



Exploring the niche of a highly effective biocalcifier: calcification of the eukaryotic microalga *Oocardium stratum* Nägeli 1849 in a spring stream of the Eastern Alps

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Abstract

Microbially mediated calcification is a major process of carbonate production, yet little is known about eukaryotic microalgal calcifiers. We describe calcification and propagation of the unicellular microalga *Oocardium stratum* in an Alpine spring stream. The spring sheds Ca–Mg–HCO₃ water with a temperature of 8–11 °C. The biota is dominated by *O. stratum* and diatoms; mosses, cyanobacteria, and filamentous eukaryotic algae are accessories. *O. stratum* colonize various substrates within the stream throughout the year. When colonizing, single cells attached to mucilage, then induced precipitation of a rim of calcite, and underwent a first division. A mature clone of *O. stratum* typically consists of single cells each housed within a calcite tube precipitated by the microalga. Upon cell division, the tubes branch, too, under retention of the optical orientation of the calcite. Continued growth, cell division, and calcification result in laminae of *Oocardium* calcite (OC) concordant with substrate shape. *O. stratum* accelerates but seems not to control calcite precipitation. A maximum vertical calcification rate of 5 mm/a was documented for a site ~25 m downstream of the spring. ‘Crystal-skeletal’ OC characterized low calcite supersaturation, whereas higher supersaturation corresponded with rhombohedral OC. Abiotic precipitation downward of the upper tips of growing calcite tubes resulted in compact spar crystals, irrespective of initial crystal habit. Diatoms that thrived on OC benefit from a large differentiated habitat. Our study confirms previous works that identify *O. stratum*, not cyanobacteria, as major biocalcifiers in some hardwater springs. Diagenetically mature *Oocardium* tufa, however, may be confused with spring limestones of other origins.

Keywords Biocalcification · Microbial · Spring limestone · *Oocardium* · Calcareous tufa

Introduction

Precipitation of calcium carbonate causally associated with life, i.e., biocalcification, is a fundamental process in the secular development of Earth (e.g., Konhauser 2007). The role of cyanobacteria as major prokaryotic calcifiers has

been identified since the late 19th century, and continues to be investigated in many studies with different research focus (e.g., Gerdes and Krumbein 1987; Merz 1992; Riding 1992; Arp et al. 2001; Pratt 2001; Kawaguchi and Decho 2002; Pentecost 2005; Jones and Renaut 2010; Riding 2011; Jones 2017). Similarly, calcification associated with uni- and multicellular eukaryotes is comparatively well documented (e.g., McConnaughey and Whelan 1997; Gattuso et al. 1998; Stanley et al. 2002; Erba 2006; Pomar and Hallock 2008; Ries 2009; Jackson et al. 2011; Granier 2012; Gutner-Hoch et al. 2017; Wood et al. 2017). With respect to tufa formation in ambient-temperature spring streams, it was often implicitly underpinned that it is largely or entirely mediated by cyanobacteria and/or by mosses and, locally, by macroalgae such as *Vaucheria*. Conversely, the potential of eukaryotic microalgae to contribute to or even to dominate the limestone-precipitating biotic assemblage of springs was much more rarely identified.

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Only over the past decade or so, an increasing number of springs became identified in which calcification is largely mediated by the unicellular eukaryotic alga *Oocardium stratum* (Sanders and Rott 2009; Rott et al. 2010, 2012; Sanders et al. 2011; Ibarra et al. 2014; Linhart and Schagerl 2015; Cantonati et al. 2016; Trobej et al. 2017; Grüniger and Günzl 2018). This taxon belongs to the coccoid zygnemataleans (or desmids), a group of freshwater algae that is placed into the green algae sensu lato together with other ancestral lineages of land plants, such as the Charophyta (Streptophyta, e.g., Gontcharov et al. 2003). Zygnematalean algae date back at least to late Cambrian to Ordovician times (Gontcharov et al. 2003), and photosynthetic eukaryotes in freshwater habitats probably exist since the Proterozoic (e.g., Strother et al. 2011; Blank 2013; Wellman and Strother 2015).

Oocardium is a highly effective biocalcifier that typically forms crystals of calcite spar up to a few millimeters in size (e.g., Sanders and Rott 2009; Rott et al. 2012). Because *O. stratum* still is little known in the geoscientific community, to enable communication, the major characteristics of growth and calcification of this microalga are summarized first (Figs. 1, 2; Table 1). In all *Oocardium* calcites observed so far, irrespective of initial calcification style along the growing tips of the calcite tubes (cf. Fig. 1g, h), continued abiotic crystallization results in large crystals of calcite spar as final product (Fig. 1e, f) (Sanders and Rott 2009; Rott et al. 2012). The potential of *O. stratum* to build extensive limestone lithosomes has been known since the early 20th century (Wallner 1933, 1934a, b, 1935), but fell largely forgotten afterwards. In consequence, the physico-chemical limits, the life habits, and the synecological relations of *O. stratum* are still poorly known (Rott et al. 2012; Linhart and Schagerl 2015; Cantonati et al. 2016; Trobej et al. 2017). To date, aside a growing number of localities in Europe, the microalga was reported from North America, China, India, and Cuba (Rott et al. 2012; Linhart and Schagerl 2015; Trobej et al. 2017).

In the field, due to the small size of unicells, the presence of *O. stratum* is not easily detected. The few more detailed studies on the calcification of *O. stratum* indicate that it may be admixed to spring biota characterized by mosses and cyanobacteria, or that it strongly prevails and builds up lithosomes of a specific type of limestone (Sanders and Rott 2009; Rott et al. 2012; Linhart and Schagerl 2015). Other aspects of the ecology of *O. stratum* and its calcification remain unclear. Open questions are, for instance, Does it hibernate? How and when does it colonize substrates? Is there a seasonal change in calcification or synecology (e.g., with diatoms)? Does calcification correlate with calcite saturation state and CO₂ concentration of the water? Here we describe the colonization and calcification of *O. stratum* over 13 months in a spring stream of the Eastern Alps.

Setting

The studied spring emerges from a wide terrace along the left side of the Inn valley, one of the major trunk valleys of the Eastern Alps (Fig. 3). The terrace is veneered by glacial till and by lacustrine and glacio-fluvial deposits (Starnberger et al. 2013). The studied spring is one of several springs and seepages that all emerge at ~580 m a.s.l. from within the glacio-fluvial deposits (Ausserlechner 2012). The springline is located on a forested N-facing slope that is shady the year over (Fig. 3c). The spring was chosen for study because (1) it is perennial, (2) it shows active calcification over the comparatively longest extent (~45 m) downstream, (3) its calcifying biota consists nearly exclusively of *O. stratum*, and (4) the water is a relatively 'simple', moderately mineralized Ca–Mg–HCO₃ water (Table 2; Figs. 3c, 4a–c). The spring emerges on a gently sloping morphological step (Fig. 3c, 'spring area' in Fig. 4a) by confluence of seepages into a single streamlet.

Even after heavy snowfalls, the spring stream remained open to the sky (Fig. 5a). Because calcification exceeds stream incision, the stream flows over a moderately steep, ramp-like ledge composed of *Oocardium* tufa (Fig. 5b). Viable *Oocardium* cells, as indicated in the field by a light-green hue, were present over the entire observation period (3.2.2015–21.3.2016) (Fig. 5c). In the upper stream sector, from the spring to site 2 (cf. Fig. 4a), dark green to blackish tufts of filamentous cyanobacteria (e.g., *Tolythrix*) are locally interspersed into *Oocardium* calcite but disappear downstream. Downstream of site 1, patches of filamentous zygnemataleans (*Zygnema*, *Mougeotia*, *Spirogyra*) are locally present, but are not identified as effective calcifiers. Besides *O. stratum*, only diatoms were recognized as important for total calcification mainly by processing new substrates for *Oocardium* settlement (see below). Along both margins of the waterrun ledge, a fringe of grass tufts and of the moss *Palustriella commutatum* is locally present (Fig. 5d). Downstream of site 3 (cf. Fig. 4a), part of the stream water re-infiltrates into the lithosome of spring limestone. Near site 4 (cf. Fig. 4a), the moss *Eucladium verticillatum* grows and calcifies within the stream. Moss calcification is due to overgrowth with *O. stratum* and is not considered separately hereunder.

Seasonal changes of biocolonization were subtle. Patches of viable *Oocardium* (Fig. 5c) were present all year over, but spread during late spring and autumn. Diatoms (mainly *Achnanthes* and *Gomphonema*) were common over the entire year. Although the diatoms were not investigated in detail, no obvious seasonal change of taxonomic composition was identified. Tufts of filamentous zygnemataleans spread in abundance during late spring. In autumn, downstream of site 3–site 4 (cf. Fig. 4a), wilted

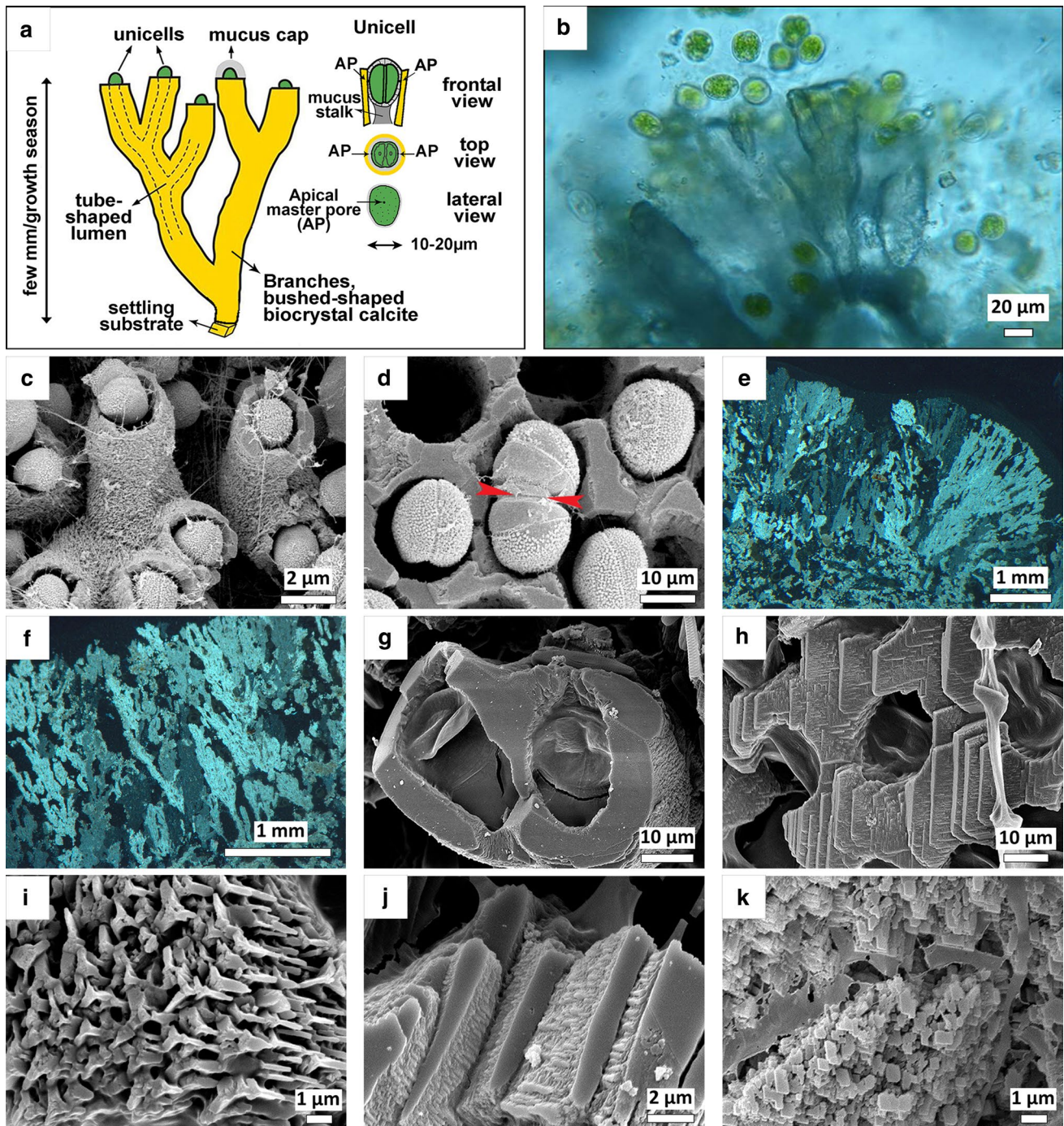


Fig. 1 Explanation of *Oocardium stratum*. **a** Scheme showing (1) branched, initially hollow calcite tubes (s) precipitated by the micro-alga, (2) position of cells (green) on a mucus stalk in the calcite tubes. Cells are fixed by holdfasts secreted from apical master pores (AP). **b** Transmitted light image of *Oocardium* calcite (OC) and green *Oocardium* cells. **c, d** *O. stratum* cells in their calcite tubes.

Red arrows in **d** point to a dividing cell. **e, f** Thin sections of *Oocardium* calcite. Note large single calcite crystals. Crossed nicols. **g–k** Terminology for shapes of calcite crystals that comprise the calcite tubes. **g** Massive; **h** stepped; **i** crystal-skeletal; **j** bladed; **k** microcrystallites. See text for further description

leaves of maples and alders locally covered up to an estimated 50–70% per square meter of the limestone surface (Fig. 5e). The waterrun upper sides of leaves provided substrates for biocolonization–calcification successions

similar to those observed on experimental substrates (Fig. 5f). The autumnal input of leaves and its potential role for the biotic assemblage is discussed in more detail below.

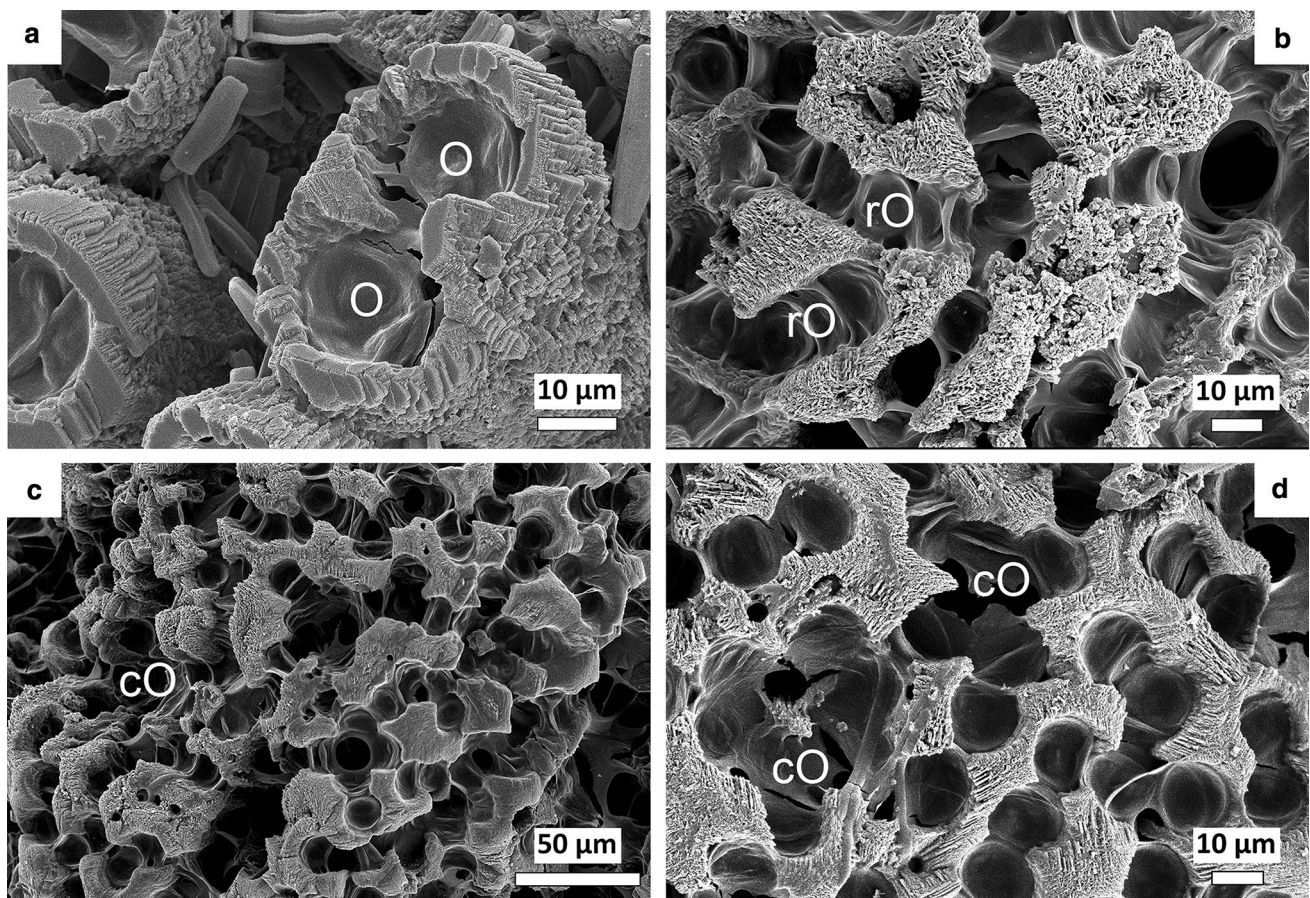


Fig. 2 Arrangement of *O. stratum* cells. **a** Two separate cells (*O*) shortly after division. **b** Rows of *Oocardium* cells (*rO*) separated by ‘walls’ of calcite. **c, d** Clusters (*cO*) and rows of *Oocardium* cells

Methods, definitions

Documentation of *Oocardium* calcification described herein was started on February 3, 2015 and was repeated every 3 weeks until March 21, 2016. Microbotanical inspection under both reflected and transmitted light of fresh samples extracted in the field provided documentation of cyanobacteria and green algae. Microbotanical samples and samples for analysis of water chemistry were stored in a coolbox immediately after extraction from the field. Thin-sections of 21 dried and resin-impregnated samples of (1) natural *Oocardium* limestone and (2) of *Oocardium* calcite grown on experimental substrates provided data on calcite petrography, rates of vertical growth, and general aspects of fabric development. The spring stream was subdivided by four sites 1–4 (Fig. 4) each providing a fixed station for field measurement of physico-chemical conditions of water, water sampling, placement of experimental substrates, and for judgment of the overall state of stream biota with changing season.

In the field, water temperature, electrical conductivity, and pH were measured with a calibrated multiparameter Combo Tester of Hannah Instruments®. On every field visit, downstream, several water samples for full chemical analyses were taken. Water samples were carefully filled into 250-ml bottles. A few hours after field sampling and storage in a coolbox, the water samples were handed over to the Chemisch-Technische Umweltschutzanstalt of the Federal Government (Innsbruck city) for certified laboratory analyses. The PHREEQC Interactive program (version 3.3.7.11094) was used to calculate the equilibria of water samples (Parkhurst and Appelo 1999; Charlton and Parkhurst 2002, 2011). Calcite saturation index (SI_{calcite}) was applied as a measure of equilibrium according to the formula: $SI = \log(IAP/KT)$, where: IAP—Ion Activity Product for ions forming minerals soluble in the given water solution, calculated according to the law of mass action for the *K*-equilibrium constant for a given reaction (Gradziński 2010). Free CO₂ causing pH lowering at site 1 was analyzed using GRAN titration of samples kept isothermal in a coolbox as soon as possible after sampling. CO₂ gas was

Table 1 Descriptive terminology for growth and calcification fabrics of *Oocardium stratum*

Criterion	Description, figure reference
External shape of <i>Oocardium calcite</i>	Lamina (laminar): laterally adjacent, vertically elongate ‘bushes’ (each from a parent cell) of <i>Oocardium calcite</i> , similar in appearance to a thicket (Fig. 1a, f) Pustule (pustular): hemispherical aggregate of tubes of <i>Oocardium calcite</i> , originated by upward growth and multiple divisions of a parent cell (Fig. 7b)
Cell arrangement	Single celled: each tube of <i>Oocardium calcite</i> houses a single cell (Fig. 1a, c) Double celled: a tube of <i>Oocardium calcite</i> houses two fully separate cells (cells after complete division) (Fig. 2a) Cell rows: straight to meandering rows of laterally adjacent <i>Oocardium</i> cells (Fig. 2b) Cell clusters: clusters of <i>Oocardium</i> cells; within the clusters, cells are not separated by calcite (Fig. 2c, d)
Calcite crystals per tube of <i>Oocardium calcite</i> (applies only to single- and double-cell arrangements)	Single crystal: calcite tube consists of a single crystal or is part of a larger crystal (Figs. 1e, f, 10b) Multicrystal: calcite tube consists of two or more crystals, including crystal twins (Figs. 8e, 11e, f)
Crystallization style (as seen on top and upper flanks of calcite tubes)	Massive-idiomorphic: smooth surfaces of calcite rhombohedron well identifiable (Fig. 1g) Stepped: growing surface of calcite crystal is compartmentalized into many steps, each delimited by crystal surfaces of identical orientation (Fig. 1h) Bladed: growing surface of calcite crystal is compartmentalized into ‘blades’ separated by high steps or by empty space between; top of blades delimited by crystallographic surface (Fig. 1j) Crystal-skeletal: growing calcite crystal consists of numerous small crystal skeletons of identical orientation (Fig. 1i) Microcrystalline (rare): growing calcite crystal consists of numerous sub-crystallites displaying rhombohedral shape (Fig. 1k)
Terminology for crystal size according to Folk (1974)	Mini-micrite: crystals < 1 μm in size Micrite: crystals 1–4 μm in size Microspar: crystals 4–10 μm in size Spar: crystals > 10 μm in size Orthospar: crystals > 10 μm in size that grew into open pore space Pseudospar: crystals > 10 μm in width that grew to final size by aqueous recrystallization

analyzed by gradual gas removal during back-titration to pH 8.3 (for further details see Rott et al. 2010). Duplicate titrations were performed to limit scatter.

To track colonization and calcification by *Oocardium*, we placed experimental substrates at sites 1–4 (Table 3). Based on earlier experiences, we used diverse natural and artificial substrates not only to provide colonization surface but also to test the effects of complex substrate space and outer day-lit versus inner dark or low-lit surfaces. Substrates were placed at different times over the observation period. Most substrates were sampled on each or every second field visit (interval of 3–6 weeks) by carefully chipping off a small piece for inspection by electron microscopy. In the laboratory, the SEM samples were air dried at room temperature. Calcification rate was determined by measurement from SEM images and from thin sections of *Oocardium calcite* grown on experimental substrates. Documentation was centered on backscattered electron microscopy of *Oocardium* colonization and calcification on diverse experimental substrates relative to the physico-chemistry of stream water at site. Imaging and analyses of samples

by scanning electron microscopy (SEM) were carried out with a JEOL Model JSM-6010 LV. The samples were mounted on carbon tape and gold sputtered. SEM analysis provided a magnification range from 20× to 30,000×, with a maximum resolution of 0.5 μm (secondary electron image), an acceleration voltage of 10 kV, and an optional high-vacuum and low-vacuum operation mode. Backscattered electron shadow images were acquired at low vacuum with an acceleration voltage of 15 kV (Perfler et al. 2015, 2016). Due to air drying, in SEM, *Oocardium* cells look similar to deflated soccer balls but remain well identifiable as such.

Herein, we use the term ‘abiotic’ for calcite crystals and fabrics thereof that apparently grew, or that continued to grow, without decisive interaction with organisms as far as identifiable in SEM at magnifications up to 15–20 k. This does not necessarily exclude that the crystals *initially* were related to bio- or organofilms. The terminology to designate crystal sizes is according to Folk (1974) (Table 1). In the following, the natural state of the spring limestone and the results from experimental substrates placed for precipitation of *Oocardium*

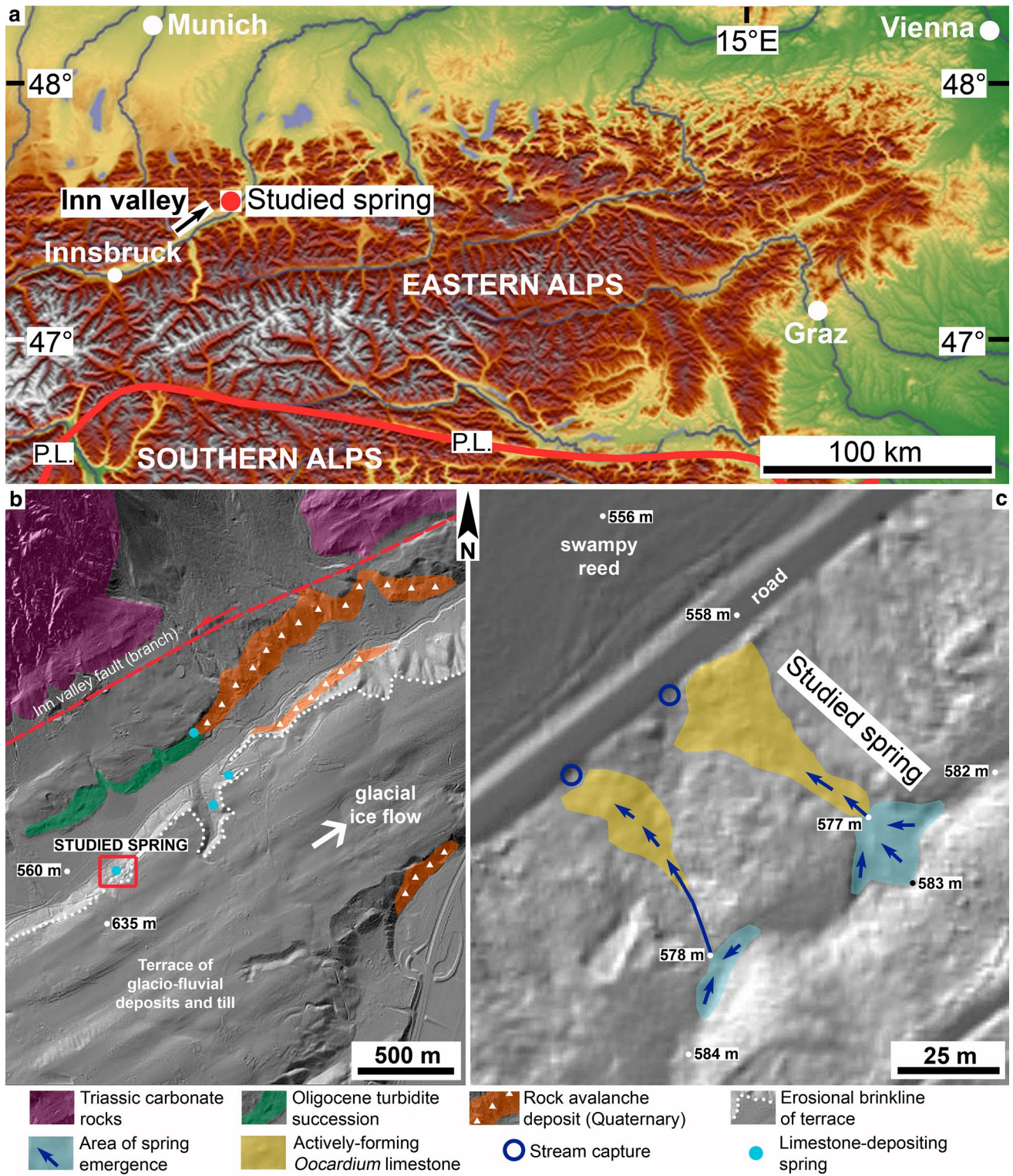


Fig. 3 **a** Position of studied spring in the Eastern Alps. **b**, **c** Local geological setting of spring. Calcification was studied at four downstream sites 1–4. Site 1: 47.520407°N/12.043665°E; site 2:

47.520439°N/12.043610°E; site 3: 47.520472°N/12.043536°E; site 4: 47.520556°N/12.043222°E. Site coordinates in decimal notation. See text for further description

Table 2 Average values and minimum–maximum range of physico-chemical parameters of the water of the studied spring

	Average ($n=58$)	Minimum/maximum
Temperature (°C)	9.03	4.87/15.63
pH	8.03	7.12/8.7
Electrical conductivity ($\mu\text{S}/\text{cm}$)	555.96	428.67/679.33
Ca^{2+} (mg/l)	84.54	61.8/99.3
Mg^{2+} (mg/l)	27.61	21.4/31.6
Mg/Ca molar ratio	0.55	0.46/0.71
Na^+ (mg/l)	2.76	2.21/3.4
K^+ (mg/l)	2.74	2.21/3.64
HCO_3^- (mg/l)	396.12	335/441.75
CO_2 (mg/l)	18.44	3.15/53.14
SI calcite (PHREEQC)	0.9	0.08/1.47
$(\text{SO}_4)^{2-}$ (mg/l)	4.4	3.6/6.46
Cl^- (mg/l)	2.79	2.14/3.65
NO_3^- (mg/l)	5.63	4.86/7.33
Total phosphorus (mg/l)	< 15	< 15/21

See Fig. 4 for downstream changes of parameters

calcite are described downstream from sites 1–4 (cf. Fig. 4; Tables 3 and 4). The term ‘green *Oocardium* calcite’ denotes that most or all of the calcite tubes (cf. Figs. 1, 2) are inhabited by living *O. stratum* cells; conversely, ‘white *Oocardium* calcite’ indicates a limestone that consists of calcite tubes as diagnostic of *O. stratum* calcification, but with only a few or no living cells within their tubes. For samples of *Oocardium* calcite that were taken from the natural stream bed, we use the term ‘natural *Oocardium* calcite’, whereas calcite precipitated on experimental substrates is briefly named ‘grown *Oocardium* calcite’ or ‘*Oocardium* calcite grown on’.

Results

Downstream calcification

Site 1

At this site, the natural stream bed was paved mainly with white *Oocardium* calcite, but interspersed with smaller patches of green *Oocardium* calcite (Table 4); the green patches persisted the entire year over. The white *Oocardium* calcite showed as empty tubes in crystal-skeletal to bladed crystallization style (Fig. 6a, b). The patches of green *Oocardium* calcite, however, typically consisted of ‘walls’ of calcite laterally separating rows of *O. stratum* cells (Fig. 2) or, locally, of ‘pillars’ of calcite within clusters of *O. stratum* cells; in both cases, the calcite also showed crystal-skeletal to bladed crystallization style (Fig. 6c, d). When- and

wherever sampled, at site 1, green *Oocardium* calcite always showed crystal to, more rarely, bladed crystal fabric.

Despite the presence of green *Oocardium* cells at site 1 over the entire year, this site remained inert with respect to colonization and calcification of experimental substrates by *O. stratum* (cf. Table 3). Substrates were colonized by biofilms of diatoms and undetermined bacteria, and with scattered nanometer- to micrometer-scale crystals of calcium carbonate (Fig. 6e). Overall, the density of calcification with small-sized crystals increased within the bacterial-diatom biofilms over the observation period (Fig. 6f). Macroscopically, the rinse fleece remained soft and uncalcified as it was when placed into the stream. In summary, except for a low rate of calcification associated with diatoms and bacteria, site 1 remained practically inert over more than a year with respect to *Oocardium* colonization and calcification.

Site 2

At site 2, green *Oocardium* cover prevailed all over the year (Fig. 5c). The natural *Oocardium* calcite sampled from this site is characterized by cell rows and clusters, and by crystal-skeletal to bladed initial calcification (Fig. 7a). Experimental substrates typically were colonized by *Oocardium* within 3 weeks after placement (see Table 3). On most substrates, *Oocardium* calcified in bladed to massive style, and with cells typically arranged in rows and clusters (Fig. 7b–d). At site 2, pustules of *Oocardium* calcite grew at a rate of 55 and 78 $\mu\text{m}/\text{month}$ ($n=2$) (Table 4).

Site 3

Here, the natural *Oocardium* calcite was characterized by massive-idiomorphic initial calcification (Fig. 7e). Whereas *Oocardium* was clearly prevalent, intercalated patches colonized by diatoms were present. The diatom patches were associated with cumulates of (sub)micron-sized calcite crystals that presumably had precipitated within the mucus of the diatom mats (Fig. 7f). At site 3, all precipitation substrates were colonized by diatoms and bacteria within 3 weeks, and first settlement and early calcification by *Oocardium* required 3–6 weeks, i.e., one to two check intervals in duration. *Oocardium* consistently calcified in massive-idiomorphic or, more rarely, bladed style all over the year (Table 3).

The different experimental substrates showed specific styles of initial *Oocardium* colonization. (a) Substrates completely overrun by water, such as rinse fleece, became more-or-less equally colonized. Three patches of rinse fleece placed during spring and winter (see Table 3 for dates) all were colonized by diatoms and *Oocardium* within 3 weeks after placement. The *Oocardium* cells settled on the fleece

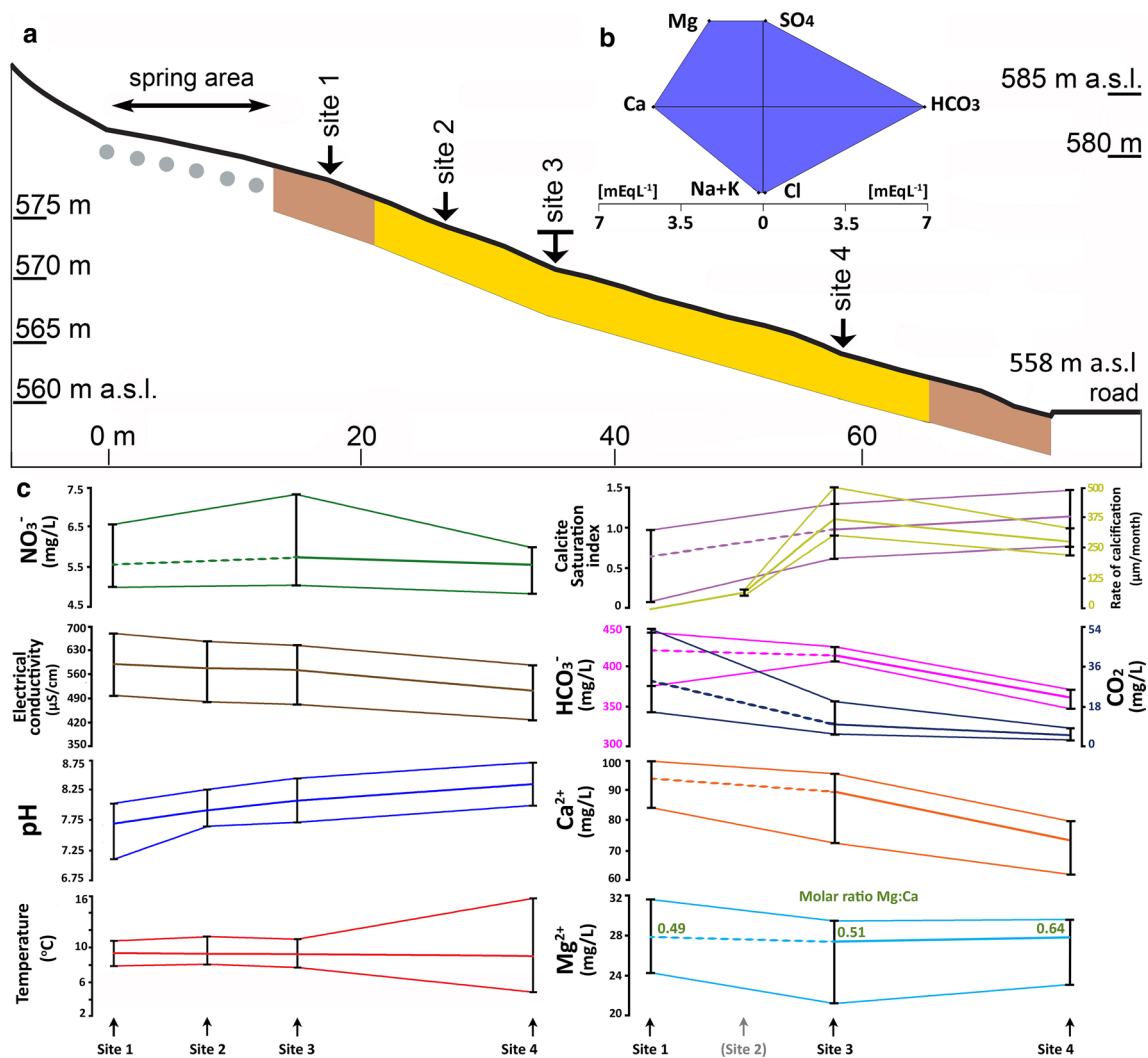


Fig. 4 **a** Section down spring stream. The physico-chemistry and the biota were mainly studied at sites 1–4. At site 3, numerous experimental substrates (see text) were distributed over a downstream

distance of ~2 m. **b** Stiff plot to indicate mean cationic/anionic chemistry of the stream. **c** Downstream changes of major physico-chemical parameters

surface coalesced into a continuous layer of massive-idiomorphic calcite (Fig. 8a, b). (b) Substrates that projected out of stream flow, such as small flowerpots (Table 3), became first colonized and calcified by *Oocardium* along the downstream (lee) side, and near the interface between stream and atmosphere. Notably, the lower fringe of the low-lit inner side of flowerpots also became colonized by *Oocardium* cells; these cells had started to calcify but died soon after settlement and early calcification (Fig. 8c–e). (c) The sharp-edged plastic strips first became colonized by diatoms and bacteria; within these biofilms, isolated crystals and crystal clusters of calcite precipitated. *Oocardium* colonization, in contrast, always started along the edges of the strips. Only later, when a crust of calcite crystals or wider crystal clusters had precipitated on the upper surface of the strips, had *Oocardium* also settled there (Figs. 8f; 9a, b). (d) Finally,

plastic cords that waved freely in shooting stream flow also became colonized by diatoms and *Oocardium* as readily as fixed substrates. *Oocardium* colonization on the cords took place also during winter, and green cells within calcite tubes of massive-idiomorphic crystallization style persisted over the winter (Fig. 9c–f).

At site 3, the documented rates of calcification were highest. SEM observations and thin-sections of experimental substrates indicate that the *Oocardium* calcite grew vertically at a rate of 300–370–500 $\mu\text{m}/\text{month}$ ($n=9$) (Table 4). In precipitation substrates with a large pore space—such as rinse fleece, loofah, or small boxes of balsa wood—(see Table 3), the low-lit (rinse fleece, loofah) or dark (wood box) pore space became filled mainly by micropeloidal grainstone to packstone whereas the daylight outer surfaces were colonized by calcifying *Oocardium* (Figs. 9a, 10b–d).

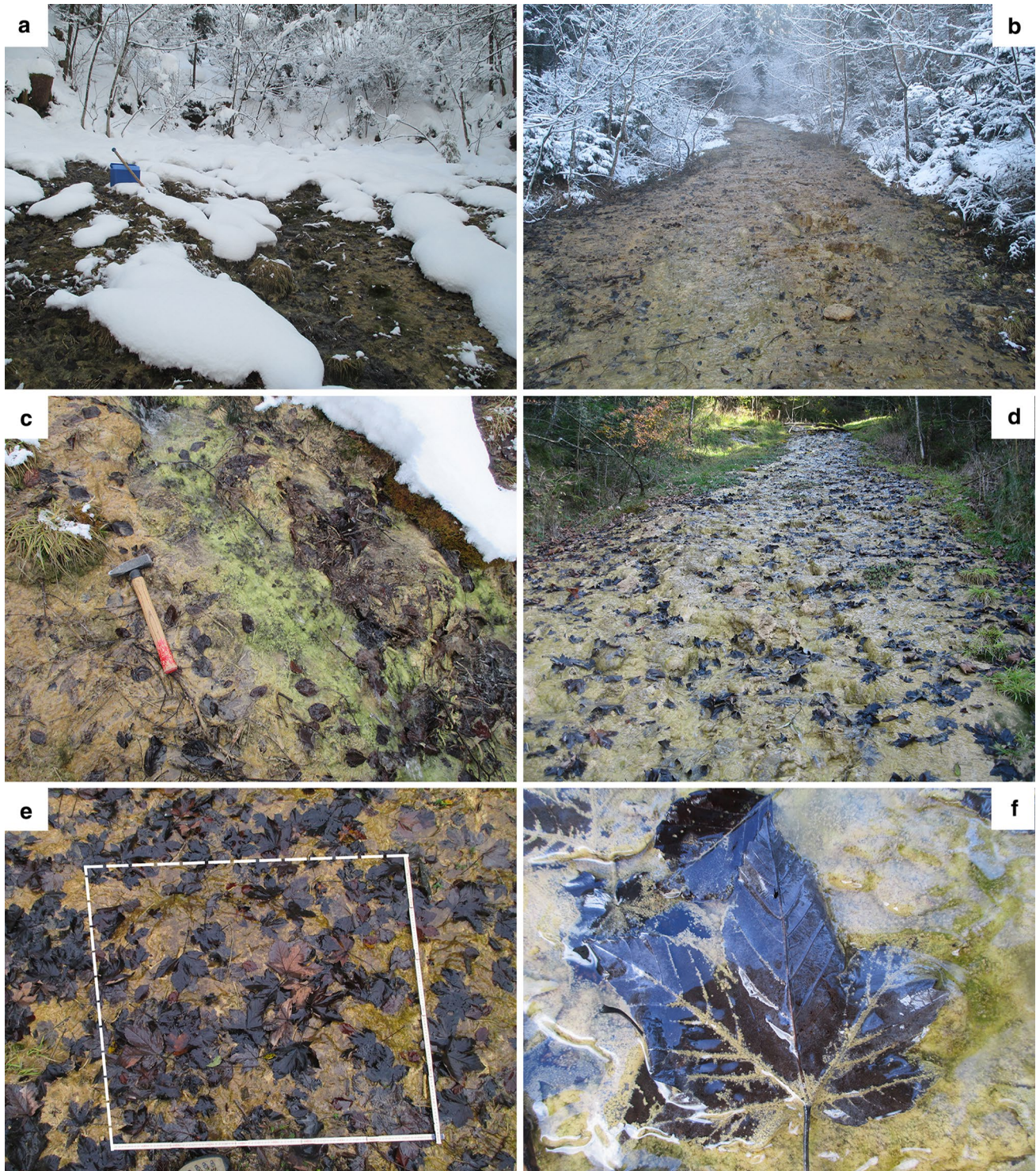


Fig. 5 **a** Spring area on 2.3.2015 after heavy snowfall. **b** View up the spring stream on 7.1.2016. **c** Active *Oocardium* calcite (green hue on stream bed), and *Oocardium* calcite with only a few viable cells (brownish hue); 3.2.2015. **d** View upstream, 6.11.2011. Note leaves

fallen into the stream, and marginal fringes of grass tufts and moss. **e** Stream bed on 12.10.2015 between site 3 and site 4 (cf. Fig. 3a), littered with wilted leaves. **f** Maple leaf on stream bed. Calcification starts along the protruding leaf veins; 23.11.2015

Table 3 Overview of main results from experimental substrates

Substrate (datum of placement)	Site 1 (installed 2015-02-03) NOC fabrics: crystal-skeletal, bladed; clustered cells typical	Site 2 (2015-06-11) NOC fabrics: crystal-skeletal, bladed, bookshelf; clustered to linear cells and single cells	Site 3 (2015-02-03) NOC fabrics: massive-idiomorph, bookshelf; single cells	Site 4 (2015-02-03) NOC fabrics: massive-idiomorph, bookshelf; single cells
Rinse fleece (2015-02-03)	Nearly no calcification over entire observation period; colonization by diatom/bacterial assemblages; rare Ooc overgrown by diatom/bacterial biofilms	–	Colonized by Ooc with bookshelf to massive calcification; Ooc colonization later quenched, perhaps during 'July–August 2015 crisis' (see text); subsequently overgrown by diatom biofilm	Scarcely colonized by Ooc 6 weeks after placement; significant Ooc colonization only 3 months after placement; diatoms continued to prevail in patches; many Ooc cells remained viable over the winter
Rinse fleece (2015-05-21)	–	–	Colonized by Ooc within 3 weeks after placement; calcification style highly variable from crystal–skeletal to massive	–
Rinse fleece (2015-06-11)	–	Colonized by Ooc mainly with bookshelf to massive calcification; clustered and linear cells	–	–
Rinse fleece (2015-12-21)	–	Colonized by Ooc mainly with massive and bookshelf (locally also bladed) calcification; single cells, few double cells	Colonized by pustular Ooc within 3 weeks after placement	–
Loofah (2015-02-03)	–	–	Colonized by Ooc within 3 weeks after placement; bookshelf to massive calcification	–
Loofah (2015-06-11)	–	Colonized by Ooc mainly with bookshelf to massive calcification	–	–
Crockery flowerpot (2015-06-11)	–	Outer surface: Crust of 'abiotic' micro-spar- to spar-sized calcite crystals colonized by Ooc; calcification style from crystal-skeletal to massive Inner surface: Patchily coated by aggregates of nm- to micron-sized calcite crystals mixed with clastic grains, bacteria, dead diatoms, and diatoms that lived when sampled	Outer surface: Colonized by pustular Ooc in massive to bookshelf calcification Inner surface: Crust of 'abiotic' microspar- to spar-sized calcite crystals colonized by Ooc; the cells died soon after settlement and initial calcification	–
Crockery flowerpot (2015-11-23)	–	Outer surface: Colonized by Ooc with massive-idiomorphic to bookshelf-banded calcification style; diatoms abundant on Ooc calcite Inner surface: Small patches composed of diatom colonies mixed with micritic to microsparitic calcite crystals	–	–

Table 3 (continued)

Substrate (datum of placement)	Site 1 (installed 2015-02-03) NOC fabrics: crystal-skeletal, bladed; clustered cells typical	Site 2 (2015-06-11) NOC fabrics: crystal-skeletal, bladed, bookshelf; clustered to linear cells and single cells	Site 3 (2015-02-03) NOC fabrics: massive-idiomorph, bookshelf; single cells	Site 4 (2015-02-03) NOC fabrics: massive-idiomorph, bookshelf; single cells
Plastic strip (2015-06-11)	–	Ooc initially settled on edge of strip; bookshelf to massive calcification; viable Ooc cells also over the winter	Ooc pustules initially settled on edge of strip; massive calcification	Ooc initially settled on edge of strip; massive calcification style; substrate partially shaded by wilted maple leave ≥ continued abiotic calcite crystallization on template of Ooc calcite; after removal of leave (by us), calcite was recolonized by Ooc and diatoms
Plastic strip (2015-11-02)	–	–	Ooc initially settled on edge of strip; substrate partially shaded by wilted maple leave ≥ continued abiotic calcite crystallization on template of Ooc calcite	Ooc initially settled on edge of strip, then abandoned and followed by (a) abiotic calcite growth, accompanied by (b) settlement of diatoms and green algae
Plastic strings (2015-03-17) (moved freely in flow)	–	–	Ooc initially settled in individual pustules; microcrystalline calcification	–
Plastic strings (2015-06-11) (moved freely in flow)	–	–	Colonized by pustular Ooc with massive to bookshelf calcification	Colonized by pustular Ooc with massive calcification; subsequent to 21.10.2015, the bundle was destroyed by trampling (deer or humans)
Plastic strings (2015-12-21) (moved freely in flow)	–	–	Colonized by Ooc 3 weeks after placement; viable Ooc with massive calcification persisted over the winter	–
Wooden box (2015-03-17)	–	–	Outer surface colonized by Ooc 9 weeks after placement; stepped rhombohedral to microcrystalline calcification; pustules to thickets with single cells and a few double cells	–

NOC 'Natural' *Oocardinum* calcite on stream bed (not grown on experimental substrates), *Ooc Oocardinum*

Table 4 Characterization of downstream sites 1–4 (see Fig. 4)

	Stream bed	<i>Oocardium</i> cell arrangement	Initial crystallization style of <i>Oocardium</i> calcite	Rates of <i>Oocardium</i> calcification (μ /month)	Remarks
Site 1	Mainly WOC, patches of GOC	Rows and clusters	Crystal-skeletal to bladed	Zero	Experimental substrates remained uncalcified by <i>O. stratum</i>
Site 2	Mainly GOC, patches of WOC	Rows and clusters	Crystal-skeletal to bladed	55 μ /month; 78 μ /month ($n=2$)	–
Site 3	Mainly GOC, patches of WOC	Single cells	Massive-idiomorphic, rarely bladed	300–370–500 μ /month ($n=9$)	–
Site 4	GOC and WOC, seasonally strongly changing	Single cells	Massive-idiomorphic	220 μ /month; 330 μ /month ($n=2$)	Stream bed was perennially overrun by a ‘sheet’ ~ 1–3 mm thick of water

For terms related to cell arrangement and initial crystallization style of *O. stratum*, see Table 1 and Fig. 1

WOC White *Oocardium* calcite, GOC Green *Oocardium* calcite. See text for further description

Site 4

Downstream of site 3, the spring stream divides and widens, and the water re-infiltrates into the body of spring limestone. As a consequence, stream flow is a sheet of water ~ 1–3 mm in thickness at site 4, yet perennial. Hence, the choice of precipitation substrates was more limited. The natural *Oocardium* calcite at site 4 showed massive-idiomorphic initial crystallization. A piece of rinse fleece placed on February 3, 2015, and overrun by a thin film of water, required 6 weeks for diatom colonization; in addition, rare *Oocardium* was found (Fig. 11a, b). Sizeable colonization by pustular clones of *Oocardium* with massive-idiomorphic crystallization occurred only 3 months after substrate placement; overall, diatoms remained the most abundant colonizers. Only slowly, and probably with increasing overall calcification, the density of *Oocardium* cover increased, but large patches colonized only by diatoms remained. Over the winter of 2015 to 2016, *Oocardium* remained in part viable, and the calcite tubes provided a substrate for cover by diverse diatoms (Fig. 11c). Conversely, the two precipitation substrates placed on June 11, 2015 (Table 3) were colonized by diatoms and calcifying *O. stratum* within the 3-week check interval after placement. At site 4, despite an overall high calcite saturation index, rates of calcification are lower (cf. Fig. 4). *Oocardium* calcified at an averaged rate of 220 and 330 μ m/month ($n=2$) (Table 4). Downstream of site 4, watershed progressively dwindles, and precipitation of spring limestone tapers out within a few meters (Fig. 4).

Oocardium settlement and early calcification

Calcite substrate was first colonized by single cells of *O. stratum*. Initially, a cell settled and held fast on what appears to be an organic ‘glue’ secreted by the cell. This phase of settlement was only rarely observed (Fig. 11d).

Subsequently, while still attached to the initial place of settling, the cells became fringed by calcite crystals (Fig. 11d, e). Next, when the calcite crystals had precipitated to size, in most cases, the cell seemed to grow up for a small vertical distance (i.e., a short mucus stalk had developed; Figs. 11f, 12a) and then underwent its first division. Upon progressive calcification and cell divisions, a multicellular pustule of *Oocardium* calcite developed by (a) vertical growth of calcite tubes, combined with (b) ongoing cell division and, hence, branching of calcite tubes. As mentioned, the optical orientation of the calcite is retained upon cell division and branching of calcite tubes, resulting in single large biocrystals (cf. Fig. 10a–d).

Downward along the tubes of *Oocardium* calcite, the mucus stalks disappeared, and the lumen of the tube was filled by calcite with identical optical orientation to its housing calcite tube. In SEM, broken calcite tubes also showed that despite the wide range in initial calcite crystallization, within at most a few tens of microns downward from the tube top, the inner part of the tube walls consisted of massive (non-porous) calcite. All types of precipitation substrates with distinct edges were first colonized by *Oocardium* along the edges (cf. Figs. 8f; 9a, b). Where wider areas were colonized by *Oocardium*, markedly different stages of growth—ranging from single cells in initial calcification to fully developed multicellular pustules as described—indicate that after the initial lag phase, colonization is continuously taking place.

Relation of *Oocardium* to bacteria and diatoms

At all sites, precipitation substrates were initially colonized by bacteria and diatoms; in no case, *Oocardium* was the first colonizer of substrates. Recall that the precipitation substrates included only ‘non-calcitic materials’ (wood, plastic, etc.) (Table 3). On calcitic surfaces, in turn, individual

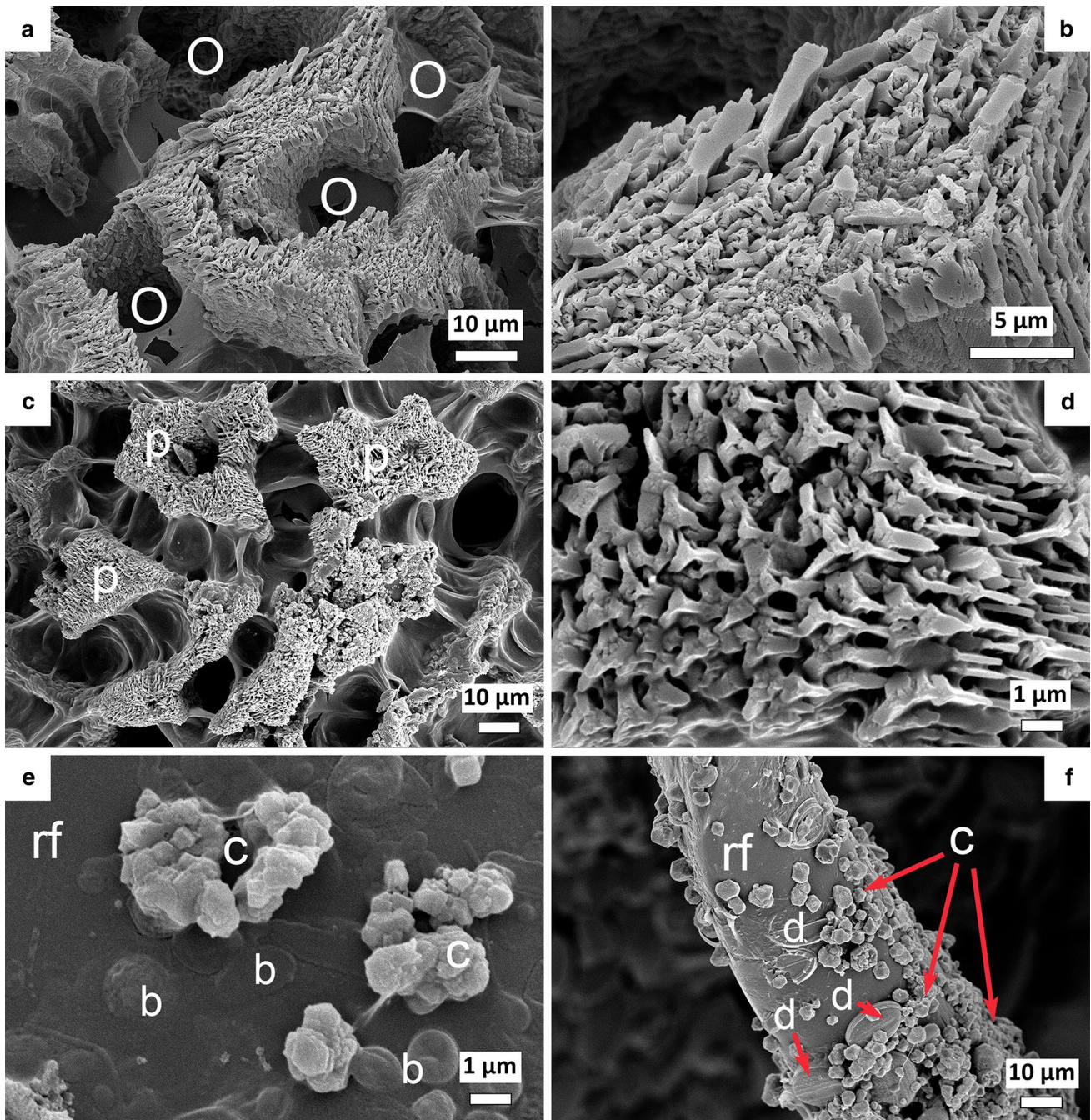


Fig. 6 **a** Site 1 (cf. Fig. 3): natural white *Oocardium* calcite in skeletal crystallization style (O: sites without *O. stratum* cells). **b** Detail of crystal-skeletal fabric of subfigure **a**. **c** Site 1: detail from a patch of natural green *Oocardium* calcite. Rows of *Oocardium* cells intercalated by calcite ‘pillars’ (p) with crystal-skeletal fabric. **d** Crystal-

skeletal fabric of subfigure **c**. **e** Site 1: experimental substrate (rf, rinse fleece) colonized by unspecified bacteria (b). Note submicron- to micron-sized calcite crystals (c). **f** Site 1: plastic fiber of rinse fleece (rf) colonized by diatoms (d), 13.5 months after placement. Note cover of calcite crystals (c)

Oocardium cells settled directly. On the precipitation substrates, the early bacterial-diatom biofilms were associated with mini-micritic to sparitic calcite crystals that were isolated and/or comprised clusters and patches (Fig. 12b; see also Fig. 6e, f).

In fully developed fabrics of *Oocardium* calcite inhabited by living cells, bacteria were rare to most commonly absent. Conversely, diatoms were widely associated with living *O. stratum*. The diatom flora observed on the *Oocardium* calcite was dominated by *Achnanthes* and *Gomphonema*; in

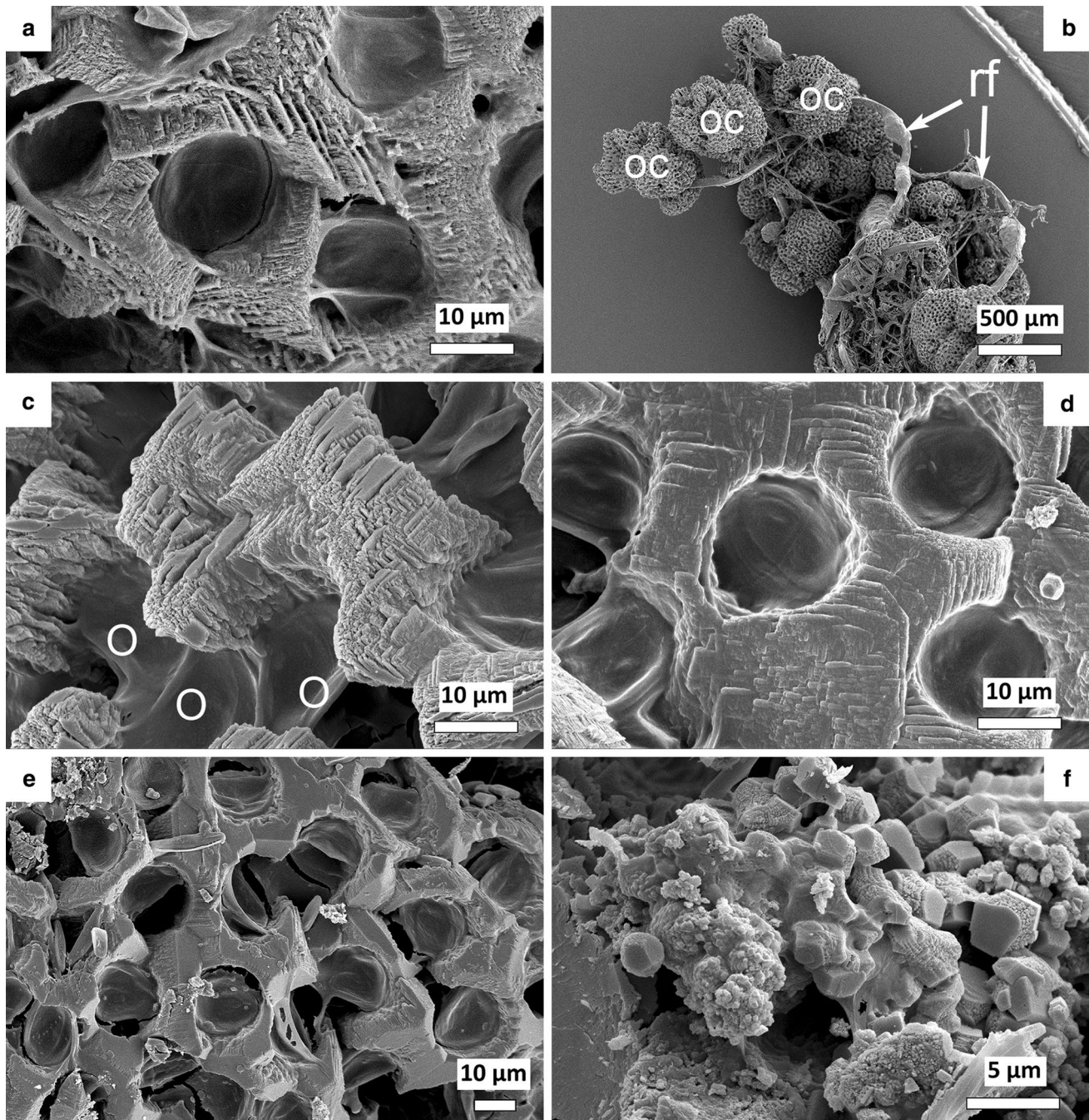


Fig. 7 Downstream changes of *Oocardium* calcification. **a** Site 2: natural green *Oocardium* calcite with crystal-skeletal calcification. **b** Site 2: rinse fleece (rf) overgrown by spherical *Oocardium* calcite (OC) each comprising numerous cells. Substrate placed 11.6.2015; sampled 2.11.2015. **c** Detail of subfigure **b**: bookshelf calcification,

and short row of *Oocardium* cells (O). **d** Site 2: green *Oocardium* calcite (grown on rinse fleece) showing stepped calcification. Substrate placed 11.6.2015; sampled 21.3.2016. **e** Site 3: natural *Oocardium* calcite in massive calcification style. **f** Site 3: detail of a patch of calcite crystallites typically associated with diatom mats

in addition, a number of other less widespread forms was present (e.g., *Navicula*, *Synedra*, *Diatoma*, *Cymbella*, *Fragilaria*, *Cocconeis*). Diatom colonization ranged from dense aggregations (Fig. 12c, d) to a few frustules scattered on and between *Oocardium* calcite tubes. The diatoms settled

on the large surface provided by the arrays of calcite tubes, and/or settled and grew up between the tubes (Fig. 12e). Even in case of very dense diatom colonization, however, provided that *Oocardium* calcite tubes were inhabited by cells when sampled, the top of the calcite tubes was free

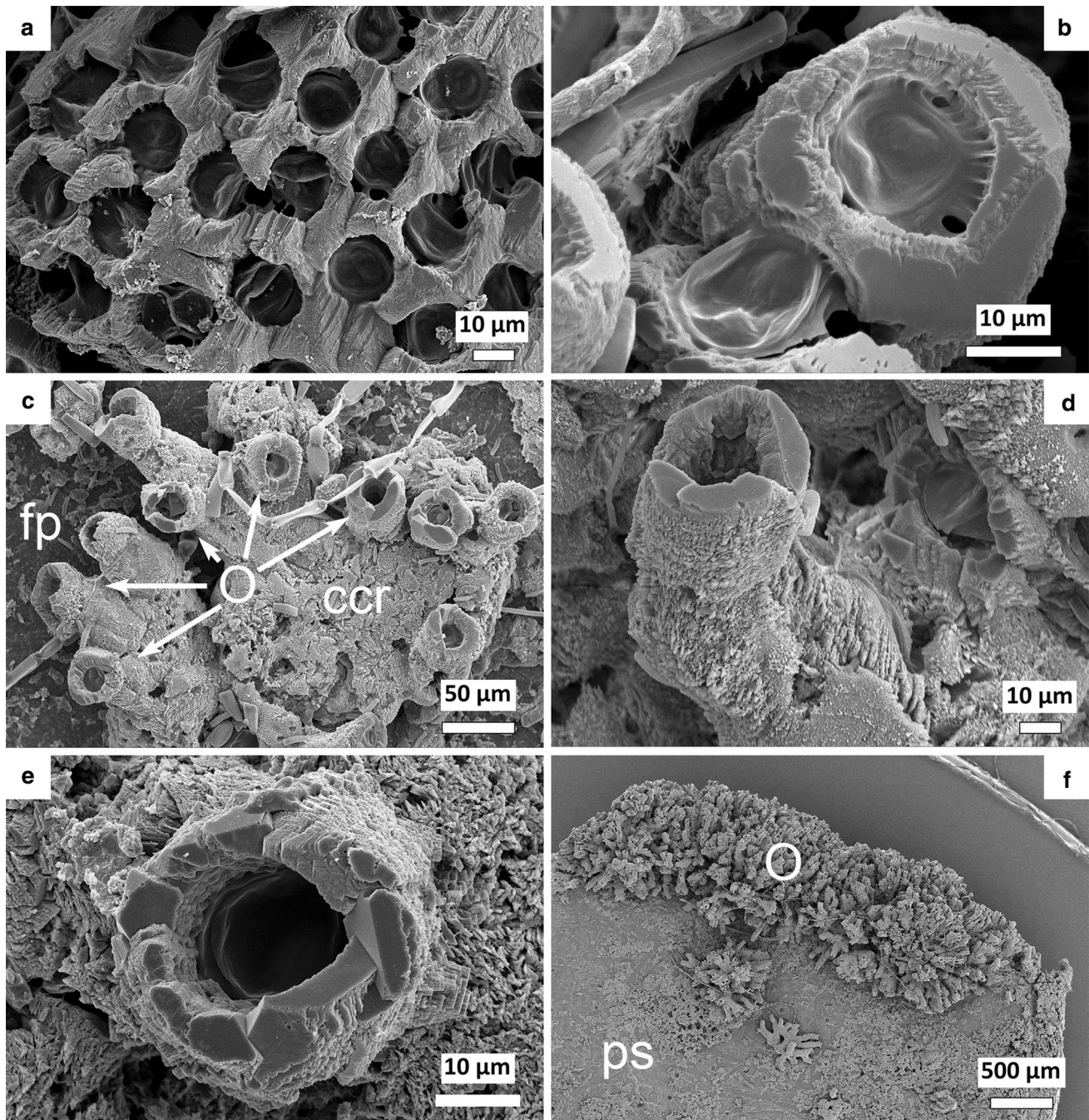


Fig. 8 *Oocardium* calcites grown on experimental substrates. **a** Site 3: *Oocardium* calcite on rinse fleece placed on 3.2.2015; sampled 30.6.2015. **b** Site 3: *Oocardium* calcite (massive calcification) on rinse fleece placed on 21.12.2015; sampled 21.3.2016. **c** Site 3: day-lit side of flowerpot (fp) colonized by *Oocardium* (O) settled on a calcite crust (ccr). Substrate placed 11.6.2015; sampled 22.9.2015.

d *Oocardium* calcite tube on the outer side of the flowerpot. **e** Site 3: abandoned *Oocardium* calcite on the inner, nearly dark side of a flowerpot fixed in the stream. Substrate placed 11.6.2015; sampled 22.9.2015. **f** Site 3: colonization of plastic strip (ps) by *Oocardium* (O) along the edges of the strip. Substrate placed 11.6.2015; sampled 2.11.2015

of diatoms; only on abandoned tubes, diatoms settled on the top (Fig. 12f). Where patches of abandoned *Oocardium* calcite became overgrown by diatom films, cumulates of

micron- to submicron-sized calcite crystals closely similar to those observed in first colonization of experimental substrates were seen.

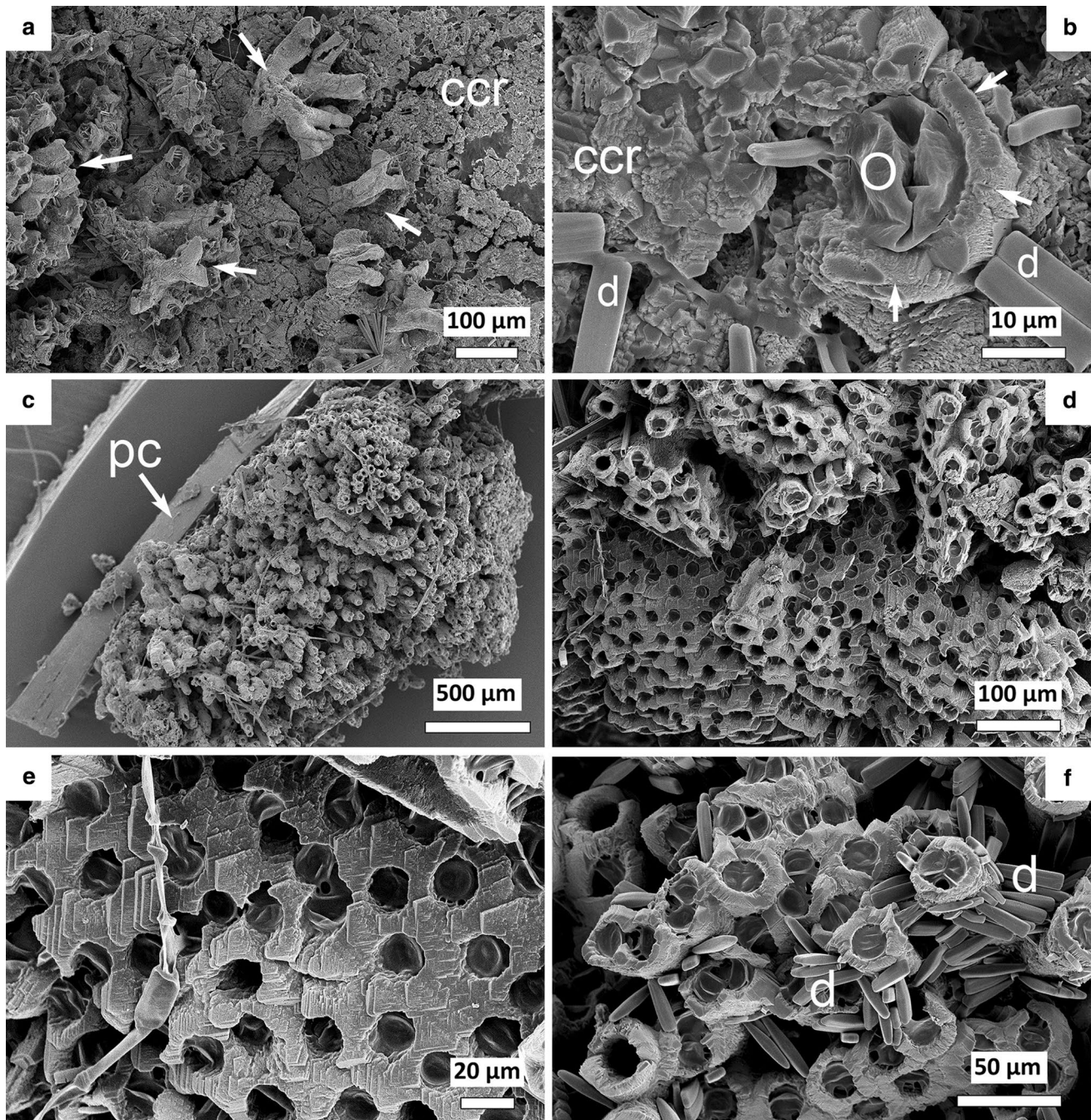


Fig. 9 *Oocardium* calcites on experimental substrates. **a** Site 3: *Oocardium* tubes (arrows) grown from a calcite crust (ccr) precipitated on a plastic strip placed on 11.6.2015; sampled 12.10.2015. **b** Detail of subfigure **a**: calcite crust (ccr), and *Oocardium* cell (O) shortly after settlement. Note diatoms (d) and calcite crystals (arrows)

around the *O. stratum* cell. **c** Site 3: plastic cord (pc) that waved freely in shooting stream flow and became overgrown by *Oocardium* calcite. Substrate placed 11.6.2015; sampled 2.11.2015. **d**, **e** Details of subfigure **c**, showing stepped calcification style. **f** Site 3: green *Oocardium* calcite associated with diatoms (d) during winter

Discussion

Viability, calcification, water chemistry

The persistence of green *Oocardium* cells over the entire year indicates that the wintery lowering of air temperature

as well as of illumination was not sufficient to extirpate the population. Because the spring stream remained open to the sky even during heavy snowfalls and unfrozen over the observation period (Fig. 5), and because stream temperature varied only up to 3° (Fig. 4), *O. stratum* cells bathed within the stream experienced a seasonal change much

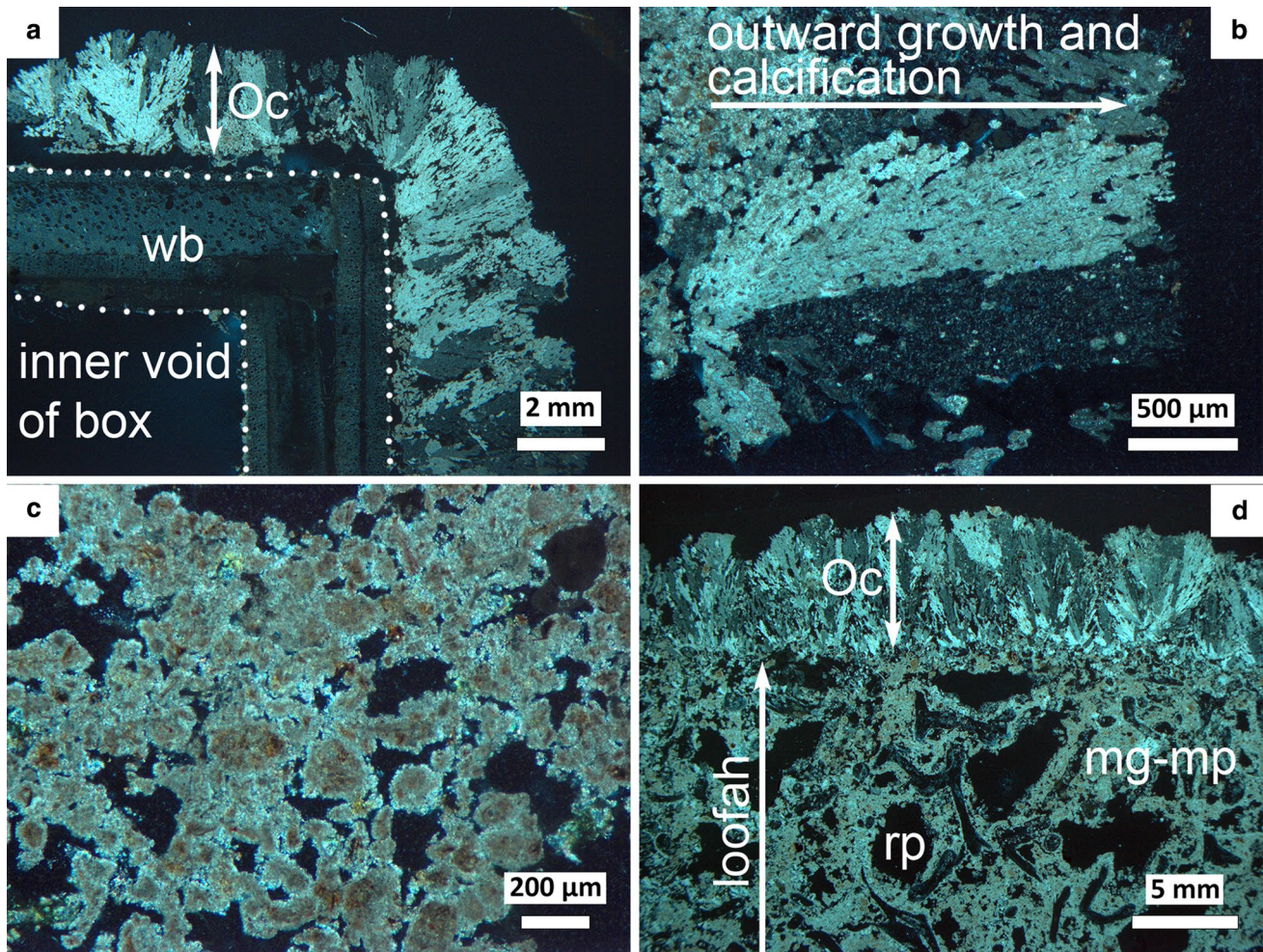


Fig. 10 *Oocardium* calcites on experimental substrates. **a** Site 3: thin-section through wooden box (wb) coated by *Oocardium* calcite (Oc). Crossed nicols. Box placed 17.3.2015; sampled 21.3.2016. **b** Detail of *Oocardium* calcite in subfigure **a**. Crossed nicols. **c** Micropeloidal grainstone formed in the dark inner void of the wooden box (cf. sub-

figure **a**). Crossed nicols. **d** Site 3: thin-section through loofah bathed in the stream. The pore space contains micropeloidal grainstone to packstone (mg-mp) around remnant pores (rp); the top is coated by *Oocardium* calcite (Oc). Crossed nicols. Loofah placed 3.2.2015; sampled 21.3.2016

smaller than that of the subaerial environment. *O. stratum* is documented to thrive over a total temperature range of 4.7–20 °C, but most streams wherein it was observed so far are in the range of 8–15 °C (see Table 5). On the other hand, the spread of green *Oocardium* cover during spring suggests that an increase in illumination and perhaps also increasing daylength favored cell division and substrate recolonization. Partly comparable results were obtained in earlier studies (in other springs) on seasonal calcification of *O. stratum*: there, *Oocardium* calcite became overgrown by diatom mats during winter; during spring, the diatoms largely disappeared and/or were outpaced in vertical growth by newly settled calcifying *O. stratum*. In this way, a seasonal lamination of the *Oocardium* limestone can be produced (Wallner 1933; Sanders and Rott 2009; Linhart and Schagerl 2015). Freezing of stream

water, longer snow cover and/or strong seasonal lowering of illumination (e.g., in higher latitudes) may indeed delimit the distribution of *O. stratum*.

From sites 1 to 3, the calcite saturation state was rising, and so did the rate of *Oocardium* calcification (Fig. 4). The sharp rise of calcification rate from sites 2 to 3, respectively, may indicate optimum conditions for growth and calcification. At site 4, in contrast, highest calcite supersaturation contrasted with a lowered rate of calcification (Fig. 4). The concentrations of Ca^{2+} and HCO_3^- at site 4 are well-within the range of concentrations measured at other springs with *Oocardium* calcification (see Table 5). At site 4, thus, the discrepancy between saturation state and calcification rate may be related to site-specific factors, perhaps (a) intermittently low CO_2 concentrations, impeding photosynthetic activity, and/or (b) potential intermittent

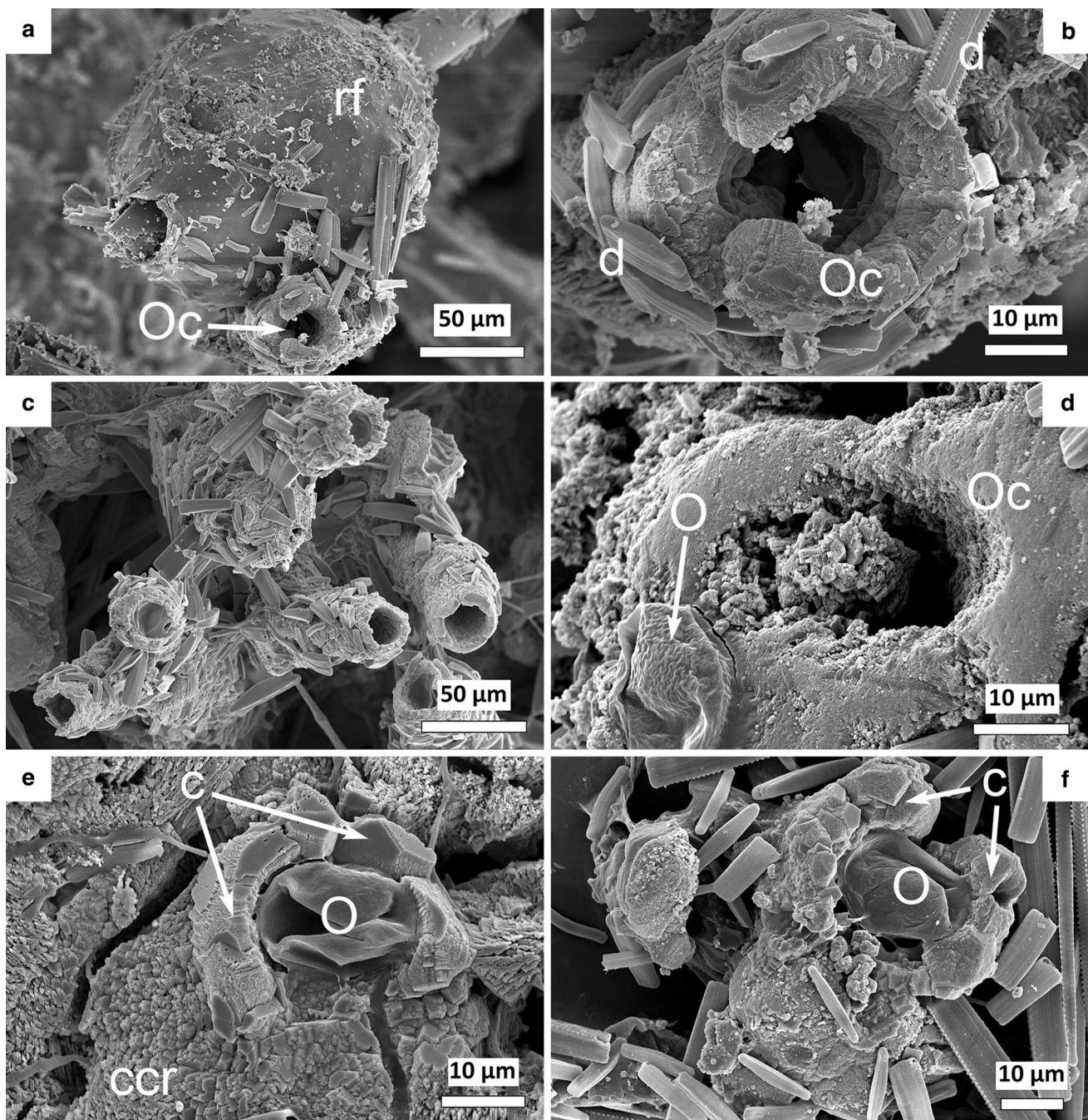


Fig. 11 Colonization and early calcification of *O. stratum*. **a** Site 4: rinse fleece (rf) overgrown by diatoms and by short tubes of *Oocardium* calcite (Oc). Substrate placed 3.2.2015; sampled 17.3.2015. **b** Detail of subfigure a. Abandoned *Oocardium* calcite (Oc) overgrown by diatoms (d). **c** Site 4: detail of *Oocardium* cover grown on rinse fleece during winter 2015–16. Many *Oocardium* cells remained viable; the calcite tubes were settled by diatoms (d). Substrate placed 3.2.2015; sampled 21.3.2016. **d** Abandoned *Oocardium* calcite (Oc),

and newly settled cell of *O. stratum* (O) still devoid of its housing calcite tube. Sample taken on 30.6.2015. **e** Site 3: *Oocardium* cell (O) settled on a calcite crust (ccr). Note calcite crystals (c) around the cell. Substrate placed 11.6.2015; sampled 12.10.2015. **f** Site 2: early calcification along the edge of a plastic strip. Note ring of calcite crystals (c) around newly settled *Oocardium* cell. Substrate placed 11.6.2015; sampled 12.10.2015

freezing of the relatively thin water film at site 4 in winter. Culture experiments showed that *O. stratum* consumes only CO₂ by passive diffusion and is therefore likely confined

to (limestone-precipitating) waters of sufficiently high CO₂ concentrations and alkalinity (Schagerl and Wukovits 2014). Linhart and Schagerl (2015) suggested that alkalinity and

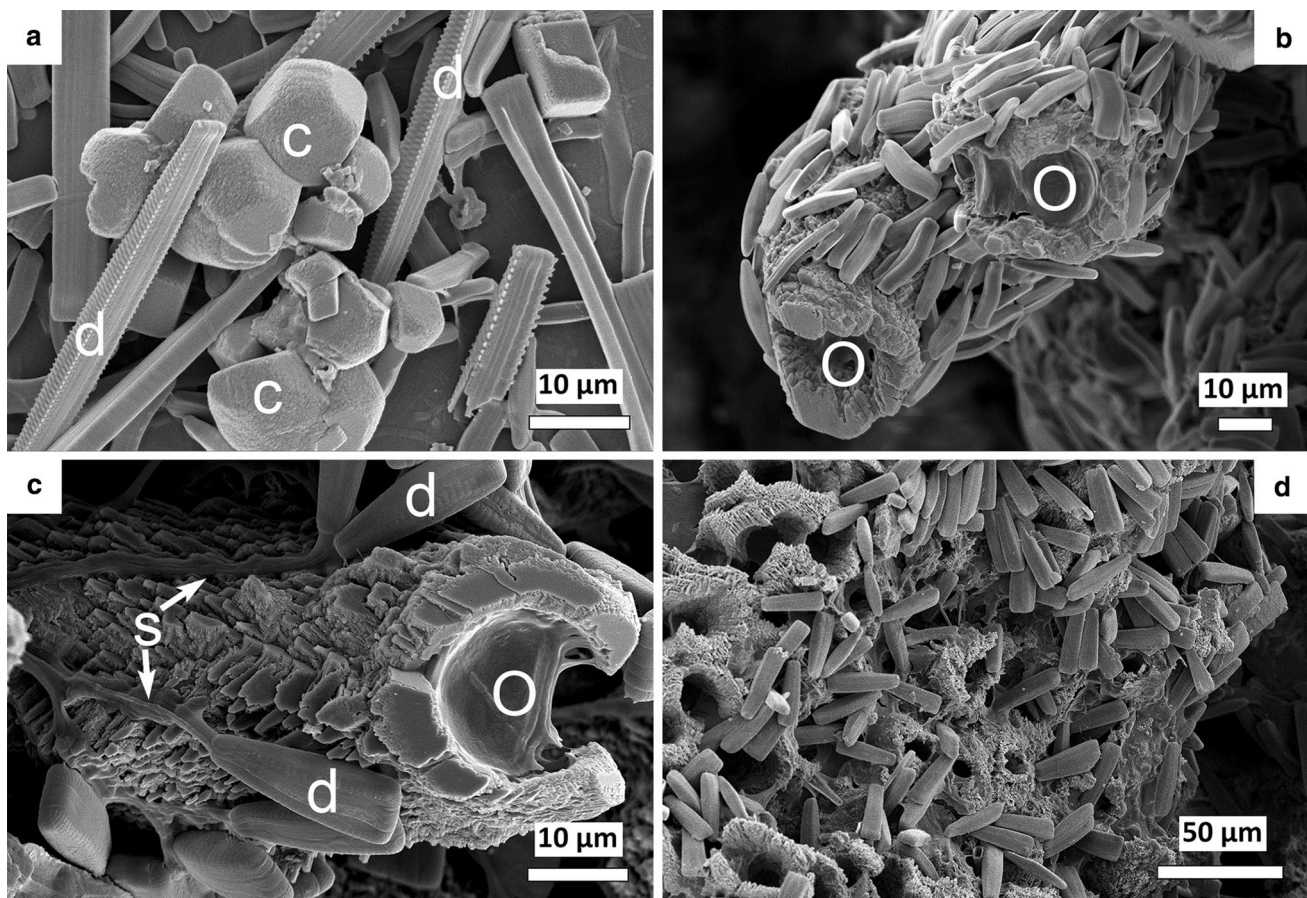


Fig. 12 Diatom colonization of experimental substrates and of *Oocardium calcite*. **a** Site 2: diatom colonization of a plastic strip. Note calcite crystals (c) associated with the diatoms (d). Substrate placed 11.6.2015; sampled 2.11.2015. **b** Diatoms settled on tubes of green *Oocardium calcite* (O). Sample taken on 21.3.2016. **c** Diatoms

(d) grown between tubes of green *Oocardium calcite* (O). Upon drying, the formerly free-standing diatom stalks (s) adhered to the calcite tube. Sample taken on 30.6.2015. **d** Diatom mat coating white *Oocardium calcite*. Sample taken on 28.4.2015

water temperature are the two most significant controls over *O. stratum* presence and activity, with an alkalinity of 4.7 meq/l and a temperature of 13 °C considered as optimal. This might imply that *O. stratum* can persist also in uncalcified form in waters that do not precipitate limestone. Springs of elevated alkalinity but of low Ca^{2+} concentrations are rare. The Ca^{2+} concentrations of springs with *O. stratum* spread over two orders of magnitude, but commonly range from a few tens to a few hundreds of mg/l (Table 5). So far, *O. stratum* was observed only in waters of low Mg/Ca molar ratio that precipitate low-magnesian calcite, and that are supersaturated for calcite at least along the stream reach with *O. stratum* (Table 5). In view of the fact that *O. stratum* settled only on substrates of calcite and started to induce precipitation of calcite early after settlement (cf. Figs. 8c–e; 9b; 11d, e; see below for further discussion), this strongly suggests that calcification is indispensable. The calcite tubes are required to support the soft mucus stalks of *O. stratum* (cf. Fig. 1a). In absence of calcite precipitation, the

mucus stalks would hardly support upward growth; upon cell division, thus, only sheet-like soft cumulates of *O. stratum* cells were possible. Together, this supports that *O. stratum* requires calcification as an integral part of its life strategy.

At site 1, which showed the highest CO_2 concentration (cf. Fig. 4c, Table 2), the practical standstill of *Oocardium* calcification combined with the lack of substrate colonization together suggest that colonization/calcification was influenced by factors additional to CO_2 concentration and alkalinity (e.g., reactive phosphate concentration). The habitat window of *O. stratum* thus may be also framed by a combination of calcite supersaturation with CO_2 concentration. As observed on the experimental substrates (flowerpots, plastic strips; Figs. 8c, e, f, 9a, 11e), their surface became coated by a calcite crust that did not show evidence for biological mediation (abiotic calcite), also in SEM. This calcite crust was settled on by *O. stratum* cells. After settlement, the tubes of *Oocardium calcite* grew upward at a much higher rate than the surrounding abiotic calcite crusts.

Table 5 Compilation of physico-chemical parameters observed in streams with actively calcifying *Oocardinum stratum*

Control (unit)	Characterization	References, remarks
Water temperature (°C)	Range: 4.7–20 Our study: 4.7–15.63	Pentecost (1991), Rűf (2006), Sanders and Rott (2009), Gesierich and Kofler (2010), Gradziński (2010), Rott et al. (2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
Generalized water chemistry	Ca ²⁺ – HCO ₃ [–] – (SO ₄ ^{2–}) water; low-magnesian calcite depositing stream	<i>Oocardinum</i> prefers well-lit waters of low-moderate concentration of dissolved major ions (Sanders and Rott 2009)
Electrical conductivity (μS/cm)	Range: 386.8–906 Our study: 428.67–679.33	Rűf (2006), Sanders and Rott (2009), Gesierich and Kofler (2010), Cantonati et al. (2012), Rott et al. (2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
pH	Range: 7.1–8.7 Our study: 7.12–8.7	Pentecost (1991), Sanders and Rott (2009), Gesierich and Kofler (2010), Gradziński (2010), Rott et al. (2010, 2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
CO ₂ concentration (mg/l)	Range: 1.44–169.4 Our study: 3.15–53.14	Pentecost (1991), Sanders and Rott (2009), Rott et al. (2010, 2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
Alkalinity (HCO ₃ [–]) (mg/l)	Range: 176.9–450.3 Our study: 335–441.75	Pentecost (1991), Rűf (2006), Sanders and Rott (2009), Gesierich and Kofler (2010), Gradziński (2010), Rott et al. (2010, 2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
Ca ²⁺ concentration (mg/l)	Range: 1.6–289.2 Our study: 61.8–99.3	Pentecost (1991), Rűf (2006), Sanders and Rott (2009), Rott et al. (2010, 2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
Water agitation	Shallow strongly turbulent flow required. <i>Oocardinum</i> prefers streams or stream reaches with high water–air exchange	Pentecost (1991), Gradziński (2010), Rott et al. (2010) and Linhart and Schagerl (2015), this study
Mg/Ca molar ratio	Range: ~0.022–0.706 Our study: 0.46–0.706	Values calculated from: Pentecost (1991), Rűf (2006), Sanders and Rott (2009), Rott et al. (2012) and Trobej et al. (2017), this study
Calcite saturation indices (SI)	0.27–0.81 (calculated from data in Pentecost 1991) – 0.22 to 1.27 (SI by PHREEQC) (Gradziński 2010) 0.26–1.0 (Langelier SI) (Trobej et al. 2017) Our study: –0.07 to 1.32 (SI by PHREEQC)	Pentecost (1991), Gradziński (2010) and Trobej et al. (2017), this study
Sulfate concentration (mg/l)	Range: 0.8–745.92 Our study: 3.6–6.46	Pentecost (1991), Rűf (2006), Sanders and Rott (2009), Gesierich and Kofler (2010), Cantonati et al. (2012), Rott et al. (2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
Nitrate concentration (mg/l)	Range: 0.055–12.69 Our study: 4.86–7.33	Pentecost (1991), Rűf (2006), Gesierich and Kofler (2010), Cantonati et al. (2012), Rott et al. (2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study
Total phosphorus concentration (TP) (μg/l)	< 15 (detection limit) to 179.1, mostly 0.2 to a few micrograms/L Our study: mostly < 15; few values 17–21 (summer 2015)	Pentecost (1991), Gesierich and Kofler (2010), Rott et al. (2012), Linhart and Schagerl (2015) and Trobej et al. (2017), this study

Under conditions of overall supersaturation, *O. stratum* thus accelerated calcite precipitation. *O. stratum* seems to be resilient against a wide range of sulfate and nitrate concentrations, i.e., over at least three orders (sulfate) and two orders of magnitude (nitrate) (see Table 5). In our study, the total phosphorus concentration in most cases was below the detection limit of 0.015 mg/l; only during summer 2015, a few values of up to 0.021 mg/l were determined (Table 2). The nitrate and phosphorus concentrations of our study and of former studies suggest that *O. stratum* prefers oligotrophic to ultraoligotrophic waters (cf. Rott et al. 2012).

Initial calcification style and cell arrangement

As described, the downstream range of initial calcification styles on the growing tips of the *Oocardium* calcite tubes was similar both on the natural stream bed and on experimental substrates. The downstream change of initial crystal fabrics and rates of precipitation, from (i) crystal-skeletal fabrics at site 1 (lowest calcite supersaturation, practically no precipitation; cf. Fig. 6a–d) to (ii) massive-idiomorphic fabrics at sites 3 and 4 (highest supersaturation, high precipitation; cf. Figs. 7e, 8b) contrasts with conventional correlations of crystal habit with degree of oversaturation. Whereas crystal skeletons form rapidly at high degrees of supersaturation, idiomorphic crystals precipitate slowly at low supersaturation (e.g., Sunagawa 2005); the physical explanation for this is that at high supersaturation, rapid crystallization proceeds along the energetically most favorable planes (intersections of main crystal surfaces) and lines (e.g., crystallographic c-axis); at low supersaturation and slow crystal growth, in contrast, there is time to occupy also the energetically less favorable inner parts of crystal surfaces, to result in an idiomorphic crystal (Sunagawa 2005).

Induced calcification in ambient-temperature spring streams, however, is different in that over a comparatively narrow range of temperature the degree of supersaturation is strongly controlled by CO₂ concentration (cf. Chen et al. 2004). The crystal-skeletal fabrics at site 1 (highest CO₂ concentration) thus suggest that supersaturation at the site of crystallization is too low to allow for occupation also of the energetically less favorable crystal surfaces. Dissolution of calcite can result in a ‘spiky’ surface (e.g., Jones and Pemberton 1987) and similar surface fabrics were produced by experimental etching of *Oocardium* calcite (Pentecost 1991). Spiky surface produced by calcite dissolution, however, differs in appearance from the crystal-skeletal fabric observed at site 1. Moreover, if the crystal-skeletal fabric resulted from dissolution, both active degradation of the calcite that formed within the EPS of the diatom-bacterial films on the experimental substrates and degradation of the natural *Oocardium* calcite at site 1 should be expected. No clear-cut evidence was seen for

active calcite dissolution. Furthermore, the arrangement of cells on the natural *Oocardium* calcite at site 1 into clusters and rows supports the hypothesis that the crystal-skeletal fabric is of primary instead of a secondary origin. The crystal-skeletal fabrics at site 1 thus seemed to result from limitation, not rapidity, of crystallization. Conversely, the massive-idiomorphic calcite fabrics at sites 3 and 4 suggest that supersaturation was sufficiently high to allow for full crystallization. The crystal-skeletal fabrics of site 1 also fundamentally differed from travertine calcite precipitated from hot springs. In hot springs, whisker crystals and diverse forms of dendritic–skeletal crystals mainly result from rapid, significant cooling combined with rapid CO₂ loss upon depressurization (Guo and Riding 1998; Jones and Renaut 2008; Jones and Peng 2012; Jones 2017). The crystal whiskers to dendrites to skeletons formed at hot springs seem to largely correspond to the conventional interpretation of crystallization fabrics (cf. Sunagawa 2005).

The style of cell arrangements of *O. stratum* also appears to correspond with calcite supersaturation and precipitation rate; at site 1, the most distinct clustered cell arrangements are positioned between widely and spaced calcite “pillars” with crystal-skeletal fabric (cf. Fig. 2b, c); at site 2, cell rows are common aside of single-celled tubes (Fig. 2d). Farther downstream, at sites 3 and 4, only single-celled calcite tubes were found (cf. Figs. 1a, c, d, g; 2a). The differences in cell arrangements may be interpreted as a reaction to degree of calcite supersaturation: under low supersaturation (site 1), it is hardly possible for a cell to precipitate a tube on its own; upon division, thus, cells become positioned in a cluster adjacent to each other. Under higher supersaturation, in contrast, each cell can induce the precipitation of its own calcite tube. Comparison to previous studies suggests that the described variations of cell arrangement, in particular, the clusters and rows, are relatively rare or had not been taken care of in documentation. In any case, the single-celled housing seems to be the most widespread. In as much the relatively low calcite supersaturation at site 1 is the prime factor or an important factor at least, for the lack of active substrate colonization by *O. stratum* cannot be assessed with the data at hand.

Oocardium ecology and calcification

On precipitation substrates with a large pore space (e.g., rinse fleece, loofah, boxes of balsa wood; Table 3) the low-lit or dark pore space became filled mainly by micropeloidal grainstone to packstone. Conversely, the day-lit outer surfaces were colonized by diatoms and *Oocardium*. A similar fabric development is observed in natural spring limestones (cf. Sanders and Rott 2009). The observation that *O. stratum* directly settled on calcite suggests that it requires calcite for settlement, be it a large single crystal or clusters of small crystals such as those

formed in bacterial-diatom biofilms that ‘prepare the ground’ for settlement (cf. Figs. 6e, f; 11a).

Fallen leaves (Fig. 5e) quench biocalcification beneath them while their upper side is recolonized by *O. stratum*, mainly along their margins and protruding leaf nerves (Fig. 5f). This ‘edge effect’ of colonization is probably related to microhabitat conditions, perhaps submillimeter-scale water turbulence facilitating CO₂ uptake (cf. Chen et al. 2004). The seasonal input of leaves might help to support the long-term stability of the diatom/*O. stratum* assemblage, mainly because diatoms and *O. stratum* are effective colonizers, and because a cover of *Oocardium* calcite may be difficult to invade by cyanobacteria and mosses (cf. Linhart and Schagerl, 2015). Moss tufts may be so densely colonized by calcifying *O. stratum* to become ultimately choked (Rüf 2006; Sanders et al. 2006; Willegger 2008; Sanders and Rott 2009). Several factors thus may combine to keep stream beds open for *O. stratum*, (1) its capability for effective colonization and rapid calcification, and (2) partial cover with fallen leaves that are quickly re-colonized.

The rate of *O. stratum* calcification increased downstream, from zero at site 1 to a maximum at site 3. At site 3, the mean calcification rate of 370 µm/month and the maximum rate of 500 µm/month rank intermediate relative to rates reported from other springs (cf. West et al. 1923; Sanders and Rott 2009; Linhart and Schagerl 2015). The highest rate of upward growth of *Oocardium* calcite observed so far amounts to 830 µm/month (or 10 mm/a, Sanders and Rott 2009). As a result of seasonal changes in calcification, spring limestones composed of *Oocardium* calcite consist of laminae typically between 3 and 8 mm in thickness (cf. Sanders and Rott 2009; Ibarra et al. 2014).

Conclusions

1. The vertical calcification of *Oocardium stratum* proceeded at a maximum rate of 500 µm/month, which is within the range of rates documented previously from other springs.
2. *Oocardium stratum* remained viable and colonized new substrates also during winter. Calcitic substrates were colonized by single *Oocardium* cells that, after settling, induced precipitation of calcite crystals around themselves; then, a mucus stalk was secreted that supported upward growth of the microalga within a housing tube of calcite. Non-calcitic substrates, in turn, were first colonized by biofilms of unspecified bacteria and diatoms. Calcite crystal aggregates formed within these biofilms later provided the substrate for *O. stratum* settlement. The crucial role of biofilms of diatoms and/or bacteria in ‘preparing the ground’ for *Oocardium* to colonize non-calcitic substrates was identified for the first time.

3. At low calcite supersaturation and relatively high CO₂ concentration (upstream), *O. stratum* cells formed atypical clusters and rows between ‘pillars’ or ‘walls’ of *Oocardium* calcite with a crystal-skeletal fabric. At higher supersaturation and lower CO₂ concentration (downstream), rhombohedral calcite initially formed along the tips of upward growing calcite tubes, each housed by a single *O. stratum* cell, as described in previous studies.
4. Because *Oocardium* calcite provides a highly differentiated substrate for settlement, diatoms benefit from being associated with *O. stratum*. Conversely, because of their photosynthesis, at least dense diatom populations settled on *Oocardium* calcite perhaps aid to increase local calcite supersaturation. In general, the role of diatoms in total spring calcification is under-investigated and requires further study.
5. The niche of *O. stratum* can be characterized as: Perennial, ambient-temperature streams with low to moderately mineralized Ca–(Mg)–HCO₃–(SO₄) water; total phosphorus is low to very low (oligotrophic–ultraoligotrophic; typically < 15 to 21 µg/l), nitrate concentrations (0.055–12.69 mg/l) and sulfate concentrations (0.8–745.92 mg/l) can be highly variable. The stream bed—in most cases of *Oocardium* calcite—is characterized by shallow, highly turbulent flow. This conclusion is based on the results of the present study and of previously published articles.
6. Fossil deposits of freshwater limestones previously thought to be entirely or largely of cyanobacterial origin may contain a hitherto unidentified “eukaryotic component”, or in some cases may even have been largely produced by eukaryotic calcifiers.

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