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Ecological and physiological aspects of some coralline algae from the Western Baltic. Calcium uptake and skeleton formation in *Phymatolithon calcareum**

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Abstract

A coralline alga from the Baltic (Belt Sea, Samsö) belonging to the genus *Phymatolithon* has been investigated. The mineral skeleton consists of magnesium calcite and shows the following composition (expressed in percent of the dry weight): 94–96% skeletal carbonates, 4–6% organic matter, 32.1–33.4% Ca, 3.3–3.5% Mg and 0.15–0.17% Sr. MgCO_3 determined by the peak shift method (X-ray diffraction) is in the region of 10–11 mol %. The kinetics of ^{45}Ca uptake consist of a fast step and a low step. The fast step is due to equilibration of isotope with the soft tissues and spaces between cells. The slow step is attributed to net deposition of calcium in the skeleton. From the rate constant of the slow step calcium net deposition was found to be $5.6 \mu\text{g Ca/g dry weight/h}$ or $14 \mu\text{g CaCO}_3/\text{g dry weight/h}$. Pulse chase experiments show that the calcification is the resultant of calcium exchange between the alga and the seawater. Light-dark calcification ratios are in the range of 1.1–1.3. The O_2 production amounts to $0.04 \text{ mg O}_2/\text{g dry weight/h}$ at an irradiance of 0.085 KW/m^2 . When expressed per unit weight of total organic matter, this assimilation rate would fall into the range commonly found for other noncalcifying Rhodophyta. The results form the basis for further work on calcification mechanism and CaCO_3 production in coralline algae.

Zusammenfassung

Ökologische und physiologische Aspekte einiger korallinen Algen aus der Westlichen Ostsee. Kalziumaufnahme und Skelettbildung von *Phymatolithon calcareum*

Eine koralline Rotlage der Gattung *Phymatolithon* aus der Ostsee (Beltsee, Samsö) wurde untersucht. Das Algenskelett besteht aus Magnesiumkalzit und hat die folgende Zusammensetzung (in Prozent bezogen auf das Trockengewicht): 32.1–33.4% Ca, 3.2–3.5% Mg und 0.15–0.17% Sr. Aus der Röntgendiffraktion ergibt sich ein MgCO_3 -Gehalt von 10–11 mol %. Die ^{45}Ca -Aufnahme erfolgt in 2 Schritten: schnelle Absättigung organischer Teile und interstitieller Räume gefolgt von langsamer ^{45}Ca -Zunahme im Skelett. Die Kalziumnettoabscheidung wurde aus der 2. Geschwindigkeitskonstanten zu $5.6 \mu\text{g Ca/g Trockengewicht/h}$ bzw. $14 \mu\text{g CaCO}_3/\text{Trockengewicht/h}$ ermittelt. Die hohen ^{45}Ca -Auswaschraten zeigen, daß die Calcifi-

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zierung des Algenskeletts nach dem Prinzip des Calciumaustausches erfolgt. Das Verhältnis der Licht/Dunkel Calcifizierungsraten liegt im Bereich von 1.1–1.3. Die Sauerstoffproduktion, gemessen bei einer Lichtintensität von 0.085 KW/m², beträgt 0.04 mg O₂/g Trockengewicht/h. Wenn man den geringen Gehalt an organischer Substanz berücksichtigt und die Assimilationsrate auf g Trockengewicht organischer Substanz umrechnet, findet man Werte, die in der gleichen Größenordnung liegen, wie sie bei nicht-calcifizierenden Rotalgen gefunden wurden. Diese Ergebnisse sollen weitere Arbeiten über den Calcifizierungsmechanismus und über CaCO₃-Produktion ermöglichen.

Introduction

The role of coralline red algae as carbonate producers is well recognized (MILLIMAN, 1974). Rates of CaCO₃ deposition are known for a few tropical species (GOREAU, 1963; ADEY & VASSAR, 1975), but little information exists for representatives from the boreal regions, none from the Baltic Sea. The mechanism of algal calcification and the interrelationship of the central reactions of carbonate, i. e. skeletogenesis, assimilation and respiration is also essentially unknown. The deposition of CaCO₃ occurs exterior to the algal cell wall in the middle lamella and intracellularly within the cell wall and the cytoplasm (FLAJS, 1977). Metabolic processes affecting Ca⁺⁺ and CO₃⁻⁻ concentrations may influence or regulate calcification and synchronize skeleton formation with cellular growth. Whether or not metabolic CO₂ is in fact utilized in the calcification, process is not clear at present. Heavy carbon isotope data (CRAIG, 1953; KEITH & WEBER, 1965) remain inconclusive. From the existing theories of algal calcification, i. e. CO₂ utilization, bicarbonate usage, organic matrices, and phosphate removal (for review see BOROWITZKA, 1977) it appears that none have been tested for *Phymatolithon*. Inorganic processes may also be involved. In coralline red algae these can occur in conjunction with cell degeneration leading to the formation of secondary precipitates within the cytoplasm (BAILEY and BISALPUTRA, 1970) and independent of metabolic activity by cementation as a result of diagenesis in the deeper part of the living alga (ALEXANDERSSON, 1974).

An interesting condition exists in the Baltic Sea in that the CaCO₃ saturation is generally low and that salinities fluctuate (WEFER, 1976). It is also noteworthy that *Phymatolithon calcareum* is one of few coralline algae occurring in the Baltic, growing at a depth range of 13 m near the light compensation point, where salinities are constant and where CaCO₃ dissolution has often been shown to occur (WEFER, 1976). Following an assessment of the elementary composition of *Phymatolithon calcareum* we have determined ⁴⁵Ca uptake and O₂ production under various conditions with the view to establish basic physiological data and to study the interrelationship between calcification and carbon metabolism.

Materials and Methods

The algae were dredged from 13 m in the Belt Sea, east of Samsö. The salinity at 13 m was 30‰. The alga is shown in Fig. 1 and has been identified as *Phymatolithon calcareum* (Pallas) nov. comb. (ADEY & McKIBBIN). Chemical analysis was as indicated in Table 1. Oxygen was determined by the WINKLER method and electrochemically in an open flow through system (see KING & SCHRAMM, 1976) The algae were incubated in 50 ml powder jars with approximately 1 µCi ⁴⁵Ca as the

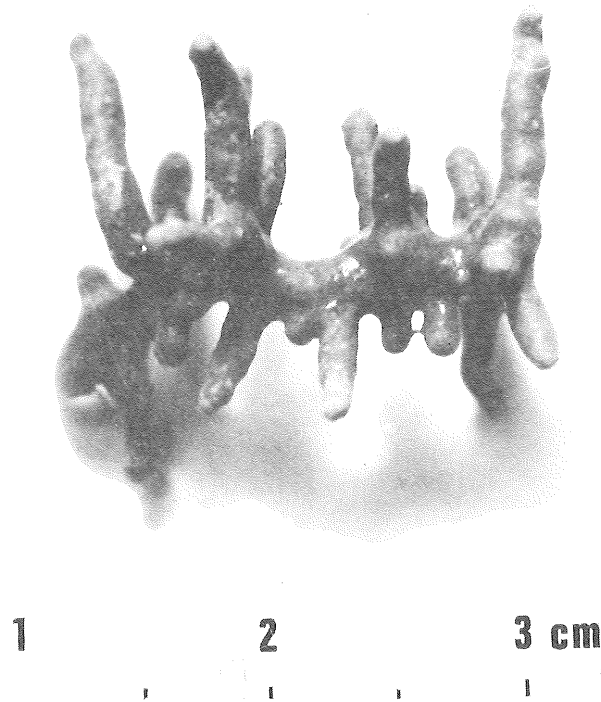


Figure 1
Phymatolithon calcareum from the Western Baltic (Samsö)

chloride (AMERSHAM) at 10°C and illuminated with fluorescent light at an irradiance of 0.085 KW/m² in a light thermostat. Sampling of seawater and rinsing of algae was carried out observing the usual precautions (BÖHM, 1978). Radioactivity in the seawater and in the skeleton was determined as previously described (BÖHM & GO-REAU, 1974) except that Minisolve (ZINSER, 6 Frankfurt 50, Postfach 501151, FRG) was employed as the cocktail.

Results and Discussion

The inorganic and mineral composition of *Phymatolithon calcareum* is presented in Table 1. The results are well within the range commonly found for coralline algae and show close agreement with data for crustose *Lithothamnium sp.* as compiled by MILLILMAN (1974). Figures given here for total skeletal carbonate content and for organic carbon are useful additional parameters which facilitate comparison of results among species.

Fig. 2 shows that light saturation of *Phymatolithon calcareum* occurs at an irradiance of ~ 0.1 KW/m². Short term experiments also showed that assimilation continues at maximum rates at irradiance levels of up to 0.4 KW/m². It is interesting to note that other noncalcifying red algae from Kiel Bay, e. g. *Dumontia incrassata* and

Table 1Elementary composition of *Phymatolithon calcareum* in per cent of dry matter

Skeletal carbonates ^{a)}	Organic matter ^{b)}	CO ₃ -C ^{a)}	Organic-C ^{c)}	Water
94–96 Mineralogy ^{e)}	4 – 6 Ca	11,6–11,9 MG	1,86– 1,96 MgCO ₃	10 –12 ^{d)} Sr
Magnesium Calcite	32,9–33,4 ^{f)} 32,1–32,7 ^{g)}	3,3– 3,5 ^{g)}	11,4 –12,1 ^{j)} 10 –11 ^{h)}	0,15– 0,17 ^{g)}

a) volumetric as CO₂ and assuming the Ca, Mg and Sr are present as carbonates only

b) by difference

c) wet oxidation

d) in per cent of wet weight

e) x-ray diffraction, Cu, K α

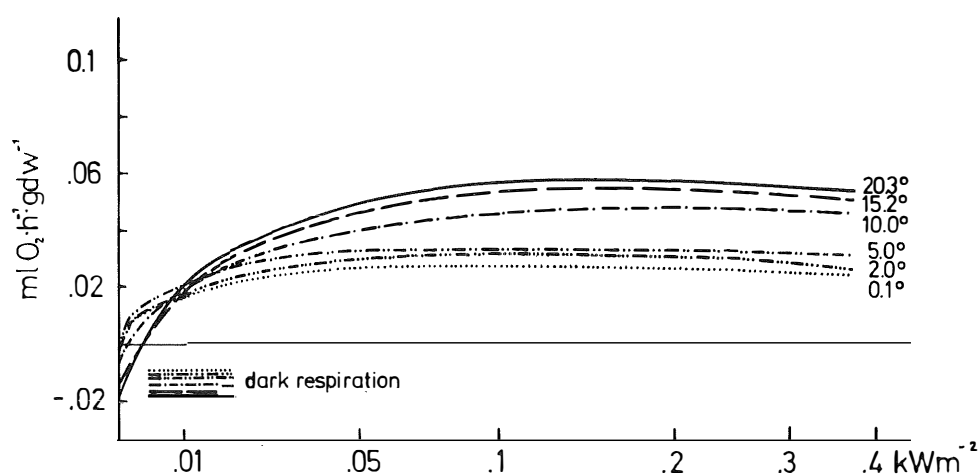
) EGTA – Titration

*) AAS

h) in mole per cent by peak shift method (x-ray-diffr.)

j) in weight per cent from Mg-determination

Porphyra leucostica also show light saturation at $\sim 0.1 \text{ kW/m}^2$ (KING & SCHRAMM, 1976). This is the more remarkable as these algae populate principally the intertidal and subtidal range, whereas the occurrence of *Phymatolithon calcareum* appears to be restricted to the subhalocline region (von WACHENFELDT, 1975). From this

**Figure 2**

Rates of O₂ exchange in ml O₂/g dw/h at various light intensities and temperatures in *Phymatolithon calcareum*, Samsö (Western Baltic) 30‰ salinity. For methodological details see King & Schramm (1976)

it seems that the distribution of *Phymatolithon calcareum* in the Baltic Sea is controlled by salinity rather than by light. ¹⁾ Temperature has also been found to be a controlling factor (ADEY & Mc KIBBIN, 1970). In view of the high light tolerance and the low light/dark calcification ratios of this alga (see below) we felt that it was safe to conduct the ⁴⁵Ca-isotope incubations at an irradiance of 0.085 KW/m² which is near the saturation light intensity. Irradiance at the surface at noon and under clear sky in this part of the Baltic 55°N) may reach values of approximately 0.5 KW/m² and may be as low as 0.05 KW/m² on cloudy days at noon in winter (DIEKMANN, HAARDT and PETERSOHN, 1976).

The kinetics of ⁴⁵Ca uptake (Fig. 3) is similar to that in other calcareous algae (BÖHM, 1978; BÖHM and SCHRAMM, 1977) consisting of a fast step and a slow step. The fast step is due to equilibration of isotope with the soft tissues and spaces bet-

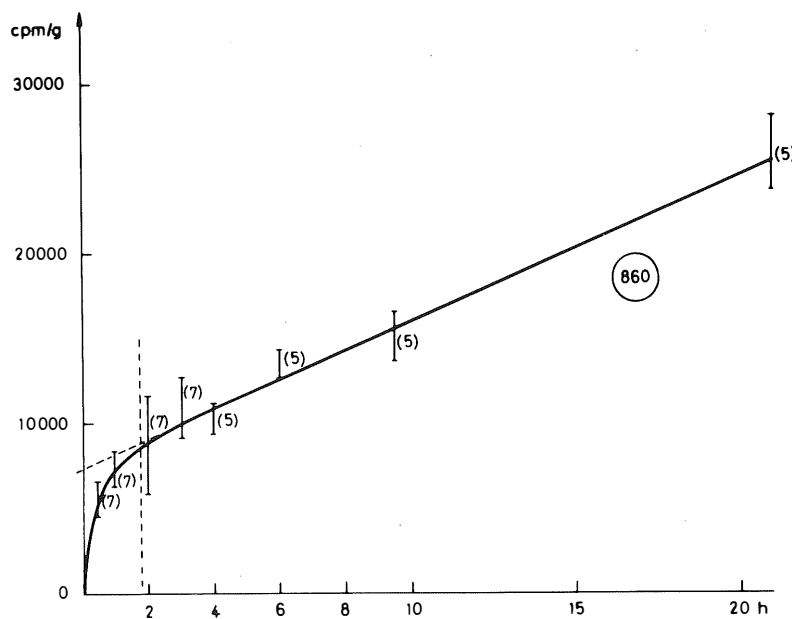


Figure 3

Time course of ⁴⁵Ca uptake in *Phymatolithon calcareum*. The circled number represents the rate constant for the linear part of the graph. All determinations were made in seawater which had a specific activity of 154 200 cpm/mg Calcium. The amount of radioactivity taken up by the alga was determined on different individuals using 10–15 mg portions of the tips and is expressed as cpm/mg algal dry weight. All counts were normalized to the 90% efficiency level. Number in brackets refer to the number of determinations made at each time interval. The Calcium net uptake calculates from the ratio 860/154 200 = 0.0056 mg Ca/g algal dry weight/hour and represents an average value

¹⁾ Measurements in Mannin Bay on the West coast of Ireland have shown that *Phymatolithon calcareum* occurs to a depth limit of 16 m where the prevailing irradiance is as high as 30% of the irradiance at the surface (BOSENCE, 1977). The importance of light and temperature as factors controlling the depth and geographic distribution of coralline algae in the northern Atlantic has previously been discussed (ADEY, 1970, ADEY & MCKIBBIN, 1970 and ADEY & ADEY, 1973). The outcome of our studies on the light, temperature and salinity tolerance of *Phymatolithon calcareum* from the Baltic Sea (Samsö) will be given elsewhere (SCHRAMM & BÖHM, 1978)

ween cells. The slow step is due to net deposition (see ref. BÖHM, 1978 for further discussion). From the rate constant of the slow step and the specific activity of the seawater the Calcium net deposition has been found to be $5.6 \mu\text{g Ca/g/h}$ or $14 \mu\text{g CaCO}_3/\text{g/h}$. Assuming a cylindrical shape of the skeletal branches, the apical branch elongation calculates to $0.5\text{--}2.7 \text{ mm/year}$. By planimetry, crusts of arctic and boreal shallow water corallines have been found to expand at a rate of $2.4\text{--}3.6 \text{ mm/year}$ (ADEY and VASSAR, 1975) whereas branches of articulated tropical species (*Neogoniolithon*) grow at a rate of $12\text{--}23 \text{ mm/year}$. This shows that the ^{45}Ca -method compares favourably with mechanical methods. Pulse-chase experiments show that $40\text{--}65\%$ of the isotope is returned to the seawater by live algae and only $7\text{--}12\%$ in dead algae, depending on the labelling time.

It therefore appears that calcification in *Phymatolithon* is by calcium exchange. Calcium exchange between seawater and alga was first demonstrated for the Chlorophyte *Halimeda opuntia* (BÖHM and GOREAU, 1973) and has since been shown to occur in other species of *Halimeda* (BOROWITZKA and LARKUM, 1976) and other calcareous algae (BÖHM, 1977; BÖHM and SCHRAMM, 1977). From the rate of ^{45}Ca incorporation it can be concluded that CaCO_3 deposition in *Phymatolithon* proceeds in the light and in the dark showing light/dark calcification ratios in the range of $1.1\text{--}1.3$. This is in agreement with the results of GOREAU who gives a mean ratio of 1.6 for various coralline algae (GOREAU, 1963).

The mean O_2 production in *Phymatolithon calcareum* amounts to $0.04 \text{ mg O}_2/\text{g/hour}$ at an irradiance of 0.085 KW/m^2 . Assuming an organic carbon concentration of $30\text{--}40\%$ in the organic dry matter this would correspond to an assimilation rate of approximately $1 \text{ mg O}_2/\text{g/h}$ and is well within the range commonly found for other noncalcifying Rhodophytes from the Baltic Sea at this light intensity (KING and SCHRAMM, 1976).

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