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AN ABSTRACT OF THE THESIS OF Dale Howard Robinson for the Masters of Science in Biology presented August 8, 1983.

Title: The Distribution and Morphology of Fucus distichus in an Estuarine Environment and the Effect of Selected Ions on the Uptake of Inorganic Carbon and Nitrate.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

John G. Rueter Jr., Chairperson

B. E. Lippert

Richard Petersen

The morphology, distribution, and habitat of dwarf and normal forms of Fucus distichus in Nehalem Bay were examined. The dwarf form lacked the holdfast and sexual structures of the normal form and was more highly branched. Examples of the dwarf form were found growing as outgrowths

of fragmented normal forms indicating that both forms are the same species. The normal form occurred attached to rocks near the mouth of the bay in waters of oceanic salinity. The dwarf form occurred as a free-living form in the salt marshes and in waters of lower salinity. These observations suggested that the occurrence of the dwarf form is related to salinity.

Nutrient uptake studies with nitrate and carbon demonstrated that both forms have similar responses to changes in salinity. The dwarf form however, was better adapted to the lower salinities than the normal form. Both forms showed a drop in carbon uptake and a slight rise in nitrate uptake as salinity was decreased, but the dwarf form maintained near maximal carbon uptake rates to a much lower salinity.

It was shown that carbon uptake is sensitive to sodium and potassium ions, and nitrate uptake is sensitive to potassium ions. Reducing the sodium ion concentration by changing the medium composition decreased the carbon uptake rate. This rate was reduced further by the addition of potassium ion. The addition of sodium and potassium specific ionophores to the medium also depressed the uptake rate of carbon. Nitrate uptake was relatively unaffected by decreased sodium concentrations, but was drastically reduced by elevated potassium levels. The potassium specific ionophore valinomycin also produced a significant drop in

the nitrate uptake rate. These data suggested that chemical potentials for sodium and potassium drive the uptake of carbon and that potassium is involved in the uptake of nitrate in *E. distichus*.

THE DISTRIBUTION AND MORPHOLOGY OF FUCUS DISTICHUS
IN AN ESTUARINE ENVIRONMENT AND THE EFFECT
OF SELECTED IONS ON THE UPTAKE OF
INORGANIC CARBON AND NITRATE

by
DALE HOWARD ROBINSON

A thesis submitted in the partial fulfillment of the
requirements for the degree of

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in
BIOLOGY

Portland State University

1983

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of
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CHAPTER I

INTRODUCTION

This investigation examines the euryhaline brown alga Fucus distichus which commonly occurs in estuarine environments (Bold & Wynne, 1978). The study is divided into three parts: 1) an ecological view of the distribution and morphology of varietal forms of F. distichus L. (Powell, 1957), 2) an examination of the physiological basis for the distribution and morphology and, 3) a more specific examination of the mechanisms of nutrient uptake operating in the species.

The significance of such a study has its basis in the importance of understanding estuarine environments. Estuaries are ecotonal areas where fresh water and salt water habitats meet. The productivity and diversity of these areas are high despite the severe and highly variable physical and chemical gradients that occur there. In addition, these regions are quite susceptible to anthropogenic disturbances. To monitor and manage such a complicated and fragile environment it is essential to be able to identify the estuarine habitats as well as to understand the physiological processes and environmental interactions. Consequently, any study which addresses the ecological or

physiological characteristics of organisms which occur in estuaries would be valuable.

A dwarf form of Fucus distichus occurs in some Oregon estuaries which is morphologically distinct from the open coast normal form. The distribution of the two forms suggests that their occurrence is related to salinity. As reported in Chapter IV, a natural system was studied to document the morphological differences between the two forms and to examine the physical evidence which addresses the salinity hypothesis. Observations confirmed the major morphological differences and also showed a correlation between salinity and varietal distribution.

Chapter V investigates the working hypothesis that low salinities hindered the plants ability to acquire nutrients, producing stunted (dwarf) forms, and so providing a physiological basis for the association of dwarf forms and salinity. Uptake studies of the major nutrients carbon and nitrogen examined under varying degrees of salinity did not support this hypothesis. The results showed an adaptation of the dwarf to the lower salinities, lending support to theories to explain dwarfism other than the working hypothesis of salinity induced nutrient limitation.

The results from Chapter V do show a similar change in nutrient uptake as salinity is decreased for both the normal and dwarf forms. However, because lowering salinity affects the concentrations of all of the components of a medium, it

was not possible to determine whether the change in uptake was the result of decreasing the concentration of a single ionic species or a combination of two or more species. Determining which ions cause this uptake effect would be an important step in understanding the mechanism of uptake. In Chapter VI, the major components of seawater were isolated and their effects on nutrient acquisition examined independently through changes made in the composition of a synthetic seawater growth medium. Combined with the use of sodium and potassium ionophores, the results clearly show a dependence on sodium concentration for carbon uptake and indicate an effect of potassium concentration on both carbon and nitrate uptake.

The results of this study characterize some aspects of the physiological and ecological processes occurring in the estuarine environment. Varietal forms of *F. distichus* are shown to be indicators of a particular habitat within the estuary. Physiological responses to salinity stress are studied through the use of synthetic media to produce conditions similar to natural salinity gradients found in estuaries. The mechanisms of uptake of the major nutrients carbon and nitrate are examined as functions of sodium and potassium concentrations. This information on the distribution and physiological responses to salinity is important in understanding the environmental impact of changes on these sensitive environments.

CHAPTER II

BACKGROUND

ENVIRONMENTAL PARAMETERS

The distribution of an aquatic organism is largely controlled by physiological responses to environmental factors. These factors vary in intensities over a range which may effect metabolic processes. The most favorable conditions are generally where the maximum growth occurs. The limits of the growth range are determined by limitation or deficiencies on one side, and excess or toxicity on the other.

The controlling environmental factors can be both physical and chemical. An example of a physical effect would be temperature, which slows metabolic reaction rates if too low, and degrades proteins and other organic molecules when too high. Copper is an example of a chemical factor, being nutritionally required in low concentrations and becoming inhibitory at high levels (Christenson, 1983; Rueter, 1979). In a natural system, complex interactions occur between these factors, and biological influences such as competition and predation further modify the effects.

In an estuary the mixing of fresh and sea waters can produce steep physical and chemical gradients which are much

more severe than those found in the open ocean. Furthermore, these gradients can shift rapidly with the tidal cycle or changes in stream flow. Under such varying conditions the limits of tolerance of many organisms may be exceeded, resulting in a community of organisms in the estuary with the ability to adapt to or tolerate the severe fluctuations (Kirk and Schatz, 1980).

ORGANISM

Fucus is an alga in the division Phaeophyta and the family Fucaceae. The genus, as well as other genera in the family, exhibits a wide range of morphological diversity within a single species. This is evidenced by the five described varieties of Ascophyllum nodosum L. (Gibb, 1957) and the 21 different subspecies of Fucus evanescens Ag. identified by Gardner (1922).

The morphological difference between the varieties range from slightly altered frond form to gross changes in size and shape. Among the most striking intraspecific forms are the free-living dwarfs, which differ greatly from the attached open coast forms. Dwarf forms were first described in the early 1800's (Gibb, 1957) and were thought to be fairly rare. Such forms are now known to occur along the North Atlantic shores of Europe (Gibb, 1957) and North America (Brinkhuis and Jones, 1976), and on the west coast of North America (Lippert, personal communication).

Morphology

Fucus is a macroscopic alga usually found growing attached in the intertidal zone. The thalli of the plants are differentiated into a holdfast, stipe, and frond. The stipe and frond are flattened, with fertile pit-like structures (conceptacles) at the apex of some swollen branches (Bold and Wynne, 1978).

Dwarf forms are generally found in estuarine environments associated with salt marshes. They exhibit a curling thallus which, when compared to the normal form, is highly branched and smaller in both blade width and total length. There is a lack of any attachment holdfast or sexual structures (Munda, 1964).

Possible causes of dwarfism

Reduced salinity has long been thought to be an important factor in the production of a dwarf form of Fucus (Gibb, 1957). Direct observations have shown that the dwarf form is found in areas of low salinity (Munda, 1964). Physiological processes are influenced by the degrees of salinity in the environment. Changes in the chemical composition of Fucus have been noted (Munda, 1964), and decreasing salinity has been shown to cause decreased photosynthesis and increased respiration (Munda and Kremer, 1977).

Many other factors have been hypothesized to effect the dwarf condition, and include nutrient limitation due to

physical barriers, breakage by wave action, and environmental stress. Studies on other brown algae have shown a two thirds reduction in carbon fixation in still waters where a nutrient boundary layer has developed (Wheeler, 1980). The still waters of an estuary may not have sufficient movement to remove a boundary layer, especially when compared to the mixing of waters exposed to the action of waves and currents.

It has been suggested that normal plants are fragmented by wave action and washed into estuaries. Adventitious branching then follows, producing the dwarf form (Brinkhuis and Jones, 1976). The weak anchoring strategy of entangled growth that is employed by the dwarf form would then limit where the plants could develop, and distribution would be a function of a suitable anchoring substrate.

Other work suggests that dwarfism may be a product of intertidal stress. The salt marsh habitat of the dwarf is high up in the estuary and subject to long periods of atmospheric exposure. Brinkhaus and Jones (1976) observed a gradient from one A. nodosum form to another as a function of vertical zonation or tidal exposure. In addition, intertidal brown algae that are exposed to longer and more severe periods of stress exhibit reduced cell size and a dwarf-like growth habit (Liddle, 1975). Bidwell and Craigie (1963) have shown that the ability of fucoids to absorb

carbon when not submerged is greatly reduced, even when the surrounding air was saturated and the plant surface wet. Slower growth rates would result and could account for the highly branched nature of dwarf forms.

NUTRIENTS AND TRANSPORT

Other than the hydrogen and oxygen in water itself, carbon and nitrogen quantitatively are the two most important elements required by brown algae. Their contributions to cell dry weight are around 50% and 10% respectively. In aquatic systems, the molecular species of these nutrients available for uptake are generally in the ionic form. In addition, their normal concentrations found in natural waters are far less than the levels found in the cytoplasm of cells. This presents two problems in the acquisition of these nutrients. The first is the need for a mechanism which can concentrate nutrients inside the cell, and the second is maintaining an electrical balance across the cell membrane.

Uptake of nutrients against a concentration gradient requires a source of energy. The hydrolysis of ATP supplies the energy for the transport of some substances. The pumping of sodium out and potassium in is a good example. No such system has been isolated yet for inorganic carbon and nitrogen compounds in algae, although Falkowski (1975)

has suggested that a chloride dependent ATPase for nitrate uptake may exist.

The energy for many uptake systems is thought to be supplied by metabolically produced chemical gradients across the plasmalemma. For example, the uptake of glucose by the fresh water alga Chlorella is linked to an actively produced hydrogen ion gradient (Komor and Tanner, 1974). In marine systems, it is thought that the driving force for many uptake systems is a sodium potential (Rees et al, 1980). Normally cytoplasmic concentrations for sodium are low relative to the external environment, and the potential energy from this concentration difference could power transport of other substances. Sodium dependent transport has been demonstrated for carbon and nitrogen compounds (Rees et al, 1980) and has been found to be tied to external potassium concentrations (Hellebust, 1978; Wheeler and Hellebust, 1981).

An ion transported into a cell carries with it an electrical charge which could produce an electrical imbalance across the membrane. It is believed that this charge buildup is prevented by the simultaneous influx (symport) of an oppositely charged ion or efflux (antiport) of a similarly charged ion accompanying nutrient uptake (Luttge and Higinbotham, 1979). The uptake systems previously described for sodium, potassium, and chloride

would also be effective in charge balance, and so are further implicated in the uptake of charged nutrients.

Ionophores

Ionophores are small to moderate molecular weight compounds which can bind cations to form lipid-soluble complexes. These complexes can insert into membranes and transport the cations across the bi-lipid layer. The turnover rate for transport is high and the selectivity of an ionophore for a particular ion can be high.

Ionophores have been used in culture work to evaluate the role of ions in nutrient transport and cell metabolism (Hellebust, 1978; Rees et al, 1980; Wheeler and Hellebust, 1981). Additions of ionophores are made to the growth media to destroy concentration gradients by increasing membrane permeability to ions. By using ion specific ionophores, the effect of selected ions can be isolated.

In this study the ionophores valinomycin and monensin were used. Valinomycin is an ionophore which is highly specific for potassium and has an extremely low affinity for sodium. Monensin has a high affinity for sodium and a markedly lower affinity for potassium (Hellebust, 1978). Consequently, the effect of monensin on a membrane would be to allow the passage of a great deal of sodium and a smaller amount of potassium, while valinomycin would pass a great deal of potassium and virtually no sodium.

CHAPTER III

MATERIALS AND METHODS

FIELD WORK

Two collection sites for the dwarf form and two for the normal form of Fucus distichus were located on the shores of Nehalem Bay, Oregon . These sites were selected for the quality and quantity of algal material found and for reasons of accessibility.

Salinity measurements were made at the collection sites twice a month, at high and low tide, from June to September 1981 and 1982. Conductivities were first checked by collecting water samples, warming them to 25°C, and taking readings with a Chemtrix type 70 conductivity meter. These data were verified and checked against salinity using a YSI model 33 salinity, conductivity, and temperature meter. A standard curve was generated to determine salinity from conductivity readings.

Thalli of the normal and dwarf forms were collected during the months of June through September 1981 and 1982. Young plants were selected which were visibly free of epiphytes and frond damage. The plants were rinsed in and transferred to Synthetic Ocean Water (SOW) (Morel et al,

1979) and transported to the lab, where they were once again rinsed and cleaned in SOW.

MEDIA

All media used were modifications of AQUIL (Morel et al, 1979). AQUIL is a chemically defined medium with a synthetic ocean water base to which nutrients, trace metals, and vitamins are added (Table I).

To vary salinity, full strength AQUIL was diluted with deionized, distilled water containing 2×10^{-3} M bicarbonate to retain a constant alkalinity and pH.

To adjust the concentrations of selected ions, changes were made in the AQUIL recipe to produce media that were free of the selected ion. Three modified media were used: one that was chloride free by replacement with sulfate and mannitol, one that was sodium free by replacement with potassium, and one that was sodium free by replacement with mannitol (Table I). By diluting normal AQUIL with a modified medium, the concentration of the selected ions could be varied without changing the other components of AQUIL or the osmotic strength.

When adjustments in the pH were made, the medium was spiked with 10^{-3} M Tris buffer (Sigma) and the pH adjusted with NaOH and HCl. The medium was allowed to equilibrate

TABLE I
COMPOSITION OF AQUIL AND MODIFICATIONS

<u>COMPONENT (mM)</u>	<u>MEDIA</u>			
	<u>AQUIL</u>	<u>Cl free</u>	<u>Na free with K</u>	<u>Na free with Mannitol</u>
Na	480	480	0	0
Cl	560	0	560	560
K	10	10	480	10
SO ₄	29	309	29	29
Mannitol	0	280	0	480
Bromide	.84	same	same	same
Borate	.49	same	same	same
Ca	11	same	same	same
Carbonate	2.4	same	same	same
Co	2.5x10 ⁻⁶	same	same	same
Cu	1.0x10 ⁻⁷	same	same	same
EDTA	5.0x10 ⁻³	same	same	same
F	7.1x10 ⁻²	same	same	same
Fe	4.5x10 ⁻⁴	same	same	same
Mg	55	same	same	same
Mn	2.3x10 ⁻⁵	same	same	same
Mo	1.5x10 ⁻⁶	same	same	same
Phosphate	1.0x10 ⁻²	same	same	same
Sr	6.4x10 ⁻²	same	same	same
Zn	4.0x10 ⁻⁶	same	same	same

for 24 hr and was retested for pH with a glass pH electrode.

ALGAL CUTTING METHOD

Uptake experiments were initially done using cuttings of the vegetative tips of both forms. However, it was determined that this method may produce erroneous results due to a plant response to wounding. Studies on the brown alga Macrocystis pyrifera L. (Wheeler, 1979), and Laminaria longicruris (Hatcher, 1977) have demonstrated a significant decrease in nutrient uptake as a result of cutting as well as an extrusion of large amounts of mucus (Wheeler, personal communication).

Results from cutting method experiments (Figure 1) show varying patterns of carbon uptake from one experiment to the next, while nitrate uptake showed quite constant uptake patterns in both experimental runs (Figure 2). The higher sensitivity of carbon uptake to wounding may be related to mucus release. The mucus is a complex polysaccharide containing a high percentage of carbon and much less nitrogen. It is likely that the production and loss of large quantities of this substance would have an affect on carbon metabolism.

In order to produce results which accurately accounted for nutrient uptake in Fucus, the cutting method was abandoned and whole plants used. However, since results

Figure 1. Carbon uptake *vs.* salinity using the cutting method. Results for the normal (□) and dwarf (Δ) forms are reported as separate plots to indicate the large variation.

Figure 2. Nitrate uptake vs. salinity using the cutting method. Results for the normal (Δ) and dwarf ($\square$) forms are reported as separate plots to indicate the small error.

from the cutting method have been presented, the materials and methods used will follow.

For the carbon uptake experiments, vials were prepared with 20 ml of medium of varying salinities containing 20 μM phosphate, 300 μM nitrate, vitamins and trace metals with EDTA as in the medium AQUIL (Morel et al, 1979) and were allowed to equilibrate for 12 hr. The vials were then spiked with 100 μl of 1 $\mu\text{Ci/ml}$ $\text{NaH}^{14}\text{CO}_3$ and allowed to equilibrate for 1 hr. Uniform cuttings of algal vegetative tips were placed in the vials and incubated in the growth chamber for 24 hr.

In the nitrate uptake experiments, flasks with 50 ml of medium were prepared as in the carbon experiments except that nitrate and radioactive carbon were omitted. The flasks were spiked with 10 μM nitrate and uniform cuttings of vegetative algal tips were added. Incubations were carried out in the same light and temperature conditions as in the carbon experiments. Samples of the media were taken at time 0 and at 24 hr and analysed for nitrate.

The growth chamber consisted of a rectangular container constructed of a colorless, transparent lucite. A water bath of distilled water was circulated through the container and cooled to 18° C by a Braun Frigomix model 1495. Illumination was provided from below by a bank of four "cool white" fluorescent bulbs, providing a photon flux density of 400 $\mu\text{E/m}^2\cdot\text{s}$.

Analysis

Chlorophyll a (chla) content was determined by the method of Strickland and Parsons (1972). Algal tissue was placed in a Wheaton 15 ml glass tissue homogenizer and ground in 90% acetone (American Scientific) with three drops of saturated MgCO_3 solution added. The homogenate was then transferred to calibrated tubes and made to ten ml with 90% acetone. The mixture was extracted for 24 hr at 4° C in the dark, then centrifuged for ten minutes at 1450 x g. The centrifugate was placed in a 12 mm Bausch and Lomb cuvette and read for fluorescence in a Turner model 111 fluorometer using a Turner number 7-60 primary filter and number 2A-15 secondary filter with the normal full range phototube.

The fluorometric method of chlorophyll analysis (Strickland and Parsons, 1972) requires calibration of the fluorometer using a spectrophotometer. This was done by extracting algal samples as described above and determining the fluorescence. The chla content of these same extracts was then calculated using the spectrophotometric method of Strickland and Parsons (1972). Absorbances of each sample were determined at 720, 660, 645 and 630 nm, using a Bausch and Lomb model 100 spectrophotometer in a ten mm Pyrex cuvette. This data was used to calculate the chla content of the sample.

The chla content value was then divided by the fluorescence value and the resulting dividends for all of

the samples were averaged to derive a "door factor" with the units of μg of chl_a/fluorometric unit. A door factor was calculated for each fluorometer door used. Chl_a content could then be measured directly from the fluorometer by multiplying the fluorescence by the door factor. For an additional check these procedures were repeated, with good agreement, using chl_a standards of known concentrations supplied by the U.S. Environmental Protection Agency.

To determine carbon uptake, the centrifugate and pellet from the chlorophyll analysis were recombined, placed in scintillation vials, and evaporated to dryness. Eight ml of scintillation liquid were added (Scintiverse, Fisher) and the activities assayed on a Bechman liquid scintillation counting system. Quench was determined by the H number method. Sample vials were exposed to an internal gamma ray source and the quench was calculated by changes in energy emitted from the vials. Corrections for quench were made automatically by the production of a standard H number curve using standards of known activity.

Nitrate was determined by the method of Strickland and Parsons (1972) but modified for smaller volumes (Rueter, 1979). 25 ml of sample were drawn from the medium and 500 μl of concentrated NH_4Cl were added. 20 ml of sample were then run through a reduction column as a wash and discarded. The final 5 ml were then run through the column and immediately spiked with 100 μl of sulphanilamide (Sigma).

After 3 minutes 100 μ l of n-(1-naphthyl) ethylenediamine dihydrochloride solution (Sigma) were added, forming a blue colored compound. After 10 minutes, color development was complete and absorbance was determined at 543 nm. Standard samples with nitrate at known concentrations were analysed to produce a standard curve.

WHOLE PLANT METHOD

Whole plants were blotted dry and wet weights were taken to 0.001 g using a Sauter 414 analytical balance. The plants were placed in media containing 10 μ M nitrate and 1 ml of 1 μ Ci/ml $\text{Na}^{14}\text{CO}_3$ and incubated for 5 hr. Salinity or individual ion concentrations were varied in the media. The plants were incubated in flasks at 20° C in an Environator environmental chamber illuminated from above by "cool white" fluorescent bulbs providing 400 $\mu\text{E}/\text{m}^2\cdot\text{s}$ of light. The flasks were stirred continuously at 100 RPM by a Fermentation Design rotary shaker. Samples of the media were taken at time zero and at 5 hr and analysed for nitrate concentration. At 5 hr the plants were removed from the media and analysed for ^{14}C .

Analysis

Analysis for ^{14}C was by the method of Lewis et al (1982). The plants were dried for 12 hr at 65° C in a Thelco model 16 incubator, weighed, and then ground in a tissue homogenizer. 2 ml of concentrated nitric acid were

added to solubilize the ground material. After 3 hr, duplicate 0.5 ml aliquots were removed and diluted with 5 ml of 0.75 M tris buffer. 1 ml of the buffered mixture was placed in a scintillation vial to which 10 ml of liquid scintillation cocktail was added. Radioactivity was measured on a Beckman liquid scintillation counting system.

Nitrate analysis was by the method of Strickland and Parson (1972) as in the algal cutting method above.

CHAPTER IV

ECOLOGICAL DISTRIBUTION AND MORPHOLOGY OF FUCUS DISTICHUS IN NEHALEM BAY

INTRODUCTION

A natural system was examined to characterize the habitats, morphologies, and taxonomic relationships of the two furoid forms. Collection of this information was necessary to determine the ecological position of both forms in relation to the environment. Nehalem Bay was selected as a suitable site because both forms occur in abundance on its shores.

Observations within the bay provided information on varietal differences in size and structure, and demonstrated a correlation between the distribution of the two forms and salinity. In addition, visual evidence which supports that the two forms are the same species was found.

DISTRIBUTION

Nehalem Bay is located on the Northern Oregon coast two miles south of the town of Manzanita (Figure 3). The Nehalem River is the major fresh water input, cutting a channel on the southern side of the bay. Large boulders have been placed on the southern shore beginning at a point

Figure 3. Maps showing the collection site locations in Nehalem Bay and the location of Nehalem Bay in Oregon.

near Wheeler Heights (Figure 3) and extending out to both jetties. The northern portion of the bay is a large shallow body of water which, during low tides, often recedes to a mud flat. A salt marsh surrounds this shallow portion of the bay.

The normal form of Fucus distichus is found attached to the boulders on the southern shore starting at a point near Fishery Point and extending out on to both jetties. The quantity of the plants was extensive, totally obscuring the upper portions of the boulders. The sample site for this form was located on the southern shore, 100 m north of the mouth of Messhouse Creek (Figure 3).

Dwarf plants occurred in the salt marshes along the northern shore. Their range extended from a point 1000 m north of Fishery Point to Dean Point (Figure 3). Stabilized dunes surround this area serving as a wind break, producing a sheltered region. The sample site for the dwarf form was located on the Northern shore 1200 m north of Fishery Point.

MORPHOLOGY AND HABIT

The two forms differ greatly in form (Figure 4) and habitat. The normal plant is fastened to the rocks by means of a disc shaped holdfast from which a narrow stipe emerges. Above the stipe is the erect portion of the plant, consisting of flattened blades which are dichotomously branched at regular intervals. The blades are from 1.5 to

Figure 4. Photo of normal (above) and dwarf (below) forms of Fucus distichus at 2/3 actual size.

2.0 cm in width, and show a conspicuous midrib throughout their entire length. Many of the blade apices form rounded, swollen receptacles which produce and hold gametes in fertile conceptacles. An average overall length for the plants is approximately 50 cm.

The dwarf form was found lying prostrate in thick mats, partially covered in mud and entwined amongst the bases of the marsh grasses. The portions of the thallus that were submerged in mud were not living and were partially covered with epiphytes. Compared to the normal form, the dwarfs were smaller, with blade widths of 1 to 2 mm and an overall length of 5 cm, and were more highly branched, not only dichotomously but trichotomously. No midrib is apparent and the plants produce no holdfasts, being held in place by the lattice they weave around the marsh grasses. There was no evidence of the production of receptacles or fertile conceptacles, indicating a lack of sexual reproduction.

Examples of dwarf forms extending from decaying larger Fucus forms with normal form morphology can be found in the marshes. This supports the idea that the dwarf form is the same species as the normal form, and indicates that the dwarfs are propagated by outgrowths of normal form fragments.

SALINITY

Periodic salinity measurements at both collection sites confirm that the dwarf forms occur in regions of lower salinity than the normal forms. At the site where the normal form was collected, salinities ranged from 28-31 parts per thousand (ppt) at high tide and from 26-30 ppt at low tide with averages of 30 ppt and 28 ppt respectively. Readings ranged from 2-36 ppt at high tide to 2-18 ppt at low tide at the dwarf site, with 11 ppt and 8 ppt averages (Table II). The dwarf site showed wider variations and lower average salinity readings than the normal site, indicating a correlation between the occurrence of the dwarf forms and the low salinity of the surrounding waters.

DISCUSSION

Two furoid forms occur in Nehalem Bay which are varieties of the same species, Fucus distichus. The forms show morphological differences in size and structure which give insight into the habit and life cycle of each variety.

The normal form grows attached near the open coast in waters of ocean water salinity, while the dwarf is restricted to a sheltered salt marsh where lower salinities are common. The marsh location may be a limitation of the dwarf morphology, requiring grass stocks with which to intertwine for attachment. Alternatively, the distribution

TABLE II
SALINITY DATA FOR THE DWARF AND NORMAL FORM
COLLECTION SITES

	Dwarf Site		Normal Site	
	<u>Average</u>	<u>Range</u>	<u>Average</u>	<u>Range</u>
Low Tide	8	2-18	28	26-30
High Tide	11	2-26	30	28-31

and/or morphology of the dwarf may have some physiological basis related to low salinity. Regardless of how these factors control distribution, it appears that the occurrence of the dwarf is an indicator of a protected salt marsh habitat and of lowered salinity.

CHAPTER V

SALINITY EFFECTS ON NITRATE AND CARBON UPTAKE IN THE NORMAL AND DWARF FORMS OF FUCUS DISTICHUS

INTRODUCTION

The working hypothesis in this chapter is that in low salinity, F. distichus growth is stunted by a reduced ability to acquire essential nutrients, producing the dwarf form. Field work in Chapter IV and by Munda (1964) has linked the distribution of dwarf fucoids to low salinity regions. In addition, physiological responses have been attributed to salinity stress (Munda 1977). Normal Fucus forms are inhibited by low salinity, demonstrating decreased photosynthesis and increased respiration (Munda and Kremer 1977). Similar growth inhibiting effects in other organisms have been noted in response to low salinity environments (Abdulrahman and Williams 1981).

Nutrient uptake of carbon and nitrate was found not to be inhibited by low salinity in the dwarf form. Uptake experiments carried out in various salinity conditions showed that rates were either unaffected or increased by lower salinity in the dwarf form. Furthermore, a comparison of the dwarf and normal uptake rates showed that the dwarfs

are well adapted to reduced salinity conditions. These results favor alternate theories accounting for dwarfism (adventitious branching and tidal exposure) rather than a physiological response to salinity.

MATERIALS AND METHODS

To vary salinity, AQUIL was diluted with deionized, distilled water containing 10^{-3} M bicarbonate. Dilutions were made to 100, 50, 25, 12.5, 6.3, and 0 percent of the salinity of full strength AQUIL. For each dilution, duplicate flasks with 100 ml of media were poured and equilibrated to the temperature in the growth chamber. Next, 1 ml of 1 $\mu\text{Ci/ml}$ of $\text{NaH}^{14}\text{CO}_3$ and 10 μM of KNO_3 were added, and the solutions mixed thoroughly. Whole normal and dwarf plants were weighed and added to the flasks and the initial samples for nitrate analysis were taken. The flasks were incubated for 5 hr in the growth chamber, then the final samples for nitrate analysis were taken. The contents of each flask were poured into a filter apparatus and washed twice with 3 ml of SOW. The filtrate was saved for analysis. The algal samples were dried and weighed, and analysed for radioactive carbon content.

To determine if extracellular carbon was released into the media, the filtrate was acidified to pH 2 with 1 N HCl and aerated to drive off inorganic carbon. This was neutralized with NaOH and 2 ml of the solution were added to

8 ml of scintillation liquid. The analysis for radioactive carbon was by liquid scintillation counting.

RESULTS

Carbon uptake in the normal form was highest at the control salinity of 100% AQUIL and steadily dropped off as the salinity was decreased (Figure 5) to 52% of control.

The dwarf form also had the highest uptake rate at the control salinity, however this high rate was maintained down to a salinity of 12% of AQUIL salinity before dropping off (Figure 5). The lowest rate was at 0% salinity, with a value of approximately 40% of control. Organic carbon release during the incubation was very near background for both forms over the entire range of salinities (Table III). This demonstrated that extracellular carbon could not account for the change in measured uptake.

In both forms, nitrate uptake was lowest at the control levels, increasing linearly to the highest rate at 0% salinity (Figure 6).

Throughout the entire range of salinities examined, the uptake rates for nitrate and bicarbonate were higher in the dwarf form than in the normal form.

DISCUSSION

The data do not support the working hypothesis that the dwarf form results from nutrient limitation brought on

Figure 5. Carbon uptake vs. salinity for the normal ($\square$) and dwarf (Δ) forms. Each point is a mean value with the range indicated.

TABLE III
RELEASE OF ORGANIC CARBON DURING CARBON
UPTAKE EXPERIMENTS

Salinity	Carbon (cpm/g-hr)	
	<u>Normal</u>	<u>Dwarf</u>
0	19.2	20.7
12.5	18.2	24.9
25	27.9	27.6
50	17.6	23.9
100	22.2	21.2
Back Ground	19.6	23.3

Figure 6. Nitrate uptake vs. salinity for the normal ($\square$) and dwarf (Δ) forms. Each point is a mean value with the range indicated.

by lower salinities. Nitrate uptake rates for both forms increased with lower salinity. Carbon uptake rates did decrease with lower salinities, but not in a manner consistent with nutrient limitation in the dwarf form. The natural salinity range of the dwarf form determined in Chapter IV (Table II) corresponds to a range of 10 to 80‰ of AQUIL salinity. The dwarf form exhibits near maximal carbon uptake rates within this range (Figure 5). These results do not indicate inhibition of the acquisition of carbon or nitrate within the natural salinity range of the dwarf form, instead they show maximal or increased uptake. The results do not rule out inhibited nutrient acquisition brought on by other factors, and suggest that other explanations for dwarfism in fucoids should be examined.

Adaptation to the low salinity environment is evident from the data. In the normal form the carbon uptake drops off with decreasing salinity, however high rates of carbon uptake are maintained in the dwarf at the lower salinities. It is possible that this may be the direct result of the morphology of the dwarf variety. The small, highly branched thallus arrangement increases the surface to volume ratio of the plants, allowing for an increased number of porter or diffusion sites. If this is true, then the morphology would not be the result of a physiological response to the environment as originally hypothesized, but rather the

physiology of uptake would be a consequence of the morphological change.

CHAPTER VI

THE EFFECTS OF SODIUM, POTASSIUM, AND CHLORIDE ON NITRATE AND CARBON UPTAKE IN FUCUS DISTICHUS

INTRODUCTION

Although the dwarf and the normal forms differ in their uptake rates at the same salinity (Chapter V), they do show similar trends in their response to salinity. In both forms carbon uptake decreases with decreasing salinity and nitrate uptake increases. These trends suggest that salinity is acting on both Fucus forms in a similar fashion and indicate that the mechanisms of uptake are the same in both forms.

A simple dilution of the media, as in Chapter V, results in reduced ionic and osmotic strength and reduces the concentration of each ionic species in the medium, making it impossible to determine which factors are affecting uptake. An approach was used in this chapter to single out selected variables by holding the ionic and/or osmotic concentration of the medium constant while changing the concentration of a single component of salinity. By using this modified medium in uptake experiments, the effect

of a single ionic species could be isolated. In addition, the use of ionophores was implemented as an effective means for studying the results of dissipating the chemical potential across the membrane.

The ions selected for the study were sodium, potassium and chloride because of their major contribution to the composition of seawater and their possible importance to nutrient uptake systems. Sodium is the major cation in seawater and chloride is the major anion. Potassium is present in smaller amounts. Sodium and potassium are known to be actively pumped by ATPase systems and the resulting chemical potentials which are generated have been linked to nutrient transport (Hellebust, 1978; Rees et al, 1980). An ATPase has also been suggested which is chloride dependent and which transports nitrate across the cell membrane (Falkowski, 1975).

MATERIALS AND METHODS

Three media were made with altered compositions: 1) chloride was replaced with sulfate and mannitol, 2) sodium was replaced with potassium and 3) to isolate sodium from the effects of potassium, sodium was replaced with mannitol. To vary the selected ion concentrations, AQUIL was diluted with one of the modified media. In each case the selected ion was diluted to 100, 50, 25, 12.5, 6.3, and 0 percent of the concentration found in full strength AQUIL. 100% AQUIL

was the control. For all cases there was a 10^{-6} M sodium contamination from added sodium EDTA and a trace contamination from the sodium bicarbonate radioisotope. Additions of bicarbonate to adjust the alkalinity were in the form of the potassium salt.

For each dilution, duplicate flasks with 100 ml of media were poured and equilibrated to the temperature in the growth chamber for 1 hr. Then 1 ml of 1 $\mu\text{Ci/ml}$ of $\text{NaH}^{14}\text{CO}_3$ and 10 μM of KNO_3 were added and the solutions mixed thoroughly. Whole normal Fucus plants were weighed wet and added to the flasks and the initial samples were drawn for nitrate analysis. The flasks were incubated for 5 hr in the growth chamber and then the final samples for nitrate analysis were taken. The contents of each flask were poured into a filter apparatus and washed twice with 3 ml of SOW. The filtrate was discarded. The algal samples were then dried and weighed, homogenized, and analysed for radioactive carbon content.

The effect of pH was examined with uptake experiments run in media buffered to selected pH values from 6.5 to 9.0, at one half pH unit intervals. Incubation and analysis for nitrate were carried out as in the above experiments.

Two ionophores were used in uptake experiments, potassium specific valinomycin (Sigma) and sodium specific monensin (Sigma). Due to the low solubility of the ionophores in water, they were first dissolved in absolute

ethanol to a concentration of 1 mg/ml. Media concentrations of 1 and 10 µg/ml were accomplished by spiking 100 ml of the medium AQUIL with 1.0 ml or 0.1 ml of the ethanol mixture. Incubation and analysis were carried out as in the above experiments. AQUIL with no ionophores was used as a control.

For each set of ion concentrations or ionophore concentrations, experiments were run in duplicate and the results reported as the average. All results were normalized to wet weight.

RESULTS

Carbon

Reducing the sodium concentration by replacement with potassium produced decreased uptake rates for carbon (Figure 7). The change in the rate is quite linear over the range of sodium concentrations. The lowest rate occurred at 0% sodium concentration and the maximum at the control concentration of 100% AQUIL.

When lowering sodium concentration by replacement with mannitol the rate of carbon uptake decreased, but much less dramatically than with potassium replacement (Figure 7). This drop in uptake was similar to the change observed with decreasing salinity and appeared to be related to similar changes in external sodium concentrations. The control concentration of 100% AQUIL produced the maximum uptake

Figure 7. Carbon uptake vs. sodium concentration of the growth medium. Sodium concentration was altered by replacement with mannitol (□) and potassium (Δ). Each point is a mean value with the range indicated.

rates, and the maximum depression of uptake occurred at the 0% sodium concentration.

Reducing the chloride concentration of the medium had little effect on uptake (Figure 8). Variations in the rate over the span of chloride concentrations were small and showed no observable trends.

Experiments using 1 $\mu\text{g/ml}$ concentrations of valinomycin produced a 50% drop in carbon uptake when compared to the AQUIL control (Table IV). Increasing the ionophore concentration by 10 fold had no additional effect. The use of 1 $\mu\text{g/ml}$ monensin had a significant effect on uptake, lowering rates to 20% of control values. A ten fold increase in monensin had some additional effect, reducing uptake to 11% of control.

Nitrate

Lowering sodium concentration by replacement with potassium dramatically lowered nitrate uptake rates (Figure 9). Values declined hyperbolically from the control levels at 100% AQUIL sodium concentrations to one third of control levels at 0% sodium concentration.

Decreasing the sodium concentration by replacement with mannitol produced a small increase in uptake, with 18% increases over control levels at sodium levels less than 50% of Aquil (Figure 9).

As with carbon, the uptake of nitrate was unaffected by the chloride concentration of the medium (Figure 10).

Figure 8. Carbon uptake vs. CI concentration of the growth medium. Each point is a mean value with the range indicated.

TABLE IV
 PERCENT REDUCTION IN CARBON AND NITRATE UPTAKE
 RATES FROM CONTROL LEVELS USING THE
 IONOPHORES VALINOMYCIN
 AND MONENSIN

	K ⁺ Specific Valinomycin		Na ⁺ Specific Monensin	
	<u>1 ug/ml</u>	<u>10 ug/l</u>	<u>1 ug/ml</u>	<u>10 ug/ml</u>
NITRATE Uptake	50	50	30	36
Carbon Uptake	46	46	80	89

Figure 9. Nitrate uptake vs. sodium concentration of the growth medium. Sodium concentration was altered by replacement with mannitol ($\square$) and potassium ($\triangle$). Each point is a mean value with the range indicated.

Figure 10. Nitrate uptake vs. Cl concentration of the growth medium. Each point is a mean value with the range indicated.

The effect of medium pH over the range of 6.5-9.0 showed a sharp optimum, with maximum uptake occurring at about pH 8. There was a steep decline in uptake on either side of the optimum, with rates falling to zero at pH 7 and 8.7 (Figure 11).

A 1 $\mu\text{g/ml}$ concentration of valinomycin reduced nitrate uptake to 50% of control rates. Uptake dropped to 56% of control rates with a 10 fold increase in the concentration of the ionophore. Monensin concentrations of 1 and 10 $\mu\text{g/ml}$ depressed uptake to 30 and 36% of control respectively (Table IV).

DISCUSSION

A summary of some of the results in this chapter and Chapter V (Table V) shows a sodium dependence for carbon uptake. In each case where the medium sodium concentration was reduced there was a corresponding decrease in the carbon uptake rate. In addition, when sodium was replaced with potassium, the drop in carbon uptake was much more severe than when sodium was replaced with mannitol. This indicated that uptake was sensitive to low external sodium concentrations and high potassium concentrations.

A link between potassium concentration and nitrate uptake is also suggested (Table V). When there was no change or a small decrease in medium potassium concentration, there was little change or a slight increase

Figure 11. Nitrate uptake vs. pH of the growth medium. Each point is a mean value with the range indicated.

TABLE V

A SUMMARY OF THE EFFECTS ON CARBON AND NITRATE
 UPTAKE AS THE SALINITY AND SODIUM
 CONCENTRATIONS ARE DECREASED

	Changing Total <u>Salinity</u>	Sodium Replaced with <u>Mannitol</u>	Sodium Replaced with <u>K</u>
Potassium Conc.	decrease	no change	increase
Sodium Conc.	decrease	decrease	decrease
Carbon Uptake	decrease	decrease	decrease
Nitrate Uptake	increase	no change	decrease

in nitrate uptake. With large increases in the potassium levels there was a dramatic drop in the uptake rate. Unlike carbon, nitrate uptake was independent of sodium concentration.

A requirement for high external sodium concentrations for substrate binding or as a cofactor in the transport reaction (Hellebust, 1978) could explain some of these results, however nutrient transport driven by concentration gradients existing for sodium and potassium across the plasmalemma could account for the observed ion dependence for the uptake of nitrate and carbon. These existing chemical and electrical gradients could serve to drive uptake through sodium cotransport or potassium antiport. The natural state of the cell cytoplasm is a low sodium and a high potassium concentration relative to the external medium. By decreasing sodium levels or increasing potassium levels in the medium, the concentration differences are equalized and the driving force for active uptake is diminished.

The results of an alternate method of dissipating the concentration gradients through the use of ion specific ionophores further support the contention that potassium potentials are linked to the transport of both of these nutrients and that sodium is linked to carbon uptake. The ionophores equalize concentration gradients across membranes by bringing internal concentrations to external levels

rather than the reverse. Valinomycin produced significant drops in both carbon and nitrate uptake, and monensin produced a large drop in carbon uptake but a small drop in nitrate uptake. The reduction in nitrate uptake with the use of monensin appears to indicate a sensitivity to sodium. This may, however, be explained by examining the relative specificities of each ionophore to sodium and potassium. Valinomycin is very specific for potassium and it is likely that most of the effects seen resulted from potassium passage through the membrane. On the other hand, although monensin prefers sodium, it does allow passage of some potassium. Consequently, the depressed nitrate uptake observed with monensin may have been the result of some increase in potassium permeability.

CHAPTER VII

CONCLUSIONS

Fucus distichus is an alga that has the ability to exist in more than one form. Propagation of the dwarf form was observed as an outgrowth of detached normal forms. Consequently, the dwarf morphology is not an expression of a smaller species phenotype, but is rather a single species' response to environmental conditions.

Which conditions cause these morphological changes has not been determined, but it is clear that the dwarf is not a result of stunted growth of the normal form caused by salinity induced nutrient limitation. It was hypothesized in Chapter V that both forms would respond similarly to lower salinities by showing significantly lower uptake rates. The normal form was found to be inhibited by low salinities, as evidenced by decreased uptake rates. The dwarf, however, showed a sustained or greater ability to take up nutrients throughout the lower salinity range. This indicates that the dwarf variety is adapted to the low salinity environment.

From these morphological and physiological studies it should be possible to predict the occurrence of the dwarf in other estuaries. This is supported by other studies which

have noted the occurrence of the dwarf form along most of the west coast of the United States and have found it to be associated with a particular sheltered low salinity salt marsh environment (Lippert, personal communication). This also suggests that it may be possible to watch for salinity changes in the estuarine environment using Fucus forms as an bio-indicator. Periodic evaluation of the dwarf population would reflect the prevailing conditions in an estuary, and would indicate if additional monitoring is required.

This study also showed that the uptake of carbon is dependent on sodium and potassium, and nitrate uptake on potassium alone. The patterns of dependence fit well with the hypothesis that uptake is driven by concentration differences that exist across the cell membrane. These results support the work of Hellebust (1978) which demonstrated sodium and potassium dependent uptake systems operating in marine organisms.

Two directions are suggested as important steps in continuing this research. The first would be experiments following the uptake of ammonia in response to sodium and potassium. Ammonium is a common source of nitrogen for algae and the charged character of the ion would pose similar transport problems as nitrate, but may show different responses due to the opposite nature of the charge. The second direction is to determine how the dwarf form adapts to low salinity environments. One hypothesis is

that the increased surface to volume ratio of the dwarf form allows for increased nutrient uptake. Uptake kinetics experiments over a range of nutrient concentrations, which use both the dwarf and normal forms and which are normalized to plant surface area as well as weight, would help to determine the role of high surface area morphology in nutrient uptake.

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