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DIPLOMARBEIT

Titel der Diplomarbeit

„Autecology of *Oocardium stratum* Naeg.
and
$CaCO_3$ precipitation of autotrophic biofilms in travertine rivulets“

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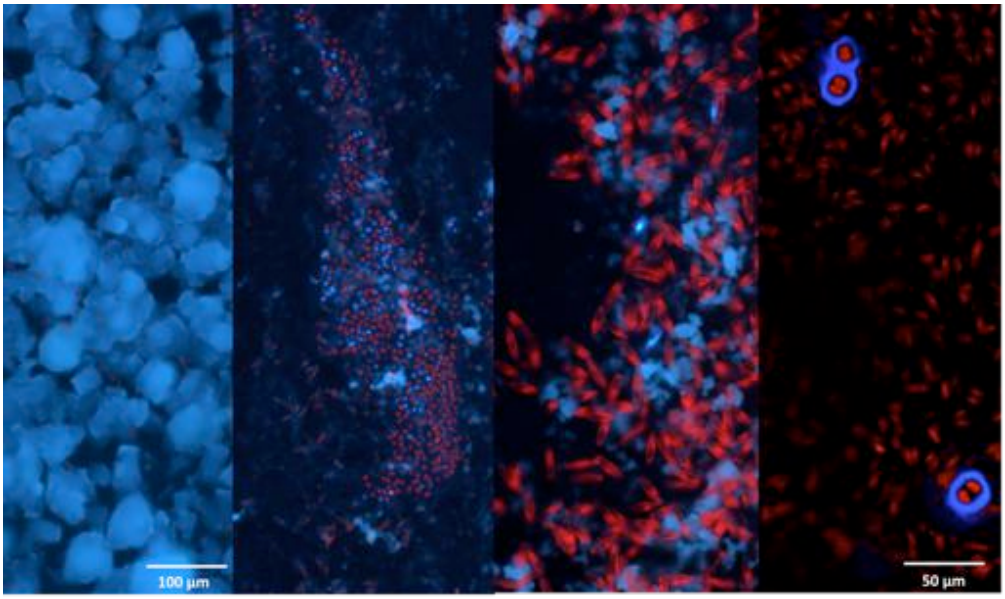

Autofluorescence of $CaCO_3$, Cyanobacteria, Diatoms and Oocardium stratum.

„Most living things are small and easily overlooked“

Bill Bryson, A short history of nearly everything

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1 Einleitung

Karbonatfällung durch Biomineralisation findet im Meer, in Seen, Flüssen und Böden statt (Martinez *et al.*, 2010). Die häufigste Form des Karbonatniederschlages ist Kalziumkarbonat ($CaCO_3$). Kalzifikation ist jene Form der biogenen Karbonatfällung, bei der Kalk in Form von Kalziumkarbonat mit geringen Mengen Magnesiumkarbonat ($MgCO_3$) abgelagert wird. Die Ablagerung des Kalziumkarbonates kann außerhalb der Zelle erfolgen (extrazellulär), zwischen den Zellen (interzellulär) oder in der Zelle (intrazellulär). Das abgelagerte Kalziumkarbonat kommt in der Natur meist als Calcit oder Aragonit vor. Diese sind verschiedene polymorphe Kristallisationsformen von $CaCO_3$, wobei Aragonit löslicher ist und mehr Magnesium (Mg) enthält als Calcit. (Bürger, 2010)

Muscheln, Schnecken, Schwämme und vor allem „gesteinsbildende" Korallen, sowie marine Mikroorganismen und makroskopische Grünalgen haben über Millionen von Jahren durch biogene Kalkausfällung Riffe und schließlich Gebirge entstehen lassen (Bürger, 2010), und sind daher hauptsächlich für die Bildung (Orogenese) von Kalkgebirgen verantwortlich, wie etwa die Dolomiten (Bosellini *et al.*, 2003, Faupl, 2003). Marine Mikroorganismen haben den größten Anteil an der Kalziumkarbonatausfällung (Martinez *et al.*, 2010, Field *et al.*, 1998). Es ist äußerst beachtlich sich vorzustellen, dass ganze Gebirgszüge wie die Kalkalpen durch Ablagerungen und Ausscheidungen von Mikroorganismen entstanden sind. Ebenso erscheint es besonders beeindruckend, dass mikroskopisch kleine Algen fähig sind mächtige Kalkschichten über mehrere 100 Meter zu bilden, wie etwa die weißen Klippen in Südengland. Selbstverständlich sind dies Prozesse, die viel Zeit und spezielle Bedingungen erfordern, aber erst dieses Wissen lässt die oftmals unscheinbaren Mikroorganismen in einem anderen Licht erscheinen. (Bürger, 2010)

In Fließgewässern wird Biomineralisation auch durch mikrobielle Biofilme beeinflusst, da sie hauptsächlich das Gleichgewicht zwischen organischem und anorganischem Kohlenstoff kontrollieren (Dupraz *et al.*, 2009b). Eine Unterscheidung zwischen organisch und anorganisch induzierter $CaCO_3$-Ausfällung ist aber oft schwerer (Merz-Preiß and Riding, 1999), sobald Kleinstlebewesen wie Cyanobakterien oder Mikroalgen beteiligt sind, da hier die Kalkablagerung hauptsächlich extrazellulär stattfindet. Dupraz *et al.* (2009) nennt drei verschiedene Typen der Biomineralisation: Biologisch induziert (Weiner and Dove, 2003, Bazylinski and Frankel, 2003), biologisch kontrolliert und biologisch beeinflusst. Eine ausschließlich abiotische Ausfällung von $CaCO_3$ kommt sowohl in Flüssen als auch in stehenden Gewässern vor, sobald sich der Partialdruck von Kohlendioxid (CO_2) verringert, bzw. wenn sich das Gewässer erwärmt. Dies bewirkt ein Ausgasen von CO_2 in die Atmosphäre. Quell- oder Bachökosysteme mit hohen $CaCO_3$

Ausfällungen werden Travertine genannt. Aufgrund der mikrobiellen Veratmung (Respiration) im Boden, wird Grundwasser mit CO_2 angereichert. Travertinsysteme, mit CO_2-Ursprung aus der Bodenatmung, werden meteogene Travertine genannt. Das CO_2 kann aber auch durch tiefere thermische Vorgänge in der Erdkruste ins Grundwasser gelangen, diese Travertinsysteme werden als thermogen bezeichnet. Das gelöste Kohlendioxid greift in beiden Fällen nun Kalkgestein an, dabei gehen Kalzium und Bikarbonat in Lösung. (Pentecost, 2005).

$$CaCO_3 + CO_2 + H_2O \longleftrightarrow Ca_2^{+} + 2\,(HCO_3)^{-}$$

Es können sich dabei in Kalkgebirgen weitgehende Kluftsysteme und Höhlen bilden (Karst).

Travertin- oder Tuffablagerungen kommen nun durch die entgegengesetzte Reaktion zustande. CO_2 übersättigtes Grundwasser tritt an die Oberfläche; durch den niedrigeren CO_2-Partialdruck in der Atmosphäre, diffundiert CO_2 aus dem Wasser in die Atmosphäre und $CaCO_3$ fällt aus. Der CO_2-Verlust ist rein abiotisch genauso wie das Ausgasen von CO_2 durch Erwärmung oder durch Wasserturbulenzen. Die Entnahme von CO_2 durch Photosynthese von aquatischen Pflanzen wäre aber ein biotischer Vorgang, wie etwa bei der Ablagerung von Seekreide.

Imposante Beispiele thermogener Travertine sind die Kalksinterterrassen in Pamukkale (Türkei), Mammoth Hot Springs im Yellowstone National Park (USA) oder die heißen Quellen in der Südtoskana (Italien). Meteogene Travertine weisen eine geringere Kalkausfällung auf und kommen vor allem im Alpenvorland vor, meist als „Bachterrassen“ oder „steinerne Rinnen“.

Travertin besteht hauptsächlich aus Kalzit und Aragonit mit niedriger Porosität zwischen den Kristallen aber hoher struktureller Porosität (Dupraz *et al.*, 2009a). Organisches Material, das in den Travertinbach fällt, wird meist binnen Tagen inkrustiert und regelrecht einzementiert. Travertine bilden oft Kaskaden unmittelbar nach Quellaustritten und besitzen eine besondere Flora und Fauna. Typische Vertreter der Travertinflora sind das Moos *Palustriella commutata* (Hedw.) Ochyra und die Zieralge *Oocardium stratum* Nägeli. *O. stratum* kommt ausschließlich an aktiven Travertinstandorten vor (Abbildung 1, 2). Die erste Beschreibung der seltenen Zieralge *O. stratum* stammt von Nägli (1849) anhand von Material aus der Schweiz. Wallner (1933, 1934, 1935) untersuchte diesen Organismus und sein Habitat intensiv in den frühen 30ern und erwähnte das Vorkommen

von *O. stratum* für rund 30 Standorte in voralpinen Gebieten Bayerns. Bis heute wurde *O. stratum* nur an einigen Stellen gefunden, diese sind aber weltweit verteilt. Fundorte wurden bereits für Nordamerika, Kuba, Indien und einige Standorte in Europa erwähnt. (Rott *et al.*, 2009, Wallner, 1934, Pfiester, 1976, Rieth, 1969, Sanders and Rott, 2009). Pfiester (1976) erwähnte bereits, dass *O. stratum* vermutlich häufiger vorkommt als angenommen, aber aktive und intakte meteogene Travertinhabitate ohne anthropogenen Einfluss sind trotz der weltweiten Verteilung selten. *O. stratum* wurde in Ostösterreich in der Nähe von Wiener-Neustadt durch Hangsgirg (1905) und von Brehm und Ruttner (1926) in der Lunzer Gegend aufgefunden. In den folgenden Jahrzehnten wurden keine weiteren Funde angegeben, bzw. wurde *O. stratum* nicht mehr entdeckt. Ebenso wird die Art in der "Desmidiaceaen Flora Österreichs" (Lenzenweger, (2003) nicht erwähnt. 2007 entdeckten Schagerl und Pröschold *O. stratum* in einem kleinen Quellbach im Mayrgraben in Lunz/See erstmals wieder (Abbildung 1). Weiters erwähnten Sanders and Rott (2009) zwei neue Fundorte in Westösterreich.

O. stratum ist durch das limitierte Vorkommen auf spezielle Tuffhabitate zunehmend gefährdet.

Durch Abholzung, Quellfassung, Drainagierungen und Nährstoffbelastungen werden aktive und intakte Tuffhabitate immer seltener. Deshalb haben Travertinsysteme durch die

Abbildung 1: Der Travertinbach in Lunz/See Mayrgraben. Steine, Blätter und Äste werden alsbald mit Kalk überzogen.

Abbildung 2: Die untere Kaskade des Lunzer Travertinbaches mit flächigem *O. stratum* Vorkommen.

Europäische Habitatrichtline einen Schutzstatus (Traxler, 2010, EC, 1992).

Die makroskopische Struktur der *O. stratum* Kulturen ähnelt grünen Stecknadelköpfen (Abbildung 3). Die Zieralge bildet kleine Kolonien von etwa 100 Zellen, die nur mikroskopisch zu unterscheiden sind (Pentecost, 1991). Die *Oocardium* Zelle besteht aus zwei Halbzellen, die durch einen Isthmus, der den Zellkern enthält, verbunden sind (Abbildung 4). Die *Cosmarium* ähnlichen Zellen (15-20 µm breit, 10-20 µm lang) sitzen am Ende von Gallertstielen, die von einer Kalkschicht umgeben sind. Diese Gallertstiele bestehen aus extrazellulären polymeren Substanzen (EPS), an denen sich besonders leicht Kalkkristalle niederschlagen (Pentecost, 1985).

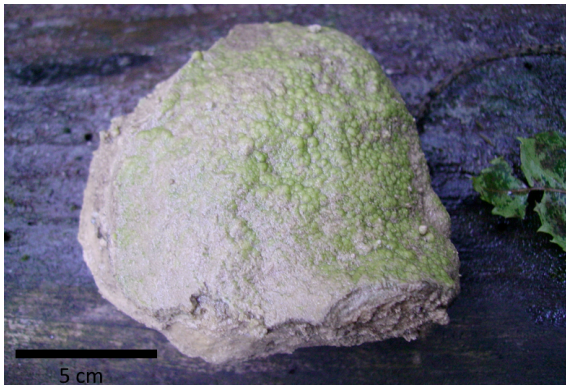

Abbildung 3: Die *O. stratum* Kolonien auf einem tuffüberzogenen Stein.

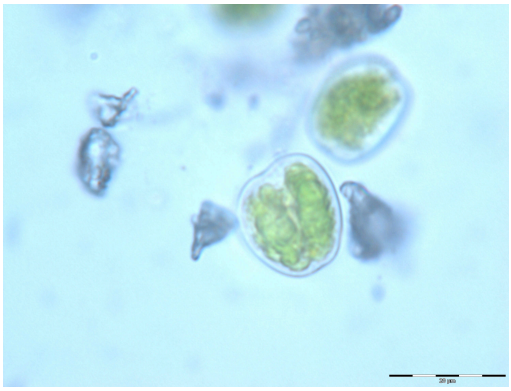

Abbildung 4: Einzelzelle von *O. stratum* mit Kalkkristallen.

Bisher war der Prozess der Kalzifizierung und der Einfluss von *O. stratum* auf die Kalziumkarbonat-Ausfällung Thema der Travertinforschung (Sanders and Rott, 2009, Rott *et al.*, 2009, Pentecost, 1991, Wallner, 1934). Dennoch gibt es bisher keine eindeutigen Beweise, ob *O. stratum* eine aktive Rolle in der Kalzifizierung ihrer Gallertstiele hat oder nicht.

Um aus Photosynthese Zucker aufzubauen, ist eine Kohlenstoffquelle notwendig. In den meisten Fällen nutzen Pflanzen oder auch Mikroalgen CO_2 als Kohlenstoff. Manche Pflanzen sind aber imstande, mittels eines speziellen Enzyms, der Carboanhydrase, auch Bikarbonat (HCO_3^-) zu nutzen. Durch die Aufnahme von CO_2 oder HCO_3^- durch Photosynthese verschiebt sich das Kalziumkarbonat Gleichgewicht und es kann zu vermehrter Kalkausfällung kommen. Im Fall von *O. stratum* ist es noch nicht klar, ob Enzyme, wie etwa externe Carboanhydrase, eine Rolle im Ausfällungsprozess spielen, oder ob durch die CO_2-Aufnahme der pH-Wert lokal rund um die Gallertstiele geändert wird (Borowitzka, 1982). Ebenso können die EPS-Stiele ausschließlich als Substrat für die abiotische Kalkausfällung dienen, da sie Ca^{2+} Ionen binden können (Somers and Brown, 1978). Bisher existieren nur wenige Informationen über die autökologischen Aspekte von

O. stratum (Wallner, 1933, Wallner, 1934, Pentecost, 1981, Pentecost, 1991, Pentecost, 1993, Pentecost, 2005, Sanders and Rott, 2009, Rott *et al.*, 2009). Somit sind Daten detaillierter chemischer und physikalischer Standortsansprüche, sowie über saisonale Biomasseschwankungen noch nicht verfügbar. Solche Informationen sind aber notwendig, um Reinkulturen von *O. stratum* anlegen zu können. Reinkulturen von *O. stratum* existieren bisher weltweit noch nicht, sind aber erforderlich, um weitere molekularbiologische Untersuchungen am Organismus durchführen zu können. Diese wären etwa die Identifizierung des Erbgutes zur genauen taxonomischen Einordnung und die Bestimmung der Zusammensetzung der zellumgebenden Gallerte.

Wallner (1933, 1934) beobachtete, dass *O. stratum* Kolonien stets erst ab einer bestimmten Distanz zur Quelle aufgefunden wurden und dass das Vorkommen der Alge sich meist über 2/3 des Tuffbachs erstreckte. Eine ähnliche Situation erwähnten Rott and Holzinger (2009); dies ist ein Anzeichen für eine sehr spezielle Nische der Alge, sogar im Travertinbach selbst. Bisher sind die speziellen Standortanforderungen, die Wachstumsbedingungen und das Vorkommen der Alge während der Jahreszeiten noch unklar. Ebenso wirft der Mechanismus der Kalzifizierung noch einige Fragen auf. So war es die Zielsetzung dieser Arbeit mehr über die speziellen Standortbedingungen von *O. stratum* herauszufinden sowie das Ökosystem „Tuffquelle“ besser kennenzulernen. Diese Diplomarbeit soll einen Beitrag zum Verständnis dieses Ökosystems liefern, um in naher Zukunft einige dieser offenen Fragen beantworten zu können. Das Hauptaugenmerk der Arbeit liegt in der chemischen und physikalischen Habitatscharakteristik und der saisonalen Sukzession von *O. stratum*. Eine Mikro- und Makrokartierung diente zur Beobachtung des saisonalen Vorkommens von *O. stratum* und aufgeraute Objektträger wurden als Substrat zur Quantifizierung des Kalkniederschlages verwendet. Der Aufwuchs der Substratplatten wurde mittels HPLC (High Performance Liquid Chromatography) auf algenspezifische Pigmente hin untersucht. Letztendlich wurden die Umweltparamenter mit den Biomassedaten verknüpft, um die Habitatsansprüche dieser speziellen Alge herauszufinden.

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3 Autecology of the rare tufa-forming desmid *Oocardium stratum* Naeg.

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3.1 Abstract

The rare calcifying desmid *Oocardium stratum* occurs exclusively on active travertine habitats; the macroscopic colonies form hemispherical structures with a diameter of 0.5 to 2.0 mm^2. Because autecology of this organism is still poorly understood, we focused on its seasonal development and related various environmental factors to the occurrence and biomass of *Oocardium.* The study was conducted in a rivulet in Lunz/See (Lower Austria) for 17 months on a weekly (growing season) to monthly (winter season) basis. *Oocardium* colonies were found throughout the whole year with maximum abundances during the mid summer months July and August. Repeated macromapping of three travertine spots with a size of 750 cm^2 each showed a maximum *Oocardium* cover of 31 % in August. Two smaller maxima occurred in early summer and late autumn with about 10 % cover. Diatom mats, dominated by *Cymbella minuta,* occurred in spring, autumn and winter with more than 74 % of cover observed on macromapping spots. Micromapping was done at a single area covering 25.5 mm^2 and revealed the same results. This seasonal succession of *Oocardium* and diatom mats in limestone precipitating springs causes a typical sequence pattern of travertine layers. Redundancy analysis revealed water temperature and bicarbonate content as the main structuring factors, which control occurrence and growth of *Oocardium*. Optimal growth conditions appeared at an alkalinity of 4.6-4.7 mmol L^{-1} and water temperatures of 13 °C. Site openess, nitrate and carbon dioxide availability were inversely related to *Oocardium* biomass, but more connected to diatoms occurrence. Other environmental factors like total ions content or soluble reactive phosphorus had no significant influence on *Oocardium stratum* occurrence.

Keywords: alga, travertine, spring, alkalinity, diatom

3.2 Introduction

Oocardium stratum Nägeli is a desmid, which occurs exclusively in active meteogene travertine springs and headstreams on various surfaces encrusted with tufa. The macroscopic colony-structure of the greenish *O. stratum* resembles green pinheads. The *Cosmarium*-like cells (width 15-20 µm, length 10-20 µm) are located at ends of gelatinous stalks, which are encrusted with lime (Wallner, 1933, Wallner, 1934, Golubić and Marčenko, 1958, Schagerl, 2007). Until now, *O. stratum* has been found only on a few locations worldwide; it has been documented for North America, Cuba, India and a few locations all over Europe (2009). Active and intact meteogene travertine habitats without anthropogenic pollution are rare, but exist in limestone areas all over the world. Because of its peculiar habitat, *O. stratum* is easily overlooked and probably more frequent than reported (Pfiester, 1976). For Eastern Austria, *O. stratum* was documented by Hangsgirg (1905) near Wiener-Neustadt and by Brehm and Ruttner (1926) in the area of Lunz/See. Later on, it seemed to have disappeared and in his desmids flora of Austria, Lenzenweger (2003) did not include this taxon. Recently, Schagerl and Pröschold (2007) rediscovered *O. stratum* in a small rivulet at the Mayrgräben in Lunz/See. Sanders and Rott (2009) mentioned two more sites in Western Austria.

The first description of *O. stratum* was given by Nägeli (1849) based on material found in Switzerland. Wallner (1933, 1934, 1935) investigated this organism and its habitat intensively in the early 1930's in Upper Bavaria and mentioned the occurrence of *O. stratum* at 30 sites in pre-alpine areas of Bavaria, Germany.

The characteristics of travertine springs are reviewed in detail by Pentecost (2005). Basically, groundwater enriched in dissolved carbon dioxide dissolves carbonates and a solution of calcium bicarbonate is formed. Reaching the surface, carbon dioxide becomes supersaturated and degasses and as a result carbonates precipitate. Pentecost (2005) classified travertine on their carrier carbon dioxide, which may originate from soil by respiration activities (meteogene travertines) or from processes in deeper earth layers where higher temperature is an additional factor for dissolving carbonates (thermogene travertines). *O. stratum* is mentioned to have a patchy distribution (Pentecost, 2005) because of its restricted occurrence on meteogene tufa habitats.

To date, there exists only rare information about autecological aspects of *O. stratum* (Wallner, 1933, Wallner, 1934, Pentecost, 1981, Pentecost, 1991, Pentecost, 1993, Pentecost, 2005, Sanders and Rott, 2009, Rott *et al.*, 2009). Wallner (1933, 1934) observed that *O. stratum* colonies can be found at a certain distance to the spring and their occurrence extends over 2/3 of the full length of the limestone-precipitating stretch. A

similar distribution was found by Rott and Holzinger (2009) and indicates a very peculiar spatial niche even in the travertine stream. Recent studies focusing on travertine formation and lamination induced by *O. stratum* have been carried out by Sanders and Rott (2009). They suggested that the seasonal change in irradiance supply control the presence/absence of *O. stratum* and therefore the seasonal layering of travertine. The travertine formation shows a high increase up to 5 mm a^{-1} during warmer periods induced by intense *Oocardium stratum* growth, and a strong reduction caused by massive development of benthic diatoms in autumn and winter (Sanders and Rott, 2009). Small amounts of green *O. stratum* cells occurred throughout the cold season.

Rott and Holzinger (2009) focused on the community composition in two travertine systems and considered also the morphology of *Oocardium*. Together with *Oocardium* they reported the filamentous cyanobacterium *Rivularia sp.* and the mosses *Palustriella commutata* (Hedw.) Ochyra and *Eucladium verticillatum* (Brid.) Bruch & Schimp. Nitrate concentration between both sites differed around 10 times (10.54 mg L^{-1} at Lingenau, approximatly 1 mg L^{-1} at Alpenzoo). The authors mentioned, that total phosphorus amounts were low, however they did not provide data. A few other studies of this peculiar taxon focused mainly on travertine formation, the ecological niche of *Oocardium* was largely neglected. Especially investigations in order to check for seasonal biomass fluctuations of *O. stratum* and studies to define key variables for the occurrence of this rare taxon have not been conducted. With our study, we wanted to shed some light into those open questions. We were mainly interested in habitat characteristics and the seasonal succession of *O. stratum*. We used macro and micro-mapping to reveal the seasonal pattern of *O. stratum* development. Environmental conditions were also considered and finally linked to the algal cover.

3.3 Material and Methods

3.3.1 Study site

The small meteogene travertine headstream "*Oocardium* rivulet" is part of the south-west faced slope Mayrgraben system in Lunz am See (47°15 N, 15°04 E), located in the lime alps of Lower Austria at 700 m above sea level (Figs. 1, 2). The geological catchment area of the spring system is part of the Lunzer beds II (Sulzbach beds) on border to the Ötscher nappe (Tollmann, 1965, Tollmann, 1966) and belongs to the geological era of Oberostalpines Mesozoikum (Faupl, 2003). (Fig. 3). This nappe system includes mainly karst formations like Gutensteiner limestone, Werfener banks and Dolomite (Götzinger, 1955). Both the *Oocardium* rivulet (= travertine rivulet) and the Mayrbach rivulet are

located in a sandstone and shale formation of the Lunzer beds (Fig. 4). The riparian vegetation at the study site comprises mainly *Fraxinus excelsior* L., *Picea abies* L. H. Karst, *Fagus silvatica* L. and *Rubus subgen. Rubus* L. Mean annual precipitation in Lunz am See is about 1545 mm a-1 (1971-2000) and the mean annual air temperature is 6.9 °C (1991-2000; www.noel.gv.at). After a storm event in 2007, broken trees were removed and as a result most of the travertine layers were destroyed leading to a collapse of the *O. stratum* population. This gave us the chance to study the succession of a travertine system including a recolonization of *O. stratum.* Four sampling sites are located in the travertine rivulet (A-D) and one more in the main Mayrbach rivulet (E) without travertine formation (Fig. 5). Site A is located nearest to the spring; here travertine depositions start to develop. It is characterized by a high amount of incoming irradiance. B and C are located in the middle stretch of the headstream; B had natural shading and on spot C artificial leaf shading was installed. D is located a few m upstream the mouth of the headstream, where the travertine spring stream discharges into the Mayrbach rivulet. It is also characterized by a high amount of incoming light; both B and D are located on travertine cascades. E is located in the Mayrbach rivulet a few m upstream the merging of the travertine stream (Fig. 2, 5). E was taken as a reference spot without travertine formation and absence of *Oocardium*.

3.3.2 Data collection

Sampling took place between March 2008 and July 2009. From March 2008 to November 2008 we sampled weekly, in the following winter season until March 2009 on a monthly basis and from April 2009 to July 2009 again in weekly intervals. At each spot, data loggers (HOBO UA-002-64 Pendant Data Logger) recorded water temperature (°C) and luminous intensity (lux) every two min. For data analyses, the weekly average of temperature (also day-min, day-max), and weekly sum of incoming light were used. On site, specific conductivity (µS cm^{-1}), water temperature (°C) and oxygen concentration (% and mg L^{-1}) were measured by using a portable multi-Meter (Hach Lange, HQ 40d, 10105). Water discharge (L s^{-1}) was estimated at a gorge by means of a bucket and a stop watch.

Sky openness was estimated by means of hemispherical photos, which were taken with the digital camera Nikon Coolpix 4500 equipped with a Nikon fisheye converter FC-E8 0.21 x. The camera was placed on a thin, levelled polystyrol plate directly in the headstream. The magnetic north was marked on the lens of the fisheye converter. The photos were post-processed using Adobe Photoshop Version 8.0.1 in order to eliminate any shading caused by the photographer. The light parameters were then calculated by the computer

program Gap Light Analyzer (GLA) Version 2.0 (Frazer *et al.*, 1999). Except for spectral fraction, which was set to 0.45 (default 0.5) to adjust for the transmitted photosynthetic active radiation, default settings were used (Frazer *et al.,* Hainz *et al.*, 2009). The length of the growing season was assumed to be 12 months, which seemed to be approximately the growing period of the *O. stratum* colonies.

The *O. stratum* cover was estimated at B, C and D (at these sites *O. stratum* was found) by macromapping of photos (Olympus µ 1030 SW) from always exact the same fragment (30 cm x 25 cm) using a fixed grid. On site C, we also analysed the benthic algae community in detail at a smaller scale. This was done by micromapping of photos of exact the same spot (65 mm x 50 mm) using a fixed tripod. Species identification was done with a Zeiss Axio Imager.M1 microscope (camera: Axio Cam MRc5, computer application: Axio Vision Release 4.7.2). The macromapping photos were post-processed with Adobe Photoshop Version 8.0.1 in order to adjust the same photographed area and resolution. Three copies of each photo were made: one for quantification of all green pixels (*Oocardium*), one for quantification of all red pixels (Diatoms); the third one was used as a reference picture (Fig. 11). The binary pictures of red and green fractions were further analyzed with respect to the amount of overgrown biomass area of *O. stratum*, moss *Cratoneuron sp.* and diatom mats, respectively, using the computer application ImageJ Version 1.43j (Rasband et al., 1997-2009). The pictures were then converted into 16 Bit greyscale pictures and adjusted to a threshold of 200 to estimate the summarized area of all particles and calculate the percentage of diatom and *Oocardium* biomass.

For water chemistry, we took triplets from each site. We used BOD bottles in order to keep the CO_2 pressure in the water samples constant until analysis. Bottles were filled with a PVC hose to avoid intense turbulences with the atmosphere and sparkling (Legler, 1988). The bottles were kept cold and dark and were brought immediately to the laboratory. From filling the water bottles until starting the water analyses in the laboratory no more than 45 min passed. Total alkalinity (m-value at pH 4.3), pH and acidity (p-value at pH 8.2) were determined titrimetrically with a Titrino 702 SM (Metrohm Ion Analysis, Switzerland) immediately after returning to the laboratory. According to Legler (1988), samples for acidity were covered with 2 ml pentan to avoid out gassing of CO_2 during titration. Soluble reactive phosphorus (SRP), nitrate-N ($N\text{-}NO_3$), nitrite-N ($N\text{-}NO_2$), ammonium-N ($N\text{-}NH_4^+$) were analysed according to the standardized Continuous Flow Method (Eberlein and Kattner, 1987, Kempers and Luft, 1988, APHA *et al.*, 1998). Magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^+), chloride (Cl^-), potassium (K^+) and sulphate (SO_4^{2-}) were analyzed by

ion chromatography (Metrohm Compact IC 761, Metrohm IC Filtration Sample Processor 788).

3.3.3 Data analyses

Statistical analyses were carried out using the computer program R 2.9.2 GUI 1.29 (R Development Core Team, 2009) including the external software packages nortest, psych and vegan. All variables were checked for normal distribution using qq-plots and Lilliefors (Kolmogorov-Smirnov) test statistics. If necessary, they were transformed using arcsin√x (for percentage data: biomass and site openness), sqrt(x) or $\log_{10}(x+1)$ transformation (Ramette, 2007, Hainz *et al.*, 2009). Since the sample size of 50 limited the number of input variables for the principal component analysis (PCA), a bivariate correlation matrix for all *Ocardium* sites was calculated including all variables in order to choose the adequate input variables for a PCA. Several factors were excluded from the PCA: the sum of ions and HCO_3^- because of correlation with conductivity, oxygen because of low variation, pH because of high correlation with free CO_2, $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ because of a very low amount and the better representation of nitrogen with $N\text{-}NO_3^-$, and precipitation because discharge has more influence on water chemistry and it contains also the snowmelt. PCA was performed including all *Oocardium* sites (ABCD) in a single model.

The synthetic gradient length of biomass data was checked with a detrended correspondence analysis (DCA, decorana within R package vegan) in order to test whether unimodal or linear methods should be used. For all tested sites (B, C, D) the gradient length of the first DCA axis was found to be smaller than 2 indicating that linear ordination methods are adequate (Leyer and Wesche, 2007, Dormann and Kühn, 2009). We chose redundancy analyses (RDA) (Havskum *et al.*, 2004, Leyer and Wesche, 2007) to get insight into species-environment relationships. RDA is a linear constrained ordination method and an extension of multiple regression and principal component analysis. In constrained ordination two different site scores exist: linear combinations scores (linear combinations of constraining variables) and weighted average (WA) species scores. The *vegan* package uses WA scores because they are more stable against random error, which are very common in environmental datasets (Oksanen, 2007, Oksanen, 2010). In order to extract the best subset of the environmental data set, the function *bioenv* (package *vegan*) was used. In this method the variable selection is based on maximum (rank) correlation of the Euclidean distance of the scaled environmental data with the species dissimilarity matrix (Oksanen *et al.*, 2007). For the final RDA model we used constraints extracted by permutation tests (at least 999 permutations). A sequential ("Type I") permutation test analysed the significance of all selected terms separately and a "Type III" test calculated

the marginal effect of each constraining variable, while each variable is removed from the model including all other variables. Also, each axis was tested with a permutation test (Oksanen, 2010). Additionally, variance inflation factors (VIF) for each variable were checked and variables with a VIF above 10 were excluded to avoid multicollinearity (Oksanen, 2007, Dormann and Kühn, 2009, Oksanen, 2010). In the final model, only significant ($p < 0.05$) and independent variables were used. Sites (B-D) and dates (1-50) were coded and treated as covariables to avoid the problem of pseudoreplication.

3.4 Results

3.4.1 Site characteristics

The karst system of the catchment area received precipitation up to a weekly maximum of 100 mm; discharge of the rivulet was approximately 1 L s^{-1}. Only two times, in March 2009 during the snowmelt and in July 2009 at heavy rainfalls, the discharge reached 6 L s^{-1} and 3 L s^{-1}, respectively (Table 2). Within the short stretch of the headstream, mean annual water temperature increased from 8.5 °C directly at the spring to 11.3 °C at the discharge into the Mayrbach rivulet. The *Oocardium* rivulet was slightly oversaturated with free CO_2 and O_2 and generally showed very low nutrient concentrations (Table 2,Table 3, Fig. 9,Fig. 6). The stretch from A to D clearly revealed decreasing gradients in free carbon dioxide (Fig. 6), conductivity, and also a slight decreasing gradient in Ca^{2+} (Table 2). The pH increased downstream and the lowest pH was related to the highest amounts of free carbon dioxide (site A). The preferring cation in *Oocardium* rivulet was Ca^{2+} with an annual average of 80.7 mg L^{-1} followed by Mg^{2+} with 6.4 mg L^{-1}. Dominant anion was HCO_3^- with 274 mg L^{-1} (Table 1). High water events like snowmelts in spring or thunderstorms in summer decreased ion concentrations: the snowmelt in spring 2009 led to a discharge of 6.0 L s^{-1} (mean 0.92 L s^{-1}) and decreased conductivity, Mg^{2+}, SO_4^{2-} and HCO_3^-, while a high water event in June 2009 (2.5 L s^{-1}) had a big impact on mainly oxygen concentration, Mg^{2+} and SO_4^{2-}. Interestingly, N-NH_4^+ concentrations decreased downstream. In spite of leaf cover, irradiance supply at the *Oocardium* rivulet was higher in summer compared to the winter season. In both years, site A displayed the highest irradiance supply from mid of April to mid of July. Both the minimum (3457 klux in October 2008) and the maximum (204924 klux in April 2009) of seasonal irradiance supply were measured at C (Table 3). The reference site E neither showed any active travertine formation nor *O. stratum* colonies. Compared to the headwater stream, free CO_2 of site E was significantly lower (t-test, $P < 0.001$, $n = 50$) and the amount of SRP was

significant higher (t-test, $P < 0.001$, $n = 50$) (Fig. 6); for details of water chemistry refer to Table 1. PCA revealed three principal components (PCs) with an eigenvalue beyond 1 explaining 71.7 % of the total variance in the data set. PC1 had maximum loadings on temperature and $N\text{-}NO_3^-$. PC2 had highest loadings on free CO_2 and PC3 on irradiance (Table 4).

3.4.2 Algal cover, seasonality and its relation to environmental variables

Colonies of *O. stratum* started to develop 65 m downstream the spring and were observed mainly on cascades down to the confluence into the Mayrbach rivulet, which is a stretch of about 20 m (Fig. 2). Besides *O. stratum*, a diatom community dominated by *Cymbella minuta* and the moss *Cratoneuron* sp. could be identified.

Macromapping and micromapping sites showed the same seasonal patterns: *O. stratum* cells were found during the whole year, but during winter months from end of November till April, its cover was reduced at macromapping spots to an amount of around 5 to 1 % (Fig. 7Fig. 9) and at the micromapping spot to less than 2 % of the travertine surface. The few colonies were mainly found in small travertine holes. In early summer (May and June), *O. stratum* growth was increasing and resulted in a first maximum of about 18 % cover until diatom mats developed in the beginning of July coinciding with a short decrease of *O. stratum*. This brief decline succeeded the summer maxima of *O. stratum* biomass in July and August. Diatom mats were absent during this time and a second *O. stratum* maximum was observed with around 31 % cover. September and October were characterised with a steep decrease of *O. stratum* biomass. Diatom mats started to replace the colonies and finally dominated the biofilm. A short increase in *O. stratum* with a maximum cover of 10% occurred in November after a breakdown of the diatom mats. Diatom mats dominated in early spring, autumn and winter, with a maximum cover of 74% on macromapping spots (Fig. 7,Fig. 9).

While colony development on macromapping spots was rather heterogenous, *O.stratum* was spread homogeneously on the micromapping site. After the colonies reached a size of about 5 mm in diameter, new colonies started to develop in parallel. Highest *O. stratum* cover on the micromapping site D was 34 % and lowest was close to 0 %. Interestingly, mosses also served as substrate for *O. stratum*. After intense diatom growth, *O. stratum* overgrew small moss seedlings, which are encrusted with fine sediment.

The significant final RDA model over sites B to D included the significant variables temperature, HCO_3^-, NO3-N and site openness and explained 25.3 % of total inertia (Table 6). Irradiance and free CO_2 turned out to be insignificant. The first RDA axis explained

24.9 % of total variance. *Oocardium stratum* occurrence and biomass was explained mainly along the temperature and the HCO_3^- gradients (Fig. 8).

3.5 Discussion

3.5.1 Site characteristics

Some studies revealed that discharge and stream flow have an influence on biofilm biomass (Blenkinsopp and Lock, 1994, Battin *et al.*, 2003, Besemer *et al.*, 2007). For this study, low water (discharge < 0.5 L s^{-1}) was frequently observed in summer and autumn and coincided with *O. stratum* biomass maxima in summer and autumn. Interestingly, RDA revealed no significant contribution of discharge to *O. stratum* occurrence, probably because this taxon is also quite resistant against high discharge through its strong incrustation and calcification. Only during high water events when even the cemented tufa is mobilized, *O. stratum* colonies are eroded. Such an event was observed e.g. in July 2009. Sanders and Rott (2009) mentioned discharge of 1 to 10 L s^{-1}, but indicated that just a part of this discharge belongs to the *Oocardium* overgrown places. Pentecost (Pentecost, 1991) referred two sites in the British Isles with discharge rates of 0.5-5 L s^{-1} and 40-200 L s^{-1} and one in Belgium with 10 L s^{-1}. These data clearly show that *O. stratum* is able to handle vigarous discharge conditions.

Data referring to the water chemistry of *O. stratum* sites are rare and available only for the British Isles (measured 4 times 1989) and Belgium (single measurement in 1989) by Pentecost (1991) and Austria (Sanders and Rott, 2009, Rott *et al.*, 2009). Temperature and pH data mentioned by Pentecost (1991) are similar to our results. Also SRP levels are comparably low, but N-NH_4^+ amounts were 5 to 10 times higher during our investigation. Ion concentrations are slightly higher at our sites compared to the studies conducted at the British Isles and Belgium, except for Ca^{2+}, which is much higher at Lunz (Table 2, 3). Sanders and Rott (2009) analysed occasionally selected parameters: compared to those sites, the *Oocardium* rivulet in Lunz/See showed only moderate levels of bicarbonate, also ions and nutrients concentrations were clearly lower. Phosphate levels are generally low in meteogene and thermogene travertine springs due to precipitation of calcium phosphate and its low solubility (Pentecost, 2005). The *Oocardium* rivulet has a SRP level close to the detection limit (Table 2) and also the *Oocardium* sites in Western Austria indicated low levels of phosphate (Rott *et al.*, 2009, Sanders and Rott, 2009).

Our reference site Mayrbach rivulet showed HCO_3^- and Ca_2^+ amounts, which were comparable to that of the *Oocardium* rivulet, but active travertine precipitation was not

detectable. A possible explanation could be the increased SRP levels, as they strongly inhibit travertine precipitation (Pentecost, 2005). The high amounts of free carbon dioxide at site A (some supersaturation was calculated) resulted in pH lower than on the remaining sites, therefore Ca^{2+} concentration was highest here and carbonate precipitation lowest (Linhart 2010, unpubl.). Downstream, degassing of free carbon dioxide and the calcification process resulted in a parallel decline of free CO_2, Ca^{2+}, and conductivity.

3.5.2 Algal cover, seasonality and its relation to environmental variables

O. stratum occurrence was mainly explained by water temperature and HCO_3^- content. HCO_3^- levels of 4.7 mmol L^{-1} and a temperature around 13.3 °C provided optimal growth conditions for *O. stratum*. Both variables had their maxima during summer (Figs. 8, 9). The fast colonization of *O. stratum* and associated calcium precipitation in summer resulted in an increase of travertine layers up to 5 mm, while diatom mats and decreased precipitation due to a better solubility of CO_2 contributed nearly nothing to the travertine increase in the colder season. The biplot of the PCA showed a clear horseshoe of sampling times, which illustrated the seasonal alignment of chosen variables. The RDA biplot also indicated a seasonal distribution (despite sampling times were defined as covaribles): high *Oocardium* amount, water temperatures and HCO_3^- levels were associated with the warmer period, while diatoms mainly occurred in the cold season related to the site openness (Fig. 8).

Interestingly, mosses were not persistent at the studied sites. One explanation could be grazing by invertebrates or that moss seedlings become overgrown by *O. stratum*. Compared to mosses, *O. stratum* seems to be resistant against grazing due to its growth strategy with calcified mucilage tubes.

3.5.3 Calcification and restricted occurrence of *Oocardium stratum*

Why is *O. stratum* restricted to active meteogene travertine springs? One answer could be found in the very peculiar habitat, where other algae are easily swept away and/or overgrown by fast $CaCO_3$ precipitation. The growth strategy of *O. stratum* by its gelatinous stalk system is perfectly adapted to active travertine springs and protects the colonies also from grazing. The mucilage tubes themselves most probably get calcified by abiotic calcification. The stalk system is essential for attachment, but also for keeping on the surface of the travertine. Increased colonization at sites with higher precipitation rates supports this hypothesis. However, until now it is still not clear if higher growth rates induce elevated calcium precipitation or if higher precipitation rates cause increased *Oocardium* growth.

Within the travertine stretch, we were able to identify a characteristic zone of *Oocardium*, which was already reported by Wallner (1934) and Rott and Holzinger (2009). It seems that *Oocardium* occurs from a specific distance to the spring over 2/3 of the full length of the active travertine depositions. We suppose that very close to the spring, the travertine precipitation exceed the maximum growth rate of *Oocardium*. Downstream, growth is possible because of the growth strategy until the precipitation becomes so small the *Oocardium* is outcompeted. We assume that *Oocardium* needs a sufficient amount of Ca^{2+} precipitation to colonize on travertine surfaces, where it is best adapted compared to other species. Moreover, *Oocardium* prefers sites with a high water-air exchange like cascade splash water zones. Travertine zones with laminar flow or pools under cascades did not show *Oocardium* colonies. This observation indicates that *Oocardium* uses mainly atmospheric CO_2 which has a much lower diffusion coefficient compared to water diffusion and lead to the conclusion that carbonic anhydrase is absent in the species.

As stated already by Pfiester (1976), *Oocardium* might be more common in active meteogene travertine systems than expected, but these habitats are rarely visited by phycologists. However, active and intact travertine springs combined with low nutrient concentrations are becoming fewer and fewer. Due to deforestation, drainage pipes, well casing, and pollution these peculiar habitats are disappearing and therefore, travertine systems have a protective status by the European Habitat (Traxler, 2010, EC, 1992). As *Oocardium* seems to be restricted to these habitats, it is increasingly endangered.

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3.8 Tables and Figures

Table 1. Abbreviations and units for all environmental variables of the study.

Environmental variable	Abbreviations and synonyms	unit
free carbon dioxide CO_2, acidity	free CO_2	mg L^{-1}
Bicarbonate, alkalinity	HCO_3	mmol L^{-1}
oxygen	O_2	%
pH (-log(H^+))	pH	
specific conductivity	conductivity	µS cm^{-1}
temperature	temp	°C
ammonium	N-NH_4^+	µg L^{-1}
nitrate	N-NO_3^-	µg L^{-1}
nitrite	N-NO_2^-	µg L^{-1}
ortho-phospate	P-PO_4^3-P, SRP	µg L^{-1}
sodium	Na^+	mg L^{-1}
potassium	K^+	mg L^{-1}
calcium	Ca^{2+}	mg L^{-1}
chloride	Cl^{-1}	mg L^{-1}
magnesium	Mg^{2+}	mg L^{-1}
sulphate	SO_4^{2-}	mg L^{-1}
Site Openness	SiteOpen	%
discharge	discharge	L s^{-1}
weekly sum of precipitation	precipitation	mm
weekly sum of light	Radiation, rad, irradiance supply	klux

Table 2. Summary of environmental variables of the 4 spots of *Oocardium* (n=50, except for nutrients n=46 and ions n=36). min (minimum), Q1 (first quartile), median, Q3 (third quartile), max (maximum). For other abbreviations and units see Table 1.

	min	Quantil1	Median	Quantil3	max
Free CO_2	0.0	1.5	2.1	2.7	5.0
HCO_3^-	s3.8	4.3	4.5	4.6	5.2
oxygen	91.1	99.1	100.3	101.3	105.2
pH	8.1	8.3	8.3	8.4	8.5
conductivity	337.0	380.0	391.0	402.0	453.0
temperature	3.2	9.3	11.6	13.4	20.5
N-NH_4^+	0.0	3.6	5.8	8.9	164.1
N-NO_2^-	0.0	0.5	0.8	1.1	4.8
N-NO_3^-	302.1	638.8	836.3	1127.8	1769.4
N_{tot}	307.2	641.3	849.3	1138.7	1777.1
SRP	0.0	0.0	0.4	1.0	6.1
Na^+	0.6	0.7	0.7	0.8	1.7
K^+	0.4	0.6	0.7	0.8	1.3
Ca^{2+}	59.5	77.4	81.0	84.1	92.7
Mg^{2+}	3.8	5.7	6.5	7.1	8.3
Cl^-	0.3	0.4	0.5	0.5	1.3
SO_4^{2-}	2.0	2.5	2.7	2.9	3.4
discharge	0.1	0.5	0.6	0.8	6.7
precipitation day	0	0	1.25	5.5	64.8
precipitation week	0.2	7.4	21.55	50	233.2
SiteOpen	13.9	28.0	22.4	36.3	50.9
temperature week mean	1.3	9.0	11.5	13.8	18.2
temperature day mean	1.1	8.7	10.9	14.1	19.9
temperature day max	2.4	12.1	17.4	22.7	40.9
temperature day min	0.1	6.3	9.1	11.2	15.5
radiation week mean	1.0	7.2	12.2	18.9	40.7
radiation week sum	3457.5	35515.6	61481.7	96394.0	204924.0
radiation day mean	0.3	5.1	10.8	19.0	43.2
radiation day sum	225.2	3457.3	6619.1	14010.4	31119.6

Table 3: Mean values and standard errors during the study, of various variables for each spot at *Oocardium* rivulet (A-D), its spring (N=5) and from Mayrrivulet (E). radiation (weekly sum of radiation,) cond. (conductivity). For abbreviations and units see Table 1.

	spring	A	B	C	D	E
CO_2	19.33 ± 2.73	3.07 ± 0.63	2.31 ± 0.56	1.61 ± 0.69	1.44 ± 0.60	1.1 ± 1.5
HCO_3^-	5.93 ± 0.04	4.54 ± 0.25	4.49 ± 0.25	4.45 ± 0.25	4.42 ± 0.25	4.7 ± 0.3
pH	7.51± 0.07	8.2 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.4 ± 0.2
cond.	516.00 ± 2.35	397.8 ± 20.4	392.5 ± 20.1	388.5 ± 20.2	386.8 ± 20.0	408.7 ± 20.3
$N\text{-}NH_4^+$	13.45 ± 11.18	12.54 ± 24.48	8.21 ± 10.43	6.71 ± 5.15	6.17 ± 4.52	10.2 ± 9.2
$N\text{-}NO_2^-$	0.38 ± 0.67	0.85 ± 0.77	0.80 ± 0.43	0.87 ± 0.41	0.86 ± 0.49	2.5 ± 2.1
$N\text{-}NO_3^-$	1278.94 ± 14.59	906.03 ± 330.89	909.89 ± 341.15	912.30 ± 347.39	907.55 ± 347.22	964.24 ± 166.34
SRP	7.41 ± 0.65	0.99 ± 1.15	0.55 ± 0.67	0.59 ± 0.88	0.50 ± 0.66	12.5 ± 6.0
Na^+	0.88 ± 0.04	0.77 ± 0.12	0.77 ± 0.19	0.75 ± 0.12	0.74 ± 0.11	1.9 ± 0.5
K^+	1.00 ± 0.09	0.74 ± 0.15	0.75 ± 0.16	0.74 ± 0.14	0.76 ± 0.17	1.4 ± 0.3
Ca^{2+}	105.77 ± 1.68	82.86 ± 4.61	81.36 ± 4.34	79.43 ± 5.64	79.33 ± 4.69	82.4 ± 6.5
Cl^-	0.63 ± 0.04	0.49 ± 0.14	0.48 ± 0.17	0.47 ± 0.10	0.48 ± 0.12	1.4 ± 0.4
Mg^+	8.84 ± 0.43	6.30 ± 1.10	6.42 ± 1.07	6.35 ± 1.05	6.37 ± 1.09	8.0 ± 1.1
SO_4^{3-}	3.19 ± 0.12	2.70 ± 0.32	2.72 ± 0.33	2.70 ± 0.31	2.73 ± 0.34	3.0 ± 0.5
radiation		84053 ± 48698	77869 ±35808	77869 ±35808	46548 ±42334	

Table 4. Loadings of transformed environmental variables of the three factors extracted by factor analysis. (loadings > 0.5 marked by an asterisk). Abbreviations: lightsum (radiation week sum), cond (conductivity), Q (discharge), temp.abs (temperature in °K). Abbreviations of the variables are as in Table 1.

Variables	Factors (% variance explained)		
	PC1	PC2	PC3
	31	24	16
$log(NO_3+1)$	0.095*	0.020	-0.050
CO_2	0.030	0.980*	0.030
sqrt(lightsum)	-0.060	0.030	0.970*
sqrt(SRP)	0.010	0.120	0.090
cond	0.060	0.110	-0.140
sqrtQ	0.150	0.040	0.180
temp.abs	-0.400	-0.200	0.160

Table 5. Weighted averages of all factors for algal taxa. (values > 0.3 marked by an asterisk). Abbreviations: temp.abs (temperature, °K), lightsum (weekly sum of radiation, klux), SiteO (Site Openness, %), for others see Table 1.

Factor	arcsin(*Oocardium*)	arcsin(Diatom)
CO_2	-0.24	0.24
HCO_3^-	0.40*	-0.44*
pH	-0.12	0.04
cond	0.35*	-0.38*
temp.abs	0.44*	-0.59*
sqrtNH_4^-	0.21	-0.21
sqrtNO_2^-	0.09	-0.11
logNO_3^-	-0.27	0.37*
sqrt(SRP)	-0.20	0.10
sqrt(Na^+)	-0.06	0.06
sqrt(Ca^{2+})	0.12	-0.24
sqrt(Cl^-)	-0.14	0.27
sqrt($Mg^{2+)}$	0.31*	-0.29
sqrt(SO_4^{3-})	-0.20	0.23
sqrt(Q)	-0.27	0.34*
sqrt(lightsum)	-0.24	0.06
log(SiteO+1)	-0.45*	0.63*

Table 6. Summary statistics and results of ANOVA for the whole model and for the first axis of redundancy analysis of all *Oocardium* spots.

Partitioning of variances				
	Inertia		Proportion	
Total	0.0142		1	
Conditioned	0.0003		0.0239	
Constrained	0.0035		0.2470	
Unconstrained	0.0104		0.7291	
Sum of all eigenvalues	0.0139		0.9761	
Anova	for whole model		for axis RDA1	
F-ratio	12.113		47.616	
P-value	0.005		0.001	
	Axes			
	RDA 1	RDA 2	PC 1	PC 2
Eigenvalues	0.0035	0.0001	0.0084	0.0020
Cumulative percentage variance	0.2487	0.2532	0.8582	1
Species-environment correlation	0.5558	0.1495		

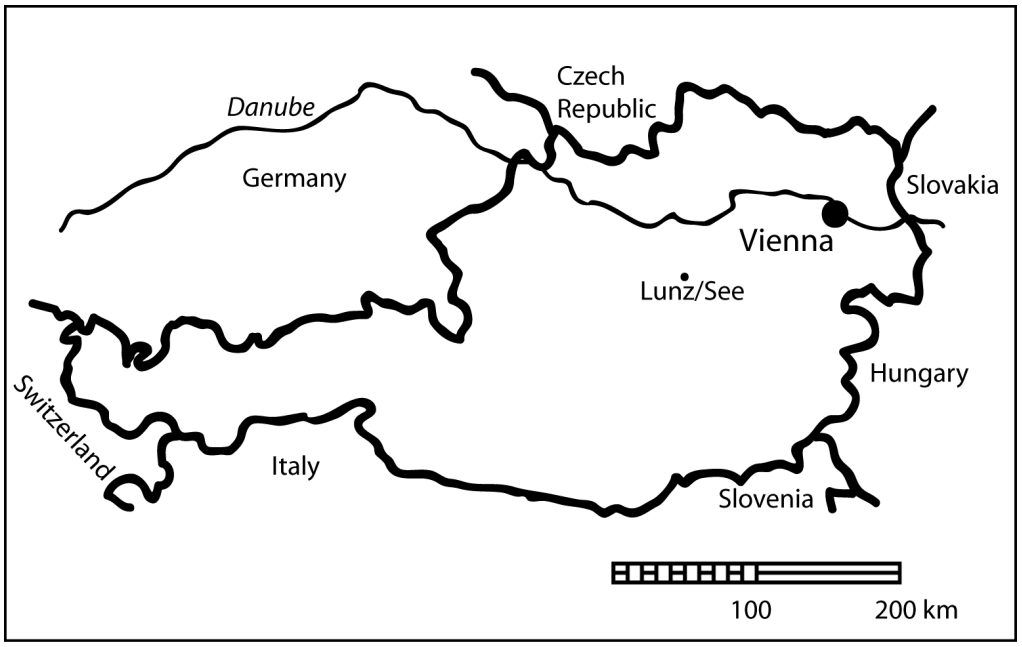

Fig. 1. Location of the sampling site in Austria

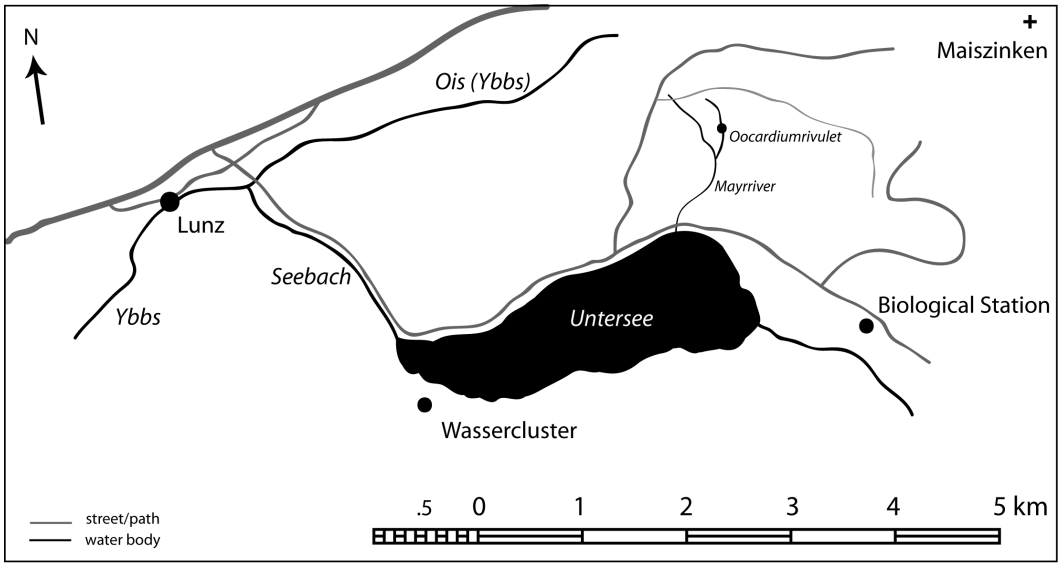

Fig. 2. Location of the sampling site in Lunz/See

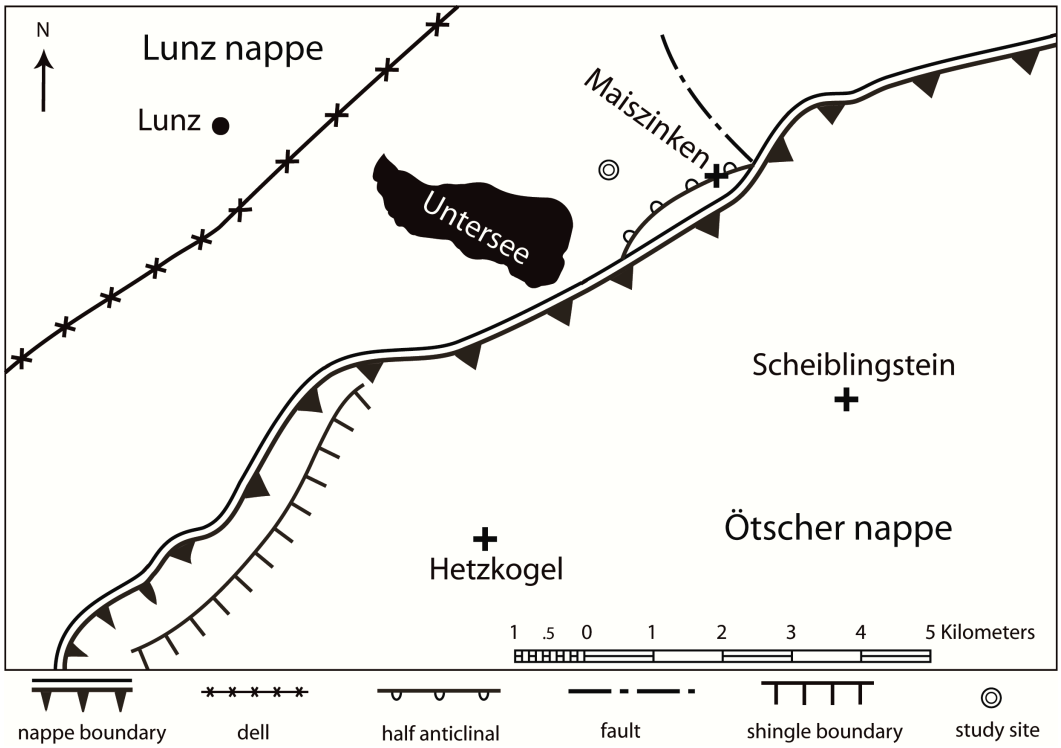

Fig. 3. Tectonic assembly of the study area. The study site is near the boarder of Lunz nappe and Ötscher nappe. (Tollmann, 1965, Tollmann, 1966)

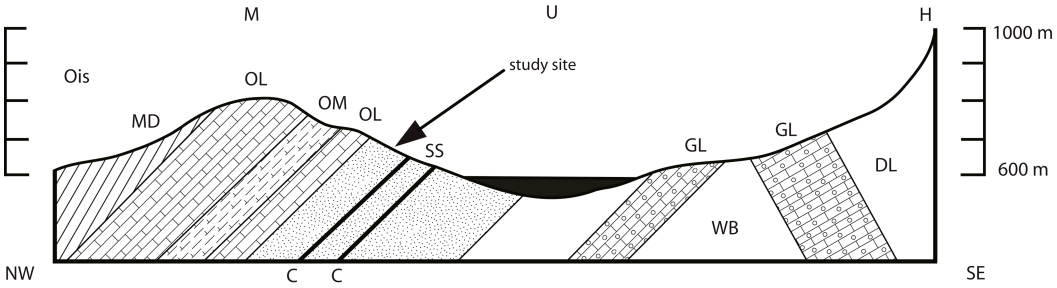

Fig. 4. Geological profile through the lake valley of Lunz from the Maiszinken (M) over the Lunz lake (U) to the Hetzkogel (H), High to length 1:75. MD (Main dolomite), OL (Opponitzer limestone), OM (Opponiter Mergel), SS (Sandstone and shale), C (Coal), GL (Gutensteiner limestone), WS (Werfener banks), DL (Dachstein limestone). (GÖTZINGER, 1910)

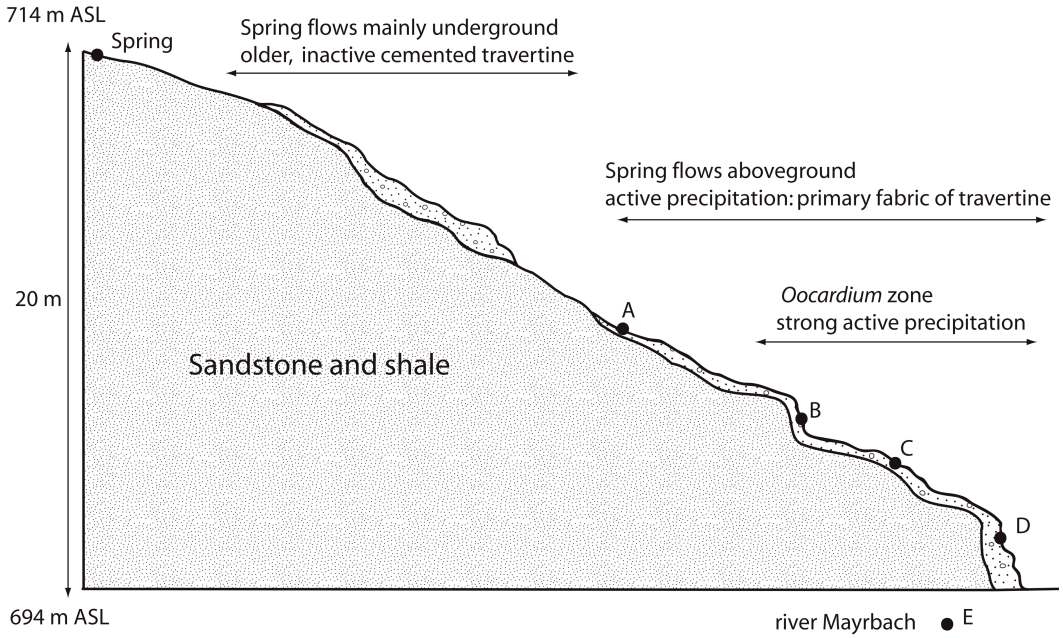

Fig. 5. Altitude profile of the study site and the positions of the sampling spots. The spring of *Oocardium* river in the Mayrgraben is 714 m above sea level. Towards a few meter of the spring, the course of the rivulet is mainly underground. Inactive and cemented travertine indicates the former spring course. At an altitude of 703 m above see level, the rivulet flows again aboveground and active precipitation starts. Between spot A and B, 65 m downstream the spring, the *Oocardium* zone starts and ends 84 m downstream into the mouth of the Mayrbach river.

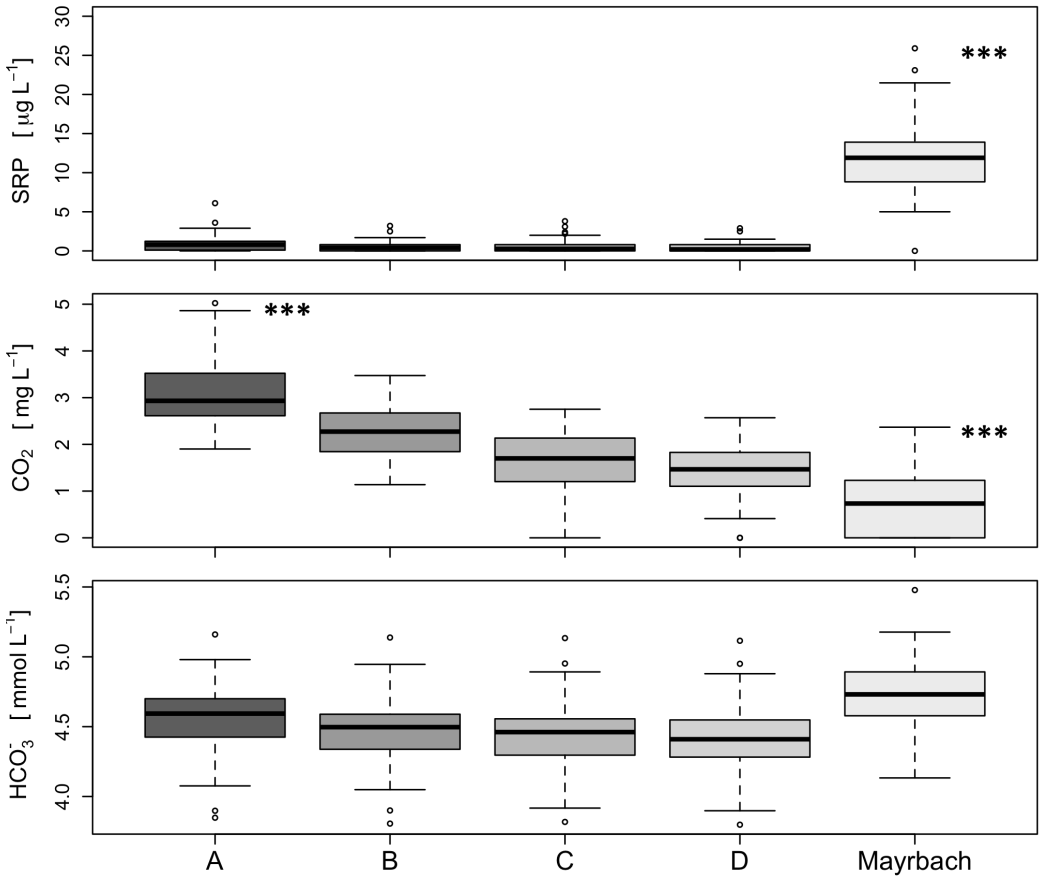

Fig. 6. SRP, free carbon dioxide (free CO_2) and bicarbonate (HCO_3^-) levels for all spots: box-blots with their means (−), standard deviations and outliers (°). Significant differences between the spots are marked with a star (*).

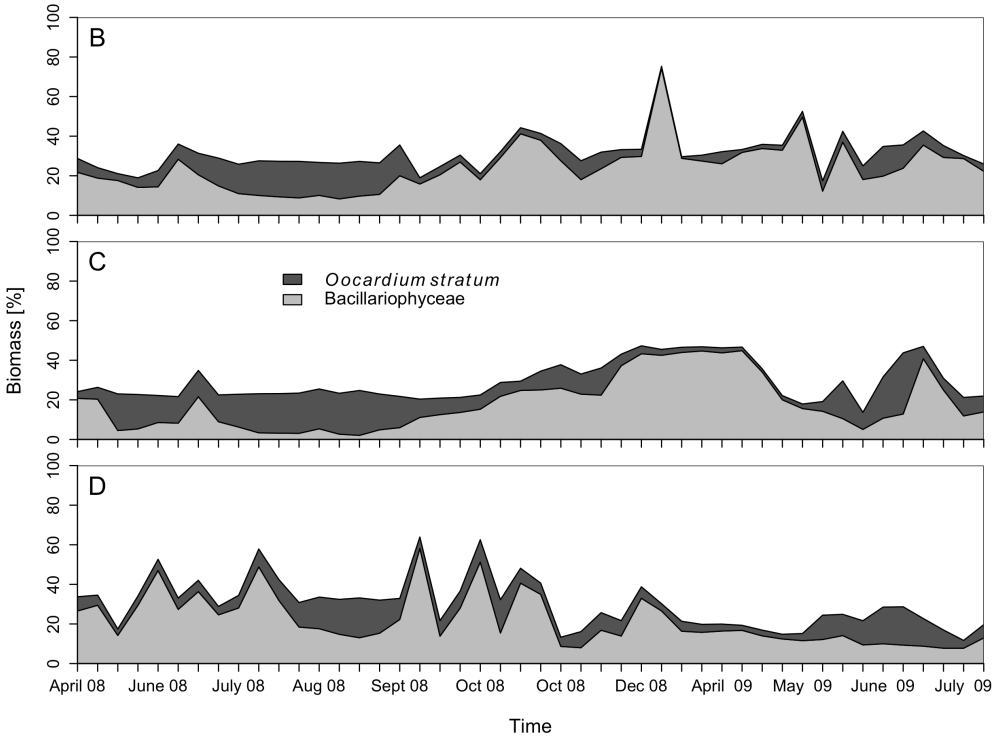

Fig. 7. Seasonal variation of *Oocardium* and Diatom biomass in percentage of overgrown travertine surface (macromapping) of spot A, B and C from 16.4.2008 till 24.7.2009.

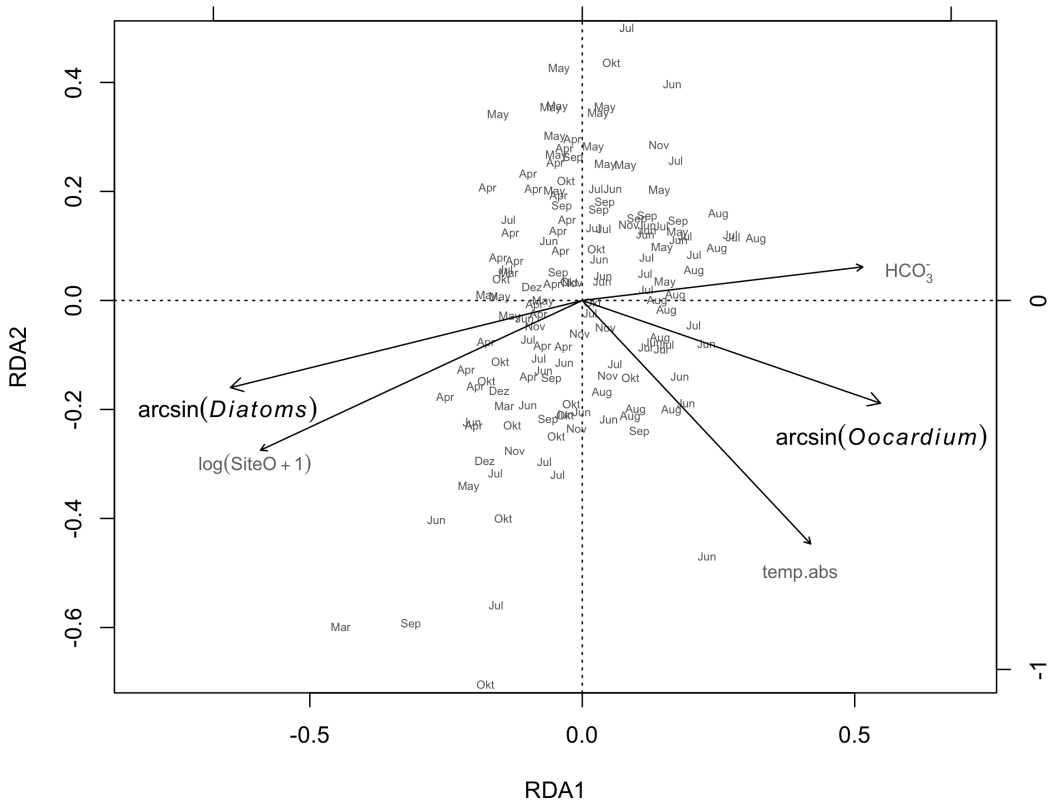

Fig. 8. Redundancy analysis biplot of algal biomass. Presented are the first two axis of the reduced model, with only significant constraints, the transformed variables bicarbonate (HCO_3^-), temperature [°K] (temp.abs) and site openness log(SiteO + 1). The investigation spots and times are represented as aberrations of sampling months, for a better representation of the season.

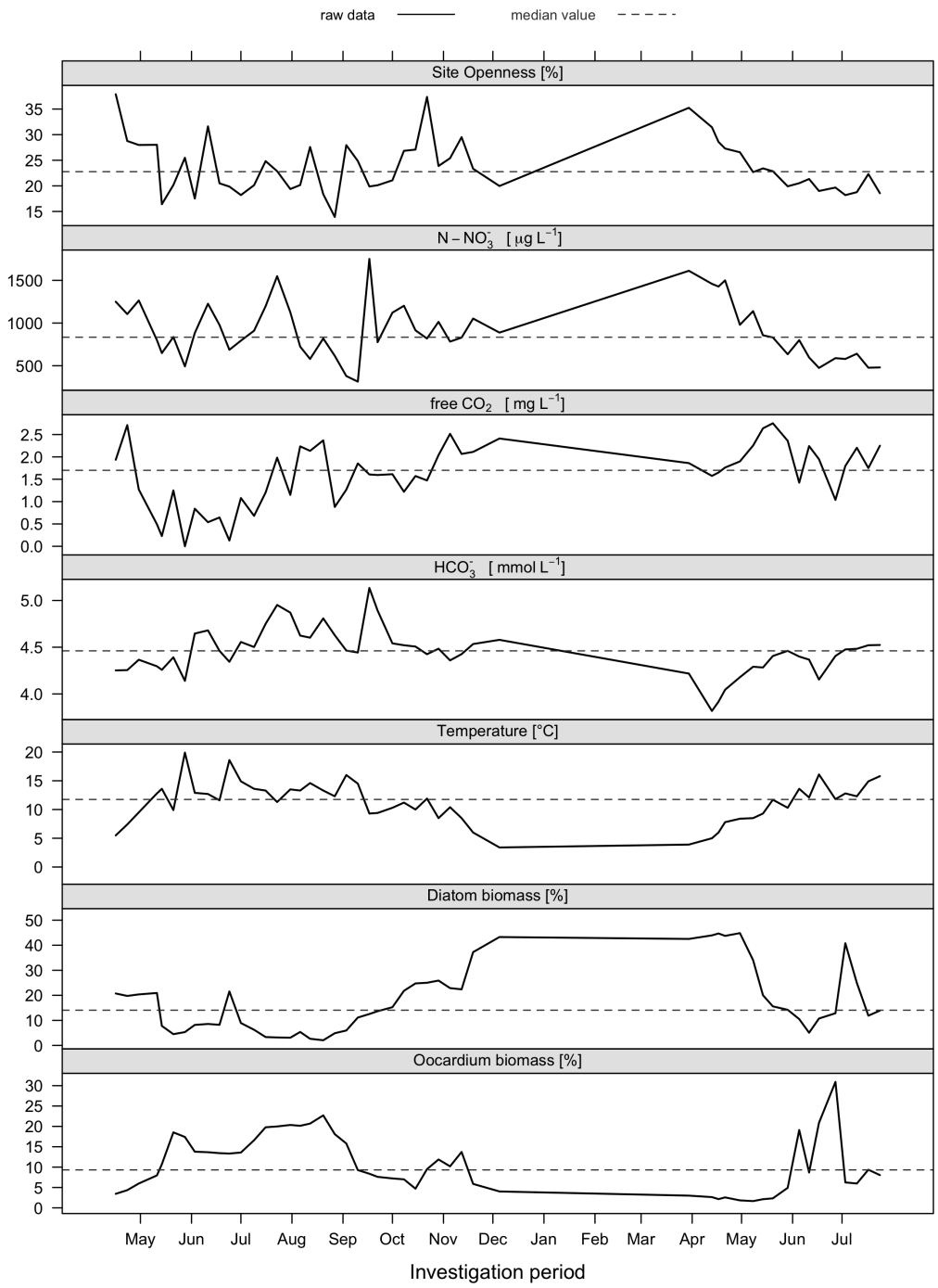

Fig. 9. Seasonal variation of *Oocardium* and Diatom biomass (macromapping) of spot C from 16.4.2008 till 24.7.2009 with significant environmental variables temperature (***), bicarbonate (***), Site Openness (**) and insignificant variables nitrate and free carbon dioxide.

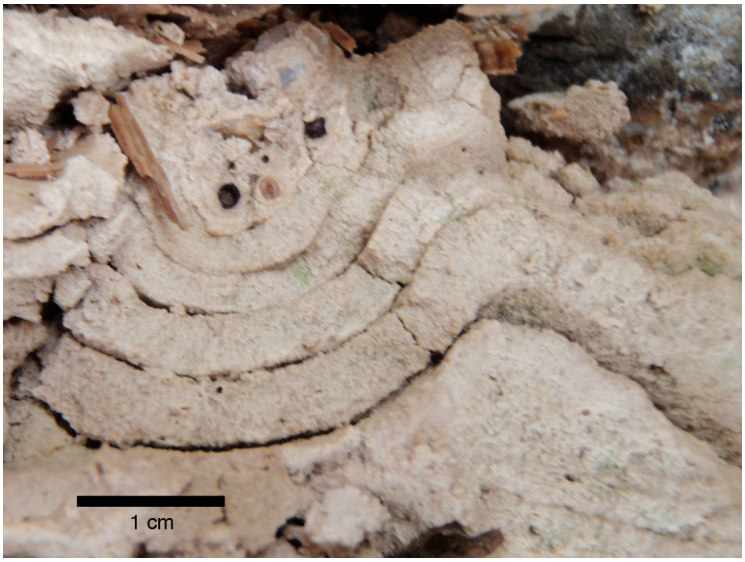

Fig. 10. Seasonal travertine lamination of primary travertine fabric at *Oocardium* rivulet in the Mayrgraben Lunz/See

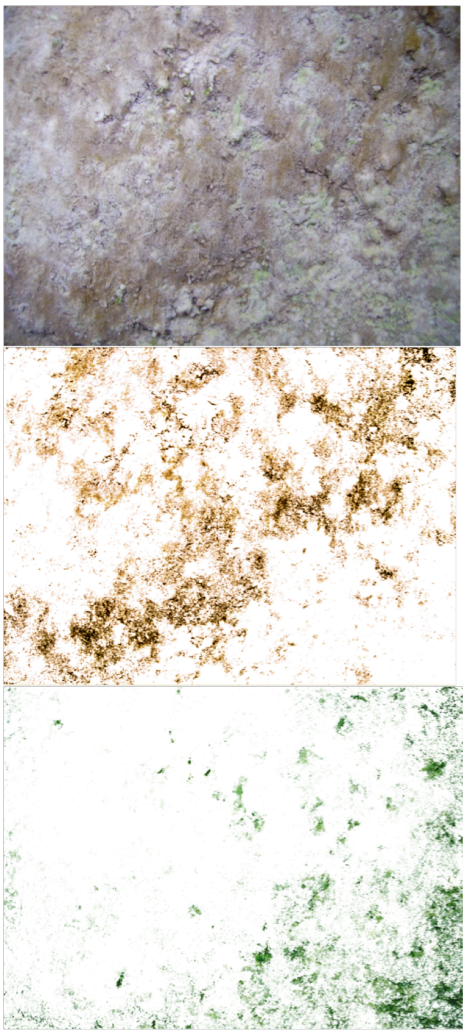

Fig. 11. Micromapping photo from 19.9.2008, with green (*O.stratum*) and red (Diatoms) channel.

4 Algae associated meteogene travertine precipitation: deposition rate and algal community composition

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4.1 Abstract

The rare desmid *Oocardium stratum* is found exclusively in travertine headstreams. We studied the succession of photoautotrophic biofilms with a special focus on *O. stratum.* The seasonal succession of *O. stratum* and diatom mats in limestone precipitating springs causes a typical sequence pattern of travertine layers, which can be recognised already with blank eyes. Our study was conducted in a travertine headstream near Lunz/See (Austria) for 17 months and included three sites. We installed frosted glass slides as artificial substrata, which were exposed between 3 to 12 weeks. Environmental variables were measured weekly; biofilm and precipitated tufa were sampled biweekly. We found a significantly lower precipitation rate near the spring because of higher free CO_2 amounts and higher pH levels than downstream. Precipitation was also higher in summer than in colder seasons. The biweekly precipitation of calcium carbonate near spring barely showed a positive rate. The amount of precipitated $CaCO_3$ near spring was in both years nearly constant without a clear accession. However further downstream situated spots showed a clear positive biweekly precipitation rate, which ranged from 5 mg cm^{-2} up to 27.5 mg cm^{-2}. The biweekly Chlorophyll *a* rate ranged at all spots from 0.07 µg cm^{-2} up to 3.9 µg cm^{-2}. The colonization of the glass slides started with the growth of diatoms and after the successfully development of a constant rough travertine layer, *Oocardium* colonies followed. The *O. stratum* colonies were after 6-7 weeks macroscopically visible and its biomass was higher in summer than in autumn and spring. The diatom mats were dominated by *Cymbella minuta*; only smaller amounts of coccoid Cyanobacteria could be detected. The distribution of taxa for the mean of all sites and dates showed that 77 % of the algae biomass were diatoms, 20 % could be related to *O. stratum* and 3 % comprised Cyanobacteria. Diatoms were negatively related to precipitation, whereas Cyanobacteria were slightly positively and *O. stratum* biomass was strongly coinciding with carbonate precipitation. From our results, we assume an induction of carbonate precipitation by *O. stratum*, but no active deposition due to photosynthesis. *O. stratum* biomass and the amount of precipitation showed parallel reactions to downstream gradients and to the seasonal changes. We therefore could not distinguish, if *O. stratum* increases the precipitation or if both, precipitation and *O. stratum* are mainly controlled by downstream chemical gradients and seasonal changes.

Keywords: *Oocardium stratum*, diatoms, succession, pigment analysis, artificial substrate, tufa

4.2 Introduction

Carbonate precipitation due to biomineralization occurs in the sea, in freshwaters and soils (Martinez *et al.*, 2010). Pelagic marine microorganisms have greatest impacts on carbonate sedimentation (Martinez *et al.*, 2010, Field *et al.*, 1998) and marine animals and seaweeds were responsible for orogenesis, like the Dolomite Alps (Bosellini *et al.*, 2003, Faupl, 2003). The most common form of carbonate precipitation is calcium carbonate ($CaCO_3$). Besides macrophytes and animals, biomineralization in freshwater is influenced by microbial mats, which mainly control the equilibrium between organic and inorganic carbon (Dupraz *et al.*, 2009b). A distinction between organically and inorganically induced $CaCO_3$ precipitation is not always obvious (Merz-Preiß and Riding, 1999), especially if the precipitation is connected to cyanobacteria or microalgae. Dupraz *et al.* (2009) listed three different types of biomineral formations: biologically induced (Weiner and Dove, 2003, Bazylinski and Frankel, 2003), biologically controlled and biologically influenced mineralization. Additionally, an exclusively abiotic precipitation of $CaCO_3$ will occur in both rivers and standing water bodies if the partial pressure of carbon dioxide (CO_2) is decreased e.g. through heating of the water column. This finally causes outgassing of CO_2 into the atmosphere. Freshwater ecosystems with high $CaCO_3$ precipitation rates are termed travertine. Basically, groundwater enriched by dissolved CO_2 because of respiration activities dissolves carbonates and as a result a solution of calcium bicarbonate is formed. Reaching the surface, carbon dioxide becomes supersaturated, degasses and consequently calcium carbonate precipitates.

In this study we focussed on $CaCO_3$ precipitation in travertines where also the biological influenced precipitation takes place. The characteristics of travertine springs are reviewed in detail by Pentecost (2005), who classified travertines by means of their carrier CO_2, which can originate from soil by respiration activities (meteogene travertines), or from processes in deeper earth layers where higher temperature is an additional factor for dissolving carbonates (thermogene travertines). Travertine mainly consists of calcite and aragonite deposits with low porosity between crystals, but high structural porousness (Dupraz *et al.*, 2009a). Organisms associated with travertines are for example the moss *Palustriella commutata* (Hedw.) Ochyra and the desmid *Oocardium stratum* Nägeli, which was the research focus of our survey. *O. stratum* occurs exclusively in active meteogene travertine springs and headstreams on various surfaces encrusted with tufa. Tufa is a type of fluvial limestone deposition and originates through the downstream out gassing of CO_2 and the following precipitation of $CaCO_3$. The first description of *O. stratum* was given by Nägeli (1849) based on material from Switzerland. Wallner (1933, 1934, 1935)

investigated this organism and its habitat intensively in the early 1930's in Upper Bavaria and mentioned the occurrence of *O. stratum* at 30 sites in pre-alpine areas of Bavaria, Germany. Until now, *O. stratum* has been found only at a few other locations worldwide; it has been documented for North America, Cuba, India and a few locations all over Europe (Rott *et al.*, 2009, Wallner, 1934, Pfiester, 1976, Rieth, 1969, Sanders and Rott, 2009). Active and intact meteogene travertine habitats without anthropogenic pollution are rare, but exist in limestone areas all over the world. Because of its peculiar habitat, *O. stratum* is easily overlooked and probably more frequent than reported (Pfiester, 1976). In Eastern Austria, *O. stratum* was documented by Hangsgirg (1905) near Wiener-Neustadt and by Brehm and Ruttner (1926) in the area of Lunz/See. In the following decades, it seemed to have disappeared and Lenzenweger (2003), in his book on the desmid floras of Austria, did not include this taxon. Recently, Schagerl and Pröschold (2007) rediscovered *O. stratum* in a small rivulet in the Mayrgraben in Lunz/See. Sanders and Rott (2009) mentioned two more sites in Western Austria.

The macroscopic structure of *O. stratum* colonies resembles green pinheads. The desmid builds small colonies of about 100 cells which can only be distinguished microscopically (Pentecost, 1991). The *Cosmarium*-like cells (width 15-20 µm, length 10-20 µm) are located at the ends of gelatinous stalks, which are encrusted with lime (Wallner, 1933, Wallner, 1934, Golubić and Marčenko, 1958, Schagerl, 2007). These stalks are extracellular polymeric substances (EPS). EPS proofed to be ideal nucleation sites for $CaCO_3$ precipitation (Pentecost, 1985). The question of how *O. stratum* is connected to $CaCO_3$ precipitation (influenced, controlled or induced) was already in the focus of travertine research (Sanders and Rott, 2009, Rott *et al.*, 2009, Pentecost, 1991, Wallner, 1934). However, until now the precipitation process with *O. stratum* has not been sufficiently investigated. It is still not known whether enzymes like carbonic anhydrase are involved, or whether the EPS stalks just serve as precipitation nucleolus.

Wallner (1935) reported a growth rate of 5 mm a^{-1} for *Oocardium* associated travertine's. According to Pentecost (2005, 1991), the mean deposition rate of meteogene travertine systems is also around 5 mm a^{-1}, and may increase to 9 mm a^{-1} if eukaryotic algae are involved to a greater extent. Precipitation rates of 2.2 mm a^{-1} for travertine systems with cyanobacteria are measured by Merz-Preiß and Riding (1999). Recent studies focusing on travertine formation and lamination induced by *O. stratum* have been carried out by Sanders and Rott (2009). At two different sites in Austria (Voralberg/Lingenau and Innsbruck/Alpenzoo) they measured a precipitation rate in the presence of *O. stratum* of 1-2 mm a^{-1} in Lingenau and 5-10 mm a^{-1} in Alpenzoo. Sanders and Rott (2009) suggested

that the seasonal change in irradiance supply controls the presence/absence of *O. stratum* and therefore the seasonal layering of travertine. The travertine formation showed a high increase up to 5 mm a^{-1} during warmer periods coinciding with intense *O. stratum* growth, and a strong reduction caused by massive development of benthic diatoms in autumn and winter. A few colonies of *O. stratum* persisted in the cold season.

The few existing studies on this peculiar taxon mainly focused on travertine formation, whereby the ecological niche of *O. stratum* was largely neglected. Especially studies investigating seasonal biomass fluctuations of *O. stratum* and searching for key variables of the occurrence of this peculiar taxon have not been conducted. Also, the influences on precipitation throughout a season and the interaction with other algae and bacteria have not been studied in detail.

With our study, we wanted to shed some light into these open questions. Like Sanders and Rott (2009), we also observed a clear seasonal lamination of the tufa. We were interested in the question, if the seasonal lamination occurs through community composition changes (biotic) or through temperature gradients (abiotic). Therefore, we were mainly interested in the precipitation rate of $CaCO_3$ associated with *O. stratum*, Diatoms and/or Cyanobacteria. Furthermore, we investigated the shifts of algal community composition throughout the seasons. We proposed that *O. stratum* is, because of its EPS stalks, linked to higher precipitation rates than diatom mats.

4.3 Material and Methods

4.3.1 Study site

The study site is a small meteogene travertine headstream at a south-west faced slope in the Mayrgraben system in Lunz/See (47°15 N, 15°04 E). It is located in the lime alps of Lower Austria at an altitude of 700 m above sea level (Figs. 1, 2). The geological catchment area of the spring system is part of the Lunzer beds II (Sulzbach beds) situated next to the Ötscher nappe (Tollmann, 1965, Tollmann, 1966); it belongs to the geological era of the Oberostalpine Mesozoikum (Fig. 3); (Faupl, 2003) and mainly consist of karst formations, like the Gutensteiner limestone, the Werfener banks and Dolomite (Götzinger, 1955). The *Oocardium* rivulet showing typical travertine formations is located in a sandstone and shale formation of the Lunzer beds II (Fig. 4). The riparian vegetation at the study site comprises mainly *Fraxinus excelsior* L., *Picea abies* L. H. Karst, *Fagus silvatica* L. and *Rubus* subgen. *Rubus* L.. Mean annual precipitation in Lunz am See is about 1545

mm a^{-1} (1971-2000) and the mean annual air temperature is 6.9°C (1991-2000; www.noel.gv.at).

After a heavy storm event in 2007, broken trees were removed and as a result most of the travertine layers were destroyed, leading to a collapse of the *O. stratum* population. This gave us the opportunity to study the succession of a travertine system including a recolonization of *O. stratum*. Four sampling sites are located in the travertine rivulet (Fig. 5). Site A is located nearest to the spring; here travertine depositions start to develop. This site is also characterized by a high amount of incoming irradiance. Sites B and C are located in the middle stretch of the headstream; an artificial leaf shading was constructed at site C. Site D is located a few m downstream towards the mouth of the headstream, where the travertine spring discharges into the Mayrbach. This site is also characterized by a high amount of incoming light; both sites B and D were located on travertine cascades.

4.3.2 Data collection

The study took place between March 2008 and July 2009. From March 2008 to November 2008 we collected weekly, in the following winter season until March 2009 on a monthly basis, and from April 2009 to July 2009 again in weekly intervals.

At each of the sites B, C and D a set of 25 frosted glass slides fixed in acrylic glass frames was installed at the travertine surface. The slides were overflown with water. Sampling took place between 30th of July 2008 and 22nd of October 2008 and between 1st of May 2009 and 27th of July 2009. Every two weeks five slides were removed randomly and replaced by new ones, in order to ensure tufa and biomass samples to be taken every second week. Before exposure, the slides were combusted (450 °C, 4 hours). The glass slides were handled only with rubber gloves to keep them free of grease. The glass slides stayed between two and twelve weeks within the spring to observe the initial state of travertine precipitation, the precipitation rate, the algal biomass and the algal community structure.

The slides were carefully removed from the acrylic glass frames and transported to the laboratory in falcon tubes. The glass slides were scraped with a scalpel and the striped material and the biofilm were washed with 50 ml aqua dest. into falcon tubes. In order to determine the precipitation rate, Chlorophyll *a* (Chl *a*) and accessory pigments, the samples were homogenized (PT 1600 E disperser) and separated into subsamples. These subsamples were gently vacuum filtrated on pre-combusted and pre-weighted glass fibre filters (Whatman GF/F). One set of GF/F subsamples was subsequently oven dried (60 °C, 24 hrs), weighted for dry mass (DM) and incinerated (450 °C, 4 hrs) to determine the ash

mass (AM) and to calculate the particulate organic matter (POM = DM - AM). AM was treated as carbonate precipitation rate. AM samples were analyzed with X-ray diffraction (XRD) in order to determine the mineral fractions.

The GF/F subsamples used for pigment analysis were kept frozen at -80 °C to aid the bursting of the cells until extraction, which took place in the Vienna laboratory. For extracting, the GF/F carrying the material was ground in 10 ml 90% acetone (homogenizer Polytron PT 1600 E) and the solution was stored for 8-12 hours in a refrigerator (4°C). After centrifugation (15 min, 3000 rpm, 10 °C), the supernatant was decanted and analyzed (HPLC system Hitachi Elite LaChrom, Diode Array Detector Hitachi L-2455, column thermostat L-2300 with temperature of 35°C, column Superspher RP — 18, 100 LIChrocart, precolumn LIChrocart rP-18 endcapped) according to a modified protocol of Wright *et al*., (1991). Peaks were quantified at 440 nm and identified through comparing retention times and spectral data with those of authentic standards (DHI Bioproducts; (Haberleitner, 2010). For peak analyses, calibration, and peak area integration, we used EZChrom Elite Client Version 3.2. In order to determine the percentages of *O*. *stratum*, Bacillariophyceae, and Cyanoprokaryotes, we first calculated and class-specific pigment ratios. For *O*. *stratum* we used species-specific pigment ratios of clone cultures. For Bacillariophyceae and Cyanoprokaryotes we used means of class specific pigment ratios from literature (Mackey *et al*., 1996, Bianchi *et al*., 1997, Schlüter *et al*., 2000, Brotas and Plante-Cuny, 2003, Schagerl and Donabaum, 2003, Lewitus *et al*., 2005); Table 5). Both the ratio matrix and the field-sample matrix were taken as input data to estimate algae class abundances relative to Chlorophyll a (Chl *a*) as a surrogate for total algal biomass. We used the R-package *BCE*, which is the Bayesian Compositional Estimator (Van den Meersche *et al.*, 2008). We also used qualitative observations of the biofilm, applying a Zeiss Axio Imager.M1 microscope (camera: Axio Cam MRc5, computer application: Axio Vision Release 4.7.2). The amount of Chl *a*was also measured spectrophotometrically (Talling, 1961) and served as a control for HPLC pigment analyses.

Additionally, at each site a full set of environmental data was collected (Linhart, 2010 unpubl.). Data loggers (HOBO UA-002-64 Pendant Data Logger) recorded water temperature (°C) and luminous intensity (Melillo *et al.*) every two minutes. For data analyses, the weekly average of water temperature (also day-min, day-max) and the weekly sum of incoming light were used. On each site, specific conductivity (µS cm^{-1}), water temperature (°C), and oxygen concentration (% and mgL^{-1}) were measured by using a portable multi-Meter (Hach Lange, HQ 40d, 10105). Water discharge (L s^{-1}) was estimated at a gorge by means of a bucket and a stop watch.

For the analysis of the water chemistry, we took sample triplets from each site. We used BOD bottles in order to keep the CO_2 pressure in the water samples constant until analysis, which took place within 45 min after sampling. Bottles were filled with a PVC hose to avoid turbulences with the atmosphere and sparkling (Legler, 1988). The bottles were kept cold and dark and were brought to the laboratory immediately. Total alkalinity (m-value at pH 4.3), pH, and acidity (p-value at pH 8.2) were determined titrimetrically with a Titrino 702 SM (Metrohm Ion Analysis, Switzerland). Soluble reactive phosphorus (SRP), nitrate-N (NO_3-N), nitrite-N (NO_2-N), and ammonium-N (NH_4^+-N) were analyzed according to the standardized Continuous Flow Method (Eberlein and Kattner, 1987, Kempers and Luft, 1988, APHA *et al.*, 1998). Magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^+), chloride (Cl^-), potassium (K^+), and sulphate (SO_4^{2-}) were analyzed by ion chromatography (Metrohm Compact IC 761, Metrohm IC Filtration Sample Processor 788).

Sky openness was determined by means of hemispherical photos, which were taken with the digital camera Nikon Coolpix 4500 equipped with a Nikon fisheye converter FC-E8 0.21 x. The camera was placed on a thin, levelled polystyrol plate directly into the headstream. The magnetic north was marked on the lens of the fisheye converter. The photos were post-processed using Adobe Photoshop Version 8.0.1 in order to eliminate any shading caused by the photographer. The light parameters were then calculated by the computer program Gap Light Analyzer (GLA) Version 2.0 (Frazer *et al.*, 1999). Except for spectral fraction, which was set to 0.45 (default 0.5) to adjust for the transmitted photosynthetic active radiation, default settings were used (Frazer *et al.,* Hainz *et al.*, 2009). The length of the growing season was assumed to be 12 months, which seemed to be approximately the growing period of the *O. stratum* colonies.

4.3.3 Data analyses

Statistical analyses were carried out using the computer program R 2.9.2 GUI 1.29 (R Development Core Team, 2009); including the external software packages *BCE, psych*, *nortest* and *vegan.*

Environmental data were transformed, if necessary using arcsin√x (for percentage data: site openness), sqrt(x) or $\log_{10}(x+1)$ transformation (Ramette, 2007, Hainz *et al.*, 2009). Since the sample size of 50 limited the number of input variables for the principal component analysis (PCA), a bivariate correlation matrix for all *Oocardium* sites was calculated including all variables in order to choose the adequate input variables for a PCA. Several factors were excluded from the PCA: the sum of ions and HCO_3^- because of correlation with conductivity, oxygen because of low variation, pH because of high correlation with free CO_2; $N\text{-}NO_2^-$, and $N\text{-}NH_4^+$ because of a very low amount and the

better representation of nitrogen with $N\text{-}NO_3^-$; and precipitation because discharge has more influence on the water chemistry and it also contains the snowmelt. PCA (principal component analysis) was performed, including all *Oocardium* sites (ABCD) in a single model.

Data for biomass (Chl *a* µg cm^{-2}), tufa ($CaCO_3$, mg cm^{-2}), and data for community composition (amount of *Oocardium stratum*, Bacillariophyceae and Cyanoprokaryotes in µg cm^{-2})were tested for normal distribution using qq-plots and the *Lilliefors* (Kolmogorov-Smirnov) test. It was necessary to use $\log_{10}(x+1)$ transformation (Burns and Walker, 2000, Ramette, 2007).

T-tests were used to test for significant differences between sites and one-sided t-tests were used to find out whether there are significant accumulations of Chl *a*, tufa, and specific taxa amounts during the growing time of the autotrophic biofilm.

Two-factor analysis of variance (ANOVA) (site*time) with Chl *a,* tufa, and biomass of *O. stratum*, Bacillariophyceae, Cyanoprokaryotes as dependent variables were performed. We used "site" as an independent factor representing the downstream gradient in water chemistry of the ANOVAs; while the factors "season" or "time" should represent changes over the year, which are mainly represent variations in water temperature, radiation and site openness. Post-hoc Tukey HSD tests were used to find out specific differences between sites and exposition-times of the glass slide samples. All data were split into the two respective years (2008, 2009) of observation. In both years, one continuous experiment over 12 weeks and one seasonal comparison experiment was performed.

4.4 Results

4.4.1 Site characteristics

Precipitation up to a weekly amount of 100 mm was readily buffered by the karst system of the catchment; therefore, the discharge was in most of the time approximately 1 L s^{-1}. Only in July 2009, the discharge reached 3 L s^{-1} through heavy rainfalls. Within the short stretch of the headstream, mean annual water temperature increased from 8.5 °C directly at the spring to 11.3 °C at the discharge into the Mayrbach rivulet. The *Oocardium* rivulet was slightly oversaturated with free CO_2 and O_2 and generally showed very low nutrient concentrations (Tables 2, 3). The stretch from site A to D clearly revealed decreasing gradients in free carbon dioxide (Fig. 6), conductivity, and also a slightly decreasing gradient in Ca^{2+} (Table 3). The pH increased downstream and the lowest pH was related to the highest amounts of free carbon dioxide (site A). The predominating cation in the

Oocardium rivulet was Ca^{2+} with an annual average of 80.7 mg L^{-1}, followed by Mg^{2+} with 6.4 mg L^{-1}. Interestingly, $N\text{-}NH_4^+$ concentrations decreased downstream. The dominant anion was HCO_3^- with 274 mg L^{-1} (Table 1). Heavy rainfalls in June 2009 (discharge 2.5 L s^{-1}) decreased ion concentrations and also had a huge impact on the oxygen concentration. In spite of canopy cover, the irradiance supply at the *Oocardium* rivulet is higher in summer than in winter. In both years, site A received the highest irradiance supply from mid of April until mid of July, whereas site D received the highest irradiance levels in high summer, autumn and winter. The minimum (3500 klux in October 2008) and maximum (205000 klux in April 2009) of seasonal irradiance supply at the travertine rivulet were both measured at site C. PCA revealed three principal components (PCs) with an eigenvalue beyond 1 explaining 71.7 % of the total variance in the data set. PC1 had maximum loadings on temperature and $N\text{-}NO_3^-$. PC2 had highest loadings on free CO_2 and PC3 on radiation (Table 4).

The differences between the sites are mainly characterized by clear downstream gradients in Ca^{2+}, CO_2, pH and also a slight gradient in HCO_3^-. Site A has significantly increased free CO_2 levels (t-test, n = 50, p-value < 0.001***) than the other sites, whereas HCO_3^- differed not significantly between sites (Fig. 6).

4.4.2 Autotrophic Biofilm and tufa development

The X-ray diffraction showed the same mineral fractions for all AM samples. The precipitated material mainly consists of calcite (60 %, $CaCO_3$) and aragonite (39 %, $CaCO_3$) and also slight amounts of quartz (1 % SiO). 30th of July 2008 and 22nd of October 2008 and between 1st of May 2009 and 27th of July 2009

Chl *a* and $CaCO_3$ precipitation in 2008 were not detectable until week 3 (Start: 30th of July2008) of exposition and in 2009 until week 4 of exposition (Start: 1st of May 2009). In general, the Chl *a* amount on the glass slides ranged from 0.004 to 5.500 µg cm^{-1}, the tufa amount ranged from 0.002 to 116.400 mg cm^{-1}. The dominant fraction of the biofilm was consisting of Bacillariophyceae with about 77 % of the total Chl *a* amount. The most abundant species was *Cymbella minuta* Hilse ex Rabenhorst. *Oocardium stratum* contributed around 20 % to the total Chl *a*, whereas Cyanoprocayotes were negligible (3 %). All detected Cyanobacteria were tiny coccoid forms.

The amount of precipitated tufa ranged from 0.002 mg cm^{-2} after 4 weeks exposition until May 2009, site A) to 116.3 mg cm^{-2} (12 weeks exposition until Oct 2008, site D). *O. stratum* biomass was positively related with the amount of precipitated $CaCO_3$ (r=0.75, n=150), whereas Cyanoprokaryotes showed less correlation (r=0.25, n=150).

Bacillariophyceae were negatively correlated to the precipitated $CaCO_3$ amount (r=-0.03, n=150).

4.4.3 Continuous colonization experiments 2008

The ANOVAs (spot*time) calculated with the datasets of Chl *a*, tufa and occurrence of *O. stratum* showed a significant influence of spot, time and their combined effect (2-way ANOVA, p-value < 0.001***). At every spot, the highest Chl *a* amount was reached after 12 weeks. Concerning the Chl *a* amount, no significant differences between all spots could be measured after 5 weeks (post-hoc TukeyHSD, n = 5, p-value > 0.05). From week 9 to 12, Chl *a* showed significant differences at all spots (post-hoc TukeyHSD, n = 5, p-value < 0.001***). The spots C and D were similar in tufa precipitation (post-hoc TukeyHSD, n = 5, p-value > 0.05) and significantly different from the tufa amount in spot A (post-hoc TukeyHSD, n = 5, p-value < 0.001***). The influence of time on precipitation was significant (post-hoc TukeyHSD, n = 5, p-value < 0.001***) in all weeks, except in week 3 to 5 and week 9 to 12 (Table 13). As tufa, the amount of *O. stratum* also depends on spot and time. The post-hoc TukeyHSD-test indicated that week 12 was significantly different from all other times (p-value < 0.001***). Also only in week 12, spots B and C differed significantly from A (post-hoc TukeyHSD, n = 5, p-value < 0.001***). Biomass of Bacillariophyeae showed no significant differences between spots, but there were significant differences between the sampling times (2-way ANOVA, p-value < 0.05*). Cyanoprokaryotes showed no differences (2-way ANOVA, p-value > 0.05, Table 14).

4.4.4 Summer – Autumn comparison 2008

The ANOVA revealed a significant influence of spot and season (p-value < 0.05*) on the Chl *a* amount and on the biomass of *O. stratum*. A significant decrease of the *O. stratum* amount from summer to autumn was only detectable at spot C (post-hoc TukeyHSD, n = 5, p-value < 0.05*; t-test, n=5, p-value < 0.007*). Spot, season and their interactions proofed to have a significant influence on the tufa precipitation (2-way ANOVA, p-value < 0.001***). Spot A was significantly different (post-hoc TukeyHSD, n = 5, p-value < 0.001***) from the spots C and D, which had a similar precipitation regime. In summer, the tufa precipitation at C and D was significantly different from autumn (post-hoc TukeyHSD, n = 5, p-value < 0.001***), while at spot A the season influence was insignificant (post-hoc TukeyHSD, n=5, p-value > 0.05). The comparison of summer and autumn after 9 weeks exposure revealed no significant influence of spot and time on the Bacillariophyceae and Cyanoprokaryotes amount (2-way ANOVA, p-value > 0.05; Table 15).

4.4.5 Continuous colonization experiments 2009

Again, the interaction ANOVA showed that spot and time had significant influences on the Chl *a* and the tufa amount (2-way ANOVA, p-value < 0.001***, Table 16). The results for Bacillariophyceae showed the same results as for Chl *a*. The *O. stratum* amount was significantly influenced by the factors spot (2-way ANOVA, p-value < 0.05*), time and their interactions (2-way ANOVA, p-value < 0.001***). Just the comparison of the *O. stratum* amounts of spot A with spot C showed that they had significantly different *O. stratum* amounts (post-hoc TukeyHSD, n = 5, p-value < 0.05*). Only spot C significantly changed in its *O. stratum* amount over time (post-hoc TukeyHSD, n = 5, p-value < 0.001***). Cyanoprokaryotes were significantly influenced by spot (2-way ANOVA, p-value < 0.01**) and time and their interactions (2-way ANOVA, p-value < 0.05 *). Again, spot A was concerning the tufa amount significantly different from C and D (post-hoc TukeyHSD, n = 5,p-value < 0.001***). Concerning time, spot A did not show significant differences, while the spots C and D changed in the tufa amount over time significantly (post-hoc TukeyHSD, n = 5, p-value < 0.001***).

4.4.6 May – July comparison 2009

In 2009 we were able to compare two 4-weeks experiments conducted in May and July, respectively. ANOVA revealed a significant influence of spot, season and their interaction (p-value < 0.001***) on Chl *a* and Baccillariophyceae amount (Table 17). The post-hoc TukeyHSD test showed same results for Chl *a* and Baccilariophyceae amount. There are no significant differences between the spots in May (p-value > 0.05). However in July, spot A was significantly different from both the spots C and D (post-hoc TukeyHSD, n = 5, p-value < 0.001**), while the spots C and D were comparable (post-hoc TukeyHSD, n = 5, p-value > 0.05). The Cyanophyceae amount showed similar characteristics. Also in tufa amount spot A was in both seasons significantly different from both spots C and D (post-hoc TukeyHSD, n = 5, p-value < 0.01***), while C and D showed similar values. All spots significantly changed in their tufa amounts over time (post-hoc TukeyHSD, n = 5, p-value < 0.001***). The *O. stratum* biomass of C and D was only significantly influenced by time (2-way ANOVA, p-value < 0.001***). Spot A did not show changes in *O. stratum* amount at all while spot C and D changes significantly (Table 12).

4.5 Discussion

4.5.1 Spot characteristics

It is obvious that biofilm biomass is higher at lower discharge (Blenkinsopp and Lock, 1994, Battin *et al.*, 2003, Besemer *et al.*, 2007). We also observed the development of thick diatom mats in connection with low discharge, especially in autumn. The high discharge ≥ 2 m s^{-1} in July 2009 mobilized even the cemented tufa. As a result, diatom mats and even *O. stratum* colonies were removed. For their study conducted in Western Austria, Sanders and Rott (2009) estimated a discharge between 1 to around 10 L s^{-1}, but they mentioned that only a part of this discharge belongs to places overgrown with *Oocardium*. (Pentecost, 1991) referred to two spots in the British Isles with discharge rates of 0.5-5 L s^{-1} and 40-200 L s^{-1} and to one in Belgium with 10 L s^{-1}. These data indicate, that *O. stratum* is able to handle a broad range of discharge. Another important variable, which is flow velocity directly at the colonies, has not been measured until now to our knowledge.

Besides our study, only a few data referring to the water chemistry of *O. stratum* spots are available, which are for the British Isles (measured 4 times), for Belgium (1 set) (Pentecost, 1991) and for Austria (Sanders and Rott, 2009, Rott *et al.*, 2009). Data belonging to the temperature regime and pH levels as mentioned by Pentecost (1991) are comparable to our data. Also nutrient conditions such as SRP and N-NH_4^+ levels were very low in both investigations, but N-NH_4^+ was 5 to 10 times higher in Lunz/See. Compared to the spots of the British Isles and Belgium, ion concentrations were slightly increased at our spot, but with the exception of Ca^{2+}, which showed significantly higher values at Lunz/See (Table 2, 3). Sanders and Rott (2009) analyzed occasionally selected parameters: compared to those spots, the *Oocardium* rivulet at Lunz/See showed a moderate level of bicarbonate. Although the pH was higher, ion and nutrient concentrations were clearly lower at the *Oocardium* rivulet at Lunz/See. Phosphate levels are generally low in most meteogene and thermogene travertine springs due to the precipitation of calcium phosphate and its low solubility (Pentecost, 2005). This was also the case at our rivulet, which showed SRP levels close to the detection limit (Table 2). The *Oocardium* spots located in Western Austria also indicated low levels of phosphate (Rott *et al.*, 2009, Sanders and Rott, 2009).

The high amounts of free carbon dioxide at spot A kept the pH lower than on the spots C and D. In accordance, the Ca^{2+} concentration was highest while the carbonate precipitation was lowest at spot A. Downstream, degassing of free carbon dioxide and the calcification process resulted in a parallel decline of free CO_2, Ca^{2+}, and conductivity.

4.5.2 Biofilm and tufa development

Obtained Chl *a* data are comparable to other studies on river biofilms. Sabater *et. al.* (1998) mentioned 0.06 - 4.2 µg cm^{-2} Chl *a* on wood and 0.03 - 1.1 µg cm^{-2} Chl *a* on tiles exposed in a forest stream after 3 - 42 days of colonisation. Bacillariophyceae dominated the biofilm with *Cymbella minuta* being the most common species on the wood. The study of Domozych *et. al.* (2008) showed for freshwater-wetland biofilms dominated by desmids Chl *a* amounts of 0.06 - 1.5 µg cm^{-2} (16 - 90 days exposure). For travertine biofilms, this is the first study providing some data: Chl *a* amounts were in the same range as already measured for other freshwater growth mats. There was however some interesting differences observed. The photoautotrophs in the *Oocardium* rivulet developed quite slow and it took more time until detectable amounts could be obtained. Instead of a few days, the photoautotrophs needed some weeks to reach certain amounts. A possible explanation could be that the biofilm is dependent on a carbonate layer in order to stick tightly to the substrate. The high velocity and low water temperature also reduce the growth rate. On contrary, the relatively high incoming irradiance given through the southwest facing slope system, could have had a positive effect. Like Domozych *et. al.* (2008) we have also data for one month exposition experiments and we also found Chl *a* amounts in the same range and biomass increase coinciding with elevated water temperatures.

We found macroscopical *O. stratum* colonies after 2 to 3 months slides exposure, which was a much shorter period than that one observed by Pentecost (1991), who mentioned a visible colonization on wood canes after 9 months. Remarkably, he also observed the major growth period mainly during winter. In our study, *O. stratum* colonies mainly developed in the warmer period.

Spot A showed the lowest amount of *O. stratum* and $CaCO_3$ precipitation.

The fluctuations of the $CaCO_3$ precipitation on spot A were in both years very low during the 12 weeks continual experiments, while the spots C and D showed high fluctuations. However, the results of the 2008 seasonal comparison between summer - autumn revealed that $CaCO_3$ precipitation declined significantly at all spots (t- test, $n = 5$, $p < 0.001$***). Now the question arises whether this decline is biologically induced or environmentally controlled. Regarding spot D in the summer - autumn comparison of 2008, results showed a clear decline in the $CaCO_3$ precipitation from summer to autumn, but *O. stratum* biomass and total Chl *a* showed no significant decline in this period (t-test, $n= 5$, $p > 0.05$). This leads to the assumption that $CaCO_3$ precipitation is more influenced by season (temperature and water level) than by the *O. stratum* biomass. Also the 2009 May – June comparison led to the same results. In July, spot A showed significantly ($p < 0.001$***)

more precipitation than in May, but with an amount of *O. stratum* less than 1%. This also supports the hypothesis that mainly higher water temperature is responsible for the increase in carbonate precipitation and not *O. stratum* growth. In July 2009, Chl *a* was significantly higher at spot A than on C and D ($p < 0.001$***). Interestingly, mainly diatoms with just a minor influence on the precipitation rate dominated the biofilm. Also an increase of carbonate precipitation at spots C and D could be detected even when no colonies of *O. stratum* were observable. We conclude from these findings, that $CaCO_3$ precipitation is mainly controlled by the downstream gradient of water chemistry, rather than by the *O. stratum* biomass.

From our results, we could not find whether the Cyanobacteria are also highly involved in precipitation. The Cyanoprokaryotes biomass was higher in 2009 (14 %) than in 2008 (2 %). Lee *et. al*. (2006) attributed to *Synechococcus* sp. a high precipitation rate, especially for the strain PCC 8806. They found out that growth of *Synechococcus* increases with increasing HCO_3^-. During a two weeks experiment in an HCO_3^- oversaturated environment (2.5 mmol L^{-1}) 3 mmol Ca^{2+} L^{-1} were removed by *Synechococcus* strain PCC 8806. Maybe coccoid Cyanoprokaryotes play a more important role in the "initial state" of colonization than previously expected. They might promote a precipitation through excretion of carbonic anhydrase, which could facilitate a first $CaCO_3$ layer, which in turn could serve as a substrate for *O. stratum* colonies.

Wukowitz *et. al.* (2011) revealed that *O. stratum* is not able to use HCO_3^-, but has a very efficient CO_2 uptake, which indicates the absence of carbonic anhydrase. The cultures of *O. stratum* showed high EPS production but no calcification was observed.

In cultures, *Oocardium* grows patchy in hemispherical colonies, but without calcificating tubes. This is another point to support the hypothesis of abiotic calcification, or induced calcification by *O. stratum*. Our results revealed, that calcification of *O. stratum* mucilage tubes originates through an abiotic precipitation of $CaCO_3$ of oversaturated source waters. Extracellular mucilage tubes are favourable spots for calcite nucleation in HCO_3^- and Ca^{2+} oversaturated source waters and facilitate the precipitation rate (Merz-Preiß and Riding, 1999, Pentecost, 1985). Pentecost (1981) described the surface of travertine environment as an area "of slow sedimentation often followed by rapid removal." Organisms have to be able to cope with both of these processes. Precipitation forces *O. stratum* to develop faster in order to avoid being buried by $CaCO_3$. Higher abiotic precipitation therefore leads to a higher excretion of EPS, and the EPS stalks are again nucleation spots for precipitation. As a consequence, *O. stratum* biomass is influenced by $CaCO_3$ and vice versa.

4.6 Conclusion

The lamination of travertine develops through seasonal alteration of the biofilm composition through thick diatom mats and high *O. stratum* growth. Precipitation is mainly of abiotic origin, but may also be favoured by increased EPS excretion (gelatinous stalks) of *O. stratum*.

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4.9 Tables and Figures

Table 7. Abbreviations and units for all environmental variables of the study.

Environmental variable	Abbreviations and synonyms	Unit
Free carbon dioxide CO_2, acidity	free CO_2	mg L^{-1}
Bicarbonate, alkalinity	HCO_3^-	mmol L^{-1}
oxygen	O_2	%
pH (-log(H^+))	pH	
specific conductivity	conductivity	µS cm^{-1}
temperature	temp	°C
ammonium	N-NH_4^+	µg L^{-1}
nitrate	N-NO_3^-	µg L^{-1}
nitrite	N-NO_2^-	µg L^{-1}
ortho-phosphate	P-PO_4^3-P, SRP	µg L^{-1}
sodium	Na^+	mg L^{-1}
potassium	K^+	mg L^{-1}
calcium	Ca^{2+}	mg L^{-1}
chloride	Cl^{-1}	mg L^{-1}
magnesium	Mg^{2+}	mg L^{-1}
sulphate	SO_4^{2-}	mg L^{-1}
Site Openness	SiteOpen	%
discharge	discharge	Ls^{-1}
weekly sum of precipitation	precipitation	mm
weekly sum of light	Radiation, rad, irradiance supply	klux

Table 8. Summary of environmental variables of the four sites of *Oocardium* (n=50, except for nutrients n=46 and ions n=36). Min (minimum), Q1 (first quartile), median, Q3 (third quartile), max (maximum). For other abbreviations and units see Table 1.

	Min	Quantil1	Median	Quantil3	max
Free CO_2	0.0	1.5	2.1	2.7	5.0
HCO_3^-	3.8	4.3	4.5	4.6	5.2
oxygen	91.1	99.1	100.3	101.3	105.2
pH	8.1	8.3	8.3	8.4	8.5
conductivity	337.0	380.0	391.0	402.0	453.0
temperature	3.2	9.3	11.6	13.4	20.5
$N\text{-}NH_4^+$	0.0	3.6	5.8	8.9	164.1
$N\text{-}NO_2^-$	0.0	0.5	0.8	1.1	4.8
$N\text{-}NO_3^-$	302.1	638.8	836.3	1127.8	1769.4
N_{tot}	307.2	641.3	849.3	1138.7	1777.1
SRP	0.0	0.0	0.4	1.0	6.1
Na^+	0.6	0.7	0.7	0.8	1.7
K^+	0.4	0.6	0.7	0.8	1.3
Ca^{2+}	59.5	77.4	81.0	84.1	92.7
Mg^{2+}	3.8	5.7	6.5	7.1	8.3
Cl^-	0.3	0.4	0.5	0.5	1.3
SO_4^{2-}	2.0	2.5	2.7	2.9	3.4
discharge	0.1	0.5	0.6	0.8	6.7
precipitation day	0	0	1.25	5.5	64.8
precipitation week	0.2	7.4	21.55	50	233.2
SiteOpen	13.9	28.0	22.4	36.3	50.9
temp. week mean	1.3	9.0	11.5	13.8	18.2
temp. day max	2.4	12.1	17.4	22.7	40.9
temp. day min	0.1	6.3	9.1	11.2	15.5
radiation week mean	1.0	7.2	12.2	18.9	40.7
radiation week sum	3457.5	35515.6	61481.7	96394.0	204924.0
radiation day mean	0.3	5.1	10.8	19.0	43.2
radiation day sum	225.2	3457.3	6619.1	14010.4	31119.6

Table 9: Mean values and standard errors of various variables for each site of the biofilm experiment at *Oocardium* rivulet (A-D) during the study. For abbreviations and units see Table 1.

	A	C	D
CO_2	3.07 ± 0.63	1.61 ± 0.69	1.44 ± 0.60
HCO_3^-	4.54 ± 0.25	4.45 ± 0.25	4.42 ± 0.25
pH	8.2 ± 0.1	8.3 ± 0.1	8.3 ± 0.1
conductivity	397.8 ± 20.4	388.5 ± 20.2	386.8 ± 20.0
$N\text{-}NH_4^+$	12.54 ± 24.48	6.71 ± 5.15	6.17 ± 4.52
$N\text{-}NO_2^-$	0.85 ± 0.77	0.87 ± 0.41	0.86 ± 0.49
$N\text{-}NO_3^-$	906.03 ± 330.89	912.30 ± 347.39	907.55 ± 347.22
SRP	0.99 ± 1.15	0.59 ± 0.88	0.50 ± 0.66
Na^+	0.77 ± 0.12	0.75 ± 0.12	0.74 ± 0.11
K^+	0.74 ± 0.15	0.74 ± 0.14	0.76 ± 0.17
Ca^{2+}	82.86 ± 4.61	79.43 ± 5.64	79.33 ± 4.69
Cl^-	0.49 ± 0.14	0.47 ± 0.10	0.48 ± 0.12
Mg^+	6.30 ± 1.10	6.35 ± 1.05	6.37 ± 1.09
SO_4^{3-}	2.70 ± 0.32	2.70 ± 0.31	2.73 ± 0.34
radiation w. sum	84053 ± 48698	77869 ±35808	46548 ±42334

Table 10. Loadings of transformed environmental variables of the three factors extracted by factor analysis (loadings >0.5 marked by an asterisk). Abbreviations: lightsum (radiation week sum), cond (conductivity), Q (discharge), temp.abs (temperature in °K). Abbreviations of the variables are as in Table 1.

Variables	Factors (% variance explained)		
	PC1	PC2	PC3
	31 %	24 %	16 %
$log(NO_3+1)$	0.095*	0.020	-0.050
CO_2	0.030	0.980*	0.030
sqrt(lightsum)	-0.060	0.030	0.970*
sqrt(SRP)	0.010	0.120	0.090
cond	0.060	0.110	-0.140
sqrtQ	0.150	0.040	0.180
temp.abs	-0.400	-0.200	0.160

Table 11. Input pigment ratios for occurring taxa to determine the community composition of the biofilm samples. chlc= Chlorohyll *c*, fuco = Fucoxanthin, neo = Neoxanthin, viola = Violaxanthin, diadino = Diadinoxanthin, diato = Diatoxanthin, lutein = Lutein, zea = Zeaxanthin, chlb = Chlorophyll *b*, chla = Chlorophyll *a*, echin= echinenon, β-caro = β - Carotin, cantha = Canthaxanthin.

	chlc	fuco	neo	viola	diadino	diato	lutein	zeax	chlb	chla	echin	β-caro	cantha
O. stratum	0.000	0.000	0.054	0.075	0.000	0.000	0.157	0.000	0.406	1.000	0.000	0.054	0.000
Bacillariophyceae	0.275	0.708	0.000	0.000	0.238	0.050	0.000	0.000	0.000	1.000	0.000	0.057	0.000
Cyanophyceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.578	0.000	1.000	0.062	0.262	0.026

Table 12. Summary statistics of the *BCE* result. min (minimum), max (maximum), SD (standard deviation)

	O. stratum [µg cm^{-2}]	Bacillariophyceae [µg cm^{-2}]	Cyanophyceae [µg cm^{-2}]	Chl *a* [µg cm^{-2}]	Tufa [mg cm^{-2}]
min	0.000	0.002	0.000	0.004	0.002
max	1.506	4.354	0.351	5.548	116.383
mean	0.099	0.385	0.017	0.504	14.625
SD	0.283	0.711	0.043	0.896	22.540

Table 13. Two-way ANOVA to compare log(x+1) transformed dependent variables (a) chlorophyll and (b) tufa for biofilms of three sites (A, C, D)after 3, 5, 7, 9 and 12 weeks from 30.7. – 22.10. 2008. Post-hoc TukeyHSD showed specific differences between sites, times and their interactions.

ANOVA	a) Chlorophyll					b) Tufa			
variable	df	SS	F	p		SS	F	p	
site	2	0.118	59.339	<0.001	***	8.512	542.289	<0.001	***
time	4	2.369	593.469	<0.001	***	3.961	126.178	<0.001	***
site : time	8	0.421	52.759	<0.001	***	1.215	19.344	<0.001	***
TukeyHSD				p-adj				p-adj	
site									
C-A				<0.001	***			<0.001	***
D-A				<0.001	***			<0.001	***
D-C				<0.001	***			0.093	.
Time									
3w-12w				<0.001	***			<0.001	***
5w-12w				<0.001	***			<0.001	***
7w-12w				<0.001	***			<0.001	***
9w-12w				<0.001	***			0.114	
5w-3w				0.003	**			0.712	
7w-3w				<0.001	***			<0.001	***
9w-3w				<0.001	***			<0.001	***
7w-5w				<0.001	***			<0.001	***
9w-5w				<0.001	***			<0.001	***
9w-7w				<0.001	***			<0.001	***
site : time									
A:3w-A:12w				<0.001	***			0.996	
A:5w-A:12w				<0.001	***			<0.001	***
A:5w-A:3w				0.165				0.014	*
A:7w-A:12w				<0.001	***			<0.001	***
A:7w-A:3w				0.880				0.017	**
A:7w-A:5w				0.995				1.000	
A:9w-A:12w				<0.001	***			0.982	
A:9w-A:3w				<0.001	***			0.369	
A:9w-A:5w				<0.001	***			<0.001	***
A:9w-A:7w				<0.001	***			<0.001	***
C:12w-A:12w				0.267				<0.001	***
C:3w-A:3w				1.000				0.199	
C:3w-C:12w				<0.001	***			<0.001	***
C:5w-A:5w				0.941				<0.001	***
C:5w-C:12w				<0.001	***			<0.001	***
C:5w-C:3w				0.969				0.096	.
C:7w-A:7w				<0.001	***			<0.001	***
C:7w-C:12w				<0.001	***			<0.001	***
C:7w-C:3w				<0.001	***			<0.001	***
C:7w-C:5w				<0.001	***			<0.001	***
C:9w-A:9w				0.004	**			<0.001	***
C:9w-C:12w				<0.001	***			0.570	
C:9w-C:3w				<0.001	***			<0.001	***
C:9w-C:5w				<0.001	***			<0.001	***
C:9w-C:7w				0.001	**			0.001	**
D:12w-A:12w				<0.001	***			<0.001	***
D:12w-C:12w				<0.001	***			1.000	
D:3w-A:3w				1.000				<0.001	***
D:3w-C:3w				1.000				0.090	.
D:3w-D:12w				<0.001	***			<0.001	***
D:5w-A:5w				1.000				<0.001	***
D:5w-C:5w				1.000				0.216	
D:5w-D:12w				<0.001	***			<0.001	***
D:5w-D:3w				0.779				0.229	
D:7w-A:7w				0.699				<0.001	***
D:7w-C:7w				0.003	**			1.000	
D:7w-D:12w				<0.001	***			<0.001	***
D:7w-D:3w				0.017	*			<0.001	***
D:7w-D:5w				0.842				0.534	
D:9w-A:9w				0.155				<0.001	***
D:9w-C:9w				0.993				1.000	
D:9w-D:12w				<0.001	***			0.082	.
D:9w-D:3w				<0.001	***			<0.001	***
D:9w-D:5w				<0.001	***			<0.001	***
D:9w-D:7w				<0.001	***			0.006	**

w = week; Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 14. Two-way ANOVA and post-hoc TukeyHSD test to compare log(x+1) transformed dependent variables (a) *O. stratum*, (b) Bacillariophyceae and (c) Cyanophyceae.(sites A, C, D after 3, 5, 7, 9 and 12 weeks from 30.7. – 22.10. 2008)

ANOVA		*a) Oocardium stratum*				b) Bacillariophycae				c) Cyanopycaea			
variable	df	SS	F	p		SS	F	p		SS	F	p	
site	2	0.117	21.727	<0.001	***	7.001	2.547	0.087	.	1.575E-03	3.072	0.054	.
time	4	0.408	37.899	<0.001	***	17.652	3.211	0.019	*	2.455E-03	2.394	0.060	.
aite:time	8	0.204	9.484	<0.001	***	29.491	2.682	0.014	*	3.259E-03	1.589	0.147	
TukeyHSD				p-adj				p-adj				p-adj	
site													
C-A				<0.001	***			0.099				0.142	
D-A				<0.001	***			0.189				0.917	
D-C				0.289				0.942				0.061	
time													
3w-12w				<0.001	***			0.909				0.080	
5w-12w				<0.001	***			0.939				0.162	
7w-12w				<0.001	***			0.953				0.422	
9w-12w				<0.001	***			0.216				0.951	
5w-3w				1.000				1.000				0.997	
7w-3w				0.406				1.000				0.901	
9w-3w				0.066	.			0.031	*			0.329	
7w-5w				0.430				1.000				0.980	
9w-5w				0.073	.			0.039	*			0.520	
9w-7w				0.882				0.045	*			0.848	
site:time													
A:3w-A:12w				1.000				1.000				1.000	
A:5w-A:12w				1.000				1.000				1.000	
A:5w-A:3w				1.000				1.000				1.000	
A:7w-A:12w				1.000				1.000				1.000	
A:7w-A:3w				1.000				1.000				1.000	
A:7w-A:5w				1.000				1.000				1.000	
A:9w-A:12w				1.000				0.007	**			0.999	
A:9w-A:3w				1.000				0.001	**			0.985	
A:9w-A:5w				1.000				0.002	**			1.000	
A:9w-A:7w				1.000				0.002	**			0.986	
C:12w-A:12w				<0.001	***			1.000				0.063	
C:3w-A:3w				1.000				1.000				1.000	
C:3w-C:12w				<0.001	***			1.000				0.024	*
C:5w-A:5w				1.000				1.000				1.000	
C:5w-C:12w				<0.001	***			1.000				0.033	*
C:5w-C:3w				1.000				1.000				1.000	
C:7w-A:7w				0.263				1.000				1.000	
C:7w-C:12w				<0.001	***			1.000				0.235	
C:7w-C:3w				0.259				1.000				1.000	
C:7w-C:5w				0.262				1.000				1.000	
C:9w-A:9w				0.356				0.003	**			1.000	
C:9w-C:12w				<0.001	***			1.000				0.697	
C:9w-C:3w				0.307				1.000				0.941	
C:9w-C:5w				0.310				1.000				0.966	
C:9w-C:7w				1.000				1.000				1.000	
D:12w-A:12w				<0.001	***			1.000				1.000	
D:12w-C:12w				1.000				1.000				0.071	
D:3w-A:3w				1.000				1.000				1.000	
D:3w-C:3w				1.000				1.000				1.000	
D:3w-D:12w				<0.001	***			1.000				1.000	
D:5w-A:5w				1.000				1.000				1.000	
D:5w-C:5w				1.000				1.000				1.000	
D:5w-D:12w				<0.001	***			1.000				1.000	
D:5w-D:3w				1.000				1.000				1.000	
D:7w-A:7w				1.000				1.000				1.000	
D:7w-C:7w				0.377				1.000				1.000	
D:7w-D:12w				<0.001	***			1.000				1.000	
D:7w-D:3w				1.000				1.000				1.000	
D:7w-D:5w				1.000				1.000				1.000	
D:9w-A:9w				0.903				0.004	**			0.998	
D:9w-C:9w				1.000				1.000				0.985	
D:9w-D:12w				<0.001	***			1.000				1.000	
D:9w-D:3w				0.868				1.000				1.000	
D:9w-D:5w				0.873				1.000				1.000	
D:9w-D:7w				0.943				1.000				1.000	

Table 15. Two-way ANOVA to compare log(x+1) transformed dependent variables (a) chlorophyll, (b) tufa, (c) *O. stratum*, (d) Bacillariophyceae and (e) Cyanophyceae for biofilms of three sites (A, C, D) after 9 weeks from 30.7. – 1.10. 2008 (summer) and from 19.9. – 22.10.2008 (autumn). Post-hoc TukeyHSD showed specific differences between sites, season interactions of them.

ANOVA		a) Chlorophyll				b) Tufa				c) *Oocardium stratum*				d) Bacillariophycae			e) Cyanopycaea		
variable	df	SS	F	p		SS	F	p		SS	F	p		SS	F	p	SS	F	p
site	2	0.383	4.691	0.019	*	4351.700	21.960	<0.001	***	0.101	4.627	0.020	*	3.192E+16	1.667	0.210	0.007	1.763	0.193
season	1	0.242	5.915	0.023	*	7058.400	71.236	<0.001	***	0.066	6.017	0.022	*	1.596E+16	1.667	0.209	2.1E-04	0.112	0.741
site:season	2	0.069	0.846	0.442		2722.200	13.737	<0.001	***	0.069	3.154	0.061	.	3.192E+16	1.667	0.210	0.004	0.949	0.401
TukeyHSD																			
site																			
C-A				0.030	*			<0.001	***			0.050	.			0.273			0.597
D-A				0.043	*			<0.001	***			0.029	*			0.273			0.167
D-C				0.986				0.971				0.966				1.000			0.647
site:season																			
C:s-A:s				0.101				<0.001	***			0.022	*			0.259			1.000
D:s-A:s				0.438				<0.001	***			0.209				0.259			0.915
A:a-A:s				0.374				0.975				1.000				0.259			0.984
D:s-C:s				0.949				0.873				0.873				1.000			0.802
C:a-C:s				0.999				<0.001	***			0.025	*			1.000			0.808
D:a-D:s				0.442				<0.001	***			0.957				1.000			1.000
C:a-A:a				0.875				0.993				1.000				1.000			0.575
D:a-A:a				0.511				0.759				0.650				1.000			0.570
D:a-C:a				0.985				0.968				0.689				1.000			1.000

s = summer, a = autumn; Signif. codes for sign. F ratios: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 16. Two-way ANOVA to compare log(x+1) transformed dependent variables (a) chlorophyll, (b) tufa, and biomass of (c) *O. stratum*, (d) Bacillariophyseae and (e) Cyanophyceae for biofilms of three sites (A, C, D) after 3, 4, 6 and 12 weeks from 1.5. – 24.7. 2009. Post-hoc TukeyHSD (n=5) showed specific differences between sites, times and their interactions.

ANOVA		a) Chlorophyll				b) Tufa				c)*Oocardium stratum*				d) Bacillariophycae				e) Cyanopycaea			
variable	df	SS	F	p		SS	F	p		SS	F	p		SS	F	p		SS	F	p	
site	2	0.031	15.476	<0.001	***	0.744	126.089	<0.001	***	1.517E-04	3.866	0.030	*	0.023	22.937	<0.001	***	2.119E-03	5.736	0.007	**
time	2	0.042	21.132	<0.001	***	2.072	351.040	<0.001	***	6.059E-04	15.438	<0.001	***	0.035	35.069	<0.001	***	1.451E-03	3.928	0.029	*
site : time	4	0.050	12.533	<0.001	***	0.262	22.202	<0.001	***	4.905E-04	6.249	0.001	***	0.036	17.681	<0.001	***	2.021E-03	2.735	0.044	*
TukeyHSD				p-adj				p-adj				p-adj				p-adj				p-adj	
site																					
C-A				<0.001	***			<0.001	***			0.023	*			<0.001	***			0.031	*
D-A				<0.001	***			<0.001	***			0.488				<0.001	***			0.009	**
D-C				0.540				0.581				0.253				0.596				0.874	
time																					
4w-12w				0.003	**			<0.001	***			<0.001	***			0.032	*			0.038	*
6w-12w				0.014	*			<0.001	***			<0.001	***			<0.001	***			0.946	
6w-4w				<0.001	***			<0.001	***			0.878				<0.001	***			0.076	
site:time																					
C:12w-A:12w				1.000				<0.001	***			<0.001	***			1.000				0.998	
D:12w-A:12w				0.995				<0.001	***			0.090	.			0.991				0.824	
A:4w-A:12w				0.385				<0.001	***			1.000				0.566				0.402	
A:6w-A:12w				<0.001	***			0.975				0.995				<0.001	***			0.779	
D:12w-C:12w				0.995				1.000				0.228				1.000				0.996	
C:4w-C:12w				0.369				<0.001	***			<0.001	***			0.871				0.849	
C:6w-C:12w				0.999				<0.001	***			<0.001	***			0.993				0.854	
D:4w-D:12w				0.840				<0.001	***			0.070	.			0.962				0.998	
D:6w-D:12w				0.997				<0.001	***			0.079	.			1.000				0.999	
C:4w-A:4w				1.000				0.004	**			1.000				1.000				1.000	
D:4w-A:4w				1.000				0.007	**			1.000				1.000				1.000	
A:6w-A:4w				<0.001	***			<0.001	***			0.996				<0.001	***			0.011	*
D:4w-C:4w				1.000				1.000				1.000				1.000				1.000	
C:6w-C:4w				0.770				<0.001	***			1.000				0.359				1.000	
D:6w-D:4w				0.998				<0.001	***			1.000				0.980				1.000	
C:6w-A:6w				<0.001	***			<0.001	***			0.996				<0.001	***			0.012	*
D:6w-A:6w				<0.001	***			0.001	**			0.992				<0.001	***			0.011	*
D:6w-C:6w				0.987				0.987				1.000				0.918				1.000	

w = week; Signif. codes for sign. F ratios: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 17. Two-way ANOVA to compare log(x+1) transformed dependent variables (a) chlorophyll, (b) tufa, (c) *O. stratum*, (d) Bacillariophyceae and (e) Cyanophyceae for biofilms of three sites (A, C, D) after 4 weeks in May and July 2009. Post-hoc TukeyHSD (n=5) showed specific differences between sites, months and their interactions.

ANOVA		a) Chlorophyll				b) Tufa				c)*Oocardium stratum*				d) Bacillariophycae				e) Cyanopycaea			
variable	df	SS	F	p		SS	F	p		SS	F	p		SS	F	p		SS	F	p	
site	2	0.044	224.700	<0.001	***	1.31	27.26	<0.001	***	1.18E-05	2.323	0.120		0.034	115.615	<0.001	***	8.41E-04	7.66	0.003	**
month	1	0.010	98.230	<0.001	***	2.799	116.468	<0.001	***	6.76E-05	26.606	<0.001	***	0.005	32.767	<0.001	***	4.17E-04	7.599	0.011	*
site: month	2	0.041	210.020	<0.001	***	0.027	0.566	0.575		2.78E-05	5.458	0.011	*	0.032	109.153	<0.001	***	8.18E-04	7.451	0.003	**
TukeyHSD																					
site																					
C-A				<0.001	***			<0.001	***			0.174				<0.001	***			0.008	**
D-A				<0.001	***			<0.001	***			0.167				<0.001	***			0.005	**
D-C				0.944				0.742				1				0.927				0.982	
Site:month																					
C:m-A:m				1.000				0.004	**			1				1				1	
D:m-A:m				0.987				0.005	**			0.919				0.999				1	
A:j-A:m				<0.001	***			<0.001	***			0.997				<0.001	***			0.001	***
D:m-C:m				0.999				1				0.954				1				1	
C:j-C:m				0.035	*			<0.001	***			0.028	*			0.046	*			1	
D:j-D:m				0.362				<0.001	***			<0.001	***			0.227				1	
C:j-A:j				<0.001	***			0.003	**			0.100				<0.001	***			0.001	***
D:j-A:j				<0.001	***			<0.001	***			0.015	*			<0.001	***			0.001	***
D:j-C:j				0.955				0.849				0.947				0.984				1	

m= May, j = July; Signif. codes for sign. F ratios: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

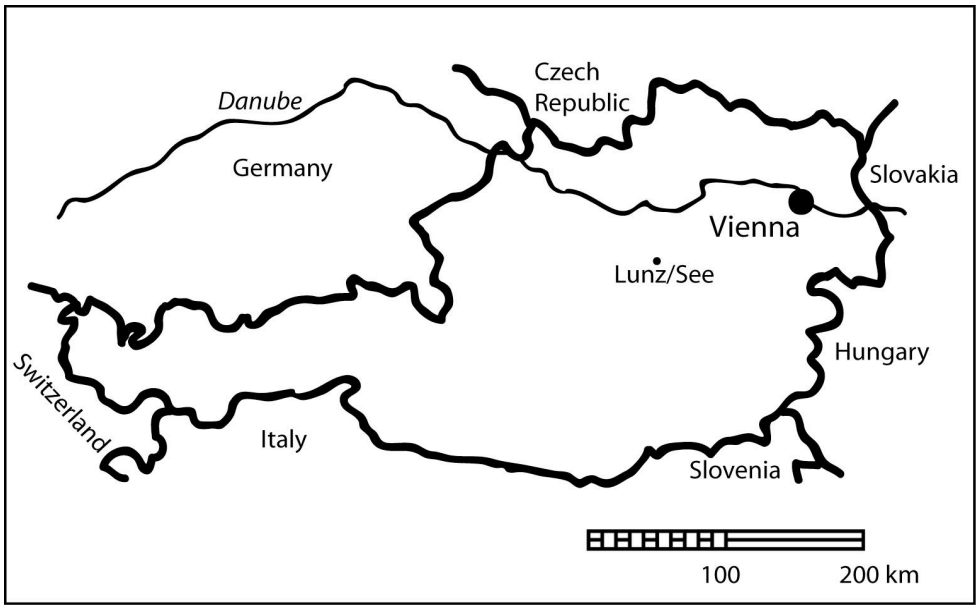

Fig. 12. Location of the sampling site in Austria

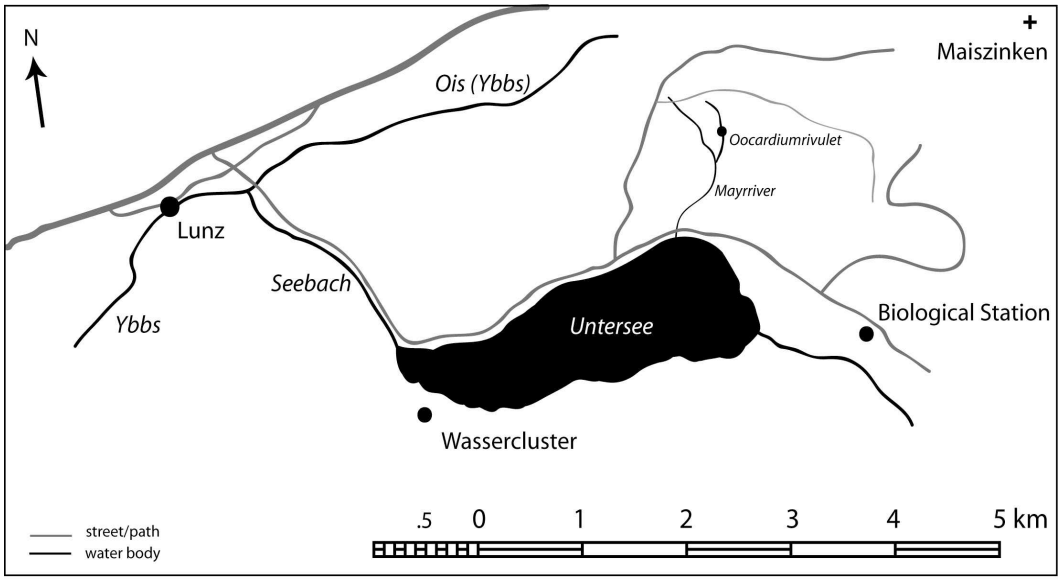

Fig. 13. Location of the sampling site in Lunz/See

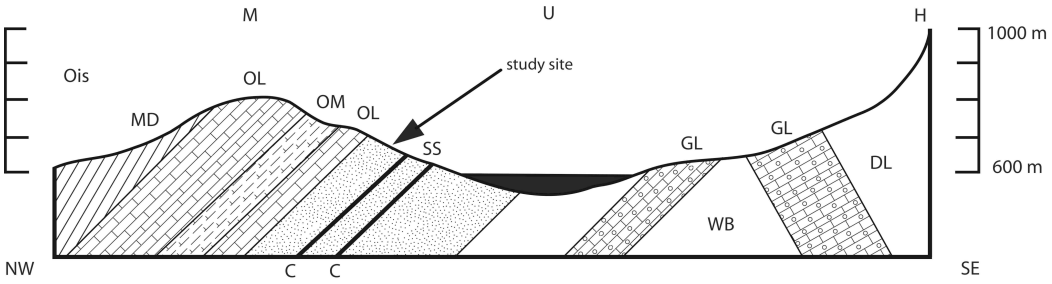

Fig. 14. Geological profile through the lake valley of Lunz from the Maiszinken (M) over the Lunz lake (U) to the Hetzkogel (H), Height to length 1:75. MD (Main dolomite), OL (Opponitzer limestone), OM (Opponiter Mergel), SS (Sandstone and shale, C(Coal), GL (Gutensteiner limestone), WS (Werfener banks), DL (Dachstein limestone)(GÖTZINGER, 1910).

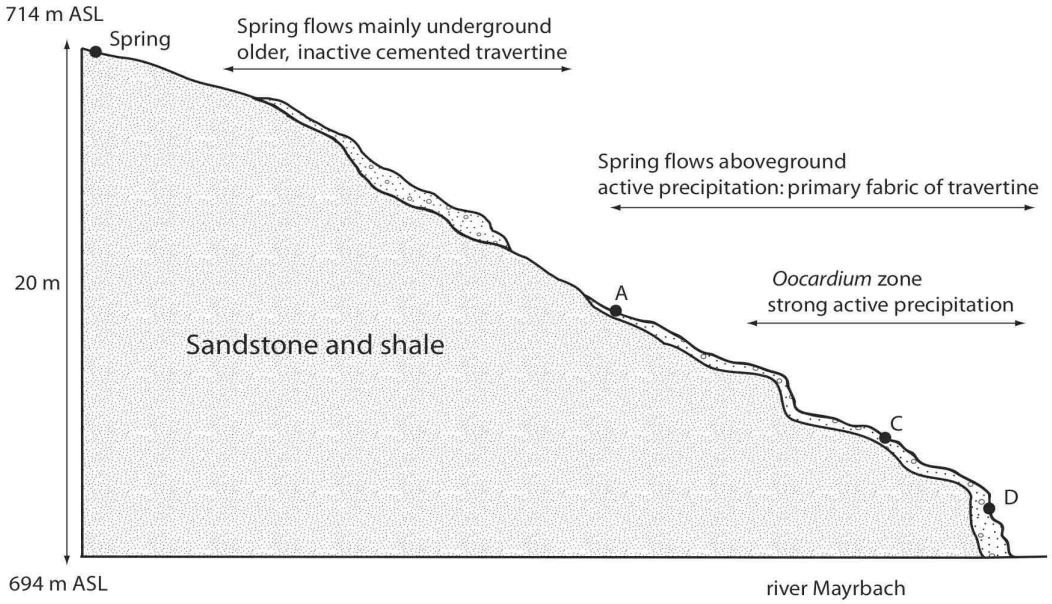

Fig. 15. Altitude profile of the study site with the positions of the sampling sites. The spring of *Oocardium* river in the Mayrgraben is 714 m above sea level. After a few meter aboveground the spring, flows again mostly underground. Inactive and cemented travertine indicates the former spring course. At an altitude of 703 m above see level, the rivulet flows again aboveground and active precipitation starts. 65 m downstream the spring, the *Oocardium* zone starts and ends 84 m downstream, when the rivulet discharges into the mouth of the Mayrbach river.

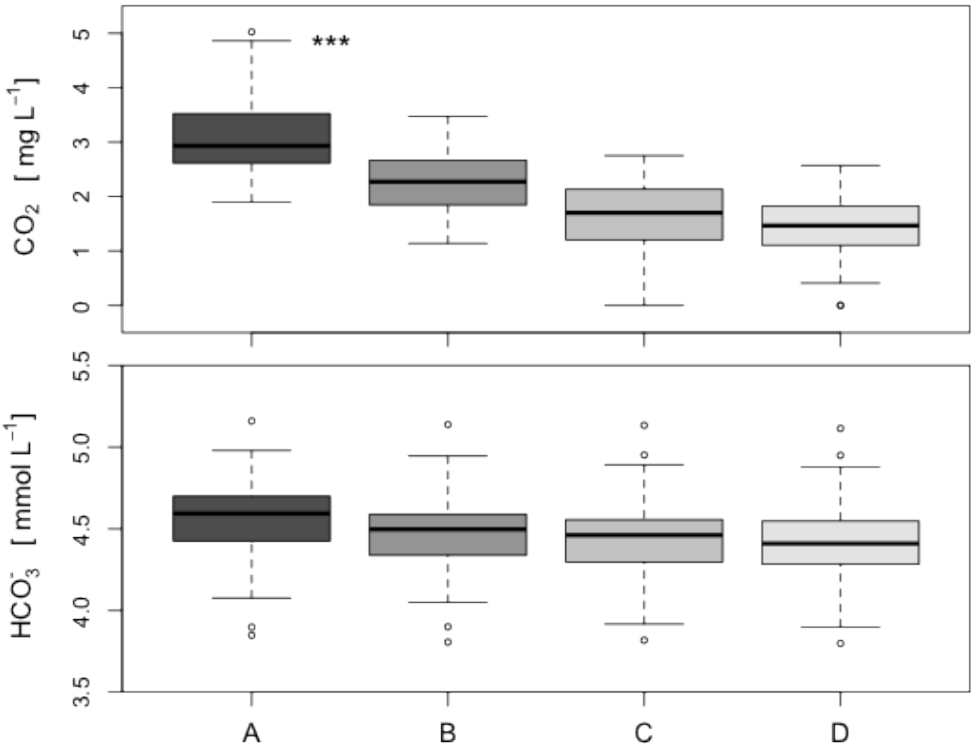

Fig. 16. Free carbon dioxide (free CO_2) and bicarbonate (HCO_3^-) levels for all sites: box-blots with their means (**–**), standard deviations and outliers (°). Highly significant differences (t-test, p-value < 0.001, n=50) between the sites are marked with stars (***).

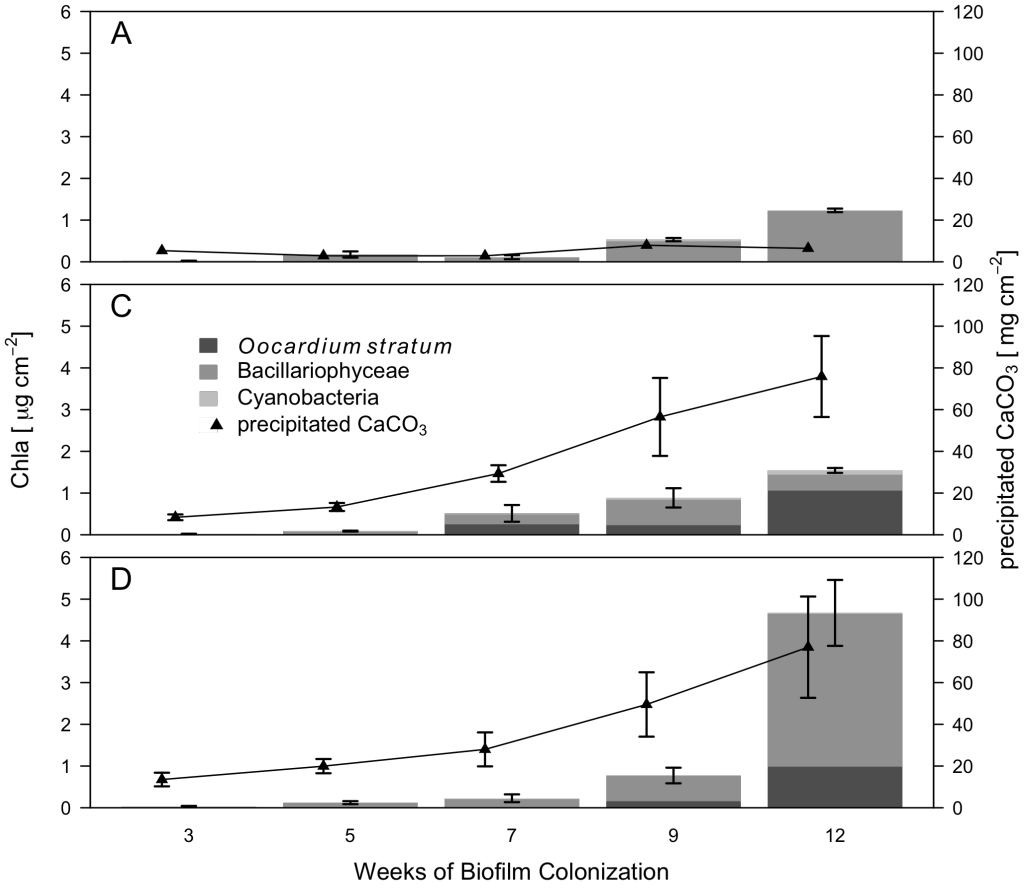

Fig. 17. Means of Chl *a* amount withmeans of related *O.stratum*, Bacillariophyceae and Cyanophyceae biomass in µgcm^{-2} and means of tufa (precipitated $CaCO_3$) amount in mgcm^{-2} as well as appropriated standard deviations of Chl *a* and tufa for sites A, C and D at the continuous growing experiment in 2008.

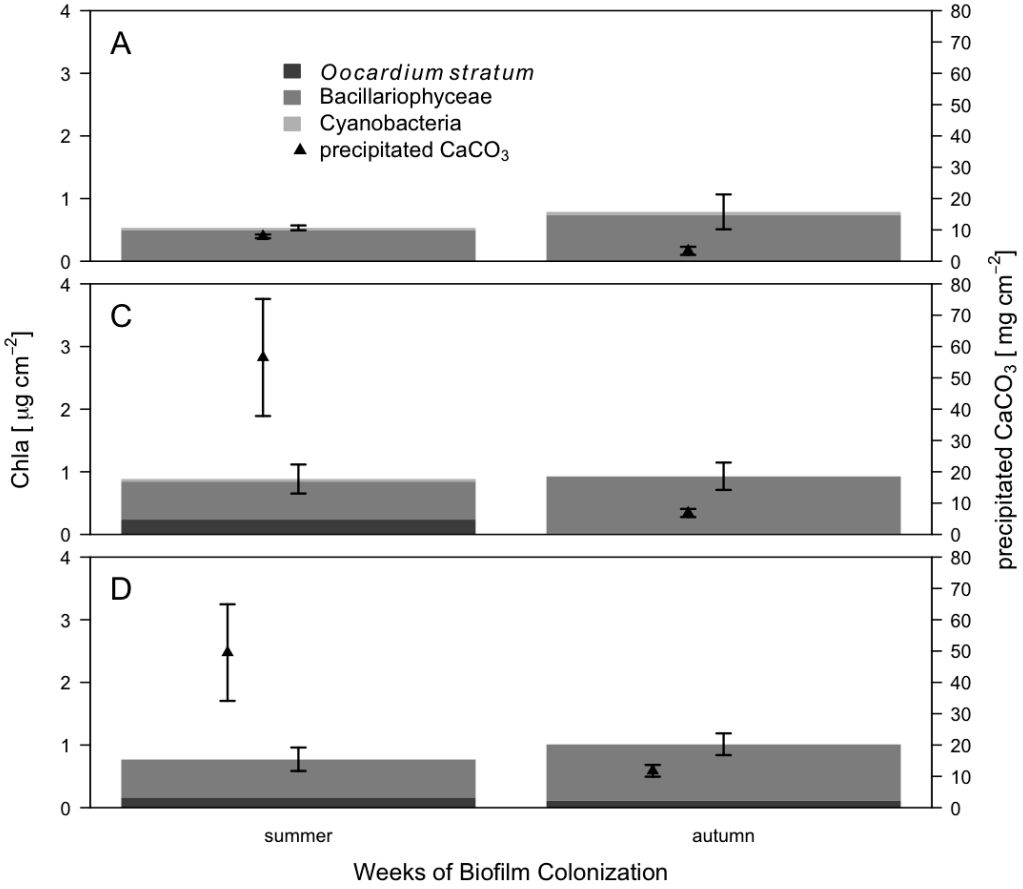

Fig. 18. Means of Chl *a* amount with means of related *O. stratum*, Bacillariophyceae and Cyanophyceae biomass in µgcm^{-2} and means of tufa (precipitated $CaCO_3$) amount in mgcm^{-2} as well as appropriated standard deviations of Chl *a* and tufa for sites A, Cand D for 9 weeks in summer and autumn 2008.

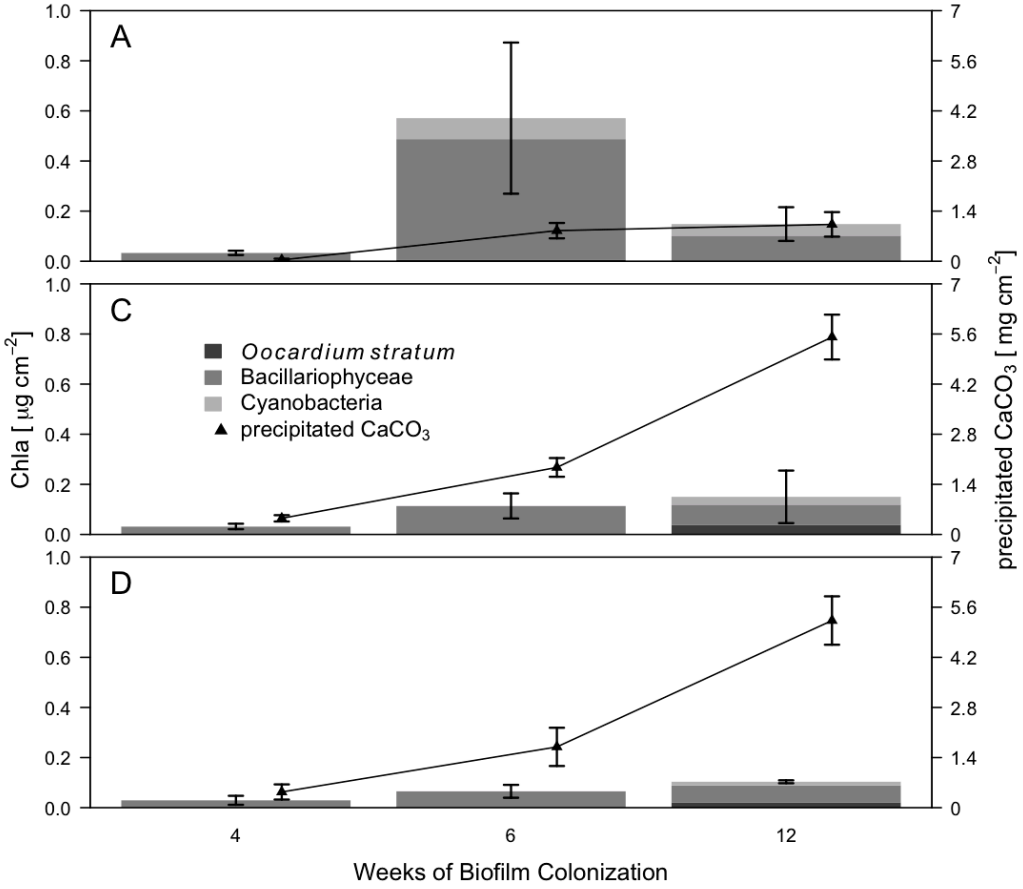

Fig. 19. Means of Chl *a* amount with means of related *O. stratum*, Bacillariophyceae and Cyanophyceae biomass in µgcm^{-2} and means of tufa (precipitated $CaCO_3$) amount in mgcm^{-2} as well as appropriated standard deviations of Chl *a* and tufa for sites A, Cand Dat the continuous growing experiment in 2009.

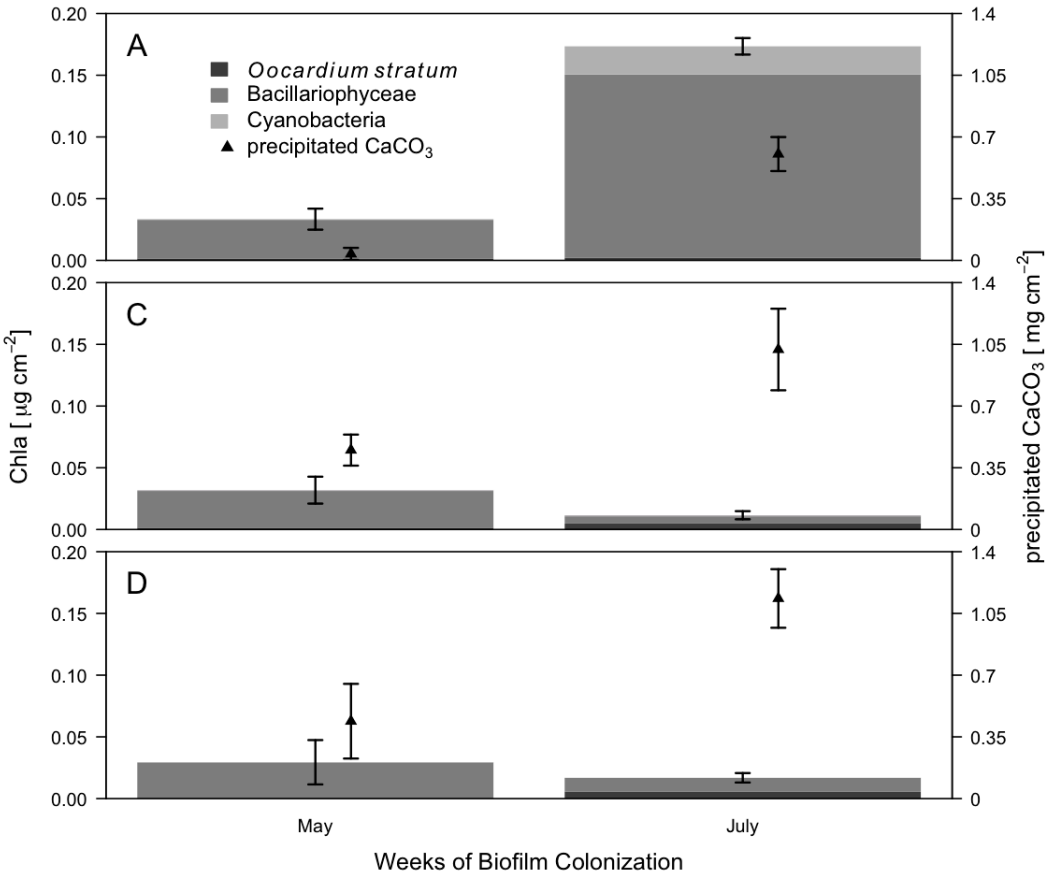

Fig. 20. Means of Chl *a* amount with means of related *O. stratum*, Bacillariophyceae and Cyanophyceae biomass in µgcm^{-2} and means of tufa (precipitated $CaCO_3$) amount in mgcm^{-2} as well as appropriated standard deviations of Chl *a* and tufa for sites A, C and D for 4 weeks in May and July in 2009.

5. Zusammenfassung

Das Vorkommen der seltenen Zieralge *Oocardium stratum,* ist ausschließlich auf aktive Tuffstandorte beschränkt. *Oocardium stratum* bildet Gallertscheiden aus, an denen sich $CaCO_3$ ablagert. Die stecknadelkopfgroßen, hellgrünen Kolonien weisen einen Durchmesser von 0.5 bis 2.0 mm auf. Da die Autökologie dieses Organismus bisher kaum erforscht war, untersuchten wir im ersten Teil unserer Studie die Saisonalität und den Einfluss von Umweltvariablen auf das Vorkommen von *Oocardium stratum*. Zu diesem Zweck beobachteten wir mittels wöchentlicher Mikro- und Makrokartierung die algenbewachsene Tuffoberfläche. Ebenso wurden wöchentlich die Wasserchemie und die Strahlungsbedingungen aufgenommen. Der zweite Teil der Studie beschäftigt sich mit der Sukzession von autotrophen Biofilmen, die *O. stratum* aufweisen, und der damit verbundenen Kalziumkarbonat-Abscheidung. Hierfür installierten wir aufgeraute Objektträger als künstliches Substrat, die zwischen 3 und 12 Wochen an den Versuchsstellen exponiert waren. Der abgekratzte Biofilm wurde anschließend auf den gesamten Chlorophyll-*a* Gehalt, den $CaCO_3$-Anteil und mittels HPLC auf die Pigmentverteilung hin untersucht. Die Studie wurde an einem Quellbach in Lunz/See (Niederösterreich) über 17 Monate hinweg durchgeführt. Umweltvariablen wurden wöchentlich und im Winter 14-tägig gemessen. Biofilm und Kalziumcarbonat wurden ebenfalls 14-tägig besammelt.

Im Zuge der Autökologiestudie wurde *O. stratum* das ganze Jahr hindurch an der Tuffquelle gefunden. Das Vorkommen der Kolonien erreichte ein Maximum im Hochsommer während der Monate Juli und August. Wiederholte Makrokartierungen mit einer Oberfläche von 750 cm^2 an drei Spots in der Travertinquelle, zeigten ein Maximum von *O. stratum* überzogenem Tuff im August mit einer Bedeckung von 31%. Zwei kleinere Maxima zeigten sich im Frühsommer und Spätherbst mit 10% *O. stratum* - Bedeckung. Dicke Matten von Diatomeen, dominiert von *Cymbella minuta*, zeigten sich im Frühling, Herbst und Winter mit mehr als 74% Tuffbedeckung an den untersuchten Spots. Eine Mikrokartierung mit einer Oberfläche von 25.5 mm^2 wurde ebenfalls an einer Stelle der Tuffquelle durchgeführt und zeigte die gleichen Ergebnisse. Die Redundanzanalyse zeigte Wassertemperatur und Bikarbonatgehalt als treibende Hauptfaktoren an, die das Vorkommen und das Wachstum von *O. stratum* kontrollieren. Optimale Wachstumsbedingungen wurden bei einer Alkalinität von 4.6-4.7 mmol L^{-1} und einer Wassertemperatur von 13 °C beobachtet. Die Parameter Belichtung, Nitrat und Kohlendioxid zeigten einen negativen Zusammenhang zur *O. stratum* Biomasse, hingegen einen positiven zum Diatomeen Vorkommen. Andere Umweltvariablen wie Ionengehalt

oder gelöster reaktiver Phosphor hatte keinen signifikanten Einfluss auf das Vorkommen von *O. stratum*.

In Quellnähe war die Niederschlagsrate von $CaCO_3$ signifikant niedriger als an weiter quellabwärts gelegenen Untersuchungsstellen. Der Grund hierfür liegt am deutlich höheren freien CO_2-Gehalt und dem niedrigeren pH- Wert in Quellnähe. Die $CaCO_3$-Niederschlagsrate war im Sommer am höchsten. Die Menge an $CaCO_3$ in Quellnähe war in beiden Untersuchungsjahren annähernd konstant und zeigte keine Zuwächse im zweiwöchigen Messabstand. Die quellabwärts gelegenen Untersuchungsstellen zeigten allerdings eine eindeutige positive zweiwöchig gemessene Niederschlagsrate, die von 5 - 27.5 mg cm^{-2} reichte. Die Chlorophyll-*a* Rate lag an allen Untersuchungsstellen zwischen 0.07 - 3.9 µg cm^{-2}. Die Objektträger wurden zuerst von dicken Diatomeen-Matten besiedelt, dominiert von *Cymbella minuta*. Ein geringer Anteil an kokkenähnlichen Cyanobacterien konnte mikoskopisch und mittels HPLC nachgewiesen werden. Erst nachdem sich eine raue Kalkschicht niedergeschlagen hatte, war auch *O. stratum* auf den Objektträgern nachzuweisen. Nach etwa 6-7 Wochen waren die *O. stratum*-Kolonien auch makroskopisch sichtbar.

Die Verteilung der Taxa gemittelt über alle Untersuchungsstellen und Aufnahmen zeigte, dass 77% der Algenbiomasse von Diatomeen stammen, 20% konnten *O. stratum* zugeordnet werden und 3% der Algenbiomassen waren Cyanobacterien. Diatomeen wiesen einen negativen Zusammenhang zur $CaCO_3$-Niederschlagsrate auf, wohingegen Cyanobacterien einen geringfügig positiven Zusammenhang zeigten und *O. stratum* eine stark positive Übereinstimmung mit dem $CaCO_3$-Niederschlag anzeigte. Unsere Resultate weisen auf eine Induzierung des Kalziumkarbonatniederschlages durch *O. stratum* hin; eine aktive, photosynthese-getriebene Kalkabscheidung durch *O. stratum* ist aber auszuschließen. Biomasse von *O. stratum* und gefälltes Kalziumkarbonat reagieren auf den chemischen, quellabwärtsgerichteten Gradienten und auf saisonale Veränderungen parallel. Dadurch ist es schwer zu unterscheiden, ob *O. stratum* die Fällung von $CaCO_3$ erhöht oder ob beide, die *O. stratum* Biomasse und der $CaCO_3$-Niederschlag, hauptsächlich durch den quellabwärts gerichteten chemischen Gradienten und durch saisonale Veränderungen kontrolliert werden.

6. Summary

The rare calcifying desmid *Oocardium stratum* occurs exclusively on active travertine habitats; the macroscopic colonies form hemispherical structures with a diameter of 0.5 to 2.0 mm. Because autecology of this organism is still poorly understood, the first part of our

study focused on its seasonal development and related various environmental factors to the occurrence and biomass of *Oocardium*. The second central theme of the study was the succession of photoautotrophic biofilms with a special focus on *Oocardium stratum*. For this we installed frosted glass slides as artificial substrata, which were exposed between 3 to 12 weeks. The study was conducted in a rivulet in Lunz/See (Lower Austria) for 17 months. Environmental variables were on a weekly (growing season) to monthly (winter season) basis; biofilm and precipitated tufa were sampled biweekly.

The Autecology study resulted that *Oocardium* colonies were found throughout the whole year with maximum abundances during the mid summer months July and August. Repeated macromapping of three travertine spots with a size of 750 cm^2 each showed a maximum *Oocardium* cover of 31 % in August. Two smaller maxima occurred in early summer and late autumn with about 10 % cover. Diatom mats, dominated by *Cymbella minuta,* occurred in spring, autumn and winter with more than 74 % of cover observed on macromapping spots. Micromapping was done at a single area covering 25.5 mm^2 and revealed the same results. Redundancy analysis revealed water temperature and bicarbonate content as the main structuring factors, which control occurrence and growth of *Oocardium*. Optimal growth conditions appeared at an alkalinity of 4.6-4.7 mmol L^{-1} and water temperatures of 13 °C. Site openness, nitrate and carbon dioxide availability were inversely related to *Oocardium* biomass, but more connected to diatoms occurrence. Other environmental factors like total ions content or soluble reactive phosphorus had no significant influence on *Oocardium stratum* occurrence. The seasonal succession of *Oocardium* and diatom mats in limestone precipitating springs causes a typical sequence pattern of travertine layers, which can be recognised already with blank eyes. We found a significantly lower precipitation rate near the spring because of higher free CO_2 amounts and higher pH levels than downstream. Precipitation was also higher in summer than in colder seasons. The biweekly precipitation of calcium carbonate near spring barely showed a positive rate. The amount of precipitated $CaCO_3$ near spring was in both years nearly constant without a clear accession. However further downstream situated spots showed a clear positive biweekly precipitation rate, which ranged from 5 mg cm^{-2} up to 27.5 mg cm^{-2}. The biweekly Chlorophyll *a* rate ranged at all spots from 0.07 µg cm^{-2} up to 3.9 µg cm^{-2}. The colonization of the glass slides started with the growth of diatoms and after the successfully development of a constant rough travertine layer, *Oocardium* colonies followed. The *O. stratum* colonies were after 6-7 weeks macroscopically visible and its biomass was higher in summer than in autumn and spring. The diatom mats on frosted glass slides were also dominated by *Cymbella minuta*; only smaller amounts of coccoid Cyanobacteria could be detected. The distribution of taxa for the mean of all sites and

dates showed that 77 % of the algae biomass were diatoms, 20 % could be related to *O. stratum* and 3% comprised Cyanobacteria. Diatoms were negatively related to precipitation, whereas Cyanobacteria were slightly positively and *O. stratum* biomass was strongly coinciding with carbonate precipitation. From our results, we assume an induction of carbonate precipitation by *O. stratum*, but no active deposition due to photosynthesis. *O. stratum* biomass and the amount of precipitation showed parallel reactions to downstream gradients and to the seasonal changes. We therefore could not distinguish, if *O. stratum* increases the precipitation or if both, precipitation and *O. stratum* are mainly controlled by downstream chemical gradients and seasonal changes.

7. Curriculum Vitae

Personal data

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Date of birth: 22nd of May 1985

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Education

September 1991 until June 1995:	Primary School Gaming
Sepember 1995 until June 1999:	Secondary school Gaming
September 1999 until June 2003:	Grammar school Scheibbs (with emphasis on art)
June 2003	Matura (final exam)
September 2003 until September 2004:	Biotechnology FH Campus Vienna
Since October 2004	Studies of Biology at the University of Vienna
March 2006:	diploma examination (first part); Studies of Ecology, focus on Limnology
Since March 2008	work in my diploma thesis; Advisor: Dr. Michael Schagerl („Autecology of *Oocardium stratum* and $CaCO_3$ precipitation of autotrophic biofilms in travertine rivulets“)
January 2010 - July 2010	Erasmus exchange term: NTNU Trondheim, main foci on Ecotoxicology and Statistics with R.

Languages:

German (first language), English (Business fluent), Norwegian (Basic knowledge)

Computer literacy:

Excellent knowledge of MS-Office (Word, Excel, PowerPoint), ArcGis, Geomedia, SPSS, Sigma Plot, Stata and **R**, Chemtax (R BCE Package und Excel-Macro), EZ-Chrom (HPLC)

Work experience:

1999 – 2003:	Biological Station Lunz/See NÖ; Respectively 3-4 weeks summer internship, mainly fieldwork.
2003-2008:	Ski- and Snowboard instructor in several Skiing schools in Lower Austria winter internship
2004:	Baxter Bioscience Vienna, summer internship, Laboratory.
2006:	eb&p Umweltbüro Klagenfurt, summer internship, Fieldwork and ArcGIS.
May-August 2007:	Niederösterreichische Umweltanalytik (NUA), minor employment status, mainly Fieldwork.
Jan. – Dec. 2009:	Umweltbüro Blattfisch, minor employment, mainly Identification of Macrozoobenthos and Fieldwork (electro fishing).
2009:	Wasserkluster Lunz, contract for services for Identification of Freshwater Algae.
Since November 2010:	Scientific collaborator at the Medicine University of Innsbruck, Department of Medical Statistics, Informatics and Health Economy (MSIG); foci on R-Programming and Statistics.

Analytical Methods:

High performance Liquid Chromatography (HPLC)

DAPI-staining, Epifluorescencmicroscopie

Titrations

Measurements of Fluorescent with Pulse Amplitude Modulation (PAM)

Additional Skills:

2002-2009 7 years active member of the Austrian Mountain Rescue Service

PADI Open Water Diving Licence

Snowboardinstructor

Übungsleiter Sportklettern (Climbing Instructor of the ÖAV, Austrian Alpine Club)

Personal Interests:

Climbing, Skiing, literature, “cold habitats”, polar- and glacier-research.