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How to cite

PLEE, Karine et al. Unravelling the microbial role in ooid formation - results of an in situ experiment in modern freshwater Lake Geneva in Switzerland. In: Geobiology, 2008, vol. 6, n° 4, p. 341–350. doi: 10.1111/j.1472-4669.2007.00140.x

This publication URL: https://archive-ouverte.unige.ch/unige:17099

Publication DOI: 10.1111/j.1472-4669.2007.00140.x

Unravelling the microbial role in ooid formation – results of an *in situ* experiment in modern freshwater Lake Geneva in Switzerland

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ABSTRACT

The microbial role in the formation of the cortex of low-Mg calcite freshwater ooids in western part of Lake Geneva in Switzerland has been suggested previously, but not demonstrated conclusively. Early work mostly concentrated in hypersaline milieus, and hence little is known about their genesis in freshwater environments. We designed an *in situ* experiment to mimic the natural process of low-Mg calcite precipitation. A special device was placed in the ooid-rich bank of the lake. It contained frosted glass (SiO_2) slides, while quartz (SiO_2) is the most abundant mineral composition of ooid nuclei that acted as artificial substrates to favour microbial colonization. Microscopic inspection of the slides revealed a clear seasonal pattern of carbonate precipitates, which were always closely associated with biofilms that developed on the surface of the frosted slides containing extracellular polymeric substance, coccoid and filamentous cyanobacteria, diatoms and heterotrophic bacteria. Carbonate precipitation peaks during early spring and late summer, and low-Mg calcite crystals mostly occur in close association with filamentous and coccoid cyanobacteria (e.g. *Tolypothrix, Oscillatoria* and *Synechococcus, Anacystis,* respectively). Further scanning electron microscope inspection of the samples revealed low-Mg calcite with crystal forms varying from anhedral to euhedral rhombohedra, depending on the seasons. Liquid cultures corroborate the *in situ* observations and demonstrate that under the same physicochemical conditions the absence of biofilms prevents the precipitation of low-Mg calcite crystals.

These results illustrate that biofilms play a substantial role in low-Mg calcite ooid cortex formation. It further demonstrates the involvement of microbes in the early stages of ooid development. Combined with ongoing microbial cultures under laboratory-controlled conditions, the outcome of our investigation favoured the hypothesis of external microbial precipitation of low-Mg calcite as the main mechanism involved in the early stage of ooid formation in freshwater Lake Geneva.

Received 5 October 2007; accepted 3 December 2007

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INTRODUCTION

Ooids are well-rounded sand grains composed of a nucleus encompassed by poorly to well-developed concentric micritic laminae. The nucleus can be of various compositions such as a quartz grain, a lithoclast or a bioclast. Most ooids are smaller than 2 mm in diameter; with many between 0.5 and 1.0 mm (Fig. 1A,B). Three main factors are involved in ooid cortex formation (Davis *et al.*, 1978): (i) a chemical factor that triggers precipitation due to the supersaturation of the environment; (ii) a physical factor related to either currents, wave action or storms, which maintain the grain in suspension or mobilize it around the lake floor; and (iii) a biological factor involving biofilms triggering direct and/or indirect $CaCO_3$ precipitation. Ooid formation often results from a combination of these different factors while their relative significance depends on the prevailing conditions at each location. Ooids are found throughout the geological record, and they currently form in various environments, and thus represent modern analogues to interpret older deposits. Ooids have been used as valuable palaeoenvironmental proxies for water depth, salinity, temperature and water energy. However, most studies of modern ooids have concentrated in marine or saline lacustrian environments. Recent lacustrine ooids have been reported in the Great Salt Lake, Utah (Halley, 1977), and in Pyramid Lake, Nevada (Popp & Wilkinson, 1983). Scarce ooids in freshwater environments have also been reported in Higgins Lake, Michigan (Wilkinson *et al.*, 1980). Despite these studies, their genesis remains

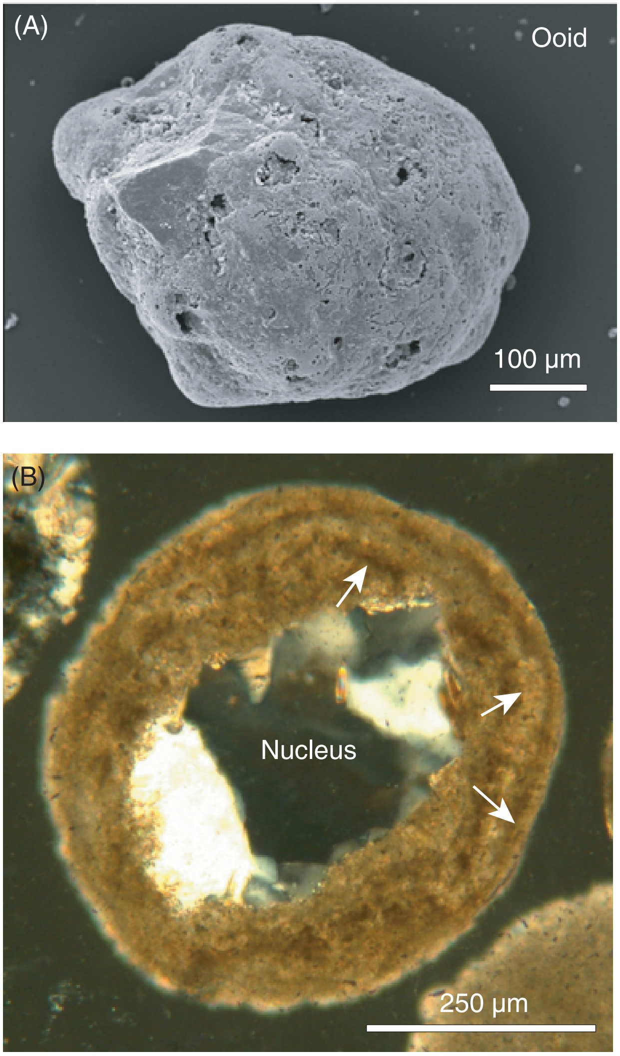

Fig. 1 Ooid from Lake Geneva: (A) scanning electron microscope microphotograph of a subspherical ooid with a discontinuous micritic coating; (B) thin section under polarized light. Poorly developed low-Mg calcite laminae surrounded a quartz nucleus. The dark laminae (white arrows) are due to the highly porous cortices resulting from the degradation of biofilms (Davaud & Girardclos, 2001).

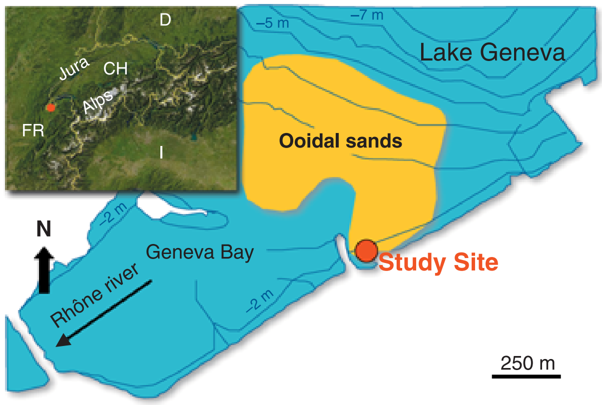

Fig. 2 Satellite image showing the location of Lake Geneva between the Jura Mountains and the Swiss and French Alps. The close up of the westernmost part of the lake shows the distribution of ooidal/oncoidal sands in the Geneva Bay (modified from Moscariello, 1996 and Corboud, 2001). The dot indicates the position of the *in situ* experiment presented here.

controversial and is generally accounted for by purely physico-chemical precipitation in middle to high-energy milieu (e.g. Cayeux, 1935; Nesteroff, 1956). However, a biogenic origin for ooids was suggested as early as the late 19th century when they were considered as unicellular algae (Rothletz, 1892; Nesteroff, 1956). Drew (1911) proposed that the bacteria '*Pseudomonas calcis*' could trigger calcium carbonate precipitation, whereas Dangeard (1941) pointed out the presence of cyanobacteria enclosing modern ooids in the Suez area, concluding that a biochemical mechanism was responsible for their formation. Nesteroff (1956) studied ooids of various ages in several regions and found organic remains indicative of a biological origin, in all cases. More recently, the role of microbial activity during the generation of coated grains has gained significant momentum. Investigations by Monty (1976), Golubic (1976), Pentecost & Riding (1986), Gerdes & Krumbein (1987) and Gerdes *et al.* (1994), among others, clearly established the essential role played by cyanobacteria during the formation of carbonate concretions such as stromatolites and oncoids. These observations are further supported by the experimental results of Adolphe & Billy (1974), Castanier *et al.* (1989), Buczynski & Chafetz (1991), and Knorre & Krumbein (2000), who precipitated calcite crystals comparable to natural crystals under laboratory conditions in the presence of micro-organisms. It should be noted that most of these studies focused on either field observations, or laboratory experiments. More recently, studies in modern environments have successfully utilized *in situ* measurements to bridge the gap between natural environments and laboratory experiments (Reid *et al.*, 2000; Visscher *et al.*, 2000).

Modern lakes are natural laboratories that provide unique environments to understand a wide variety of biogeochemical processes (e.g. Hsü & McKenzie, 1985). Recent investigations in the 'Petit Lac' of western Lake Geneva indicate that more than 90% of the shallow water sediments are composed of ooids (see Fig. 2; Moscariello, 1996; Corboud, 2001). In contrast to the marine environment, physical factors in Lake Geneva are not the most important mechanism that triggers carbonate precipitation. Meteorological and physical time-series data of the water column indicate that the area containing ooids is usually reworked only five to 10 times during winter (Girardclos, 1993). Previous detailed microscopic observations of these low-Mg calcite ooids discovered the presence of biofilms in depressions within the surfaces of their nuclei (Fig. 1A; Davaud and Girardclos, 2001). These biofilms appear to be the starting point for the development of low-Mg calcite ooid cortex. The aim of this paper is to focus on this early stage of ooidal cortex formation by studying biofilms present in the 'Petit Lac' where ooids have been deposited and to evaluate the role of the biological factor during this stage. Therefore, an *in situ* experiment was conducted throughout an entire annual cycle in western Lake Geneva, Switzerland. This experiment bridges the gap between field observations and laboratory experiments in an attempt to provide new evidence of microbial mediation during the complex process of ooid formation in freshwater lacustrine basins.

STUDY SITE – MODERN LIMNOLOGY

Lake Geneva is located in the western end of Switzerland (Fig. 2). Secluded between the Jura and Alps mountain ranges, it is the largest freshwater lake in Central Europe with a surface of 582 km^2. The lake comprises two separate basins: the 'Grand Lac' with a maximum depth of ~310 m and the 'Petit Lac' reaching 76 m (Zahner, 1984). This second basin is monomictic, mesotrophic and waters are always well oxygenated and well mixed at the place of the experiment. This is due to the shallow water depth and episodically strong hydrodynamic processes related to wind-induced wave action. Long-term meteorological data show that dominant wind blowing from the north-east can be particularly strong for about 5–10 days per year, reworking sediments down to 10-m water depth in the Geneva Bay area (Girardclos, 1993).

The exceptional availability of limnological data allows the reconstruction of the annual cycle in the water column. The CIPEL (International Commission for Lake Leman Water Protection) monitors the water quality of the lake throughout the annual cycle. Hence, physicochemical parameters are regularly measured every 2.50 m along the depth of the water column at two selected sites in the 'Grand Lac' and the 'Petit Lac', respectively. In this paper only the upper 10-m dataset is considered due to the shallow character of the study site.

IN SITU EXPERIMENT

Moscariello (1996), Corboud (2001) and Davaud & Girardclos (2001) have previously described the presence of an ooid-rich zone in the western part of the 'Petit Lac' (see Fig. 2 for location). Such zones are rare in freshwater environments, and provide us with an optimum environment to closely follow the precipitation of carbonate throughout the year. A special device was designed to reproduce the conditions on the surface of the nucleus of ooids in the natural environment under actual water column conditions. The device was set at 2.5-m water depth within the ooid-rich bank (Fig. 2). It contains 12 glass slides that are dispersed along both sides of a plastic arm, with a simple mechanism to allow a diver to unscrew and easily remove each slide (Fig. 3). The slides were placed vertically to minimize the quantity of particles settling on their surfaces. The glass slides were previously frosted with quartz grains under a press to provide an attractive substrate to attach biofilms, similar to what is found as on the surfaces of ooid nuclei. Since quartz grains

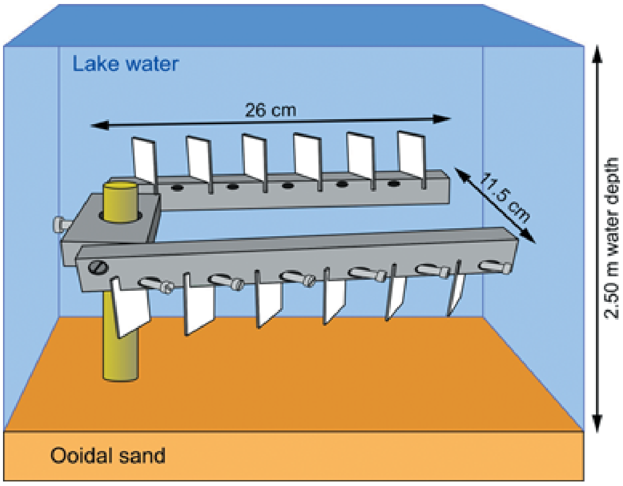

Fig. 3 The experimental device has been set at 2.5-m water depth with a pile steel foundation and contains a total of 12 slides arranged on both sides of a plastic arm. Frosted slides are set vertically to avoid sediment accumulation either from the epilimnion and/or by remobilization during storms.

are most often the natural nucleus in Lake Geneva ooids, we chose an irregular silica slide for our experiment with the rational of having a good analogue for initial ooid cortex formation.

We present results acquired from observations and measurements of material on slides of three independent devices, which were placed at once and collected during monthly dive expeditions to the test site from summer 2002 to summer 2003. Thus, the presented data refer to cumulative changes in carbonate precipitation over a variable number of months. The slides were immediately taken to the laboratory for analysis after they were removed from the lake.

MATERIALS AND METHODS

Untreated samples were analysed directly after being removed from the lake using a Leica M420 microscope (LEICA Microsystems, Heerbrugg, Switzerland) under natural and ultraviolet light. Autoflorescence analyses were performed at the Laboratory of Geomicrobiology, ETH-Zürich. Elemental distribution including high-resolution profiling and mapping of the fresh samples was achieved using an X-ray microfluorescence EAGLE III apparatus (INSIDIX Innovative Technology for Interface Evaluation, Seyssins, France). The samples were inspected under atmospheric conditions with a 50-µm diameter beam. Prior to analyses with a scanning electron microscope (SEM), the samples were fixed and dehydrated using the Critical Point Drying Method (CO_2 substitution), with 2.5% glutaraldehyde, 0.1 mol Na-cacodylate, and with increasing concentrations (30% to 100%) of both ethanol (1 mol) and amyl-acetate (1 mol) to preserve their structure. They were gold-coated prior to imaging with a Jeol JSM 6400 SEM (University of Geneva, Geneva, Switzerland). Elemental distribution at this scale was achieved with an X-ray INCA 300 (Oxford Instruments, Sarl,

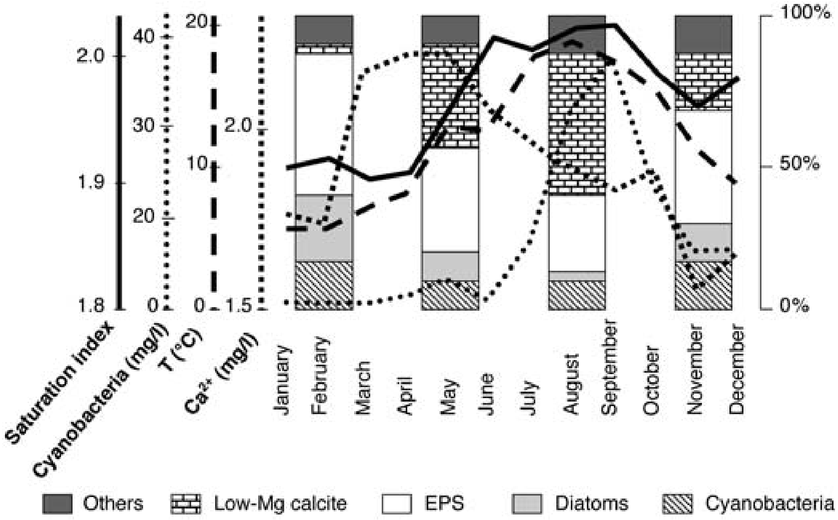

Fig. 4 Lake Geneva surface water showing a typical pattern of temperature evolution throughout the year. The summer/early fall interval (i.e. maximum water temperature) that coincides with the increasing development of cyanobacteria and saturation index (log $[Ca^{2+}]*[CO_3]/Ks$) and resulting decrease of dissolved Ca^{+2} in the water column. Conversely, all parameters mirror the previously described pattern during the remaining seasons (data included are a 1998–2002 average series courtesy of CIPEL). In the background, result of random point-counting on biofilms formed during the *in situ* experiment, using JMicroVision program (Roduit, 2007). Low-Mg calcite is present throughout the year with a clear increase in spring and a maximum during summer.

Table 1 BG11 media (Rippka *et al.*, 1979) used for *in situ* biofilms cultures

	Stock g L^{-1}	Solution mL L^{-1}
1 $NaNO_3$	150	10
2 $K_2HPO_4*3H_2O$	40	1
3 $MgSO_4*7H_2O$	75	1
4. $CaCl_2*2H_2O$	36	1
5 Citric acid +	6	1
Ferric ammonium citrate	6	1
6 EDTA	1	1
7 Na_2CO_3	20	1
8 Trace metal solution	1	
H_3BO_3	2.86	
$MnCl_2*4H_2O$	1.81	
$ZnSO_4*7H_2O$	0.222	
$NaMoO_4*5H_2O$	0.390	
$CuSO_4*5H_2O$	0.079	
$Co(NO_3)2*6H_2O$	0.0494	

Saclay, France) analyser. Percentage estimation of both organic matter and carbonate precipitates was obtained from SEM images by random point counting using the JMicroVision software (Roduit, 2007). The mineral determination of single crystals retrieved from *in situ* frosted glass slides was determined with a Gandolfi Camera (PW 1743, Philips, Eindhoven, the Netherlands). Biofilms developed during the *in situ* experiment were cultured under laboratory conditions using a specific BG11 media for freshwater cyanobacteria (Table 1; Rippka *et al.*, 1979).

RESULTS

Statistical analyses of the existing meteorological time-series dataset indicate an average of five to 10 storms per year that would remobilize the sediments in the area of ooid formation (Girardclos, 1993). This mixing occurs during the colder months when the calcite saturation index (log $[Ca^{2+}]*[CO_3^{2-}]/Ks$) (Skirrow, 1975), which ranges from 1.90 to 2.10 during the year, is at its lowest (Fig. 4). Figure 4 shows the *in situ* cyanobacterial productivity, temperature, Ca^{2+} concentration and saturation index of low-Mg calcite throughout the year. Cyanobacterial mass displays a bimodal distribution with the most prominent peak during the summer months. Lake water temperature varies seasonally between 5.7 °C and 18.5 °C. Ca^{2+} concentration of lake waters ranges between 1.77 and 2.21 mg L^{-1}, attaining the highest values during early spring, whereas the saturation index ranges between 1.90 during the winter and 2.10 in the summer (Fig. 4). A gradual decrease in Ca^{2+} concentration characterizes late spring and the summer seasons, corroborating the increasing cyanobacterial productivity, temperature and saturation index.

Microscopic observations of freshly retrieved samples display a patchy development of biofilms containing extracellular polymeric substances (EPS) and numerous micro-organisms. Although occurring on both sides of the slide, a dominant biocolonization is obvious on the frosted side. Their size and composition vary according to the yearly variation in their productivity (Figs 4 and 5A). Autofluorescence observations showed that these biofilms contain mostly photosynthetic organisms (Fig. 6A,B). A more detailed examination of the microbial community under the microscope reveals at least five different species of filamentous and coccoid cyanobacteria, preliminary identified as *Synechoccus* and *Anacystis*, and *Tolypothrix* and *Oscillatoria*, respectively, which are dominant in spring and summer (Fig. 6C,D). Cyanobacteria productivity peaks in late spring and fall, and heterotrophic bacteria bloom immediately after these peaks, whereas diatoms are dominant during the winter.

Additional SEM observations of the same samples confirmed a patchy pattern for both the micro-organisms colonizing the glass slides of the *in situ* experiment and also revealed a close

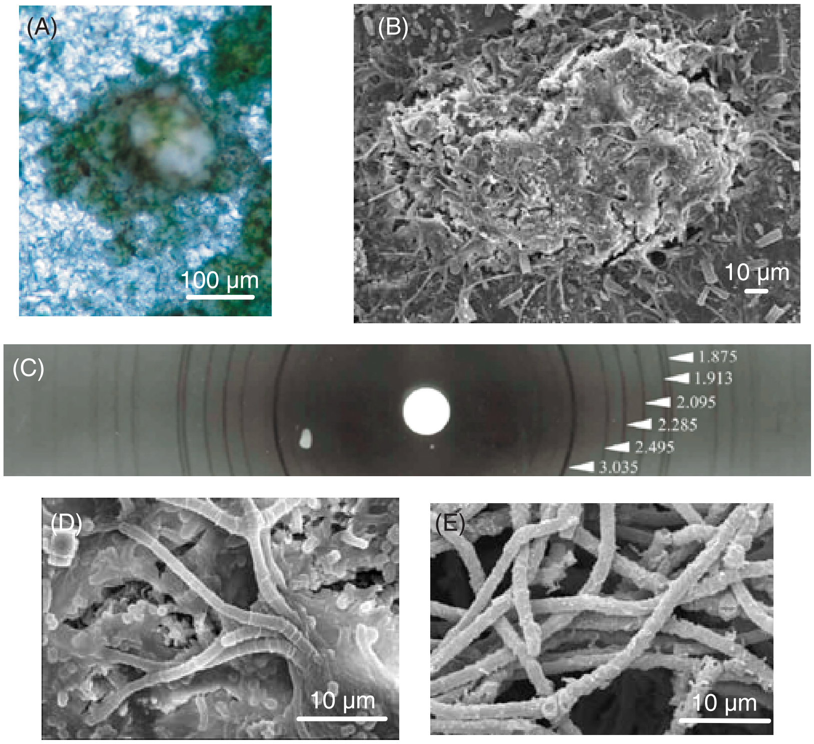

Fig. 5 (A) Biofilm developed on the surface of an *in situ* frosted slide, under natural light, composed of low-Mg calcite and micro-organisms (greenish); the latter produce the EPS that contains both mineral and biological phases. (B) SEM micrograph of a similar biofilm developed on the surface of a frosted slide showing the low-Mg calcite aggregate within the EPS. (C) X-ray film displaying the position of the main reflections indicating the low-Mg calcite composition of the precipitates. (D) Detailed SEM view of the white square indicated in (B) showing the close relationship within the EPS between both coccoid and filamentous cyanobacteria (diameter: 1.5–2 μm) and the freshly precipitated carbonate. (E) SEM micrograph of an ooid after impregnation with low-viscosity epoxy and etching during 10 h with HCl vapours (Davaud & Girardclos, 2001). Once that the carbonate coatings are completely dissolved, a dense network of filaments (diameter: 1.5–2 μm) are revealed. Notice the striking similarity in size and structure of the filaments observed in both the *in situ* experiment and the ooid.

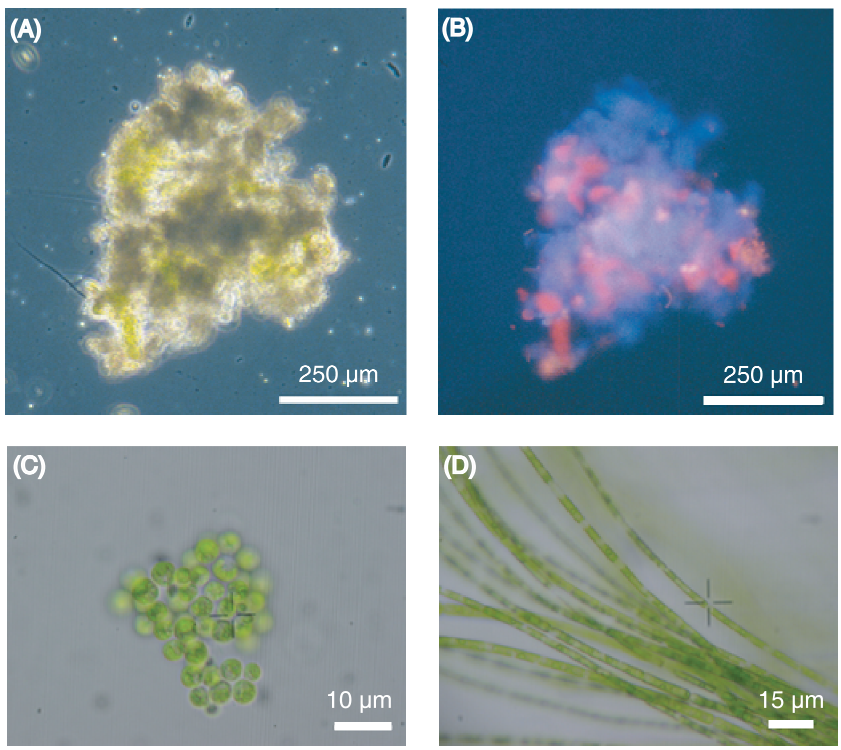

Fig. 6 Slide covering the April to September interval. (A) Biofilm composed of low-Mg calcite intimately associated with micro-organisms under natural light. (B) Autofluorescence of the same aggregate showing in red the concentration of photosynthetic micro-organisms; liquid BG11 cultures enriched from *in situ* samples: (C) ~3 μm diameter coccoid cyanobacteria under natural light preliminary determined as *Synechococcus* and *Anacystis*; and (D) cyanobacteria filaments (*Tolypothrix* and *Oscillatoria*) under natural light.

association with crystal precipitation (Fig. 5A,B). Mineralogical analyses of the precipitates clearly indicate that, as in the ooid cortex, low-Mg calcite is the dominant mineral phase as shown by a conspicuous peak distribution in the Gandolfi X-ray analyses (Fig. 5C). Inspection of the glass slides using X-ray microfluorescence also shows a patchy distribution of the precipitated low-Mg calcite during the *in situ* experiment that corresponds to the biofilm distribution. Several chemical elements

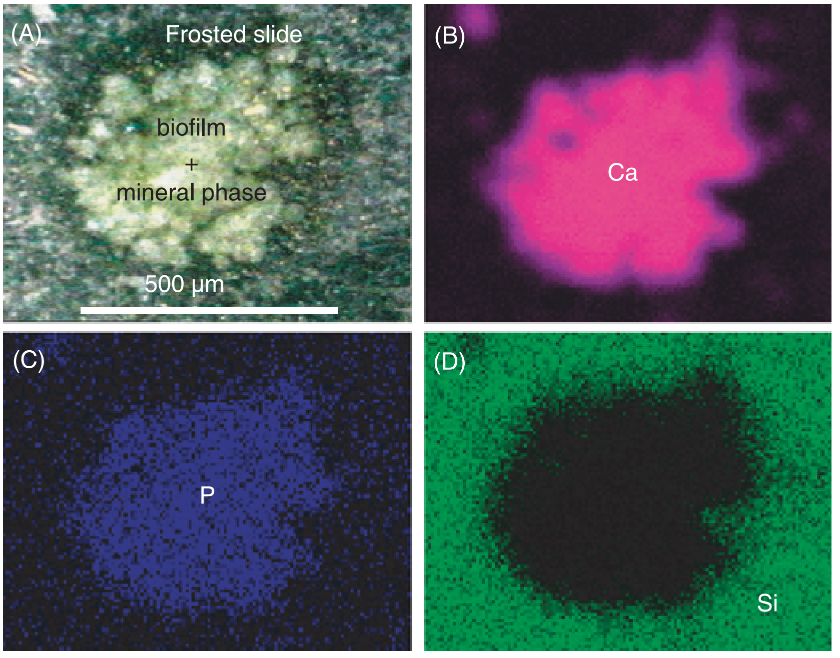

Fig. 7 (A) Microscopic picture under natural light of a biofilm developed on the *in situ* frosted slides. The ultra-high resolution mapping of a selected section of the aggregate (same scale) shows variations in elemental concentrations throughout the analysed section. Lighter tones are indicative of comparatively higher concentrations, whereas dark areas correspond to very low abundance of a given element. Notice the excellent correlation between Ca (B) and P (C) even at very high resolution. The opposite distribution of Si (D) (i.e. frosted glass) allows to detect areas without either organic remains and/or precipitated carbonates.

were mapped at a micrometric scale showing variable concentrations throughout the slides (Fig. 7). A patchy biofilm (Fig. 7A) similar to Fig. 5(B) shows a conspicuous elemental distribution indicating that Ca and P are concentrated in, and within, the microbial/carbonate mass (Fig. 7B,C). Increasing Si (Fig. 7D) and decreasing Ca intensities are indicative of areas without both organic remains and associated low-Mg calcite precipitates, respectively, providing an outstanding method to spot and further quantify areas of mineral precipitation.

Random point counting of SEM microphotographs using the JMicroVision software (Roduit, 2007) indicates seasonal changes in both quantity and morphology of low-Mg calcite precipitates. These changes follow variations in the physico-chemical parameters and biological productivity of the water column (Fig. 4). Carbonate precipitation peaked twice during the study cycle; initially, during springtime with dominant subhedral to euhedral rhombohedric crystals, and subsequently during the summertime when the major peak of anhedral to subhedral crystals occurred (Fig. 8). Low-Mg calcite precipitation decreased during the fall, producing very different crystal shapes of snowy clusters and compact aggregates (Fig. 5B), with very few or noncrystallized particles. Carbonate precipitates were rare to absent during the winter, and comprise poorly developed crystals with frequently dissolved surfaces (Figs 4 and 8). Carbonate aggregates produced during the *in situ* experiment display a distinctive microbial imprint, either as a coating on the surface showing cyanobacterial filaments within biofilms (Fig. 9A), and/or as the imprint of former filaments after they have vanished from the ooid cortex (Fig. 9B). Figure 5(D) displays a detailed SEM microphotograph of the zone indicated by the white square in Fig. 5(B). Notice the close association between both coccoid and filamentous cyanobacteria forming the biofilm and the precipitated low-Mg calcite within EPS. An identical structure containing a network of filaments is also observed in the interior of a present day ooid, after etching (Fig. 5D,E).

The presence of coccoid cyanobacteria in SEM microphotographs is not as clear since they are often entombed within the EPS or low-Mg calcite, as observed within *in situ* experiment biofilms and ooid surfaces (Figs 5D and 9C, respectively). EDAX analyses of the same SEM image clearly shows, however, comparatively higher concentrations of Ca^{2+} in the areas containing higher concentration of bacteria.

The comparison of modern water column chemical data and carbonate precipitation at the experiment site indicates a positive correlation between the lake cyanobacterial cycle and the amount and shape of low-Mg calcite precipitation. Carbonate production in the slides increased during the spring and summer months, peaking during the early spring and late summer, followed by a significant reduction during June and the end of September/October. Carbonate production also coincides with a reduction in the lake water of Ca^{2+} concentration and an increase of the saturation index. Conversely, carbonate precipitation diminished substantially during the cold winter months.

DISCUSSION AND OUTLOOK

Field observations indicate that the ooids are in suspension during the winter, when the low-Mg calcite saturation index is at its lowest. Conversely, the development of a dense underwater flora fixes the sediments, preventing major wave action, and therefore sediment mixing during the rare windy periods in

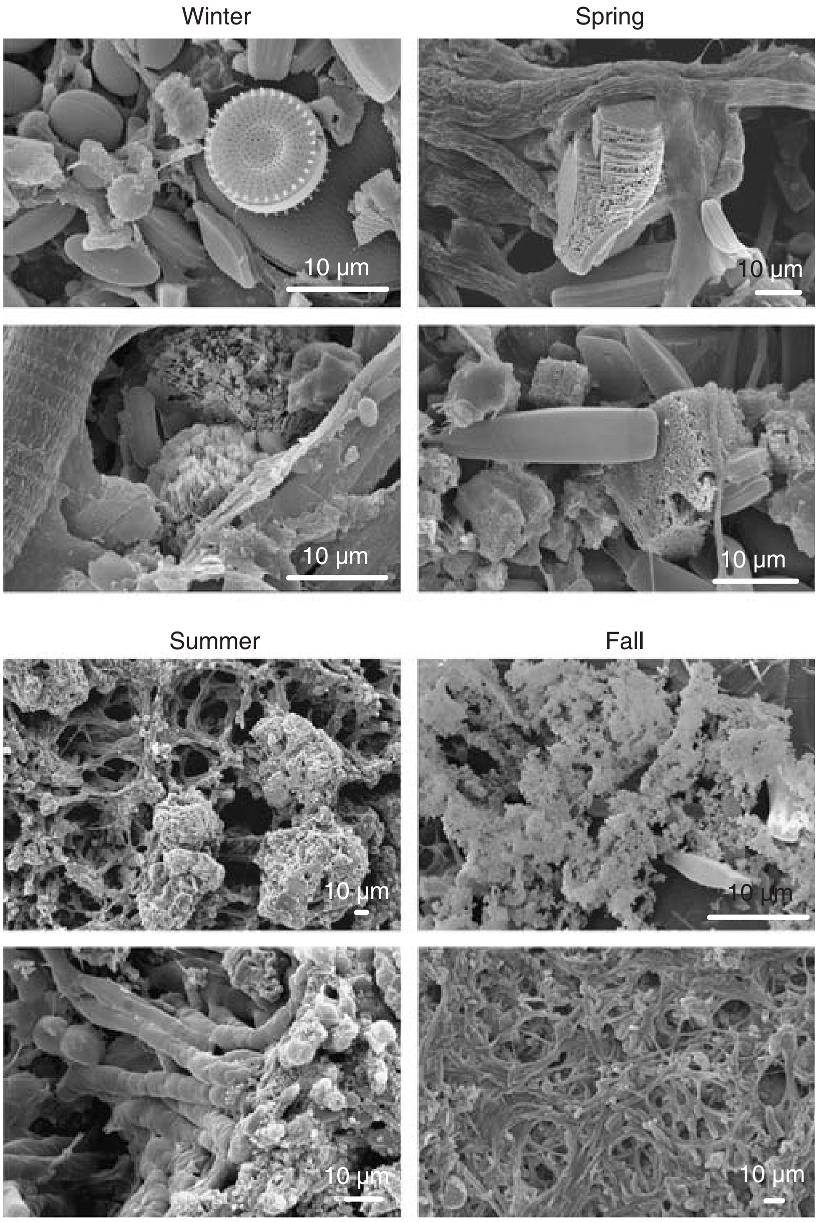

Fig. 8 SEM microphotographs highlighting sequence of changes in crystal form and size through annual cycle. See text for details.

the summer. These data indicate that ooidal cortex formation mainly occurs during the quietest periods, and thus the biological factor dominates ooid cortex formation in Lake Geneva. Further laboratory experiments at 20 °C with mixed cultures from biofilms harvested from the *in situ* experiment indicate that low-Mg calcite precipitation only occurs in the presence of biofilms. Conversely, no crystal formation was observed in cultures undergoing continuous shaking, as in a high-energy environment. Furthermore, carbonate precipitation occurs in both sides of the slides although it is more important on the frosted side (easy to colonize by microbes). The latter provides an additional argument that favours our hypothesis that immobile substrates can be an analogue to ooid formation. The surface of the slide is more than two times larger than the average surface of a nucleus of an ooid, indicating that slides in our experiment enhance the natural process of microbial colonization.

The mineral composition of the precipitates of the *in situ* experiment is identical to that developed on the depressions of the ooid's nucleus surface (Fig. 10A,B, respectively). The diversity of the micro-organisms forming the biofilms that are the starting point of the ooid cortex formation is further compared to both the amount of low-Mg calcite and the water column geochemical dataset (Figs 4 and 8). As previously mentioned, there is a good correlation between the abundance of coccoid and filamentous cyanobacteria, and the amount of low-Mg calcite in the slides, corresponding to spring and summer. This correspondence follows the annual variations in temperature, dissolved Ca^{2+} and the saturation index in the water column.

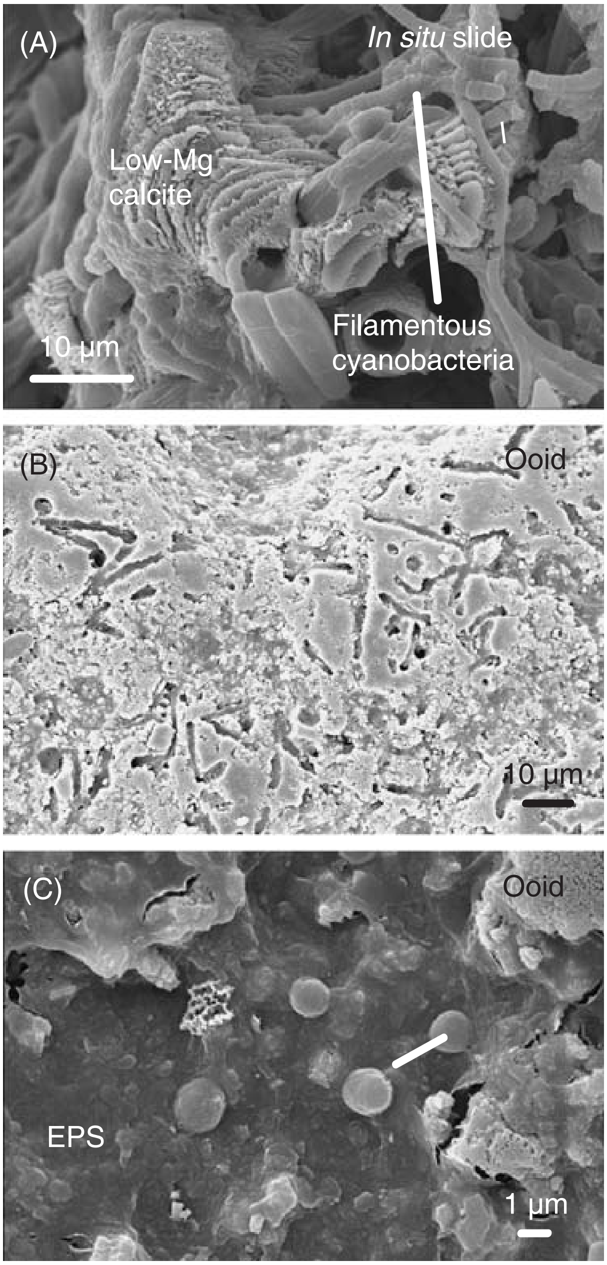

Fig. 9 Comparison of the biofilms developed on the *in situ* frosted slides and the surface depression of the ooid. (A) Carbonate coating on cyanobacterial filaments within a biofilm developed on the *in situ* frosted slide. Cyanobacterial filaments are clearly preserved in the carbonate precipitated in the ooids in the same fashion during the *in situ* experiment that presently formed in the lake as shown by Davaud & Girardclos (2001); (B) ooid cortex surface with filaments fingerprints; (C) coccoid cyanobacteria (diameter: 1 µm) in a ooid depression that seems to be entombed within the EPS or low-Mg calcite and are much smaller than the filaments (notice the 10 fold difference in the scale between images).

Different micro-organisms are known to promote carbonate precipitation, e.g. sulphate-reducing bacteria (Van Lith *et al.*, 2003; Dupraz *et al.*, 2004; Ludwig *et al.*, 2005) and cyanobacteria. Indeed it has been suggested that cyanobacteria trigger the precipitation of low-Mg calcite in their sheath or outermost zone of cells (Pentecost & Bauld, 1988; Ferris *et al.*, 1994). They can induce precipitation through two different, but often combined mechanisms: (i) these micro-organisms excrete hydroxyl ions as a by-product of photosynthesis, and consequently increase the pH of their microenvironment (Neumeier, 1998; Merz-Preiss, 2000; Thompson, 2001; Konhauser, 2007); and (ii) they contain chelates that can bind Ca^{2+} cations, promoting $CaCO_3$ precipitation on their cell surface. Cyanobacteria and most of the submerged micro-organisms produce EPS, which forms a continuous film, rich in amino acids, carboxyhydrates, proteins, lipids and nucleic acids as well as mono- and polysaccharides (Reimann *et al.*, 1966; Grady *et al.*, 1999; Lawrence *et al.*, 2003). EPS serve as a nutrient (Wolfaard *et al.*, 1995) permitting the growth of both chemoorganotrophic and chemolithotrophic bacteria. Alkalophilic bacteria may absorb carbon derived from the organic component of the mucigel and even dissolve part of the early precipitated authigenic carbonates. On the other hand, other chemolithotropic bacteria might simultaneously drive the pH to the alkaline range, triggering the (re)precipitation of low-Mg calcite.

Coccoid cyanobacteria are generally covered by layers of low-Mg calcite or EPS that can eventually mask their presence. EDAX analyses of coccoid cyanobacteria indicated higher Ca^{2+} concentration, suggesting that they are ideal sites for carbonate nucleation and precipitation. These microbes could further induce active precipitation by causing $CaCO_3$ saturation on their surface. Thus, the actual mineral nucleation would take place on the surface of the membrane (Castanier, 1987; Vasconcelos *et al.*, 1995; Neumeier, 1998; Thompson, 2001). This may explain why a coccoid cyanobacteria often appears 'entombed' in the freshly precipitated low-Mg calcite or EPS, in both the experimental and the natural ooids (Fig. 9C). Some bacteria also accumulate Ca^{2+} internally during sporulation as it has been observed in the samples. This locally enhanced Ca^{2+} concentration can act as a potent microenvironment where the ion product $[Ca^{2+}]*[CO_3^{2-}]$ greatly exceeds saturation on a submicron scale. According to Folk (1993), this can trigger crystallization within and around the bacterial body, irrespective of the overall composition of the surrounding waters.

Despite the fact that the photosynthetic activity of diatoms can passively induce carbonate precipitation, a clearly negative correlation has been observed between their abundance and the amount of precipitated carbonate throughout the annual cycle. Figure 4 shows that diatom remains in the slides are higher during the coldest months, coinciding with the lowest carbonate precipitation.

Bahamian ooids are mobile during significant periods of their formation, maintained in suspension by turbulence under supersaturated water that finally induces abiotic aragonite layers precipitation. This is not the case in freshwater lake Geneva. Our data and observations imply that in our example ooids are maintained in suspension only during episodically strong hydrodynamic processes related to wind-induced wave

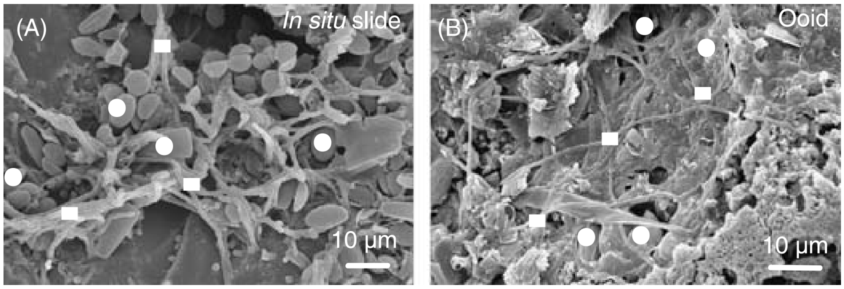

Fig. 10 (A) SEM micrograph from the *in situ* experiment showing freshly formed biofilms composed of filamentous and coccoid cyanobacteria (rectangles and circles, respectively) and diatoms (dots). (B) Close-up view of an ooid surface at the same scale for a comparison, displaying identical biofilms colonizing depressions, with filamentous and coccoid cyanobacteria (arrows and circles, respectively) and diatoms (dots). This biofilm underwent a first stage of diagenesis as shown by the resulting decrease in quantity of micro-organisms, increasing amount of entombed coccoid cyanobacteria as well as broken diatom frustules.

action caused by storms affecting Geneva Bay for about 5–10 days per year. This occurs principally during winter, when water chemistry is not favourable for low-Mg calcite precipitation. Hence, in Lake Geneva initial ooid formation occurs during static stages that justifies the use of our immobile device as an analogue. In summary, the biofilms responsible for the initial growth of low-Mg calcite ooid cortex were successfully harvested in our *in situ* experiment and in laboratory cultures (Table 1). The low-Mg calcite precipitation observed in the frosted slides is always associated with biofilms and particularly to cyanobacterial filaments and coccoid cyanobacteria, which bloom during the spring and summer. Consequently, this *in situ* experiment is representative of at least the earliest stage of actual ooid cortex formation, i.e. lacustrine biofilm in the natural environment. A comparison of the timing and quantity of these carbonate precipitates with the water column physicochemical parameters reveals a dependence of mineral precipitation on seasonal changes in light penetration, water temperature and saturation index, and therefore cyanobacteria development during the spring and summer. Microbial biofilms have been harvested from the frosted glass slides and successfully grown in BG11 solid and liquid cultures. DNA extraction on ooid cortex, *in situ* biofilms and biofilm cultures for precise identification of biofilm communities involved in low-Mg calcite ooid cortex formation is associated with ongoing experiments under laboratory-controlled conditions, which will help to constrain the actual microbial role during carbonate precipitation in freshwater ooids (Plée *et al.*, 2006). These controlled experiments will not only shed new light on the spatial distribution of ooids in modern Lake Geneva, but may also prove to be a valuable analogue to other freshwater ooidal deposits at various geographical and temporal scales. Furthermore, the combined natural and laboratory-based experiments can provide critical information to constrain the proposed changes in water chemistry and cyanobacterial physiology resulting from variations in atmospheric composition during the Proterozoic and Phanerozoic (Riding, 2006).

ACKNOWLEDGEMENTS

We would like to thank the staff of the Laboratory of Geomicrobiology, ETH-Zürich, for their hospitality and scientific discussions. We are grateful to Rolf Warthmann from the same laboratory for preliminary microbe identification and to Laurie Mauclaire for help with autofluorescence microscopy. Cris Vasconcelos is kindly acknowledged for his encouragement, fruitful discussions and a critical review of an early version of this manuscript. We thank Richard Spikings and Richard Williams for their suggestions concerning English expression and written style. Jacques Metzger helps with graphics is kindly acknowledged. We thank three anonymous reviewers and Kurt Konhauser for helpful comments.

This research was partially funded by the Augustin Lombard fellowship to K. Plée.

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