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Are Zoochlorellae from Lake Baikal Sponges Shade Adapted?

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Abstract

Zoochlorellae were extracted from the fresh water sponge *Lubomirska baikalensis* from Lake Baikal, Siberia, Russia. Samples were taken from 5 and 28 m. Morphometric analysis of the ultrastructure of zoochlorellae revealed no significant difference between algae from the two depths except for the presence of a larger lipid storage droplet in the deeper ones. For both depths the ratio of chloroplast to cell volume, surface area of thylakoids per cell volume and per chloroplast volume are all typical of cells under light limitation.

We suggest that this photoacclimation to low light results from heavy self shading among the cells at both depths.

Keywords: Zoochlorellae, Lake Baikal, light adaptation, sponges

1. Introduction

Many members of the Porifera are known to form mutualistic associations with photosynthetic organisms and bacteria (Wilkinson, 1978). In marine sponges it was found that the photosynthetic partners are cyanobacteria (Wilkinson, 1983), whereas fresh water sponges are inhabited by green algae (Gilbert and Allen, 1973), called "zoochlorellae".

The fresh water sponge *Lubomirska baikalensis* is widespread in dense stands in the benthos of shallow waters of Lake Baikal from 5 m to a depth of about 30 m. No sponges were found in extremely shaded niches. The sponges are bright green in color, due to the presence of zoochlorellae. *L. baikalensis* retains its green color even in the depth unlike the North American freshwater sponge, *Spongiella lacustris* which is white due to the loss of zoochlorellae when it grows at low light intensities, on undersides of rocks or logs (Williamson, 1979).

As a result of light attenuation with depth we expected to find some light-shade adaptations of the zoochlorellae in the deeper samples, like those of the zooxanthellae of symbiotic corals (Dubinsky et al., 1984; Berner et al., 1987). The study was aimed at exploring the differences between zoochlorellae from the two depths, mainly on the ultrastructural level.

2. Materials and Methods

The sampling took place during 20–26 August 1990 at the Limnological Institute in Listvianka, on the Eastern shore of Lake Baikal. Fresh sponges (*Lubomirska baikalensis*) were collected from depths of 5 and 28 m by scuba divers.

Algal cells were obtained by squeezing the sponge until it became white. Cell counts were made with an improved Neubauer haemocytometer. Surface area of the sponge was measured by wrapping it with thin aluminium foil, after which the total area was calculated (Marsh, 1970).

Chlorophyll *a* analysis was carried out on cells that were filtered through glass fiber paper (Whatman GF/C), which was then ground in 90% acetone in a glass-Teflon homogenizer and then filtered. Chlorophyll *a* was determined according to the equations of Jeffrey and Humphrey (1975), following spectrophotometric analysis of the filtrates on a Phillips spectrophotometer. Sections of sponges were prepared for SEM according to the GTGO procedure (Gamliel et al., 1983). Samples were examined on a JEOL JSM 840.

Squeezed animal cells and zoochlorellae were fixed for TEM in 2.5% glutaraldehyde. After post fixation in OsO_4, samples were dehydrated by serial transfers through progressive aqueous-ethanol series and finally embedded in Spurr's resin (Spurr, 1969). Sections were cut and stained with uranyl acetate (Stempak and Ward, 1964), followed by lead citrate (Reynolds, 1963) and were observed in a JEOL TEM 1200x operating at 80 kV.

Cell diameter was measured from EM micrographs. Morphometric analysis of the relative volume of chloroplasts, nucleus and lipid storage bodies to cell

volume, and the surface density of thylakoids were all calculated by the superimposition of an array of short lines on the TEM photographs (Weibel et al., 1966; Freere and Weibel, 1967).

Light penetration through the zoochlorellae (Table 3) was estimated as follows:
Knowing the extremely oligothropic nature of Lake Baikal, (Back et al., 1991) and the small (3 μm diameter) size of the cells, we used the k_c values of 0.015 and 0.020 $m^2\ mg^{-1}$ ch *a* at 5 and 28 m, respectively. These values are based on Fig. 1 in Atlas and Bannister (1980), for *Chlorella* in Crater Lake type water.

From areal chlorophyll concentration and k_c it is possible, using a derivation of the Beer-Lamberth Law, to estimate the fraction of light incident on an algal layer, which is absorbed by this layer (eq. 1).

$$I_z = I_0 e^{-kc\ chl} \tag{1}$$

I_z — irradiance passing through the algal layer
I_0 — light incident on the algal layer
chl — chlorophyll *a* ($mg\ m^{-2}$)

$$I_z/I_0 = e^{-kc\ chl} \tag{2}$$

3. Results and Discussion

The sponge *L. baikalensis* grows on the edges of Lake Baikal up to a depth of 30 m. Each sponge can reach a height of about 50 cm. The symbiotic system includes the sponge, green algae-zoochlorellae and bacteria. The zoochlorellae occur in the ameboid archeocytes of the sponge, and each such host cell usually shows more than ten algal cells in any one section and 3–8 cells in a row (Fig. 1). The algal cells are about 3 μm in diameter and each one occurs intracellularly in a single-membrane vacuole. The single chloroplast is cup shaped with densely packed thylakoids. Starch grains appear within the chloroplast, whereas a large lipid storage body or a few lipid droplets occur in the cytoplasm. No pyrenoid has been observed (Fig. 2).

The morphometric analysis shows that there is no significant difference between zoochlorellae from shallow- and deep-water sponges, except for the lipid droplets, which are twice as large at 28 m as they are at 5 m (Table 1). The relative volume of the chloroplast per cell and the surface density of thylakoids to chloroplast and to cell volume are typical of shade adapted microalgae (Berner et al., 1989; Sukenik et al., 1989). No pseudograna have been identified.

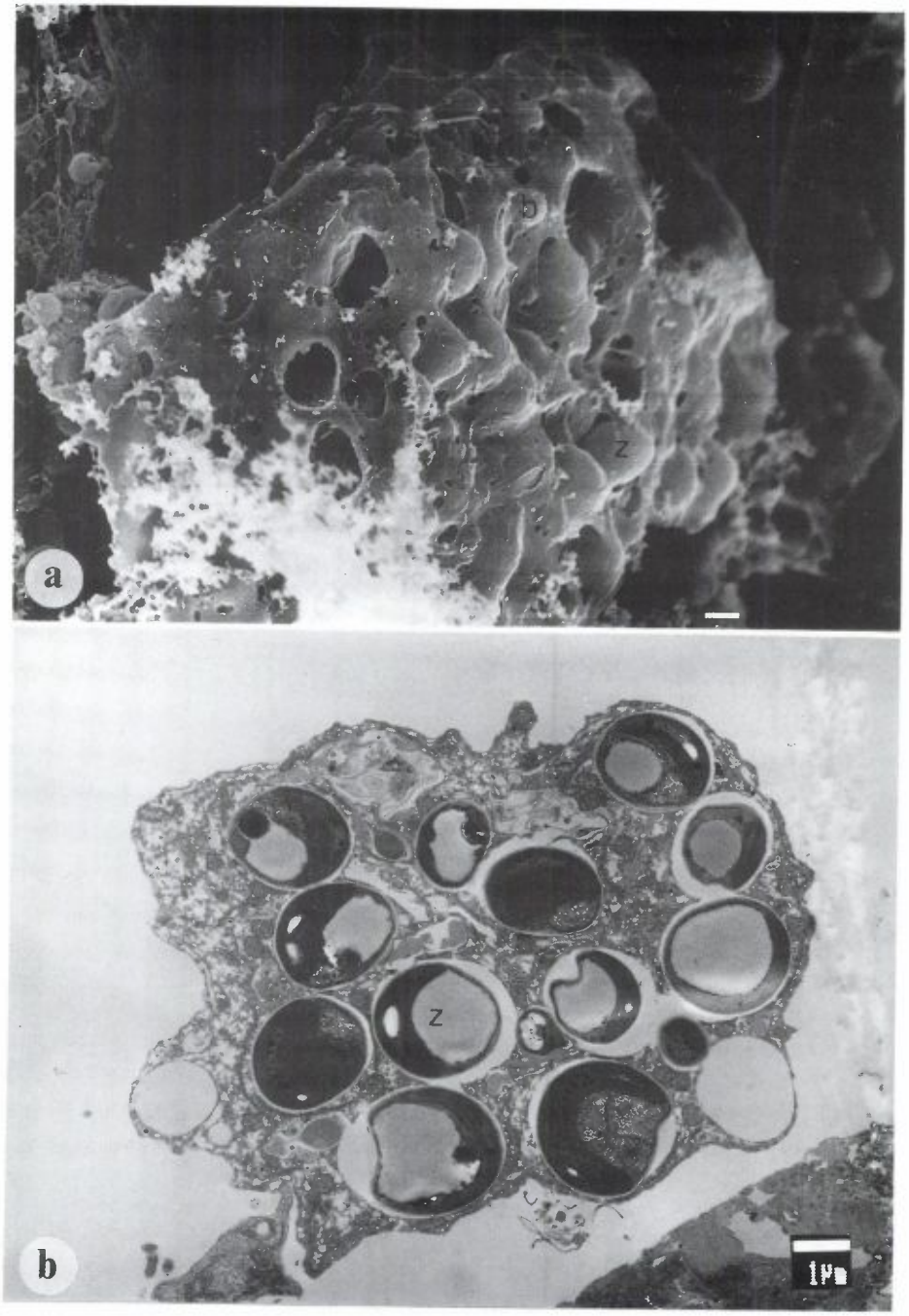

Figure 1. Ultrastructure of ameboid archeocyte containing zoochlorellae (a-SEM, b-TEM) z-zoochlorellae, b-bacteria. Scale bar = 1 μm.

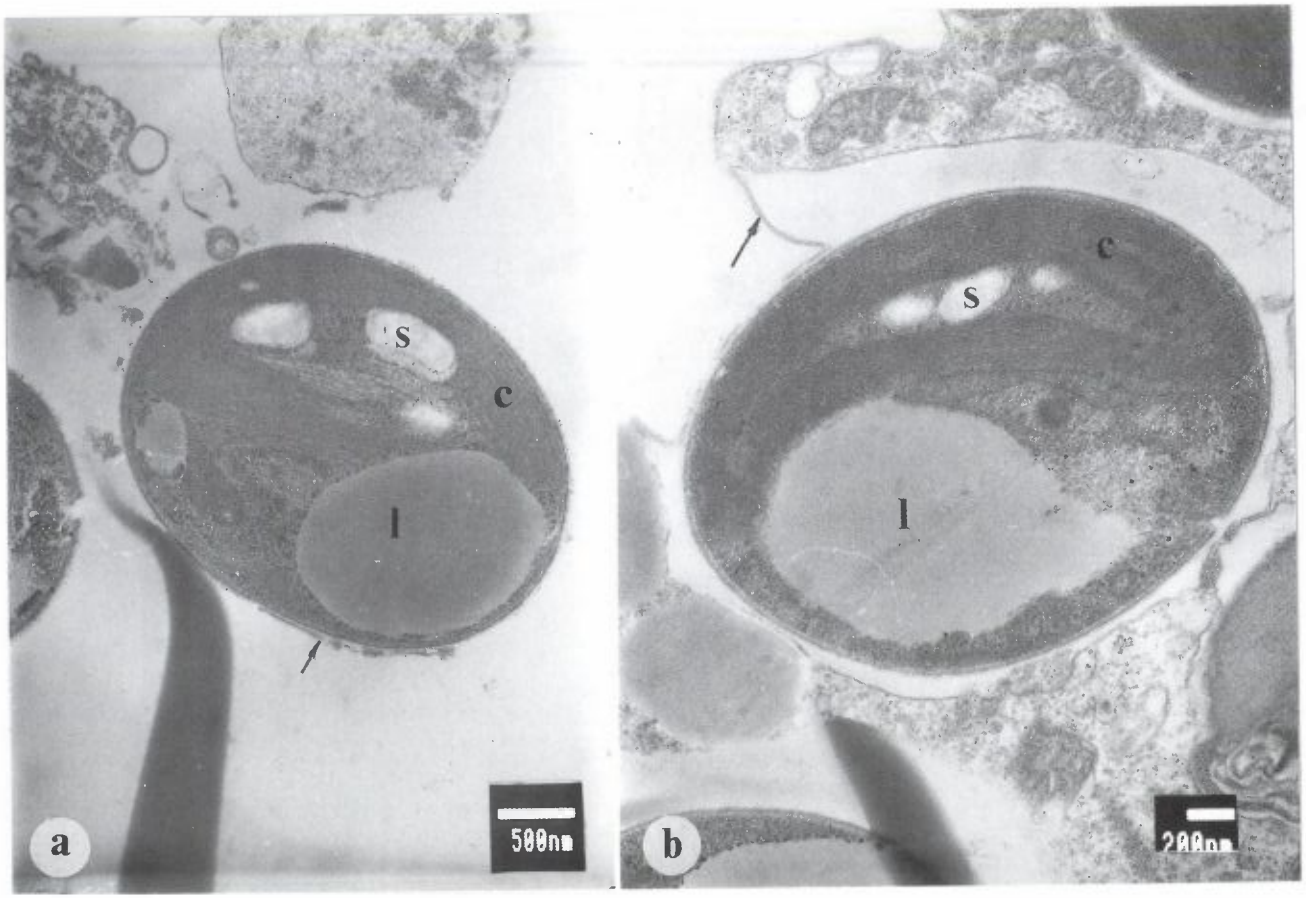

Figure 2. TEM micrograph of zoochlorellae from 5 (a) and 28 m (b) depth. l-lipid body, c-chloroplast, s-starch grain, arrow-membrane of vacuole surrounding each zoochlorella.

Table 1. Morphometric analysis of zoochlorellae

Parameter	Depth 5 m (n=30)	28 m (n=30)
Chloroplast (relative volume)	45.00±2.3	42.13±2.12
Nucleus (relative volume)	3.83±0.85	2.33±1.05
Lipid droplets (relative volume)	14.69±2.8	29.58±4.58
Thylakoid/chloroplast (surface density $mm^2 \cdot mm^{-3}$)	1.79±0.135	1.87±0.12
Thylakoid/cell (surface density $mm^2 \cdot mm^{-3}$)	0.88±0.09	0.79±0.07

Table 2. Comparison of zoochlorellae in sponges from 5 m and 28 m

Parameter	Depth 5 m	28 m
10^8 cell number$\cdot cm^{-2}$	10.17±2.2	9.36±1.01
Chlorophyll *a* (pg$\cdot$cell^{-1})	.047	.027

The similarity in cell number per surface area (Table 2) as in most other morphometric parameters between the two samples, indicates that self shading occurs among the zoochlorellae population. The response to this self shading within the host sponge overrides any possible differences between zoochlorellae from the two depths, which might result from the difference in underwater irradiance.

There is hardly any difference between sponges growing in the shallow or the deep layers in terms of areal density of the algae. However, there is a significant difference in chlorophyll content (Table 2). In the zooxanthellae Red Sea coral *Stylophora pistilata*, at lower light intensity, chlorophyll content per cell was found to be higher than in shallow water (Dubinsky et al., 1984). There is, however, evidence that in some zooxanthellae of deep growing corals a bleaching-like phenomenon may take place, and actually a decrease in chlorophyll per cell with depth occurs (Titlyanov, 1991). This seems to be the case with the zoochlorellae of the sponge *L. baikalensis*.

From our calculations, the areal projection of zoochlorellae is 7 μm^2 (diameter 3 μm), which means that over 1.4×10^7 cells may be accommodated on one

cm^2 as a monolayer. The actual number we found is two orders of magnitude higher.

In order to estimate whether the zoochlorellae are likely to be light limited we calculated the light absorbed by the algal layer (Table 3). When the light penetration is estimated it becomes obvious that I_z/I_0 is very low, indicating that the zoochlorellae in the inner parts of the sponge are virtually in effective darkness. Although the value of I_z/I_0 is higher in the deep layers due to the lower chlorophyll content, the absolute light penetrating to 28 m is very low. It therefore seems that most of the cells are in the dark rather than in the shade. In this case, part of the chlorophyll may be degraded, especially in the sponges growing in the deeper layers.

The morphometric analysis shows that accumulation of lipids in the zoochlorellae growing in the depth, doubles, when compared to those from the shallow layers (Table 2). Chemical analysis of accumulation rate of ^{14}C in lipids is about twice as high at 28 m as it is at 5 m (0.8 and 1.7 ng atom $^{14}C \cdot cm^{-2}$ of sponge surface, respectively (C. Bil, pers. comm.). Since temperature decreases with depth (Table 4), it is to be expected that respiration rate would be lower. There is evidence that under low temperatures respiration rate is slowed down to a lesser extent than is photosynthesis (Tilzer and Dubinsky, 1987), resulting in storage of photosynthates. It is possible that lipids, derived from photosynthates, are the way by which energy-rich compounds are translocated from the algae to their host, as has been shown to be the case

Table 3. Comparison of zoochlorellae and light penetration between sponges from 5 m and 28 m

Parameter \ Depth	5 m	28 m
Cell diameter (μm)	2.95±0.07	3.02±0.08
k_c (m^2 mg Chl^{-1})	0.015	0.020
Chl·*a* (mg m^{-2})	478.0	252.7
I_z/I_0	0.00077	0.0064

Table 4. Physical conditions during the observation period

Parameter \ Depth	5 m	28 m
Temperature (°C)	10–12	4
Light (% of PAR)	50–70	2–5

in sea anemones (Kellogg and Patton, 1983). We speculate that due to the reduced metabolic rate at lower temperature, less lipids are transferred to the animal, resulting in lipid accumulation.

We conclude that part of the zoochlorellae population in the sponge *L. baikalensis* from Lake Baikal is shade adapted whereas the remainder grows in the dark, probably, at least in part, heterotropically.

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