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Iron Uptake and Translocation by *Macrocystis pyrifera*[1]

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ABSTRACT

Parameters of iron uptake have been determined for blade tissue of *Macrocystis pyrifera* (L.) C. Ag. These include the effects of iron concentration, light, various inhibitors, and blade type. All experiments were conducted in the defined artificial seawater Aquil. Iron uptake is light independent, energy dependent, and dependent on the reduction from Fe^{3+} to Fe^{2+}. Iron is concentrated in the sieve tube exudate; exudate analysis revealed the presence of other micronutrients. Iron and other micronutrient translocation is discussed.

Iron uptake and translocation by higher plants have been extensively studied (2). Investigations using higher plants have determined that root tissue releases reductants which reduce Fe^{3+} to Fe^{2+} outside the cell prior to cellular uptake (3) and that transport of iron into the xylem is metabolically controlled (5). No information exists on iron uptake by marine macroalgae.

Macrocystis sporophytes can reach 40 m in length. Blades absorb nutrients from seawater to support rapid growth primarily from their basal (frond producing) and apical (blade producing) meristems. Much is known about nitrogen and phosphorus uptake and translocation (8, 23, 24, 26). The effect of blade morphology on water motion and thus on macronutrient uptake has been determined (26). This paper presents some parameters of iron uptake and demonstrates the translocation of iron in *Macrocystis pyrifera*. This study includes the use of artificial media, Aquil, for the uptake experiments. The chemical species of iron present in seawater are poorly defined because of complex organic and inorganic interactions. Chemical speciation in Aquil is completely definable (16).

MATERIALS AND METHODS

Culturing. Juvenile sporophytes of *M. pyrifera* (L.) C. Ag. (5 to 8 cm in length), cultured in flowing seawater from spores that had been attached to ropes, were preconditioned in offshore surface seawater renewed daily and supplemented with 20 μM NH_4Cl and 2 μM K_2HPO_4; the water was vigorously recirculated at 15 C in a 40-liter Plexiglas aquarium (18). Plants were discarded when they attained their first secondary division. Microscopic examination of tissue revealed no algal or fungal contamination. Light intensity was maintained at 3×10^4 ergs·cm^{-2}·s^{-1} (Cool White fluorescent lamps (General Electric Co., Cleveland, OH)). Discs (diam. of 1.01 cm, total tissue area 1.602 cm^2) were routinely cut 4 to 6 cm from the base of the blade even though iron uptake was found independent of distance from the blade base (14). Discs were placed in aerated Aquil batch cultures for 16 h at 15 C before uptake experiments.

Adult Plant Material. Blades from adult sporophytes were obtained from Crystal Cove, Orange County, CA. They consisted of immature (1 m from apex), mature (3 m from apex), and senescing blades from a canopy frond. Three discs were cut from each blade 6 cm from base and placed in batch culture as described above. A canopy frond was collected off Main Beach in Laguna Beach, CA, on November 17, 1980, for the STE[2] labeling study. An adult plant (25 fronds) was collected at the latter site on December 8, 1980, for the collection of STE.

Artificial Seawater. The artificial seawater used for preconditioning tissue discs and for uptake experiments was Morel's (16) Aquil, modified by Kuwabara as an optimal medium for *M. pyrifera* gametophytes (Table I) (11). Iron:EDTA ratio was maintained for each Fe concentration used.

Uptake Experiments. Clean procedures were used to minimize contamination. Uptake experiments were performed in a hooded isolated area with clean and covered surfaces. All containers (linear polyethylene or polystyrene) for Aquil or diluted radionuclide were treated to prevent contamination from leaching (15). Tissue discs were handled with Teflon forceps. Uptake experiments utilized ^{59}Fe as $FeCl_2$ in 0.5 N HCl, 13.6 mCi/mg Fe (ICN).

Discs from batch culture were transferred to 4 ml Fe-free Aquil for 5 min, then to 4 ml of stirred Aquil (pH 8.1, 10 to 12 C) of known total Fe containing ^{59}Fe. Surface illumination was 1.3×10^4 ergs·cm^{-2}·s^{-1}. After 10 min (within linearity, Fig. 1A), the tissue was dipped in 4 ml of 10 μM FeEDDHA in Aquil (wash solution), placed in a second 4 ml stirred wash solution (4 to 6 C) for 30 min (juvenile tissue) or 40 min (adult tissue) to remove >90% free space iron (Fig. 1, A and B), and then counted. Analysis by ICP (Vetter Research Labs, Costa Mesa, CA) of the uptake solution revealed no contamination after an experiment minus tissue and micronutrient. Adsorption of Fe on beaker surface was negligible.

Effects of light, darkness, BPDS, DNP, and DCMU (Sigma) were also determined. Discs were preconditioned in Aquil batch culture with DNP (50 and 100 μM) or DCMU (50 μM) for 0, 15, 30, and 60 min before uptake experiments in Aquil. Discs were not preconditioned in BPDS. Iron analysis by ICP of BPDS, DNP, and DCMU showed some contamination; 28.5 mg iron per kg sample, 17.5 mg/kg and 402 mg/kg, respectively. The contamination was accounted for in the calculations.

Measurements of Photosynthesis and Respiration. Blade tissues (about 0.4 to 0.6 g fresh weight) directly from juvenile sporophytes growing as rope cultures were placed in 50 ml Aquil, stirred in a Plexiglas cell at 15 C with blade surfaces perpendicular to the light

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[2] Abbreviations: STE, sieve tube exudate, EDDHA, ethylenediamine-di (o-hydroxyphenylacetic acid); ICP, inductively coupled plasma; BPDS, bathophenanthroline disulfonate; DNP, dinitrophenol; ESCR, external standard channel ratio.

Table I. *The Optimal Micronutrient Composition of Aquil for Macrocystis Gametophytes*[a]

Micronutrient	Analytical Concentration	Major Species	%
	nM		
Fe^{3+}	350	FeEDTA[b]	100
Mn^{2+}	30	MnEDTA	65
Co^{2+}	70	CoEDTA	99
Cu^{2+}	10	CuEDTA	99
Zn^{2+}	170	ZnEDTA	100
MoO_4^{2-}	100	Free ion	100
I^-	100	Free ion	100
EDTA	6×10^3	CaEDTA	89

[a] According to Kuwabara (11).

[b] Actual free ion concentration 7×10^{-11} nM; other species <0.5 nM.

(2×10^5 ergs·cm^{-2}·s^{-1}). Light-dark O_2 evolution and consumption were measured with a galvanic cell O_2 analyzer. Concentrated solutions of inhibitors in 0.2 ml of 95% ethanol were added by syringe after O_2 evolution or consumption achieved a constant rate.

Exudate Labeling, Collection, and Analysis. A canopy frond was cut 140 cm from the apical blade and placed in a shallow tray containing 20 liters of offshore surface water and ^{59}Fe (25 μl of FeCl stock, 4.69 mCi/mg) yielding an added iron concentration of 13.2 nM. Fluorescent lamp illumination was 1.4×10^4 ergs·cm^{-2}·s^{-1}. Exudate was collected from the cut stipe, after the technique of Schmitz and Srivastava (23), for 10 h at 10 min and later 30 min intervals. The stipe was recut (1 cm back) periodically to ensure exudate flow. Subsequently, an adult plant was submerged in Newport Harbor, California, with the holdfast placed on the Kerckhoff Marine Laboratory dock and exudate (10 ml) collected from glass-cut stipes. Before collection, the cut ends were rinsed with deionized and distilled H_2O and the first few drops of exudate were discarded. Exudate was analyzed for trace elements by ICP and for TCA precipitated protein after Lowry *et al.* (12).

Measurement of Fe^{3+} EDTA Reduction. Reduction of FeEDTA was measured spectrophotometrically by following $Fe^{2+}(BPDS)_3$ formation as described by Chaney *et al.* (3). Nine tissue discs (1.60 cm^2/disc) were placed in 20 ml Aquil (FeEDTA = 350 nM) to which BPDS was added (final concentration, 1 μM). Absorbance at 535 nm was determined on periodically removed aliquots. Three controls incorporated Aquil only, Aquil plus BPDS, and plant plus Aquil.

Tissue Preparation/Counting Procedure. After uptake experiments, tissue discs were held for 12 to 15 h in capped borosilicate glass scintillation vials with 3 ml of NCS tissue solubilizer (Amersham Corp., Arlington Heights, IL) in a heated water bath at 50 C. A thin, transparent, disc-shaped cell wall debris remained which disintegrated with mild agitation. Samples were bleached by adding 1 ml of 10% w/w benzoyl peroxide in toluene, followed by exposure to direct sunlight for 5 to 7 h. They were then heated in a water bath (35 to 40 C) and dark adapted for 24 h to eliminate chemiluminescence and phosphorescence. Ten milliliters of OCS scintillation cocktail (Amersham) was then added and the sample was counted. Quench correction for tissue samples was determined by production of quenched standards. A known amount of radionuclide solution was placed on top of a series of 10 discs, to which 3 ml of NCS was added after 30 min. The tissue was then treated as described above. Average counting efficiency was 85 ± 2%. Solutions were then quenched and counting efficiency was determined by ESCR. This procedure is an approximation because ESCR measures solution efficiency only. It is assumed that the radionuclide is totally eluted from the solid phase into solution. The variation in counting efficiencies of experimental material did not exceed 5%.

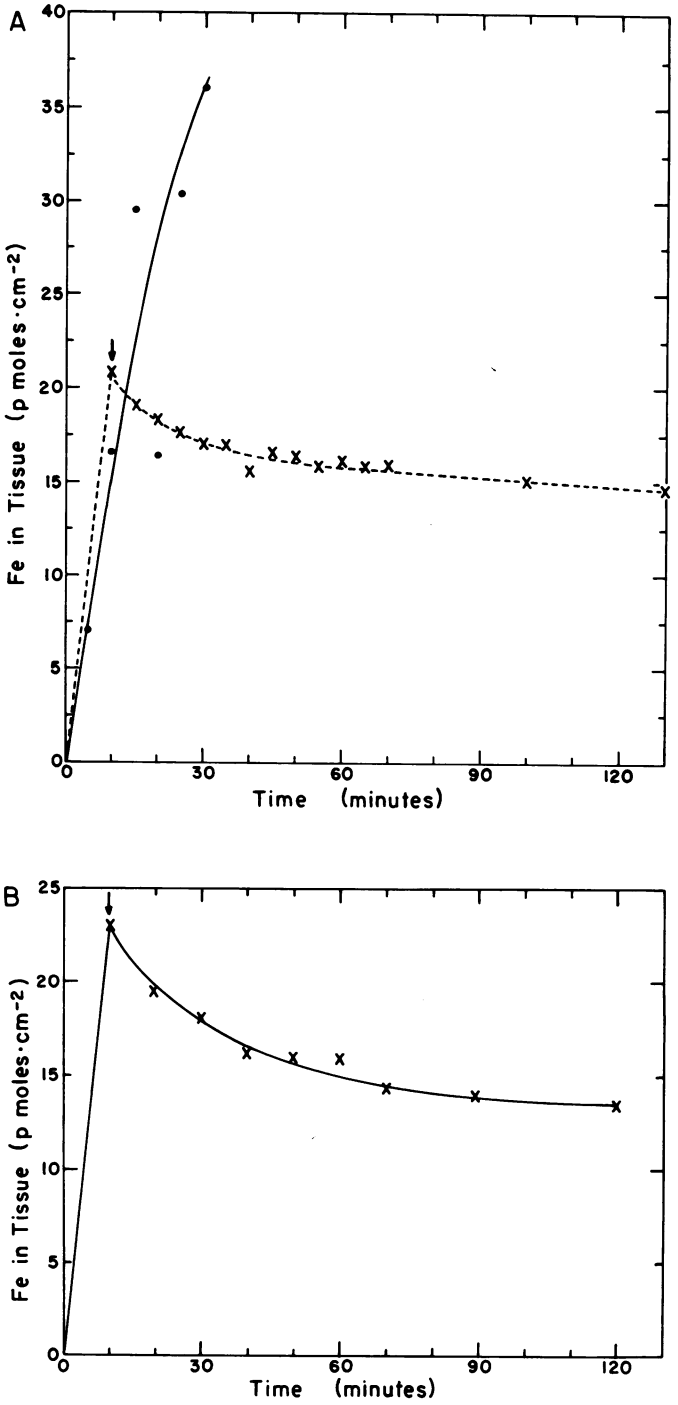

FIG. 1. Iron uptake into tissue discs in Aquil *versus* time. A, juvenile plant tissue, total Fe, 350 nM; ×—× 10 min uptake followed by wash (↓) to remove free space iron; (●—●) uptake after 30 min wash. B, adult blade tissue total Fe, 250 nM; 10 min uptake followed by wash (↓).

A second technique modified from the procedure of Gagne *et al.* (7) totally dissolved the tissue. Chemiluminescence and quenching from both techniques were similar. Tissue discs were pretreated with 0.5 ml H_2O_2 (30% v/v) at 50 C for 18 h followed by addition of 4 ml NCS at 50 C for 16 h in the dark. OCS (10 ml) was then added and the sample was counted. This second technique was employed to a limited extent in this study since it was learned of after the bulk of this work was completed. Advantages are apparent and the technique will be preferred for future studies.

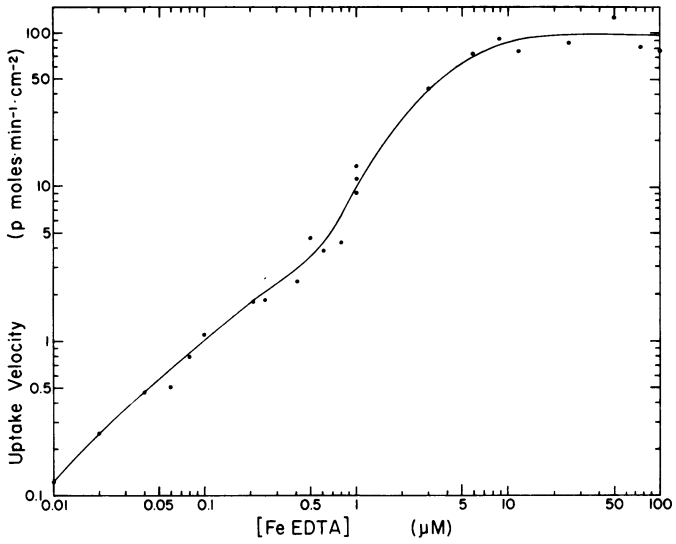

FIG. 2. Iron uptake rate *versus* iron concentration in Aquil for juvenile plant tissue discs.

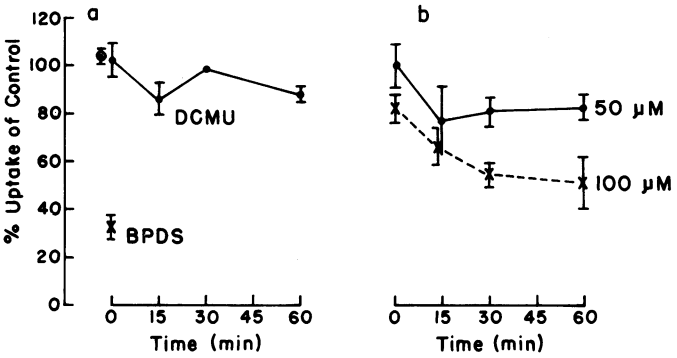

FIG. 3. Effects of darkness and various inhibitors on iron uptake in Aquil by juvenile tissue discs (based on three replicates). a, (●—●) DCMU, 50 μM; × BPDS, 50 μM; ⊙, dark. b, DNP, (●—●) 50 μM, (×—×) 100 μM. The control experiment was performed without inhibitor and in the light; the inhibitor experiments in the light. Brackets indicate mean deviation.

Radioactive solutions and STE were counted by liquid scintillation in Aquasol-2 cocktail (New England Nuclear). Quench correction was determined by ESCR. The maximum counting efficiency obtained was 88.8%.

RESULTS AND DISCUSSION

Labeled iron exchanged relatively slowly from the free space (Fig. 1, A and B). Divalent metals were exchanged from the free space (>90%) of juvenile tissue with 10 μM $PbNO_3$ in Aquil in 20 min while a similar wash required 50 min to remove free space iron (14). The slow exchangeability of iron may reflect the higher affinity of the cell wall and intercellular constituents, alginic acid and "fucoidan," for Fe^{3+}. Mature blade tissues exchanged iron at a slower rate than tissue from immature blades or juvenile plants, perhaps reflecting a change in amounts and composition of alginic acid and fucoidan as well as increased tissue thickness.

Iron uptake *versus* its concentration (Fig. 2) displayed saturation kinetics with a curious inflection over the range of 0.2 to 0.8 μM. The inflection may be an experimental artifact because determinations were replicated only at 1.1 μM. Such a curve, if real, does have the appearance of positive cooperativity suggesting a single multiphasic carrier. A Hanes-Woolf plot of the points above 0.8 μM yielded a correlation coefficient of +0.979, K_m = 2.9 μM and V_{max} = 84.0 pmol·min^{-1}·cm^{-2}. A Woolf-Augustinsson-Hofstee plot of the same points yielded: correlation coefficient, −0.556; K_m, 3.48 μM; V_{max}, 98.7 pmol·min^{-1}·cm^{-2}.

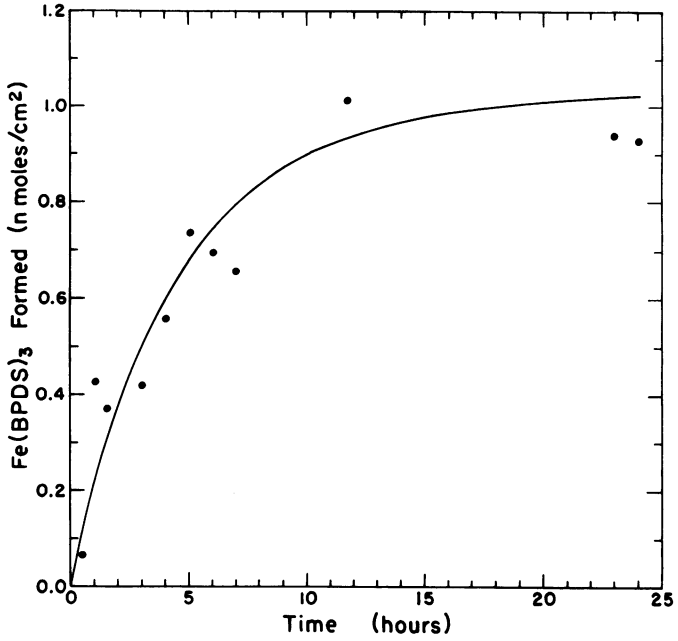

FIG. 4. Reduction of Fe^{3+} EDTA by juvenile tissue discs as measured by the appearance of Fe^{2+} $BPDS_3$ with time. Initial FeEDTA, 350 nM; BPDS, 1 μM.

Table II. *Effect of Inhibitors on Photosynthesis and Respiration by Macrocystis*

Compounds were added in ethanol solution.

Compound	Final Concn	% of Control	
		Photosynthesis	Respiration
DCMU	50 μM	0, 3.8 min	100, 3.8 min
DNP	50 μM	97, 30 min	106, 7 min; 111, 15 min
	100 μM	91, 3.8 min; 59, 1 h	176, 7 min
Ethanol	0.4% (v/v)	100, 3.8 min	105, 7.5 min

Uptake inhibition by BPDS was immediate and drastic (Fig. 3). This effect had been initially described for soybeans and demonstrated the prereduction of Fe^{3+} prior to uptake (3). The formation of $FeBPDS_3$ increased with time (Fig. 4) and demonstrated Fe^{3+} reduction by *Macrocystis* tissue. The addition of BPDS to cultures of *Chlorella sorokiniana* induced chlorosis, inhibited growth, and formed $FeBPDS_3$ (20). The reduction of Fe^{3+} before uptake appears to be a mechanism widely used in the plant kingdom.

The Fe^{2+} formed is in equilibrium with free EDTA and the carrier system (3) but may also be in equilibrium with free space polysaccharides of *Macrocystis*. Fucoidan from *Ascophyllum nodosum* has a high affinity for Fe^{2+} as compared with most of the other divalent metal ions (21). The relative affinity of Fe^{2+} to alginic acid has not been determined although an affinity series for other divalent metal ions has been determined (9). Because Fe^{2+} is readily oxidized to Fe^{3+} in Aquil and could potentially be adsorbed to free space polysaccharides, any Fe^{2+} formed must be rapidly absorbed into the cell.

Iron uptake rates differed for blades of different ages (total Fe, 245 nM): immature, 0.96 ± 0.13 (mean deviation); mature, 2.11 ± 0.04; senescent (basal) 1.50 ± 0.23 pmol·min^{-1}·cm^{-2}. This com-

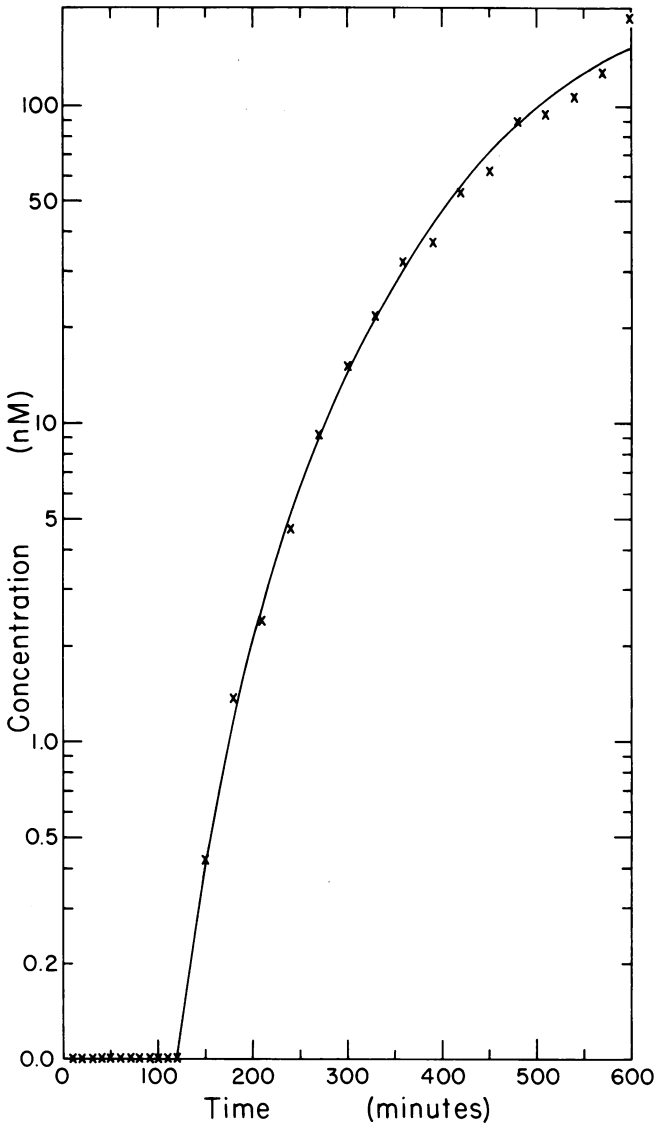

FIG. 5. ^{59}Fe accumulation into STE *versus* time. Initial [^{59}Fe], 13.2 nM in offshore surface water.

pared to 1.83 pmol·min^{-1}·cm^{-2} for juvenile plant tissue. This pattern was similar to that seen for photosynthetic capacity (26).

Iron uptake in the light was not significantly different from that in the dark and was not significantly inhibited by DCMU (Fig. 3). Immediate and complete inhibition of the Hill reaction did occur with the addition of DCMU (Table II). Slight respiratory stimulation occurred with the addition of DNP (Table II) and is consistent with DNP acting as an uncoupler. The partial inhibition of iron uptake by DNP also occurred, increasing with time and with an increase in the DNP concentration (Fig. 3). This effect is probably due to the direct action of DNP on the plasmalemma causing the depolarization of the potential difference across the membrane by shuttling protons across the membrane (6, 10). The potential of the cytoplasm to the outside of a marine algal cell is approximately −100 mv (13). This electrical potential may be greater than the opposing concentration gradient and, therefore, may be the driving force of Fe^{2+} uptake. Iron uptake would thus be described as passive but energy dependent.

Labeled iron was accumulated against a concentration gradient into the STE (Fig. 5). The concentration of ^{59}Fe in the STE eventually become 11-fold more concentrated than the initial solution concentration. The concentration of total iron in the STE

Table III. *Concentrations of Some Inorganic Constituents of STE, as Determined by ICP*

Element	Concentration	
	In STE	In seawater (1)
	μM	
Al	21.7	0.074
As	290	0.050
B	34,900	410
Ba	0.218	0.150
Ca	1,030	10,200
Cd	4.13	0.001
Co	<0.424	0.0008
Cr	0.385	0.0057
Cu	3.79	0.008
Fe	5.10	0.035
Li	4.18	26.0
Mg	4,480	53,200
Mn	1.11	0.0036
Mo	66.4	0.010
Na	19,000	468,000
Ni	2.52	0.028
Pb	2.92	0.0002
Se	44.3	0.0025
Si	11.3	71.0
Sr	6.61	91.0
Zn	17.3	0.076

Table IV. *A Comparison of Growth Parameters, Iron Assimilation, and Iron Uptake for Fronds of Macrocystis*

See text for assumptions used for calculating iron uptake and assimilation.

Productivity, g fresh wt·day^{-1}·frond^{-1}	135[a]	40[b]	14[b]	4.2[b]
Length, m	12[a]	12[b]	5[b]	1.5[b]
Area, m^2	3.8[a]	2[b]	0.5[b]	0.06[b]
% Increase in length	3.6[a]	2.3[b]	4.0[b]	6.3[b]
Iron assimilation, mg·day^{-1}·frond^{-1}	1.05	0.31	0.11	0.03
% Mature blade area, estimated	90	90	60	40
Iron uptake, mg·day^{-1}·frond^{-1}	1.94	1.0	0.23	0.02
Uptake:assimilation	1.80	3.2	2.1	0.67

[a] Based on a single frond, Wheeler 1978 (26).

[b] Calculated from equations based on large sample size, North 1971 (17).

was also far greater than that reported for seawater (Table III). The need for iron translocation is not immediately evident because *Macrocystis* in the ocean presumably resides in a nutrient medium free of a vertical gradient of iron. Uptake apparently supplies more than enough iron for mature but not for juvenile fronds (Table IV). Therefore, translocation to juvenile fronds may be necessary; that is, a source-sink relationship exists. Certain assumptions were made in order to generate Table IV: (a) The concentration of available iron in coastal seawater is 35 nM. The reported values for the total amount of iron (dissolved plus particulate) in seawater vary, which reflects analytical and geographic variation. Sugimura *et al.* (25) reported values of 72 to 108 nM for coastal seawater, half of which was considered dissolved iron. Other reported values are significantly lower, such as: 31 nM (19) and 35 nM (1), the former value having been determined for surface water south of Newport Bay. The species of iron available for uptake by *Macrocystis* is unknown; if only a fraction of the total iron is available (*i.e.*, total dissolved, about 17 nM), then the uptake:assimilation ratios will be lower. (b) The uptake kinetics of

available iron in seawater is similar to the uptake kinetics of FeEDTA in Aquil. (c) Water motion is continually saturating in a kelp bed. A velocity of 3 to 5 $cm \cdot s^{-1}$ is saturating for macronutrient uptake, but such flows may not continually exist in a kelp bed (26). (d) The average amount of iron in kelp tissue, including free space iron, is 78.12 μg Fe·g·dry weight (19).

Net productivity values used (b in Table IV) were based on population averages while uptake rates represented optimal conditions. Individual and seasonal variations certainly exist, and net productivity would be higher under optimal conditions, leading to a lower uptake:assimilation ratio. The iron uptake rate of a frond increases at a faster rate with increased frond length than does the iron assimilation rate because the uptake rate is a function of frond surface area while the assimilation rate is a function of net productivity. Net productivity is defined in terms of fresh weight per day and should not be confused with photosynthetic or gross productivity. Gross productivity does not take into account loss due to respiration, translocation, or tissue damage (26). As a frond grows, its net productivity can decrease and eventually cease while photosynthesis continues supplying material for translocation (17, 26).

Iron translocated in the STE may be chelated with some organic compound. In higher plants, Fe^{2+} is oxidized in the xylem and chelated with citrate in the plant fluid (2, 4). Analysis of [^{14}C]-labeled products of the STE of *Macrocystis* has not revealed the presence of organic acids (23); *Laminaria* STE, however, contains 2% malate (22). Phosphate, aspartate, and glutamate are all present in the STE (23, 24) and are candidates for chelation in *Macrocystis*. Also, the protein present in the STE (1.34 mg/ml, TCA precipitated) may bind and transport iron.

Analysis of the STE revealed the presence of a number of elements, although concentrations may have been lowered by protein precipitated during cold storage prior to analysis (Table III). This protein loss represented 8.3% of the total protein. The concentrations of Mg and Na were significantly lower than the previously reported values (23) and considerably lower than in seawater (1). Concentrations of the micronutrients Zn, Cu, Mo, and Mn were well above reported values for seawater. The high values of Zn, Cu, Cd, Cr, Ni, and Pb may partially reflect their higher concentration in harbor water (27). The binding and/or chelation of the other trace metal micronutrients in the STE may occur. The STE protein, being a polyelectrolyte, may be the important carrier.

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