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Autor: Ariztegui, Daniel / Plee, Karine / Farah, Rédha
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Bridging the gap between biological and sedimentological processes in ooid formation:

Crystalizing F.A. FOREL's vision

Daniel ARIZTEGUI¹, Karine PLEE^{1,a}, Rédha FARAH¹, Nicolas MENZINGER¹, and Muriel PACTON²

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Abstract

Today, biomineralisation s.l. processes constitute a growing branch of research within the fast evolving field of geomicrobiology. These processes include those influenced and/or induced by microorganisms referring as organomineralization (Dupraz et al. 2009). The study of these processes was already among the multiple scientific interests of F.-A. Forel ! As early as the second half of the 19th century he confirmed some of the findings of other contemporaneous Swiss naturalists. In the third volume of his renowned book "Le Léman" he mentioned ... les naturalistes neuchâtelois ont étudié les sculptures admirablement développées sur les galets de la rive des lacs du pied du Jura, et les ont attribuées à l'action d'une algue Cyanophycée... From this starting point he further speculated about the nature of the bio-chemical processes involved in the precipitation of these lacustrine carbonates. At the time many of the advanced hypothesis remained as such due to the lack of detailed knowledge about these organisms, their metabolisms and the associated chemical reactions that they may produce. This was due to methodological limitations that did not allow the observation and monitoring of these microorganisms neither in situ nor in the laboratory. How far have we gone since Forel's early observations ? In this contribution we summarize the results of very recent investigations carried out in the shallow area of western Lake Geneva (Switzerland) that demonstrate, as forecasted by Forel and other contemporaneous naturalists, the critical role of cyanobacteria in the formation of ooids or carbonate coated sand grains.

Keywords: Petit-Lac, ooids, biomineralisation, microbes

Introduction

Ooids are well-rounded sand grains composed of a nucleus surrounded by frequently concentric laminae most often of carbonate composition (Fig. 1). They contain a nucleus of different nature such as a quartz grain, lithoclast or bioclasts. Although of variable size most ooids falls in the millimetre diameter. They can be found throughout the geological record as well as in modern environments. Most studies of recent ooids are, however, focused in marine or saline lacustrine environments. Modern lacustrine ooids have been described in Great Salt Lake (Halley 1977) and in Pyramid Lake (Popp and Wilkinson 1983). Ooids in

freshwater environments have been also reported in Higgins Lake, Michigan (Wilkinson *et al.* 1980) but they are scarce. Their genesis remains still controversial and has been for a long time based on a purely physico-chemical precipitation in middle to high-energy settings (e.g., Cayeux 1935; Nesteroff 1956). A biological origin of ooids, however, was early suggested by Delawaque (1888) that considered them as unicellular algae (referred in Nesteroff, 1956). A few years later Rothletz (1892) attributed the formation of ooids in Great Salt Lake to blue-green algae, i.e., cyanobacteria. Analogously, F.A. FOREL suggested a strong biological role precipitating the carbonate coats observed in coarse rubbles of Lake Geneva

¹ Section of Earth & Environmental Sciences, University of Geneva, Geneva, Switzerland; E-mail: daniel.ariztegui@unige.ch

² Geologisches Institut, ETH-Zürich, Zürich, Switzerland.

^a Present address: Addax Petroleum, Geneva, Switzerland.

shores (Forel 1904). At the beginning of the last century several scientists also proposed a biological origin for their formation. In 1911 Drew specified 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 a biochemical mechanism for their formation. Nesteroff (1956) further study ooids coming from various regions and diverse ages founding in all cases organic remains indicative of an algal origin. Recently, the fast progress of geomicrobiological studies has triggered a lot of interest to understand the role of microbial activity generating coated grains. Investigations by Monty (1976), Golubic (1976), Pentecost and Riding (1986), Gerdes and Krumbein (1987) and Gerdes et al. (1994), among others, have clearly established the essential role played by cyanobacteria in the formation of carbonate concretions like stromatolites and oncoids. These observations are further supported by experimental results of Adolphe and Billy (1974), Castanier et al. (1989), Buczynski and Chafetz (1991) and Knorre and Krumbein (2000) who precipitated calcite crystals close to the natural ones under laboratory conditions. Most of these studies focused either on field observations or laboratory experiments.

The "Petit-Lac" as called the western part of Lake Geneva (Switzerland) contains a sand bank with more than 90% of ooids down to ca. 10 m water depth (Girardclos 1993; Moscariello 1996; Corboud 2001). More recently Farah (2011) has estimated that more than 95% of coated grains define the ooidal bank between 2 and 6 m water depth. A detailed microscopic observation of these low-Mg calcite ooids (Fig. 1) first pointed the presence of biofilms filling up depressions in the surfaces of their nuclei (Davaud and Girardclos 2001). They were interpreted as clearly representing the starting point for the development of low-Mg calcite ooid cortex.

The goal of this contribution is to summarize the outcome of recent studies applying cutting edge microscopical, microbiological and geochemical techniques to reproduce the early stage of ooids cortex formation (Plee et al. 2008; Ariztegui and Plee 2009; Plee et al. 2010; Pacton et al. 2012). The combined results of an *in situ* experiment with laboratory investigations provide unequivocal evidence of the microbial mediation in the complex process of ooid formation in this freshwater lacustrine basin. Furthermore, lake current model experiments were specially tailored to test the physical role in carbonate precipitation (Menzinger 2011). These modelling results imply that lake currents have little impact in the precipitation process. Thus, as early forecasted by F.A. FOREL, these compiled evidence confirm the critical role of Cyanobacteria and other photosynthetic microbes in the formation of western Lake Geneva carbonate coated sand grains.

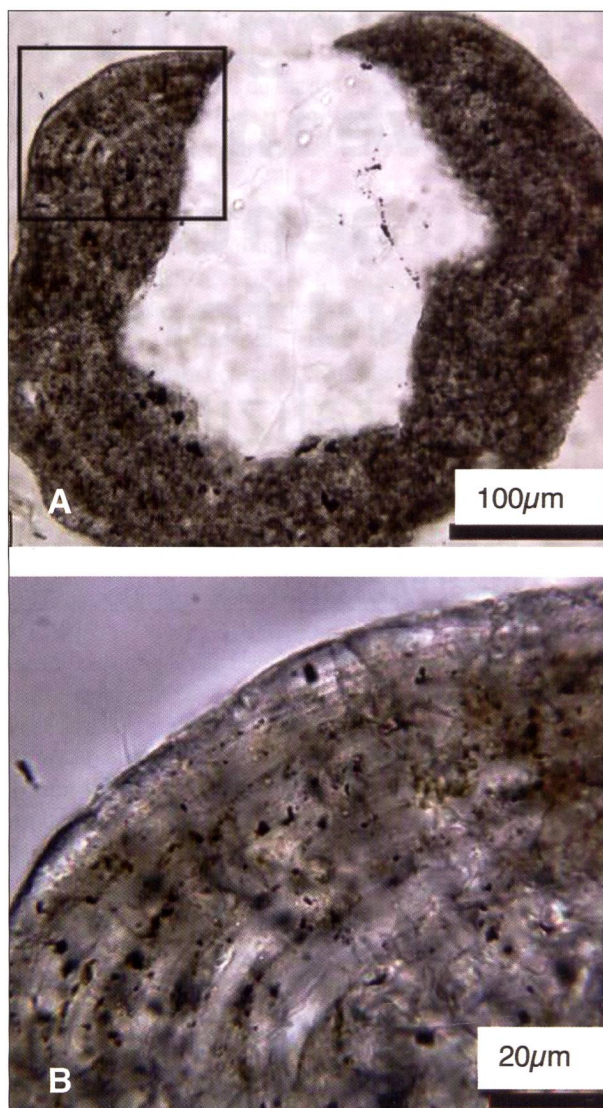


Fig. 1. A: Typical concentric ooid from Lake Geneva with a quartz nucleus under natural light; B: Zoom up of the black square in A displaying preserved organic matter between low Mg-calcite laminae.

Study site and approach

Lake Geneva 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 ooidal bank and where the *in situ* experiment was set (Fig. 2). This is due to the shallow water depth and episodically strong hydrodynamic processes related to wind-induced wave action. Long term meteorological data shows 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).

Girardclos (1993), Moscariello (1996), Corboud (2001) and Davaud and Girardclos (2001) have previously described the presence of an ooid rich zone in the western part of the “Petit-Lac”. Farah (2011) further mentioned the observation of ooids downstream of the water fountain (“Jet d’Eau”) in Geneva city in the area of the locally known Banana Canyon (see Fig. 2 for location). It provides us with an optimum environment to closely follow the precipitation of low Mg-calcite 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. Twelve glass slides were disposed along both sides of a plastic arm, with a simple mechanism to allow a diver to unscrew and easily remove each slide (Fig. 3). They 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 many ooid nuclei (Fig. 1). More details about the device and the experimental conditions can be found in Plee et al. (2008). Biofilms developed during the *in situ* experiment were sampled at different intervals. These biofilms were further cultured under laboratory conditions and several experiments were carried out using these cultures (Plee et al. 2010). Low Mg-calcite precipitation was followed regularly with microscopical observations and pH and Ca^{2+} measurements on separate tubes. Precipitates were inspected using confocal microscopy, SEM and TEM whereas their DNA was characterized using a PCR-DGGE approach.

Three sedimentary cores were retrieved in 2009 in the - estimated - thickest part of the ooidal bank at ca. 2 m water depth. The latter is close to the area where the *in situ* experiment was carried out to evaluate the plausible impact of early diagenesis throughout the entire ooidal bank (Fig. 2; Farah 2011). The sedimentological study of these cores provides new light into the development of early diagenesis. Furthermore, nano-scale elemental mapping combined with electron microscopy using Nano-SIMS in selected samples have now permitted to propose an alternative model to explain carbonate precipitation in this freshwater lake.

Finally, a two dimensional modelling experiment of the Petit-Lac was carried out to investigate the relationship between meteorological parameters, lake

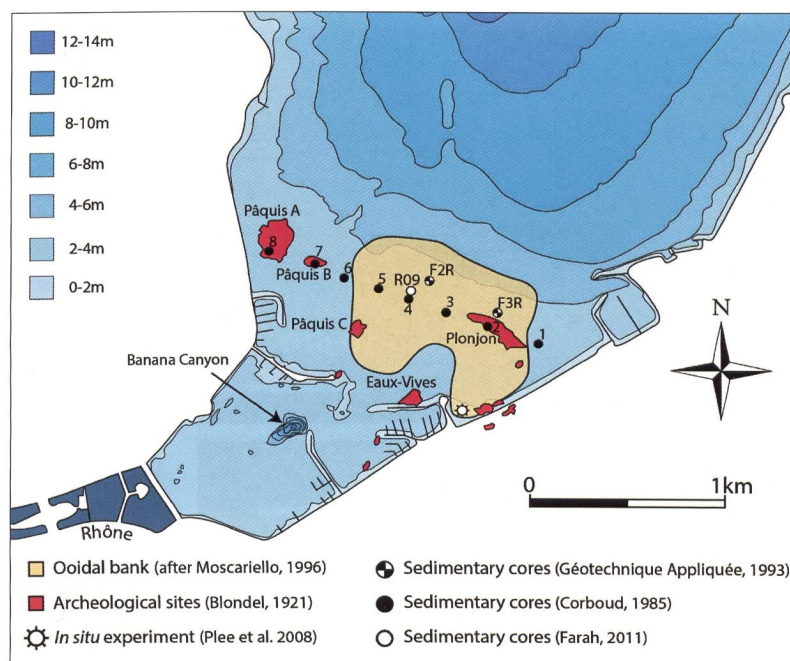


Fig. 2. Map of western Lake Geneva (Petit-Lac) showing the location of the ooidal bank as well as the various coring and study sites that have been retrieved by different researchers. Notice that some of the main archaeological sites (in red) coincide with the present location of the sand bank.

currents and the presence of ooids in the western part of Lake Geneva (see Fig. 1 for location). A digital model of the bottom of the Petit-Lac was obtained using the combination of a NURBS and CAD models (after Taruffi et al. 2008). Currents were simulated using the Gambit® and Fluent® commercial softwares to buildup the mesh, solve the Navier-Stokes equations and complete the processing of the data,

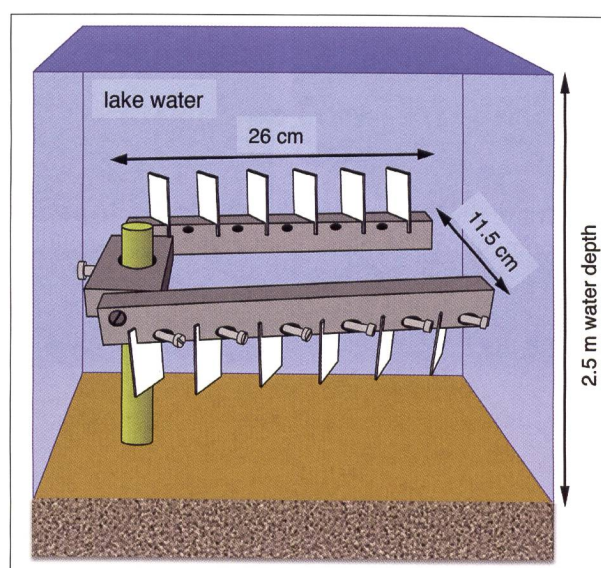


Fig. 3. Schema of the *in situ* experimental device (modified from Plee et al. 2008).

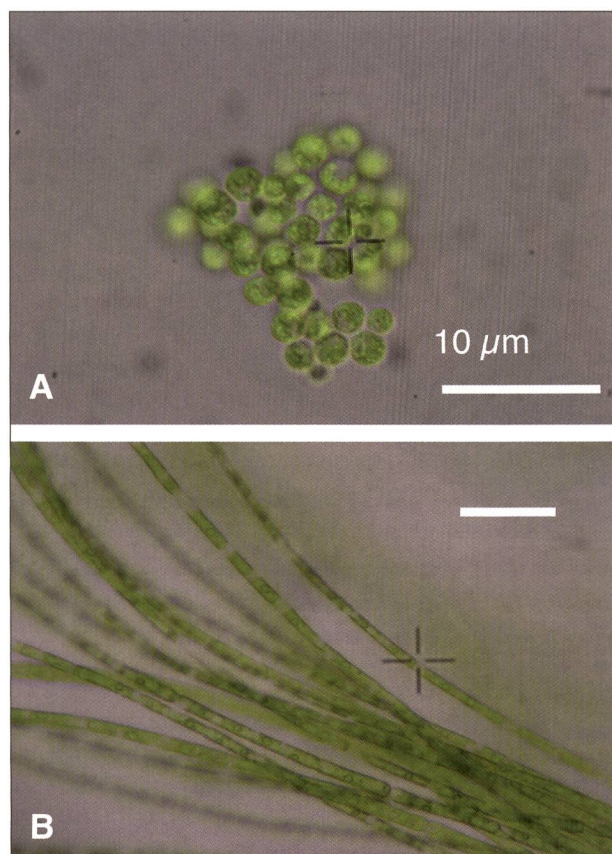


Fig. 4. Several species of cyanobacteria are known in Lake Geneva with main blooming peaks in spring and autumn (Plee et al. 2008). A: ~3 μ m diameter coccoid cyanobacteria under natural light (*Synechococcus* and *Anacystis*); B: cyanobacteria filaments (*Tolypothrix* and *Oscillatoria*) under natural light.

respectively. More details about the methodological approach and results can be found elsewhere (Menzinger 2011).

Results of the in situ experiment

Microscopic observations of freshly retrieved samples from the experimental site display a patchy development of biofilms containing extra-cellular polymeric substances (EPS) and numerous microorganisms. The size and composition of the bio colonization varied according to the yearly variation in productivity. Autofluorescence observations of the slides showed that these biofilms contain mostly photosynthetic organisms. A more detailed examination of the microbial community under the microscope reveals at least five different species of filamentous (*Synechococcus* and *Anacystis*) and coccoid (*Tolypothrix* and *Oscillatoria*) cyanobacteria, which are dominant in spring and summer (Fig. 4). Cyanobacteria productivity peaks in late spring and fall, and

heterotrophic bacteria bloom immediately after these peaks, whereas diatoms are dominant during the winter.

More detailed SEM observations of the same samples confirmed a patchy pattern for both the microorganisms colonizing the glass slides of the in situ experiment and also revealed a close association with crystal precipitation. Mineralogical analyses of the precipitates clearly indicate that, as in the ooid cortex, low-Mg calcite is the dominant mineral phase (Plee et al. 2008). Furthermore, Fig. 5 shows SEM images of both a modern ooid and a glass slide colonized by cyanobacteria displaying identical shapes and structures. The latter confirm that the experimental data is truly reproducing the process occurring in the ooid's surface.

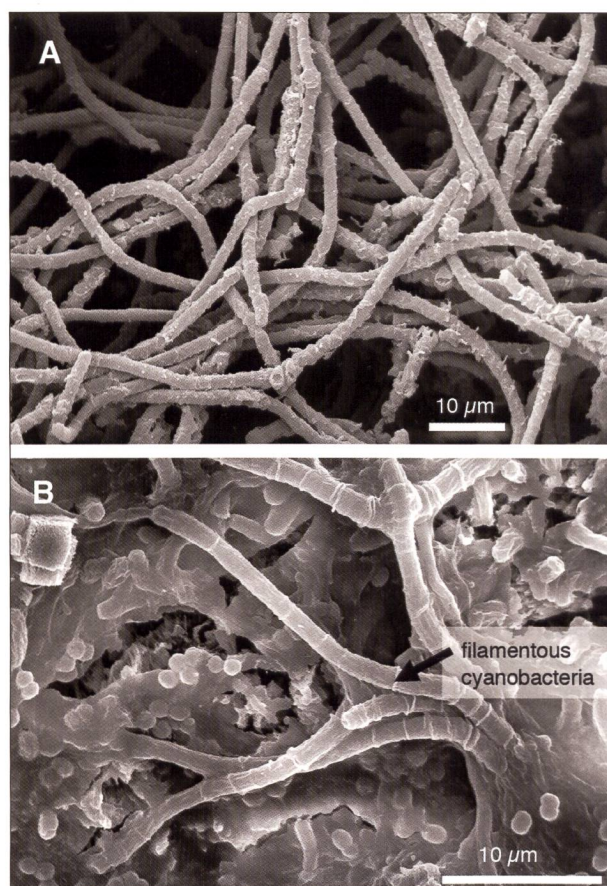


Fig. 5. SEM microphotographs showing filament remains of cyanobacteria in an actual ooid cortex that are comparable to those of living cyanobacteria shown in Fig. 4B. A: modified from Davaud and Girardclos 2001); and B: a carbonate precipitate in a glass slide from the in situ experiment.

Results of microbial culture experiments

The biofilms retrieved from the *in situ* experiment described in the previous section were further harvested and used to plan laboratory-controlled exper-

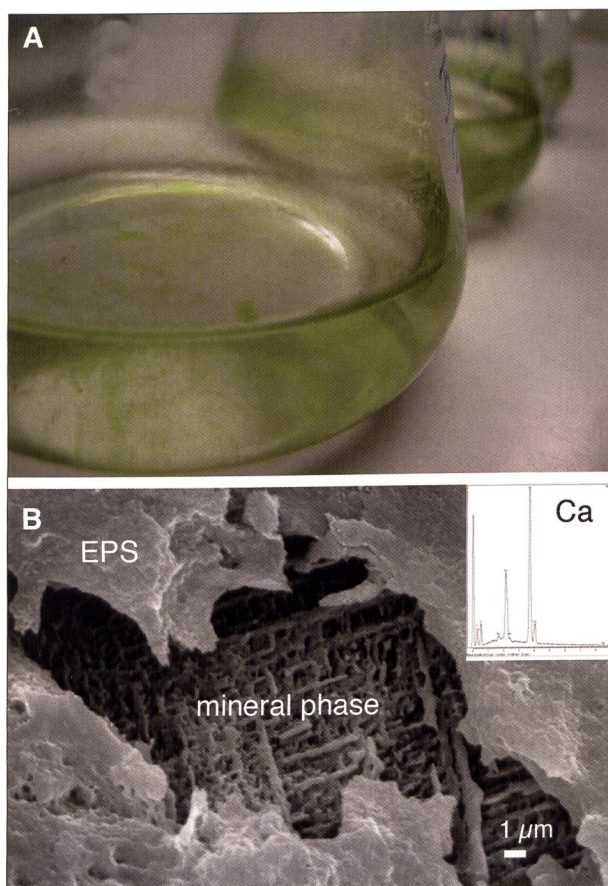


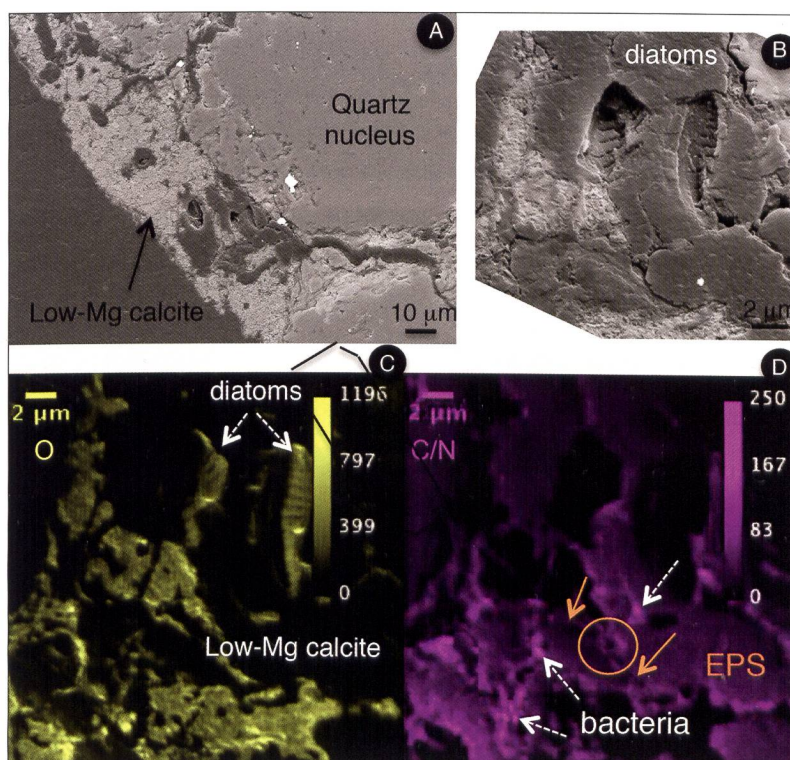
Fig. 6. A: Liquid culture of cyanobacteria harvested from the in situ experiment; B: SEM microphotograph of a cracked biofilm displaying a mineral phase embedded within EPS. Elemental analysis of this mineral phase indicates a high content of Ca^{2+} corresponding to low-Mg calcite as further confirmed by X-ray diffractometry (Plee et al. 2008).

tion with green algae and diatoms all embedded within the EPS (Fig. 6). Analogously, low-Mg calcite crystals, formed in the laboratory experiments under light conditions, are found within the EPS and always associated with photosynthetic microorganisms. The latter in concert with the lack of evidence for the presence of sulphate-reducers and other heterotrophic microbes, allowed Plee et al. (2010) to postulate that photosynthetic activity through increasing pH in EPS is the main factor triggering low-Mg calcite precipitation in the ooidal bank of western Lake Geneva.

Very recently, Pacton et al. (2012) showed that photosynthetic microbes not only enhance early carbonate precipitation around the ooid nucleus but also control the formation of their entire cortex. Nano-scale elemental mapping combined with electron microscopy using Nano-SIMS show that microbial EPS are first per-silicified as amorphous magnesium silicates (amMg-Si) before being calcified (Fig. 7). In situ carbon isotope data showing a ca. 5-6‰ depletion of ^{13}C in ooid cortices compared to both bulk values and carbonate nuclei support this photosynthetic

iments. They were designed to unequivocally identify the microorganisms responsible for low-Mg calcite precipitation within freshwater photosynthetic biofilms through microscopical and physicochemical data. Plee et al. (2010) compared biofilms lining ooid depressions in the natural environment with those grown under laboratory conditions in a culture media (BG11). Carbonate precipitates in the natural environment show that cyanobacteria dominate the microbial community in associa-

Fig. 7. A: Correlated SEM-NanoSIMS imaging showing the main components of an ooid cortex; B: SEM image showing location of NanoSIMS analysis within the ooid cortex; C: NanoSIMS ion images of oxygen (^{16}O) showing the low-Mg calcite crystals (high intensity in ^{16}O -image only) in which diatoms have been trapped. D: Clusters of bacteria are present within the EPS showing high intensities of $^{12}\text{C}/^{14}\text{N}$. Calibration bars show ion intensity variations, where brighter colours indicate higher intensity.



microbial mechanism and argue against contributions from sulphate reducing bacteria (SRB) or methanogenesis. These data have significant implications for paleoenvironmental studies since photosynthetic microbes now provide a clear alternative to turbulent hydrodynamic conditions in the formation of freshwater ooids.

Results of the lake currents model experiment

Menzinger (2011) used a two-dimensional model to simulate water column currents and their impact on the sediments in order to find independent arguments towards the relatively small influence of physical factors in the formation of these ooids. The mesh used represents a global volume of $\sim 22 \times 10^6 \text{ m}^3$ and is composed of ca. 860,000 cells, 960,000 nodes and 2,695,328 faces (Fig. 8A). In this model the uppermost 250 cm of the profile corresponds to the water column and the lowermost 50 cm represents the

ooidal bank. The model was run using variable wind velocities from null to 12 m/s allowing the observation of a variable impact of currents on both ooids distribution and sediment dynamics at different current velocities (contour scale in m/s on the left in Fig. 8B). This 2-D modelling exercise has shown a limited impact of lake currents on ooids formation that at first glance appears to be more realistic than 3-D models that were also tested. However, existing measurements in the lake water column under the most prevailing weather condition do not record the extremely high current velocities predicted by the model. In addition, on site observations indicate that the bottom of the study area has a dense macrophyte cover during the carbonate precipitating season (summer) that would hamper the wind impact throughout depth. Thus, it appears that under average meteorological conditions lake currents in the Petit-Lac would play a secondary role in the formation and even the distribution of ooids. The outcome of these modelling exercises is hence consistent with a dominant biological component governing today's early stages of ooidal cortex formation. Different current regimes might have prevailed during past lake level stands (Corboud 2001) and, thus, a diverse role of the currents than today cannot be excluded. However, the similarity of the dominant textural features of the ooids under SEM throughout depth (e.g., filaments, EPS remains, etc.) point towards comparable precipitation processes to those prevailing today. Other models and particularly current measurements are still necessary to fully validate these conclusions.

Results of the downcore sedimentological analyses

A detailed sedimentological description of the ooidal sand bank was carried out in sedimentary cores comprising its entire thickness (refer to Fig. 2 for core's location) in order to

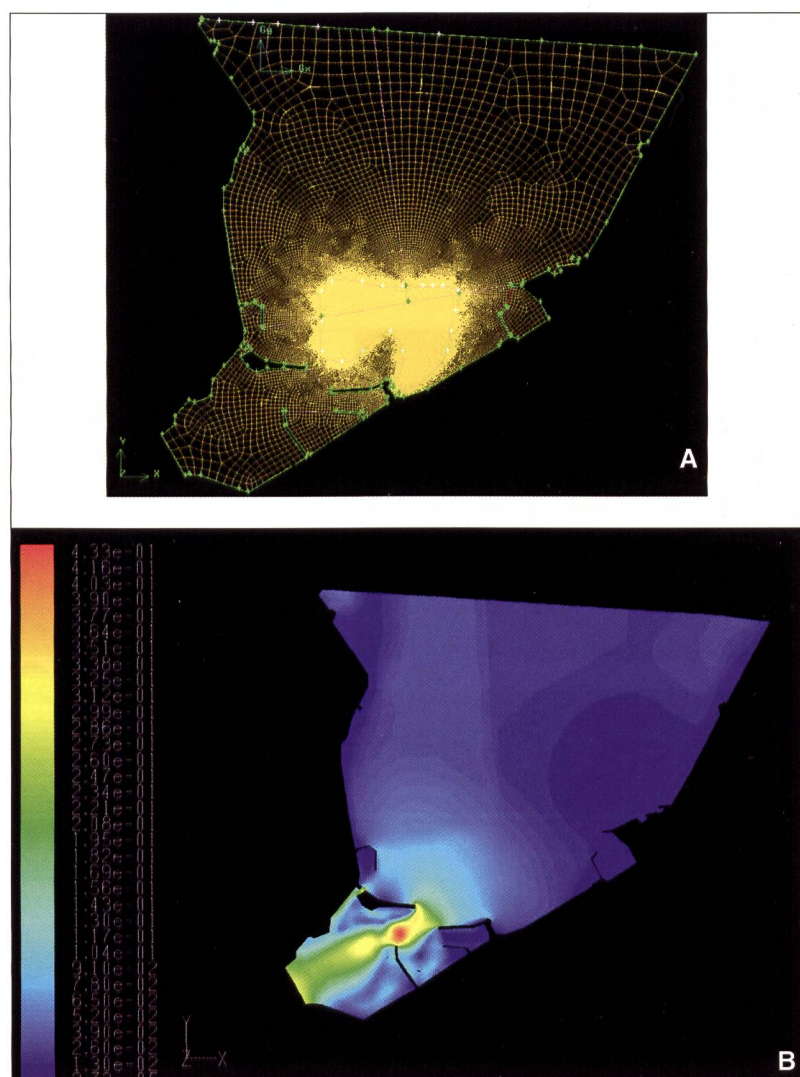


Fig. 8. A: Model mesh used to simulate the currents. The location of the ooidal bank is shown in yellow; B: Simulated current distribution in summer under dominant present wind conditions (colours indicate velocity in m/sec according to the scale shown on the left). Note that the outcome of the model does not show special turbulence around the ooidal bank.

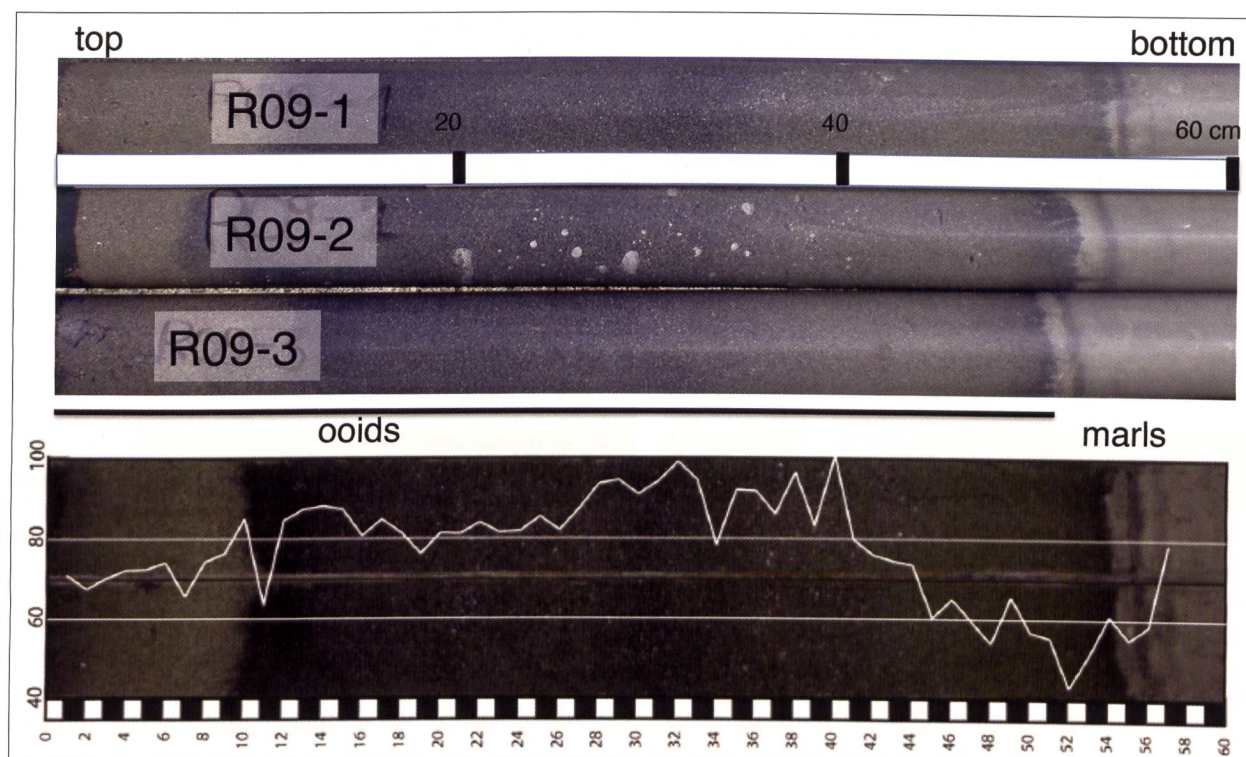


Fig. 9. Photographs of three sedimentary cores retrieved in 2009 clearly showing the thickness of the ooidal bank. The lowermost photograph display the opened core R09-1 and the carbonate percentage that shows relative variations throughout the bank.

identify early diagenetic processes (Farah 2011). Three gravity cores were studied showing a quite uniform sedimentary sequence comprising a lacustrine chalk at the bottom covered by a ca. 60 cm of sands mostly composed of carbonate coated grains (Fig. 9). A combined petrophysical, sedimentological and geochemical study has shown no major changes in the nature of the coated grains throughout depth suggesting a comparable origin. The average carbonate content varied between 55 and 95% throughout depth depending on the dominant nature of the nucleus (Fig. 9). However, the detailed inspection of thin-sections under the petrographic microscope shows preliminary signs of cementation all along the sedimentary core (Fig. 10A, B). Furthermore, in this phreatic environment the development of calcareous meniscus of micritic composition between grains appears to be triggered by microbial activity within EPS. Thus, signs of cementation suggest that early diagenetic processes in this part of Lake Geneva may have started as early as ca. 3000 years ago.

Girardclos (1993) dated the starting of the ooidal bank in Lake Geneva at 2900 radiocarbon years. In a recent review of the existing literature in central European lakes Farah (2011) noticed that these carbonate rich sands and concretions are often located close to archaeological sites of Neolithic age (e.g., Magny 1993; Davaud and Girardclos 2001; Magny 2004). Hence, the presence of Neolithic settlements in lacustrine shallow areas might suggest a plausible

causal link between the formation of ooidal grains and early human activities. This linkage may be related to either the introduction of cyanobacteria to the system by these Neolithic populations and/or due to changes in the lake water chemistry resulting from the increasing input of organic matter. The latter may be in turn due to increasing domestic cattle activities in the catchment during the late Bronze Age that may have increased soil erosion. Despite that Farah (2011) suggestion is mostly based in archaeological reports and publications that often do not contain an exhaustive sedimentological description but are the only available, this linkage appears to be worth to be further investigated.

Outlook

The formation of freshwater ooids was historically attributed to purely physico-chemical processes dominating high energetic sedimentary environments. However, early observations by F.A. FOREL in Lake Geneva shores already pointed towards a biological component playing a major role either forming or inducing the precipitation of these carbonates. To tackle this problem and precise the relative influence of these different processes in freshwater ooid formation we developed a research strategy from the field to the laboratory using cutting edge technology.

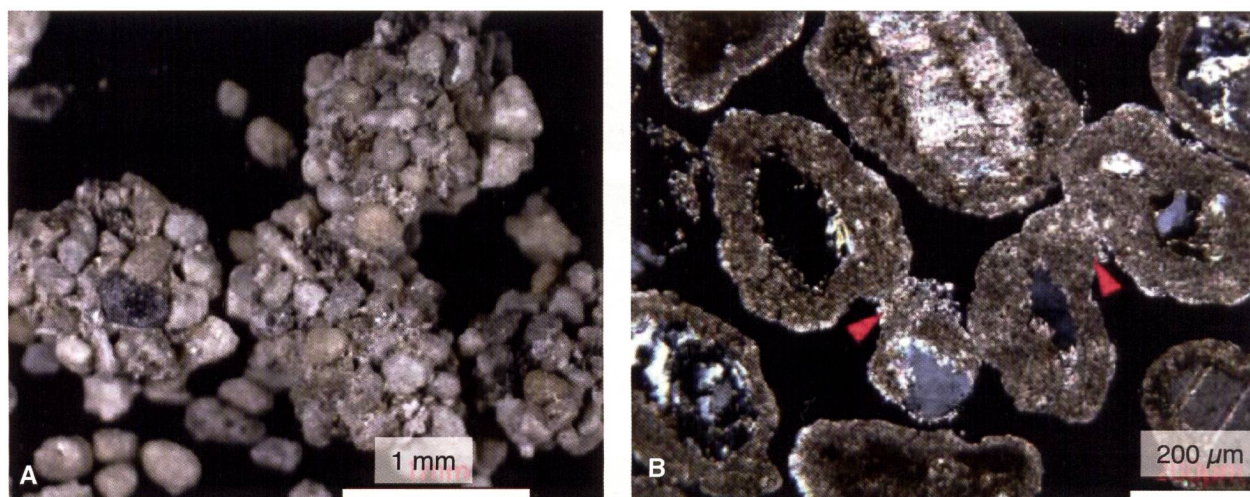


Fig. 10. A: General view of the sand grains at 38 cm sediment depth under a binocular microscope showing incipient carbonate cementation; B: Thin-section under cross-polarized light displaying the initial stage of a grapestone formation at 46 cm sediment depth. Red arrows indicate starting points of carbonate cement precipitation (micrite).

Results of *in situ* and microbial culture laboratory experiments were combined with detailed microscopical observations in the most recent sediments of the ooidal bank in modern Lake Geneva. They indicate that initial ooids formation occurs during static stages and that low-Mg calcite precipitation in the frosted slides is always associated with biofilms and particularly to cyanobacteria. Laboratory experiments in microbial cultures harvested from the *in situ* experiment further indicate that these biofilms are principally composed of photosynthetic microorganisms (such as *Chlorella* sp., *Clamydomonas* sp. and *Synechococcus* sp.) promoting low-Mg calcite precipitation. Carbonate crystals are mainly developed within EPS produced by living microorganisms and not surrounding cell walls. Extracellular Ca^{2+} released from microbial cells would induce this precipitation mediated by photosynthetic microorganisms. The latter is confirmed by the absence of reaction of heterotrophic microorganisms even when they are solicited during laboratory experiments. Furthermore, nanoscale geochemical and microscopical data using Nano-SIMS indicate that EPS produced by photosynthetic microbes serves as a template for carbonate precipitation, triggering amMg -silica permineralization that in turn creates the nanoporosity preceding calcification.

Microscopical and geochemical analyses of sedimentary cores clearly document the effects of early diagenesis affecting the coated grains in Lake Geneva since the late Holocene. They further point towards the microbial impact on early diagenetic processes that can be useful understanding the origin of similar deposits at various temporal and geographical scales. Finally, the outcome of a two-dimensional lake currents model also suggests a limited influence of high energy in ooidal formation at least under present day

conditions. Although different models taking in account past lake-level changes and detailed current measurements are still necessary to fully validate these conclusions, the combined evidence of our investigations indicate that physical accretion in turbulent conditions is certainly not a prerequisite for early cortex precipitation. In early-populated regions such as Lake Geneva shores they may be instead related to increasing microbial populations often linked to human activities. Hence, some previous palaeoenvironmental reconstructions based on ooid-rich sediments may need to be re-visited. Finally, these results endorse early forecasted views by F.A. FOREL and other contemporaneous naturalists, that identified the critical role of biomineralisation processes in the formation of ooids and other carbonate coated sand grains in freshwater Lake Geneva.

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