FACIES	26	81-102	Taf. 19-20	7 Abb.	2 Tab.	ERLANGEN 1992

The Biology of Carbonate Precipitation by Cyanobacteria

Martina U. E. **Merz**, Marburg

KEYWORDS: CYANOBACTERIA – *SCHIZOTHRIX, SCYTONEMA* – CALCIFICATION – STABLE ISOTOPES – FRESHWATER CARBONATE – EVERGLADES (FLORIDA) – RECENT

CONTENT

SUMMARY

In the freshwater areas of the Everglades, Florida, U.S.A., carbonate is precipitated in dense cyanobacterial mats. Precipitation is linked with photosynthesis in the mats in a quantitative relationship.

On ground of field observations and experiments a model for precipitation in the filamentous cyanobacteria *Scytonema* is proposed, which links precipitation to bicarbonate use in photosynthesis and subsequent release of OH^- ions.

Besides supersaturation of the water with respect to carbonate and photosynthetic bicarbonate use, precipitation requires a suitable sheath structure and composition. The characteristics of the sheath seem to be responsible for a distinct crystal morphology in the two genera *Scytonema* and *Schizothrix*, as well as for the restriction of calcification to the outer sheath in *Scytonema*. In the immediate vicinity of the trichom precipitation seems to be inhibited.

Comparison of this form of calcifying cyanobacteria with calcification in calcareous algae shows many similarities and rises the question of the biological significance of calcification or precipitation.

The precipitated carbonate shows equilibrium precipitation in its δ oxygen values, while it is enriched in ^{13}C relative to the ambient water. This agrees with a model of precipitation in which the carbonate derives from the water immediately surrounding the filament. There the water is depleted in ^{12}C which is preferably taken up for photosynthesis. No respiratory carbon is involved in precipitation.

From measurements of the amount of precipitation in the field and in experiments the annual sedimentation rate is estimated to be 0.024 to 0.24 mm. These values fall within the range of laminae thicknesses in fossil algal laminites.

1 INTRODUCTION

From the Precambrian-Cambrian boundary until the end of the Cretaceous, calcifying cyanobacteria frequently occur in normal marine environments. After the end of the Cretaceous, however, they seem to be restricted to non-marine settings. In the Modern, calcification of cyanobacteria occurs almost exclusively in freshwater, and hypersaline or brackish waters. Because the precipitation is extracellular, this change in environmental distribution is believed to reflect changes in sea water chemistry. It has been proposed that the calcium/magnesium ratio (RIDING, 1982) or the degree of supersaturation with respect to carbonate (KEMPE & KAZMIERCZAK, 1990) of the oceans changed, leading to less favourable conditions for precipitation. To determine, what other environmental factors may have affected this change in geological distribution, it is neccessary to know which mechanisms of calcification exist in cyanobacteria and how the mutual influence of organic and inorganic factors in precipitation might vary in different environments.

Photosynthesis and photosynthetic uptake of bicarbonate

Address: Dr. M. Merz, Institut für Geologie und Paläontologie, Hans Meerwein Str., Lahnberge, D-3550 Marburg

have been shown to be the driving mechanism for the calcification in many calcareous algae (BOROWITZKA, 1990). The same was suggested for cyanobacteria (for example: GOLUBIC, 1972; BRAITHWAITE et al., 1989). Conclusive evidence from field observations or experimental data, however, are scarce. It has been shown by GLEASON that in the Florida Everglades the timing of carbonate precipitation in cyanobacterial mats coincides with photosynthetic activity and that inorganic factors seem to play a neglegible role in precipitation (GLEASON, 1972; GLEASON & SPACKMAN, 1974). The Florida Everglades, therefore, have been chosen as an excellent location for field experimentation on the mechanism of carbonate precipiation.

2 INTRODUCTION TO CYANOBACTERIA

2.1 Photosynthesis

Photosynthetic activity is an important factor in the calcification of calcareous algae. By its effect on water composition and especially pH, it creates an environment where carbonate precipitation can easily be induced.

Photosynthesis transforms light energy into chemical energy, which can be used for the formation of organic molecules from CO_2 and other inorganic substances. The photosynthetic apparatus of plants and cyanobacteria, which absorbs the energy, consists of two photosystems (PS I and PS II) in which photosynthetically active pigments, generally chlorophylls, absorb light of an appropriate wavelength. The absorbed light energy is then transformed into chemical energy (NADPH and ATP) via two electron transport chains. Thereby water is reduced in PS II. While the hydrogen is used in the electron transport chains, the oxygen is released by the cells into the medium. The energy stored in NADPH and ATP is used for the synthesis first of a hexose, and subsequently of various other substances.

The synthesis of these substances begins with the attachment of CO_2 to RuBP ('carboxylation'). The reaction is catalyzed by the enzyme RUBISCO-carboxylase. In this reaction the CO_2 can be substituted by O_2, which leads to the unintentional oxidation of RUBISCO instead of its carboxylation. Photosynthesis is more effective, the higher the ratio is of CO_2 to O_2, because less energy is lost by the oxidation of RUBISCO. Plants and cyanobacteria developed various techniques to increase the concentration of CO_2 relative to O_2. The RUBISCO of cyanobacteria has an especially low affinity for CO_2 (RAVEN & LUCAS, 1985). Nevertheless cyanobacteria photosynthesize rapidly even at low CO_2-concentrations. They posses a mechanism which allows them to increase the intracellular concentration of inorganic carbon (DIC) up to 1000-fold above that of the ambient water (LUCAS, 1983; VOLOKITA et al., 1984; KAPLAN et al., 1987). The DIC concentrating mechanism consists of an energy consuming pump-system, which actively takes up DIC and transports it into the cells (KAPLAN et al., 1987; BADGER & PRICE, 1989). CO_2 is taken up preferentially, but bicarbonate is taken up as well, especially at high pH-values (MILLER & COLMAN, 1980; PRICE & BADGER, 1989 a; MILLER et al., 1990). The species arriving at the inside of the cell seems to be always HCO_3^- (BADGER et al., 1985; OGAWA & KAPLAN, 1987; REINHOLD et al., 1987). In the cell interior, the conversion of HCO_3^- to CO_2 is catalyzed by the enzyme carboanhydrase (CA). Whichever carbon species was taken up, only CO_2 is finally used for the carboxylation of RuBP. Nevertheless, in the following 'HCO_3^- use in photosynthesis' will be used if the original species taken up was bicarbonate.

The energy required to run the DIC-pump is balanced by the increase in the rate of photosynthesis under low-CO_2 conditions. In environments where CO_2 supply rather than the available light energy limits the rate of photosynthesis, the active uptake of HCO_3^- gives an advantage over organisms which have to rely on CO_2 uptake alone. Under high O_2 concentrations, as frequently found in microbial mats, the intracellular accumulation of DIC might help to suppress oxydation of RuBP (PRINS & ELZENGA, 1989; RAVEN & LUCAS, 1985).

Another positive effect of the DIC concentration could be as a protection from photoinhibition (BADGER & ANDREWS, 1982; KAPLAN, 1985; KRAUSE, 1988). Photoinhibition occurs when more light energy is absorbed by the photosystems than can be used for the formation of NADPH and ATP. Some of the excessive energy is released in the form of fluorescence or heat, but the rest might irreversibly damage the photosynthetic apparatus. An active DIC pump allows higher rates of carbon fixation, so that higher light levels could be utilized. In addition some of the potentially damaging energy could directly be used to run the pump (KAPLAN, 1981).

2.2 Carbonate Precipitation Associated with Cyanobacteria

Cyanobacteria can live as single cells (coccoid cyanobacteria) or several cells may be linked to form cell rows, called trichom. If the trichom is surrounded by a mucilagenous sheath, the structure is called a filament. The main constituents of the sheath are polysaccharides. The function of the sheath is still unclear, but it seems to be involved in the ability of many benthic cyanobacteria to glide towards optimum light conditions.

The occurence of cyanobacteria is frequently linked to the deposition of carbonate. By trapping sediment between the filaments, they are involved in the formation of stromatolites in both sea water and freshwater. Cyanobacteria can also calcify when carbonate is precipitated in association with the organism. The result of precipitation is often a micritic tube surrounding the filament or the trichom. Similar tubes can be found in the fossil record in marine environments from the Precambrian-Cambrian boundary until the end of the Cretaceous. Various names are given to these fossils depending on the tube diameter, possible branching, and overall growth form. While some of the names might unite several biological species under the same fossil name (RIDING, 1977 b), and others might be due to organisms other than cyanobacteria, there is little doubt that most of them do represent calcified cyanobacteria. From the Cretaceous onward, calcification seems basically restricted to non-marine

environments (PENTECOST & RIDING, 1986).

In the Modern, calcification occurs in a variety of environments, often in shallow water poor in nutrients (GLEASON, 1972; SABATER, 1989), and nearly always non marine. In freshwater, calcifying cyanobacteria can frequently be found in spring tufas or oncolites. It has been estimated that in tufas the photosynthetic activity of the cyanobacteria can only account for 1 to 2 % of the precipitated carbonate while the rest of the carbonate is precipitated because of equilibration of the water with the atmospheric conditions (PENTECOST, 1978). Calcification of filaments also occurs in shallow hypersaline environments, which might be exposed to periods of emersion or freshwater influx (GOLUBIC, 1983; HORODYSKI & VONDER HAAR, 1975) and in desert crusts or caves where the cyanobacteria are living in a subaerial environment (COX et al., 1989; JONES & KAHLE, 1986; KRUMBEIN & GIELE, 1979). Calcification of living cyanobacteria in a normal marine setting is only described by GOLUBIC & CAMPBELL (1981), as a species specific calcification of various *Rivularia* species. The degradation of dead cyanobacterial material also seems to promote calcification in cyanobacterial mats (DEFARGE et al., 1985; LYONS et al., 1984), although it is not always clear whether cyanobacteria have been calcified alive or post mortem (BRAITHWAITE et al., 1989). It seems unlikely that the mechanism of calcification and the degree of biologic influence is the same in all these environments.

In calcification of modern cyanobacteria, a distinction has to be made between two different forms of calcification because they may reflect two different ways of precipitation concerning the degree of influence by the cyanobacteria. One is the encrustation of the sheath with carbonate, where the crystals nucleate outside on the sheath surface. This form has been called 'encrusted sheath' by RIDING (1977 a). The result is a carbonate tube with an inner diameter reflecting the diameter of the filament. This form of calcification often occurs in environments such as tufas, where mostly inorganic factors such as CO_2 degassing or temperature increase lead to precipitation. An influence of photosynthesis on tufa deposition could not be shown (GOLUBIC, 1972; PENTECOST, 1980; PENTECOST & RIDING, 1986).

The other form of calcification is the impregnation of the sheath with carbonate crystals. In this form, which is termed 'impregnated sheath', are the crystals precipitated completely within the sheath (RIDING, 1977 a). The outer diameter of the resulting micrite tube corresponds to the outer diameter of the filament, while the inner diameter might have the same or a larger diameter than the trichom. It is this second form of calcification which seems to be controlled by the organism, with precipitation being closely tied to the physiological activity of the cyanobacteria.

The calcification of the sheath also seems to depend on taxa-specific characteristics (PENTECOST & RIDING, 1986). *Plectonema* shows similar forms of calcification in different environments such as stromatolitic crusts in the Borrego Desert, California, (KRUMBEIN & POTTS, 1979) and temporary freshwater ponds on the Aldabra Atoll (RIDING, 1977 a). On the other hand, do different species calcify differently in the same environment (KRUMBEIN & GIELE, 1979; GOLUBIC & CAMPBELL, 1981; LEINFELDER, 1985; OBENLÜNESCHLOSS & SCHNEIDER, 1990). The different morphology of the crystals is probably due to differences in the chemical composition and the arrangement of the polysaccharides in the sheaths. The polysaccharides probably provide a suitable surface for nucleation. They absorb calcium ions, which consequently serve as binding sites for carbonate ions.

In both cases, encrustation and impregnation of the sheath, the mineralogy of the precipitated carbonate corresponds to the chemistry of the ambient water. Aragonite is precipitated in sea water, high-magnesian calcite in brackish water, and low-magnesian calcite in freshwater (PENTECOST & RIDING, 1986).

3 METHODS

Temperature and pH were measured in the field with a portable Orion pH/T/volt meter (model SA 230) and an Orion combination electrode. For measurements of the pH in the cyanobacterial mats an Ingold combination electrode with a tip diameter of 3.5 mm was used. Temperature was measured with a resolution to 0.1 C, accuracy +/-1.0 C, pH with a resolution to 0.01, accuracy ±0.02.

Oxygen was measured in the field with a portable oxygen-meter from YSI, model 52, with a resolution to 0.1 ppm. The electrode was calibrated against air, following the procedure described in the YSI manual.

Water depth was measured against an arbitrary scale with a zero mark at the sediment surface. The light intensity was measured in the air with a portable lux meter from Lutron, model LX 101.

For measurements of calcium and chloride concentration and of alkalinity, water samples were taken to the laboratory. For the calcium and alkalinity measurements, 30 ml samples were filtered in the field (Whatman G/F glasfiber filters) and poisened with one drop of concentrated $HgCl_2$ to prevent alteration by biological activity. For chloride determination filtered 15 ml samples were taken but no $HgCl_2$ was added.

Alkalinity was titrated in 10 ml samples with 0.1 or 0.05n HCl, following the method by GRAN (1952), using a Corning combination electrode to measure the pH changes after the addition of acid. Reproducibility of the results was better than 0.02 meq. 1, 2, and 3 meq laboratory standards of $NaCO_3$ were used for standardization.

Calcium was titrated with 10 or 1 mmol EGTA according to the method of GIESKES (1986). Reproducibility was 1 ppm (0.025 mmol). A 50 ppm $CaCO_3$ solution was used as a standard.

Chloride was also titrated following the method by GIESKES (1986). The titrant was diluted to 0.01 mol. Reproducibility was better than 5 %. Better results could not be determined because of the very low chloride concentrations in the samples.

The amount of dissolved inorganic carbon (DIC) was calculated from the measurements of pH, temperature, alkalinity, and calcium and chloride concentration, using the equilibrium constants by MILLERO (1979), corrected for the ionic strength as described by HELDER (1988). The amount of precipitation was calculated by the changes in calcium

concentration and alkalinity, with the decrease in calcium being equal to, and the change in alkalinity being twice the amount of precipitation. For the cyanobacteria the change in calcium reflects the amount of precitated carbonate while the change in alkalinity reflects precipitation as well as the amount of DIC being concentrated inside the cells. Normally, however, the amount of intracellular DIC can be assumed to be very small as compared with the amount of precipitated carbonate. Total DICs were calculated from pH, temperature, and water analyses before and after the experiments. The photosynthetic CO_2 uptake was calculated assuming that the total decrease of DIC in the water, minus that removed by precipitation, reflects the DIC used in photosynthesis.

Cyanobacterial mats, water, sediment, and gastropod shells were measured for their carbon and oxygen isotopic composition. All samples were measured with a Finnigan-MAT mass spectrometer, model MAT 251.

Oxygen isotopes in the water were measured following the method by EPSTEIN & MAYEDA (1953).

Carbon isotopic composition of the DIC in the water was measured in CO_2 liberated from the water, following the addition of 0.5 ml concentrated phosphoric acid to a 5 ml water sample. The samples taken at the beginning of the wet season (7/16/89 to 9/26/89) were allowed to equilibrate with 10 ml of air. This leaves oxygen isotopic composition basically unaffected, carbon isotopic composition, however, might have changed towards equilibrium values with the atmosphere. As the measured values are well below equilibrium values, the original composition must have been even lighter.

Samples of the sediment and gastropods were always collected from the same horizon of a core to obtain samples of the same age that probably precipitated from a water of comparable isotopic composition. The shells were carefully cleaned from the attached sediment before being measured. The isotopic composition was measured in the CO_2 produced after treatment with phosphoric acid.

All laboratory work was done under supervision of Dr. P.K.Swart in the Stable Isotope Laboratory of the Rosenstiel School of Marine and Atmospheric Sciences, University of Miami.

Sediment cores of the Holocene carbonate muds were taken manually with 8 cm diameter aluminum tubes. They were frozen, slabbed, and oven dried. For thin sections and polished slabs, the cores were vacuum-impregnated with resin.

Cyanobacteria samples for microscopic observations were fixed for several hours by the addition of 3.5 % glutaraldehyde in filtered water from the sampling site to preserve organic structures. The samples were then gradually transferred into a 70 % alcohol solution. Samples for the electron microscope were critical point dried. This method minimizes the disruption of organic structures by dehydration. First the samples have to be transferred into ethyleneglycol then into 100 % acetone. The acetone is then replaced by liquid CO_2. Finally the samples are dried at the critical temperature and pressure conditions of CO_2, when the liquid and the gaseous phase coexist. Drying under these conditions causes minimal shrinkage in the organic material. The storage in alcohol, however, leads to a certain volume change.

For observations of the carbonate crystals in the sheaths, the specimen were treated with sodiumhypochlorite (Clorox) to disintegrate the organic material.

The amount of organic versus inorganic material was determined by ashing the samples at 55° C for 3 hours. The change in weight reflects the amount of organic material, whereby an amount of 7 % ash was assumed (WOLK, 1973).

For the experiments, 0.4 to 1.0 g (dry weight) of cyanobacterial mats covering floating parts of submerged plants were used, after being cleaned from plant material. They were incubated in beakers holding up to 600 ml of sampling-site water. The experiments were generally started in the early morning, before the onset of photosynthesis, and run until the late afternoon when decreasing oxygen concentrations indicated the cease of photosynthesis. Some experiments were run over 24 hours or even over several days, to measure dissolution as well as precipitation. The experiments were run in sets of 2 to 5 replicates and the same experimental setup was repeated on several days.

Experiments were made with dichlorphenyldimethylurea (DCMU), an inhibitor for the activity of PS II, and ethoxyzolamide, an inhibitor for bicarbonate use in photosynthesis. DCMU was dissolved in methanol and added to the incubated samples to give final concentrations of 10^{-5}, $5x10^{-5}$, and 10^{-6} mol DCMU. Ethoxyzolamide was dissolved in Dimethylsulfoxide (DMSO) and appropriate amounts were added to give final concentrations of $2x10^{-4}$, $4x10^{-4}$, and $6x10^{-4}$ mol ethoxyzolamide (PRICE & BADGER, 1989 b).

As the effect of the inhibitors was variable on different days, each set of experiments included 2 to 5 untreated controls consisting of cyanobacteria incubated in pure sampling-site water. The controls always showed significantly higher amounts of precipitation than the cyanobacteria treated with an inhibitor.

In most experiments, no attempt was made to inhibit gas exchange with the atmosphere in order to prevent a buildup of high oxygen concentrations and therefore favouring photooxidation. Only when it was intended to measure the amount of photosynthesis were the experiments carried out in tightly covered beakers or in sealed Erlenmeyer flasks. While pH, temperature, and oxygen were generally measured every hour, measurements in the closed containers were only taken at the beginning and end of every experiment.

A slight underestimation of the amount of precipitation due to an increase of ion concentration by evaporation in the uncovered beakers does not affect the interpretation of the experimental data, because the results are always interpreted in relation to untreated controls of the same day.

To allow a comparison of the amount of precipitation in the various containers, precipitation was calculated, knowing the amount of incubated cyanobacterial material, as the theoretical amount of carbonate precipitated by 1 g cyanobacteria out of 1 l of water in 10 hours (mmol/g/l/10h).

4 CYANOBACTERIAL MATS IN THE FLORIDA EVERGLADES

4.1 The Environment

The Everglades are situated at the southernmost tip of the Florida mainland. The most important environmental feature is the seasonal runoff of freshwater to the south and southwest over six to eight months per year, temporarily covering extensive areas in the Everglades with shallow water. Where the freshwater mixes with the marine waters of Florida Bay, a mangrove belt is developed protecting the areas to the north from marine influence. The typical sediment is a mangrove peat of varying thickness. Northward, in the freshwater prairies, there is a vegetation which can withstand several months of complete dessication, consisting mostly of sawgrass (*Cladium jamaicensis*), a few scattered mangroves, and extensive cyanobacterial mats. Carbonate muds accumulate in these areas. GLEASON has shown that the carbonate is precipitated during the day, within the cyanobacterial mats, rather than being caught between the filaments (GLEASON, 1972; GLEASON & SPACKMAN, 1974). Inorganic factors are unlikely to play a significant role in precipitation in the Everglades. As the water flows over large distances and is very shallow (up to 50 cm), it is well equilibrated with the atmosphere except for a temporal disequilibrium caused by the biologic activity of the microbial mats. During times of complete water cover, flow velocities up to 25 cm per second (8/27/91) were estimated.

The sampling site is located in the freshwater prairies, east of Paurotis Pond, along the road from Florida City to Flamingo (fig. 1). It is covered by water from about July to January or February. During this time cyanobacterial mats develop, covering the sediment surface and submerged parts of plants. Well-developed mats are one to two centimeters thick. The surface of the mats is greyish brown or yellow because of a high content of protective carotenoids. Most of the chlorophyll is concentrated in the deeper layers of the mats, causing a deeply green colour.

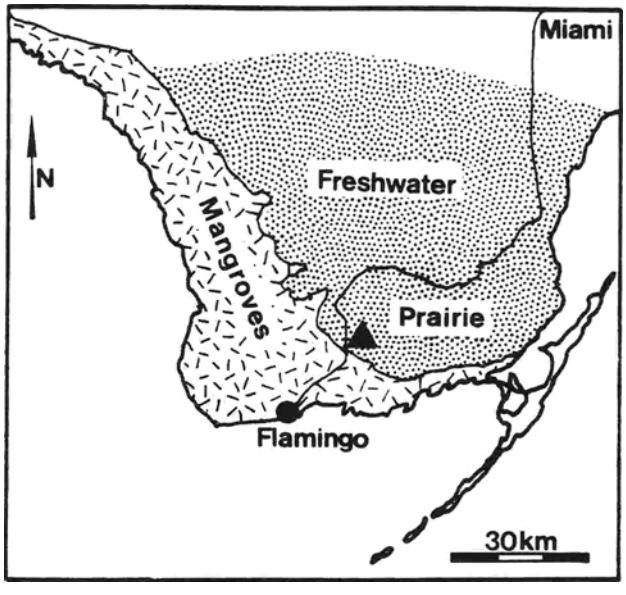


Fig. 1. The sampling site, marked with a black triangle, is located in the freshwater areas of the Everglades National Park, east of Paurotis Pond, at the road from Florida City to Flamingo.

The mats are dominated by calcifying species of filamentous cyanobacteria, *Schizothrix* and *Scytonema* (determination by courtesy of S.GOLUBIC).

4.1.1 The Water

The 1989/90 wet season (e.g. period of complete water cover at the sampling site) was relatively short due to little rainfall in this and the previous season. It lasted from the first week of July to the end of December. By this time the site was completely dry again. During the wet season the alkalinity of the water rose from 1.5 meq in July to 2.5 meq at the end of December. The calcium concentration rose in the same time from 1.1 to 1.25 mmol. Only in the first week of July were both concentrations higher, with an alkalinity of 1.7 meq and a calcium content of 4.5 mmol. Both alkalinity and calcium showed their lowest concentrations in September and October, when the water level was highest. As can be seen in fig. 2, the concentrations show a distinct peak in the middle of August, possibly related to a change in the flow direction of the water (in the figure indicated by arrows). At low water level (less than 5 cm), the water at the sampling site flows to the east, while at higher water levels it flows westward over the sampling site. With the changing flow directions, water from slightly different source areas might reach the site.

The concentration of chloride varied during the wet season between 1.0 and 7.5 mmol after it dropped from 18 mmol to 1.3 mmol in the first three weeks of water cover. The extremely high chloride concentration at the beginning of the season might be caused by the dissolution of easily soluble minerals that were precipitated during the period of dessication.

The measured water temperature at the sampling site showed extremes of more than 41°C in August to around 10°C at the end of December. The average temperature lies around 25° C (SWART et al., 1990; MERZ, 1990).

While most of the seasonal variations in water composition are due to the effects of evaporation and run-off, alkalinity, calcium concentration, oxygen and pH also show strong daily variations caused by the activity of the microbial mats. The concentration of chloride is neither influenced by physiological activities nor by carbonate precipitation and was therefore measured as a reference to determine the importance of biological factors.

Daily variations:

In the first week of complete water cover, before the cyanobacterial mats were developed at the sampling site, alkalinity, pH, and calcium concentration were measured over 24 hours. The concentration of calcium rose slightly during the day (from 4.69 mmol at 9 am to 4.79 mmol at 7 pm), alkalinity showed a week maximum in the midmorning (1.89 meq at 7 am, 1,90 meq at 11 am, and 1,68 meq at 7 pm). The pH varied unsystematically between 6.93 and 7.75. Later in the season, over well-developed cyanobacterial mats, temperature, pH, oxygen, calcium concentration, and alkalinity fluctuate systematically within 24 hours (Fig. 3).

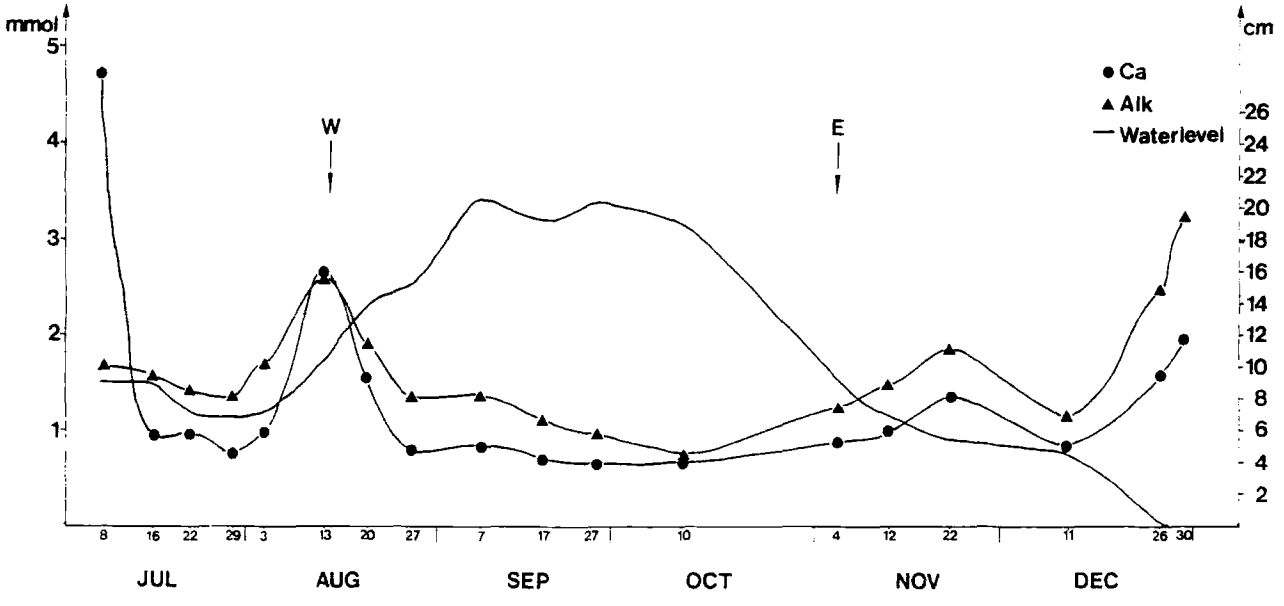


Fig. 2. shows the changes in calcium concentration, alkalinity, and water level from the first week of complete water cover at the sampling site (7/8/89) until the site was dried out at the end of December. All samples were taken around 6 pm. The arrows indicate changes in flow direction, to the East in the beginning of the wet season, to the West from August to November, and then, with decreasing water level, again to the East.

The pH can rise during day time more than one unit, up to values above pH 9. The lowest measured value was 7.04 in the early morning (8/20/89). The oxygen concentration, together with the pH, rises during daytime above equilibrium values with the atmosphere. In the evening it begins to fall. The minimum values of oxgen are measured in the early morning.

The concentration of chloride is constant throughout a day, indicating that the changes in calcium and oxygen concentration, alkalinity, and pH are caused by the biological activity in the microbial mats.

Both pH and oxygen concentration depend on the photosynthetic and respiratory activity of the microbial mats. Photosynthetic CO_2 uptake causes the pH to rise. Simultaneously, oxygen is released by the cells. Respiration is reflected by a decrease of oxygen concentration and pH, because of the release of CO_2.

Calcium concentration and alkalinity show, 1-2 hours out of phase, the reverse rhythm from oxygen concentration and pH. They decrease during the day, indicating carbonate precipitation during photosynthesis. The lowest values are reached about one hour before sunset. During the night they increase, when carbonate is dissolved by the respiratory activity in the microbial mats.

Several times measurements have been made of the water in the mat. During the daytime the pH was always significantly higher than in the water above the mats, while in the night it was lower. Although it was not possible to take samples of the mat water without any contamination of the overlying water, the calcium concentration and the alkalinity were always significantly higher in the mats.

4.1.2 The Sediment

The Holocene freshwater carbonate muds in the Everglades reach a thickness of up to 50 cm, depending on the relief of the underlying Pleistocene carbonates. In push cores it can be seen that the first few centimeters have a dark brown colour, rapidly fading with depth, because of a decrease in the content of organic material. Most of the sediment is a light brown to grey, unstructured carbonate mud, frequently containing plant roots and freshwater snails. The carbonate tubes of *Scytonema* appear to disintegrate rapidly as they could not be found in either thin section or by scanning electron microscopy.

While other carbonate muds associated with cyanobacterial mats often exhibit a distinct lamination (Andros Island, Bahamas, Monty, 1972; Hardie & Ginsburg, 1977), lamination in the sediment cores from the Everglades are restricted to the top few centimeters. The laminations are rapidly obscured with depth. This could be due to a different structure of the mats themselves, to a less pronounced periodicity in sediment accumulation, or to a higher rate of bioturbation in the Everglades.

Despite the proximity of the marine environment to the freshwater carbonate muds, no marine fossils are found. This fact is probably due to the protection by the mangrove belt which inhibits landward transport of marine sediments. In the fossil record, the carbonates of the Everglades would appear as unstructured micrites with a low fossil diversity, containing only a few species of freshwater snails.

4.2 Microscopic Observations

In the Everglades, the trichoms of *Scytonema* are surrounded by a sheath that is differentiated into two distinct layers. The inner sheath is dense, structured, and birefringent under crossed nicols and is always uncalcified (Pl. 19/1-3). The inner sheath is surrounded by a less dense outer sheath. The outer sheath is impregnated by micritic calcite crystals and encloses the inner sheath like bark a tree. In samples that have been critical point dried the outer sheath might break

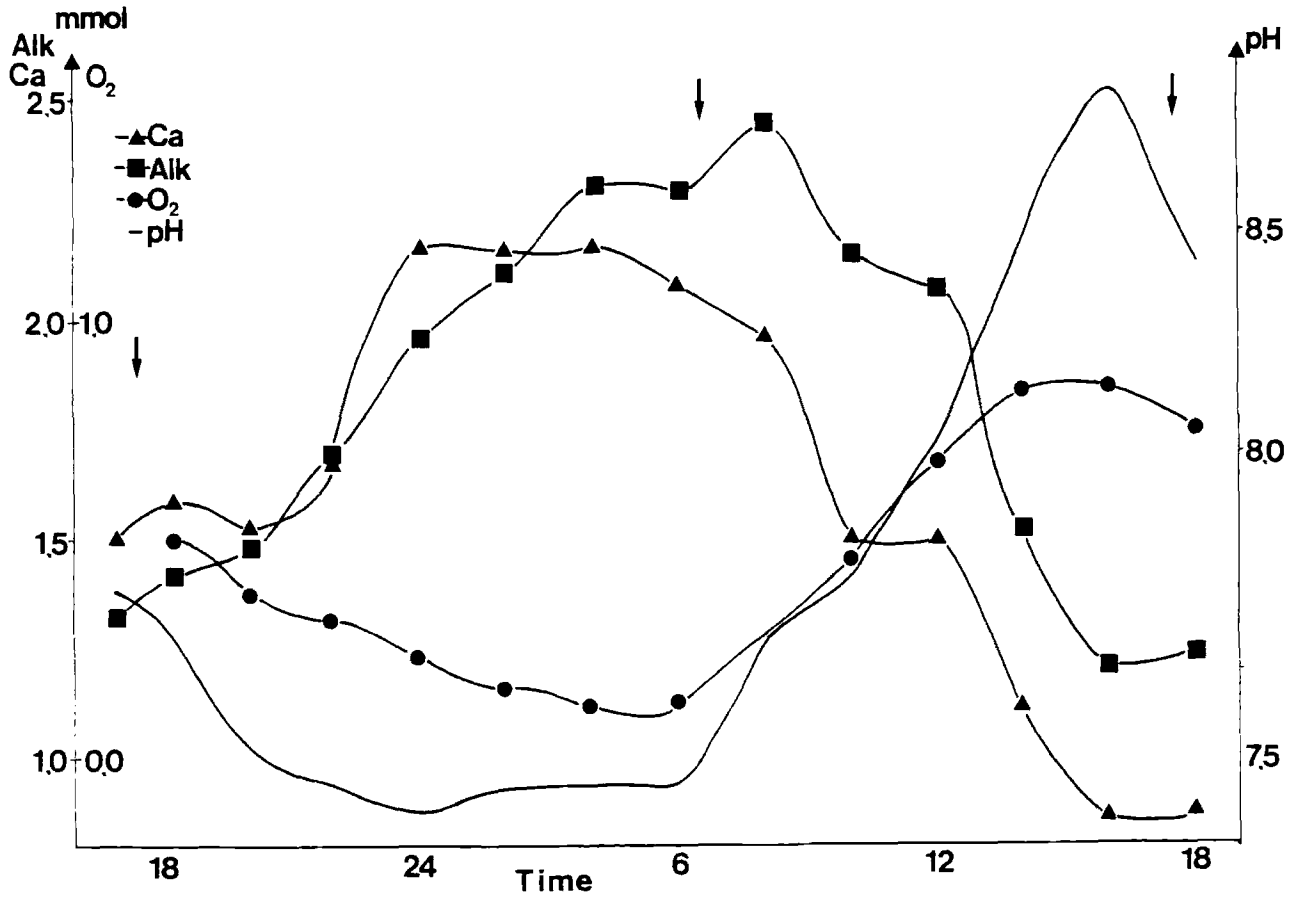


Fig. 3. Oxygen concentration and pH decrease after sunset (indicated by the arrow), while calcium concentration and alkalinity rise. After sunrise (arrow), the increase in oxygen concentration and pH indicates the onset of photosynthesis with an simultaneous decrease of calcium concentration and alkalinity. The time is given in 24 hours.

off and exhibit the smooth surface of the uncalcified inner sheath (Pl. 19/7). The crystals in the outer sheath may be as small as 2-3 µm, but are generally 5-7 µm long (Pl. 19/5).

The two sheaths seem to differ in structure as well as in chemical composition. Treatment with sodiumhypochlorite (Clorox) leads to the rapid disintegration of the outer sheath and the trichom, while the inner sheath remains preserved for an extended period of time. In most of the *Scytonema* filaments, the impregnation of the outer sheath leads to the formation of a dense carbonate tube (Pl. 19/6). The outer diameter of the tube is constant along the filament. As there is no precipitation in the inner sheath, the inner diameter of the tube reflects the diameter of the trichom plus the inner sheath. Close to the filament tip the diameter of the tube decreases. The filament tip itself is always uncalcified (Pl. 19/1). Calcification never exceeds the outer sheath. This is shown by the constant tube diameter, as well as by the fact that in critical point dried samples, the organic material of the sheath covers the outer surfaces of the crystals (Pl. 19/8). Treatment with Alizarin Red S leads to a staining of the organic material in the outer sheath. The crystals remain unstained which indicates that they are covered by organic material and therefore are not accessible to the stain.

Some of the *Scytonema* filaments show a completely different mode of calcification. Calcification is restricted to single rings within the outer sheath (Pl. 20/2-4). Some of them have the shape of straight cylinders (Pl. 20/3), while others are funnel shaped (Pl. 20/2, 4). The occurrence of the rings is independent of any morphological features of the filament. Rarely are the rings produced by the alternation of denser and less densely calcified zones of a continous tube.

Observations indicate that calcification of *Scytonema* and *Schizothrix* in the Everglades is different. These differences are similar to those reported by GLEASON & SPACKMAN (1974). Due to the small size of the filaments of *Schizothrix*, the structure of the sheath and the location of crystals within it is not as easily observed as in *Scytonema*. It can be seen, however, that the filaments are not as densely calcified. The calcification is generally restricted to single crystals or crystal conglomerates, that are probably embedded in sheath material (Pl. 20/1).

The morphology of the single crystals of the two taxa, as well as of the two different forms of calcification in *Scytonema* is distinctly different. Typical crystals of the *Scytonema* tubes are well developed rhombohedra that do not show any preferred orientation with respect to the trichom. The crystal faces in contact with the inner sheath are sometimes flattened, probably due to dissolution (Pl. 19/2). The crystals may form a tight tube, in which case the crystal morphology is not as distinct (Pl. 19/6).

The crystals in the carbonate rings of *Scytonema* have another morphology. They consist of single needles, or possibly dendritic crystals (Pl. 20/8). The morphologic difference is obvious even under the light microscope where

the crystals have a star-like appearance (Pl. 20/3). They appear to be embedded in organic material with a denser structure than the outer sheath of the other filaments. Due to this organic coating, the boundaries between single crystals are invisible (Pl. 20/8).

The calcite crystals in *Schizothrix* also have a distinct morphology. Their most remarkable feature is the dendritic growth which leads to the development of aggregates of calcite needles, following the crystallographic directions of calcite (Pl. 20/5-7). Some of them have a triangular form and seem to be oriented with their base parallel to the trichom (Pl. 20/7). The crystals may coalesce to form larger aggregates of dendrites enclosing short segments of the filament.

4.3 Carbonate Precipitation, Sedimentation Rates

Sedimentation rates of a freshwater carbonate mud, precipitated by cyanobacteria, can be estimated from the field and experimental data obtained in the Everglades. The alkalinity of water is not changed by photosynthetic CO_2 uptake and in freshwater (total) alkalinity is about equal to carbonate alkalinity (DREVER, 1988). Therefore it can be assumed that changes in alkalinity and in calcium concentration of a water both reflect carbonate precipitation or dissolution.

In this way the amount of precipitated and dissolved carbonate was calculated for 12 and 24 hour sampling periods of the 1989/90 wet season. Precipitation was calculated by subtracting the minimal values of alkalinity and calcium measured in the evening from the maximum values in the morning. Dissolution was calculated accordingly, by subtracting the maximum values in the morning from the minimum values of the previous evening. Because the maximum and minimum values were taken, the calculated timespans are slightly different at the different days. Tab. 1 shows the amount of precipitation, calculated for various days. On the 8/20 and 8/27 only precipitation has been measured, while on the 9/25, 10/10, 11/4, and 12/12/89 dissolution has been measured as well.

As can be seen in Tab. 1, the amounts of precipitation and dissolution per day vary considerably, but do not show any seasonal pattern. Precipitation varies from 0.09 to 0.46 mmol (assuming that the value of 1.10 mmol (11/4/89), estimated by the change in calcium concentration, is due to an analytical error). During the night, some of the carbonate, 0.05 to 0.5 mmol, is redissolved.

By subtracting the amount of dissolved carbonate from the amount of carbonate precipitated during the day, the net amount of precipitation from 1 l water in 24 hours can be estimated. As there seems to be hardly any sediment influx other than the carbonate precipitated by the cyanobacteria the estimated net precipitation reflects the amount of sedimentation. When the net sedimentation is calculated with field data it can be seen that there is net precipitation in two out of four days, while at the other two days the values indicate net dissolution.

From these data, 0.015 mmol $CaCO_3$ per liter water in 24 hours is taken as an average amount of precipitation throughout the wet season. In 1989 the duration of water cover at the sampling site lasted about 180 days, from the first week of July to the last week of December. As this wet season was comparatively short, because of a succession of especially dry summers, a normal duration of water cover of 200 days will be assumed for calculations of the annual sedimentation. The average water depth at the sampling site is taken to be 10 cm, estimated from the recorded water depth in 1989 (Fig. 2).

For a daily net precipitation of 0.015 mmol and a density of calcite of 2.7, a pore space free volume of 111 mm^3 $CaCO_3$ per wet season can be calculated. This gives an annual sediment layer with a thickness of 0.011 mm per

Plate 19 Light microscopical and SEM photographs of calcified *Scytonema* filaments, Everglades, Florida. All samples were taken in the freshwater algal marshes east of Paurotis Pond.

Fig. 1. Uncalcified filament tip of *Scytonema*. The structured inner sheath is clearly visible. The trichom is shorter than the sheath, which might be an artefact because of shrinkage during preparation. The outer sheath is vaguely visible. Sample fixed in 3.5 % glutaraldehyde. x 460

Fig. 2. Calcified filament of *Scytonema*. The inner sheath, surrounding the central trichom, appears dark. Single crystals are located in the outer sheath. The sides of the crystals facing the inner sheath are flattened, which might be a dissolution feature.Sample fixed in 3.5 glutaraldehyde. x 230

Fig. 3. Calcified filament of *Scytonema*. The layered structure of the uncalcified inner sheath is clearly visible, while in the outer sheath there are well developed calcite rhombohedra. Sample fixed in 3.5.% glutaraldehyde. x 460

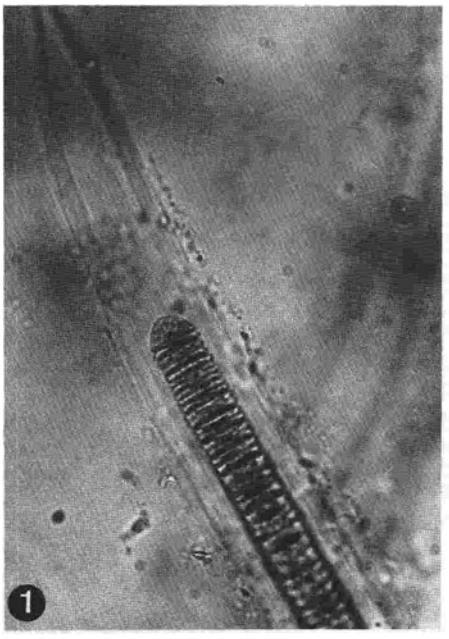
Fig. 4. Filament of *Scytonema*, being enclosed in carbonate crystals. The single crystal rhombohedra are still clearly visible. Sample fixed in 3.5 % glutaraldehyde. x 460

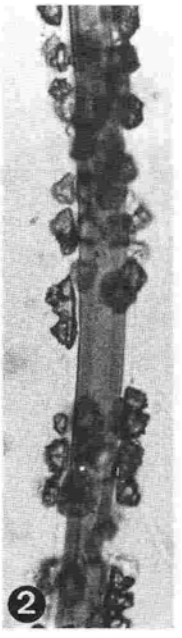
Fig. 5. SEM photograph of a *Scytonema* filament, treated with sodiumhypochlorite. Well developed calcite rhombohedra are laying on the surface of the undisintegrated inner sheath.

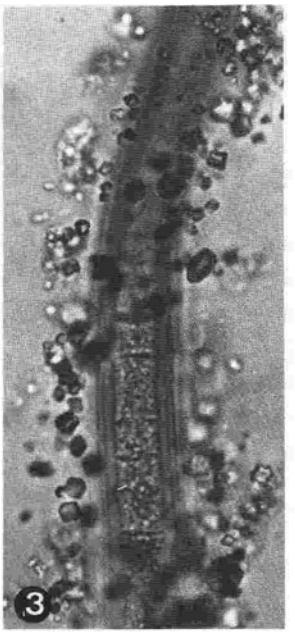
Fig. 6. SEM photograph of a calcified *Scytonema* filament. The carbonate crystals coalesce to form a dense calcite tube around the trichom and the inner sheath. Sample treated with sodiumhypochlorite.

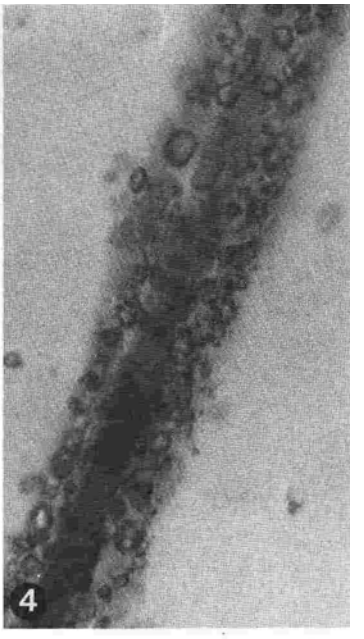
Fig. 7. SEM photograph of a calcified *Scytonema* filament. The outer sheath, heavily impregnated with carbonate crystals, broke off and exposed the smooth surface of the uncalcified inner sheath. Sample fixed in 3.5 % glutaraldehyd, critical point dried.

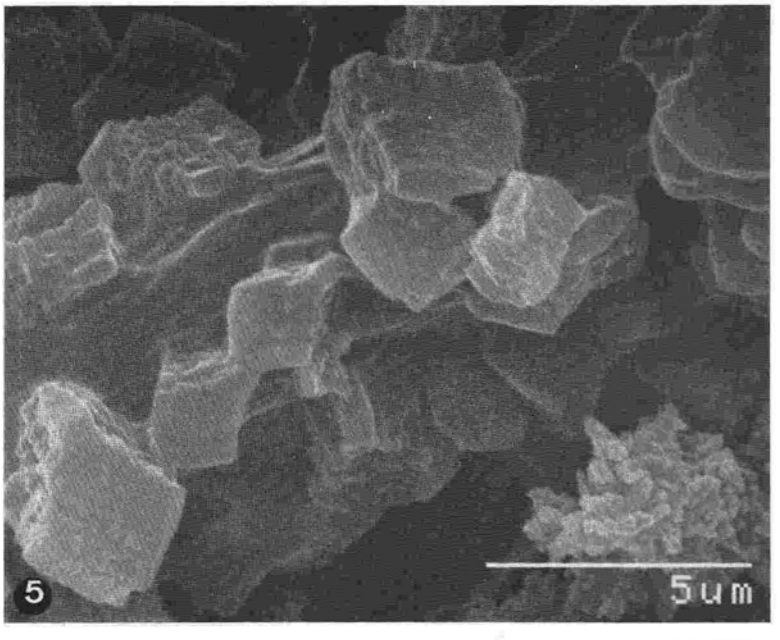
Fig. 8. SEM photograph of a calcified *Scytonema* filament. The organic material of the dried and collapsed outer sheath completely covers the calcite crystals. Sample fixed in 3.5 % glutaraldehyd, critical point dried.

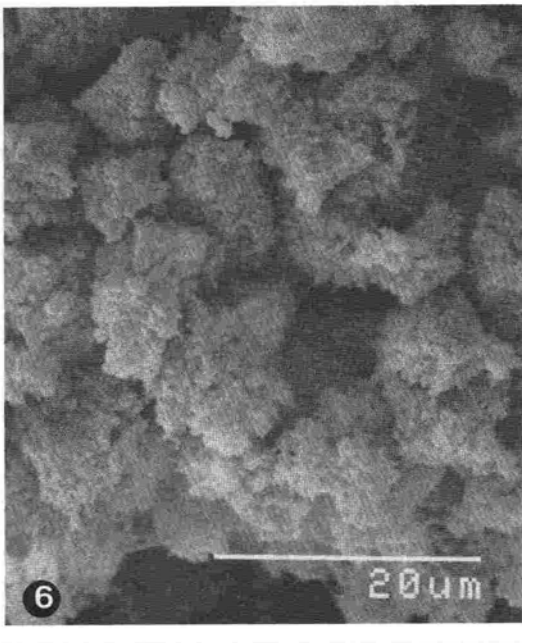

1

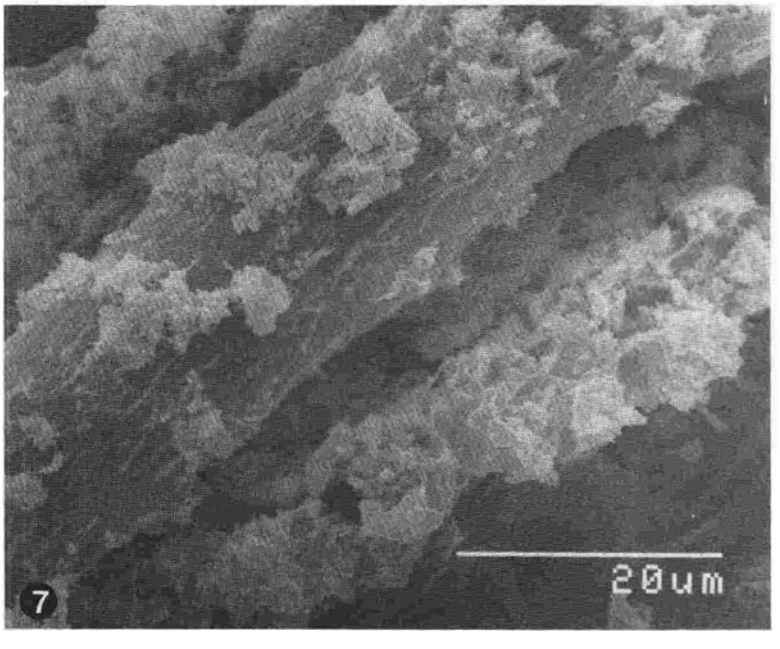

2

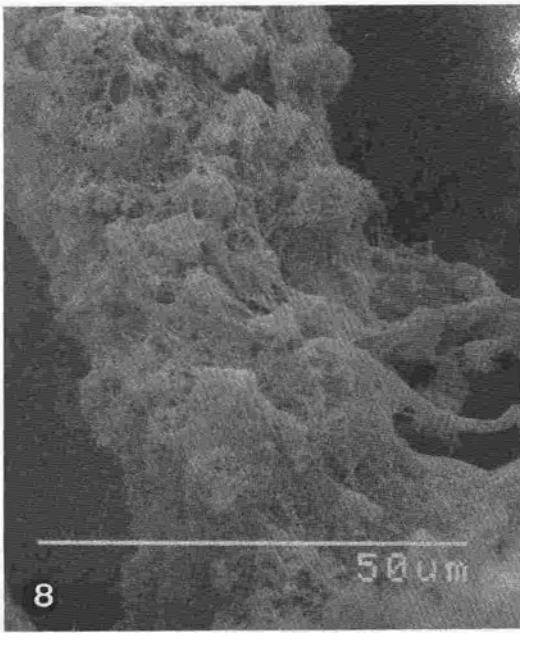

3


4


5
5um


6
20um


7
20um


8
50um

Date	20.8.89	27.8.89	25.9.89	10.10.9	4.11.89	12.12.89
Precipitation						
Time	7-19:00	8-18:00	8-18:00	9-18:00	8-16:00	8-15:30
Ca^{2+}	0,340	0,350	0,194	0,111	1,105	0,456
Alk	0,090	0,325	0,185	0,150	0,615	0,460
Dissolution						
Time			18-8:00	18-9:00	8-16:00	15:30-7:30
Ca^{2+}			-0,212	-0,048	-0,467	-0,474
Alk			-0,230	-0,085	-0,565	-0,485
Net						
Ca^{2+}			-0,018	0,063	0,638	-0,018
Alk			-0,045	0,065	0,050	-0,025

Tab. 1. Amount of carbonate precipitation in mmol measured at various days during the 1989/90 wet season.

square dm (= precipitation out of 1 l of water with an average water depth of 10 cm).

The same calculations were made with the data obtained from cyanobacteria precipitating over 24 or 36 hours in experiments in 500 ml beakers. Again, the amount of net precipitation varied on different days. Experiments performed on 12/30/89 gave a net amount of 0.080 mmol/24 hrs from 1 liter, while experiments performed the 11th through 13th of January gave values of 0.200 mmol/24 hrs/liter. Taking the calculated values again as average values for precipitation throughout the wet season, annual sediment thicknesses of 0.60 to 0.148 mm are estimated. Because they were measured in closed containers rather than in running water, these values should be more exact than the data obtained from the field measurements.

Assuming 60% pore space the thickness of the annually accumulated sediment layer would be 0.018 mm (1.8 cm/1000 yrs) for the field data, and 0.1 and 0.24 mm (10 and 24 cm/1000 yrs) for the experimental results. These calculations are made ignoring a possible contribution by organic material (30 % dry weight of the cyanobacteria).

Scholl et al. (1969) estimated the sedimentation rates in the Everglades from ^{14}C-data to be 1.6 cm/1000 yrs, with higher rates immediately after flooding of the platform about 4000 yrs b.p. and slowly decreasing later (1.2 mm/yr in the first 1000 yrs). Their estimates give sedimentation rates that are 10 times higher than the ones calculated in this study from the field data, but they fall within the range calculated from the experimental data (for the representativity of the experimental data see Chapter 4. 6.1).

When comparing the data from the Everglades with the laminae thicknesses of fossil laminites, it must be kept in mind that the volume changes during diagenesis might be considerable and highly variable. It is difficult to estimate how much of the original porosity will be destroyed by compaction and how much might be preserved by early cementation. The data from the Everglades, however, lie well within the range of laminae thicknesses given by Müller-Jungbluth for the Hauptdolomite, Triassic, Northern Calcareous Alps. He measured laminae of 0.08 to 1.0 mm thickness in the Middle Hauptdolomite, and 0.1 to 5 mm in the Lower Hauptdolomite (Müller-Jungbluth, 1968; 1970).

4.4 Organic Material

The field observations show a close temporal relationship between photosynthesis and the precipitation of car-

Plate 20 Lightmicroscopical photographs of *Scytonema* and *Schizothrix*, SEM photographs of calcification in *Schizothrix*. All samples were taken in the freshwater algal marshes east of Paurotis Pond, Everglades, Florida.

Fig. 1. *Schizothrix* filament in the light microscope. The crystals appear star like in the light microscope. The tiny filament is vaguely visible between the carbonate crystals. It apparently is surrounded by a voluminous unstructured sheath. Sample fixed in 3.5 glutaraldehyde. x 460

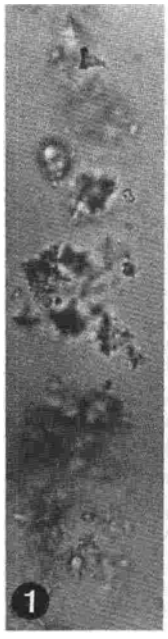
Fig. 2.-4. Ring calcification in *Scytonema*. The rings can be funnel shaped (2), cylindrical (3), or look like shallow bowls (4). The morphology of the crystals in the rings is similar to the crystal morphology in *Schizothrix*. As in the continously calcified *Scytonema* filaments (Plate 19) remains the inner sheath always uncalcified. Under the light microscope it is clearly visible because of its darker colour and layered structure. Samples fixed in 3.5 % glutaraldehyde.

Fig. 5. SEM photograph of a single dendritic *Schizothrix* crystal. Sample treated with sodiumhypochlorite.

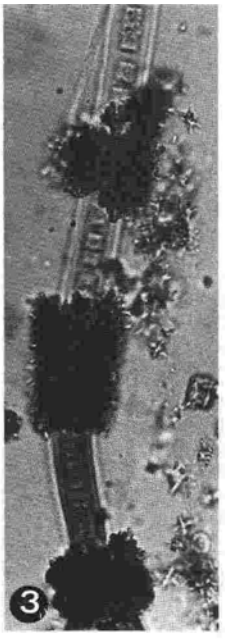
Fig. 6. SEM photograph of a calcified *Schizothrix* filament, densely encrusted by dendritic calcite crystals. Sample treated with sodiumhypochlorite.

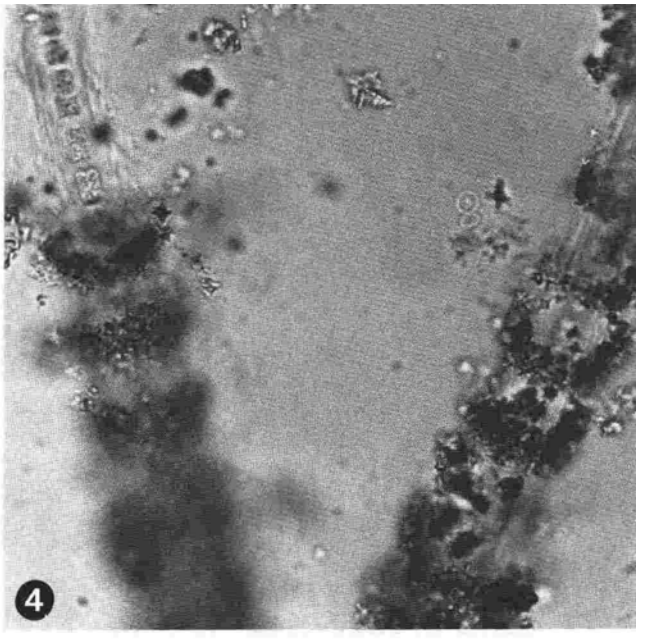
Fig. 7. SEM photograph of a calcified *Schizothrix* filament. The dendritic crystals are triangular and their base seems to be oriented parallel to the filament.

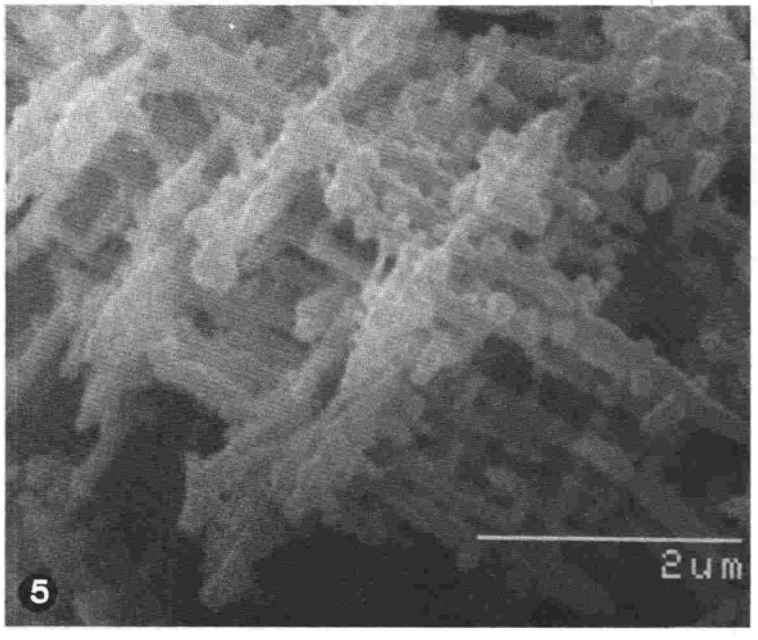
Fig. 8. SEM photograph of ring calcification in *Scytonema*. The crystals, possibly dendritic, seem to be embedded in organic material denser than the outer sheath of the continously calcified *Scytonema* filaments.

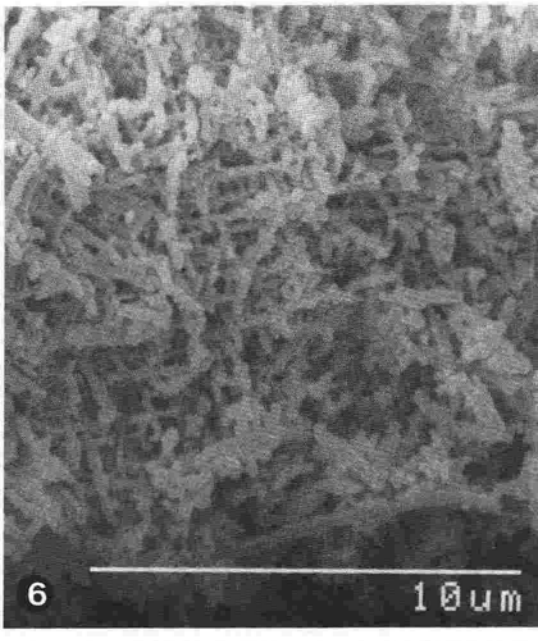

1

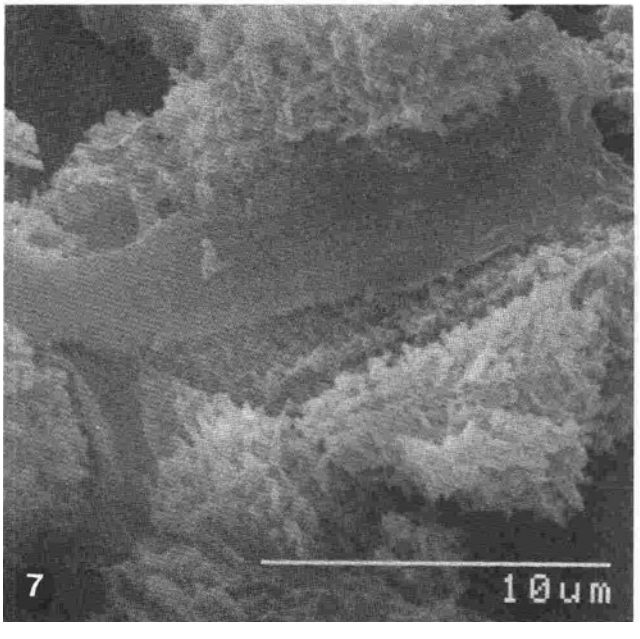

2

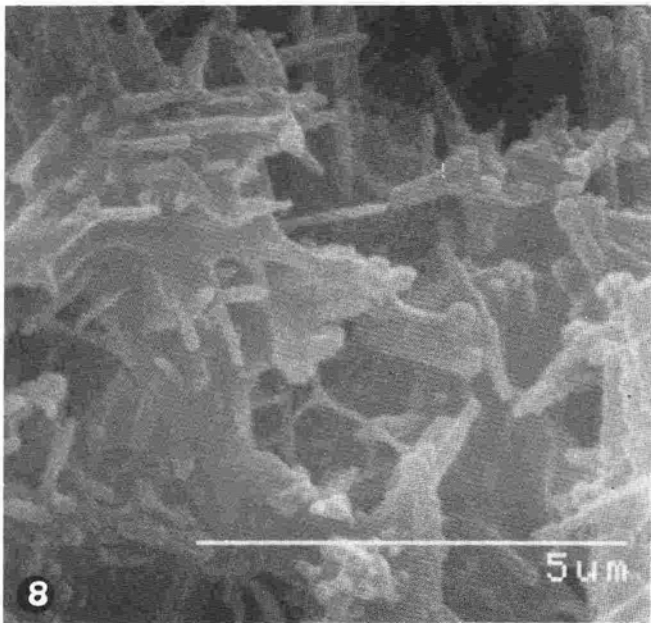

3


4


5
2um


6
10um


7
10um


8
5um

bonate. They do not allow, however, deductions to be drawn about how actively the carbonate is precipitated by the cyanobacteria. Precipitation could simply be a byproduct of photosynthesis, which the organism is unable to inhibit. If this is the case, the amount of precipitation would be expected to depend on environmental conditions, such as the ion concentrations, temperature, and- biologically influenced- the pH. But if precipitation is a more active process, it should have a stronger relation to the rate of photosynthesis than to environmental factors.

To distinguish between these two possibilities, the amount of photosynthesis versus the amount of precipitated carbonate has been measured. Different methods were used, each showing constant ratios between photosynthesis and precipitation of 1 mol precipitated carbonate for 1 to 2 mol organically fixed carbon.

Measuring the percentage of organic material in 29 samples of cyanobacterial mats gives values close to 32 %. This percentage is constant throughout the season, independent of the time when the sample was taken, showing that there is no stronger calcification in older cyanobacteria. In order to compare the amount of precipitation with the amount of organically fixed carbon, the amount of carbon in the organic material was calculated, assuming that the organic material of cyanobacteria contains 46 % carbon (Wolk, 1973). The amount of carbon fixed organically and the amount precipitated in carbonate shows a constant relationship with an average ratio of 1.86 mmol organic carbon to carbonate.

While this methods gives an average ratio of photosynthesis to precipitation over several weeks or months, the ratio can also be directly measured over a few hours. This has been done in closed containers, in which the amount of photosynthesis was calculated either by measuring CO_2 uptake or by measuring O_2 release. The CO_2 uptake was measured in beakers which were tightly covered to inhibit gas exchange with the atmosphere. As oxygen equilibrates with the atmosphere much quicker, oxygen was measured with an electrode in tightly sealed Erlenmeyer beakers. Precipitation was again measured by the decrease of alkalinity and calcium concentration. In all the containers cyanobacteria were incubated for several hours.

The equation

(DIC - precipitation)/precipitation = V

gives the ratio V of photosynthesis versus precipitation. It can be seen from the equation that the method of calculation increases any possible analytical error in the determination of precipitation. On the other hand, alkalinity is used for the calculation of the amount of photosynthesis as well as for the calculation of precipitation, which leads to a weak artificial correlation. The ratios calculated in this way should therefore be taken as estimates rather than exact values. They do, however, indicate a constant relationship between photosynthesis and precipitation. Around 1.15 mol carbon is fixed organically for every mol precipitated carbonate (1.10 to 1, according to the estimates by Ca^{2+}, 1.20 to 1, according to the estimates by alkalinity), (Tab. 2).

Despite these inaccuracies, the calculated results show fairly good accordance with the ratios found by direct measurements of photosynthesis by measuring oxygen evolution. For every mol of CO_2 fixed in photosynthesis, one mol of O_2 is released. The amount of oxygen therefore corresponds exactly to the amount of carbon fixed. The ratios of oxygen to precipitated carbonate are given in Table 2. The values are slightly lower than the previously calculated ones, indicating that for every mol CO_2 used in photosynthesis, one mol of carbonate is precipitated. The low value of sample 890 is probably caused by a loss of O_2, due to a defect seal on the beaker. The low value for sample 891 also occurs in the Ca^{2+}-calculation for the CO_2-uptake calculations, indicating an analytical error in the titration of calcium.

Irregardless of which method was used, it can be said that for one to two mol carbon fixed organically there is one mol

Sample	Ci	Ca2+	Alk	CO2/Ca2+	CO2/Alk	O2/Ca2+	O2/Alk
Control							
888	0,484	0,227	0,225	1,13	1,15	0,93	0,94
889	0,508	0,254	0,235	1,00	1,16	0,86	0,93
890	0,597	0,332	0,280	0,80	1,13	0,47	0,56
891	0,433	0,296	0,200	0,46	1,17	0,67	0,99
892	0,587	0,256	0,275	1,29	1,13	0,93	0,87
893	0,439	0,178	0,200	1,46	1,19		
894	0,215	0,111	0,095	0,94	1,27		
895	0,352	0,182	0,160	0,95	1,20		
896	0,324	0,121	0,145	1,68	1,23		
897	0,379	0,143	0,170	1,65	1,23		
898	0,457	0,210	0,210	1,18	1,18		
899	0,428	0,210	0,195	1,04	1,19		
900	0,386	0,165	0,175	1,34	1,20		
901	0,412	0,189	0,190	1,18	1,17		
902	0,720	0,328	0,340	1,19	1,12		
995	0,395	0,219	0,175	0,08	1,26		
996	0,322	0,134	0,145	1,40	1,22		
997	0,238	0,153	0,105	0,55	1,27		
Ethoxyzolamide							
998	0,051	0,012	0,010	3,24	4,08		
999	0,214	0,061	0,085	2,51	1,52		
1000	0,094	0,076	0,025	0,024	2,77		

Tab. 2. Ratio of photosynthesis to precipitation in untreated samples and samples treated with the inhibitor ethoxyzolamide. Photosynthesis was calculated from the amount of CO_2 uptake and measured from the amount of O_2 released by the cells.

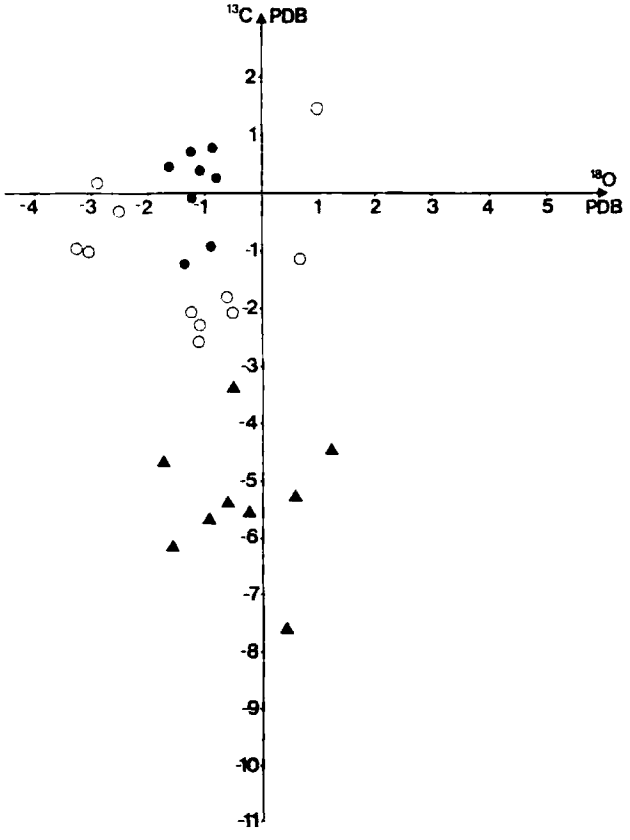


Fig. 4a. Isotopic composition of carbonate precipitated during the 1989/90 wet season (circles), carbonate sediment from push cores (solid dots), and gastropod shells (triangles). Data are all given versus PDB.

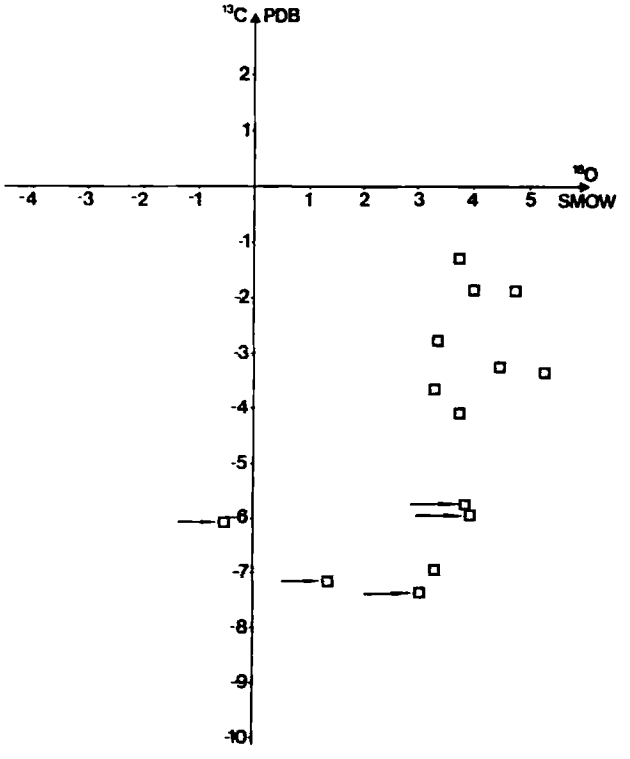


Fig. 4b. Isotopic composition of water, measured throughout the wet season 1989/90. The arrows mark samples which are possibly slightly to heavy in 13C due to storage before measuring. Carbon data are given versus PDB, oxygen data versus SMOW.

of carbonate precipitated. This is a constant relationship, apparently relatively independent of inorganic factors. The amount of carbonate never exceeded the amount of organically fixed carbon in either the experiments or in the cyanobacterial mats.

4.5 Stable Isotopes

Stable carbon (versus PDB) and oxygen (versus SMOW) isotopic compositions of the water in the Everglades have been measured throughout the duration of the wet season. In addition stable isotopic compositions have been determined for calcite precipitated in the water during the same time span, for carbonate mud sampled from the sediment cores, and for gastropod shells sampled from the same horizons in the cores as the carbonate mud (carbon and oxygen versus PDB). The carbon data of the water samples from the beginning of the wet season are possibly depleted, in ^{13}C due to equilibration with 10 ml of air (see chap. 3). In fig. 4 b these data are marked with an arrow.

Carbon and oxygen isotopic compositions of gastropod shells have been measured as a reference for equilibrium precipitation. The gastropods were used because it has been shown that molluscs precipitate in equilibrium with both the carbon and the oxygen compositions of the surrounding waters (Epstein et al., 1951; Fritz & Poplawski, 1974; Mook & Vogel, 1968).

The oxygen isotopic composition of carbonate precipitated by cyanobacteria during the 1989/90 wet season indicates precipitation in equilibrium with the ambient water. The same is true when comparing the values of the carbonate mud from the cores and the gastropods. According to the equation given by O'Neil (1969, in McConnaughey, 1989) carbonate, precipitated from water of $\delta^{18}O$=5.269 (12/26/89) to $\delta^{18}O$= -1.882 (7/8/89) should have a composition of $\delta^{18}O$=3.499 to -3.652. This is assuming an average temperature of 25° C, which is the average temperature given by Swart et al. (1989) for Florida Bay following temperature data from a weather station in Key West. It is confirmed by calculating the average temperature for three days in September, November, and December of 1989, when the temperature at the sampling site was measured over 24 hours. These data give an average temperature at the sampling site of 26.6° C.

The measured oxygen data of the carbonates fall within the range of the lighter calculated values. Equilibrium precipitation is also supported by a comparison of the isotopic data obtained from gastropod shells and the carbonate sediment from the cores. Taking into account that calcite is about 0.6 permil lighter than coprecipitated aragonite, the $\delta^{18}O$ values of the shells and the sediment fall within the same range.

The carbon isotopes, however, show an enrichment of ^{13}C in the cyanobacterial carbonate relative to the water. An average value of -5.5 permil $\delta^{13}C$ was assumed for the water. The fractionation of carbon is independent of the temperature of precipitation. In equilibrium precipitation carbonate is always about 2 permil heavier in ^{13}C than the DIC of the water it precipitated from. Carbonate in equilibrium with the

water during the 1989/90 wet season should therefore have a composition of about -3.5 permil. The sediment values, however, range between 0.25 to -2.6 permil. The sediment is therefore heavy (i.e. enriched in ^{13}C), relative to the values calculated from the water isotopic composition.

Comparing again the $\delta\ ^{13}C$ values of the gastropod shells with the sediment composition, it can be seen that the sediment is considerably heavier than equilibrium precipitation. The aragonite shells show $\delta^{13}C$ values between -1.25 and 0.7 permil. In addition, a fractionation between aragonite and calcite has to be taken into account, with calcite being 1.8 permil depleted in ^{13}C relative to aragonite (Rubinson & Clayton, 1969). The sediment is therefore 6-7 permil heavier than the water it was precipitated from, the water composition being reflected by the gastropod shells.

The ^{13}C enrichment in the carbonate precipitated by the cyanobacteria is understandable when considering the carbon fractionation in photosynthesis. The enzyme RUBISCO (Chapter 2.2.1) is generally discriminating against ^{13}C up to -27 permil (Swart, 1983; Sharkey & Berry, 1985). In cyanobacteria, the degree of discrimination depends on the environmental DIC concentration. It varies between -18 permil and 0 permil, being reduced by active DIC uptake (Calder & Parker, 1973; Estep, 1984).

The enrichment of ^{13}C in the carbonate is an effect of preferential ^{12}C uptake during photosynthesis, which increases the ^{13}C concentration in the immediate vicinity of the filaments. This enrichment indicates that the precipitated carbon is derived completely extracellular, and that no respiratory carbon is incorporated.

The same effect, i.e a ^{13}C enrichment in carbonate due to photosynthetical ^{12}C uptake, can be seen in some calcareous algae and in corals with a high density of zooxanthellae and consequent high rates of photosynthesis (Keith & Weber, 1965; Borowitzka, 1986; Swart, 1983; McConaughey, 1989; Cummings & McCarty, 1982). In contrast, some coralline red algae show relatively light $\delta^{13}C$ values because of incorporation of respiratory carbon in the skeleton (Borowitzka, 1986 and compilation by Swart, 1983, Fig. 1).

Stuiver (1970) measured the isotopic composition of various lacustrine carbonates and found an enrichment of ca. 5 permil relative to mollusc shells in ^{13}C, which he explained by photosynthetic ^{12}C depletion by phytoplankton. A ^{18}O enrichment, which he found in his sediments, can not be confirmed from the Everglades data.

In comparison with fossil cyanobacteria carbonates it has to be considered that in addition to the water composition, active DIC uptake might change the degree of carbon fractionation in photosynthesis and hence the isotopic composition of the residual carbon pool for precipitation.

4.6 Experiments on Precipitation

Experimental data on carbonate precipitation by cyanobacteria are scarce, probably due to difficulties in inducing precipitation under laboratory conditions (Pentecost, 1978). The same phenomenon, i.e. a weaker or lacking calcification in laboratory cultures as compared with field grown specimens, is described from calcareous algae (Giraud & Cabioch, 1979). The experiments have therefore been conducted in the field. An advantage of field experiments are

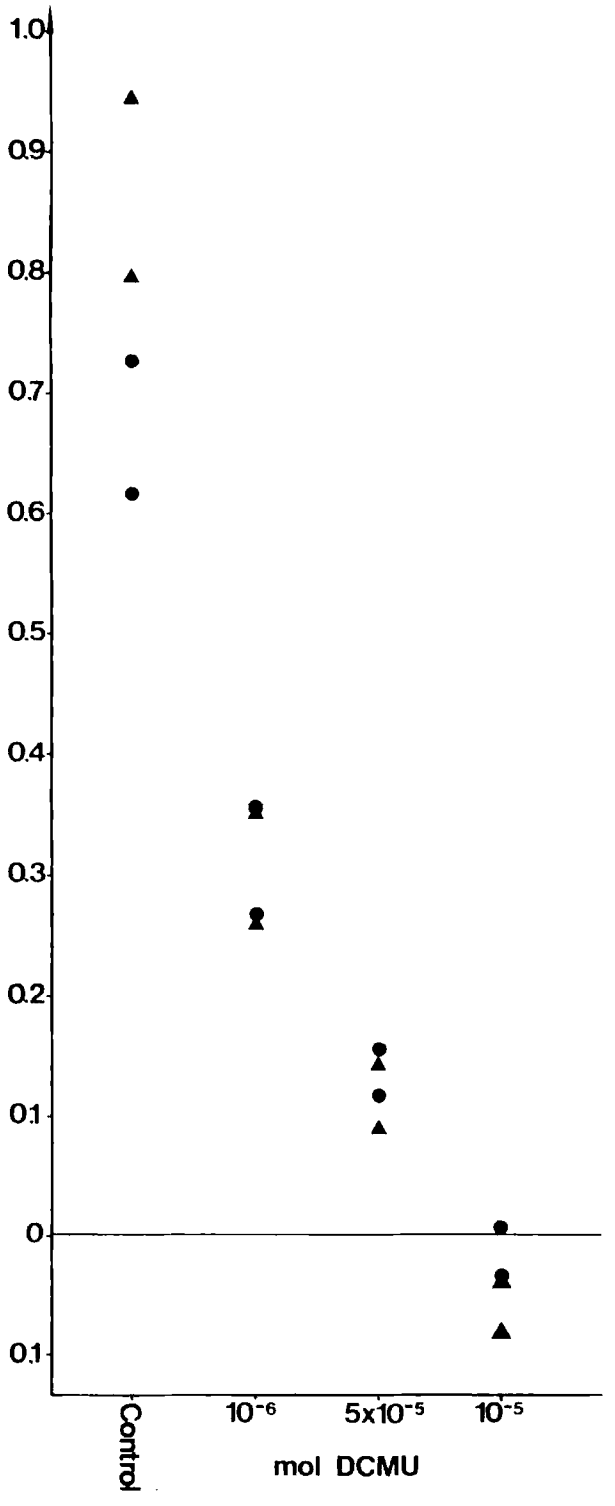


Fig. 5. gives the amount of carbonate (in mmol) precipitated in experiments with various amounts of the photosynthesis inhibitor DCMU and an untreated control experiment (10/11/89). The amount of precipitated carbonate decreases with an increasing inhibitor concentration. At a concentration of 10^{-5} mol DCMU the net result is carbonate dissolution, because the amount of (uninhibited) respiration in the cyanobacteria exceeds the amount of photosynthesis. Precipitation was estimated from changes in calcium (solid circles) and alkalinity (triangles).

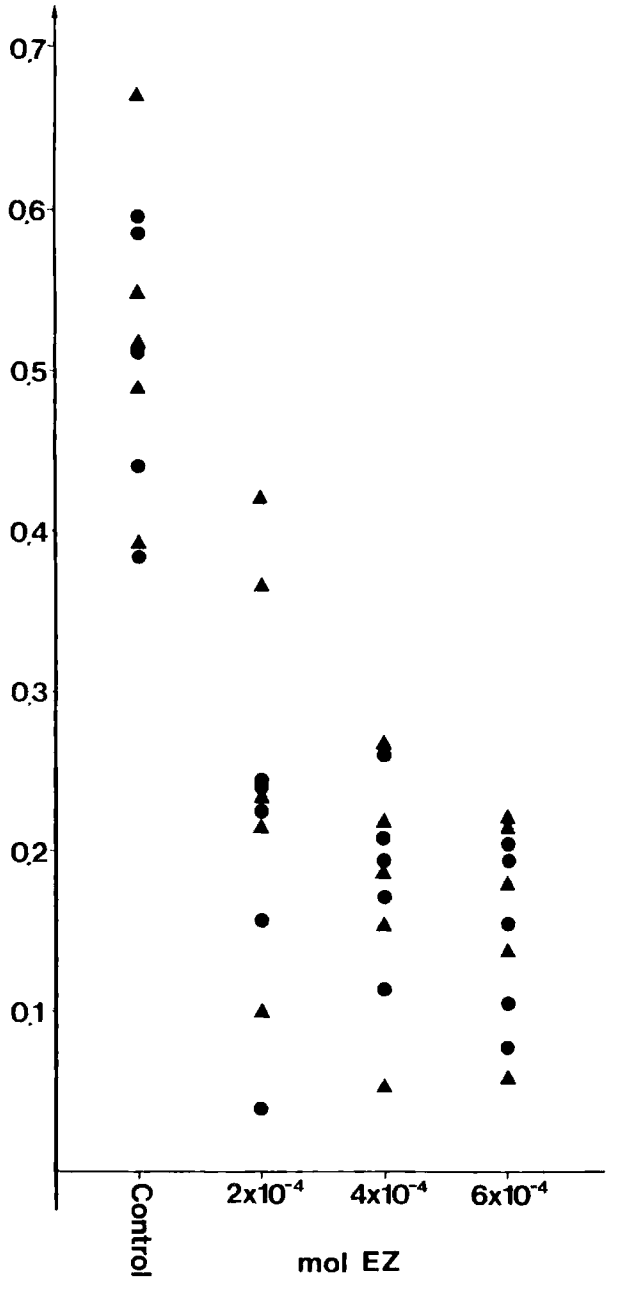


Fig. 6. gives the amount of precipiation in experiments with various amounts of the inhibitor ethoxyzolamide and an untreated control experiment. Precipitation decreases with an increasing concentration of the inhibitor. Precipitation was estimated from changes in calcium (solid circles) and alkalinity (triangles).

the natural conditions in temperature, illumination, and water composition. A disadvantage is that these factors cannot be controlled and are subject to both daily and seasonal variations.

4.6.1 Untreated Cyanobacteria

The amount of precipitation in containers with untreated controls was not only used as a reference for the inhibitor experiments, but also allows the evaluation of how closely the amount of precipitation measured in the experiments resembles the amount of precipitation under natural conditions. As the cyanobacteria start to grow soon after the site is completely covered by freshwater, the age of the cyanobacteria is about the same as the duration of the wet season. The cyanobacteria used in the experiments on January 11th to 13th had a carbonate content of 1.3 to 1.9 g. They precipitated this amount in 180 days, from July to December 1989. This gives a daily amount of precipitation of 7.2 to 10.5 mg carbonate. In the experiments they precipitated an net amount of 15 mg (0.150 mmol, January 11./12.) and 5 mg (0.050 mmol, January 12./13.). This calculation shows that at least in experiments of several hours or a few days the amount of precipitated carbonate corresponds to precipitation under natural conditions.

4.6.2 Experiments with Inhibitors

Precipitation starts in the morning with the onset of photosynthesis and ends in the evening when photosynthesis ceases. To actually prove, however, that precipitation depends on photosynthesis, cyanobacteria were treated with the inhibitor DCMU (Dichlorphenyldimethylurea). This inhibitor blocks the electron transport from photosystem II to photosystem I in photosynthesis. For *Synechococcus* a 10^{-5} molar DCMU solution was shown to completely inhibit photosynthesis (Badger & Andrews, 1982).

Because of the poor solubility of DCMU in water, appropriate amounts of a concentrated stock solution of DCMU in methanol (0.012 g in 50 ml methanol) were added to sampling site water to get the desired final DCMU concentrations of 10^{-5}, $5x10^{-6}$, and 10^{-6} mol DCMU. The maximum methanol concentration of 1 % showed no negative effect on the viability of the cyanobacteria.

Fig. 5 shows the results of an experiment with various concentrations of DCMU (10^{-6}, $5x10^{-6}$, 10^{-5}; 10/11/89). It is obvious that the amount of precipitated carbonate decreases with the increasing concentration of the inhibitor, corresponding to the decreasing photosynthetic activity. When transferred from the DCMU solution to pure water, the cyanobacteria immediately began to photosynthesize and carbonate was precipitated.

Ethoxyzolamide

Ethoxyzolamide (EZ) is an inhibitor for the activity of the enzyme carboanhydrase (CA), which catalyses the conversion of HCO_3^- to CO_2 (Chapter 2.2). In which way EZ affects photosynthesis of cyanobacteria is yet not clear (5.1). Appropriate amounts of a stock solution of EZ in DMSO (Dimethylsulfoxide) were added to incubated cyanobacteria to give final inhibitor concentrations of $2x10^{-4}$, $4x10^{-4}$, and $6x10^{-4}$ mol. As a consequence of the addition of EZ, the pH dropped in the containers 0.04 to 0.1 pH units. All the inhibitor experiments therefore inevitably have a slightly lower initial pH.

Fig. 6 shows the amount of carbonate precipitated by controls and by cyanobacteria treated with various EZ concentrations. It can be seen that the amount of precipitation decreases with the increasing concentration of the inhibitor.

Comparing the amounts of dissolution occurring over night in all the samples, it can be seen that there is as much dissolution in the inhibitor experiments as in the control

samples, because the inhibitor does not affect the pentose-phosphate pathway and thus CO_2 release and dissolution. The net result in 24 hours is therefore precipitation in the controls, but dissolution for the EZ treated cyanobacteria.

Calculating the ratio of photosynthesis to precipitation for the EZ experiments, gives values significantly different from the ones in the control samples Tab. 2). While in the controls the ratio lies around 1.15 to 1 are the values for the EZ experiments considerably higher, up to 4 to 1. The inhibitory effect on precipitation is stronger than on photosynthesis. The ratio of respiration to dissolution occuring over night, shows, as expected, no differences between the untreated cyanobacteria and the EZ samples. This ratio is, however, always slightly more variable than the ratio of photosynthesis to precipitation, varying beween 1 to 2 mol respired carbon for every mol dissolved carbonate.

DMSO

PRICE & BADGER (1989 b) found a slightly negative effect on photosynthesis by DMSO concentrations above 0.2%. Therefore some experiments were run to test the effect of the DMSO concentrations used in the EZ experiments. In these tests, no influence of pure DMSO were found, neither on photosynthesis nor on carbonate precipitation.

5 DISCUSSION

PENTECOST (1988) concluded that the high amount of carbonate as compared to organic material in the green alga *Gongrosira* sp. was an indicator of a high incidence of inorganic precipitation. In tufas the amount of organic material derived from cyanobacteria is estimated to be as low as 1 to 0.3-0.4 % (PENTECOST, 1978; 1985). RAVEN et al. (1986) measured the ratio of organic material versus carbonate in characeans and found more organic carbon than carbon in carbonate. They interpreted this as a reflection of little inorganic influence on precipitation.

The ratio of organic versus inorganic carbon seems to be an indicator of the relative percentage of organic and inorganic influences on precipitation. A ratio of more than 1:1 is the result of organic dominated precipitation, while a higher amount of carbonate than organic carbon (e. g. a ratio below 1) reflects the influence of inorganic factors. The crystal morphology can in both cases be specifically influenced by the organic material, which might provide the nucleation sites for precipitation.

In the Everglades, the amount of carbon precipitated in carbonate is always lower than the amount of organic carbon. The restriction of calcification to the sheath also indicates biological precipitation. If inorganic factors were the cause of precipitation, calcification would be expected to exceed the outer sheath, because of continous growth following crystal nucleation. All these observations suggest active carbonate precipitation by cyanobacteria.

5.1 The Mechanism of Precipitation

Cyanobacteria grown under high CO_2 conditions (above equilibrium with the atmosphere) have the ability to actively take up CO_2 and transport it into the cells. When grown under low CO_2 conditions (equilibrium with the atmosphere and less) they activate a mechanism which allows them to take up bicarbonate as well. Bicarbonate use causes the release of equivalent amounts of OH^- ions, which are produced by the conversion of HCO_3^- to CO_2 (KAPLAN, 1985; SCHERER et al., 1988 a; PRINS & ELZENGA, 1989). It was shown by MILLER & COLMAN (1980) for *Coccochloris* sp. that the amount of OH^- ions released into the medium exactly equals the amount of $NaHCO_3$ provided for photosynthesis. The mechanism of DIC uptake is presently discussed, and there are still considerable uncertainties. It is not yet clear, if one or two transport systems are involved in the uptake of the two carbon species, and how the pumps work. The pump, however, appears to have a carboanhydrase-like moiety in the cell wall (BADGER et al., 1985; BADGER & PRICE, 1989; ESPIE et al., 1989; MILLER & COLMAN, 1980; PRICE & BADGER, 1989 a;). EZ might inhibit the uptake mechanism by impeding the CA-moiety of the pump and/or by inhibiting the intracellular conversion of bicarbonate to CO_2 and OH^- (PRICE & BADGER, 1989 a,b). The experimental results show, however, different effects of EZ in different species, possibly indicating differences in the structure of the pump systems. There also may be variations caused by environmental conditions, with cells grown in the presence of low CO_2 concentrations using a more complex mechanism than those functioning in an environment more rich in CO_2 (PRICE & BADGER, 1989 b). VOLOKITA et al. (1984) found a stronger inhibition of CO_2 uptake than of HCO_3^- uptake in *Anabaena variabilis*. According to PRICE & BADGER, *Synechococcus* posses one pump, transporting CO_2 as well as HCO_3^- into the cells. In their experiments EZ affects the uptake of both species equally, which they interpret as an indication of inhibition of the pump system. An additional effect of EZ on the intracellular CA activity is possible (PRICE & BADGER, 1989 b). ESPIE et al. (1989; *Synechococcus*), however, propose an uptake mechanism consisting of two pump systems because, according to the authors, the uptake of each of the two carbon species can be specifically inhibited.

The experimental results from the Everglades suggest preferential inhibition of bicarbonate uptake. An equal inhibition of both bicarbonate and CO_2 uptake should lead to an equal decrease in photosynthesis and precipitation. The preferential decrease in precipitation shows that the two processes can, to a certain degree, be separated. Taking into account the considerable differences between the experimental conditions and the natural conditions existing in the Everglades, and, in addition, the different species involved, differences are not surprising.

The quantitative relationship of photosynthesis and precipitation and the preferential inhibition of precipitation by EZ, suggest a model in which precipitation is linked to photosynthetic bicarbonate use rather than general DIC uptake. Fig. 7 shows the proposed model in *Scytonema*. HCO_3^- is taken up, CO_2 is used for photosynthesis, and OH^- ions are released from the cells. In the sheath they either react with another HCO_3^- ion to form water and a carbonate ion, or they are neutralized by the uptake of H^+ ions (among others: ESPIE et al., 1989). Either way, there are carbonate ions formed in the immediate vicinity of the cell. As the percent-

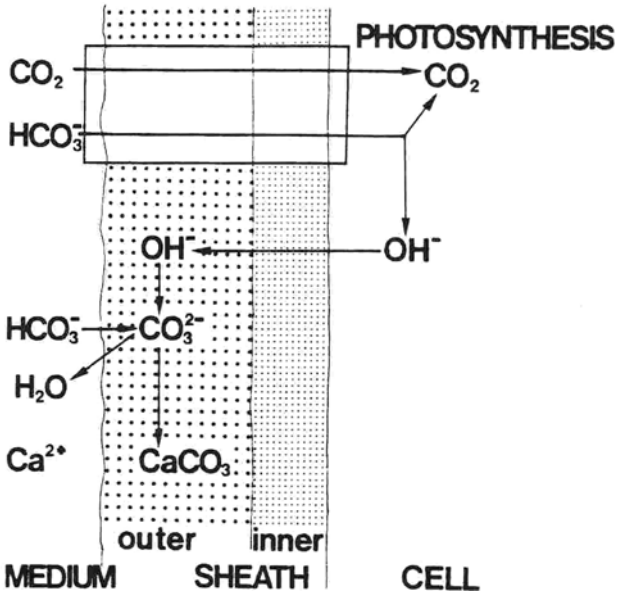


Fig. 7. Schematic model for precipitation in *Scytonema*. DIC is taken up, either in form of CO_2 or HCO_3^-, by an active pump system. The pump system is indicated by the frame. It's structure is presently discussed. When HCO_3^- is the carbon species used for photosynthesis, hydroxyl ions are liberated. Either they are released by the cells, or H^+ ions are taken up for neutralization. Either way there are carbonate ions quantitatively formed in the outer sheath, subsequently leading to precipiation by attracting calcium ions.

age of HCO_3^- use in photosynthesis increases, more carbonate ions are formed. In cyanobacteria the sheath acts as a diffusion barrier resulting in a local increase in the concentration of carbonate ions. The carbonate ions react with calcium being absorbed by the polysaccharides of the sheath, and calcium carbonate is precipitated. When CO_2 is the carbon species taken up, no OH^- ions are formed and no precipitation occurs. Therefore the ratio of organically to inorganically fixed carbon depends on the relative percentage of HCO_3^- in photosynthesis. If the percentage is low, due to low pH values or the inhibition of HCO_3^- use, the amount of photosynthesis is relatively high as compared to the amount of precipitation. This is demonstrated by the results of the EZ experiments, in which up to four times as much carbon was taken up for photosynthesis than was precipitated in carbonate. If the percentage of HCO_3^- used in photosynthesis is high, then a ratio of photosynthesis to precipitation of 1 can result.

The result of this purely biological calcification might be an impregnation of the sheath, while an encrustation indicates inorganic precipitation. In this case, sheath impregnation should preferentially occur in environments where photosynthetic bicarbonate use occurs.

5.2 Biological Influence

The restriction of calcification to the outer sheath in *Scytonema* and the different crystal morphologies in the two taxa, despite all the natural variations in ion concentration, temperature, and probably crystallization rate throughout the wet season, suggest a taxa specific influence of the cyanobacteria over calcification.

The crystal morphology can be strongly influenced by the rate of crystallization (ADDADI & WEINER, 1985), which depends on the rate of ion supply as well as on the rate of ion incorporation into the crystal lattice. Variations in sheath structures and compositions, by allowing different diffusion rates for Ca^{2+}-ions, might lead to different crystallization rates, subsequently resulting in differences in crystal morphology. For example, higher crystallization rates in *Schizothrix* might have lead to the growth of dendritic crystals, while the rhombohedral crystals in *Scytonema* might be due to a relatively slower growth rate. It has been demonstrated that the polysaccharides of the sheaths can serve as an interface for mediating and controlling crystal nucleation because of their high affinity for Ca^{2+} ions (SOMERS & BROWN, 1978), onto which CO_3^{2-} ions are subsequently absorbed (Ionotrophy; GREENFIELD et al., 1984). In addition, WECKESSER et al. (1988) showed a different capacity for calcium absorption in different cyanobacterial genera. Those differences could also be reflected in the distinct crystal morphologies observed in *Scytonema* and *Schizothrix* (MERZ & ZANKL, submitted).

5.3 Environmental Influence

Calcifying cyanobacteria only occur in waters supersaturated with respect to carbonate, but even there calcification is restricted to certain genera (*Homoethrix, Phormidium, Plectonema, Rivularia, Schizothrix, Scytonema;* PENTECOST & RIDING, 1986). None of these genera, however, are obligate calcifiers.

The occurrence of calcified and uncalcified cyanobacteria living under the same environmental conditions indicates that supersaturation is not always sufficient for calcification.

The results from this study show that two other factors can be of major importance. One is bicarbonate uptake by cyanobacteria, and the other is the suitability of the sheath for carbonate nucleation. Any environmental factor that influences either one of these factors will influence the cyanobacteria's ability to calcify.

The bicarbonate pump is induced when the CO_2 supply from the ambient water is low relative to the potential rate of photosynthesis. It should be active in an environment where CO_2 concentrations are low, either due to low total DIC concentrations or to high pH values. But also in environments where high light intensities sustain or even require especially high rates of photosynthesis, bicarbonate uptake might be advantageous for the reduction of photoinhibition (Chapter 2.2). In these environments photosynthetic bicarbonate use could then lead to carbonate precipitation.

Structure and composition of the sheath also seem to be essential for calcification. Differences among species in their affinity for calcium were described by WECKESSER et al. (1988). But the affinity for calcium might also depend on environmental conditions (WHEELER & SIKES, 1984; GREENFIELD et al., 1984), thus possibly changing the suitability of the sheath for nucleation. The thickness of the

sheath as a diffusion limited site for precipitation is also influenced by environmental conditions (TUFFERY, 1969).

5.4 Possible Biological Significance of Calcification

The question about the environmental factors influencing calcification is closely linked to the question about possible benefits for the cyanobacteria. In principle there seem to be no differences in the calcification of cyanobacteria and of calcareous algae. In algae, calcification depends on the activity of the enzyme carboanhydrase, the uptake of bicarbonate for photosynthesis (LUCAS, 1979; SIKES & WILBUR, 1982; BOROWITZKA, 1990), or on the presence of a polysaccharide matrix. Green algae calcify extracellulary, either in diffusion limited intracellular spaces (*Halimeda*; BOROWITZKA & LARKUM, 1977), or within sheath material containing polysaccharides (*Penicillus*, *Udotea*; BOROWITZKA, 1986). DCMU inhibits precipitation in *Halimeda*, as it does in cyanobacteria, while diamox, an inhibitor for carboanhydrase activity, greatly reduces calcification (BOROWITZKA & LARKUM, 1976). As in cyanobacteria seem the polysaccharides in *Halimeda* to control the orientation of the crystals to a certain degree. It was shown that in characeans (chlorophyceae, chlorophyta) precipitation occurs in localized bands of OH^- release, which are formed by the use of bicarbonate in photosynthesis (LUCAS, 1979; BOROWITZKA, 1982). There seems to be no organic material involved in precipitation by characeans.

In the calcification of corallinaceans (rhodophyta) and coccolithophoridae, obviously under biologic control, polysaccharides play an important role. Corallinaceans precipitate within the cell wall. The orientation of the crystals is regulated by organic molecules.

These comparisons show that some forms of calcification in cyanobacteria and calcareous algae may basically be the same. Therefore there is, for cyanobacteria as for calcareous algae, the question, if there is any beneficial effect from calcification or precipitation for the organism as is suggested by the restriction of calcification to certain taxa and the control calcifying cyanobacteria can exert on precipitation.

5.4.1 Light Protection

When there is more light energy absorbed than can be used in photosynthesis for the formation of organic molecules, a severe damage of the reaction centers in the photosynthetic apparatus might occur. The rate of photosynthesis can be reduced or the reaction centers might be irreversibly damaged. Some of the carotenoids in cyanobacteria absorb the potentially hazardous radiation and thus protect the photosynthetically active pigments. It has been proposed that the calcification of some cyanobacteria and algae might have a light shading function (Cyanobacteria, VAN LIERE & WALSBY, 1982; Corallinaceae, LITTLER, 1976; Characeae, RAVEN et al., 1986). The same has been proposed for the fossil calcifying cyanobacteria *Renalcis* and *Epiphyton* (ROWLAND & GANGLOFF, 1988).

Calcifying cyanobacteria occur, besides being common in tufa deposits and similar environments, often in areas of extremely shallow water where they are exposed to very high light intensities. Comparing the measured light intensities in the Everglades with literature data on the intensity optimum for photosynthesis by cyanobacteria it can be seen that the optimum values are exceeded more than ten fold. For some strains WALSBY (1968) and HÄDER (1987) give values of 500 to 1000 lx. For mat forming Oscillatoriaceans in a natural environment the light optimum is about 3000 lx, while they show photophobic reactions when the irradiance exceeds 10000 lx (PENTECOST, 1984). CASTENHOLTZ (1982) describes phototactic movements up to 10000 lx. Generally, cyanobacteria prefer low light intensities.

There are two ways, how a strong light irradiance might induce conditions favourable for carbonate precipitation.

The stress of living in high light environments could induce secretion of sheath material neccessary for precipitation (KAZMIERCZAK et al., 1990; SCHERER et al., 1988 b), and a calcified sheath might physically shade the filament. But high light intensities might also affect precipitation via an induction of the bicarbonate pump. Some of the absorbed light energy has to be used for the maintenance of the pump, and would thus not be able to damage the photosynthetic apparatus. Calcification would then occur as a secondary consequence of the high radiation.

5.4.2 Ion Sink

Precipitation might help to buffer the pH rise caused by CO_2 uptake during photosynthesis. This was proposed by RAVEN et al., (1986) for characeans and by PAASCHE (1964) and SIKES et al. (1980) for coccoliths. The hydroxyl ions would lead to rapid increase of pH, if they would be released into a weakly buffered medium. By their reaction with an extracellular bicarbonate ion and the subsequent precipitation of carbonate, the pH rise is reduced. Therefore carbonate precipitation might allow higher rates of photosynthesis in weakly buffered waters. The occurrence of calcifying cyanobacteria, however, does not seem to be restricted to environments with a low natural buffer capacity.

Carbonate precipitation could also be used as a sink for carbonate ions being inevitably formed as a consequence of hydroxyl ion release. Carbonate ions cannot be used in photosynthesis. Therefore they may have a negative effect on bicarbonate uptake when they get attached to the pump and block it (LUCAS, 1983). The carbonate precipitation might be a way to protect the pump from the carbonate ions, thus ensuring a constant high rate of photosynthesis by unimpaired DIC uptake. The effect would be similar to the spatial separation of bicarbonate uptake and hydroxyl release in characeans (LUCAS, 1983).

Although there are too few experimental data on the calcification of cyanobacteria to decide, what factors finally cause precipitation, it can be seen that there are various ways how the organisms may benefit from it.

ACKNOWLEDGEMENTS

This paper is a summary of a PhD thesis supervised by Prof. H. Zankl, Marburg. I thank Prof. R.N. Ginsburg, Miami, for hospitality at Fisher Island, Prof. P.K. Swart,

Miami, for generously allowing me to use his lab facilities and for measuring the stable isotopes, Prof. S. Golubic, Boston, for valuable discussions and determination of the cyanobacteria, Prof. L. Brand, Miami, for his support, A. Titze, Marburg, for the SEM photographs, Dr. G.M. Grammer, Miami, for proof reading the English text, and the Everglades National Park Service for the sampling permit.

The research was founded with a stipend for graduate students by the German Academic Exchange Service to M. Merz and a research grant to H. Zankl by the German Science Foundation (Za 22/33-1). An anonymous reviewer gave hints to impove the manuscript.

REFERENCES

ADDADI, L. & WEINER, S. (1985): Interactions between acidic proteins and crystals: Stereochemical requirements in biomineralization. - Proc. Natl. Acad. Sci. U.S.A., **82**/6, 4110-4114, 5 Figs., Washington

BADGER, M.R. & ANDREWS, T.J. (1982): Photosynthesis and inorganic carbon usage by the marine cyanobacterium, *Synechococcus* sp.. - Plant Physiol., **70**/2, 517-523, 9 Figs., 1 Tab., Lancaster, Pa.

BADGER, M.R., BASSETT, M. & COMMINS, H.N. (1985): A model of HCO_3^- accumulation and photosynthesis in the cyanobacterium *Synechococcus* sp.. - Plant Physiol., **77**/2, 465-471, 7 Figs., Lancaster, Pa.

BADGER, M.R. & PRICE, G.D. (1989): Carbonic anhydrase activity associated with the cyanobacterium *Synechococcus* PCC 7942. - Plant Physiol., **88**/1, 51-60, 2 Tabs., 5 Figs., Lancaster, Pa.

BOROWITZKA, M.A. (1982): Mechanisms in algal calcification. - In: ROUND, F.E., CHAPMAN, D.J. (eds.): Progress in Phycological Research, vol. **1**, 137-178, 11 Figs., 2 Tab., Amsterdam (Elsevier Biomedical Press)

-- (1986): Physiology and biochemistry of calcification in the Chlorophyceae. - In: LEADBEATER, B.S.C., RIDING, R. (eds.): Biomineralization in Lower Plants and Animals. – The Systematic Association, Spec. Vol. **30**, 400 pp., 107-124, 2 Figs., 1 Tab., Oxford (Clarendon Press)

BOROWITZKA. M.A. (1989): Carbonate calcification in algae-Initiation and control. - In: MANN, S., WEBB, J., WILLIAMS, R.J.P. (eds.): Biomineralization: chemical and biochemical perspectives. – 541 pp., 63-94, 10 Figs., 4 Tabs., Weinheim (VCH Verlagsgesellschaft mbH)

BOROWITZKA, M.A. & LARKUM, A.W.D. (1976): Calcification of the green algae *Halimeda*; IV. The action of metabolic inhibitors on the photosynthesis and calcification. - J. Exp. Bot., **27**, 894-907, 5 Figs., 4 Tabs., Oxford

-- & -- (1977): Calcification in the green algae *Halimeda*; I; An ultrastucture study of thallus development. - J. Phycol., **13**/1, 6-16, 24 Figs., New York

BRAITHWAITE, C.J.R., CASANOVA, J., FREVERT, F., WHITTON, B.A. (1989): Recent stromatolites in landlocked pools on Aldabra, western Indian Ocean. - Paleogeogr., Paleoclimat., Paleoecol., **69**/3-4, 145-165, 4 Pls., 23 Figs., Amsterdam

CALDER, J.A., PARKER, P.L. (1973): Geochemical implications of induced changes in 13C fractionation by blue-green algae. - Geochim. Cosmochim. Acta, **37**/1, 133-140, 2 Figs., 3 Tabs., New York

CASTENHOLZ, R.W. (1982): Motility and taxes. - In: CARR, N.G. & WHITTON, B.A. (eds.): The Biology of Cyanobacteria. – 688 pp., 414-439, Oxford (Blackwell)

COX, G., JAMES, J.M., LEGGETT, K.E.A., OSBORNE, R., ARMSTRONG, L. (1989): Cyanobacterially deposited speleothems: subaerial stromatolites. - Geomicrobiol. J., **7**, 245-252, 7 Figs., New York

CUMMINGS, C.E., MCCARTHY, H.M. (1982): Stable carbon istope ratios in *Astrangia danae:* evidence for algal modification of carbon pools used in calcification. - Geochim. Cosmochim. Acta, **46**/6, 1125-1129, 2 Figs., 2 Tabs., New York

DEFARGE, C., TRICHET, J., SIN, P. (1985): First data on the biogeochemistry of Kopara deposits from Rangiroa Atoll. - Proceed. 5th Int. Coral Reef Congress, Tahiti, **3**, 365-370, 6 Figs., 2 Tabs., Moorea

DREVER, J. (1988): The Geochemistry of Natural Waters. - 2nd Ed., 437 pp., Englewood Cliffs (Prentice Hall)

EPSTEIN, S. & MAYEDA, T. (1953): Variation of ^{18}O content of waters from natural sources. - Geochim. Cosmochim. Acta, **4**/5, 213-224, 3 Figs., 1 Tab., New York

ESPIE, G.S., MILLER, A.G. & CANVIN, D.T. (1989): Selective and reversible inhibition of active CO_2 transport by hydrogen sulfide in a cyanobacterium. - Plant. Physiol., **91**/1, 387-394, 7 Figs., Lancaster, Pa.

ESTEP, M.F. (1984): Carbon and hydrogen isotopic compositions of algae and bacteria from hydrothermal environments, Yellowstone National Park. - Geochim. Cosmochim. Acta, **48**/3, 591-599, 6 Figs., 10 Tabs., New York

FRITZ, P., POPLAWSKI, S. (1974): 18O and 13C in the sheaths of freshwater molluscs and their environments. - Earth Planet. Sci. Lett., **24**, 91-98, 6 Figs., 1 Tab., Amsterdam

GIESKES, J. (1986): Water chemistry procedures aboard Joides Resolution - some coments. - Ocean Drilling Program, Technical Note No. **5**, 46 pp., College Station

GIRAUD, G. & CABIOCH, J. (1979): Ultrastructure and the elaboration of calcified cell-walls in the coralline algae (Rhodophyta, Cryptonemiales). - Biol. Cellulaire, **36**, 81-86, Paris

GLEASON, P.J. (1972): The origin, sedimentation and stratigraphy of a calcitic mud located in the southern fresh-water Everglades. - PhD-Thesis, Pennsylvania State Univ., 355 pp., University Park

GLEASON, P.J. & SPACKMAN, W.JR. (1974): Calcareous periphyton and water chemistry in the Everglades. - In: GLEASON, P.J. (ed.): Environments in South Florida, Present and Past. – 146-181, 34 Figs., 9 Tab., Miami

GOLUBIC, S. (1972): The relationship between blue-green algae and carbonate deposits. - In: CARR, N.G. & WHITTON, B.A. (eds.): The Biology of Blue-Green Algae. – 676 pp., 434-472, 19 Figs., Oxford (Blackwell)

-- (1983): Stromatolites, fossil and recent: a case history. - In: WESTBROEK, P., DE JONG, E.W. (eds.): Biomineralization and biological metal accumulation. – 313-326, 6 Figs., Dordrecht (Reidel)

GOLUBIC, S. & CAMPBELL, S.E. (1981): Biogenically formed aragonite concretions in marine *Rivularia*. - In: MONTY, C. (ed.): Phanerozoic Stromatolites: Case histories. – 249 pp., 209-229, 3 Pls., 2 Figs., 1 Tab., Berlin (Springer)

GRAN, G. (1952): Determination of the equivalence point in potentiometric titrations, Part I. - Analyst, **77**, 661-671, London

GREENFIELD, E.M., WILSON, D.C. & CRENSHAW, M.A. (1984): Ionotropic nucleation of calcium carbonate by molluscan matrix. - Am. Zool., **24**/4, 925-932, 7 figs., Bloomington, Indiana

HÄDER, D.-P. (1987): Photomovement. - In: FAY, P., VAN BAALEN, C. (eds.): The Cyanobacteria. – 325-345, 10 Figs., 1 Tab., Amsterdam (Elsevier)

HARDIE, L.A. & GINSBURG, R.N. (1977): Layering: The origin and environmental significance of lamination and thin bedding. - In: HARDIE, A. (ed.): Sedimentation on the Modern Carbonate Tidal Flats of NW Andros-Island, Bahamas. – Johns Hopkins Univ. Studies in Geol., **22**, 203 pp., 50-123, 94 Figs., 16 Tabs., Baltimore

HELDER, R.J. (1988): A quantitative approach to the inorganic carbon in aqueous media used in the biological research; dilute solutions isolated from the atmosphere. - Plant Cell Environment, **11**, 211-230, 4 Figs., 5 Tabs., Oxford

HORODYSKI, R.J. & VONDER HAAR, S.P. (1975): Recent calcareous stromatolites from Laguna Mormona (Baja California) Mexico. - J. Sed. Petrol., **45**/4, 894-906, 7 Figs., Tulsa

JONES, B. & KAHLE, C.F. (1986): Dendritic calcite crystals formed

by calcification of algal filaments in a vadose environment. - J. Sed. Petrol., **56/2**, 217-222, 7 Figs., Tulsa

KAPLAN, A. (1981): Photoinhibition in *Spirulina platensis*: Response of photosynthesis and HCO_3^- uptake capability to CO_2-depleted conditions. - J. Exp. Bot., **32/2**, 669-677, 4 Figs., 1 Tab., Oxford

-- (1981): Photoinhibition in *Spirulina platensis*: Response of photosynthesis and HCO_3^- uptake capability to CO_2-depleted conditions. - J. Exp. Bot., **32/2**, 669-677, 4 Figs., 1 Tab., Oxford

-- (1985): Adaptation to CO_2 levels: Induction and the mechanism for inorganic carbon uptake. - In: LUCAS, W.J. & BERRY, J.A. (eds.) Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms,. – Am. Soc. Plant Physiol., 325-338, 9 Figs., Rockville, MD.

KAPLAN, A., MARCUS, Y., ZENVIRTH, D., OMATA, T., REINHOLD, L. & OGAWA, T. (1987): The mechanism of inorganic carbon uptake by cyanobacteria: energetization and activation by light. - In: BIGGINS, J. (ed.): Progress in Photosynthesis Research, vol. IV, 6.301-6.307, 8 Figs., Dordrecht (M.Nijhoff Publ.)

KAZMIERCZAK, J., ITTEKOT, V. & DEGENS, E.T. (1985): Biocalcification through time: environmental challenge and cellular response. - Paläontolog. Zeitschr., **59/1-2**, 15-33, 6 Figs., Stuttgart

KEITH, M.L. & WEBER, J.N. (1965): Systematic relationships between carbon and oxygen isotopes in carbonates deposited by modern corals and algae. - Science, **150**, 498-501, 2 Figs., 1 Tab., Washington

KEMPE, S. & KAZMIERCZAK, J. (1990): Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. - In: ITTEKKOT, V, KEMPE, S., MICHAELIS, W., SPITZY, A. (eds.): Facets of modern biogeochemistry, 433 pp., 161 Figs., 255-278, 4 Figs., 4 Tabs., Berlin (Springer)

KRAUSE, G.H. (1988): Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. - Physiol. Plant., **74/3**, 566-574, 2 Figs., Copenhagen

KRUMBEIN, W.E. & GIELE, C. (1979): Calcification in a coccoid cyanobacterium associated with the formation of desert stromatolites. - Sedimentology, **26/4**, 593-604, 9 Figs., Amsterdam

Krumbein, W.E. & Potts, M. (1979): Girvanella-like stuctures formed by Plectonema gloeophilum (Cyanophyta) from the Borrego desert in Southern California. - Geomicrobiol. J., **1/3**, 211-217, 4 Figs., 1 Tab., New York

LEINFELDER, R.R. (1985): Cyanophyta calcification, morphotypes and depositional environments (Alenquer oncolite, Upper Kimmeridgian?, Portugal). - Facies, **12**, 253-274, 2 Pls., 3 Figs., 2 Tab., Erlangen

LITTLER, M.M. (1976): Calcification and its role among macroalgae. - Micronesia, **12/1**, 27-41, 7 Figs., 3 Tabs., Agana

LUCAS, W.J. (1979): Alkaline band formation in Chara corallina. - Plant Physiol., 63/2, 248-254, 9 Figs., 1 Tab., Lancaster, Pa.

LUCAS, W.J. (1983): Photosynthetic assimilation of exogenous HCO3- by aquatic plants. - Ann. Rev. Plant Physiol., **34**, 71-104, 5 Figs., Palo Alto, Pa.

LYONS, B.W., LONG, D.T., HINES, M. E., GAUDETTE, H.E., ARMSTRONG, P. B. (1984): Calcification of cyanobacterial mats in Solar Lake, Sinai. - Geology, **12/10**, 623-626, 1 Fig., 2 Tabs., Boulder

MCCONAUGHEY, T. (1989): ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. patterns. - Geochim. Cosmochim. Acta, **53/1**, 151-162, 15 Figs., 2 Tabs., New York

MERZ, M.U.E. (1990): Karbonatfällung durch Cyanobakterien im Süßwasserbereich der Everglades, Florida. - unpubl. PhD-Thesis, Univ. of Marburg, 71 pp., 19 Figs., 7 Tabs., Marburg

MERZ, M.U.E. & ZANKL, H. (submitted): The influence of the sheath on carbonate precipitation by cyanobacteria. - submitted to Boll. Soc. Pal. Italiana, Modena

MILLER, A.G. & COLMAN, B. (1980): Evidence for HCO_3^- transport by the blue.green alga (cyanobacterium) *Coccochloris peniocystis*. - Plant Physiol., **65/2**, 397-402, 7 Figs., Lancaster, Pa.

MILLER, A.G., ESPIE, G.S. & CANVIN, D.T. (1990): Physiological aspects of CO_2 and HCO_3^- transport by cyanobacteria: a review. - Can. J. Bot., **68/6**, 1291-1302, 10 Figs., Ottawa

MILLERO, F.J. (1979): The thermodynamics of the carbonate systems in seawater. - Geochim. Cosmochim. Acta, **43/10**, 1651-1661, 9 Figs., 9 Tabs., New York

MONTY, C.L.V. (1972): Recent algal stromatolitic deposits, Andros Island, Bahamas, Preliminary report. - Geol. Rdsch., **61**, 742-783, 32 Figs., 1 Tab., Stuttgart

MOOK, W.G. & VOGEL, J.C. (1968): Isotopic equilibrium between shells and their environment. - Science, **159**, No. 3817, 1 Fig., Washington

MÜLLER-JUNGBLUTH, W.-U. (1968): Sedimentary petrologic investigation of the Upper Triassic 'Hauptdolomit' of the Lechtaler Alps, Tirol, Austria. - In: MÜLLER, G. & FRIEDMAN, G. (eds.) Recent Developments in Carbonate Sedimentology in Central Europe. – 255 pp., 228-239, 14 Figs., Berlin (Springer)

-- (1970): Sedimentologische Untersuchung des Hauptdolomit der Östlichen Lechtaler Alpen, Tirol. - Festbd. Geol. Inst. 300-J.-Feier Univ. Innsbruck, 255-308, Innsbruck

OBENLÜNESCHLOSS, J. & SCHNEIDER, J. (1990): Ecology and calcification patterns of Rivularia (Cyanobacteria). - In: ANAGNOSTIDIS, K., HICKEL, B. & KOMAREK, J. (eds.)Proc. 11th Symp. IAC, – Arch. Hydrobiol., Suppl. Vol, 'Algological Studies', Berlin

OGAWA, T. & KAPLAN, A. (1987): A model for inorganic carbon accumulation in cyanobacteria. - In: BIGGINS, J. (ed.): Progress in Photosynthesis Research, vol. IV. – 6.297-6.300, 3 Figs., 1 Tab., Dordrecht (M.Nijhoff Publ.)

PAASCHE, E. (1964): A tracer study of the inorganic carbon uptake during coccolith formation and photosynthesis in the coccolithophorid Coccolithus huxleyi. - Physiol. Plant. (suppl.) III, 1-82, Copenhagen

PENTECOST, A. (1978): Blue-green algae and freshwater carbonate deposits. - Proc. R. Soc. London, Ser. B, **200**, 43-61, 1 Pl., 11 Figs., 10 Tab., London

-- (1980): Calcification in plants. - Int. Rev. Cytol., **62**, 1-26, 2 Tabs., New York

-- (1984): Effects of sedimentation and light intensity on a mat-forming Oscillatoriaceae with particular reference to *Microcoleus lyngbyaceus* GOMONT. - J. Gen. Microbiol., **130**, 983-990, 4 Figs., London

-- (1985): Association of cyanobacteria with tufa deposits: Identity, enumeration, and nature of the sheath material revealed by histochemistry. - Geomicrobiol. J., **4/3**, 286-297, 7 Tab., New York

-- (1988): Observations on the growth and calcium carbonate deposition in the green alga Gongrosira. - New Phytol., **110/2**, 2 Figs., 7 Tab., 249-253, London

PENTECOST, A. & RIDING, R. (1986): Calcification in cyanobacteria. - In: LEADBEATER, B.S.C., RIDING, R. (ed.) The Systematic Association, Spec. Vol. 30, Biomineralization in Lower Plants and Animals. – 400 pp., 73-90, 6 Figs., Oxford (Clarendon Press)

PRINS, H.B.A. & ELZENGA, J.T.M. (1989): Bicarbonate utilization: Function and mechanism. - Aquatic Bot., **34/1**, 59-83, 3 Figs., 1 Tab., Amsterdam

PRICE, G.D. & BADGER, M.R. (1989 a): Ethoxyzolamide inhibition of CO2 uptake in the cyanobacterium Synechococcus PCC 7942 without apparent inhibition of internal carbonic anhydrase activity. - Plant Physiol., **89/1**, 37-43, 8 Figs., Lancaster, Pa.

PRICE, G.D. & BADGER, M.R. (1989 b): Ethoxyzolamide inhibition of CO_2-dependent photosynthesis in the cyanobacterium Synechococcus PCC 7942. - Plant Physiol., **89/1**, 44-50, 3 Figs., 8 Tabs., Lancaster, Pa.

RAVEN, J.A. & LUCAS, W.J. (1985): Energy costs of carbon acquisition. - In: LUCAS, W.J., & BERRY, J.A. (eds.): Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. - Am. Soc. Plant Physiol., 305-325, Rockville

RAVEN, J.A., SMITH, F.A. & WALTER, N.A. (1986): Biomineralization in the Charophyceae sensu lato. - In: LEADBEATER, S.C., RIDING, R. (eds.) The Systematic Association, Spec. Vol. 30, Biomineralization in Lower Plants and Animals. – 400 pp., 125-139, 1 Tab., Oxford (Clarendon Press)

REINHOLD, L., ZVIMAN, M. & KAPLAN, A. (1987): Inorganic carbon

fluxes in cyanobacteria: A quantitative model. - In: BIGGINS, J. (ed.): Progress in Photosynthesis Research, vol. IV, 6.289-6.296, 6 Figs., Dordrecht (M.Nijhoff Publ.)

RIDING, R. (1977 a): Calcified *Plectonema* (blue-green algae), a recent example of *Girvanella* from Aldabra Atoll. - Palaeontology, **20**/1, 33-46, 1 Pl., 5 Figs., London

-- (1977 b): Problems of affinity in Paleozoic calcareous algae. - In: FLÜGEL, E. (ed.): Fossil algae, recent results and developements. – 375 pp., 202-211, 2 Tabs., Berlin (Springer)

-- (1982): Cyanophyte calcification and changes in ocean chemistry. - Nature, **299**, No. 5886, 814-815, 1 Fig., London

ROWLAND, S.M. & GANGLOFF, R.A. (1988): Structure and paleoecology of Lower Cambrian reefs. - Palaios, **3**, Reefs Issue, 111-135, 18 Figs., Tulsa

RUBINSON, M. & CLAYTON, R.N. (1969): Carbon-13 fractionation between aragonite and calcite. - Geochim. Cosmochim. Acta, **33**/8, 997-1002, 3 Tabs., New York

SABATER, S. (1989): Encrusting algal assemblages in a mediterranean river basin. - Arch. Hydrobiol., **114**/4, 555-573, 5 Figs., 6 Tabs., Berlin

SCHERER, S., RIEGE, H. & BÖGER, P. (1988 a): Light-induced proton efflux of the cyanobacterium *Anabaena variabilis*. - In: ROGERS, L.J., GALLON, J.P. (eds.) Biochemistry of the algae and cyanbacteria. – 121-129, Oxford (Clarendon Press)

SCHERER, S., CHEN, T.W. & BÖGER, P. (1988 b): A new UV-A/B absorbing pigment in the terrestrial cyanobacterium Nostoc commune. - Plant Physiol., **88**/2, 1055-1057, 4 Figs., 1 Tab., Lancaster, Pa.

SCHOLL, D.W., CRAIGHEAD, F.C. & STUIVER, M. (1969): Florida submergence curve revised: its relation to coastal sedimentation. - Science, **163**, No. 3867, 562-564, 3 Figs., Washington

SHARKEY, T.D. & BERRY, J.A. (1985): Carbon isotope fractionation of algae as influenced by an inducible CO_2 concentrating mechanism. - In: LUCAS, W.J. & BERRY, J.A. (eds.) Inorganic carbon uptake by aquatic photosynthetic organisms. – Am. Soc. Plant Physiol., 389-403, 4 Figs., 1 Tab., Rockville

SIKES, C.S., ROER, R.D. & WILBUR, K.M. (1980): Photosynthesis and coccolith formation: Inorganic carbon sources and net inorganic reaction of deposition. - Limnol. Oceanogr., **25**/2, 248-261, 7 Figs., 2 Tabs., Baltimore

SIKES, C.S. & WILBUR, K.M. (1982): Functions of coccolith formation. - Limnol. Oceanogr., **27**/1, 18-26, 3 Figs., 3 Tabs., Baltimore

SOMERS, G.F. & BROWN, M. (1978): The affinity of trichomes of blue-green algae for calcium ions. - Estuaries, **1**, 17-28, 2 Figs., 7 Tabs., Solomons

SWART, P.K. (1983): Carbon and oxygen isotope fractionation in scleractinian corals: a review. - Earth Sci. Rev., **19**, 51-80, 10 Figs., Amsterdam

SWART, P.K., STERNBERG, L.D.S.L., STEINEN, R. & HARRISON, S.A. (1989): Controls on the oxygen and hydrogen isotopic composition of the waters of Florida Bay, U.S.A.. - Chem. Geol., Isotope Geosci., **10**, 113-125, 8 Figs., 2 Tabs., Amsterdam

TUFFERY, A.A. (1969): Light and electron microscopy of the sheath of a blue-green alga. - J. Gen. Microbiol., **57**/1, 41-50, 5 Plts., London

VAN LIERE, L. & WALSBY, A.E. (1982): Interactions of cyanobacteria with light. - In: Carr, N.G., Whitton, B.A. (eds.):The Biology of Cyanobacteria. – Bot. Monographs, Vol. **19**, 688 pp., 9-45, 13 Figs., 2 Tabs., Oxford (Blackwell)

VOLOKITA, M., ZENVIRTH, D., KAPLAN, A. & REINHOLD, L. (1984): Nature of the inorganic carbon species actively taken up by the cyanobacterium Anabaena variabilis. - Plant Physiol., **76**/3, 599-602, 5 Figs., Lancaster

WALSBY, A.E. (1968): Mucilage secretion and the movement of blue-green algae. - Protoplasma, **65**/1-2, 223-238, 17 Figs., Wien

WECKESSER, J., HOFMANN, K., JÜRGENS, U.J., WHITTON, B.A. & RAFFELSBERGER, B. (1988): Isolation and chemical analysis of the sheaths of the filamentous cyanobacteria *Calothrix parietina* and *C. scopulorum*. - J. Gen. Microbiol., **134**/3, 629-634, 1 Fig., 2 Tabs., London

WHEELER, A.P. & SIKES, S. (1984): Regulation of carbonate calcification by organic matrix. - Amer. Zool., **24**/4, 933-944, 3 Figs., 1 Tab., Bloomington, Indiana

WOLK, P.C. (1973): Physiology and cytobiological chemistry of blue-green algae. - Bacteriological Rev., **37**/1, 32-101, 16 Figs., 11 Tabs., Baltimore

Manuscript received October 7, 1991
Revised manuscript accepted November 28, 1991