Hanisak 1983). This type of tissue analysis has been used for aquatic angiosperms to assess the degree of nutrient limitation (Gerloff and Krumbholtz 1966) and has recently been applied to macroalgae (e.g. Hanisak 1979, Gordon et al. 1981, Probyn and Chapman 1983). Ideally, the relationship between tissue composition and growth rate should be determined for each species before elemental composition is used to monitor the nutrient status of field populations. However, data from field populations can be compared to elemental composition of other algae for which the relationship to growth rates has been established.

The relative elemental composition, or C:N:P ratio is also useful in determining nutrient status. For phytoplankton, deviations from the Redfield ratio of 106:16:1, C:N:P (Redfield et al. 1963) indicate nutrient stress (e.g. Goldman et al. 1979). The relationship between C:N:P ratio and nutrient status has not been established for macroalgae, although the Redfield ratio appears to be inappropriate (Atkinson and Smith 1983). Variations among the

available data may be due to taxonomic differences and environmental history (Niell 1976, Atkinson and Smith 1983, Hanisak 1983). Despite these problems, elemental ratios in macroalgae tissue may be useful for comparisons between different populations and cultures.

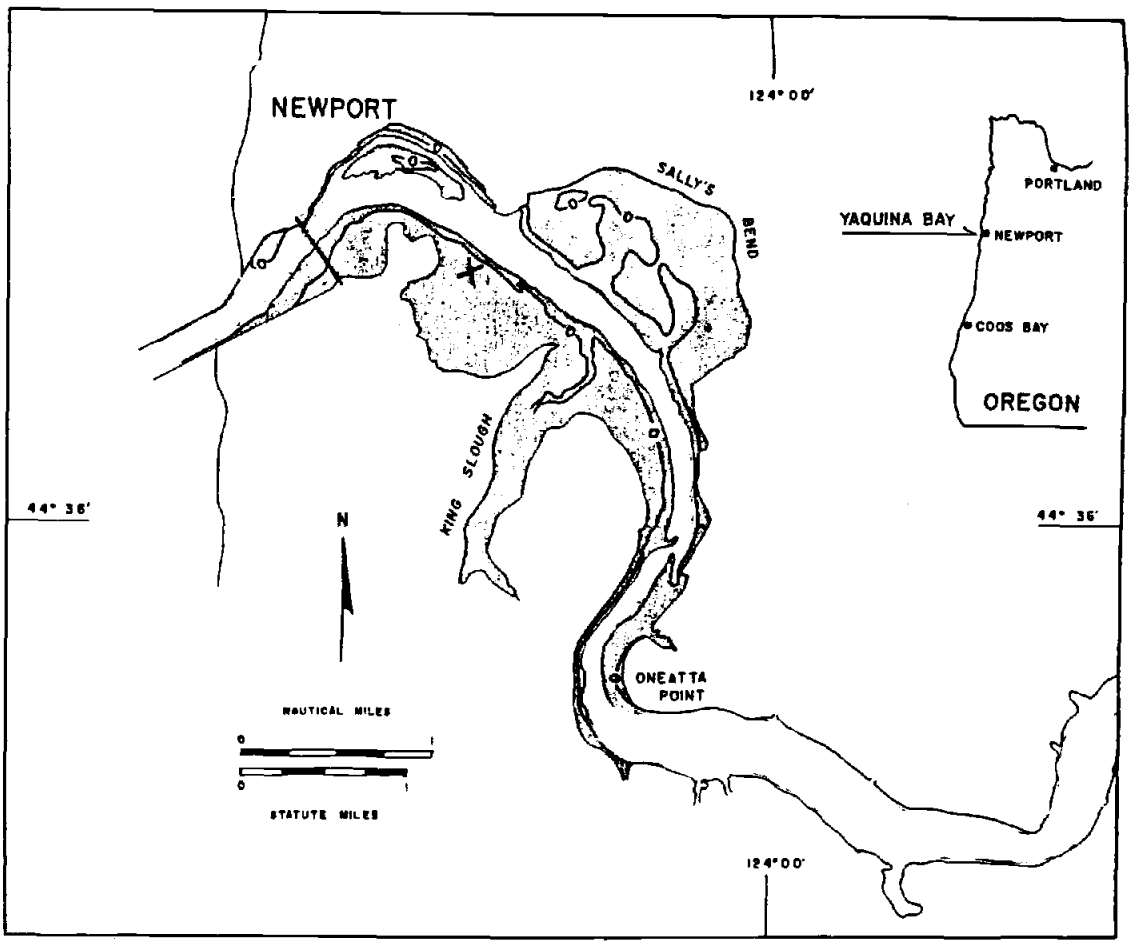
In most coastal environments, nitrogen is assumed to be the element that limits plant growth (Ryther and Dunstan 1971) and consequently, most investigators are concerned with this element. The relationship between phosphorus content and growth and the C:P and N:P ratios of macroalgae are poorly documented. Because estuaries represent a transition between fresh and seawater, it is possible that phosphorus can limit estuarine plant growth at certain times of the year (Taft and Taylor 1976, Nowicki and Nixon 1985, Short et al. 1985).

METHODS

All plants were collected from the Idaho Point mudflat within a radius of 50 m from the location shown in Figure II.1. From 3 to 7 samples were collected from damp or submerged mats at near the 0 tide height approximately biweekly between April and October, 1985 and at longer intervals in the fall, winter and early spring. Only bright green epiphyte-free algae were chosen. The elemental composition of thalli maintained in low-nutrient seawater was also measured. These thalli were maintained at 12 - 14^{o}C at light intensities of 400 $\mu E\ m^{-2}\ sec^{-1}$ or in an outdoor culture facility with ambient light and temperature. The algae maintained under artificial conditions

Figure II.1. Yaquina Bay, Oregon (from Kulm 1965). Mudflats are shaded. Collection site is given by X.

Figure II.1


NEWPORT
124° 00'
SALLY'S
BEND
YAQUINA BAY
PORTLAND
NEWPORT
COOS BAY
OREGON
KING SLOUGH
44° 36'
N
ONEATTA
POINT
NAUTICAL MILES
STATUTE MILES

were held in 2.7 L jars and low-nutrient seawater was replaced once day^{-1}. The outdoor culture facility consisted of a series of ca. 11.5 L tanks filled with seawater which had been pumped from Yaquina Bay and stripped of nutrients. The salinity in the tanks ranged from 30.9 to 31.9°/oo and temperature ranged from 15 to 20°C. The flow rate was 1 - 2 turnovers day^{-1}. Tissue content of these samples provided an indication of the lower limit of nitrogen and phosphorus content for E. prolifera.

After collection, the algae were kept on ice until they were returned to the laboratory where they were rinsed with filtered seawater, frozen, and dried for 24 h at 60°C. Dried samples were stored under vacuum until analyzed. Ash content was measured for 13 samples by combusting tissue of known dry weight at 500°C for 2 h. Carbon and nitrogen were determined in duplicate for each sample by combustion using a Perkin Elmer Model 240 Elemental Analyzer. Phosphorus was determined for each sample in duplicate after persulfate digestion of the tissue (Grasshoff 1976). A known amount of dried, pulverized tissue (ca. 5 mg) was digested with 200 mg of potassium persulfate in 24 ml of deionized water at 10 psi pressure for 30 min. After digestion the sample was centrifuged and the soluble reactive phosphate concentration of the supernatant measured. To account for errors in handling and dilution, the percent recovery of phosphorus was determined by digesting a standard KH_2PO_4 solution with the samples. Recovery averaged about 96%. All tissue composition data are expressed as % of dry wt.

RESULTS

Means and standard deviations for carbon, nitrogen and phosphorus content of tissue samples are given in Figure II.2. For all three elements, seasonal trends were evident although there were no significant differences (Student's t-test, $P \leq 0.05$) between the mean C, N or P content of algae collected from May through August and those collected in the winter and fall. The small parallel changes of elemental abundances observed during the summer may have been due to the variable ash content. Ash was found to comprise 19.0 - 33.1% of dry wt, but was not measured routinely. Carbon content ranged from 18.8 to 35.5% while nitrogen ranged from 2.28 to 5.43%. Both carbon and nitrogen were higher and less variable in the winter than in the summer. Phosphorus content ranged from 0.121 to 0.337%, a 2.8 fold change. There was considerable variation among data for each sampling date and the coefficients of variation were often greater than 25%. The highest phosphorus values were observed in the summer rather than in winter, i.e. the opposite of the seasonal pattern observed for carbon and nitrogen.

Seasonal variation in the elemental ratios were greater than variations in elemental content. The C:N, N:P and C:P ratios are given in Figure II.3. The C:N ratio increased from 7.5 to 12.2 during early spring 1985 as a result of a decrease in N content. For the remainder of the year, the C:N ratio fluctuated between 8 and 10. N:P ratios were greater than 40 in winter and fall, and lower, between 20 and 30, during the spring and summer. The variation in

Figure II.2. Carbon (A), nitrogen (B) and phosphorus (C) content as % of dry weight of Enteromorpha prolifera. Error bars are ± one standard deviation. The number above the point is the number of plants used to calculate the mean. Plants were collected from the Idaho Point mudflat between August 1984 and November 1985. Dashed lines are explained in text.

Figure II.2

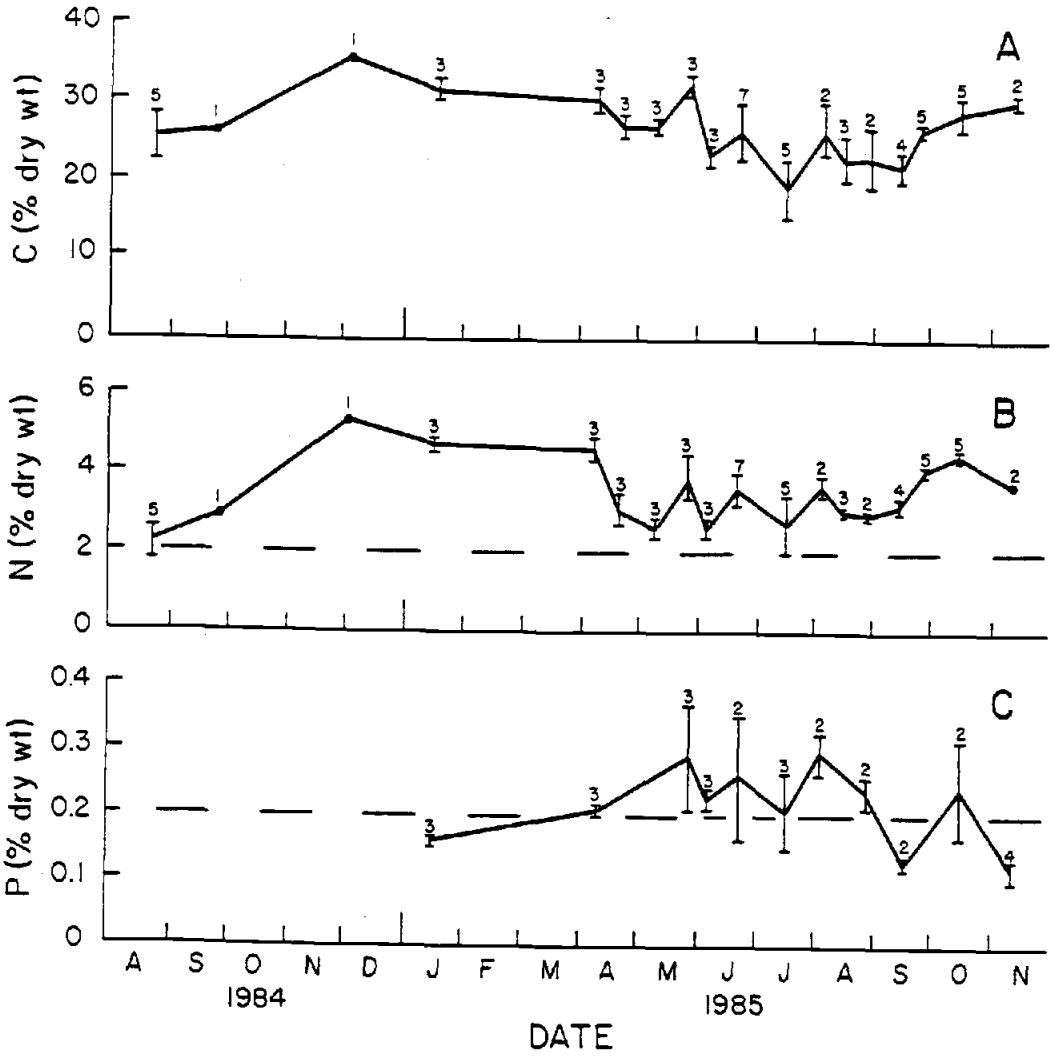
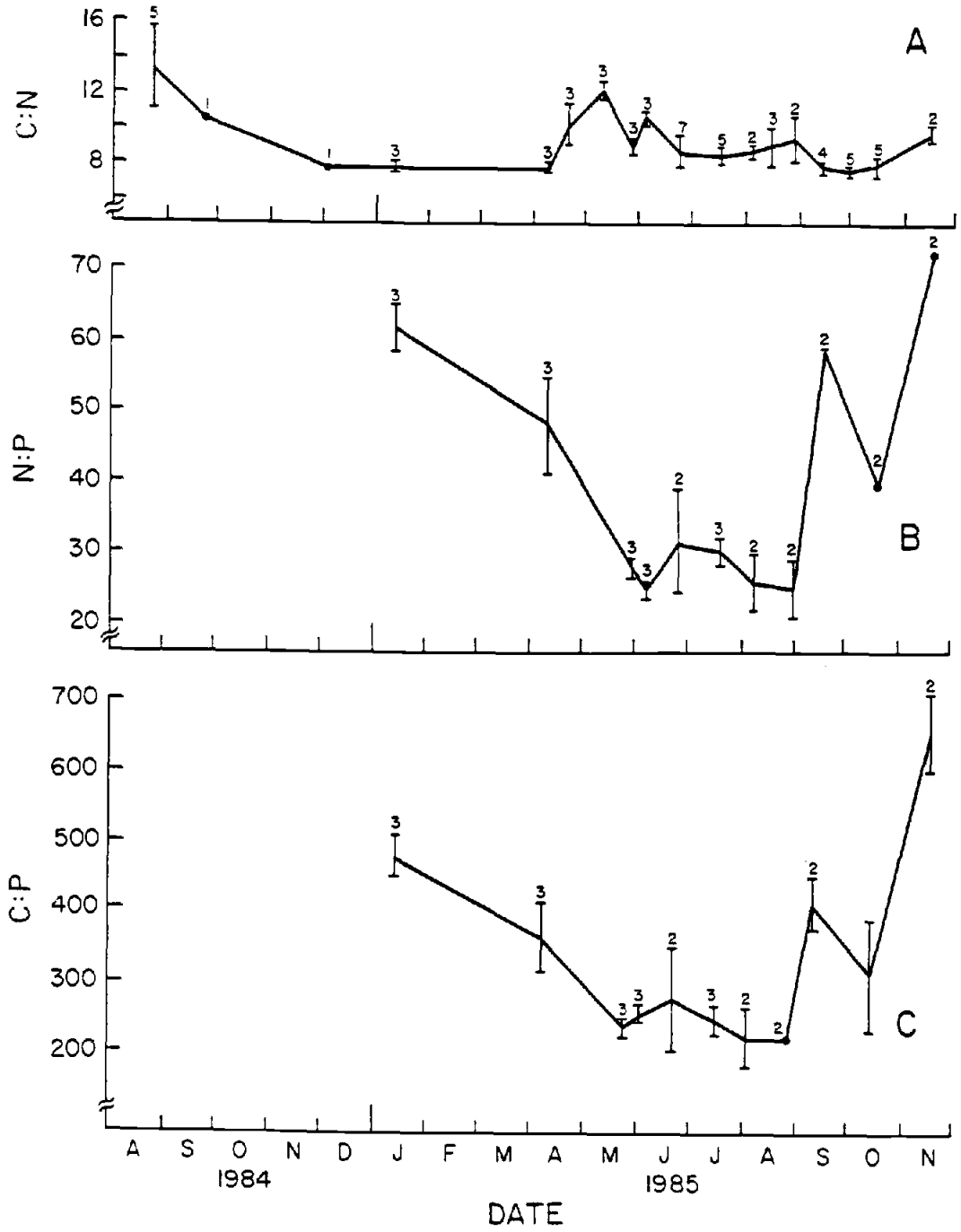

A
B
C
C (% dry wt)
N (% dry wt)
P (% dry wt)
40
30
20
10
0
6
4
2
0.4
0.3
0.2
0.1
0
A S O N D J F M A M J J A S O N
1984
1985
DATE

Figure II.3. C:N (A), N:P (B) and C:P (C) ratios by atoms of Enteromorpha prolifera. Error bars are ± one standard deviation. Plants were collected from the Idaho Point mudflat between August 1984 and November 1985. The number above the point is the number of samples used to calculate the mean.

Figure II.3


C:N
16
12
8
A
N:P
70
60
50
40
30
20
B
C:P
700
600
500
400
300
200
C
A S O N D J F M A M J J A S O N
1984
1985
DATE

the N:P ratio was high throughout the year; generally this was due to fluctuations in phosphorus content. The patterns of seasonal variation in the C:P ratio were similar to those in N:P, with ratios generally higher than 300 in the winter, and lower in the summer. The highest and lowest C:P ratios observed were 661 and 220, respectively. The average N:P and C:P ratios for the period from May through August 1985 were significantly lower ($P \leq 0.05$) than the average of winter/early spring and fall ratios. There was no significant difference between the C:N ratios for these periods.

On three occasions algae were maintained in nutrient-free water and their elemental composition monitored to assess changes in composition during starvation. Table II.1 presents tissue composition data for these algae before and after starvation. In two experiments, the nitrogen content decreased by about 50%, and the C:N ratios increased to ca. 20. This level of tissue-N depletion was never observed in the field populations. In the third experiment, 1/23/85, tissue N decreased only 12%. Phosphorus decreased only 14% in both incubations, and the ending tissue-P was low when compared to the field populations, but within the range of values observed. Similarly, the N:P ratios were high but within the range of those observed in the field.

Table II.1. Average nitrogen and phosphorus content (% dry wt) and average C:N, N:P and C:P ratios (by atoms) of E. prolifera grown under low nutrient conditions. ND = no data.

DATE		NITROGEN mean	NITROGEN SD	PHOSPHORUS mean	PHOSPHORUS SD	C:N	N:P	C:P
12/20/84	Initial	5.43	ND	ND	ND	7.63	ND	ND
	Final	1.86	ND	ND	ND	21.8	ND	ND
1/23/85	Initial	4.67	0.06	0.169	0.0121	7.78	61.6	476
	Final	4.11	0.23	0.153	0.0087	10.6	59.4	627
9/23/85	Initial	3.41	0.29	0.124	0.0040	7.56	56.6	427
	Final	1.78	0.29	0.102	0.0067	19.4	38.6	749

Table II.2. Ranges of carbon, nitrogen, and phosphorus content (% dry wt) of some representative marine macrophytes. ND = no data.

SPECIES	C	N	P	SOURCE	REFERENCE
Enteromorpha spp.	18.8-35.5	2.28-5.43	0.121-0.337	estuary	this study
	13.5-23.2	0.57-3.85	ND	salt marsh	Fujita 1985
	17. -23.	0.5 -3.2	ND	estuary	Owens & Stewart 1983
Cladophora spp.	ND	1.2 -4.1	0.05-0.31	estuary	Birch et al. 1981
	ND	1.3 -7.5	0.08-0.89	culture	Gordon et al. 1981
	ND	ND	0.08-0.87	culture	Schramm & Booth 1981
Ulva spp.	ND	0.2 -0.8	ND	rocky intertidal	Rosenberg & Ramus 1982
	25.6-30.2	1.02-3.59	ND	salt marsh	Fujita, 1985
	ND	2.99-4.65	ND	culture	Lapointe & Tenore 1981
Chordaria flagelliformis	ND	2.3 -3.9	ND	field	Probyn & Chapman 1983
	ND	0.82-4.02	ND	culture	Rosenberg et al. 1984
	ND	1.01-1.56	ND	field	Rosenberg et al. 1984
Macrocystis spp.	ND	3.1	0.12-0.53	culture	Manley & North 1984
	23.2-30.5	0.83-2.96	0.2 -0.6	kelp bed	Rosell & Srivastava 1984, 1985
Nereocystis leutkeana	19.8-27.9	0.93-2.91	0.2-0.7	kelp bed	Rosell & Srivastava 1984, 1985
Porphyra tenera	ND	3.1	0.17	unknown	Raven 1981[1]
Gracillaria tikvahiae	13.1-21.3	1.29-2.34	ND	field	Friedlander & Dawes 1985
	26.4-28.5	1.99-2.76	ND	salt marsh	Fujita 1985

[1] data given as ratios, content based on C content of 25% dry wt.

DISCUSSION

The tissue content of nitrogen or phosphorus in marine macrophytes decreases with increasing nutrient stress. The most common pattern observed is reduced levels in the summer when external nutrients are low and growth is highest (Hanisak 1983). The C, N and P content of macroalgae from a variety of habitats is shown in Table II.2. The ranges of tissue composition observed for E. prolifera are similar to the ranges observed for these species. Nitrogen content of E. prolifera tends to be slightly higher than that of algae from rocky intertidal areas, but similar to those from other estuaries or in culture. Asare and Harlin (1983) measured tissue-N for five macroalgal species and found that winter values were 2 to 3 fold higher than summer values. The seasonal fluctuations of E. prolifera tissue-N also follow this pattern and are within the ranges observed for these algae. There are less data available for phosphorus. Phosphorus levels of E. prolifera were similar to those of other algae collected from field populations, but lower than those of algae grown in culture (Table II.2).

Atkinson and Smith (1983) summarized the C:N:P ratios of 92 species of marine macroalgae and give a median ratio of 550:30:1. This yields a median C:N of 18.3, which is higher than that observed for E. prolifera. Niell (1976) observed that the Chlorophyta and Rhodophyta tended to have a lower average C:N (10) than the Phaeophyta, mainly due to their higher nitrogen content. The C:N of E. prolifera averaged 9.1 (SD = 1.7) for 1984-85, close to Niell's

average ratio. Using Niell's (ibid) C:N ratio (10:1) and an N:P ratio of 30:1 (Atkinson and Smith 1983), the average C:N:P ratio of E. prolifera can be approximated as 300:30:1.

In phytoplankton studies, deviations from the Redfield et al. (1963) ratio of 106:16:1 are used to infer nutritional status of the plants (Goldman et al. 1979). The ratios of E. prolifera can be examined in this manner, using an average ratio of 300:30:1. Hanisak (1983) suggested an upper limit of 15 for the C:N of non-nitrogen-limited algae. For most of the year, the C:N ratio of E. prolifera fluctuated around 10, thus nitrogen was probably not limiting plant growth in 1985. The C:N ratio increased sharply during the spring to a maximum of 12.1. This increase was due to a decrease in nitrogen content and suggests that the nitrogen uptake rate was slower than the growth rate at this time. The nitrogen content of Ulva spp. has been observed to decrease with increasing light intensity due to a decrease in pigment content (Rosenberg and Ramus 1982, Duke et al. 1986). This may have occurred in E. prolifera during the spring of 1985. The seasonal patterns of C:N may change from year to year, since the mean C:N in August 1984 was 13, somewhat higher than the C:N observed during the summer of 1985.

Deviations of N:P and C:P ratios from the mean value were more extreme than was the C:N ratio. Phosphorus-limitation is indicated by N:P or C:P ratios greater than 30 or 300, respectively. The average C:N:P for May through September 1985 was 285:32:1. During the winter/early spring and late fall 1985, the average C:N:P was

467:55:1. E. prolifera biomass and growth rates are highest during late spring and summer (Davis 1981) and this corresponds to the period during which N:P was less than or equal to 30 and C:P was less than 300. Deviations from the average ratio of 300:30:1 indicate that phosphorus may have limited the growth of E. prolifera during the spring and fall of 1985.

Evaluation of nutrient limitation from elemental tissue composition requires the determination of a critical level for that element (Gerloff and Krumbholtz 1966). This level has been defined as the minimum amount of a particular element necessary in plant tissue to sustain maximum growth (ibid). Elemental contents less than and greater than the critical level indicate limitation and surplus, respectively. When the growth rate is zero, the tissue content of the limiting nutrient is at the minimum, defined as q_o (Droop, 1973). The average critical nitrogen level (C_N) for the algae listed in Table II.3 is about 2% and is indicated on Figure II.2b by the dashed line. If this is a reasonable estimate for E. prolifera, then growth was not nitrogen limited during 1985, although in August 1984 and July 1985 the nitrogen content of a few algae was less than 2%. The average critical phosphorus level (C_P) for algae in Table II.3 is 0.2% and is shown on Figure II.2c. During the winter and again in the late fall, the phosphorus content of E. prolifera was well below this level suggesting P-limited growth. During the summer, the average phosphorus content was above C_P although several plant specimens had lower phosphorus contents. The period during which the tissue phosphorus was greater than 0.2% is

Table II.3. Estimates of subsistence quota (q_o) and critical levels (C_N or C_P) as % dry wt for nitrogen and phosphorus in cultures of marine algae. ND = no data.

SPECIES	NITROGEN		PHOSPHORUS		REFERENCE
	q_o	C_N	q_o	C_P	
Cladophora albida	1.2	2.1	0.05	0.33	Gordon et al. 1981
Cladophora spp.	-	1.5	-	0.16	Wong & Clark 1976[1]
Codium fragile	0.8	2.1	ND		Hanisak 1979
Chordaria flagelliformis		0.7	ND		Rosenberg et al. 1984
	0.4	1.2	ND		Probyn & Chapman 1983[2]
Laminaria saccharina	1.3	-	ND		Chapman et al. 1978
Macrocystis pyrifera	0.7	-	ND		Wheeler & North 1980[3]
	ND		0.08	0.20	Manley & North 1984[4]
Fucus distichus	0.6		ND		Rosenberg et al. 1984
Angiosperms	-	1.3	-	0.13	Gerloff & Krumbholz 1966[5]

1
2 Growth estimated from photosynthetic rates in streams.
3 Nitrogen q_o is the average of values for growth on different N sources.
4 Growth on nitrogen was not saturated.
5 Phosphorus q_o is exptrapolated from Figure 3b in Manley & North 1984.
Average C_N and C_P for 5 freshwater angiosperms.

the same as that during which the C:N:P is less than 300:30:1. The atomic ratio (N:P) of a plant containing the critical levels of nitrogen and phosphorus indicated by the literature is 22.1, which is lower than the measured average of 30 for E. prolifera. Thus the estimated levels of C_N and C_P may not be entirely appropriate for E. prolifera; either C_N is higher than 2% or C_P is lower than 0.2%. If the actual C_P for E. prolifera is 0.15%, the algae collected in the spring and fall of 1985 would still be approaching phosphorus limitation.

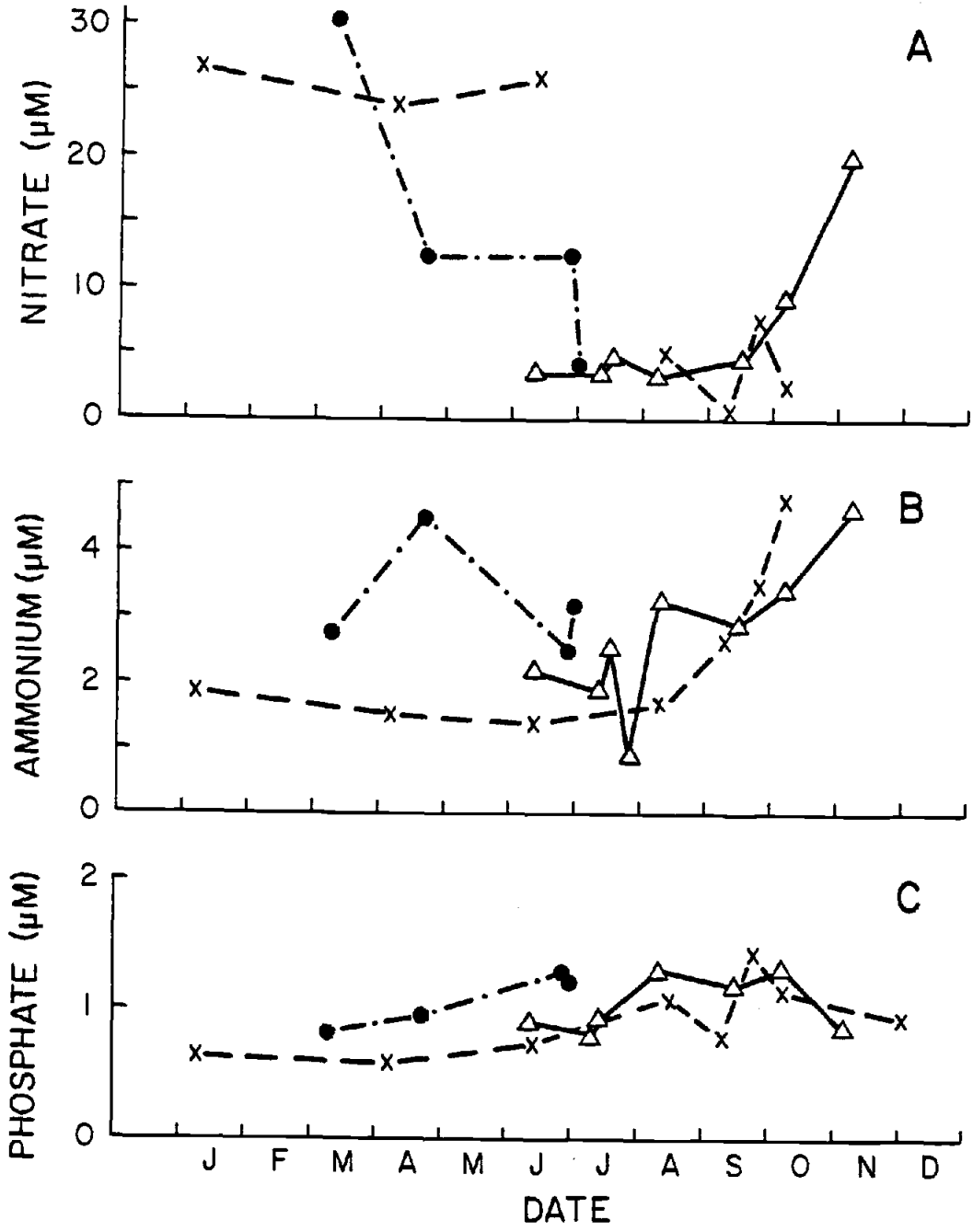
The tissue content of algae after nutrient starvation (Table II.1) provides an estimate of the subsistence quota, or q_o, for E. prolifera. Nitrogen and phosphorus subsistence quotas for E. prolifera were higher than those observed for other algae (Table II.3). The field samples of E. prolifera were always higher in nitrogen than starved algae, but the phosphorus content of field samples collected during winter and fall approached that of starved algae (note 18 January, 14 September and 12 November, Fig II.2). During winter and fall, C:P ratios of 600 to 700 or N:P ratios greater than 50 were also observed (Fig II.3). For the January and September starvation experiments, phosphorus content was already low, and further decreases were small (Table II.1). The changes in N:P were due mainly to changes in nitrogen content. Neither nitrogen nor phosphorus decreased by more than 12% and growth was very slow during the January experiment. Since winter is not a period of active growth (Davis 1981), these algae may have been in a dormant phase; alternatively, some other factor limited growth. Approximately 1 μM

NH_4^+ was present during this incubation, but the PO_4^{3-} concentrations were not measured.

The average concentrations of dissolved nitrogen and phosphorus in surface and bottom water from September 1983 to July 1985 (Garber et al. 1984, Collins 1987, Garber et al. in prep.) are presented in Figure II.4. The coefficients of variation were up to 50% and the highest variation was in the NO_3^- data from winter and spring. Nitrate concentrations are highest (>20 μM) in the bay from late fall through spring and are the result of high runoff (Garber et al. 1984). During the summer and early fall, concentrations are lower, usually <5 μM. There is very little seasonal variation in NH_4^+ or PO_4^{3-} concentrations, although both nutrients tend to be higher during the late summer and fall. The drop in plant tissue phosphorus is not correlated with a drop in dissolved PO_4^{3-}. Collins (1987) estimated the supply of PO_4^{3-} from the Yaquina River and from remineralization on the mudflat and compared these to potential macroalgal utilization calculated from fluxes in enclosed chambers. He found that PO_4^{3-} is mainly supplied by remineralization from the sediments, which was highest in the late summer and fall. Except for one sample in July, total PO_4^{3-} supply was greater than macroalgal utilization during 1984. Additional PO_4^{3-} was probably supplied to the bay by upwelling events in the coastal water (Collins 1987). Assuming that similar processes were occurring in 1985, it is difficult to find environmental data to support the apparent phosphorus limitation suggested by the tissue content data.

Figure II.4. Nitrate (A), ammonium (B) and phosphate (C) concentrations in Yaquina Bay from September 1983 to June 1985. Δ——Δ, 1983; X——X, 1984; ●—·—●, 1985. From Collins (1987), Garber et al. (1984) and Garber et al. (in prep.).

Figure II.4


NITRATE (μM)
30
20
10
0
A
AMMONIUM (μM)
4
2
0
B
PHOSPHATE (μM)
2
1
0
C
J F M A M J J A S O N D
DATE

It is possible that the lower tissue phosphorus observed during the fall and winter was not due to phosphorus limitation, but was the result of senescence or environmental stress. Whyte et al. (1981) found that inorganic molecules were easily removed from kelp tissue by leaching with fresh water. After 2 hours, 57 and 46% of tissue phosphorus was lost from Macrocystis integrifolia and Nereocystis luetkeana, respectively, and loss continued with subsequent leaching. Only 22% of N was lost from these algae during the same period. They concluded that small molecules not organically bound were most easily removed. The mudflats in Yaquina Bay are likely to be exposed to freshwater during the fall and winter. The salinity of the surface water drops to ca. $15^{o}/oo$ during the winter due to increased rainfall, and the mudflat is exposed directly to rainfall during low tide. Efflux of PO_4^{-3} is known to occur from the leaves of Elodea and Nitella (Bielski 1973, cited in Nalewajko and Lean, 1980). However, Myriophyllum released very little phosphorus unless it was senescent. Since Enteromorpha spp. has been observed to store polyphosphates (Kuhl 1962) intracellular PO_4^{3-} is likely to be present. E. prolifera has been shown to lose significant amounts of organic carbon after desiccation, especially if resubmerged in freshwater (Pregnall 1983) and perhaps PO_4^{3-} is lost as well. Finally, although plants chosen for this study appeared to be healthy, their growth is highly seasonal and plants may have been dormant or senescent during fall and winter.

In estuaries, phosphorus and nitrogen are of equal importance due to the transitional position of estuaries between fresh and

seawater. Evaluation of elemental composition of E. prolifera growing in Yaquina Bay suggested that growth may have been phosphorus-limited during the fall of 1985. However, this conclusion was not supported by the available data on rates of phosphorus supply. This disparity may have been due to interannual differences in the nutrient regime. Differences between C:N ratios during August 1984 and 1985 also suggested that seasonal patterns may change from year to year. Alternatively, concentrations of phosphorus in algal tissue during the fall may have been lower because these plants were senescent rather than phosphorus-limited. Knowledge of the importance of phosphorus at different life history stages would aid the evaluation of tissue composition data.

CHAPTER III

SHORT TERM UPTAKE OF NUTRIENTS BY *ENTEROMORPHA PROLIFERA*

The conventional method for measuring uptake of nutrients by macroalgae has been to determine changes in nutrient concentration in the incubation medium over time (Harlin and Wheeler 1985). In such experiments, incubations must be long enough for significant changes in concentration to occur and control incubations without plants are necessary.

Isotopes have been used extensively for measuring uptake of nutrients by phytoplankton (reviewed by Harrison 1983), but have only recently been applied in experimental studies with macroalgae. Williams and Fisher (1985) and Owens and Stewart (1983) used $^{15}NH_4^+$ to investigate uptake kinetics of *Caulerpa cuppressiodes* and nutrient cycling in macroalgal mats, respectively. Accumulation of isotopic tracers in plant tissue allows the direct measurement of nutrient uptake by the plants. Because the measurement of isotope incorporation is of higher sensitivity than the measurement of external nutrient concentration, shorter incubation periods are required.

Drawbacks to the use of stable isotopes for uptake experiments include the necessity of expensive equipment and time consuming sample preparation and analysis. Furthermore, in studies with natural assemblages of phytoplankton, not all of the added isotope is

recovered at the end of the experiment (Glibert et al. 1982, Laws 1984). Possible explanations for incomplete recovery of isotope include uptake by particles not captured by the filters or uptake by the plant and subsequent loss as labelled organic nitrogen. Williams and Fisher (1985) found considerable discrepancy between rates calculated from isotope accumulation in Caulerpa cuppressiodes and by disappearance of NH_4^+. No attempts have been made to trace the possible fate of ^{15}N tracers during incubations with macroalgae. I conducted tracer experiments with Enteromorpha prolifera to determine if ^{15}N could be recovered in NH_4^+ and NO_3^- uptake experiments and to compare uptake rates determined by ^{15}N accumulation in plant tissue and by disappearance of nutrient from the medium.

The kinetic parameters K_s, V_{max} and V_{max}/K_s are used to compare the uptake abilities of different algal species, to estimate the relative utilization of different nutrients, or to make inferences about the relationships between nutrient availability and plant distributions (Topinka 1978, Hanisak 1983). Although species of Enteromorpha are of great ecological importance due to their high productivity and tendency to proliferate under eutrophic conditions (FitzGerald 1978, Shellem and Josselyn 1982, Pregnall and Rudy 1985), little is known of the effects of nitrogen speciation, life history stage or nitrogen status on nitrogen uptake. I investigated the effects of nitrogen status on the uptake of NH_4^+ and NO_3^- by E. prolifera.

In many phytoplankton species NH_4^+ concentrations higher than about 1 μM inhibit the uptake of NO_3^- (McCarthy 1981). This inhibition has been observed to some extent in species of Gracilaria (D'Elia and DeBoer 1978) and Macrocystis (Haines and Wheeler 1978) but not in Fucus (Topinka 1978). The effect of NH_4^+ on the relative utilization of NO_3^- and NH_4^+ in estuarine environments could be important, since the flux of NH_4^+ from sediments may result in locally high concentrations of NH_4^+. I conducted one experiment to determine if high concentrations of NH_4^+ supressed uptake of NO_3^-.

There is evidence that phosphorus rather than nitrogen may limit the growth of plants in some estuaries (Birch et al. 1981, Short et al. 1985). The tissue composition of E. prolifera (Chapter II) indicated that this alga may be growing under phosphorus limitation in Yaquina Bay. However, very little is known about PO_4^{3-} utilization by macroalgae. I measured the simultaneous uptake of nitrogen and phosphorus by E. prolifera and compared these rates to the N and P status of the plants.

METHODS

For all experiments, algae were collected from the Idaho Point mudflat at the location shown in Fig. II.1 and maintained at 12-14°C at light intensities of 400 μE m^{-2} sec^{-1} (in air) provided by G. E. cool white bulbs or in an outdoor culture facility with ambient light and temperature. The outdoor culture facility consisted of a series of ca. 11.5 L tanks filled with seawater pumped from Yaquina Bay.

The salinity in the tanks ranged from 30.9 to 31.9°/oo and temperatures ranged from 15 to 20°C. Nutrient concentrations were controlled by mixing enriched seawater with seawater previously stripped of nutrients. The flow rate was >10 turnovers day^{-1} and tanks were aerated by bubbling with compressed air. Most experiments were carried out 12 to 48 hours after the plants were collected. For the N-limited experiments, plants were maintained in the outdoor tanks described above with low nutrient seawater at 1 - 2 turnovers day^{-1} until tissue nitrogen content was less than 2% dry wt, the assumed critical nitrogen level for this alga (see Chapter II). Nitrogen depletion required approximately 2 weeks and growth rates were monitored during this period.

Water from Yaquina Bay was used for experimental incubations after filtration through 1.0 um Millipore cartridges and stripping of ambient nutrients (if necessary) by incubations with E. prolifera or Ulva spp. In the laboratory, water was filtered a second time through Whatman GF/F filters or a Gelman 0.2 um filter cartridge and pooled into one carboy before the uptake experiments. All incubations were carried out in 2 L glass jars or 2.7 L polycarbonate bottles containing one or 1.5 L of seawater. The plant density was 300 - 500 mg wet wt L^{-1}. All incubations were carried out at 12 to 14°C and a light intensity of 400 $\mu E\ m^{-2}\ sec^{-1}$. Photosynthesis of Enteromorpha spp. is saturated at this light intensity (Davis 1981, Shellem and Josselyn 1982). Nitrogen content of triplicate or duplicate samples of plant tissue was measured with a Perkin Elmer

elemental analyzer. Phosphorus content was measured as described in Chapter II.

Recovery of tracer and comparison of rate measurements

Experiments were conducted with $^{15}NH_4^+$ and $^{15}NO_3^-$ to determine if all of the isotope added to incubation bottles could be recovered at the end. Ammonium uptake experiments were conducted with both filtered and filtered/autoclaved water as an incubation medium since NH_4^+ is taken up rapidly by microorganisms, as well as by microalgae. Only filtered water was used for NO_3^- experiments since NO_3^- is used more slowly by microorganisms (Kaplan 1983).

For the $^{15}NH_4^+$ experiment, filtered seawater (Whatman GF/F filters) was dispensed to each of 12 polycarbonate bottles and six of these were autoclaved at 20 lbs pressure for 30 minutes and allowed to cool. For about 30 minutes just before the experiment, the seawater was mixed by bubbling with acid-scrubbed air so that gases could re-equilibrate. Aseptic conditions were not maintained during the experiment. Ammonium (15 atom % ^{15}N) was added to each bottle for a final concentration of 5 μM. Approximately 500 mg wet weight of E. prolifera was added to two bottles of each treatment (autoclaved and unautoclaved), and two additional bottles of each treatment served as controls without plant tissue. The medium was mixed by bubbling with acid-washed air. The remaining bottles were used to determine the initial atom % enrichment of dissolved NH_4^+. At the end of the incubation (ca. 5 h), the plants were strained from the water which was collected in 500 ml bottles and frozen (T = 0^oC).

For the $^{15}NO_3^-$ experiment, 300 to 500 mg of E. prolifera was added to 6 bottles and 3 bottles served as controls.

Isotopic enrichment (atom % ^{15}N) of the plant tissue and of the dissolved NH_4^+ was measured for all NH_4^+ incubations. The plant tissue was frozen, dried at 60°C for 24 hours and weighed. Approximately 2 mg of dried plant was ground with Cuprox (Coleman Reagent), placed in 3 mm ID pyrex tubes containing CaO, sealed under vacuum (< 2 μm Hg) and combusted for 2 hours at 500°C. Triplicate tubes were prepared for each sample. Atom % enrichment was measured by emission spectrometry as described by Fiedler and Proksch (1975). Four or five scans were used for each ratio determination, and each sample was analyzed in triplicate. If the coefficient of variation exceeded 5%, additional sample tubes were prepared and analyzed.

The medium (800 ml) was stored frozen for 1 to 3 days before NH_4^+ was collected by steam distillation (Glibert et al. 1982). If the NH_4^+ concentration was less than 3 μM, an additional 5 μM NH_4^+ (6 atom % ^{15}N) was added as a carrier. The distillate was collected in 0.1 N HCl (Ultrex), boiled down to approximately 200 μl and spotted on a precombusted GF/F filter. Filters were ground with Cuprox and ^{15}N ratio determined as for plant tissue. The atom % enrichment of the NO_3^- was assumed to be constant during these incubations.

Samples for determinations of NH_4^+ or NO_3^- concentrations were collected in duplicate from each incubation container at the beginning and end of the experiment. Analyses were carried out

within 2 hours. Ammonium was determined by the phenolhypochlorite reaction (Solorzano 1969) and NO_3^- according to Grasshoff (1976).

Uptake kinetics

The kinetics of NH_4^+ and NO_3^- uptake by nutrient-sufficient and nutrient-deficient plants were examined and compared to tissue composition. In addition, N:P uptake ratios of nutrient-sufficient and deficient plants were compared.

Kinetics of NH_4^+ and NO_3^- uptake were measured independently for the same plant on two consecutive days. For all experiments, only the nitrogen source of interest was supplied to the plant; the concentration of the other form of nitrogen was less than 1 μM. Phosphate was added to maintain a N:P ratio of 10 in the medium. The multiple flask method (DeBoer 1981, Harlin and Wheeler 1985) was used for all kinetic experiments. Duplicate incubations with plant tissue and a single control (no plant tissue) were run for each nutrient concentration. The plant density was approximately 500 mg wet wt L^{-1}. Nutrients were measured 4 - 5 times during the incubation and uptake rates were calculated from the rate of removal of nutrient from the medium. The experiments lasted from 45 minutes to 2 hours and mixing was provided by aeration with acid-washed air. Nutrient samples were refrigerated and analyzed within eight hours using a Technicon AutoAnalyzer II with a modification of the methods of Atlas et al. (1971).

Ammonium-nitrate interactions

Algae were incubated with 15 uM NO_3^- and 1, 7 or 15 uM NH_4^+. At each nutrient level, 2 bottles contained E. prolifera and 1 bottle served as a control. Phosphate was added at a final concentration of 2 μM to prevent phosphorus limitation during the experiment. Uptake rates were calculated as described above.

CALCULATIONS

Net uptake

Equation 1 shows the net uptake rate calculated from the difference between initial and final amounts of nutrient in the medium (Harlin and Wheeler 1985).

$$\mu\text{mol N g dry wt}^{-1}\ \text{h}^{-1} = \frac{[\mu\text{mol Nutrient}]_i - [\mu\text{mol Nutrient}]_f}{\text{length of incubation (h)}} \cdot \frac{1}{\text{g dry wt plant}} \quad (1)$$

Removal of nutrient in the control jars should be subtracted from the change in the plant jars.

Accumulation of isotope

Uptake can also be calculated by appearance of ^{15}N in the plant (Williams and Fisher 1985).

$$\rho = \mu\text{mol N g dry wt}^{-1}\ \text{h}^{-1} =$$

$$\frac{[\text{atom \% }^{15}\text{N}]_f - [\text{atom \% }^{15}\text{N}]_i}{\text{length of incubation (h)}} \cdot \frac{1}{R} \cdot \frac{\mu\text{mol N plant}}{\text{g dry wt plant}} \qquad (2)$$

where R is the atom % enrichment of the dissolved nutrient and is calculated from the expression:

$$R = \frac{\mu\text{mol }^{15}\text{N added L}^{-1}}{\mu\text{mol [ambient + added] N}} \cdot 100 \qquad (3)$$

If the initial and final atom % enrichment of the dissolved nutrient are measured, then $R_{t1/2}$ is used in place of R and ρ becomes P:

$$P = \mu\text{mol N g dry wt}^{-1}\ \text{h}^{-1} =$$

$$\frac{[\text{atom \% }^{15}\text{N}]_f - [\text{atom \% }^{15}\text{N}]_i}{\text{length of incubation (h)}} \cdot \frac{1}{R_{t1/2}} \cdot \frac{\mu\text{mol N plant}}{\text{g dry wt plant}} \qquad (4)$$

$R_{t1/2}$ is the exponential average between the initial (R_o) and final (R_t) isotope enrichment of the dissolved nutrient (Glibert et al. 1982).

$$R_{t1/2} = \frac{1}{kt} \cdot R_o(1 - e^{-kt}) \qquad (5)$$

Isotope dilution and changes in nutrient concentration

Finally, uptake can be calculated by the disappearance of dissolved NH_4^+ after correction for $^{14}NH_4^+$ produced (Blackburn 1979). The rates i (for incorporation) and d (for dilution) are determined by Equations 6 and 7, respectively.

$$i = \mu mol\ N\ incubation^{-1}\ h^{-1} = d - \frac{P_t - P_o}{T} \quad (6)$$

$$d = \mu mol\ N\ incubation^{-1}\ h^{-1} = \frac{\ln R_t/R_o}{\ln P_t/P_o} \cdot \frac{P_o - P_t}{T} \quad (7)$$

P_o and P_t are the initial and final amounts of NH_4^+ in the medium and T is the length of the incubation in hours. To correct the uptake rate for variable amounts of plant biomass in the incubations, i is divided by the dry wt of plant tissue to yield the rate in units of $\mu mol\ N\ g\ dry\ wt^{-1}\ h^{-1}$.

Discrepancies between measurements of uptake calculated by net NH_4^+ removal and by accumulation of ^{15}N in plant tissue could result from 1) uptake of nutrients by microorganisms, 2) isotope dilution of the dissolved nutrient or 3) release of nitrogenous metabolites from plant tissue after nutrient uptake. Net removal of nutrients (Eq. 1) provides an accurate estimate of nutrient uptake only if uptake by microorganisms is either insignificant or subtracted from total uptake and NH_4^+ is not excreted. Assimilation of ^{15}N (p or P) will provide an accurate estimate of gross nutrient uptake by plants if

isotope dilution is insignificant or monitored during the experiment and there is no release of labelled nitrogenous metabolites. All of the methods will underestimate gross nutrient uptake if $^{15}NH_4^+$ is excreted during the experiment.

Discrepancies between P (accumulation of ^{15}N) and i (loss of dissolved ^{15}N and changes in NH_4^+ concentration) could be caused by bacterial uptake or uptake by the alga and subsequent release of labelled nitrogenous metabolites. If the discrepancy is due to uptake and subsequent loss of metabolites, then P reflects the net incorporation into tissue during the incubation and i reflects gross uptake. If the discrepancy is due to uptake by bacteria, then i reflects the uptake by bacteria + algae and P, the uptake by the algae alone.

In the recovery experiments, initial and final ^{15}N content of dissolved nutrient and plant tissue were compared. These were calculated by multiplying the total nitrogen content of the algae or the dissolved nutrient by the ^{15}N atom % enrichment/100. Since each of these measurements has an associated standard deviation, the standard deviation of the sums and products were calculated according to Bevington (1969).

In the kinetic experiments, jars were subsampled at several time points; consequently, uptake rates could be calculated by disappearance of nutrient in two ways. First, uptake rates were determined for each time interval according to Equation 1. Rates were also determined from a regression of nutrient concentration

against time, yielding an average rate for the entire incubation. Kinetic parameters, V_{max} and K_s, were determined by a nonlinear fitting program (Wilkinson 1961) or from the Wolfe transformation (Dowd and Riggs 1965).

RESULTS

Recovery of tracer

Recovery of ^{15}N for uptake experiments with NH_4^+ in filtered and filtered/autoclaved seawater is presented in Tables III.1 and III.2, respectively. The initial and recovered ^{15}N are compared in Fig. III.1. About 25% of the initial ^{15}N was not recovered as $^{15}NH_4^+$ in the filtered seawater controls (Table III.1). This loss was significant ($P \leq 0.05$) and occurred in both replicates. No loss of ^{15}N occurred in filtered/autoclaved controls or in either treatment when plants were present (Fig.III.1b-d).

The recovery of ^{15}N in the NO_3^- incubations with plants is presented in Table III.3. The initial concentration was 15 μM, and this decreased by 1.8 to 5.5 μM. The atom % enrichment of the plant tissue ranged from 0.877 to 1.321% ^{15}N at the end of the experiment. There was no significant loss of NO_3^- in the controls (data not shown). Assuming constant isotopic enrichment of dissolved NO_3^-, essentially all of the ^{15}N removed from the medium was recovered in the plant tissue. The discrepancy between the initial and final amounts of ^{15}N averaged 3%.

Table III.1. Recovery of ^{15}N for NH_4^+ incubations with and without algae in filtered seawater. Columns A and B denote replicate incubations. Standard deviations for measured quantities are given in parentheses and 95% confidence limits are given for calculated values. Incubation lasted about 5 hours.

	FILTERED SEAWATER		FILTERED SEAWATER + ALGAE	
	A	B	A	B
INITIAL				
Ammonium (µmol)	7.38 (0.079)	7.78 (0.171)	6.92 (0.136)	7.48 (0.385)
Enrichment (atom % ^{15}N)	11.67 (0.385)	11.67 (0.385)	11.67 (0.385)	11.67 (0.385)
^{15}N IN AMMONIUM (µmol)[1]	0.862 ± 0.092	0.908 ± 0.089	0.807 ± 0.077	0.873 ± 0.089
FINAL				
Ammonium (µmol)	6.43 (0.069)	7.20 (0.159)	2.56 (0.045)	2.78 (0.445)
Enrichment (atom % ^{15}N)	10.75 (0.285)	8.91 (0.202)	11.08 (0.520)	9.84 (0.118)
^{15}N IN AMMONIUM (µmol)[1]	0.700 ± 0.050	0.641 ± 0.050	0.284 ± 0.035	0.274 ± 0.109
Algal Nitrogen (µmol)	-	-	159.7 (9.8)	147.9 (9.1)
Enrichment (atom % excess)[2]	-	-	0.352 (0.020)	0.429 (0.013)
^{15}N IN ALGAE (µmol)[1]	-	-	0.562 ± 0.117	0.634 ± 0.107
RECOVERED ^{15}N (µmol)[3]	0.700 ± 0.050	0.641 ± 0.050	0.846 ± 0.122	0.908 ± 0.153
RECOVERED ^{15}N (% of initial ^{15}N)	81.2%[4]	70.6%[4]	104.8%	104.0%

1 ^{15}N = (µmol N) * (atom % ^{15}N / 100).
2 atom % excess = final atom % ^{15}N - measured natural abundance ^{15}N (0.449).
3 RECOVERED ^{15}N = ($^{15}N_{algae}$) + ($^{15}NH_4^+$).
4 difference between initial and final isotope significant ($P \leq 0.05$).

Table III.2. Recovery of ^{15}N for NH_4^+ incubations with and without algae in filtered/autoclaved seawater. Columns A and B denote replicate incubations. Standard deviations for measured quantities are given in parentheses, and 95% confidence limits are given for calculated values. Incubation lasted about 5 hours.

	FILTERED/AUTOCLAVED SEAWATER		FILTERED/AUTOCLAVED SEAWATER + ALGAE	
	A	B	A	B
INITIAL				
Ammonium (µmol)	7.20 (0.082)	6.96 (0.240)	7.47 (0.180)	7.50 (0.738)
Enrichment (atom % ^{15}N)	11.67 (0.385)	11.67 (0.385)	11.67 (0.385)	11.67 (0.385)
^{15}N IN AMMONIUM (µmol)[1]	0.840 ± 0.072	0.812 ± 0.097	0.872 ± 0.089	0.876 ± 0.226
FINAL				
Ammonium (µmol)	8.07 (0.204)	6.99 (0.153)	3.16 (0.463)	3.78 (0.105)
Enrichment (atom % ^{15}N)	11.59 (0.093)	10.94 (0.077)	2.62 (0.039)	5.29 (0.124)
^{15}N IN AMMONIUM (µmol)[1]	0.936 ± 0.062	0.765 ± 0.045	0.083 ± 0.030	0.200 ± 0.019
Algal Nitrogen (µmol)	-	-	166.4 (10.2)	153.3 (9.4)
Enrichment (atom % excess)[2]	-	-	0.423 (0.021)	0.386 (0.003)
^{15}N IN ALGAE (µmol)[1]	-	-	0.704 ± 0.138	0.592 ± 0.132
RECOVERED ^{15}N (µmol)[3]	0.936 ± 0.062	0.765 ± 0.045	0.787 ± 0.141	0.792 ± 0.133
RECOVERED ^{15}N (% of initial ^{15}N)	111.4%[4]	94.2%	90.2%	90.4%

1 ^{15}N = (µmol N) * (atom % ^{15}N / 100).
2 atom % excess = final atom % ^{15}N - measured natural abundance ^{15}N (0.449).
3 RECOVERED ^{15}N = ($^{15}N_{algae}$) + ($^{15}NH_4^+$).
4 difference between initial and final isotope significant ($P \leq 0.05$).

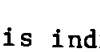
Figure III.1. Initial and final ^{15}N in seawater and algal tissue in uptake experiments with E. prolifera in filtered and filtered/autoclaved seawater. A) filtered seawater control; B) filtered seawater + algae; C) filtered/autoclaved seawater control; D) filtered/autoclaved seawater + algae. The 95% confidence interval for the sum of dissolved nutrient + algal ^{15}N is indicated.  , $^{15}NH_4^+$;  , algal ^{15}N. Replicate incubations are denoted by a and b.

Figure II.1

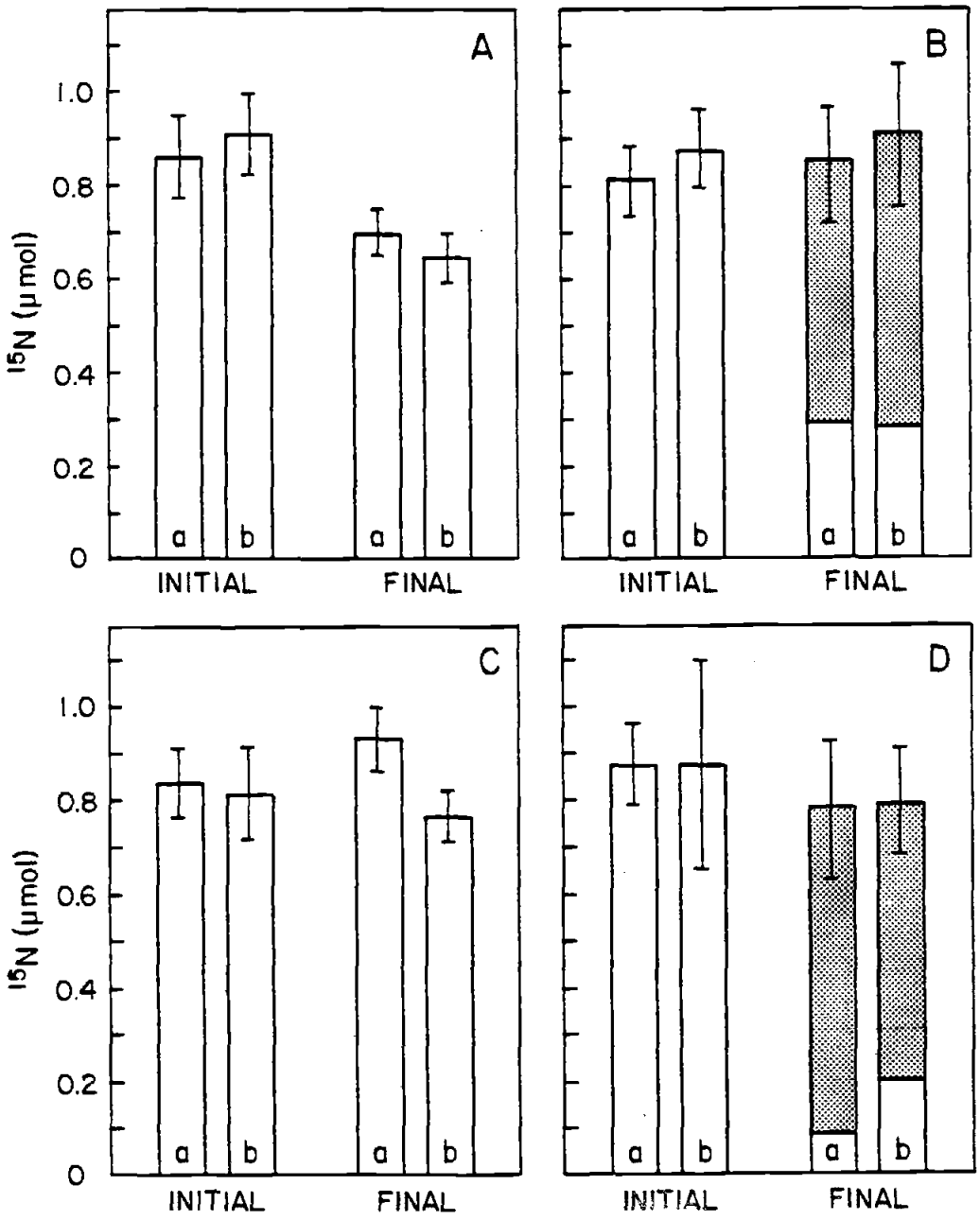


Table III.1. Recovery of ^{15}N for six NO_3^- incubations of algae in filtered seawater. Standard deviations for measured quantities are given in parentheses and 95% confidence limits are given for calculated values. Incubations lasted about 3 hours.

	A	B	C	D	E	F
INITIAL						
Nitrate (µmol)	13.32	14.00	14.24	14.26	14.08	14.26
Enrichment (atom % ^{15}N)	14.08 (0.211)	13.40 (0.201)	13.16 (0.197)	13.15 (0.197)	13.32 (0.200)	13.15 (0.197)
^{15}N IN NITRATE (µmol)[1]	1.876 ± 0.055	1.876 ± 0.055	1.876 ± 0.055	1.876 ± 0.055	1.876 ± 0.055	1.876 ± 0.055
FINAL						
Nitrate (µmol)	9.76	8.50	11.77	11.20	11.50	11.91
Enrichment (atom % ^{15}N)	14.08 (0.211)	13.40 (0.201)	13.16 (0.197)	13.15 (0.197)	13.32 (0.200)	13.15 (0.197)
^{15}N IN NITRATE (µmol)[1]	1.374 ± 0.040	1.139 ± 0.034	1.549 ± 0.046	1.473 ± 0.043	1.532 ± 0.045	1.566 ± 0.046
Algal Nitrogen	68.4 (4.36)	80.4 (5.13)	54.5 (3.48)	60.7 (3.87)	48.7 (3.11)	46.4 (2.96)
Enrichment (atom % excess)[2]	0.628 (0.035)	0.958 (0.019)	0.553 (0.022)	0.529 (0.023)	0.514 (0.035)	0.549 (0.019)
^{15}N IN ALGAE (µmol)[1]	0.430 ± 0.071	0.771 ± 0.101	0.301 ± 0.044	0.321 ± 0.049	0.250 ± 0.040	0.255 ± 0.037
RECOVERED ^{15}N (µmol)[3]	1.804 ± 0.145	1.910 ± 0.200	1.850 ± 0.098	1.794 ± 0.105	1.782 ± 0.091	0.821 ± 0.087
RECOVERED ^{15}N (% of initial)	96.2%	111.8%	98.6%	95.6%	95.0%	97.1%

1 ^{15}N = (µmol N) * (atom % ^{15}N / 100).
2 atom % excess = final atom % ^{15}N - measured natural abundance ^{15}N (0.404).
3 RECOVERED ^{15}N = ($^{15}N_{algae}$) + ($^{15}NO_3^-$).
4 difference between initial and final isotope significant ($P \leq 0.05$).

Comparison of rates calculated by nutrient removal from the medium and 15N accumulation in plant tissue

Ammonium uptake by E. prolifera during the experiment described above was calculated from Equations 1, 2, 4 and 6 and these are given in Table III.4. Rates of NH_4^+ loss and remineralization in the control incubations are also included. For all incubations, the nutrient removal rates of E. prolifera were about 13% lower than those determined from ^{15}N accumulation (ρ). However, the discrepancies between rates calculated by nutrient removal and ^{15}N accumulation (P) were 30 and 55% for incubations in filtered and filtered/autoclaved seawater, respectively, and were due to NH_4^+ regeneration in the jars containing plants. The rates calculated by equations 4 and 6 ($\hat{P}$ and i, respectively) agreed well in all incubations since both of these equations have accounted for regeneration of NH_4^+. Isotope dilution rates were lower in the filtered/autoclaved controls than in the filtered seawater controls, but higher in the filtered/autoclaved seawater when plants were present.

Ammonium uptake by E. prolifera was also determined over a wide range of ammonium concentrations for comparison of net nutrient removal and ^{15}N accumulation (Table III.5). The ^{15}N accumulation rates in this experiment were not corrected for isotope dilution. The water had been filtered through 0.2 μm Gelman cartridges before use. It is unlikely that regeneration rates would have been high enough to significantly dilute the isotope, especially at the higher concentrations. Total nutrient removal and ^{15}N accumulation rates

Table III.4. Summary of ammonium uptake and remineralization rates at initial ammonium concentrations of 5 µM, April 1985. Rates were calculated from the equations given in the text and data are in Tables III.1 and III.2. Standard deviations are given in parentheses. A and B are replicate incubations.

	FILTERED SEAWATER		FILTERED/AUTOCLAVED SEAWATER	
	A	B	A	B
INCUBATIONS WITH ALGAE				
UPTAKE: µmol NH_4^+ g dry wt^{-1} h^{-1}				
Eq. 1: NUTRIENT REMOVAL MINUS CONTROL UPTAKE	14.0 (1.35)	16.5 (2.48)	14.9 (1.88)	14.3 (2.96)
Eq. 2: ^{15}N ACCUMULATION (p)	15.9 (1.77)	19.5 (1.90)	17.8 (3.74)	16.3 (2.65)
Eq. 4: ^{15}N ACCUMULATION (P) CORRECTED FOR ISOTOPE DILUTION	19.2 (1.71)	24.9 (1.74)	43.8 (4.80)	28.3 (2.60)
Eq. 6: NUTRIENT REMOVAL AND ISOTOPE DILUTION (i)	18.3 (0.56)	23.0 (2.02)	43.3 (2.10)	30.2 (2.92)
REMINERALIZATION: µmol NH_4^+ L^{-1} h^{-1}				
Eq. 7: ISOTOPE DILUTION (d)	0.042 (0.003)	0.093 (0.019)	0.967 (0.234)	0.494 (0.144)
Eq. 5: $R_{t1/2}$	11.39 (0.327)	10.73 (0.188)	5.54 (0.424)	7.87 (0.160)
CONTROL INCUBATIONS				
UPTAKE: µmol NH_4^+ L^{-1} h^{-1}				
Eq. 1: NUTRIENT REMOVAL	0.12 (0.026)	0.07 (0.029)	0.10 (0.025)	0.005 (0.033)
REMINERALIZATION: µmol NH_4^+ L^{-1} h^{-1}				
Eq. 7: ISOTOPE DILUTION (d)	0.067 (0.151)	0.254 (0.103)	-0.006 (0.002)	0.051 (0.486)
Eq. 5: $R_{t1/2}$	11.20 (0.237)	10.20 (0.205)	11.63 (0.198)	11.30 (0.190)

Table III.5. Ammonium uptake by E. prolifera on November 1985 calculated by accumulation of ^{15}N and by removal of nutrient from the medium. Nitrogen content of plant was 3.8% dry wt, the length of the incubation was about 0.80 h and the enrichment of the dissolved ammonium was approximately 14 atom %. Standard deviations are given in parentheses. Uptake by nutrient removal is given with and without the uptake in the control jars subtracted from the uptake in experimental jars.

INITIAL AMMONIUM CONCENTRATION (μM)	replicate	FINAL ATOM % ^{15}N EXCESS IN ALGAL TISSUE	UPTAKE RATE ($\mu mol\ NH_4^+\ g\ dry\ wt^{-1}\ h^{-1}$) NUTRIENT REMOVAL TOTAL	NUTRIENT REMOVAL -CONTROL	^{15}N ACCUMULATION	ACCUM:REMOVAL
6	b	0.388 (0.026)	5.62	5.62	5.52 (0.78)	0.982
25	a	0.481 (0.0040)	45.8	39.7	30.6 (1.51)	0.771
25	b	0.464 (0.0073)	35.2	29.8	26.19 (1.46)	0.879
40	a	0.481 (0.0125)	52.7	26.5	28.55 (1.97)	1.08
40	b	0.473 (0.0050)	63.7	39.4	27.00 (0.38)	0.685
75	a	0.572 (0.0136)	71.5	ND	46.60 (3.07)	ND
75	b	0.514 (0.0018)	40.7	ND	34.14 (1.60)	ND

were comparable at the 6 μM level, but not at the higher concentrations. The agreement was improved for the 25 and 40 μM incubations after the uptake in the control jars was subtracted. At high concentrations, it appeared that there was significant uptake of ammonium by microorganisms or adsorption onto the container.

Nitrate uptake rates calculated by net nutrient removal and ^{15}N accumulation in the plant tissue are shown in Tables III.6 and III.7. In the June experiment, the rates are comparable for the two methods, although the ^{15}N accumulation rates tend to be slightly lower that the nutrient removal rates. In the second experiment (Table III.7), uptake rates were determined for a range of concentrations. There was little or no uptake at the lower concentrations; this was supported by both the ^{15}N incorporation data and the nutrient removal calculations. There may have been some uptake by the plants, but the final atom % enrichment was not significantly different from the natural abundance of ^{15}N in plant tissue. This experiment (Table III.7) was similar to the NH_4^+ experiment (Table III.5) in that the nutrient removal rates agreed with the ^{15}N incorporation rates more closely after control uptake was subtracted. The difference between the NO_3^- uptake rates calculated by nutrient removal and by ^{15}N accumulation in Tables III.6 and III.7 was about 8.2 and 9%, respectively, after the nutrient removal rates had been corrected for uptake in the control jars.

Table III.6. Nitrate uptake (at 15 μM) by E. prolifera in June 1985 calculated by nutrient removal (Eq. 1) and accumulation of ^{15}N in plant tissue (Eq. 2). Data used for calculations can be found in Table III.3. Units are μmol NO_3^- g dry wt^{-1} h^{-1}. Standard deviations are given in parentheses.

	A	B	C	D	E	F
Eq. 1: NUTRIENT REMOVAL	41.1 (3.76)	54.3 (3.20)	36.3 (4.76)	38.9 (4.12)	39.2 (4.90)	37.7 (5.16)
Eq. 1: NUTRIENT REMOVAL MINUS CONTROL UPTAKE	39.1 (4.49)	52.4 (3.81)	33.2 (5.62)	36.3 (4.88)	36.1 (6.15)	34.4 (6.15)
Eq 2: 15N ACCUMULATION (ρ)	35.5 (3.05)	56.9 (3.90)	32.3 (2.47)	30.9 (2.44)	28.5 (2.66)	30.9 (2.67)
ACCUMULATION RATE:REMOVAL RATE	0.91	1.09	0.97	0.85	0.79	0.90

Table III.7. Nitrate uptake by E. prolifera in November 1985 calculated by accumulation of ^{15}N in plant tissue and by removal of nutrient in the medium. Nitrogen content of plant tissue was 3.80% dry wt, the length of the incubation was approximately 1 h and the enrichment of the dissolved nitrate was approximately 20 atom %. Standard deviations are given in parentheses. Uptake by nutrient removal is given with and without the uptake in the control jars subtracted from the uptake in experimental jars.

INITIAL NITRATE CONCENTRATION (μM)	replicate	FINAL ATOM % ^{15}N EXCESS IN ALGAL TISSUE	UPTAKE RATE (μmol NO_3^- g dry wt^{-1} h^{-1}) NUTRIENT REMOVAL TOTAL	-CONTROL	^{15}N ACCUMULATION	ACCUM:REMOVAL
10	a	0.384 (0.008)	0	0	2.98 (0.084)	ND
10	b	0.310 (0.069)	0	0	0	ND
25	a	0.370 (0.081)	0	0	0	ND
40	a	0.490 (0.0492)	23.8	23.8	17.9 (1.83)	0.752
40	b	0.506 (0.0213)	26.3	26.3	18.8 (1.56)	0.715
60	a	0.486 (0.019)	27.2	17.4	18.8 (0.82)	1.08
60	b	0.610 (0.026)	46.4	35.1	37.3 (1.72)	1.06

Uptake kinetics

The kinetic curves for NH_4^+ and NO_3^- uptake by E. prolifera are illustrated in Figs. III.2 and III.3, respectively and the kinetic parameters are summarized in Table III.8. The uptake data can be found in Appendices A and B. In each figure, panel A shows uptake by thalli maintained in nitrogen-free seawater until the tissue nitrogen content was less than 2% dry wt (see Chapter II for rationale). Panels B and C show uptake by field collected plants.

For the 1985 experiments, determination of nutrient concentrations in each bottle over a time course permitted the calculation of uptake rates in two ways: the mean rate of change over the entire time course or the changes occurring during discrete intervals. For the September 1985 experiments there was no difference between kinetic parameters calculated by the two methods and the rates presented in Figs. III.2b and III.3b are from the mean rate of change over the entire incubation. In the November 1985 experiments (Figs. III.2c and III.3c), uptake of both nutrients was irregular during the course of the incubations so the rates used to calculate the kinetic parameters are from only those intervals where uptake occurred.

Ammonium uptake could be described by Michaelis-Menten kinetics. There was no significant ($P \leq 0.05$) difference between the kinetic parameters for NH_4^+ uptake for plants with 2.93 and 1.78% tissue-N (Table III.8). The maximum NH_4^+ uptake rate for the September 1984 experiment may have been overestimated if the high rates at 40 μM are

Figure III.2. Ammonium uptake vs. substrate concentration. A) low tissue-N, September 1985; B) high tissue-N, September 1984; C) high tissue-N, November 1985.

Figure III.2

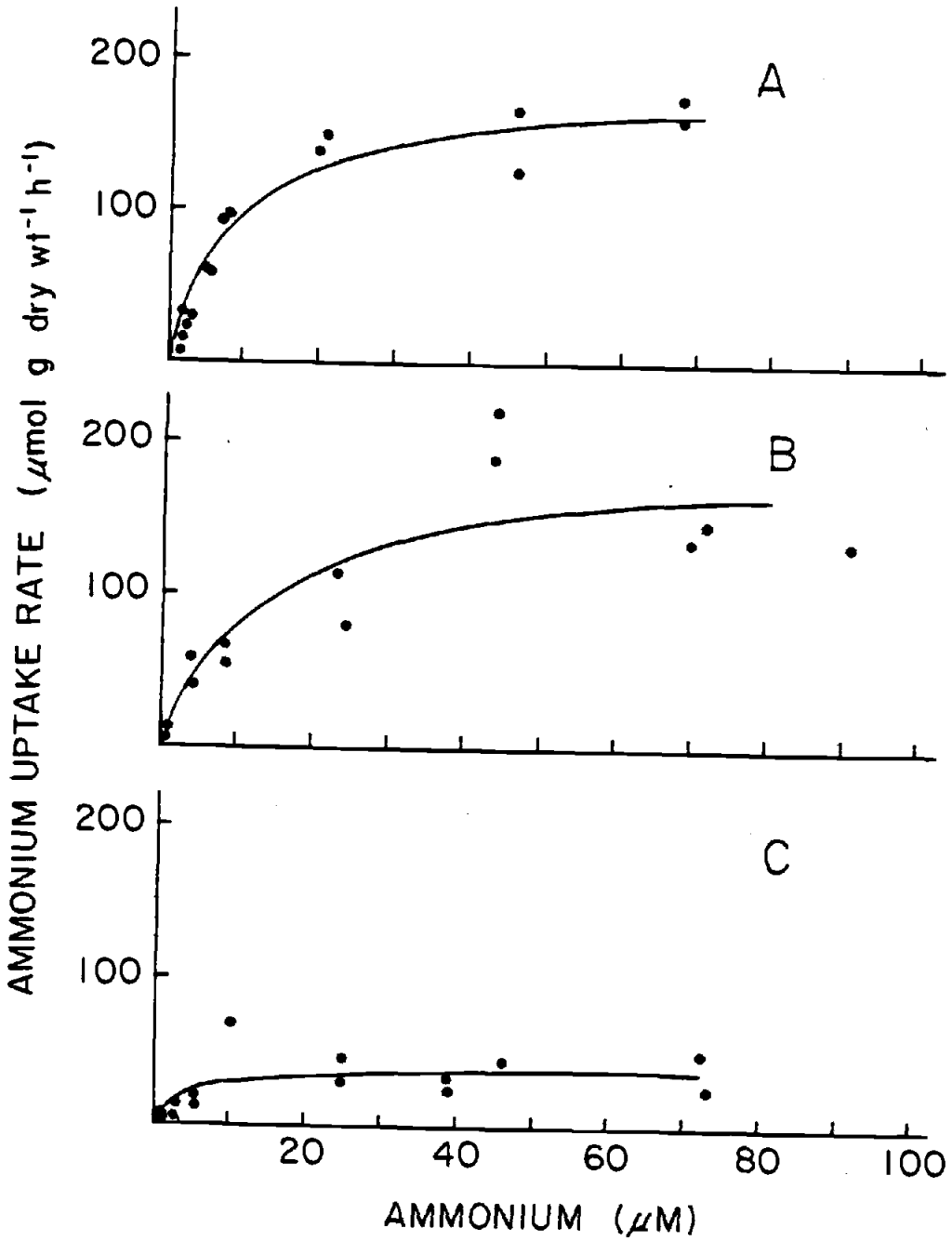

AMMONIUM UPTAKE RATE (μmol g dry wt⁻¹ h⁻¹)
200
100
A
200
100
B
200
100
C
20
40
60
80
100
AMMONIUM (μM)

Figure III.3. Nitrate uptake vs. substrate concentration. A) low tissue-N, September 1985; B) high tissue-N, May 1985; C) high tissue-N, November 1985.

Figure III. 3

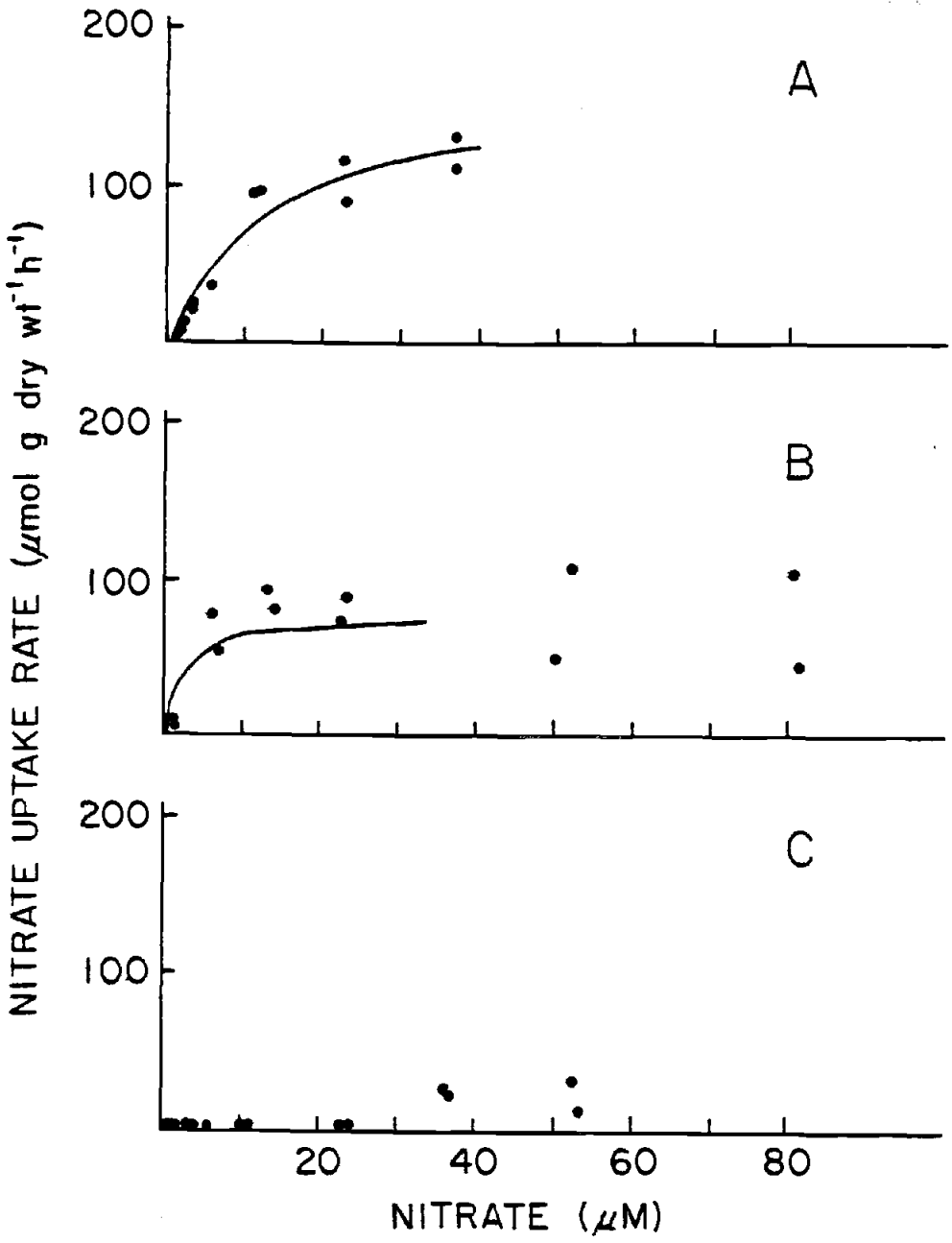

200
100
A
200
100
B
200
100
C
NITRATE UPTAKE RATE (μmol g dry wt⁻¹h⁻¹)
20
40
60
80
NITRATE (μM)

Table III.8. Kinetic parameters for uptake of NH_4^+ and NO_3^- by E. prolifera. All parameters were determined using a nonlinear fit (Wilkinson 1960), except the 5/85 NO_3^- experiment. Standard deviations are given in parentheses.

DATE	N CONTENT	C:N	V_{max} µmol N g dry wt^{-1} h^{-1}		K_s µM		V_{max}/K_s	
NH_4^+								
9/85	1.78	19.8	188.	(12.9)	9.3	(2.00)	20.2	(4.67)
9/84	2.93	10.4	188.	(30.3)	13.4	(7.35)	14.0	(7.98)
11/85	3.74	9.5	39.2	(7.45)	2.9	(2.70)	13.3	(12.4)
NO_3^-								
9/85	1.78	19.8	169.	(19.3)	13.3	(3.6)	12.7	(3.73)
5/85[1]	2.65	12.6	75.4		2.31		32.6	
11/85	3.74	9.5	-		-		-	

[1] determined by Wolfe transformation, $r^2 = 0.7624$.

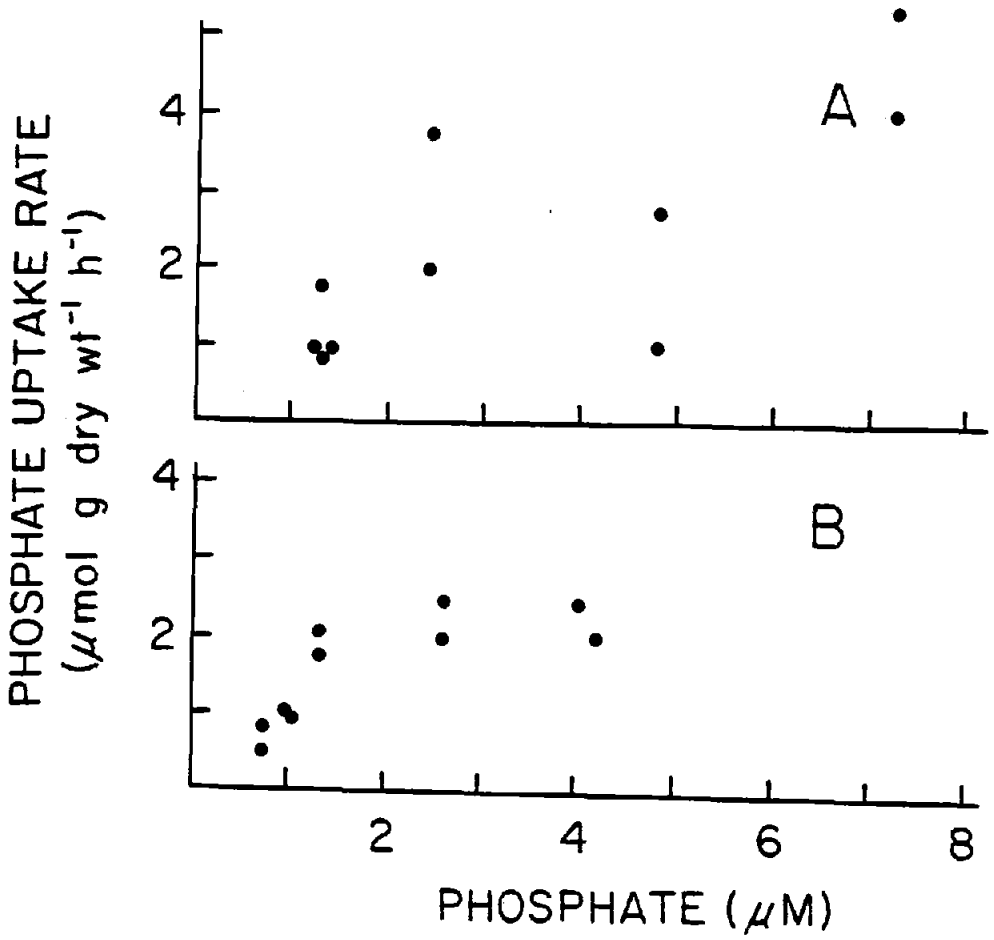
anomalous. If these rates are left out, then K_s = 11.43 (SD = 2.71) and V_{max} = 153. (SD = 10.97). The rates from the November 1985 (Fig. III.2c) experiment were much lower than rates from the other two experiments at all concentrations.

Of the three NO_3^- experiments, only the May 1984 experiment (Fig. III.3b) clearly showed saturable uptake kinetics. Only data up to the 50 μM level were used to estimate kinetic parameters because uptake rates at higher concentrations were extremely variable. In the September 1985 experiment (Fig. III.3a), uptake began to level off at the two highest concentrations but did not reach V_{max}. In contrast, the November data showed no NO_3^- uptake up to concentrations of 25 μM. Above this concentration, the uptake rates were approximately 30 μmol NO_3^- g dry wt^{-1} h^{-1}. This rate may be an indication of V_{max}, but a half-saturation constant cannot be determined. The V_{max} for NO_3^- uptake by low tissue-N algae was about double that of high tissue-N algae (Table III.8) and the half saturation constant, K_s, was also higher. Below 15 μM, the high tissue-N plants took up NO_3^- faster than the low tissue-N plants, and above 15 μM the reverse was true. The initial slopes of the curves in Fig. III.3a and b were 10.5 and 32.6, respectively, reflecting the differences between the shapes of the curves.

Rates of PO_4^{3-} uptake by algae maintained in nutrient-free water are presented in Fig. III.4. Kinetic parameters could not be accurately estimated, but the maximum uptake rate is approximately 2 μmol PO_4^{3-} g dry wt^{-1} h^{-1}. The relationship between phosphorus and

Figure III.4. Phosphate uptake vs. substrate concentration, September 1985, tissue-P content was 0.102% (SD = 0.007).
A) NH_4^+ as the nitrogen source; B) NO_3^- as the nitrogen source.

Figure III.4


PHOSPHATE UPTAKE RATE
(μmol g dry wt⁻¹ h⁻¹)
A
B
4
2
4
2
2
4
6
8
PHOSPHATE (μM)

nitrogen uptake by algae maintained in nutrient-free seawater is illustrated in Fig. III.5a. Phosphate uptake was more variable when plants were incubated with NH_4^+ than with NO_3^-. Most of the N:P ratios of uptake ranged between 40 and 60 and averaged 58.6 (SD = 31.6) for NH_4^+:PO_4^{3-} and 48.3 (SD = 10.9) for NO_3^-:PO_4^{3-}. The NO_3^-:PO_4^{3-} ratio was more similar to the N:P ratio of the plant tissue than was the NH_4^+:PO_4^{3-} ratio.

Phosphate and NO_3^- uptake by field collected plants with an average N:P ratio of 69.2:1 is presented in Fig. III.5b. The average N:P ratio for uptake was 9.77 (SD = 2.68), much lower than the average N:P ratio of plant tissue.

Ammonium-nitrate interactions:

There was some suppression of NO_3^- uptake at NH_4^+ concentrations between 1 and 15 μM NH_4^+ (Fig. III.6). These nutrient concentrations were chosen to reflect concentrations that might be present on the mudflat. The expected NO_3^- uptake rate at 15 μM NO_3^- is about 80 μmol NO_3^- g dry wt^{-1} h^{-1} in the absence of NH_4^+ (Fig. III.3a). In the presence of 1 μM NH_4^+, the uptake of NO_3^- was reduced by about 40% and in the presence of 7 and 15 μM NH_4^+, was reduced by 50%. Ammonium uptake rates in this experiment were about the same as those predicted by the kinetics curves (Fig. II.2a and b).

Figure III.5. Simultaneous uptake of nitrogen and phosphorus by E. prolifera. A) Nutrient-deprived plants, September 1985. Nitrogen and phosphorus contents of plant tissue were 1.78 and 0.102%, respectively. Average N:P ratio was 42.8:1. B) Field-collected plants, November 1985. Nitrogen and phosphorus contents of plant tissue were 3.74 and 0.123%, respectively. Average N:P ratio was 69.2:1. Open circles, $NH_4^+ + PO_4^{3-}$; solid circles, $NO_3^- + PO_4^{3-}$.

Figure III.5

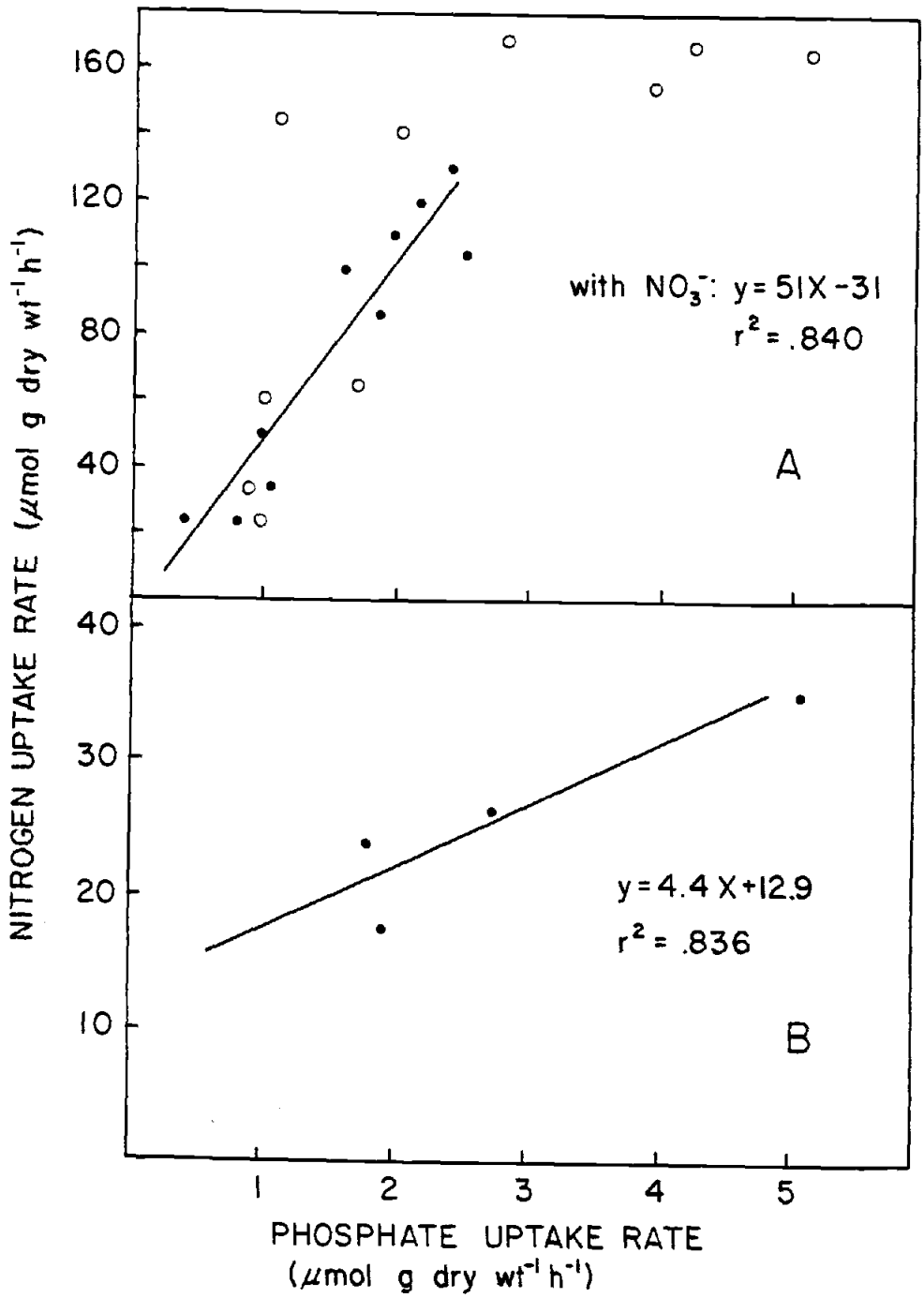
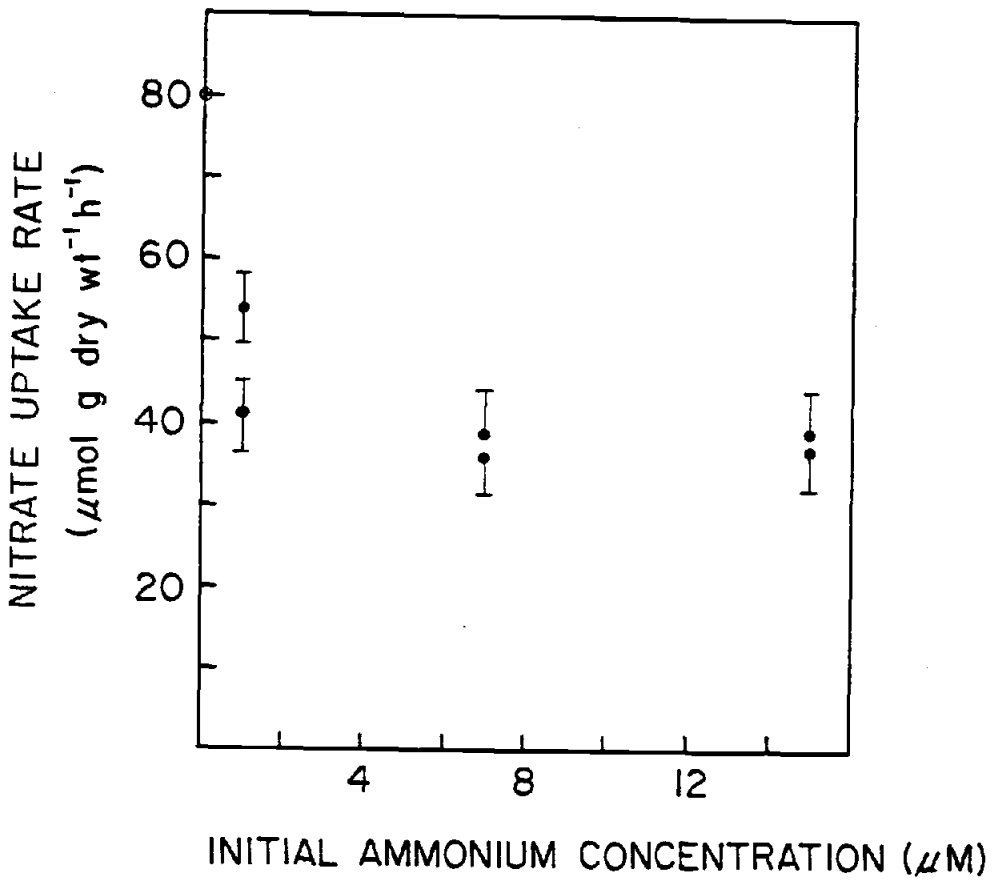

160
120
80
40
40
30
20
10
1
2
3
4
5
NITROGEN UPTAKE RATE (μmol g dry wt⁻¹ h⁻¹)
PHOSPHATE UPTAKE RATE
(μmol g dry wt⁻¹ h⁻¹)
with NO3⁻: y=51X-31
r²=.840
A
y=4.4X+12.9
r²=.836
B

Figure III.6. Nitrate uptake by E. prolifera with both NO_3^- and NH_4^+ available in seawater. Initial NO_3^- concentration was 15 μM. Nitrate uptake (open circle) in the absence of NH_4^+ was estimated from Figure III.3a. The error bars are ± one standard deviation.

Figure III.6


NITRATE UPTAKE RATE
(μmol g dry wt⁻¹ h⁻¹)
80
60
40
20
4
8
12
INITIAL AMMONIUM CONCENTRATION (μM)

DISCUSSION

Isotope recovery and uptake calculations in ammonium experiments

The recovery experiments with $^{15}NH_4^+$ (Tables III.1 and III.2) indicated the importance of isotope dilution in tracer incubations with macroalgae, and showed that complete recovery of the added isotope at the end of the incubation is possible. Essentially all of the isotope was recovered in the autoclaved/filtered controls, suggesting that uptake by free bacteria did not occur. The increase in final ^{15}N in one of these controls was due to an increase in dissolved NH_4^+ and may have been due to contamination of the sample after collection. In addition, there was no indication of NH_4^+ regeneration in the filtered/autoclaved seawter, because the atom % enrichment of the NH_4^+ in seawater did not change. The decrease in the NH_4^+ concentration and atom % enrichment in the filtered seawater controls implied that microorganisms were active in this water and that NH_4^+ was being taken up and also produced.

Although steam sterilization improved the recovery of ^{15}N, it appeared to introduce other artifacts. Regeneration rates were very high in the autoclaved/filtered seawater when plants were present. This may have resulted from the breakdown of large organic molecules during autoclaving, and subsequent remineralization by epiphytic bacteria. The high NH_4^+ uptake rates by *E. prolifera* in filtered/autoclaved water may have been a result of the increased availability of NH_4^+ due to this remineralization. Increased

concentrations of NH_4^+ were not detected because samples were collected only at the beginning and end of the experiment.

Problems in interpretation of results have been noted for uptake experiments with natural assemblages of phytoplankton using $^{15}NH_4^+$. If isotope dilution is not taken into account, rates of NH_4^+ uptake can be underestimated by a factor of 2 (Glibert et al. 1982). Furthermore, up to 56% of the isotope present initally is not recovered in particulate nitrogen or dissolved NH_4^+ at the end of the incubation (Laws 1984). Consequently, uptake rates calculated by net nutrient removal, accumulation of ^{15}N in algal tissue ($\mathcal{P}$ and ρ) and the loss of dissolved ^{15}N and NH_4^+ concentration change (i) do not always agree (Glibert et al. 1982, Laws 1984). Discrepancies of up to ca. 50% were observed between rates calculated by nutrient removal and by ^{15}N accumulation ($\mathcal{P}$ or ρ) in the present experiments. These discrepancies were in different directions and appeared to have different causes in the two NH_4^+ experiments (Tables III.4 and 5).

In the experiment in which ρ and nutrient removal rates were compared for a range of concentrations (Table III.5), ^{15}N accumulation rates averaged 67% of total uptake. The subtraction of control uptake rates improved the agreement between the ^{15}N accumulation rates and the nutrient removal rates. After uptake in the controls had been subtracted, ^{15}N accumulation rates averaged 88% (SD = 16%) of net uptake rates. Here, the discrepancy between rate calculations appeared to be due to bacterial uptake or loss to the walls of the container. Highest control uptake rates occurred at the

higher concentrations and comprised an average of 44% of total uptake. At concentrations below 25 uM NH_4^+, control uptake rates were lower, averaging 9.6% of total uptake. Overall, the average control uptake rate was 23% of total uptake (SD = 20.3%).

Williams and Fisher (1985) compared NH_4^+ uptake rates of Caulerpa cupressiodes calculated by accumulation of ^{15}N in the alga (ρ) to rates calculated by loss of nutrient at NH_4^+ concentrations up to 300 uM. Uptake calculated by ^{15}N accumulation averaged 47% of the nutrient removal rates. In my experiment (Table III.5), discrepancies of similar magnitude and direction were observed between the two rate calculations only at the highest NH_4^+ concentrations and the average discrepancy between rates calculated by the two methods was much less. Williams and Fisher (ibid) did not monitor either isotope dilution or changes in NH_4^+ concentration in control incubations, thus they could not determine the cause of the discrepancies between rates calculated by the two methods. If the difference was due to bacterial uptake then the nutrient removal rates were overestimates of actual uptake. If isotope dilution occurred, then ^{15}N rates underestimated the Caulerpa uptake.

Even when the control loss is subtracted from the loss in the algae containers, uptake rates calculated by nutrient removal do not account for possible regeneration of nutrient during the experiment or exchange between algal tissue and the medium, both of which result in dilution of ^{15}N. The error incurred when NH_4^+ regeneration is not taken into account is apparent when P (rate corrected for isotope

dilution), is compared to ρ (with no correction) and nutrient removal rates in the recovery experiment (Table III.4). Uncorrected rates (ρ) underestimated actual uptake (P and i) by about 17 and 52% in filtered and filtered/autoclaved seawater, respectively.

In the recovery experiment (Table III.4), net nutrient removal rates averaged 70% of ^{15}N accumulation rates (P) in filtered seawater. Gross plant uptake can be estimated by adding the NH_4^+ regeneration rate to the net nutrient removal rate. Estimated gross uptake was equivalent to 80% of the ^{15}N accumulation rate. Thus, approximately one-third of the difference between the two rate calculations can be attributed to NH_4^+ regeneration.

In the incubations with filtered seawater + algae (Table III.4), it appeared that neither uptake by bacteria nor release of labelled nitrogenous metabolites occurred since 1) all ^{15}N was recovered, 2) the rates P (accumulation of ^{15}N) and i (loss of dissolved ^{15}N and changes in NH_4^+ concentration) agreed, and 3) the total uptake rates (nutrient loss without control uptake subtracted) corrected for NH_4^+ regeneration agreed closely with those calculated by accumulation of ^{15}N (P and i). It is uncertain why bacterial uptake occurred in the control incubations but apparently not in the plant incubations. Bacteria in the filtered seawater may have become associated with the thalli. Alternatively, bacterial uptake may have been inhibited in plant incubations. Ulva has been observed to contain some antibacterial substances that were assumed to prevent the attachment of baterial epiphytes (Hornsey and Hide 1976, cited in Lobban et al.

1985). Whether these or similar compounds can also prevent uptake by bacteria is unknown.

Isotope recovery and uptake calculations in nitrate experiments

Nitrification rates are usually about one order of magnitude lower than NH_4^+ regeneration rates (Kaplan 1983) and consequently, the atom % enrichment of dissolved NO_3^- was assumed not to have changed significantly during these experiments. The high recovery of ^{15}N (Table III.3) and low uptake in the controls also suggested that microbial metabolism of NO_3^- was slow. Almost all of the ^{15}N lost from the dissolved NO_3^- was recovered in the plant tissue.

The difference between the NO_3^- uptake rates calculated by nutrient removal and by ^{15}N accumulation expressed as a percent of the nutrient removal rate (Tables III.6) was slightly higher than the fraction of ^{15}N that was not recovered (Table III.3). This disparity may have been due to an overestimate of initial ^{15}N tracer in dissolved NO_3^- or to an underestimate of the total nitrogen content of plant tissue. For all incubations, the same value of added ^{15}N was used to calculate 'R', and the same tissue nitrogen value was used. An analytical error in either of these two values could have resulted in a systematic underestimate of uptake rates.

Uptake kinetics

In the experiments of November 1985, NH_4^+ uptake was relatively low at all concentrations and no uptake of NO_3^- occurred at concentrations below 36 μM. It was assumed that uptake would be a function of tissue composition since the light and temperature were

held constant. However, other factors such as age of tissue, season or temperature in the field may have contributed to the differences in uptake kinetics observed in the November experiments (Harlin 1978, Topinka 1978, Gerard 1982, Rosenberg et al. 1984, Thomas and Harrison 1985). At this time, the temperature of the water over the mudflat had dropped from a summer average of about 16°C to 11 - 13°C (Collins 1987) and light levels may have been low enough to limit plant growth on the surface of the mud (Davis 1981). Although the plants had been acclimated to laboratory conditions, they may have still been in a dormant or overwintering phase induced by environmental conditions. A similar trend was observed in January 1985 when field-collected plants had low growth rates during subsequent experiments (Chapter II). Rosenberg et al. (1984) found that V_{max} for NH_4^+ uptake by Fucus distichus during the winter was 11% of that during the summer.

Little is known about the relationships between life history of E. prolifera and environmental conditions. Since the age of a thallus is difficult to distinguish in the field, it is possible that the algae used in November were mature or slowly growing plants. Several studies have shown that mature thalli have nitrogen uptake rates 50 - 80% lower than uptake rates of juveniles (Topinka 1978, Haines and Wheeler 1978, Gerard 1982, Thomas et al. 1985).

The reduction of NO_3^- uptake rates of fall/winter collected plants was more severe than reduction of NH_4^+ uptake, possibly a result of light limitation. Harlin (1978) found that uptake of NO_3^- by E. intestinalis and E. linza decreased rapidly after being removed

from light. Since NO_3^- uptake and reduction is an energy demanding process, algae growing under light limitation may not be able to metabolize NO_3^- (Davison and Stewart 1984). I will not consider the November results in the comparison of kinetic parameters because of likelihood that factors discussed above were affecting uptake.

Uptake of NH_4^+ could be described by Michaelis-Menten kinetics on all three dates. Fujita (1985) observed saturable kinetics for Enteromorpha spp. only for algae that had been cultured at high nitrogen concentrations. Under other conditions, uptake was variable. His measurements were carried out with the single flask method, and uptake was often influenced by previous exposure to high concentrations. Kautsky (1982) observed a much lower value of V_{max} for E. compressa during four day incubations in enclosures of unfiltered seawater. However, NH_4^+ may have been regenerated from decomposing material thus reducing the apparent loss due to algal uptake. For many algae, uptake of NH_4^+ is more rapid than uptake of NO_3^- at the same concentration (reviewed by Hanisak 1983). This was true for E. prolifera at concentrations higher than ca. 23 µM. At low concentrations, the uptake rates of NH_4^+ and NO_3^- were similar.

The kinetic parameters for nitrogen uptake by marine algae from a variety of habitats and experimental conditions are summarized in Table III.9. Both V_{max} and K_s observed for E. prolifera in this study are comparable to other values observed for this genus and for other ephemeral green algae (Ulva and Cladophora), but tend to be

Table III.9. Ranges of kinetic parameters for nitrate and ammonium uptake by marine macroalgae. When only one value is given, its standard deviation is included if available.

SPECIES	V_{max} μmol g dry wt^{-1} h^{-1}	K_s μM	V_{max}/K_s	NITROGEN SOURCE NO_3^-	NITROGEN SOURCE NH_4^+	REFERENCE	NOTES
Enteromorpha prolifera	74.5 - 169	2.3 - 13.3	12.7 - 32.6	X		this study	A
	39.2 - 188	2.9 - 13.4	13.3 - 20.0		X	"	A
Enteromorpha compressa	36.8	24	1.5		X	Kautsky, 1982	B
Enteromorpha spp.	120 - 996	4.1 - 24.6	29.3 - 40.5		X	Fujita, 1985	A, B
	129	17	7.6	X		Harlin, 1978	C
Ulva lactuca	138 - 144	14.7 - 40.7	3.4 - 9.8		X	Fujita, 1985	A, B
Cladophora spp.	42.1 ± 5.7	1.4 ± 0.7	30.1	X		Gordon et al, 1981	G
	130 ± 22.1	20.7 ± 11.4	6.3		X	"	G
Codium fragile	2.8 - 10.9	1.2 - 7.6	1.3 - 4.1	X		Hanisak & Harlin, 1978	D
	13. - 28	1.4 - 2.1	8.9 - 20.0		X	"	D
Caulerpa cupressoides	8.7 ± 3.0	48 ± 10	0.18		X	Williams & Fisher, 1985	E
Chordaria flagelliformis	5.9 - 13.4	0.8 - 6.3	1.2 - 8.2	X		*Probyn, 1984*	
	145	0.8	181		X	Probyn & Chapman, 1983	F
Fucus spiralis	4.3 - 20.4	5.6 - 7.8	2.6 - 2.7	X		Topinka, 1978	D
	6.2 - 31.5	6.4 - 9.6	2.5 - 3.2		X	"	D
Macrocystis pyrifera	30.5 ± 1.7	13.1 ± 1.6	2.3	X		Haines & Wheeler, 1978	A
	23.8	5.3 ± 1.0	4.5		X	"	A
Agardhiella subulata	10.7 - 12.6	2.1 - 2.7	4.7 - 5.1	X		D'Elia & DeBoer, 1978	A, B, H
	5.6 - 30.0	2.3 - 4.9	2.4 - 6.7		X	"	A, B, H
Gracilaria foliifera	3.4 - 16.3	0.2 - 3.8	3.8 - 17.0	X		D'Elia & DeBoer, 1978	A, B
	23.8	1.6	14.9		X	"	A, B
Gracilaria tikvahiae	120 - 216	16.9 - 37.6	5.7 - 8.5		X	Fujita, 1985	A, B
Hypnea musciformis	28.5 ± 7.9	4.9 ± 3.9	5.8	X		Haines & Wheeler, 1978	A

NOTES:
A. Range of values reported for N-limited and N-replete algae.
B. Single flask method used.
C. Coefficient of variation for V_{max} = approximately 30%.
D. Range of values reported for a range of temperatures.
E. Rates measured by incorporation of ^{15}N.
F. Data calculated from consumption at apparent steady state.
G. Data from several experiments combined.
H. Formerly Neoagaradhiella baileyi (Kraft and Wynne 1979).

higher than those of most brown or red algae. It has been suggested that high maximum uptake rates are characteristic of species with an opportunistic growth strategy (Rosenberg and Ramus 1982, 1984). The ratios V_{max}/K_s of Enteromorpha spp. are among the highest observed for any alga, mainly due to the high values for V_{max}. This ratio has been suggested to be the best indicator of an alga's affinity for nutrients at low concentrations because it takes into account both the half-saturation constant and potentially high maximum uptake rates (Healey 1980).

When phytoplankton are starved for nitrogen, the short term uptake of NH_4^+ often increases to a rate much higher than the steady state growth rate (McCarthy and Goldman 1979). Transient increases in uptake have been investigated for some macrophytes with varying results. Rosenberg et al. (1984) observed that rates of transient uptake of NH_4^+ by Chordaria flagelliformis and Fucus distichus were 3 - 4.8 times higher than steady state uptake rates. The magnitude of the increase was inversely correlated with thallus nitrogen content. However, Thomas et al. (1985) observed no changes either in kinetic parameters for NO_3^- or NH_4^+ uptake when 3 week old germlings of F. distichus were starved for 24 hours, or in NH_4^+ uptake by adults after 4.5 days in N-free medium. Four and one-half days is probably not sufficient to reduce the reserves of adult F. distichus (Rosenberg et al. 1984) and the time required to starve germlings is not known. Thomas et al. (1985) did not measure tissue nitrogen concentrations. D'Elia and DeBoer (1978) saw no correlation between the tissue C:N ratio and either K_s or V_{max} for NO_3^- uptake by

Gracilaria foliifera. However, NH_4^+ uptake by G. foliifera and Agardiella subulata increased linearly with tissue C:N ratio up to a C:N of 10, and then remained constant (ibid). Fujita (1985) grew Gracilaria tikvahiae (formerly G. foliifera var. angustissima, McLachlan 1979), Enteromorpha spp. and Ulva lactuca in N-free medium after acclimation to high and low nutrient flux. All three algae took up NH_4^+ at higher rates after starvation and Enteromorpha spp. had the highest rates. In my study, there was no difference between V_{max}, K_s, or V_{max}/K_s for NH_4^+ uptake by low and high tissue-N thalli. It is assumed that low tissue-N plants were nitrogen-limited since tissue-N was less than 2% dry wt (see Chapter II) and growth rates were near zero.

Some of the variations in the occurrence of transient uptake may be due to adaptations of algae to the environments from which they were collected. Among the algae discussed above, those found in environments which are characterized by generally low nutrient concentrations punctuated by pulses of high concentration exhibited transient high NH_4^+ uptake. Chordaria flagelliformis and Fucus distichus from Nova Scotia had maximum growth rates during the summer when ambient levels of dissolved nitrogen were essentially zero (Rosenberg et al. 1984). The algae investigated by Fujita (1985) were from a lagoon in which NO_3^- concentrations were always low and NH_4^+ concentrations fluctuated between zero and 35 μM depending on tidal and drainage patterns. The F. distichus examined by Thomas et al (1985) were collected from a seawall in Vancouver, British Columbia and may have been acclimated to an adequate nitrogen supply

by runoff from a populated area. In Yaquina Bay, the concentrations of total dissolved inorganic nitrogen are ca. 8 μM during the summer and, as far as is known, there are not extreme peaks in NH_4^+ concentrations.

In contrast to NH_4^+ uptake, the uptake of NO_3^- for low tissue-N plants was very different from that of high tissue-N plants (Figure III.3a, b). At low concentrations, the low tissue-N plants took up NO_3^- very slowly. The low tissue-N plants had been deprived of nitrogen just prior to the experiment and possibly, NO_3^- uptake systems did not remain fully active when NO_3^- was not available. There is some indication that this occurs in both macroalgae and phytoplankton under nitrogen deprivation although uptake can be induced after 15 or 20 minutes by a pulse of NO_3^- (Dortch 1982, Thomas and Harrison 1985). If the uptake system was activated by the presence of NO_3^-, a lag would have occurred in the uptake of NO_3^- early in the time course. This was not apparent at any concentration in my experiments. The ratio V_{max}/K_s was higher for the high tissue-N plants, indicating that these had higher uptake rates at low concentrations of NO_3^-. However, the V_{max} of the low tissue-N algae was about 2 times higher than that of the high tissue-N algae, suggesting that N-limited algae are potentially able to take up pulses of NO_3^-.

Phosphate uptake

Since phosphorus is usually not considered to be the limiting nutrient in coastal waters (Ryther and Dunstan 1971) there are few

studies of PO_4^{3-} uptake by marine macroalgae. DeBoer (1981) reported a V_{max} of 0.47 μmol PO_4^{3-} g dry wt^{-1} h^{-1} and a K_s of 0.4 μM for PO_4^{3-} uptake by Agardhiella subulata. For the chlorophyte, Cladophora albida, Gordon et al. (1981) reported a V_{max} of 3.5 μmol PO_4^{3-} g dry wt^{-1} h^{-1} and a K_s of 0.67 μM. As for nitrogen uptake, the maximum PO_4^{3-} uptake rate by E. prolifera was similar to that of C. albida but much higher than that of the red alga, A. subulata.

The uptake of phosphorus relative to nitrogen may be related to the depletion of these elements in the plant tissue. The tissue of algae used in the September experiments (Fig. III.5a) were depleted in both nitrogen and phosphorus, while those from the November experiment (Fig. III.5b) were depleted in phosphorus only. In the first experiment, the ratios of uptake rates reflected the tissue content, while in the second experiment, P:N uptake was much higher than P:N composition. Birch et al. (1981) observed a similar trend for phosphorus-limited Cladophora albida. The N:P ratio of uptake at saturating concentrations was about one-tenth the ratio of the tissue.

Estimation of in situ uptake

During the summer, E. prolifera on the Yaquina Bay mudflat has an average nitrogen content of 3.2% (Chapter II) or ca. 2.3 μmol N mg dry wt^{-1}. The maximum summer growth rate has been reported to be 0.27 day^{-1} (Davis 1981). To maintain this internal N concentration and growth rate, a thallus requires 0.62 μmol N mg dry wt^{-1} day^{-1} or

26 μmol N g dry wt^{-1} h^{-1}. During June and July, parts of the mudflat may be exposed for up to 8 hours a day. Assuming that nitrogen is not available when an alga is exposed, the nitrogen demand for the remaining 16 hours increases to 39 μmol N g dry wt^{-1} h^{-1}. According to rates measured in this study, the uptake rate at a total dissolved nitrogen concentration of 3 - 4 μM is 30 - 40 μmol N g dry wt^{-1} h^{-1}. During the summer, concentrations of NO_3^- and NH_4^+ in Yaquina Bay are about 5 and 3 - 4 μM, respectively (Chapter II, Collins 1987). Assuming that uptake rates are constant for the entire period of submergence, ambient levels of dissolved nitrogen are high enough to support the uptake required for growth.

E. prolifera appears to be utilizing only a small part of its potential uptake capacity. If an alga was to take up nitrogen continuously at its maximum rate (ca. 175 μmol N g dry wt^{-1} h^{-1} of NH_4^+ or NO_3^-), it would require about 3.5 hours to meet its nitrogen requirement for growth during a day. If the local nutrient supply is not sufficient to meet growth requirements, E. prolifera may rely on pulses of nitrogen. The high values for V_{max} indicate that it could take advantage of any pulses that may occur.

A similar calculation can be conducted for PO_4^{3-} uptake. The average phosphorus content of E. prolifera tissue was 0.226% or 73 μmol g dry wt^{-1} (Chapter II). Using the assumptions stated above, the average hourly uptake rate is approximately 1.2 μmol PO_4^{3-} g dry wt^{-1} h^{-1}. This rate would occur at a PO_4^{3-} concentration of about 1 uM. E. prolifera is capable of higher PO_4^{3-} uptake rates at

higher nutrient concentrations. Since short-term uptake rates exceed maximum observed growth rates, E. prolifera could obtain sufficient nutrients in pusles if steady-state levels are low.

Utilization of NO_3^- by E. prolifera may be lower than that calculated above because NO_3^- uptake is dependent on light and NH_4^+ concentrations as well as on NO_3^- concentration. Harlin (1978) observed than dark uptake of NO_3^- by E. linza and E. intestinalis was reduced to 9.6% of the light uptake. Uptake of NH_4^+ by most algae is not as severely affected by light (Topinka 1978, Hanisak 1983). Nitrate uptake was reduced by ca. 40% at NH_4^+ concentrations above 1 uM (Fig. III.6). Based on the kinetic data and these observations, the rates of NO_3^- uptake may be up to 20 µmol g dry wt^{-1} h^{-1} during the day and ca. 2 µmol g dry wt^{-1} h^{-1} at night, or an average of ca. 10 µmol g dry wt^{-1} h^{-1}. At NH_4^+ concentrations of 3 - 4 µM, an uptake rate of 25 - 50 µmol NH_4^+ g dry wt^{-1} h^{-1} is expected from Figure III.2. Total nitrogen uptake, then, is 35 - 60 µmol N g dry wt^{-1} h^{-1} with NO_3^- uptake accounting for 15 - 30%. This prediction of total nitrogen utilization is close to the nitrogen demand calculated from the tissue nitrogen content and plant growth rates (39 µmol g dry wt^{-1} h^{-1}).

Collins (1987) measured the in situ uptake of NO_3^-, NH_4^+ and PO_4^{3-} by algal mats on the Yaquina Bay mudflats during 1984 and 1985 using a bell jar technique. His estimates of uptake are summarized in Table III.10. The rates of NO_3^- and PO_4^{3-} uptake estimated above agree well with Collins' data. The differences may be due, in part,

Table III.10. *In situ* uptake of NO_3^-, NH_4^+ and PO_4^{3-} by algal mats on the Yaquina Bay mudflat from September 1984 to July 1985. From Collins (1987).

	CONCENTRATION		UPTAKE RATE			
	μM		μmol g dry wt^{-1} h^{-1}			
NUTRIENT	min	max	min	max	ave	SD
NO_3^-	1	14	1.1	35	10.8	11.4
NH_4^+	2	4	2	16	~6	-
PO_4^{3-}	0.7	1.4	0.01	1.8	0.64	0.62

to interspecific differences since Collins measured uptake by Ulva as well as by Enteromorpha. In addition, much of his data was collected during early spring and fall when the plants were not likely to be growing at maximum rates. However, Collins' NH_4^+ uptake rates are 3 to 10 times lower than the rates determined in this study. He included controls with no plant material, but these were not filtered. Consequently, the lower rates are probably due in part to regeneration of NH_4^+ during the incubations. Owens and Stewart (1983) estimated the in situ uptake of NH_4^+ by Enteromorpha spp. using $^{15}NH_4^+$ and observed rates of 3.2 to 15.5 μmol N g dry wt^{-1} h^{-1}. These rates are in the same range as those observed by Collins (1987) and also may have been underestimated because isotope dilution was not determined. In the present experiments, regeneration of NH_4^+ in filtered seawater led to estimates of uptake by nutrient removal that were 70% of actual uptake. Errors are likely to be higher in unfiltered seawater.

E. prolifera uses 3 to 7 times more NH_4^+ than NO_3^-. Wallentinus (1984) found that NO_3^- accounted for about two-thirds of total nitrogen uptake of an intertidal commumity in the Baltic Archipelago. Topinka (1978) estimated that Fucus spiralis received 59% of its total nitrogen from NO_3^-. In both these areas, availability of NO_3^- is much greater than that of NH_4^+. Collins (1987) estimated the maximum rate of NH_4^+ flux on the intertidal mudflats of Yaquina Bay to be 200 μmol m^{-2} h^{-1}. If this is applicable to the entire bay, then the baywide supply of NH_4^+ is 7.6×10^4 mol h^{-1}. Nitrate supplied from the Yaquina River during

the summer averages ca. 200 mol h^{-1} (Collins 1987), about 0.3% of NH_4^+ supplied by regeneration from the sediments. Nitrate entering the estuary from the ocean has not been considered here, but will probably raise the contribution of NO_3^- to the total available nitrogen.

In summary, NH_4^+ removal rates differed from $^{15}NH_4^+$ accumulation rates (P or p) by 17.3% (SD = 15.8%) in all incubations. The discrepancies in different incubations were not always in the same direction and may have been due to either bacterial uptake or NH_4^+ regeneration. Nitrate uptake rates calculated by the two methods agreed more closely than did NH_4^+ uptake rates, and ^{15}N accumulation rates were usually lower than nutrient removal rates, averaging 91.2% (SD = 13.7%). Control uptake was significant in most cases, ranging from 13.3 to 50.3% and from 3.5 to 36.0% of total nutrient removal in NH_4^+ and NO_3^- incubations, respectively. The maximum uptake rates observed for E. prolifera were among the highest reported for algae. There was no difference between uptake kinetic parameters measured during the summer for NH_4^+ uptake by low tissue-N and high tissue-N thalli. However, the K_s and V_{max} for NO_3^- uptake by low tissue-N thalli were higher than for uptake by high tissue-N thalli. The estimates of in situ utilization from kinetic data agree well with observations from bell jar experiments and with estimates based on growth rates and tissue phosphorus and nitrogen content. There is some indication that NH_4^+ remineralization may represent a significant error in in situ incubations.

CHAPTER IV

EFFECT OF DESICCATION ON NUTRIENT UPTAKE BY ENTEROMORPHA PROLIFERA

Since algae growing in intertidal habitats are subjected to intermittent exposure to air, responses to desiccation may determine the limits of species distribution (Schonbeck and Norton 1980). Many species have been classified as "desiccation sensitive" or "desiccation tolerant" based on investigations of the photosynthetic rates of emerged and submerged plants. Photosynthetic rates of algae growing in high intertidal areas are generally unaffected or even augmented by desiccation, while those of algae from low intertidal areas are reduced. Species of Enteromorpha and Ulva are considered to be desiccation sensitive (Wiltens et al. 1978, Beer and Eshel 1983a, Pregnall 1983), although in some environments Ulva is able to resist extreme dehydration (Jenik and Lawson 1967).

Thomas and Turpin (1980) found that nitrogen uptake by Fucus distichus was enhanced when plants were resubmerged after 20 - 40% desiccation. Since these plants were likely to be growing under nitrogen limitation when collected, Thomas and Turpin hypothesized that uptake increased in response to a nitrogen deficit caused by continued photosynthesis while the plant was emerged. When algae were maintained in PO_4^{3-}-free water, uptake of PO_4^{3-} was also enhanced after desiccation and resubmergence.

Desiccation appears to have a dramatic effect on carbon assimilation by Enteromorpha prolifera. Pregnall (1983) investigated the effect of desiccation on primary production of this species on a mudflat of Coos Bay, Oregon and found that the photosynthetic rate decreased 75% when only 10 - 30% of internal water was lost. In addition, up to 15% of recently fixed carbon was lost when desiccated plants were resubmerged. The current study describes the effects of desiccation on the uptake of NH_4^+, NO_3^- and PO_4^{3-}. A time course of uptake was examined to 1) determine if uptake was linear when plants were rehydrated and 2) determine if inorganic nutrients, like recently fixed carbon, are lost after dehydration.

METHODS

For all experiments, plants were collected from the Idaho Point mudflat at the location shown in Figure II.1 and maintained in an outdoor culture facility under ambient light and temperature conditions. The culture facility consisted of a series of ca. 11.5 L tanks which were filled with seawater pumped from Yaquina Bay and filtered through a 1.0 um Millipore cartridge. The salinity in the tanks ranged from 30.9 to 31.9°/oo and temperature, from 15 to 20°C. Nutrient concentrations were controlled by adding nutrients to seawater previously stripped of nutrients by incubations with Ulva. The flow rate was >10 turnovers day^{-1} and tanks were aerated by bubbling with compressed air. Experiments were carried out 12 to 48 hours after the plants were collected. Seawater used for experiments was from the same source as the culture seawater and was filtered

through a Gelman 0.2 um cartridge immediately before use. All experiments were carried out in 2 L glass jars at 12 to 14°C and at a saturating (400 $\mu E\ m^{-2}\ sec^{-1}$) light intensity (Davis 1981, Shellem and Josselyn 1982). The plant density was approximately 500 mg wet wt L^{-1}. After the experiments, the carbon and nitrogen content of triplicate or duplicate samples of plant tissue was measured with a Perkin Elmer elemental analyzer. Phosphorus concentrations in duplicate samples was measured as described in Chapter II.

Since Enteromorpha is considered to be desiccation-sensitive (Wiltens et al. 1978), an experiment was conducted to find the desiccation level above which plants did not recover. Pregnall (1983) showed that the extent of desiccation of Enteromorpha on the mudflats was related to the standing crop, which may reach a maximum of ca. 5000 g wet wt m^{-2} in Yaquina Bay (Davis 1981). Freshly collected plants were placed on duplicate trays of damp sediment in amounts that corresponded to ca. 200, 400, 1000, and 1400 g wet wt m^{-2}. The trays were placed outside at midday and the wet weights of the algae monitored for several hours. At the end of the exposure period, the plants were placed in the culture tanks described above and wet weight monitored for 10 days. Growth rates were calculated according to DeBoer et al. (1978):

$$\text{growth rate } (day^{-1}) = \frac{\ln (ww_{t2}/ww_{t1})}{t_2 - t_1} \qquad (1)$$

where ww_{t1} and ww_{t2} are wet weights at time 1 (t_1) and time 2 (t_2), respectively.

The desiccation level was calculated according to Wiltens et al. (1978):

$$\text{water loss (\%)} = \frac{(\text{wet wt} - \text{exposed wt})}{(\text{wet wt} - \text{dry wt})} \cdot 100 \qquad (2)$$

The desiccation procedure used prior to the uptake experiments kept water loss throughout the thallus as uniform as possible. A minimum of biomass was dried to avoid the mediating effects of a mat. The thalli were dried on polyethylene trays without sediment so that air could circulate around the thallus.

Due to variations observed between replicates in earlier uptake experiments, both intra-plant and inter-plant variations were examined. In the August 1985 experiments, plants from three different sites within the sampling area were dried to two levels of desiccation. A third portion of each plant was maintained in water as an undesiccated control. All plants were in damp or submerged mats when collected. To examine intra-plant variation, one plant was divided into three parts and dried to two levels of desiccation (approximately 25 and 50%). It was assumed that thalli were part of the same plant if all strands appeared to be growing from the same base. However, the mats are formed of many twisted strands of algae

and single plants may not have always been sampled. Experiments were conducted during September and October 1985.

Uptake was measured by loss of nutrient from the medium. Experiments lasted for 2 or 4 h, and samples were taken at 15, 30, 60, 120, and 240 min. A subsample of seawater (50 ml) was removed at each time point, refrigerated and analyzed within 10 hours using a Technicon AutoAnalyzer II and methods adapted from Atlas et al. (1971). Experiments were carried out independently for each nitrogen source; NH_4^+ and PO_4^{3-} uptake were measured on the first day, and NO_3^- and PO_4^{3-} uptake on the second day. The initial nitrogen concentrations were 7 or 12 μM NH_4^+ and 22 μM NO_3^-. These concentrations were chosen because they are ecologically realistic for water over the mudflat. Phosphate was present at a concentration at least 1/10 that of nitrogen. Rates were calculated for each sampling interval and as a mean rate for the entire incubation. Differences between rates of different treatments were determined using Student's t-test (Snedecor and Cochran 1980).

Potential loss of inorganic nutrients after desiccation was determined by measuring an increase in concentrations of NH_4^+, NO_3^- and PO_4^{3-} without allowing the plants to take up the nutrient subsequently. Two thalli, one desiccated and one control, were held in 250 μm Nitex baskets and moved sequentially through a series of 4 jars containing 200 ml of nutrient-free filtered seawater. The plant was submerged in the first jar of seawater for 10 sec, and in the second, third and fourth jars, for 5, 10 and 15 min, respectively.

Concentrations of nutrients were measured in each jar before and after it contained a plant, and the loss from the plant calculated from the difference in concentration. Nutrient concentrations were also measured in control jars with no plants. The experiment was repeated for a second thallus.

During the summer of 1985, tissue composition of algae from exposed habitats on the mudflat, mainly hillocks or mounds, was compared to that of algae growing in submerged or damp mats. For these comparisons, the appearance of the alga was not considered and some of the thalli were light green, easily broken, or obviously desiccated. Carbon, nitrogen and phosphorus of these samples were measured as described in Chapter II.

RESULTS

After two hours, the algae exposed to air at four levels of standing crop fell into two groups. Thalli exposed at 200 and 400 g wet wt m^{-2} lost 91.8% (SD = 6.12, n = 4) of tissue water and those exposed at 1000 and 1400 g wet wt m^{-2} lost 62.8% (SD = 7.01, n = 4). Algae within each group were combined to monitor subsequent growth after resubmergence. Both groups of algae recovered after 10 days. The growth rate of the algae desiccated to 62.8% was about 0.08 day^{-1} for the first five days, and increased to 0.15 - 0.20 day^{-1} for the last five days. The wet weight of the more severely desiccated thalli remained constant for four days, and then increased slowly. During the last three days, the growth rate was 0.21 day^{-1}. In both

treatments, bleached strands were observed, and in the more severely desiccated treatment, there were many fragmented thalli.

A summary of the uptake experiments conducted and algal tissue composition is given in Table IV.1. The algae used on 30 and 31 August were replete in both nitrogen and phosphorus, while those in later experiments appeared to be depleted in phosphorus. The tissue compositions were similar to the composition observed for these dates in Chapter II. Nutrient concentrations in the medium during two experiments are presented in Figures IV.1 and IV.2. At NH_4^+ and NO_3^- concentrations up to ca. 20 μM, uptake rates are dependent on nutrient concentration (see Chapter III). Later in the time course, variable uptake rates (determined from rate of nutrient depletion) resulted in increasing variation among the average nutrient concentrations of replicates. In most cases, rates from only the first interval have been compared.

Initial NH_4^+ uptake rates during all experiments have been summarized in Table IV.2. The uptake rates of desiccated and undesiccated thalli were different for only the first experiment. For the October experiment, the uptake rates have also been calculated for the entire incubation and these are presented in Table IV.3. When these rates were compared, the mean uptake rates of the more severely desiccated thalli were lower than those of the other two treatments. The uptake rates of the unexposed algae were about 50% higher during the September experiment (Table IV.2b) than those

Table IV.1. Tissue composition (% of dry wt) and the C:N:P ratio (by atoms) of *E. prolifera* used in desiccation experiments. ND = no data.

EXPERIMENT	DATE	PLANT	TISSUE CONTENT % DRY WT C	N	P	C:N:P
Uptake	30-31 Aug	I	ND	ND	0.330	ND
Inter-algae		II	20.2	2.83	0.264	197:23.7:1
variations		III	26.1	2.99	0.234	287:28.2:1
Uptake	17 Sept		31.7	3.15	0.193	420:33.4:1
Intra-alga	18 Sept		32.0	3.08	0.192	356:36.6:1
variations						
Uptake	13 Oct		27.0	3.69	0.182	395:46.4:1
Intra-alga	14 Oct		25.9	3.44	0.195	341:39.4:1
variations						
Loss, indepen-	14 Sept	I	20.5	3.17	0.124	429:58.8:1
dent of uptake		II	23.8	3.66	0.124	496:65.3:1

Figure IV.1. Changes in concentrations of NO_3^- (A-C) and PO_4^{3-} (D-F) after resubmergence of three algae on 31 August. ●, plant I; ×, plant II; □, plant III.
A,D) undesiccated algae; B,E) 26% water loss;
C,F) 47.8% water loss.

Figure IV.1

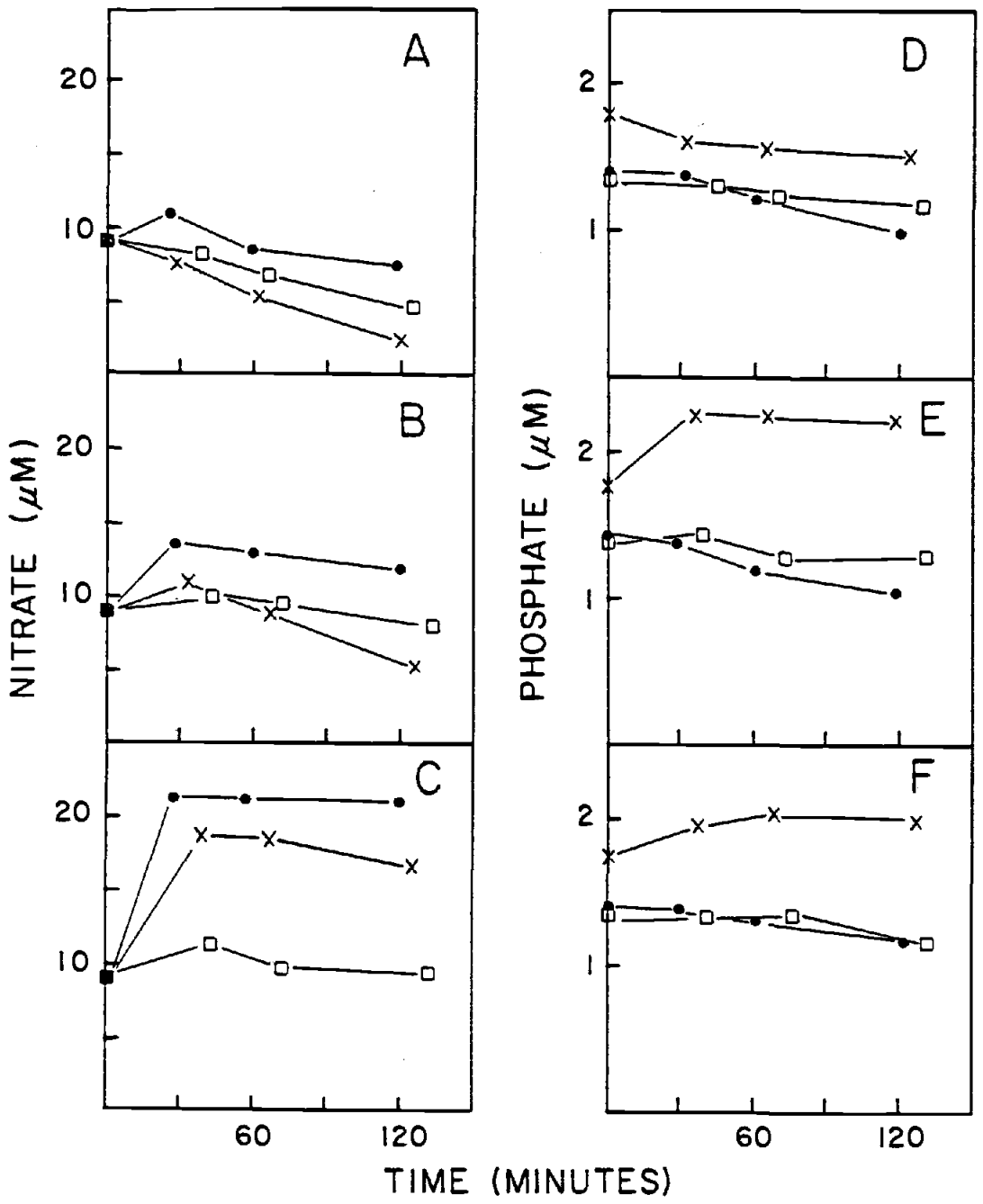


Figure IV.2. Changes in concentrations of NH_4^+ (A-C) and PO_4^{3-} (D-F) after resubmergence of three replicates of one thallus on 13 October. A,D) undesiccated algae; B,E) 36.7% water loss; C,F) 63.5% water loss. Symbols (●, ×, □) represent replicates A, B and C, respectively.

Figure IV.2

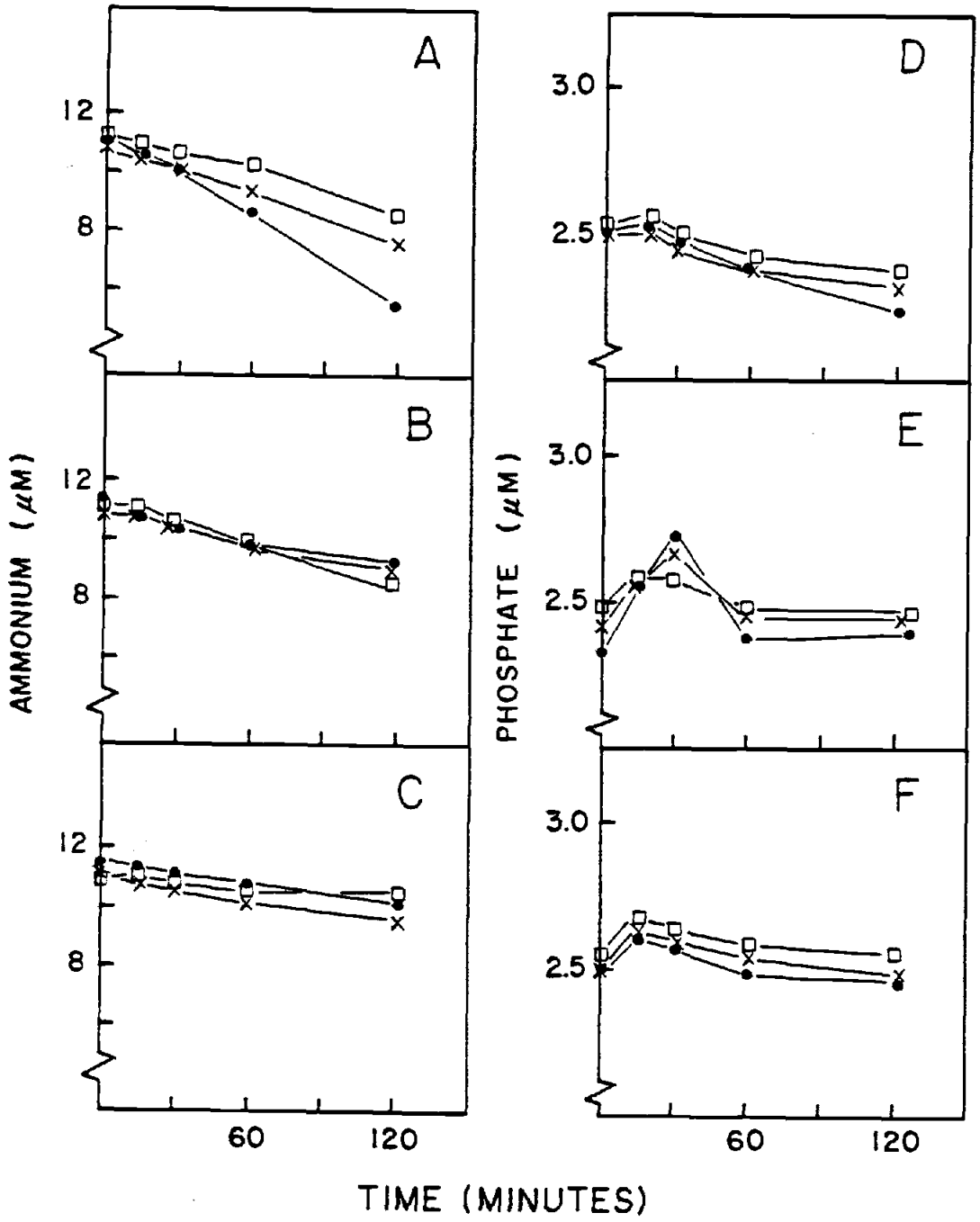

A
B
C
D
E
F
12
8
3.0
2.5
60
120
AMMONIUM (μM)
PHOSPHATE (μM)
TIME (MINUTES)

Table IV.2. Effect of desiccation on NH_4^+ uptake by E. prolifera. A) uptake by thalli from 3 sites, 30 August, average NH_4^+ concentration = 5.8 µM (SD = 0.47), rate during first 15 minutes after resubmergence. B, C) uptake by 3 replicates of one thalli, rate during first 30 minutes after resubmergence. B) 17 September, average NH_4^+ concentration = 8.9 µM (SD = 1.75). C) 13 October, average NH_4^+ concentration = 10.8 µM (SD = 0.32).

WATER LOSS %	UPTAKE RATE µmol g dry wt^{-1} h^{-1}	SD	COEFFICIENT OF VARIATION %
A			
0	25.7	6.44	25.1
25.4	13.4[1]	2.65	19.8
40.7	17.4[2]	5.47	31.4
B			
0	152	44.9	29.5
28.8	159	25.7	16.2
58.2	147	19.7	13.4
C			
0	33.6	16.4	48.8
36.7	35.5	10.5	29.6
63.5	25.9	8.9	34.4

[1] difference from unexposed plants is significant ($P \leq 0.05$)
[2] difference from unexposed plants is significant ($P \leq 0.12$)

Table IV.3. Ammonium uptake by unexposed and exposed thalli of *E. prolifera* on 13 October. NH_4^+ Rates were calculated from the slopes of the linear regression of NH_4^+ loss against time for the entire incubation (ca. 2hr). For each rate, n = 5. Average ammonium concentration = 10.4 µM (SD = 0.48). Standard deviation of rate is calculated from error of slope of regression line. A - C denote replicates.

	UPTAKE RATE µmol g dry wt^{-1} h^{-1}	SD	r^2
UNEXPOSED ALGAE			
A	42.3	0.40	0.9979
B	34.5	0.30	0.9982
C	17.3	2.83	0.6075
MEAN	31.4[1]	12.8	
36.7% WATER LOSS			
A	24.2	1.31	0.9557
B	32.3	0.58	0.9947
C	32.8	0.49	0.9962
MEAN	29.7[2]	4.87	
63.6% WATER LOSS			
A	19.1	0.82	0.9665
B	20.1	0.59	0.9837
C	9.84	0.72	0.9042
MEAN	16.3	5.66	

[1] significantly different from plants with 63.6% water loss ($P \leq 0.11$)
[2] significantly different from plants with 63.6% water loss ($P \leq 0.01$)

predicted by the kinetics curves (see Chapter III). During October, the rates were about one-half of the predicted rates.

Nitrate uptake during the first 15 minutes after desiccation was extremely variable and NO_3^- was released from the thallus during two experiments. Figure IV.3 shows rates of NO_3^- uptake and release during the first 15 minutes after resubmergence. During the 17 September experiment, the uptake rates of desiccated algae were significantly lower than those of the control plants ($P \leq 0.005$). The uptake rates of unexposed algae were about 50% higher than the rates predicted by the kinetics curves for these concentrations (Chapter III). On 30 and 31 August, NO_3^- was released from the thalli. Both the rates of loss and the variance of the mean of replicates tended to increase with increasing desiccation. In some cases, released NO_3^- was reassimilated (Figure IV.1a-c). The differences between the responses of desiccated and control algae during these experiments were of low significance due to the high variability in the rates of nutrient release for desiccated algae.

Like NO_3^-, PO_4^{3-} was often released from thalli after desiccation. In addition, PO_4^{3-} was lost from control thalli in about one-half of the incubations. Figure IV.4 shows PO_4^{3-} uptake and release by thalli during the initial time interval after resubmergence. In most cases, rates of release were higher and more variable at the more extreme desiccation level. The highest losses of PO_4^{3-} usually occurred when the nitrogen source was NH_4^+ rather than NO_3^-, however, PO_4^{3-} was reassimilated in incubations with NH_4^+

more often than in incubations with NO_3^-. Due to the variation between replicates, responses of exposed and unexposed thalli were significantly different ($P \leq 0.05$) for only three experiments, on 17 and 18 September and 13 October. On 18 September, the uptake rates of unexposed algae as well as the initial uptake rates of desiccated algae after loss (Figure IV.4), were 1.5 to 2 times higher than the maximum uptake rate observed during the experiments in Chapter III.

The patterns of nutrient release were also examined on shorter time scales, i.e. 10 sec to 15 min (data not shown). An increase in the concentration of NO_3^- was observed in all jars with desiccated algae indicating that release of NO_3^- may continue for up to 30 minutes. The release of PO_4^{3-} from desiccated algae was observed only during the first 5 minutes after resubmergence. No significant increases were observed in the jars containing undesiccated algae. The cumulative losses (as % of total tissue content) were lower than those observed in the first time intervals of the uptake experiments. A total of 0.20 and 0.32% of total phosphorus and 0.1% of nitrogen (both plants) was released.

Table IV.4 shows the tissue content of algae collected from exposed habitats during 1985. Phosphorus content of exposed algae tended to be lower than that of submerged algae, but there was no significant ($P \leq 0.05$) difference between the phosphorus or carbon content of these algae and those from the same dates collected from damp mats. On one date (22 June) the nitrogen content of exposed

Figure IV.3. Uptake and release of NO_3^- by desiccated and undessicated algae during the first 15 minutes after resubmergence. 31 August, 3 plants; 18 September, 14 October, 3 replicates of one thallus.

Figure IV.3

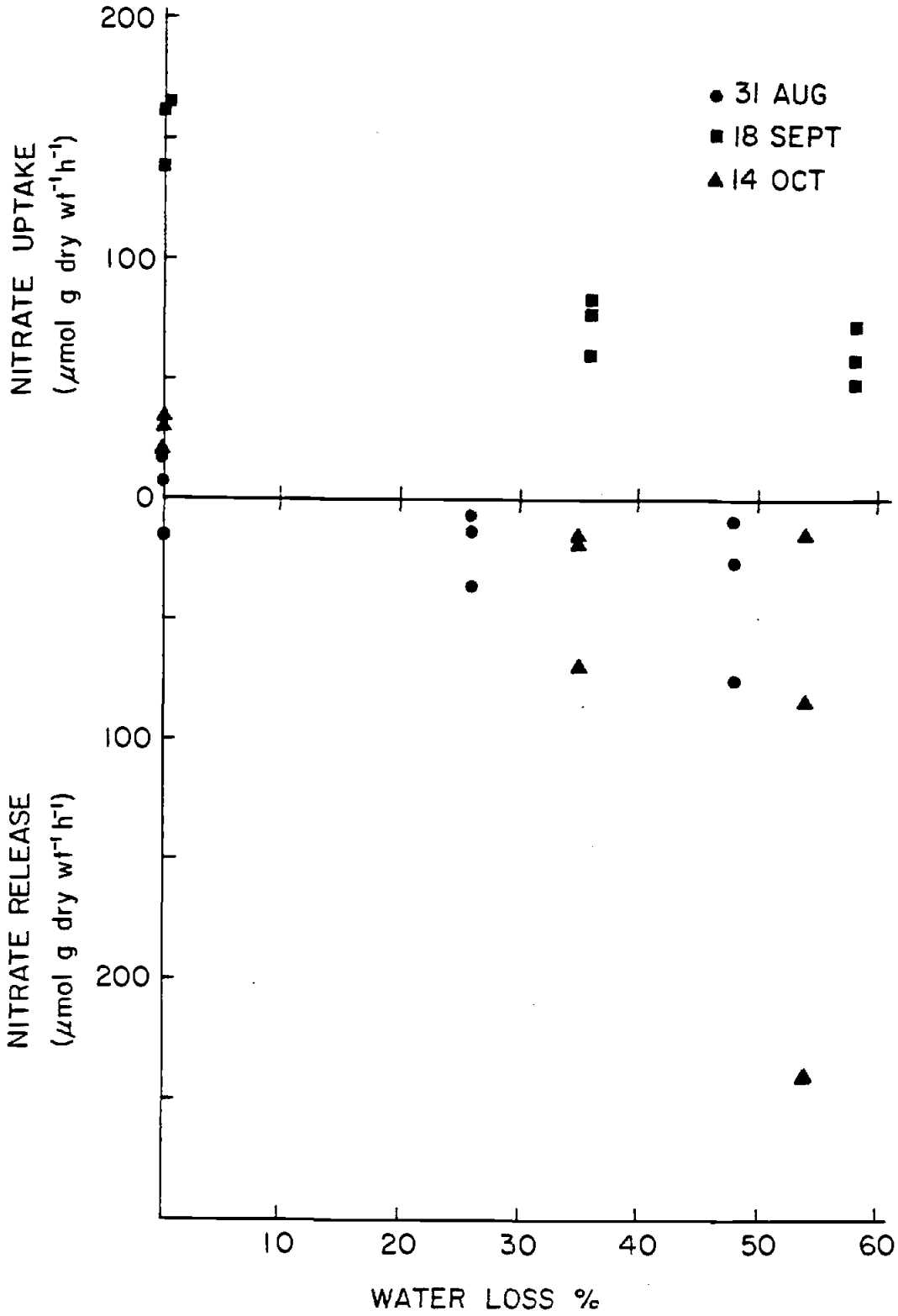

200
100
0
100
200
NITRATE UPTAKE
(μmol g dry wt⁻¹ h⁻¹)
NITRATE RELEASE
(μmol g dry wt⁻¹ h⁻¹)
31 AUG
18 SEPT
14 OCT
10
20
30
40
50
60
WATER LOSS %

Figure IV.4. Uptake and release of PO_4^{3-} by desiccated and undessicated algae during the first 15 minutes after resubmergence. 30 and 31 August, 3 plants; all other dates, 3 replicates of one thallus. Open symbols, N-source was NH_4^+; closed symbols, N-source was NO_3^-.

Figure IV.4

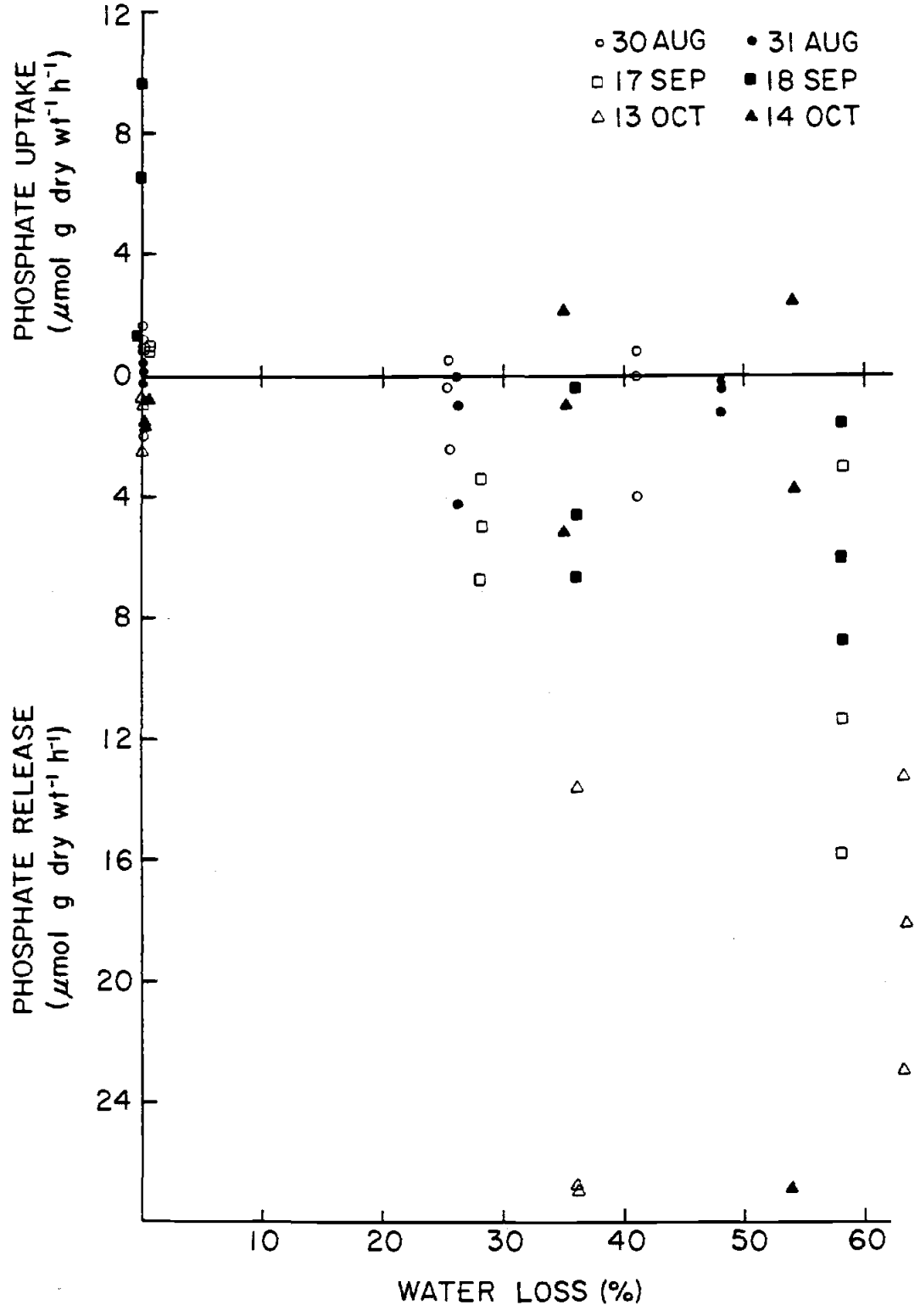

30 AUG
31 AUG
17 SEP
18 SEP
13 OCT
14 OCT
PHOSPHATE UPTAKE
(μmol g dry wt⁻¹ h⁻¹)
PHOSPHATE RELEASE
(μmol g dry wt⁻¹ h⁻¹)
12
8
4
0
4
8
12
16
20
24
10
20
30
40
50
60
WATER LOSS (%)

Table IV.4. Carbon, nitrogen and phosphorus content (% of dry wt) and the average C:N:P ratio (by atoms) of E. prolifera from exposed habitats and unexposed habitats on the Idaho Point mudflat during 1985. ND = no data.

DATE	CARBON mean	CARBON SD	NITROGEN mean	NITROGEN SD	PHOSPHORUS mean	PHOSPHORUS SD	C:N:P
EXPOSED HABITATS							
28 May	33.0	-	3.33	-	0.203	-	421:36.3:1
22 June	21.4	2.44	2.16	0.29	0.169	0.004	326:28.1:1
18 July	17.1	2.00	2.38	0.35	ND		8.4:1
5 Aug	25.4	3.9	3.58	0.27	0.286	0.027	231:28.0:1
16 Aug	21.1	6.19	2.95	0.86	ND		8.8:1
UNEXPOSED HABITATS							
28 May	32.1	0.75	4.01	0.59	0.306	0.090	229:27.0:1
22 June	26.4	3.75	3.62	0.37	0.273	0.097	267:31.4:1
18 July	18.8	3.84	2.69	0.72	0.214	0.065	247:30.1:1
5 Aug	28.6	3.9	3.90	0.42	0.337	0.013	219:25.7:1
16 Aug	22.7	3.21	3.03	0.16	ND		8.7:1

algae was significantly ($P \leq 0.05$) less than that of emergent, damp algae.

DISCUSSION

The responses of E. prolifera to desiccation observed here were different than those reported for E. intestinalis (Thomas 1983). Thomas found that uptake of NH_4^+ and NO_3^- was 10 - 30% higher by plants that had been subjected to a water loss of about 10%. For higher levels of desiccation, uptake was similar to or lower than that of undesiccated plants. In the current study, the desiccation levels were between 20 and 60%, so an enhancement of uptake would not have been observed had it occurred at desiccation levels below 20%.

The variation in the nitrogen uptake rates of Enteromorpha is evident in both Thomas' (ibid) and my data. Uptake rates of E. intestinalis at any desiccation level varied from 1.5- to 8-fold and the variation tended to increase with increasing desiccation. In the present study, the coefficients of variation were up to 50% and a 2-fold difference between rates was required before it could be considered significant. In both studies, the response of the algae was not consistent between experiments.

An important effect of desiccation on E. prolifera was the initial release of NO_3^- and PO_4^{3-}. The rates ranged from 0.23 to 28.3 μmol PO_4^{3-} g dry wt^{-1} h^{-1} and from 10.3 to 240 μmol NO_3^- g dry wt^{-1} h^{-1}. During the first 15 minutes, this release represented 0.1 - 12.2% and 0.1 - 2.4% of total tissue phosphorus and nitrogen, respectively. Most release rates were not this extreme; usually 1 to

2% of tissue phosphorus and less than 1% of tissue nitrogen was lost. Rosenberg and Ramus (1982) found that inorganic NO_3^- comprised up to ca. 7% of the total tissue nitrogen of Ulva sp. Very little is known about the inorganic PO_4^{3-} content of marine algae but it has been found to represent a significant portion of total phosphorus in Macrocystis (Manley 1983). Thomas (1983) also observed that thalli of E. intestinalis and Fucus distichus occasionally lost NO_3^- after resubmergence.

Dehydration of plant cells is accompanied by changes in the permeability of the cytoplasm, electric charge across the membrane and enzyme activities (reviewed by Henckel, 1964). All of these processes affect transport across the cell membrane. Without primary active transport, net efflux of permeable anions is likely to occur (Raven 1984). A change in the activity of porter proteins in the membrane would also alter the observed uptake. It is often assumed that regulation of osmotic pressure is confined to the vacuole (Bisson and Gutknecht 1980) and many algae store NO_3^- and PO_4^{3-} in the vacuole as well (Kuhl 1962, Hanisak 1983). Consequently, release of ions during osmotic stress may be accompanied by the release of the dissolved NO_3^- and PO_4^{3-} pools.

The release of NO_3^- or PO_4^{3-} rarely continued beyond the first 15 or 30 minutes after resubmergence, after which, net uptake usually resumed. Pregnall (1983) found that organic carbon loss from desiccated E. prolifera decreased markedly after 15 minutes. Wiltens et al. (1978) observed that fluorescence induction curves of

desiccation tolerant algae recovered most of their original features within 10 - 20 minutes of rehydration. Based on rates of photosynthesis in air, most investigators do not consider species of Enteromorpha or Ulva to be desiccation tolerant (Johnson et al. 1974, Wiltens et al. 1978, Quadir et al. 1979). However, when growth was monitored after desiccation, thalli of E. prolifera recovered from an average water loss of over 90%. Since bleached and broken patches appeared over time, it is likely that all parts of the thalli were not equally dehydrated. Net growth did not occur for several days, possibly this was the time required for the growth of less damaged areas to replace the dead regions. The fact that estuarine species of Enteromorpha are tolerant of salinities ranging from 0 to $40^{o}/oo$ (FitzGerald 1978, Shellem and Josselyn 1982) indicates effective osmoregulation. This may aid recovery after desiccation.

Thomas (1983) found that the degree of enhanced uptake was related to the tidal height at which an alga was found and consequently, to the frequency of emergence. Although E. prolifera is considered a mid-intertidal species (Abbott and Hollenberg 1976), its response to desiccation was similar to that of the low-intertidal species tested by Thomas (1983). This disparity may be the result of the ameliorating effects of the algal mat on desiccation. The algae may be subjected to less dehydration than a single thallus growing at the same tidal height. Retention of water between the fronds of Ulva spp. and inside the thallus of Colpomenia peregrina was observed to limit dehydration of these algae (Beer and Eshel 1983a, Oates 1985). Pregnall (1983) found that when standing crops of E. prolifera were

less than about 100 g dry wt m^{-2}, thalli lost more than 50% of their internal water after one hour. At higher plant densities, water loss slowed dramatically. Since the standing crop is 200 - 400 g dry wt m^{-2} between June and October (Davis 1981), desiccation may not be significant during most of the growing season. Since mats are not securely attached to the sediment, a thallus may not be repeatedly desiccated during a series of low tides.

The loss of PO_4^{3-} and NO_3^- may represent up to ca. 10% of the total tissue N or P, but this difference is within the variation observed for healthy plants (Chapter II). Consequently, the tissue content of algae from exposed habitats (Table IV.4) was no different from that of algae in mats. However, during the fall when the standing crop is greatly reduced or when thalli are anchored to the sediment, desiccation may represent a significant stress.

The uptake rates of NH_4^+ and NO_3^- observed during the September experiments were much higher than the rates predicted by the kinetic curves presented in Chapter III, in fact the rates were very close to the calculated V_{max}. It is possible that these plants were temporarily starved for nutrients. They were collected and maintained in an outdoor culture facility with ambient nutrient concentrations for about 24 hours before the experiment. At the flow rates used and with total nitrogen concentration at least 1 μM, the nutrient flux should have been sufficient for growth. Later, I observed that the concentrations of NH_4^+, NO_3^-, and NO_2^- in the incoming seawater were undetectable, and the concentration of PO_4^{3-}

was 0.4 μM. The response of the algae was consistent with what might be expected during nitrogen limitation; there was no reduction of NH_4^+ uptake or release of NO_3^-, but release of PO_4^{3-} still occurred after desiccation. The ratios of NH_4^+:PO_4^{3-} and NO_3^-:PO_4^{3-} uptake by unexposed plants during the first 30 minutes averaged 162.5 and 63.2, respectively. The N:P ratio of the algal tissue was 33.6. High nitrogen uptake relative to phosphorus indicates that these plants were probably nitrogen-limited. However, there were also high rates of PO_4^{3-} uptake both by unexposed algae and by desiccated algae when uptake resumed which cannot be explained.

These observations are consistent with those of Thomas and Turpin (1980). Desiccation-enhanced uptake of NH_4^+ and NO_3^- by Fucus distichus occurred in freshly collected algae that were probably nitrogen-limited. Desiccation-enhanced uptake of PO_4^{3-} occurred only when plants were preconditioned in PO_4^{3-}-free water.

The release of NO_3^- in some cases and uptake in others is not contradictory; it must be remembered that the increase of NO_3^- and PO_4^{3-} in the medium represents only the net flux across the membrane. Cells were still able to take up these nutrients after desiccation, although in some cases this was accompanied by release. This may in part expain the high variability in the net responses of extremely desiccated algae.

CHAPTER V

SUMMARY

Potential nutrient limitation of Enteromorpha prolifera was investigated by examining tissue composition of field-collected plants and uptake rates of nutrient-replete and nutrient-limited algae. Since exposure at low tide may either stress the alga or result in a short term nutrient deficit, the response of nutrient uptake to dehydration was also examined.

Evaluation of elemental composition of E. prolifera on the Idaho Point mudflat over a seasonal cycle suggested that growth was not nitrogen-limited during 1985, but that growth may have been phosphorus-limited during the fall and winter. High rates of PO_4^{3-} uptake relative to nitrogen uptake by field-collected plants were observed in late fall and provided further evidence for phosphorus-limitation in Yaquina Bay. However, phosphorus supply rates inferred from river flow and sediment flux measured during the preceding year did not imply phosphorus-limitation. The discrepancy between these conclusions could be due to 1) interannual variations in nutrient regimes, 2) errors in evaluating the sufficiency of nutrient supplies from tissue composition or 3) errors in the inferences drawn from nutrient uptake rates as a function of nutrient concentrations.

Previously, phosphorus has not been considered a limiting nutrient in marine environments, but phosphorus and nitrogen should be considered of equal importance to algal growth in studies of

estuarine nutrient utilization since either one is potentially limiting. Distinguishing between nitrogen- and phosphorus-limitation from elemental composition of field populations requires that the relationship between growth rate and internal concentrations of both elements be determined more precisely than was possible in this study. Patterns of elemental tissue composition and nutrient uptake observed in this study may have been related to factors other than nutrient stress such as life cycle or light history and these factors should be more thoroughly investigated. Phosphate uptake kinetics were not well delineated in this study. However, the evidence obtained for possible phosphorus-limitation suggests that a more detailed analysis of PO_4^{3-} uptake and the effect of nutrient limitation on PO_4^{3-} utilization would be particularly important for E. prolifera.

Uptake of nitrogen by E. prolifera was related to nutrient concentrations. Highest uptake rates occurred during the summer, and during late fall, nitrogen uptake rates of field collected plants decreased by as much as 75%. Kinetic parameters for NO_3^- uptake were different for thalli with low and high tissue-nitrogen. However, during the summer there was no difference between NH_4^+ uptake by thalli with less than and greater than 2% tissue-nitrogen. This also suggested that this alga may not be subjected to nitrogen limitation since nitrogen-limited algae usually show a capacity to take up NH_4^+ at high rates. Nitrate provided a relatively small proportion of total nitrogen utilization despite its presence at concentrations equal to or greater than those of NH_4^+.

Ammonium uptake rates measured by changes in dissolved NH_4^+ and ^{15}N accumulation were comparable only if certain precautions were taken. When NH_4^+ remineralization occurred, measurements of tracer accumulation corrected for isotope dilution were more accurate estimates of plant uptake than rates based on loss of nutrient from the medium. The importance of bacterial uptake in NH_4^+ incubations was extremely variable. These observations are probably applicable to ^{15}N incubations with other macroalgae species as well. Seawater used for incubations should be filtered as finely as possible just before the incubation to remove potentially contaminating microorganisms. Uptake in control incubations and NH_4^+ regeneration in plant incubations should be monitored to obtain the most accurate estimates of algal uptake. The contribution of epiphytes to regeneration and total nutrient loss was not investigated, but may account for some of the observed variation in measurements of total nutrient loss and regeneration.

Calculated *in situ* uptake of NH_4^+ by *E. prolifera* was up to 10 times higher than that measured in the field. This was probably due in part to regeneration of NH_4^+ during *in situ* incubations. Isotopic methods have been developed for the measurement of nitrogen accumulation in macroalgae and for the measurement of regeneration of NH_4^+. Application of isotopic methods to *in situ* incubations are essential for more accurate estimates of NH_4^+ utilization by macroalgae.

After desiccation, NO_3^- and PO_4^{3-} uptake rates decreased and both were often released initially from the plant tissue. Although release of inorganic nutrients was considerable, thalli were able to resume growth even after an average internal water loss of 90%. Simultaneous uptake and release of nutrients may have occurred after desiccation and more detailed analyses will be needed to understand the physiological responses of algae to desiccation and the potential ecological importance of simultaneous uptake and release. Future investigations may be able to quantify these fluxes by simultaneously monitoring accumulation of isotopic tracers in plant tissue and dilution of the appropriate tracers in the medium.

The major findings of this study are 1) possible phosphorus-limitation of macrophyte production in Yaquina Bay, 2) demonstration of the necessity of ^{15}N tracer methodology for obtaining accurate estimates of NH_4^+ utilization and 3) the occurrence of both uptake and release of nutrients after desiccation. Past estimates of NH_4^+ utilization by macrophytes have probably been low, especially for *in situ* measurements. Isotopic methods, including isotope dilution, are essential to accurate estimates if microbial activity cannot be eliminated. Measurements of isotope dilution and accumulation also provide an ideal way to approach questions concerning simultaneous uptake and release of nutrients by macrophytes after desiccation. Phosphorus-limitation of primary production has been postulated for estuarine environments. Results from this study suggest that the growth of *E. prolifera* in Yaquina Bay would be an ideal system for testing this hypothsesis.

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APPENDICES

Table A.1. Ammonium concentrations (S), uptake rates (V) and results of Wolfe transformation and Wilkinson nonlinear fit for ammonium uptake kinetics experiment, September, 1985.

RESULTS FOR FILE : NH41185A

S	V	1/S	1/V	S/V
0.32	1.9800	3.1250	0.5051	0.1616
0.30	3.6200	3.3333	0.2762	0.0829
1.93	17.7000	0.5181	0.0565	0.1090
1.85	6.9000	0.5405	0.1449	0.2681
5.56	13.6000	0.1799	0.0735	0.4088
5.32	19.9000	0.1880	0.0503	0.2673
9.61	27.8000	0.1019	0.0359	0.1464
22.80	40.4000	0.0439	0.0248	0.5644
22.90	29.7000	0.0437	0.0337	0.7710
38.79	22.8000	0.0258	0.0439	1.6974
38.50	38.8000	0.0260	0.0296	1.1391
72.10	49.0000	0.0139	0.0204	1.4714
72.90	26.4000	0.0137	0.0379	2.7614

WOLFE TRANSFORMATION RESULTS:

KS = 3.9697 VMAX = 34.9885 R SQUARED = 0.8482

RESIDUALS:

PT	S	S/V	YCAL	RESIDUAL
1	0.3200	0.1616	0.1226	-0.0390
2	0.3000	0.0829	0.1220	0.0392
3	1.9300	0.1090	0.1686	0.0596
4	1.8500	0.2681	0.1663	-0.1018
5	5.5600	0.4088	0.2724	-0.1365
6	5.3200	0.2673	0.2655	-0.0018
7	9.6100	0.1464	0.3938	0.2474
8	22.8000	0.5644	0.7651	0.2007
9	22.9000	0.7710	0.7680	-0.0031
10	38.7000	1.6974	1.2195	-0.4778
11	38.5000	1.1391	1.2138	0.0748
12	72.1000	1.4714	2.1741	0.7027
13	72.9000	2.7614	2.1970	-0.5644

RESULTS OF WILKINSON NONLINEAR FIT AFTER 13 ITERATIONS:

KS = 2.9452 VMAX = 39.2136

STDEV KS = 2.7043 STDEV VMAX = 7.4519

95 % CONFIDENCE LIMITS

KS 2.9452 +/- 5.8927

VMAX 39.2136 +/- 16.2378

Table A.2. Ammonium concentrations (S), uptake rates (V) and results of Wolfe transformation and Wilkinson nonlinear fit for ammonium uptake kinetics experiment, September, 1984.

RESULTS FOR FILE : NH4SEP84

S	V	1/S	1/V	S/V
0.72	8.1100	1.3889	0.1233	0.0888
0.94	16.5000	1.0638	0.0606	0.0570
4.05	56.7000	0.2469	0.0176	0.0714
3.98	40.0000	0.2513	0.0250	0.0995
8.18	67.7000	0.1222	0.0148	0.1208
9.24	53.9000	0.1214	0.0186	0.1529
24.20	79.0000	0.0413	0.0127	0.3063
28.59	114.0000	0.0424	0.0088	0.2061
43.90	220.0000	0.0228	0.0045	0.1995
43.50	190.0000	0.0230	0.0053	0.2289
71.60	145.0000	0.0140	0.0069	0.4938
70.00	133.0000	0.0143	0.0075	0.5263
91.30	132.0000	0.0110	0.0076	0.6917

WOLFE TRANSFORMATION RESULTS:

KS = 10.2005 VMAX = 162.4104 R SQUARED = 0.9061

RESIDUALS:

PT	S	S/V	YCAL	RESIDUAL
1	0.7200	0.0888	0.0672	-0.0215
2	0.9400	0.0570	0.0686	0.0116
3	4.0500	0.0714	0.0877	0.0163
4	3.9800	0.0995	0.0873	-0.0122
5	8.1800	0.1208	0.1132	-0.0077
6	8.2400	0.1529	0.1135	-0.0393
7	24.2000	0.3063	0.2118	-0.0945
8	28.5000	0.2061	0.2075	0.0014
9	43.9000	0.1995	0.3331	0.1336
10	43.5000	0.2289	0.3306	0.1017
11	71.6000	0.4938	0.5037	0.0099
12	70.0000	0.5263	0.4938	-0.0325
13	91.3000	0.6917	0.6250	-0.0667

RESULTS OF WILKINSON NONLINEAR FIT AFTER 8 ITERATIONS:

KS = 13.4486 VMAX =187.6091

STDEV KS = 7.3437 STDEV VMAX = 30.3118

95 % CONFIDENCE LIMITS

KS 13.4486 +/- 16.0018

VMAX 187.6091 +/- 66.0495

Table A.3. Ammonium concentrations (S), uptake rates (V) and results of Wolfe transformation and Wilkinson nonlinear fit for ammonium uptake kinetics experiment, November, 1985.

RESULTS FOR FILE : NH40985B

S	V	1/S	1/V	S/V
1.47	8.9100	0.6803	0.1122	0.1650
1.49	14.0000	0.6711	0.0714	0.1064
2.35	24.2000	0.4443	0.0413	0.0930
2.86	30.6000	0.3497	0.0327	0.0935
5.28	58.4000	0.1894	0.0171	0.0904
6.62	68.4000	0.2146	0.0158	0.0735
7.85	99.7000	0.1274	0.0100	0.0787
7.48	94.5000	0.1337	0.0106	0.0792
20.60	152.0000	0.0485	0.0066	0.1355
19.30	141.0000	0.0518	0.0071	0.1369
45.90	124.0000	0.0218	0.0081	0.3702
46.00	166.0000	0.0217	0.0060	0.2771
67.70	158.0000	0.0148	0.0063	0.4285
67.90	170.0000	0.0147	0.0059	0.3994

WOLFE TRANSFORMATION RESULTS:

KS = 14.4142 VMAX = 198.8506 R SQUARED = 0.9133

RESIDUALS:

PT	S	S/V	YCAL	RESIDUAL
1	1.4700	0.1650	0.0799	-0.0851
2	1.4900	0.1064	0.0800	-0.0264
3	2.2500	0.0930	0.0838	-0.0092
4	2.8600	0.0935	0.0869	-0.0066
5	5.2800	0.0904	0.0990	0.0086
6	4.6600	0.0735	0.0959	0.0224
7	7.8500	0.0787	0.1120	0.0332
8	7.4800	0.0792	0.1101	0.0310
9	20.6000	0.1355	0.1761	0.0406
10	19.3000	0.1369	0.1695	0.0327
11	45.9000	0.3702	0.3033	-0.0668
12	46.0000	0.2771	0.3038	0.0267
13	67.7000	0.4285	0.4129	-0.0155
14	67.9000	0.3994	0.4140	0.0145

RESULTS OF WILKINSON NONLINEAR FIT AFTER 11 ITERATIONS:

KS = 9.2795 VMAX =188.3135

STDEV KS = 2.0023 STDEV VMAX = 12.8731

95 % CONFIDENCE LIMITS

KS 9.2795 +/- 4.3250

Table B.1. Nitrate concentrations (S), uptake rates (V) and results of Wolfe transformation and Wilkinson nonlinear fit for nitrate uptake kinetics experiment, September, 1985.

RESULTS FOR FILE : N0309858

S	V	1/S	1/V	S/V
0.30	7.4000	3.3333	0.1351	0.0405
0.90	7.7000	3.3333	0.1299	0.0390
1.30	10.6000	0.7692	0.0943	0.1226
1.30	11.8000	0.7692	0.0847	0.1102
2.80	25.0000	0.3571	0.0400	0.1120
2.80	23.7000	0.3571	0.0422	0.1181
6.00	34.1000	0.1667	0.0293	0.1760
5.30	50.6000	0.1887	0.0198	0.1047
12.60	95.9000	0.0794	0.0104	0.1314
11.80	96.0000	0.0847	0.0104	0.1229
22.80	116.0000	0.0439	0.0086	0.1966
22.90	89.9000	0.0437	0.0111	0.2547
36.80	131.0000	0.0272	0.0076	0.2809
36.60	112.0000	0.0273	0.0089	0.3268

WOLFE TRANSFORMATION RESULTS:

KS = 13.5783 VMAX = 165.5544 R SQUARED = 0.8538

RESIDUALS:

PT	S	S/V	YCAL	RESIDUAL
1	0.3000	0.0405	0.0838	0.0433
2	0.3000	0.0390	0.0838	0.0449
3	1.3000	0.1226	0.0899	-0.0328
4	1.3000	0.1102	0.0899	-0.0203
5	2.8000	0.1120	0.0989	-0.0131
6	2.8000	0.1181	0.0989	-0.0192
7	6.0000	0.1760	0.1183	-0.0577
8	5.3000	0.1047	0.1140	0.0093
9	12.6000	0.1314	0.1581	0.0267
10	11.8000	0.1229	0.1533	0.0304
11	22.8000	0.1966	0.2197	0.0232
12	22.9000	0.2547	0.2203	-0.0344
13	36.8000	0.2809	0.3043	0.0234
14	36.6000	0.3268	0.3031	-0.0237

RESULTS OF WILKINSON NONLINEAR FIT AFTER 8 ITERATIONS:

KS = 13.3345 VMAX =168.5890

STDEV KS = 3.6478 STDEV VMAX = 19.2789

95 % CONFIDENCE LIMITS

KS 13.3345 +/- 7.8792

Vmax 168.5890 +/- 41.6424

Table B.2. Nitrate concentrations (S), uptake rates (V) and results of Wolfe transformation and Wilkinson nonlinear fit for nitrate uptake kinetics experiment, May, 1985.

RESULTS FOR FILE : NO30585A

S	V	1/S	1/V	S/V
1.00	9.9600	1.0000	0.1116	0.1116
1.00	6.5500	1.0000	0.1527	0.1527
7.40	56.3000	0.1351	0.0178	0.1314
6.20	78.7000	0.1613	0.0130	0.0808
13.40	92.6000	0.0746	0.0108	0.1447
14.60	78.7000	0.0685	0.0127	0.1855
22.20	99.5000	0.0450	0.0101	0.2231
28.30	72.3000	0.0429	0.0138	0.3223
53.00	51.0000	0.0189	0.0196	1.0392
52.40	106.0000	0.0191	0.0094	0.4943

WOLFE TRANSFORMATION RESULTS:

KS = 2.3098 VMAX = 75.405: R SQUARED = 0.7624

RESIDUALS:

PT	S	S/V	YCAL	RESIDUAL
1	1.0000	0.1116	0.0439	-0.0677
2	1.0000	0.1527	0.0439	-0.1088
3	7.4000	0.1314	0.1288	-0.0027
4	6.2000	0.0808	0.1129	0.0320
5	13.4000	0.1447	0.2083	0.0636
6	14.6000	0.1855	0.2243	0.0387
7	22.2000	0.2231	0.3250	0.1019
8	28.3000	0.3223	0.3396	0.0174
9	53.0000	1.0392	0.7335	-0.3057
10	52.4000	0.4943	0.7255	0.2312

RESULTS OF WILKINSON NONLINEAR FIT AFTER 20 ITERATIONS:

KS = 0.0000 VMAX = 0.0000

STDEV KS = 2.4834 STDEV VMAX = 14.2575

95 % CONFIDENCE LIMITS

KS 0.0000 +/- 5.6173

VMAX 0.0000 +/- 32.2594

BONFERRONI JOINT 95% C.I.:

KS 0.0000 +/- 6.7746