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Chemical Composition and Physiological Properties of Fucoids under Conditions of Reduced Salinity*

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Abstract

Thallus segments of *Fucus serratus* L. and *F. vesiculosus* L. (Phaeophyceae, Fucales) were transferred into seawater media with a salinity range from 32.65 to 2.25‰ and maintained for at least 2 weeks. Several parameters of chemical composition as well as rates of photosynthetic and respiratory oxygen exchange, ^{14}C-assimilate patterns, and release of ^{14}C-assimilates into the culture medium have been investigated. Compared to controls, in both species dry weight, ash, chloride, and mannitol contents distinctly decline proportionally to reduction of salinity in the incubation media, whereas content of total N (in terms of protein content) remarkably increase. Respiratory O_2-consumption is markedly increased at lower salinities, whereas rate of photosynthetic O_2-evolution shows some depression. Relatively little effects of salinity changes are observed in distribution of photosynthetically assimilated ^{14}C among the major groups of photosynthates. Release of ^{14}C-assimilates into the incubation medium never exceeds 2% of total ^{14}C-uptake, but is stimulated in media of reduced salt content. The results are discussed with emphasis on phenomena of long-term adaptation and osmoregulation in the marine fucoid species.

Introduction

Some species of the Phaeophycean order Fucales, in particular the species of the genus *Fucus*, largely occupy the intertidal zone of northern rocky shores – an environment which is generally characterized by periodic fluctuations of several ecological parmeters during the tidal cycle. On the other hand, some of the Fucales (i.e., fucoids) form extensive plant communities even in estuaries, where the typical marine environment is heavily modified by the salinity factor. Previous investigations have revealed that the chemical composition of fucoids is highly dependent on the salinity of their habitats (Haug and Larsen, 1958a, b; Munda, 1964a, 1967). Differences in the chemical composition between estuarine and marine fucoids include water and electrolyte contents as well as contents of total nitrogen, reducing compounds, assimilatory pigments, and mannitol. Specimens of the genera *Ascophyllum* and *Fucus* transplanted from estuarine to marine habitats and *vice versa* followed the same trend as the original populations (Munda, 1967). Short-term changes were found in the water and electrolyte contents as well as in the accumulation product mannitol. Long-term changes were observed in the total nitrogen, β-carotene, and reducing compounds.

Conspicuous changes in the water and electrolyte contents of certain fucoids in media of different salinities were also investigated under culture conditions (Munda, 1963, 1964b). From the results of these investigations the question arose as to how much the nitrogen contents, besides other chemical parameters, would change under the influence of the salinity factor *in vitro*. Hence, it is the aim of the present study to investigate specific changes in the chemical composition of *Fucus* spp. thallus pieces under conditions of reduced salinity in culture experiments and to correlate the well-documented alterations in composition with some physiological

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and biochemical properties of the plants such as ^{14}C-assimilation and photosynthetic or respiratory oxygen exchange, respectively. For this purpose *Fucus* spp. plants from Helgoland were kept in aerated media of different salinities for various periods of time.

Materials and Methods

Plant Material

Adult plants of *Fucus serratus* L. and *F. vesiculosus* L. were collected in the eulittoral zone of the rocky shores near Helgoland (North Sea) throughout the year. Random samples from the population were chosen for the experiments. The upper branches of the same individuals were divided into approximately 10 g (fresh weight) portions and held in 3 l Erlenmeyer flasks filled with culture media of defined salinities. Controls were kept in normal seawater. The media were prepared by dilution of natural seawater with distilled water. All cultures were continuously aerated and kept at 12°C under constant illumination (1500 to 2000 lux).

Chemical Analyses

After appropriate periods of preadaptation samples were taken for the determination of dry weight, ash, mannitol and total N (expressed as protein content). The specimens were dried at 90°C, and combustion was performed at 400°C for the determination of ash content. Mannitol was quantitatively determined according to the method of Cameron *et al.* (1948). Total N was measured using the routine Kjeldahl procedure. Content of Cl^- was determined argentometrically.

Oxygen-Exchange Measurements

Photosynthetic oxygen evolution and dark respiratory oxygen consumption by preadapted *Fucus* spp. samples were measured polarographically (Clark-type O_2-electrode WTW EO 12) in a thermostatisized plexiglass chamber (1.5 ml). Calculations of oxygen solubility in the different media used were done using the tables of Green and Carritt (1967). Polarographic measurements of photosynthetic oxygen release were also performed for monitoring the vitality of long-term incubated thallus samples.

Photosynthetic ^{14}C-Assimilation

Experiments on photosynthetic ^{14}C-assimilation were run in media of different salinities additionally containing 100 µCi $NaH^{14}CO_3$/50 ml. Further procedures such as sample fixation, extraction, ^{14}C-measurements, and thin-layer chromatographic analysis of the ^{14}C-assimilates were performed as described previously (Kremer, 1975a). For the determination of ^{14}C-assimilate release into the culture medium, the thallus samples were first allowed to photosynthesize ^{14}C from $H^{14}CO_3^-$ for 30 min in a medium of normal salinity (pulse labelling), thoroughly washed, and then post-illuminated in defined media of reduced salt content for 96 h (chase experiment). Aliquots of these media were acidified with 6% $HClO_4$ and directly counted in a scintillation spectrometer for acid-stable content of ^{14}C-compounds.

Table 1. *Fucus vesiculosus*. Changes in chemical composition of summer material following long-term incubation in seawater media of reduced salinity. Duration: 19 days

Media (‰ S)	Dry weight in % of fresh weight	Percent dry weight of: Ash	Mannitol	Protein
31.03[a]	17.45	22.60	14.57	15.67
20.94	15.70	22.50	6.61	15.31
15.53	13.21	18.20	5.81	15.54
10.67	12.50	16.20	5.38	16.31
5.15	10.80	15.900	3.18	17.74

[a]Control samples.

Results

Salinity-Induced Changes in Chemical Constitution

Experiments with *Fucus vesiculosus* were started in July 1975 with material originating from the same thallus in order to avoid individual variation in the chemical constitution (cf. Kremer, 1975 b). Plant material was kept for 19 days in a salinity series ranging from 31.03 to 5.15‰ (Table 1). Analyses of the thallus samples kept in these seawater media revealed a distinct decrease in the dry weight and in the ash and mannitol contents, depending on salinity. On the other hand an increase in the total N (i.e., protein) content was found in samples which were maintained at 10.67 and 5.15‰ S. These findings agree with previous observations from fucoids under field conditions in estuaries (Munda, 1967). The decrease in the total mannitol content of the samples kept at 5.15‰ S was about 11% on a dry weight

Table 2. *Fucus vesiculosus*. Changes in chemical composition of winter material following long-term incubation in seawater media of reduced salinity. Duration: 19 days

Media (‰ S)	Dry weight in % of fresh weight	Percent dry weight of: Ash	Mannitol	Protein
32.65[a]	20.00	22.90	14.57	6.63
22.50	17.14	22.11	11.01	7.57
16.26	17.10	21.37	10.81	8.26
11.23	16.23	19.32	10.13	7.81
5.89	15.55	15.36	8.15	10.83
2.75	14.22	13.01	6.00	7.09

[a]Control samples.

Table 3. *Fucus serratus*. Salinity-induced changes in some chemical parameters following long-term exposure in seawater media of reduced salinity. Duration: 5 days

Media (‰ S)	Dry weight in % of fresh weight	Percent dry weight of: Ash	Cl^-	Protein
32.06[a]	17.15	27.76	3.61	16.45
21.66	17.49	23.43	1.99	15.35
14.02	15.38	22.33	1.92	15.92
10.20	14.41	20.75	1.60	19.00
5.95	14.02	18.71	0.95	19.84
2.73	13.46	18.52	0.80	21.54

[a]Control samples.

basis as referred to the polyol amount in the controls. The corresponding increase in the total N amounted to about 2% (Table 1).

In a further series carried out during December 1975, experiments were run under the same conditions including a further dilution to 2.75% S. In this winter material of *Fucus vesiculosus* the total N was considerably lower than in the fertile summer material, while the mannitol content remained at the same level. Generally, the same trends of changes became evident with decreasing salinities after 19 days exposure (Table 2). The increase of total N was more pronounced, while the decrease in mannitol content was only about one half of that found in the summer material (6% on a dry weight basis) when the same salinity range is considered. In the extremely diluted 2.75% S-medium, the increase in N content was less prominent than from 20.94 to 5.15% S (Table 1), but the mannitol content exhibited a further decrease with dilution, as did the dry weight and ash contents. Similar data on specific deviations from control material yielding the same range of salinity-dependent changes in chemical composition were not only observed in different morphological forms of *F. vesiculosus* (data not presented here), but also in samples of *F. serratus* which was included for comparison. Our interest was first of all focussed on changes in the N content of *F. serratus* (Table 3). The content of N-constituents in this species was prominently increased even over an incubation period of only 5 days by about 5%. Possibly due to the fruiting activity at the time of sampling, the ash content of the thallus pieces was very high. The changes in Cl^- follow the results on electrolyte alterations from previous work (cf. Munda, 1964b).

Oxygen-Exchange Measurements

Randomly selected pieces, cut from thallus branches which were preadapted to various seawater media of lowered salinities for at least 10 days, were investigated for their oxygen exchange potentials in the light and in the dark. Average values (each comprising averages from 5 replicates including deviations of the determination) of these polarographic measurements are compiled in Table 4. It may be seen that in the range between 32.06 and 10.20‰ S, both *Fucus serratus* and *F. vesiculosus* tend to show increasing rates of dark respiration with decreasing degrees of salinity. Increased uptake of O_2 in the dark is more pronounced in *F. serratus* than in *F. vesiculosus*. In both species the respiratory rates are less increased at 5.96‰ S than at 10.20‰ S.

Similarly, there are obvious effects of reduced salinity on photosynthetic O_2-evolution. Compared to the controls, rates of photosynthesis as based on polarographic determinations are lower at reduced salinities. Photosynthetic O_2-release is obviously more heavily affected in *Fucus serratus* than in *F. vesiculosus*. Generally, the respiratory processes seem to be stimulated at lowered salinities, whereas the energy-conserving photosynthetic reactions are depressed: if the relations of respiration and photosynthesis are considered (Table 4), the ratios of both oxygen exchange rates are shifted.

^{14}C-Assimilate Patterns

Thallus samples of *Fucus serratus* and *F. vesiculosus* were allowed to photosynthesize ^{14}C from $H^{14}CO_3^-$ in different media of a salinity series after long-term adaptation. Since polymeric compounds

Table 4. *Fucus serratus* and *F. vesiculosus*. Oxygen exchange of long-term adapted thallus samples in seawater media of different salinities. Values expressed as nmoles O_2/10 min/100 mg dry matter. Average values of 5 replicates and relative deviation

Media (‰ S)	*Fucus serratus*			*Fucus vesiculosus*		
	Respiration	Photosynthesis	Ratio	Respiration	Photosynthesis	Ratio
32.06[a]	240.8 ± 4.3	1449.5 ± 26.0	1:5.7	566.4 ± 10.1	1503.0 ± 27.0	1:2.7
21.66	347.8 ± 6.3	1404.9 ± 25.2	1:3.9	628.9 ± 11.3	1456.9 ± 26.2	1:2.3
14.02	383.6 ± 6.8	1329.0 ± 23.9	1:3.4	613.7 ± 11.0	1409.4 ± 25.3	1:2.2
10.20	374.7 ± 6.7	1119.4 ± 20.1	1:3.0	678.0 ± 12.2	1338.0 ± 24.0	1:2.0
5.95	263.1 ± 4.8	972.3 ± 17.4	1:3.7	593.2 ± 10.6	1329.0 ± 23.8	1:2.2

[a]Control samples.

Table 5. *Fucus serratus* and *F. vesiculosus*. Distribution of photosynthetically assimilated ^{14}C among different groups of assimilates in % of total ^{14}C-labelling after 30 min photosynthesis. Average values of 3 replicates

Media (‰ S)	*Fucus serratus*				*Fucus vesiculosus*			
	Phosphate esters	Amino acids	Organic acids	Mannitol	Phosphate esters	Amino acids	Organic acids	Mannitol
32.06[a]	7.0	23.4	7.8	61.9	6.1	22.0	4.3	67.4
21.66	7.3	22.5	3.8	66.4	5.8	20.8	2.3	71.8
14.02	11.7	30.6	3.7	54.3	6.3	26.2	3.0	64.5
10.20	11.7	24.2	5.3	58.7	11.0	31.8	3.5	53.7
5.95	16.0	29.5	3.0	51.6	13.4	30.2	4.6	51.7
2.25	15.8	25.6	1.8	56.7	12.5	31.8	2.2	53.5

[a]Control samples.

Table 6. *Fucus serratus* and *F. vesiculosus*. Release of photosynthetic ^{14}C-assimilates into culture medium during chase-experiments (30 min photosynthesis in $H^{14}CO_3^-$-seawater and 96 h post-illumination in non-radioactive media of reduced salinities. Average values (dpm/100 mg dry weight) of 3 replicates

Media (‰ S)	*Fucus serratus*		*Fucus vesiculosus*	
	Total ^{14}C assimilated	Acid-stable ^{14}C released	Total ^{14}C assimilated	Acid-stable ^{14}C released
32.06[a]	6.236×10^6	29700 = 0.48%	5.453×10^6	40600 = 0.74%
21.66	8.378×10^6	41800 = 0.50%	4.532×10^6	64200 = 1.42%
14.02	6.352×10^6	63900 = 1.01%	3.743×10^6	58900 = 1.67%
5.95	6.005×10^6	92400 = 1.54%	4.561×10^6	88800 = 1.95%

[a]Control samples.

are ^{14}C-labelled to a negligibly low extent in short-term incubations up to 30 min (cf. Kremer, 1975a), only low-molecular weight assimilates are regarded here.

After extraction and two-dimensional thin-layer chromatographic separation of the soluble compounds, the ^{14}C-assimilates were autoradiographically localized on the chromatograms and each spot was quantitatively counted. The distribution of ^{14}C among the major groups of labelled photosynthates after 30 min photosynthesis is presented in Table 5. In all experiments mannitol is the most strongly ^{14}C-labelled compound, thus proving to be the main accumulation product of photosynthetic carbon assimilation. In both species little variation is observed in percentage of labelling over the range of different salinities investigated: labelling rates generally exceed 50% of total ^{14}C-incorporation, but are somewhat lower under conditions of highly diluted seawater media. Compared to control material, the phosphorylated compounds show a higher labelling percentage after photosynthesis in media of lowered salt content. Such effects have previously been observed in experiments with *Fucus* spp. plants assimilating $^{14}CO_2$ from the atmosphere (Kremer and Schmitz, 1973). Amounts of ^{14}C recovered from amino acids and organic acids are not markedly correlated with the experimental conditions (Table 5).

Release of ^{14}C-Assimilation

Thallus samples of *Fucus serratus* and *F. vesiculosus* which had photosynthesized ^{14}C from $H^{14}CO_3^-$ for 30 min in a natural seawater medium (32.06‰ S) were transferred into non-radioactive media of reduced salinity and further illuminated for 96 h. After this treatment the post-illumination media contained certain amounts of ^{14}C-activity which could be driven out partly as $^{14}CO_2$ by adding 6% $HClO_4$ before counting. The remaining ^{14}C-activity is considered to be due to ^{14}C-labelled organic compounds which have been released from the samples. The rates of release of assimilates into the culture medium are shown in Table 6. On the average, release of assimilates from control samples is <0.5 to <1% of total ^{14}C-incorporation. Depending on salinity reduction, however, this rate is distinctly increased by a factor of 2 (*F. vesiculosus*) or 3 (*F. serratus*). Hence, the release of organic carbon compounds seems not to be a usual physiological feature of fucoids, but may be induced by exposure to low-salinity environments.

Discussion

The salinity spectrum for *Fucus vesiculosus* in natural habitats ranges from seawater to about 2‰ S. Adaptations to estuarine growth conditions are followed by changes in the chemical composition and morphology (Munda, 1967). *F. serratus*, on the other hand, has a narrower salinity spectrum and is usually absent from the innermost estuaries. We might assume that adaptation of fucoids to dilute media in brackish and estuarine habitats represents a non-genetic adaptation which involves immediate as well as long-term changes in the physiological processes and in the structure (cf. Munda, 1964a, b, 1967). The present study yielded additional evidence for such changes (Tables 1-3) and proved that a rather short-term increase of total N takes place in diluted media (cf. Munda, in press).

The remakable changes in mannitol content of the samples investigated may be partly explained by altered respiratory-assimilatory activities (cf. Table 4). This observation is consistent with earlier findings (Hoffmann, 1929; Kesseler, 1962; Ogata and Matsui, 1965; Nellen, 1966). Since it is evident that mannitol in particular serves as a quickly available energy source for respiration (Kremer, 1975a), the shifting of the respiratory:assimilatory ratios must affect the absolute amounts of the hexitol present in the thalli. More intense respiration of mannitol at lower salinities may be overlaid by a second effect: the reduced rates of mannitol biosynthesis, as indicated by a lower percentage of ^{14}C-labelling (Table 5), may also account for decreasing amounts of total mannitol content. Since exudation of organic carbon by marine brown algae, especially under conditions of environmental stress, has previously been demonstrated by Moebus and Johnson (1974), a salinity-dependent release of assimilitory accumulation products such as mannitol may also be taken into consideration (cf. Table 6). The question arises, however, whether these effects are sufficient to explain the whole range of changes in chemical composition. It may be assumed that concomitant fluctuations such as water and electrolyte content are also parameters which influence the reference system, i.e., the dry weight basis chosen in the present study. Therefore, changes in the total N content (protein) might only be apparent.

Fucoids represent a type of macroalgae which are found in a habitat of very different water potentials. In view

of this fact, it seemed likely that various intertidal species of fucoids may be capable of osmoregulation. Mannitol, as an abundant constituent of the soluble, low-molecular weight fraction in the Phaeophyceae, has been especially considered as an osmoregulatory principle (cf. Hellebust, 1976).

Earlier investigations on *Fucus serratus* have shown that there is no effect of the salt content in the incubation medium on the ^{14}C-labelling rate of mannitol (Kremer, 1973), if short-term adapted material is used for the experiments. Provided that a possible osmoregulatory mechanism may be acting via the intracellular pools of this hexitol, distinct effects on rates of mannitol biosynthesis are generally to be expected (cf. Kirst, 1975). Rather uniform labelling percentages of polyols over a wide range of salinities are also observed in short-term adapted segments of *Pelvetia canaliculata* (Kremer, 1976). On the basis of these data and the results of Table 5, we might exclude the role of mannitol in osmoregulation and assume the different contents of mannitol proportional to a salinity series (Tables 1-3) to be due to a variety of indirect factors.

Although numerous experiments have dealt with the phenomena of osmoresistance (e.g. Höfler, 1931; Schwenke, 1958, 1960), there is still little information about long-term adaptations of benthic algae to estuarine conditions or even to diluted seawater media *in vitro* and the underlying physiological and structural changes. Such changes depending on salinity can only be described partly in terms of osmoregulation. Might the specific alterations in chemical composition and physiological behavior therefore be an expression of some kind of passive tolerance rather than progressive adaptation? Nevertheless, this is the biochemical basis which fits the fucoids to an ecologically fluctuating environment.

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Literature Cited

Cameron, M.C., A.G. Ross and E.G.V. Percival: Routine estimation of mannitol, alginic acid, and combined fucose in seaweeds. J. Soc. chem. Ind., Lond. *67*, 161-164 (1948)

Green, E.J. and D.E. Carritt: New tables of oxygen saturation of seawater. J. mar. Res. *25*, 140-147 (1967)

Haug, A. and B. Larsen: Chemical composition of the brown alga *Ascophyllum nodosum* (L.) Le Jol. Nature, Lond. *181*, p. 1224 (1958a)

- - Influence of habitat on the chemical composition of *Ascophyllum nodosum*. Nature, Lond. *181*, p. 1225 (1958b)

Hellebust, J.A.: Osmoregulation. A. Rev. Pl. Physiol. *27*, 485-505 (1976)

Hoffmann, C.: Die Atmung der Meeresalgen und ihre Beziehung zum Salzgehalt. Jb. wiss. Bot. *71*, 214-268 (1929)

Höfler, K.: Hypotonietod und osmotische Resistenz einiger Rotalgen. Öst. bot. Z. *80*, 51-71 (1931)

Kesseler, H.: Beziehungen zwischen Atmung und Turgorregulation von *Chaetomorpha linum* in Abhängigkeit von Salzgehaltsänderungen und spezifischen Ionenwirkungen. Helgoländer wiss. Meeresunters. *10*, 73-90 (1962)

Kirst, G.O.: Beziehungen zwischen Mannitkonzentration und osmotischer Belastung bei der Brackwasseralge *Platymonas subcordiformis* Hazen. Z. PflPhysiol. *76*, 316-325 (1975)

Kremer, B.P.: Untersuchungen zur Biochemie und Physiologie von Mannit in benthischen marinen Algen, Dissertation, Bonn 1973

- Mannitmetabolismus in der marinen Braunalge *Fucus serratus*. Z. PflPhysiol. *74*, 255-263 (1975a)
- Physiologisch-chemische Charakteristik verschiedener Thallusbereiche von *Fucus serratus*. Helgoländer wiss. Meeresunters. *27*, 115-127 (1975b)
- Distribution and biochemistry of alditols in the genus *Pelvetia* (Phaeophyceae, Fucales). Br. phycol. J. *11*, 239-243 (1976)
- and K. Schmitz: CO_2-Fixierung und Stofftransport in benthischen marinen Algen. IV. Zur $^{14}CO_2$-Assimilation einiger litoraler Braunalgen im submersen und emersen Zustand. Z. PflPhysiol. *68*, 357-363 (1973)

Moebus, K. and K.M. Johnson: Exudation of dissolved organic carbon by brown algae. Mar. Biol. *26*, 117-125 (1974)

Munda, I.: Kulturversuche mit *Ascophyllum nodosum* (L.) Le Jol. und *Fucus vesiculosus* L. in Medien von verschiedenem Salzgehalt. Botanica mar. *5*, 84-95 (1963)

- Observations on variation in form and chemical composition of *Fucus ceranoides* L. Nova Hedwigia *8*, 403-414 (1964a)
- Water and electrolyte exchange in the brown algae *Ascophyllum nodosum* (L.) Le Jol., *Fucus vesiculosus* L. and *Fucus ceranoides* L. Botanica mar. *6*, 158-188 (1964b)
- Der Einfluß der Salinität auf die chemische Zusammensetzung, das Wachstum und die Frukti-

fikation einiger Fucaceen. Nova Hedwigia *13*, 471-508 (1967)
- Differences in amino acid composition of estuarine and marine fucoids. Aquat. Bot. (In press)

Nellen, U.R.: Über den Einfluß des Salzgehalts auf die photosynthetische Leistung verschiedener Standortformen von *Delesseria sanguinea* und *Fucus serratus*. Helgoländer wiss. Meeresunters. *13*, 288-313 (1966)

Ogata, E. and T. Matsui: Photosynthesis in several marine plants of Japan as affected by salinity, drying and pH with attention to their growth habitats. Botanica mar. *8*, 199-217 (1965)

Schwenke, H.: Über die Salzgehaltsresistenz einiger Rotalgen der Kieler Bucht. Kieler Meeresforsch. *14*, 11-22 (1958)
- Neuere Erkenntnisse über die Beziehungen zwischen den Lebensfunktionen mariner Pflanzen und dem Salzgehalt des Meer- und Brackwassers. Kieler Meeresforsch. *16*, 28-47 (1960)

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