

Production and role of hyaline hairs in *Ceramium rubrum**

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Abstract

Light intensity within the range of 40 to 210 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ had relatively little effect on the abundance, length or distribution of *Ceramium rubrum* unicellular, hyaline hairs. External NH_4^+ concentrations less than 0.5 μM stimulated hair initiation and growth in apical regions of the thalli. Ammonium concentrations in excess of 20 μM inhibited hair formation on all regions of the thalli. Ammonium uptake velocities of plants with hairs were approximately twice those of plants without hairs. These hairs may be adaptive to take advantage of intermittent bursts of nutrients by increasing the plant's surface area and, hence, the number of nutrient uptake sites.

Introduction

Hairs occur in Cyanophycean, Chlorophycean, Phaeophycean, and Rhodophycean algae (Fritsch, 1935, 1945). "Hair", in the eukaryotic algae, generally refers to a sterile, filamentous outgrowth from the surface of the thallus. Hair formation in the algae is environmentally influenced in those species genetically predisposed to hair formation. Berthold (1882) and Boergesen (1920) postulated that hair development was promoted by high light intensity. West (1971) considered light intensity to be a major factor for the development of hairs in *Acrochaetium proskaueri*. Mshigeni (1976) reported that hair development occurs even under low light intensities in certain red algae. Yarish (1976) suggested that setae in *Bulbocoleon piliferum* were more abundantly produced at low light intensities, whereas in the cases of *Ochlochaete histrix*, *Entochaete taylori*, and *E. ramulosa* they were stimulated by higher light intensities. Shyam and Sarma (1980) found a higher

production of hairs in *Stigeoclonium pascheri* under low light intensity as compared to high light intensity. Gibor (1973) reported that hair growth in *Acetabularia* sp. is light dependent and that hairs are abscised in about ten days if the plants are placed in the dark.

Berthold (1882) and Boergesen (1920) postulated that hairs might serve as a light-screen which protects actively growing areas from illumination damage. Sauvageau (1896) and Rosenvinge (1911) hypothesized that algal hairs function in nutrient absorption. Gibor (1973) demonstrated that sterile whorls of *Acetabularia* sp. absorb and accumulate vital-dyes. He speculated that the whorls increase the surface area of the cell, facilitating the uptake of soluble substances from the environment.

Boney (1959) observed that *Pulmaria elegans* shed the hairs formed in nutrient-poor cultures when it was transferred to nutrient-rich conditions. Transferring *Phaeophila dendroides* from enriched medium into seawater induced hair formation, whereas plants kept in the enriched medium did not form hairs (Nielsen, 1972).

Enhanced hair production in several green and blue-green algae has been attributed to deficiencies in nitrogen (Adamich *et al.*, 1975; Yarish, 1976; Whitton and Harding, 1978; Shyam and Sarma, 1980), phosphorus (Yarish, 1976; Sinclair and Whitton, 1977; Whitton and Harding, 1978), and magnesium, sulfur and calcium (Sinclair and Whitton, 1977; Whitton and Harding, 1978).

Hairs occur near the apical tips of *Gracilaria tikvahiae*, *Agardhiella subulata*, *Hypnea musciformis*, *Ceramium rubrum* and *Gracilariopsis sjoestedtii* (DeBoer and Ryther, 1977; DeBoer *et al.*, 1978; D'Elia and DeBoer, 1978; DeBoer, 1979). The present study investigates the effects of light intensity and nitrogen availability on the formation of hairs in *C. rubrum* and tests the nutrient absorption hypothesis for algal hair function.

Material and methods

Ceramium rubrum (Hudson) C. Agardh was obtained from the waste recycling – polyculture raceways at the Environ-

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mental Systems Laboratory of the Woods Hole Oceanographic Institution (DeBoer and Ryther, 1978). The alga grew detached in the system and as an epiphyte on *Gracilaria tikvahiae*. The plants were isolated and maintained in 1 000-l continuous-flow containers maintained at $16^{\circ}\text{C} \pm 3^{\circ}\text{C}$ in a greenhouse (DeBoer and Ryther, 1978).

Visual observations were made using a Zeiss stereo dissecting microscope or a Zeiss compound microscope fitted with ocular micrometers. Hair length was measured to the nearest 0.1 mm on 25 randomly chosen hairs. Surface area of plants was calculated from photomicrographs of plants with and without hairs.

The apical region of *Ceramium rubrum* is defined as that portion of the thallus extending from the branch tips to the base of these forcipate branches. The subapical region is defined as that region below the apical region extending down to the next branch. The remainder of the plant thallus is termed the basal region.

Growth experiments were conducted in 15-l. continuous-flow chambers (DeBoer *et al.*, 1978). Filtered seawater was pumped through the chambers at a dilution rate of ten culture volumes per day. Water temperature was $15.0^{\circ}\text{C} \pm 1.6^{\circ}\text{C}$ with a salinity of 28 to 31‰ S.

Artificial illumination on a 12hL:12hD photoperiod was provided by Luxor[®] fluorescent tubes. Photosynthetically active radiation (400–700 nm) was measured 1 cm below water surface using an RP-90 Digital Radiometer-Photometer (International Light, Inc., Newburyport, Massachusetts, USA).

Each growth chamber was stocked with 25 g wet wt of *Ceramium rubrum*. Growth was measured by wet weight determinations twice each week. Growth rates were calculated as in DeBoer *et al.* (1978).

Ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) were determined on filtered samples using the methods of Strickland and Parsons (1972). Carbon-nitrogen analysis of lyophilized tissue samples, was conducted with a Perkin-Elmer model 240 Elemental Analyzer. Chlorophyll was measured according to MacKinney (1941).

In the first growth experiment, plants were first maintained in high external nitrogen and phosphorus concentrations and vigorous aeration for three weeks. Following this period the thalli were heavily pigmented and virtually free of hyaline hairs. Plants were then distributed into the 15-l chambers receiving unenriched seawater under one of six irradiance levels ranging from darkness to $210 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Each experimental condition was run in duplicate.

In the second growth experiment, plants were first maintained in $2.0 \pm 1.5 \mu\text{M} \text{NH}_4^+$ for three weeks and then subjected to nitrogen starvation for 3 d. Following this period the thalli had short (0.5 mm) hairs on the apical portions of the thalli and a N/C ratio of 0.142. Plants were grown under a saturating irradiance of $165 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Influent NH_4^+ in the twelve chambers ranged from environmental seawater (ca $0.25 \mu\text{M} \text{NH}_4^+$) to $40.0 \mu\text{M} \text{NH}_4^+$. Each chamber received an enrichment of H_2PO_4^- at a N:P ratio of 8:1.

Ammonium uptake measurements were made by the perturbation method (D'Elia and DeBoer, 1978) using an 8.3-l chamber containing 25.0-g fresh weight of *Ceramium rubrum*. Thalli with and without hairs, but otherwise seemingly identical, were prepared by culturing plants under vigorous aeration in seawater containing ca $0.5 \mu\text{M} \text{NH}_4^+$ for five days prior to uptake measurements. This procedure removed most hairs on subapical and basal portions of the thallus and inhibited, somewhat, their formation in the apical region. The chamber received unidirectional irradiance at the rate of $165 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ from cool white fluorescent bulbs. The chamber temperature was maintained at 15°C . Kinetic coefficients were calculated using the S/V versus S linear transformation of the data following depletion analysis (via segmental linear regression analysis) and correction for a diffusive component (D'Elia and DeBoer, 1978).

Results

Cytological observations

Under conditions of low water movement and low external nitrogen concentrations *Ceramium rubrum* frequently possessed abundant hyaline (colorless) hairs (Fig. 1). These elongate, unicellular hairs attained a length of 6 mm in some instances with lengths of 2 to 3 mm very common. The cylindrical hairs possessed thin walls and a large central vacuole. Cytoplasmic streaming was very active in the young apical hairs but decreased in the basal hairs. Chromatophores occasionally were apparent in short hairs. Nuclei were not observed.

Effect of light intensity on hair formation

Growth rates of *Ceramium rubrum* increased with increasing irradiance up to approximately $120 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Fig. 2). Below $100 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ the plants decreased in biomass. Plants maintained in total darkness died after approximately two weeks. The chlorophyll and nitrogen concentration of the plants remained relatively high in those plants having low growth rates but decreased in plants having higher growth rates (Fig. 2).

By the third day, plants grown under all light regimes possessed short (ca 0.5 mm) hairs on the apical portion of the thalli and a few plants had hairs on the subapical portion of the thalli (Fig. 3). By the seventh day, plants grown under all but the two lowest irradiance levels possessed subapical as well as apical hairs. Plants grown under the two highest irradiance levels possessed hairs on the basal portion of the thalli as well. Hair development on plants grown in darkness was indistinguishable from those grown at $40 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for the first seven days. By the seventeenth day, plants grown in the light possessed hairs on all regions of the thalli with the longest hairs found on the apical regions and the shortest hairs found

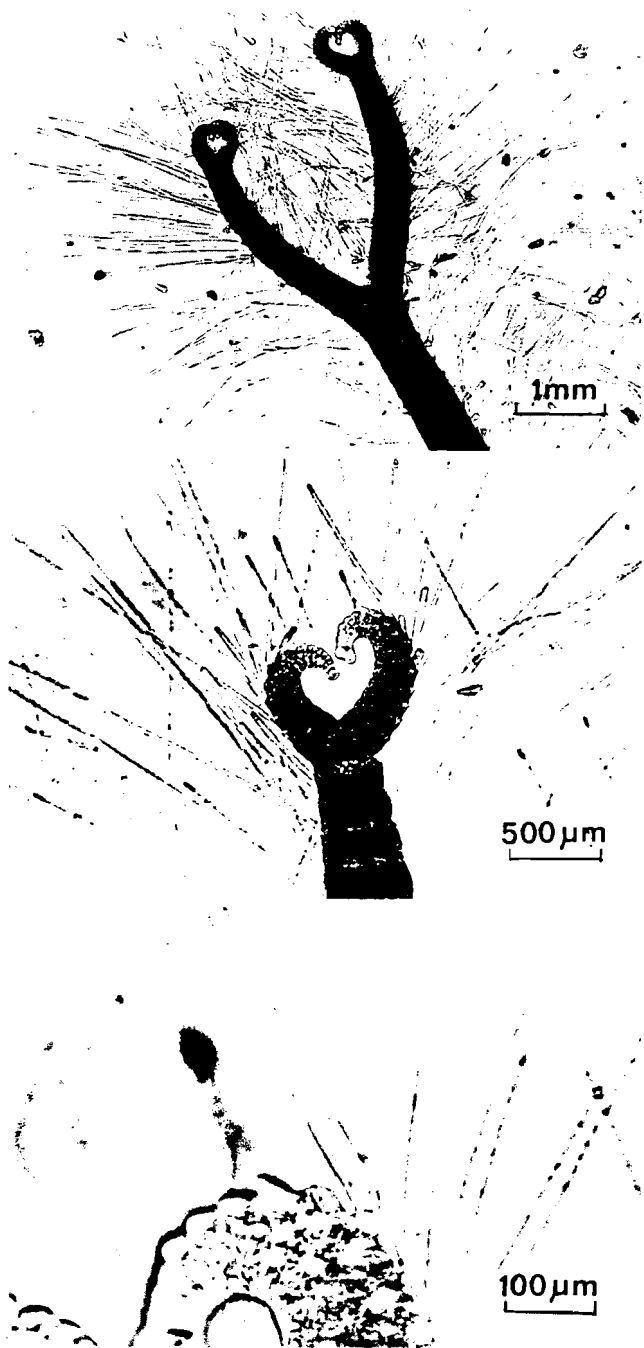


Fig. 1. *Ceramium rubrum*. Hyaline hairs on plants cultured in low N medium

on the basal regions. The frequency of hairs on basal portions was always lower than that on apical regions.

Effect of ammonia concentration on hair formation

Growth rates of *Ceramium rubrum* increased with increasing ammonium concentration up to a residual N level of ca $0.5 \mu\text{M NH}_4^+$. The maximum growth rate was ca $6\% \cdot \text{d}^{-1}$ (Fig. 4). Internal nitrogen and chlorophyll content increased with increasing N concentration up to ca $5 \mu\text{M}$

NH_4^+ with little change at higher external N concentrations (Fig. 4).

By the third day, plants grown at the three lower N concentrations exhibited hair formation on all regions of the thalli, whereas plants grown at the three higher N concentrations possessed hairs only on their apical tips (Fig. 5). Hair development observed on the tenth and twentieth days were similar. Hairs were the most fully developed on plants receiving unenriched seawater. Plants receiving increasingly higher N enrichments had increasingly shorter hairs primarily limited to apical tips. Plants grown at the highest NH_4^+ concentration had lost all hairs.

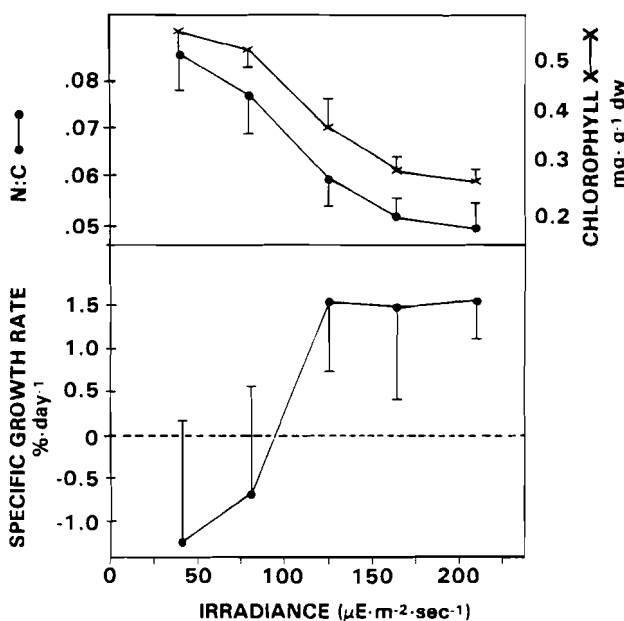


Fig. 2. *Ceramium rubrum*. Growth rate, N/C, and chlorophyll content as a function of irradiance levels. Bars represent 1 SD

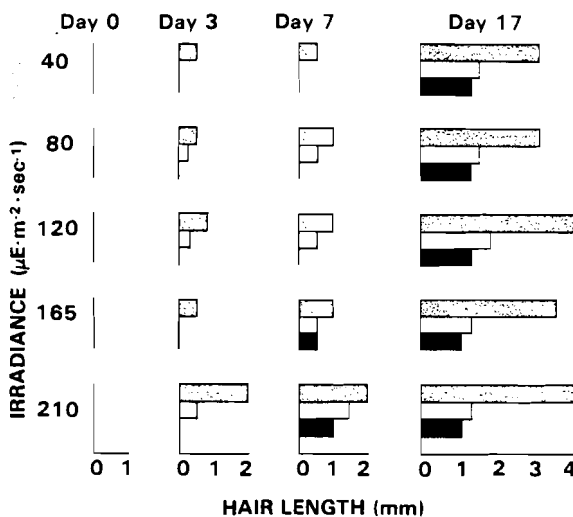


Fig. 3. *Ceramium rubrum*. Mean length of 25 randomly selected hairs in apical (stippled bar), subapical (open bar) and basal (solid bar) regions as a function of irradiance level

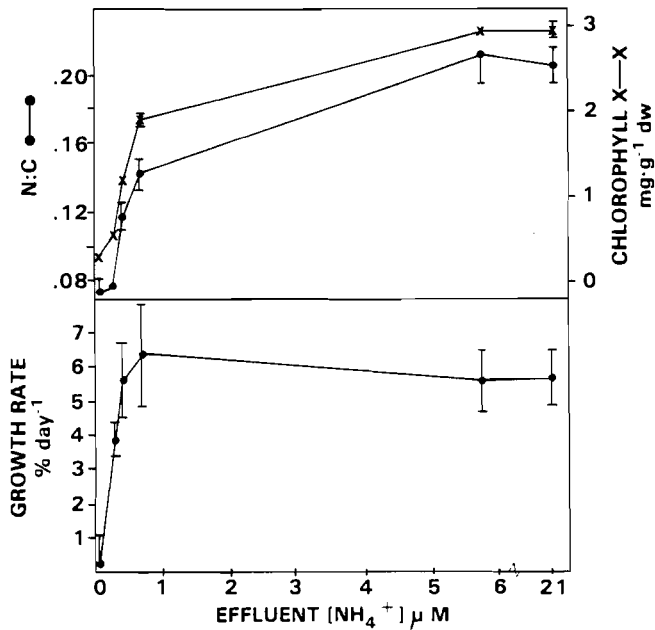


Fig. 4. *Ceramium rubrum*. Growth rate, N/C, and chlorophyll content as a function of NH_4^+ concentration. Bars represent ± 1 SD

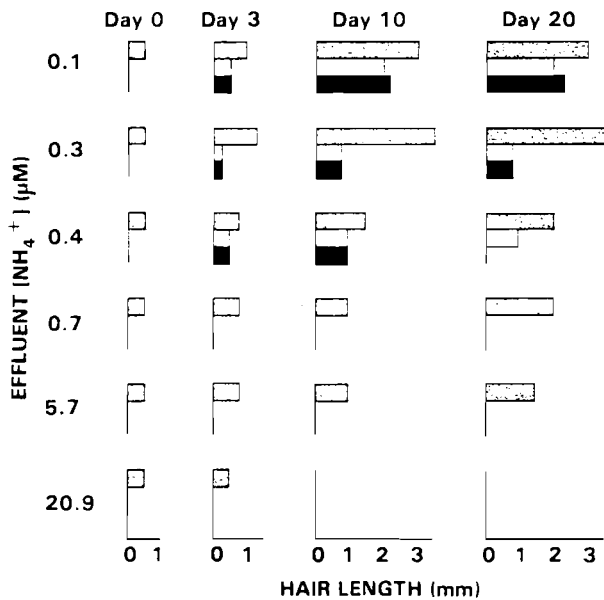


Fig. 5. *Ceramium rubrum*. Mean length of 25 randomly selected hairs in apical (stippled bar), subapical (open bar) and basal (solid bar) regions as a function of NH_4^+ concentration

Ammonium uptake kinetics

Ammonium uptake in *Ceramium rubrum* with and without hairs exhibits unsaturable kinetics at concentrations near $40 \mu\text{M NH}_4^+$ (Fig. 6). The curve is drawn assuming that, below a concentration of ca $10 \mu\text{M NH}_4^+$, a high affinity component (component 1) predominates, while above that value a strong diffusive component (component 2) predominates. The slope of the curve above $10 \mu\text{M NH}_4^+$ gives the value of the diffusive component (K_D). The uptake kinetic coefficients (Table 1) represent those experiments in which the correlation coefficients (r) for K_D and for the S transformations both exceeded 0.90.

K and V_{\max} of component 1 uptake (the low K system) are unaffected by the presence or absence of hairs ($P > 0.05$, Student's t -test). Plants with hairs had K_D values approximately five times as high as plants without hairs ($P < 0.01$, Student's t -test).

Plants with abundant hairs had as much as six times the surface area of plants lacking hairs. Some of the hairs were devoid of protoplasts. Based on live portions of the thallus, we estimate that plants with abundant hairs have 2–3 times the surface area of plants without hairs.

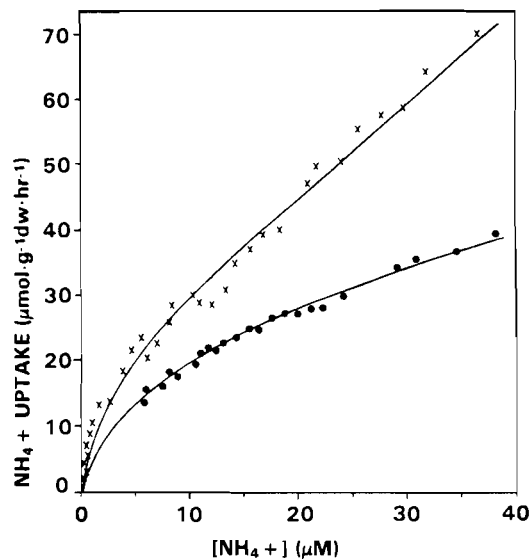


Fig. 6. *Ceramium rubrum*. Nitrogen uptake with (x-x) and without (●-●) hairs. The data correspond to trial no. 4 (with hairs) and trial no. 5 (without hairs) in Table 1

Table 1. *Ceramium rubrum*. The effect of hairs on NH_4^+ uptake coefficients. Half-saturation coefficient (K) is in μM , V_{\max} is in $\mu\text{mol N} \cdot \text{g}^{-1} \text{ dry wt} \cdot \text{min}^{-1}$, K_D is in $\text{l} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ and probabilities in Student's t -tests (NS = not significant; ** = $P < 0.01$) are given for the mean values of groups with and without hairs

| | Trial | | | | | | | | Mean values of trials | | |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-----------------------|-------|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 1-4 | 5-8 | p |
| Hair presence | + | + | + | + | - | - | - | - | + | - | |
| N/C | 0.040 | 0.048 | 0.046 | 0.045 | 0.040 | 0.058 | 0.040 | 0.060 | 0.045 | 0.050 | NS |
| K | 2.2 | 2.5 | 5.5 | 3.9 | 3.2 | 4.5 | 2.9 | 4.2 | 3.5 | 3.7 | NS |
| V_{\max} | 22.7 | 19.2 | 37.7 | 24.3 | 28.3 | 21.0 | 22.8 | 25.7 | 26.0 | 24.4 | NS |
| K_D | 2.9 | 1.4 | 1.9 | 1.9 | 0.3 | 0.5 | 0.5 | 0.2 | 2.0 | 0.4 | ** |

Discussion

Light intensity in the range of 40 to 210 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ had relatively little effect on hair formation in *Ceramium rubrum*. Under steady-state irradiances sufficient to keep the plants alive, the abundance, length and distribution of hairs were unrelated to irradiance under low external nitrogen conditions. The more rapid formation of hairs in plants subjected to high irradiances is probably related to higher metabolic rates. Our data for *C. rubrum* do not support the hypothesis that hyaline hairs function as light shields.

Hair initiation, growth and life span in *Ceramium rubrum* are correlated with external nitrogen concentration. External NH_4^+ levels less than ca 0.5 μM stimulate hair initiation and growth in apical and subapical regions of the thalli. Ammonium concentrations in excess of 20 μM inhibit hair formation on all regions of the thalli and hasten the shedding of hairs previously formed. The frequency of hairs is always highest in apical regions, irrespective of nitrogen concentration. We attribute this to the differential rate of formation and shedding of hairs on apical, subapical and basal portions of the thalli.

Our data support findings (Adamich *et al.*, 1975; Yarish, 1976; Sinclair and Whitton, 1977; Schonbeck and Norton, 1979; Shyam and Sarma, 1980) that deficiencies in nutrients such as nitrogen and phosphorus enhance hair formation and that nutrient concentrations in excess of the plant's needs inhibit new hair formation and may hasten the shedding of hairs.

A two-component model can be used to fit ammonium uptake by *Ceramium rubrum* at external N concentrations less than 40 μM . At concentrations less than 10 μM NH_4^+ a high-affinity uptake system (component 1) predominates and at high concentrations a strong diffusive component (component 2) predominates. The diffusive component may not be "diffusive" but rather a component with a high K and high V_{max} (D'Elia and DeBoer, 1978). Ammonium uptake is undoubtedly much more complex than this and may involve more components at high NH_4^+ concentrations. Nonetheless, this two-component model is useful in comparing NH_4^+ uptake in plants with and without hairs.

The mean values of K (ca 3.6 μM NH_4^+) and V_{max} (ca 25 $\mu\text{mol} \cdot \text{g}^{-1} \text{ dry wt} \cdot \text{min}^{-1}$) for *Ceramium rubrum* coincide with values calculated for other red algae (DeBoer, 1981). Diffusive component (K_D) values for *C. rubrum* (0.41 $\cdot \text{g}^{-1} \cdot \text{h}^{-1}$ without hairs and 2.01 $\cdot \text{g}^{-1} \cdot \text{h}^{-1}$ with hairs) fall within the range of K_D values reported for *Agardhiella subulata* and *Gracilaria tikvahiae* (D'Elia and DeBoer, 1978). The lowest K_D value reported for *A. subulata* (0.31 $\cdot \text{g}^{-1} \cdot \text{h}^{-1}$) was obtained from thalli lacking hairs (DeBoer, unpublished data). Those results were consistent with those obtained with *C. rubrum*.

Our data indicate that component-1 uptake is independent of the presence of hairs, whereas component-2 uptake is enhanced by hair presence (Table 1). We postulate that perhaps the most important function of hairs in *Ceramium rubrum* is to enhance nutrient assimila-

tion when external nutrient concentrations are critically low and/or water motion is restricted (Neushul, 1972). Hairs may function by increasing the number of uptake sites and the turbulence at the cell surface.

We have estimated that the surface area of the live portion of the thalli is increased by a factor of 2 or 3 in plants possessing abundant hyaline hairs. Using mean values, NH_4^+ uptake velocities of plants with hairs exceeded those of plants without hairs by 1.3 to 2.7 at NH_4^+ concentrations ranging from 0.1 to 30 μM .

By increasing turbulence at the immediate cell surface, the diffusion layer would be reduced, resulting in enhanced nutrient uptake especially at constantly low external nutrient concentrations. If this were the function of the hairs, one would expect to find an apparent decrease in K and an apparent increase in V_{max} . Neither were observed in *Ceramium rubrum* for NH_4^+ uptake. Therefore we postulate that hairs function primarily by increasing the number of uptake sites for the component-2 system. The number of sites for component-1 does not appear to increase with the presence of hairs. This suggests separate sites for components 1 and 2. If this reasoning is correct, hairs are probably adaptive to take advantage of intermittent bursts of nutrients.

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