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Allgemeine Einleitung

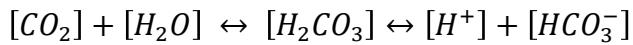
Quellen und Quellbäche

Quellen und Quellbäche stehen am Anfang eines jeden Oberflächengewässers und werden als grundwasserabhängige Ökosysteme bezeichnet (Kløve *et al.*, 2011). Sie interagieren durch geomorphologische, hydrologische und biologische Prozesse mit dem Umland. Quellbäche sind meistens sehr klein und werden daher oft in Karten vernachlässigt. Trotzdem haben sie einen großen Einfluss auf flussabwärts gelegene Ökosysteme. Sie verbinden terrestrische Habitate mit stehenden und fließenden Gewässern, weshalb Quellen und Quellbäche als Ökotone (=Saumbiotope) bezeichnet werden (Barquín & Scarsbrook, 2008; Kløve *et al.*, 2011). Quellbäche bieten verschiedene Ökosystemdienstleistungen; neben sauberem Trinkwasser gewähren sie Erholungsmöglichkeiten und durch ihre ökologische Autonomie weisen sie eine hohe Biodiversität auf (Kløve *et al.*, 2011). Die in den Quellbächen lebenden Organismen haben keinen Einfluss auf das sie umgebende Wasser, die Veränderungen durch Photosynthese und Respiration werden sofort flussabwärts transportiert und durch neues Wasser ersetzt. Die Hydrologie und Chemie von Quellbächen ist sehr konstant und bietet immer die gleichen Bedingungen, unabhängig von der Jahreszeit (Odum, 1961).

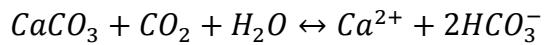
Travertin

Travertin entsteht, wenn Grundwasser an die Oberfläche tritt und dadurch starke Änderungen der chemischen Gleichgewichte verursacht werden. Gelöstes Kalziumhydrogenkarbonat fällt durch die Diffusion von Kohlendioxid (CO_2) in die Atmosphäre als Kalziumkarbonat (=Kalk) aus. Im deutschen Sprachraum verwendet man auch das Wort Tuff für diese spezielle Gesteinsformation. Pentecost (1993) und Pentecost & Viles (1994) verwenden den Begriff Travertin für sämtliche Kalkablagerungen in Quellen, wobei sie zwischen meteogenen und thermogenen Travertin unterscheiden. Meteogener Travertin bezeichnet alle Ablagerungen, bei denen das im Grundwasser

gelöste CO₂ aus der Boden- und Wurzelatmung stammt und ursprünglich aus der Atmosphäre fixiert wurde. Diese Travertine sind weit verbreitet und werden von Algen, Makrophyten, Evertebraten und Bakterien besiedelt. Thermogener Travertin beinhaltet als CO₂-Ressource die Hydrolyse und Oxidation von reduziertem Kohlenstoff. Diese Quellen haben meistens eine sehr hohe Wassertemperatur und beinhalten keine Makrophyten oder Evertebraten (Ford & Pedley, 1996). In Österreich ist nur meteogener Travertin bekannt. Im Grundwasser überwiegen respiratorische Prozesse, weshalb das Grundwasser gegenüber der Atmosphäre stark mit CO₂ angereichert ist. CO₂ verbindet sich mit Wasser zur Kohlensäure und dissoziiert in Abhängigkeit zum pH zu Hydrogenkarbonat und/oder Karbonat.



Fließt das Grundwasser durch Kalkgestein, kann die Kohlensäure Kalk lösen und bildet das im Wasser sehr gut lösliche Kalziumhydrogenkarbonat, wobei ein Teil der Kohlensäure als Gleichgewichtskohlensäure erhalten bleibt.



Kommt das Wasser an die Oberfläche, beinhaltet es viel mehr CO₂ als die Atmosphäre. Durch Unterschiede im CO₂-Partialdruck und der Temperatur beginnt die Gleichgewichtskohlensäure aus dem Wasser auszugasen und Kalziumhydrogenkarbonat fällt in weiterer Folge als Kalk (=Travertin, Tuff) aus (Stumm, 1995).

Turbulenzen und die aktive Entfernung des CO₂ durch Photosynthese begünstigen diesen Prozess. Von einem anfänglich physikalischen Prozess verlagert sich die Kalkfällung flussabwärts zu einem biogenen Prozess (Merz & Zankle, 1991). Durch die Kalkfällung können sich Kaskaden, Dämme, Wälle und Terrassen bilden z.B. „Plitvicer Seen“ (Ford & Pedley, 1996).

Algen

Unter Algen versteht man einen Sammelbegriff von oxygene Photosynthese betreibenden Organismen, welche eine sehr einfache Struktur aufweisen und daher von den Gefäßpflanzen abgegrenzt werden. Unter diesem Begriff werden sowohl Prokaryoten (Cyanobakterien) als auch Eukaryoten zusammengefasst. Algen bilden einen essentiellen Bestandteil des Nahrungsnetzes und leisten zirka 45% des Sauerstoffeintrages in die Atmosphäre (Graham & Wilcox, 2000).

Im Laufe der Erdgeschichte entwickelten sich vor 3,6 Milliarden Jahren die ersten Organismen, welche im Rahmen der oxygenen Photosynthese O₂ bildeten. Diese Cyanobakterien waren es, die die ehemals O₂-freie Atmosphäre mit O₂ versorgten und so die Grundlage für höheres Leben auf der Erde schufen (Catling & Claire, 2005; Raven & Giordano, 2014). Algen tragen einen wichtigen Teil zur Kohlenstofffixierung auf der Erde bei. Zwischen 36,5 und 50,2 Gigatonnen Kohlenstoff pro Jahr werden alleine nur im Ozean fixiert, welches fast die Hälfte des globalen Kohlenstoffes ist (Longhurst *et al.*, 1995; Antoine *et al.*, 1996; Field *et al.*, 1998).

Neben der Kohlenstofffixierung und der Sauerstoffproduktion haben Algen noch weitere wichtige Eigenschaften, die sich der Mensch zunutze machen kann. Zum Beispiel werden Algen gezielt in der Abwasserreinigung eingesetzt, um Nährstoffe aus dem Wasser zu entfernen (Hoffmann, 1998). Erhöhte Nährstoffkonzentrationen (Eutrophierung) können in Gewässern zu Problemen führen. Es kommt zu einer Massenentwicklung von Algen, was einerseits zu einer massiven Sauerstoffzerrung beim Abbau dieser Biomasse führt und andererseits können einige Cyanobakterien Toxine produzieren, welche eine Gefahr für Mensch und Tier darstellen (Dokulil & Teubner, 2000; Falconer & Humpage, 2005).

Eine weitere Anwendung von Algen ist die Produktion von Biotreibstoffen. Algen können sehr einfach kultiviert werden; sie benötigen geringere Ressourcen und stehen mit Feldfrüchten wie Raps oder Sojakulturen nicht in Flächenkonkurrenz (Chisti, 2007; Mata

et al., 2010). Algen werden auch als Nahrungsergänzungsmittel verwendet, da sie hohe Protein-, Vitamin- und Spurenelementgehalte aufweisen. Weiteres werden Karotinoide und Phycobiliproteine aus Algen gewonnen, welche als Lebensmittelfarbe in der Kosmetikindustrie und in pharmazeutischen Produkten verwendet werden. Zusätzlich werden aus Algen Omega-3 Fettsäuren gewonnen, welche essentiell für die Gesundheit des Menschen sind (Milledge, 2011).

Algen sind so anpassungsfähig, dass sie auch außerhalb von aquatischen Lebensräumen vorkommen. Sie besiedeln auch diverse Extrembiotope wie trockene, heiße oder kalte Standorte. Sie können in Höhlen, auf und in Steinen, auf Gletschern, oder auch auf Tieren und Pflanzen leben (Hoffmann, 1989; Graham & Wilcox, 2000).

Algengemeinschaften in Quellbächen

Quellbäche sind extreme Standorte und haben eine sehr hohe Artenvielfalt im Vergleich zu anderen aquatischen Ökosystemen. Diese Bäche bieten eine große Anzahl an verschiedenen Mikrohabitaten zur Besiedelung und weisen ein großes Nahrungsangebot auf (Stanford *et al.*, 1994). Sie beherbergen Grund- und Oberflächengewässerarten, terrestrische Arten und Feuchtbiotoporganismen. Kløve *et al.* (2011) weisen darauf hin, dass die Arten abhängig vom Grundwasser sind und das Puffervermögen dieser Habitate bezüglich Trockenheit und extremen Temperaturen zu ihrem Vorteil nutzen. Cantonati *et al.* (2001) ermittelten, dass Kieselalgen und Cyanobakterien die dominierenden Algengruppen sind. Weiteres stellte er fest, dass der pH, die Leitfähigkeit, der anorganische Stickstoff, die Korngröße des Substrates und die Beschattung die wichtigsten Parameter sind, welche die Algengemeinschaft formen. Kieselalgen, Cyanobakterien, heterotrophe Bakterien und mikroskopische Grünalgen wie die Zieralge *Oocardium stratum* Nägeli bilden Biofilme, um der Kalkfällung zu entgehen (Cantonati *et al.*, 2006; Golubić *et al.*, 2008).

Oocardium stratum

Oocardium stratum ist eine seltene Zieralge, welche sehr gut an die Kalkfällung angepasst ist und ausschließlich in Travertinbächen vorkommt. In Österreich sind bislang lediglich 4 Standorte mit ihrem Vorkommen bekannt. Hansgirg (1905) beschrieb *Oocardium* aus dem Piestingtal (Niederösterreich), jedoch kommt diese Alge dort nicht mehr vor. Brehm & Ruttner (1926) entdeckten *Oocardium* im Lunz Gebiet (Niederösterreich). Der Standort existiert heute nicht mehr (Wiesendrainagierung), das Taxon wurde aber in den frühen 2000er in der Nähe der ehemaligen Fundstelle wiederentdeckt (Schagerl & Pröschold, 2007). Die anderen drei Standorte sind Lingenau (Vorarlberg), das Tuffbachl nahe dem Alpenzoo Innsbruck und die Hochtalalm (beide in Tirol) (Sanders & Rott, 2009; Rott *et al.*, 2010; Rott *et al.*, 2012). *Oocardium* wurde das erste Mal von Nägeli (1849) beschrieben. Es kommt an Standorten überall auf der Welt vor und es werden laufend neue Standorte entdeckt. *Oocardium* tritt niemals in stillen Gewässern auf, sondern immer in schnell fließenden, kalkreichen Quellbächen: das Vorkommen ist auf einen bestimmten Abstand von der Quelle beschränkt. Es kann jedes Substrat besiedeln und bildet Kalkröhren. In diesen ist es mit einer Gallerte verankert. Durch physiologische Aktivität werden die Röhren verlängert (Wallner, 1933; 1934).

In Europa sind Travertinbäche der einzige Quelltyp (7220, Kalktuffquellen, Cratoneurion), welcher in der Fauna-Flora-Habitat-Richtlinie (EU HD, 1992) geschützt ist, während andere Quelltypen und kleine Bäche (< 10 km² Einzugsgebiet) in der Wasserrahmenrichtlinie (EU WFD, 2000) vernachlässigt werden. Für das Vorkommen von *Oocardium stratum* und der Algengemeinschaft ist es wichtig, diese einzigartigen Habitate zu untersuchen und zu schützen.

Hypothesen

In der vorliegenden Arbeit wurden Algengemeinschaften von meteogenen Travertinbächen auf Unterschiede hin untersucht. Sind eventuell auftretende Differenzen auf

unterschiedliche Umweltbedingungen zurückzuführen? Ein weiterer Schwerpunkt der Arbeit stellte das Vorkommen von *Oocardium stratum* dar. Welche Umweltfaktoren sind ausschlaggebend für das Vorkommen dieser Art? *O. stratum* ist von freiem CO₂ abhängig und hat vermutlich auch Vorteile wegen der besonderen Wuchsform in Travertinabschnitten. Es wurde weiters angenommen, dass die Algengemeinschaft auf die ständige Kalkfällung spezialisiert ist. Die Organismen müssen Überlebensstrategien entwickelt haben, um der Kalkausfällung entgegenzuwirken und nicht von Travertin überdeckt zu werden. Wir erwarteten vergleichbare Algengemeinschaften in den Travertinbächen; aufgrund von abiotischen Umweltfaktoren werden kalzifizierende, rheophile Arten selektiert.

Die vorliegende Studie trägt dazu bei, Schlüsselvariablen zu identifizieren, welche die Gemeinschaften formen und das Vorkommen von *O. stratum* ermöglichen. Es wurden dazu 14 Travertinbäche in Österreich auf ihre abiotischen Faktoren und ihre Algengemeinschaft hin untersucht. Mit Hilfe von multivariater Statistik wurden Umweltfaktoren mit der Artenzusammensetzung kombiniert, um bedeutende Parameter für die Algengemeinschaft zu entdecken.

Literaturverzeichnis

- Antoine D., André J.-M., & Morel A. 1996. Oceanic primary production: 2. Estimation at global scale from satellite (Coastal Zone Color Scanner) chlorophyll. *Global Biogeochemical Cycles*: 10, 57 - 69.
- Barquín J. & Scarsbrook M. 2008. Management and conservation of coldwater springs. *Aquatic Conservation: Marine and Freshwater Ecosystems*: 18, 580–591.
- Brehm V. & Ruttner F. 1926. Die Biocönosen der Lunzer Gewässer. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*: 16, 281-391.
- Cantonati M., Gerecke R., & Bertuzzi E. 2006. Springs of the Alps – Sensitive ecosystems to environmental change: From biodiversity assessments to long-term studies. *The International Journal of Aquatic Sciences*: 562, 59-96.
- Catling D. C. & Claire M. W. 2005. How Earth's atmosphere evolved to an oxic state: A status report. *Earth and Planetary Science Letters*: 237, 1-20.
- Chisti Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*: 25, 294-306.
- Dokulil M. & Teubner K. 2000. Cyanobacterial dominance in lakes. *Hydrobiologia*: 438, 1-12.
- EU HD. 1992. Council Directive 92/ 43/ EEC of 21 May 1992 on the conservation of natural habitat and of wild fauna and flora. *Official Journal of the European Communities*: L 206, 7-50.
- EU WFD. 2000. Directive 2000/ 60/ EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*: L 327, 1-73.
- Falconer I. & Humpage A. 2005. Health Risk Assessment of Cyanobacterial (Blue-green Algal) Toxins in Drinking Water. *International Journal of Environmental Research and Public Health*: 2, 43-50.
- Field C. B., Behrenfeld M. J., Randerson J. T., & Falkowski P. 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science*: 281, 237-240.
- Ford T. D. & Pedley H. M. 1996. A review of tufa and travertine deposits of the world. *Earth Science Reviews*: 41, 117-175.
- Golubić S., Violante C., Plenković-Moraj A., & Grgasović T. 2008. Travertines and calcareous tufa deposits: an insight into diagenesis. *Geologija Croatica*: 61, 363-378.
- Graham L. E. & Wilcox L. W. 2000. Algae. Prentice Hall, Upper Saddle River, NJ, 640 pp.
- Hansgirg A. 1905. Grundzüge der Algenflora von Niederösterreich. *Beihefte zum Botanischen Centralblatt*: XVIII, 417-522.

- Hoffmann J. P. 1998. Wastewater treatment with suspended and nonsuspended algae. *Journal of Phycology*: 34, 757-763.
- Hoffmann L. 1989. Algae of Terrestrial Habitats. *Botanical Review*: 55, 77-105.
- Kløve B., Ala-aho P., Bertrand G., Boukalova Z., Ertürk A., Goldscheider N., Ilmonen J., Karakaya N., Kupfersberger H., Kværner J., Lundberg A., Mileusnić M., Moszczynska A., Muotka T., Preda E., Rossi P., Siergieiev D., Šimek J., Wachniew P., Angheluta V., & Widerlund A. 2011. Groundwater dependent ecosystems. Part I: Hydroecological status and trends. *Environmental Science and Policy*: 14, 770-781.
- Longhurst A., Sathyendranath S., Platt T., & Caverhill C. 1995. An estimate of global primary production in the ocean from satellite radiometer data. *Journal of Plankton Research*: 17, 1245-1271.
- Mata T. M., Martins A. A., & Caetano N. S. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*: 14, 217-232.
- Merz M. & Zankle H. 1991. The influence of the sheath on carbonate precipitation by cyanobacteria. *Bollettino della Società Paleontologica Italiana*: 1, 325-331.
- Milledge J. J. 2011. Commercial application of microalgae other than as biofuels: A brief review. *Reviews in Environmental Science and Biotechnology*: 10, 31-41.
- Nägeli C. 1849. Gattungen einzelliger Algen, physiologisch und systematisch bearbeitet. *Neue Denkschriften der Allgemeinen Schweizerischen Gesellschaft für die Gesammten Naturwissenschaften*: 10, 1-139.
- Odum E. P. 1961. Fundamentals of ecology. Saunders, Philadelphia, 546 pp.
- Pentecost A. 1993. British travertines: a review. *Proceedings of the Geologists' Association*: 104, 23-39.
- Pentecost A. & Viles H. 1994. A Review and Reassessment of Travertine Classification. *Géographie Physique et Quaternaire*: 48, 305-314.
- Raven J. A. & Giordano M. 2014. Algae. *Current Biology*: 24, 590-595.
- Rott E., Holzinger A., Gesierich D., Kofler W., & Sanders D. 2010. Cell morphology, ultrastructure, and calcification pattern of *Oocardium stratum*, a peculiar lotic desmid. *Protoplasma*: 243, 39-50.
- Rott E., Hotzy R., Cantonati M., & Sanders D. 2012. Calcification types of *Oocardium stratum* Nägeli and microhabitat conditions in springs of the Alps. *Freshwater Science*: 31, 610-624.
- Sanders D. & Rott E. 2009. Contrasting styles of calcification by the micro-alga *Oocardium stratum* Nägeli 1849 (Zygnematophyceae) in two limestone-precipitating springs of the Alps. *Austrian Journal of Earth Sciences*: 102, 34-49.

- Stanford J. A., Ward J. V., & Ellis B. K. 1994. Ecology of the alluvial aquifers of the flathead river, Montana. In: Gibert J., Danielopol D. L., & Stanford J. A. (Eds), Groundwater ecology. 367–390. Academic Press, London.
- Stumm W. 1995. Aquatic chemistry chemical equilibria and rates in natural waters. Wiley, New York, 1022 pp.
- Wallner J. 1933. *Oocardium stratum* Naeg., eine wichtige tuffbildende Alge Südbayerns. *Zeitschrift für wissenschaftliche Biologie*: 20, 287-293.
- Wallner J. 1934. Über die Verbreitungsökologie der Desmidiacee *Oocardium*. *An International Journal of Plant Biology*: 23, 249-263.

Characterization of algae communities in spring-associated limestone habitats of Austria

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Abstract

Travertine-depositing headwaters provide a unique hydro-chemical and geomorphic environment with specialized biological communities. Algae communities were investigated in spring and autumn 2014 at 14 sites. In addition, several environmental parameters were analysed. We found typical spring-associated limestone (SAL) species such as the diatoms *Delicata delicatula*, *Denticula tenuis*, *Encyonopsis microcephala*, *Cymbopleura austriaca*, *Encyonopsis cesati*, *Gomphonema lateripunctatum*, *Brachysira calcicola* ssp. *pfisteri* and the green alga *Oocardium stratum*. *Oocardium stratum* is a rare desmid algae occurring exclusively in travertine depositing headwaters. *Oocardium stratum* was known to be present at four sites and we were able to discover three additional *O. stratum* locations. Multivariate statistics did not reveal any seasonal difference between microphytobenthos communities. Instead, a geographical pattern was obtained resulting in seven groups. An indicator species analysis identified 22 indicator species for these groups. Canonical correspondence analysis showed that anthropogenic disturbance, total phosphorus, pH, conductivity, bicarbonate concentration, Langelier saturation index, sky openness, chloride concentration and carbonate precipitation rate are relevant for species distribution. *Oocardium stratum* occurs exclusively in intact headwaters with low anthropogenic disturbance and decreased total phosphorus amounts. Furthermore it exists at higher calcium carbonate precipitation rates; one location showed also elevated sulphate amounts.

Keywords: spring, headwater, travertine, tufa, algae, *Oocardium*

Introduction

Springs are defined as groundwater dependent ecosystems (Kløve *et al.*, 2011). They combine hydrologic, geomorphic and biological processes. Although headwater streams are commonly quite small but have a great impact on the downstream systems. Because of their small size, they are often incorrectly displayed in maps. They were formerly seen as structurally and functionally simple habitats, but they link lentic, lotic and semi-terrestrial habitats (Kløve *et al.*, 2011). Therefore springs are regarded as ecotones (Barquín & Scarsbrook, 2008). They create an ecological unit and are hydrologically independent (Gomi *et al.*, 2002; Lowe & Likens, 2005). Headwater streams have several ecosystem services, such as drinking water and nutrient removal, recreational opportunities and through their ecological autonomy a very high biodiversity. Organisms inhabiting the headwaters have little influence on the surrounding water, as changes through photosynthesis and respiration are transported immediately further downstream. Headwaters are very stable in their hydrology and chemical composition over the year (Odum, 1961).

Travertine is the result of limestone precipitation caused by CO₂ loss from water into the atmosphere. Pentecost (1993) and Pentecost & Viles (1994) used the term travertine for all carbonate deposits. They proposed using “meteogene travertine” for all deposits, where CO₂ originates from soil and root respiration, which was originally fixed from the atmosphere. This sort of travertine is widely distributed and hosts micro- and macrophytes, invertebrates and bacteria. Furthermore, they introduced the term “thermogene travertine” for all deposits, where the CO₂ originates from hydrolysis and oxidation of reduced carbon. These springs are often hot and contain neither macrophytes nor invertebrates (Ford & Pedley, 1996). In addition, Sanders *et al.* (2011) introduced another term for meteogene travertine, which is spring-associated limestone (SAL). The formation of travertine underlies the process of the dissociation of CO₂. Through respiration and decomposition

processes in the soil, the groundwater is highly enriched with CO₂ and finally dissolved into carbonic acid, which lowers the pH and dissolves carbonate bedrocks. After groundwater reaches the surface at springs, CO₂ is released into the atmosphere and as a result CaCO₃ precipitates (Stumm, 1995). Turbulences and the active removal of CO₂ through photosynthesis accelerate this process, which causes a shift from an inorganic process at the source exit to a biogenic process downstream (Merz & Zankle, 1991). Through the carbonate deposition process, dams, walls, terraces and cascades can be built; e.g. "Plitvice Lakes" (Ford & Pedley, 1996).

Springs have a very high biodiversity in relation to other aquatic ecosystems because they offer various substrates for colonization and high food availability (Stanford *et al.*, 1994). They harbour groundwater and surface water species, terrestrial and wetland species. Kløve *et al.* (2011) indicated that also organisms that take advantage of the buffering capacity of the groundwater against dryness and extreme temperatures are also found in springs. Cantonati *et al.* (2001) found diatoms and cyanobacteria being the most dominant groups and that pH, conductivity, inorganic nitrogen, substrate particle size and shading are key parameters for species occurrence. In SALs, diatoms, cyanobacteria, heterotrophic bacteria and microscopic chlorophytes like the desmid *Oocardium stratum* are essential for biofilm development; they provide crystallization nuclei for calcium carbonate precipitation (Cantonati *et al.*, 2006; Golubić *et al.*, 2008). Diatoms are the dominant group at travertine depositing headwaters and were first identified at travertine headwaters (Porter, 1861). Diatoms are dependent on the supply of silicate, which is usually not limited ($> 0.10 \text{ mg l}^{-1}$) in travertine depositing headwaters (Cantonati *et al.*, 2006). Reichardt (1994;1995) and Cantonati *et al.* (2012a;2012b) investigated SAL communities in Germany and Italy and extracted species typical of these spring type from the data set (see Table 1). Reichardt (1994;1995) mentioned 15 diatom species, whereas Cantonati *et*

al. (2012a) listed 13 diatom taxa and Cantonati *et al.* (2012b) described 8 species of cyanobacteria and non-diatom algae.

Oocardium stratum is a desmid and seems to be perfectly adapted to elevated carbonate depositions. Before this study was conducted, only four *Oocardium*-sites were known for Austria. Hansgirg (1905) described *O. stratum* in Lower Austria near Wiener Neustadt, but unfortunately it seems to have disappeared from this site. Brehm & Ruttner (1926) discovered *O. stratum* in the Lunz area (Lower Austria). The original site was destroyed, but *Oocardium* was discovered in the early 2000s at a nearby location (Schagerl & Pröschold, 2007). The other three locations are Lingenaу (Vorarlberg), Tuffbachl near the Alpenzoo Innsbruck and Hochtalalm (both Tyrol) (Sanders & Rott, 2009; Rott *et al.*, 2010; Rott *et al.*, 2012). *Oocardium* is occurring worldwide in SAL habitats, but it is found only sporadically. Croatia (Matoničkin & Pavletić, 1961), South of Bavaria (Schwäbisch Alp) (Wallner, 1933), USA (Mathews *et al.*, 1965), the British isles (West & West, 1901; Pentecost, 1981), Ireland (Adams, 1908) and Belgium (van Oye & Hubert, 1937) are examples for its occurrence. The rare occurrence was however already scrutinized by Pfiester (1976), who mentioned that those habitats are seldom visited by phycologists; therefore *Oocardium* might have been overlooked in the past.

O. stratum is exclusively found in fast-flowing calcareous spring-fed streams in a certain distance from the source. It colonizes two-thirds of the whole longitudinal extension of the stream with active carbonate deposition and covers/encrusts every subject in the streambed. It builds calcareous tubes and is anchored with gelatinous stalks in these tubes (Fig. 1A). The tubes are extended at the fringe of the calcareous tubes, which are surrounded with mucus. The calcite is formed along the outer surface of this mucus (Wallner, 1933; 1934; Sanders & Rott, 2009). Wallner (1935) described another species *Oocardium depressum* Wallner which differs in cell size, but Golubić & Marčenko (1958)

rebutted this taxon because no differential characteristics exist to justify another species description.

In Europe, travertine headwaters are the only spring type (7220 Petrifying springs with tufa formation, Cratoneurion) that is registered in the European Habitat Directive (EU HD, 1992), whereas in the Water Framework Directive (EU WFD, 2000), other spring types and small headwaters (catchment < 10 km²) are not considered. It is essential to gain more detailed insight into key variables responsible for species patterns, for protecting the highly threatened SAL habitats (Weigand, 1998; Cantonati *et al.*, 2006; Ilmonen *et al.*, 2012). We searched for differences in the algae community composition of various SAL headwaters. Are species patterns related to certain environmental conditions? We moreover searched for key factors responsible for the occurrence of the rare desmid *Oocardium stratum*. We hypothesize that *O. stratum* is dependent on free CO₂ and has some advantage in travertine stretches because of its special growth form. We further hypothesize that the algae community is specialized to cope with the ongoing deposition of calcium carbonate. They need to develop survival strategies to escape to be buried in carbonate. We expect similar community compositions in the travertine springs and that the environment selects calcifying, rheophilic species. Our survey provides detailed information to identify key parameters that shape algae communities and that enable occurrence of *Oocardium stratum*.

Materials and Methods

This study was a cooperation with Jean-Pierre Bednar (2015), who explored the macroinvertebrate communities at the same sites.

Study sites

In total, 25 Austrian travertine headwaters were visited. Out of these, 14 proved to be suitable in terms of active travertine formation and were studied in detail. One site is located in Vorarlberg (Lin), two in Tyrol (Hoc, Zoo), one in Carinthia (Lap), three in

Upper Austria (Mar, Dan, Edl) and seven are located in Lower Austria (Woe, Lut, Lud, Alm, Poe, Tes, Pre) (Fig. 2). The sites are located in 6 different aquatic landscape units (Wimmer *et al.*, 2000) (Table 2).

In-situ measurements

The 14 headwaters were visited twice in spring and autumn 2014 (Fig. 2, Table 2). For sampling, a representative section within the carbonate deposition stretch was chosen. Within this section, the per cent of the 3 predominant substrates (travertine, coarse particulate organic matter = cPOM, fine particulate organic matter = fPOM) was estimated and a choriotope sketch was drawn. For all measurements triplicates were carried out. Specific conductivity, water temperature (WTW LF197i), pH (WTW pH 330i/ Metrohm combined glass electrode 6.0253.100) and O₂ concentration (WTW Oxy 197i) were measured on site. The concentration of free carbon dioxide (CO₂) and bicarbonate (HCO₃⁻) was analysed on site with unfiltered water via titration with HCl (0.1 N) and NaOH (0.01 N) to the pH endpoints 3.4 and 8.2, respectively. The concentrations of free CO₂ and HCO₃⁻ were calculated after Hütter (1994). To estimate the sky openness, photos were taken with a levelled camera positioned towards the sky and equipped with a fisheye lens (NIKON Coolpix 4500; Nikon fisheye converter FC-E8 0,21x objective). The calculation of the sky openness was done with the gap light analyzer (GLA, v 2.0) (Frazer *et al.*, 1999).

For estimating the calcium carbonate precipitation rate (ccp), artificial substrata (nails with washers) were mounted in spring and collected in autumn. The calcium carbonate layer was then measured under the binocular (ZEISS SteREO Lumar.V12) as following: the washers were vertically clamped with a paper clip and pictures were taken. Then travertine thickness was measured with the ZEISS ZEN software (blue edition, 2011).

The slope was measured with a flexible tube water level, water velocity was estimated with methylene blue (Pomeisl, 1953). Discharge was calculated from the width, depth and the

velocity of the water. Last but not least, the anthropogenic disturbance was characterized on a relative scale from 0 (undisturbed, e.g. no access, no channelling, no human activities) to 5 (highly disturbed, e.g. total water obstruction for drinking water supply).

Chemical analysis

Water samples (3 unfiltered, 6 filtered) were taken and stored cold and dark for chemical analysis in the laboratory. The water was filtrated on site with syringe-filter holders (Whatman GFF, Glass microfiber filters, 47 mm Ø). Calcium (Ca^+), magnesium (Mg^+), sodium (Na^+), chloride (Cl^-), potassium (K^+), sulphate (SO_4^{2-}) and nitrate (NO_3^-) were analysed by ion chromatography (Metrohm Compact IC 761, Suppressor-modul MSM, Metrohm 853 CO₂-Suppressor, Metrohm IC Filtration Sample Processor 788; anions Metrohm Metrosep A Supp 5 150 x 4.0 mm ID with suppression; cations Metrohm Metrosep C2 150 x 4.0 mm ID) (OENORM EN ISO 10304-1:2012-06-01; OENORM EN ISO 14911:1999-11-01). Ammonium (NH_4^+), nitrite (NO_2^-), phosphate (PO_4^{3-}) and total phosphorous (TP) were analysed spectrophotometrically (Hach-Lange DR 2800) (DIN EN 26777:1993-04; OENORM EN ISO 6878:2004-09-01; OENORM ISO 7150-1:1987-12-01). Dissolved organic carbon (DOC) was analysed after the UV/difference method (Sievers 5310 C) (DIN EN 1484). All the parameters except for TP were analysed with the filtrated water. The Langelier Saturation Index (LSI) was calculated with the online calculator Lenntech (1998-2015); a $\text{LSI} > 0$ indicates supersaturation with calcium-hydrogencarbonate and high probability of carbonate precipitation, a $\text{LSI} < 0$ indicates supersaturation of carbonic acid and high probability of corrosion.

Biological samples

Algae samples were taken from each of the 3 main choriotopes with a self-made sampling device: from a plastic bottle with a wide opening, the bottom was cut and the bottleneck placed towards the substratum to define an exact sampling area. For each choriotope, three replicates were taken within the section and unified to one sample. For the travertine

choriotope, the bottleneck was placed on the surface and the outline was drawn with a knife. Then the knife was used to scrape off the defined area. cPOM was sampled in an equal manner. For fPOM, a syringe was connected to a tube, which was placed inside the bottleneck of the sampling device to exhaust the area. Half of the material was preserved with ethanol. For diatom identification, both wet combustion (HCl , HNO_3 + H_2SO_4) (Niedermayr & Schagerl, 2010) and dry combustion were accomplished. The slides were mounted in Naphrax after placing them in 5% HCl followed by rinsing with MilliQ-water to remove carbonate layers which would otherwise disturb the microscopical studies. Identification was done with a compound microscope (ZEISS Axio Imager.M1, AxioCam MRc5 60 N-C 2/3" 0,63x, AxioCam MRm 60N-C 1" 1,0x). Abundance was estimated on the relative DAFOR scale from 1 = sporadic to 5 = massive occurrence (Kent & Coker, 1992). For algae identification following taxonomic literature were used: Komárek & Anagnostidis (2005;2013), Krammer (1997a;1997b), Krammer & Lange-Bertalot (2004;2007a;2007b;2008), Hofmann *et al.* (2011), Ettl & Rieth (1980) and Eloranta *et al.* (2011). For the bryophyte communities, the identification key of Frahm & Frey (1992) was used.

Statistics

The statistical analyses were carried out with the R package (version 3.1.3, R development Core Team, Vienna, Austria) using the packages vegan, MASS, xlsx, gclus, pvclust and labdsv (Borcard *et al.*, 2011). First of all, a dissimilarity matrix with the species data using the Bray-Curtis index was calculated using the function vegdist, which was then used to prune groups with the function hclust using the agglomeration method UPGMA. With the function cophentic, the best agglomeration method was chosen. A mantel test was carried out to find the optimum number of clusters. Afterwards, an analysis of similarities with the function anosim was used to test if there is a significant difference between the groups. A non-metric multidimensional scaling (nMDS) was calculated with the function metaMDS

using the dissimilarity matrix, using two dimensions, the number of random starts was 100, the function for the MDS was monoMDS, the maximum number of iterations was set to 2000 and the start searched for the best previous solution. This procedure was carried out four times. In addition, an indicator species analysis with the function indval was calculated. For linking species patterns with the environment, a direct gradient analysis was performed. First, a detrended correspondence analysis was carried out; the gradient lengths (axis 1: SD = 2.68, axis 2: SD = 2.79) suggested that constrained correspondence analysis (CCA) is suitable (ter Braak & Prentice, 1988). With the function cca, a constrained correspondence analysis (CCA) was calculated using the species data and the z-standardized environmental data. An automatic forward selection was carried out with the function ordistep. A second time the CCA was calculated by using only the parameters, which turned out significant in the forward selection. Only variables with inflation factors < 10 were considered for further interpretation. Through permutation tests (function anova.cca), the significance of the model, of the first axis and of the selected environmental parameters were tested. Graphs were created in R (Murrell, 2006), Windows Excel (version 14.4.9, 2011) and SigmaPlot (version 11.0) and edited in Adobe Illustrator CS6 (version 16.0.0).

Results

The environment

Most of the sampling sites are located either below 500 m asl or between 600 and 700 m asl (Table 2) with most of the sites slightly anthropogenic disturbed (Fig. 3A). Sky openness ranged between 5% and 35% (Fig. 3B); CCP ranged between 0.8 and 2.0 mm a⁻¹ (Fig. 3C), which was in agreement with the LSI > 0 indicating the tendency towards calcium carbonate precipitation (Fig. 3D). pH was within a narrow range between 8.2 and 8.4; specific conductivity showed values of 500 to 600 $\mu\text{S cm}^{-1}$ with chloride concentrations between 0.5 and 5 mg l⁻¹ and low TP amounts between 5 and 15 $\mu\text{g l}^{-1}$ (Fig.

3E to 3H). HCO_3^- concentrations were at most sites between 200 and 400 mg l⁻¹ (Fig. 3I), free CO₂ was below 2 mg l⁻¹ (Fig. 3J). The relative ion composition based on mg l⁻¹ revealed Ca²⁺ (40 to 100 %) and Mg⁺ (up to 40%) as dominant cations, K⁺ and Na⁺ were of minor importance (Fig. 4A). HCO_3^- was the dominant anion; at some sites, also SO₄²⁻ was detected in higher quantities (Fig. 4B).

Species patterns

Among the main choriotopes, only travertine contained higher algae amounts; cPOM and fPOM contained only a few dead cells and organic matter. If cPOM was present, it obviously entered the system only shortly before; the longer it was exposed in the water, the more it was coated with carbonate. Within a short time, cPOM is transformed to the travertine choriotope. For further studies, we therefore focused on travertine (see below).

In total, 93 taxa were found (Table 3). The most abundant algae group was the Bacillariophyceae comprising 76 taxa, which represent 75 to 94 % of the relative frequency of occurrence (Fig. 5). Samples collected in spring showed higher biodiversity compared to the autumn samples; Poe had the highest taxa number (32 species) in spring followed by Lut and Lud (Fig. 6). Fig. 7 shows the most abundant species for spring and autumn considering only those species that occurred at least at seven locations.

Achnanthidium minutissimum, *Navicula* cf. *tripunctata* (Fig. 8M), *Denticula tenuis* (Fig. 8I), *Diploneis* cf. *krammeri*, *Cocconeis placentula* var. *lineata* and *Encyonopsis microcephala* (Fig. 8D) were found at 10 to 13 locations in spring; in autumn they were less frequent. *Oocardium stratum* was found 6 times in spring and 8 times in autumn. In spring *Oocardium stratum* (Fig. 9B, C) was found at Lut and Lud, Lin, Zoo and Hoc and at Alm (new *O. stratum* site). In autumn it was also discovered at Poe and Dan (new sites).

Linking species patterns to the environment

Based on similarities of the algal communities, spring and autumn samplings of the sites were placed together (Fig. 10) and 7 groups were classified. An analysis of similarities
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confirmed significant differences between groups ($R = 0.9305$, $p < 0.001$; Fig. 11). Groups of the dendrogram identified by a mantel test revealed that the locations of Western Austria (Lin, Hoc, Zoo and Lap) clustered together in 2 groups, whereas all locations of Upper Austria are placed in a single group. The locations Woe and Pre are placed alone in one group. Both Lunz sites (Lut, Lud) are clustered in the same group and the sites Alm and Poe are grouped together. nMDS based on species occurrences displays that the sites located in Western Austria are separated from the other one.

Table 4 lists groups with their environmental ranges; group 7 is characterized by higher disturbance and higher TP concentrations. Group 5 had the lowest TP amounts and the highest CCP. Group 6 had both the lowest LSI and the lowest CCP. *Oocardium stratum* was dominant in 3 groups (2, 4, 5) and the cyanoprokaryote *Phormidium nigrum* in 4 groups (1, 2, 6, 7). The bryophyte identification resulted in 16 species occurring at the 14 sites. The most common bryophyte was *Palustriella commutata*, which occurred in 6 groups.

The indicator species analysis resulted in 22 indicator species (Table 5), some of them highly indicative for groups (Fig. 12B) such as *Nitzschia monachorum*, which occurred exclusively in group 1 and only with high abundances (indicator value IV 100%). *Fallacia* cf. *lenzii* was only found in group 7, but with a patchy distribution resulting in a lower IV of 63%. *Brachysira calcicola* ssp. *pfisteri* (Fig. 8A) occurred in group 4 and 5 and had a higher IV than *Epithemia adnata*, which occurred only in group 4. *Navicula* cf. *tripunctata* is indicative for group 7, but occurred also in all other groups, although in low abundance (IV of 29.1%).

CCA resulted in a significant model; the first four axes explained 34% of the total variance in the data set ($p = 0.001$) (Table 6). 25 environmental parameters were included and nine were significantly contributed to the species pattern ($p < 0.05$): disturbance ($F = 3.55$), CCP ($F = 2.61$), HCO_3^- ($F = 2.02$), sky openness ($F = 2.11$), pH ($F = 2.35$), LSI ($F = 1.94$), TP (TP) ($F = 1.78$), conductivity ($F = 1.91$) and Cl^- ($F = 1.89$). Fig. 12A displays the first two

canonical axes with all species (93 species) considering their affiliation to the 7 groups (identified by cluster analysis and indicator species analysis) and the significant environmental parameters. Taxa representing group 1 are located at increased HCO_3^- concentrations but low conductivity, taxa of group 7 occur at higher disturbance and increased TP concentrations. Group 4 is found at higher conductivity and group 5 at higher Cl^- , CCP and low TP concentrations. Group 2 is distributed around the axes origin indicating no special preference towards selected parameters. *Oocardium stratum*, arranged in group 2, occurs at higher CCP, but low TP concentrations and disturbance (Fig. 12A). *Oocardium stratum* did not show a distinct pattern concerning cation ratios, but most of the *O. stratum* were found at elevated HCO_3^- concentrations and one location with increased SO_4^{2-} concentration (Fig. 4B).

Discussion

The environment

The chemical and physical characteristics of headwater streams are based on the lithology of the aquifer. In this study, almost all headwater streams are based on carbonate substrate (Woe and Lin are based on tertiary/quaternary sediments; Alm is based on crystalline rocks), which is reflected by the environmental parameters such as alkalinity and pH. pH is influenced by lithology, dissolved carbon dioxide, nitrate and sulphate and is well buffered in carbonate systems (Cantonati *et al.*, 2006). In this study, pH was within a small range and comparable to other studies in carbonate systems (Cantonati & Ortler, 1998; Arp *et al.* (2001). In general springs and headwaters are characterized through an oligotrophic status, which is defined by a TP concentration below $10 \mu\text{g l}^{-1}$ (Cantonati *et al.*, 2006; Kociolek & Stoermer, 2009). Cantonati & Ortler (1998) analysed TP amounts between 1 to $26 \mu\text{g l}^{-1}$ at springs in Italy. We measured even higher TP of around $105 \mu\text{g l}^{-1}$ at the site Dan which can be attributed to the run-off from gardens and agricultural land. Elevated SO_4^{2-} concentrations were found at the sites Zoo, Mar, Lap and Edl. Sulphate is either geogenic

or anthropogenic in origin. Hobiger *et al.* (2007) investigated geogenic hotspots of higher sulphate background values in Austria, which corresponded with our study sites. Sanders & Rott (2009) assumed that higher amounts of the major ions (Mg^{2+} , Ca^{2+} , HCO_3^- , SO_4^{2-}) inhibit growth of *Oocardium stratum*. Nevertheless, we found *O. stratum* tolerating elevated sulphate concentrations (Fig. 4B).

Rott *et al.* (2012) investigated calcification types of *Oocardium stratum* and the conditions of the microhabitat. The authors discovered that out of 5 different SAL habitats, Lingenaу had the highest calcification rate and the highest amount of free CO_2 . Rott *et al.* (2012) investigated the natural monument in Lingenaу and we analysed a headwater upstream the monument, our study revealed a similar picture: Lin had the highest CCP and the second highest free CO_2 concentration amongst the different sites investigated (Table 2). Rott *et al.* (2012) measured values twice as high for Ca^{2+} concentration and higher Mg^{2+} concentrations at Hoc and Zoo than in this study, which could be due to different sampling points: Rott *et al.* (2012) considered the spring mouth, in the centre of the *Oocardium* zone and the downstream limit of the calcification, whereas in this study only the central *Oocardium* zone was measured.

An increase in chloride can be an indicator for anthropogenic impact, because it neither is involved in biological processes nor in chemical reactions or it gets washed out of the aquifer through the high solubility of chloride (Bakalowicz, 1994; Cantonati *et al.*, 2006).

Wilhelm (1956) investigated different headwater streams in Germany and identified 3 groups with different Cl^- concentrations. Springs of the alps with a Cl^- concentration of 4 $mg\ l^{-1}$, groundwaters of diluvial and alluvial gravel with 8 $mg\ l^{-1}$ and springs of the tertiary area with Cl^- concentrations above 14 $mg\ l^{-1}$. He described that the Cl^- concentration fluctuates seasonally. For instance a Cl^- maximum in spring, because of the snowmelt and a minimum in dry months. In this study the highest Cl^- concentration was measured at Lin with around 16 $mg\ l^{-1}$ determined by heavy rain. DOC in springs is usually $< 1\ mg\ l^{-1}$ and

differences are mainly related to snow melt and heavy rain events (Cantonati *et al.*, 2006).

Before the autumn sampling started, heavy rains took place, which obviously resulted in elevated DOC values (spring: 0.83 to 3.61 mg l⁻¹; autumn: 1.14 to 4.17 mg l⁻¹) at sites Woe, Poe, Lin and Pre.

Species patterns

Cantonati *et al.* (2012a) investigated different spring habitats, one of them SALs. The authors identified 13 SAL diatom species; 9 were also found in this study (Table 1). The most abundant species at our sites are *Achnanthidium minutissimum*, *Navicula* cf. *tripunctata* and *Denticula tenuis* (Fig. 7). *Achnanthidium minutissimum* is a widespread epilithic species of running waters with no ecological preference. *Navicula* cf. *tripunctata* - also listed in Pentecost (1991a) - is an indicator for slight eutrophication (Krammer & Lange-Bertalot, 2007a). *Denticula tenuis* is a character species of SAL with a worldwide distribution (Reichardt, 1994; Penecost & Zhang, 2000); we found this taxon at 13 sites in spring, but only at 5 locations in autumn (Table 3). Reichardt (1994;1995) investigated SAL habitats in Germany and identified 15 typical diatom taxa for calcareous rich water, some of them in only low abundances. From the 15 species, 9 were also identified in this study (Table 1). *Cymbopleura austriaca* (Fig. 8C) was found 12 times, followed by *Caloneis bacillum* (6) (Fig. 8E), *Caloneis alpestris* (5) (Fig. 8L), *Cymbopleura subaequalis* and *Diploneis minuta* (4 times each). *Brachysira calcicola* ssp. *pfisteri* was discovered only at Zoo, Hoc and Lap in spring and autumn. For cyanobacteria and algae others than diatoms, Cantonati *et al.* (2012b) specified 8 SAL species. Out of these, we only found *Oocardium stratum*. Additionally, 17 taxa that are not belonging to diatoms were identified in the headwaters in higher abundances; at some sites, only a single cyanobacterial taxon occurred in very high abundance covering everything in the streambed (e.g. *Phormidium nigrum*).

In addition to the sites, which are already known for occurrence of *Oocardium stratum*, we discovered 3 more locations, which are new reports for Austria. Two of them were identified in autumn, which points at the need of repeated site visits. Sanders *et al.* (2011) and Pentecost (2005) mentioned some important taxa that are involved in the calcium carbonate precipitation (*Vaucheria*, *Navicula*, *Meridion*, *Gomphonema*, *Scytonema*, *Oocardium*), but all these taxa except *Oocardium* are also found in non-travertine depositing headwaters. *Oocardium* is the only genus which is prevalent exclusively in SAL headwaters (Pfiester, 1976; Pentecost, 2005).

Linking species patterns to the environment

Both the cluster analysis (Fig. 10) and the nMDS (Fig. 11) revealed that there is no seasonal differentiation in the communities, which is in accordance to findings of Cantonati (1999). Headwaters can be seen as islands in the landscape with low interactions to each other, which was also reflected in the nMDS, which resulted in a geographical pattern rather than in a seasonal pattern. The sites in Western Austria (Lap, Zoo, Hoc, Lin) are significantly different and clearly separated from the other locations in Eastern Austria. Larned (2010) described 5 general classes of environmental variations for the community composition and the growth of periphyton: disturbances, stressors, resources, hydraulic conditions, and biotic interactions. For spring periphyton, these parameters can be narrowed down to nutrients such as TP, geogenic factors (pH, conductivity, HCO_3^- , SO_4^{2-}) and light supply (Cantonati, 2008; Gesierich & Kofler, 2010; Cantonati *et al.*, 2012b). Sabater & Roca (1990) mentioned that the mineral content of the water is the main parameter, which influenced the diatom community in the Pyrenean springs. This is in accordance to Soininen (2007), who found ion concentration and nutrient availability as important factors for freshwater diatom distribution. In this study, the ionic composition was less important, as we focused on a single habitat.

For characterizing different spring types, Cantonati *et al.* (2012a;2012b) considered TP, shading, current velocity, nitrate-N, temperature and altitude: SAL species occurred at increased temperatures and low TP concentrations. This pattern was also found in our study highlighting the importance of these parameters. Cantonati *et al.* (2012b) found that SAL habitats also offer increased pH and conductivity compared to other spring types. We focused exclusively on SAL headwaters, so some of the parameters such as pH, conductivity and water temperature showed only very low variations. For *Oocardium stratum*, Linhart & Schagerl (2015) identified water temperature and HCO₃⁻ concentration being key parameters influencing growth at a single site. Our study revealed that *Oocardium stratum* occurs at sites with lower TP concentration and higher CCP.

According to Biggs & Smith (2002), flood disturbance and nutrients strongly influence the algae community; an extended period of hydrological stability can enhance the algae richness. Conditions like these are met in springs: the environmental variation is quite small in springs and headwaters, they are also stable in hydrological terms. Altering these ecosystems will change the community composition, which also means a loss of rare species and the reduction of ecosystem services (Covich *et al.*, 2004). The importance of the parameter disturbance clearly reveals that it is not only the conservation of these ecosystems themselves, but also the protection of the surrounding land, which is essential for keeping these sensitive habitats intact.

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References

- Adams J. 1908. A synopsis of Irish Algae, freshwater and marine. *Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science*: 27, 11-60.
- Antoine D., André J.-M., & Morel A. 1996. Oceanic primary production: 2. Estimation at global scale from satellite (Coastal Zone Color Scanner) chlorophyll. *Global Biogeochemical Cycles*: 10, 57 - 69.
- Arp G., Wedemeyer N., & Reitner J. 2001. Fluvial tufa formation in a hard-water creek (Deinschwanger Bach, Franconian Alb, Germany). *Facies*: 44, 1-22.
- Bakalowicz M. 1994. Water geochemistry: water quality and dynamics. *Groundwater ecology*: 1, 97-127.
- Barquín J. & Scarsbrook M. 2008. Management and conservation of coldwater springs. *Aquatic Conservation: Marine and Freshwater Ecosystems*: 18, 580–591.
- Bednar J. P. 2015. Characterization of macroinvertebrate communities in meteogene travertine-depositing headwaters in Austria. Masterthesis. University of Vienna, Vienna, 72 pp.
- Biggs B. J. F. & Smith R. A. 2002. Taxonomic richness of stream benthic algae: Effects of flood disturbance and nutrients. *Limnol. Oceanogr.*: 47, 1175-1186.
- Boch R., Spoetl C., Reitner J. M., & Kramers J. 2006. A lateglacial travertine deposit in eastern Tyrol (Austria). *Mitteilungen der Oesterreichischen Geologischen Gesellschaft - Austrian Journal of Earth Sciences*: 98, 78-91.
- Borcard D., Gillet F., & Legendre P. 2011. Numerical ecology with R. Springer, New York, 306 pp.
- Brehm V. & Ruttner F. 1926. Die Biocönosen der Lunzer Gewässer. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*: 16, 281-391.
- Cantonati M. & Ortler K. 1998. Using spring biota of pristine mountain areas for long-term monitoring. *International Association of Hydrological Sciences*: 248, 379-385.
- Cantonati M. 1999. Distribution and seasonality of the phytobenthos along two mountain spring streams in catchments of contrasting lithology. *Bollettino Del Museo Civico Di Storia Naturale Di Venezia*: 49, 357-362.
- Cantonati M., Gerecke R., & Bertuzzi E. 2006. Springs of the Alps – Sensitive ecosystems to environmental change: From biodiversity assessments to long-term studies. *The International Journal of Aquatic Sciences*: 562, 59-96.
- Cantonati M. 2008. Cyanoprokaryotes and algae other than diatoms in springs and streams of the Dolomiti Bellunesi National Park (Northern Italy). *Algological Studies*: 126, 113-136.

- Cantonati M., Angeli N., Bertuzzi E., Spitale D., & Lange-Bertalot H. 2012a. Diatoms in springs of the Alps: spring types, environmental determinants, and substratum. *Freshwater Science*: 31, 499-524.
- Cantonati M., Rott E., Spitale D., Angeli N., & Komárek J. 2012b. Are benthic algae related to spring types? *Freshwater Science*: 31, 481-498.
- Catling D. C. & Claire M. W. 2005. How Earth's atmosphere evolved to an oxic state: A status report. *Earth and Planetary Science Letters*: 237, 1-20.
- Chisti Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*: 25, 294-306.
- Covich A. P., Austen M. C., Barlocher F., Chauvet E., Cardinale B. J., Biles C. L., Inchausti P., Dangles O., Solan M., Gessner M. O., Statzner B., & Moss B. 2004. The role of Biodiversity in the functioning of freshwater and marine benthic ecosystems. *Bioscience*: 54, 767-775.
- DIN EN 1484. Wasseranalytik - Anleitungen zur Bestimmung des gesamten organischen Kohlenstoffs (TOC) und des gelösten organischen Kohlenstoffs (DOC).
- DIN EN 26777:1993-04. Bestimmung von Nitrit, Spektrometrisches Verfahren (ISO 6777:1984).
- Dokulil M. & Teubner K. 2000. Cyanobacterial dominance in lakes. *Hydrobiologia*: 438, 1-12.
- Eloranta P., Kwandrans J., & Kusel-Fetzmann E. 2011. Süßwasserflora von Mitteleuropa. Rhodophyta and Phaeophyceae. Spektrum Akademischer Verlag, Heidelberg, 155 pp.
- Ettl H. & Rieth A. 1980. Süßwasserflora von Mitteleuropa. Xanthophyceae. Fischer, Stuttgart, 147 pp.
- EU HD. 1992. Council Directive 92/ 43/ EEC of 21 May 1992 on the conservation of natural habitat and of wild fauna and flora. *Official Journal of the European Communities*: L 206, 7-50.
- EU WFD. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*: L 327, 1-73.
- Falconer I. & Humpage A. 2005. Health Risk Assessment of Cyanobacterial (Blue-green Algal) Toxins in Drinking Water. *International Journal of Environmental Research and Public Health*: 2, 43-50.
- Field C. B., Behrenfeld M. J., Randerson J. T., & Falkowski P. 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science*: 281, 237-240.
- Ford T. D. & Pedley H. M. 1996. A review of tufa and travertine deposits of the world. *Earth Science Reviews*: 41, 117-175.

- Frahm J.-P. & Frey W. 1992. Moosflora. Ulmer, Stuttgart, 538 pp.
- Frazer G. W., Canham C. D., & Lertzman K. P. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York, 36 pp.
- Gesierich D. & Kofler W. 2010. Epilithic diatoms from rheocrene springs in the Eastern Alps (Vorarlberg, Austria). *Diatom Res.*: 25, 43-66.
- Golubić S. & Marčenko E. 1958. Zur Morphologie und Taxonomie der Desmidiaceengattung *Oocardium*. *Schweizerische Zeitschrift für Hydrologie*: 20, 177-185.
- Golubić S., Violante C., Plenković-Moraj A., & Grgasović T. 2008. Travertines and calcareous tufa deposits: an insight into diagenesis. *Geologia Croatica*: 61, 363-378.
- Gomi T., Sidle R. C., & Richardson J. S. 2002. Understanding processes and downstream linkages of headwater system. *Bioscience*: 52, 905.
- Graham L. E. & Wilcox L. W. 2000. Algae. Prentice Hall, Upper Saddle River, NJ, 640 pp.
- Hansgirg A. 1905. Grundzüge der Algenflora von Niederösterreich. *Beihefte zum Botanischen Centralblatt*: XVIII, 417-522.
- Hobiger G., Klein P., Haslinger E., & Pirkl H. 2007. Hydrochemical geogenic background values of near-surface groundwater bodies. In: Hydrologischer Atlas Österreich. Federal Ministry of Agriculture, Forestry, Environment and Water Management, Vienna.
- Hoffmann J. P. 1998. Wastewater treatment with suspended and nonsuspended algae. *Journal of Phycology*: 34, 757-763.
- Hoffmann L. 1989. Algae of Terrestrial Habitats. *Botanical Review*: 55, 77-105.
- Hofmann G., Werum M., & Lange-Bertalot H. 2011. Diatomeen im Süßwasser-Benthos von Mitteleuropa. Gantner, Rugell, 908 pp.
- Hütter L. A. 1994. Wasser und Wasseruntersuchung. Otto Salle, Frankfurt am Main, 515 pp.
- Ilmonen J., Mykrä H., Virtanen R., Paasivirta L., & Muotka T. 2012. Responses of spring macroinvertebrate and bryophyte communities to habitat modification: community composition, species richness, and red-listed species. *Freshwater Science*: 31, 657-667.
- Kent M. & Coker P. 1992. Vegetation description and analysis; A practical approach. CRC Pr., Boca Raton, Fla., 363 pp.

- Kløve B., Ala-aho P., Bertrand G., Boukalova Z., Ertürk A., Goldscheider N., Ilmonen J., Karakaya N., Kupfersberger H., Kværner J., Lundberg A., Mileusnić M., Moszczynska A., Muotka T., Preda E., Rossi P., Siergieiev D., Šimek J., Wachniew P., Angheluta V., & Widerlund A. 2011. Groundwater dependent ecosystems. Part I: Hydroecological status and trends. *Environmental Science and Policy*: 14, 770-781.
- Kocielek J. P. & Stoermer E. F. 2009. Oligotrophy: the forgotten end of an ecological spectrum. *Acta Bot. Croat.*: 68, 465-472.
- Komárek J. & Anagnostidis K. 2005. Süßwasserflora von Mitteleuropa. Cyanoprokaryota. 2. Teil: Oscillatoriales. Fischer, Stuttgart, 759 pp.
- Komárek J. & Anagnostidis K. 2013. Süßwasserflora von Mitteleuropa. Cyanoprokaryota. 3. Teil Heterocytous genera. Fischer, Stuttgart, 1130 pp.
- Krammer K. 1997a. Encyonema part., Encyonopsis and Cymbellopsis. Cramer, Berlin 469 pp.
- Krammer K. 1997b. Allgemeines und Encyonema Part. Die cymbelloiden Diatomeen eine Monographie der weltweit bekannten Taxa. Cramer, Berlin, 382 pp.
- Krammer K. & Lange-Bertalot H. 2004. Süßwasserflora von Mitteleuropa, Achnanthaceae. Fischer, Stuttgart, 468 pp.
- Krammer K. & Lange-Bertalot H. 2007a. Süßwasserflora von Mitteleuropa. Bacillariophyceae. Naviculaceae. Fischer, Stuttgart, 876 pp.
- Krammer K. & Lange-Bertalot H. 2007b. Süßwasserflora von Mitteleuropa. Bacillariaceae, Epithemiaceae, Surirellaceae. Fischer, Stuttgart, 610 pp.
- Krammer K. & Lange-Bertalot H. 2008. Süßwasserflora von Mitteleuropa. Centrales, Fragilariaeae, Eunotiaceae. Fischer, Stuttgart, 598 pp.
- Larned S. T. 2010. A prospectus for periphyton: recent and future ecological research. *J. N. Am. Benthol. Soc.*: 29, 182-206.
- Lenntech B. V. 1998-2015 Langelier Saturation Index Calculator. <http://www.lenntech.com/calculators/langelier/index/langelier.htm>. Zugriff am 17.02.2015.
- Linhart C. & Schagerl M. 2015. Seasonal succession of the travertine-forming desmid *Oocardium stratum*. *Journal of Phycology*, in print.
- Longhurst A., Sathyendranath S., Platt T., & Caverhill C. 1995. An estimate of global primary production in the ocean from satellite radiometer data. *Journal of Plankton Research*: 17, 1245-1271.
- Lowe W. H. & Likens G. E. 2005. Moving headwater streams to the head of the class. *Bioscience*: 55, 196-197.

- Mata T. M., Martins A. A., & Caetano N. S. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*: 14, 217-232.
- Mathews H. L., Prescott G. W., & Obenshain S. S. 1965. The genesis of certain calcareous floodplain soils of Virginia. . *Proceedings - Soil Science Society of America*: 29, 729-732.
- Matoničkin I. & Pavletić Z. 1961. Epibiontische Verhältnisse auf den Kalktuffwasserfällen des Flusses Krka in Dalmatien. *The International Journal of Aquatic Sciences*: 18, 219-224.
- Merz M. & Zankle H. 1991. The influence of the sheath on carbonate precipitation by cyanobacteria. *Bollettino della Società Paleontologica Italiana*: 1, 325-331.
- Milledge J. J. 2011. Commercial application of microalgae other than as biofuels: A brief review. *Reviews in Environmental Science and Biotechnology*: 10, 31-41.
- Müller H. J. 1991. Ökologie. G. Fischer, Jena, 415 pp.
- Murrell P. 2006. R graphics. Chapman & Hall, CRC, Boca Raton, Fla., 301 pp.
- Nägeli C. 1849. Gattungen einzelliger Algen, physiologisch und systematisch bearbeitet. *Neue Denkschriften der Allgemeinen Schweizerischen Gesellschaft für die Gesammten Naturwissenschaften*: 10, 1-139.
- Niedermayr R. & Schagerl M. 2010. Structuring factors of the phytobenthos community along a mountain headwater (Kalkalpen National Park, Austria). *Fundamental and Applied Limnology/Archiv für Hydrobiologie*: 177, 93-104.
- Odum E. P. 1961. Fundamentals of ecology. Saunders, Philadelphia, 546 pp.
- OENORM EN ISO 6878:2004-09-01. Bestimmung von Phosphor - Photometrisches Verfahren mittels Ammoniummolybdat.
- OENORM EN ISO 10304-1:2012-06-01. Bestimmung von gelösten Anionen mittels Flüssigkeits-Ionenchromatographie - Teil 1: Bestimmung von Bromid, Chlorid, Fluorid, Nitrat, Nitrit, Phosphat und Sulfat (ISO 10304-1:2007 + Cor 1:2010).
- OENORM EN ISO 14911:1999-11-01. Bestimmung der gelösten Kationen Li⁺, Na⁺, NH4⁺, K⁺, Mn2+, Ca2+, Mg2+, Sr2+, und Ba2+ mittels Ionenchromatographie - Verfahren für Wasser und Abwasser (ISO 14911:1998).
- OENORM ISO 7150-1:1987-12-01. Bestimmung von Ammonium, manuelle spektrophotometrische Methode.
- Penecost A. & Zhang Z. 2000. The travertine flora of Juizhaigou and Munigou, China, and its relationship with calcium carbonate deposition. *Cave & Karst Science*: 27, 71-79.
- Pentecost A. 1981. The tufa depositis of the Malham district. *Field Studies*: 5, 365-387.

- Pentecost A. 1991a. Algal and bryophyte flora of a Yorkshire (U.K.) hill stream: a comparative approach using biovolume estimations. *Archiv für Hydrobiologie*: 121, 181-201.
- Pentecost A. 1991b. A new and interesting site for the Calcite-encrusted desmid *Oocardium stratum* Naeg. in the British Isles. *British Phycological Journal*: 26, 297-301.
- Pentecost A. 1992. Carbonate chemistry of surface waters in a temperate karst region: the southern Yorkshire Dales, UK. *Journal of Hydrology*: 139, 211-232.
- Pentecost A. 1993. British travertines: a review. *Proceedings of the Geologists' Association*: 104, 23-39.
- Pentecost A. & Viles H. 1994. A Review and Reassessment of Travertine Classification. *Géographie Physique et Quaternaire*: 48, 305-314.
- Pentecost A. 2005. Travertine. Springer, Berlin, 445 pp.
- Pfiester L. A. 1976. *Oocardium stratum* a rare (?) desmid (Chlorophyceae). *Journal of Phycology*: 12, 134.
- Pomeisl E. 1953. Der Mauerbach. *Wetter und Leben: Sonderheft II.*, 103 - 121.
- Porter H. 1861. The Geology of Peterborough and its Vicinity, Peterborough, UK, 126 pp.
- Raven J. A. & Giordano M. 2014. Algae. *Current Biology*: 24, 590-595.
- Reichardt E. 1994. Zur Diatomeenflora (Bacillariophyceae) tuffabscheidender Quellen und Bäche im Südlichen Frankenjura. *Berichte der Bayerischen Botanischen Gesellschaft*: 64, 119-133.
- Reichardt E. 1995. Die Kieselalgenflora (Bacillariophyceae) des Wachsenden Steins von Usterling. *Berichte der Bayerischen Botanischen Gesellschaft*: 65, 87-92.
- Rott E., Holzinger A., Gesierich D., Kofler W., & Sanders D. 2010. Cell morphology, ultrastructure, and calcification pattern of *Oocardium stratum*, a peculiar lotic desmid. *Protoplasma*: 243, 39-50.
- Rott E., Hotzy R., Cantonati M., & Sanders D. 2012. Calcification types of *Oocardium stratum* Nägeli and microhabitat conditions in springs of the Alps. *Freshwater Science*: 31, 610-624.
- Sabater S. & Roca J. R. 1990. Some factors affecting distribution od diatom assemblages in Pyrenean springs. *Freshwater Biology*: 24, 493-507.
- Sanders D. & Rott E. 2009. Contrasting styles of calcification by the micro-alga *Oocardium stratum* Nägeli 1849 (Zygnematophyceae) in two limestone-precipitating springs of the Alps. *Austrian Journal of Earth Sciences*: 102, 34-49.
- Sanders D., Wertl W., & Rott E. 2011. Spring-associated limestones of the Eastern Alps: overview of facies, deposystems, minerals, and biota. *Facies*: 57, 395-416.

- Schagerl M. & Pröschold T. 2007. Rediscovery of *Oocardium stratum* Näg. in Austria and its preliminary taxonomic position within the Desmidiales. *Proceedings of Biology and Taxonomy of Green Algae, V. Smolenice-Castle.*
- Soininen J. 2007. Environmental and spatial control of freshwater diatoms - a review. *Diatom Research*: 22, 473-490.
- Stanford J. A., Ward J. V., & Ellis B. K. 1994. Ecology of the alluvial aquifers of the flathead river, Montana. In: Gibert J., Danielopol D. L., & Stanford J. A. (Eds), *Groundwater ecology*. 367–390. Academic Press, London.
- Stumm W. 1995. Aquatic chemistry chemical equilibria and rates in natural waters. Wiley, New York, 1022 pp.
- ter Braak C. J. F. & Prentice I. C. 1988. A Theory of Gradient Analysis. *Advances in Ecological Research*: 18, 271-317.
- van Oye P. & Hubert B. 1937. Recherches sur les 'crons' du Jurassique Belge. *Biologische Jaarboek Dodonaea*: 4, 231-236.
- Wallner J. 1933. *Oocardium stratum* Naeg., eine wichtige tuffbildende Alge Südbayerns. *Zeitschrift für wissenschaftliche Biologie*: 20, 287-293.
- Wallner J. 1934. Über die Verbreitungsökologie der Desmidiacee *Oocardium*. *An International Journal of Plant Biology*: 23, 249-263.
- Wallner J. 1935. Zur Kenntnis der Gattung *Oocardium*. *Hedwigia*: 75, 130-136.
- Weigand E. 1998. Limnologisch-faunistische Characterisierung von Karstquellen, Quellbächen und unterirdischen Gewässern nach Choriotopen und biozönotischen Gewässerregionen Nationalpark o.ö . Kalkalpen, Österreich. Molln, Austria. 173 pp.
- West G. S. & West W. 1901. The alga-flora of Yorkshire: a complete account of the known freshwater Algae of the county, with many notes on their affinities and distribution. Taylor, Leeds, 239 pp.
- Wilhelm F. 1956. Physikalisch-chemische Untersuchungen an Quellen in den bayrischen Alpen und im Alpenvorland. *Münchener geographische Hefte*: 10, 1-97.
- Wimmer R., Chovanec A., Gruber O., Moog M. H., & Fink D. 2000. Abiotic Stream Classification as a Basis for a Surveillance Monitoring Network in Austria in Accordance with the EU Water Framework Directive. *Acta Hydrochimica et Hydrobiologica*: 28, 177-184.

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Table 3. Continued.

Taxon	Spring						Autumn					
	Edi	Tes	Dam	Mar	Lap	Im	Pre	Zoo	Hoc	Lim	Pre	Zoo
	Woe	Lut	Poe	Lud	Poe	Lut	Woe	Alm	Hoec	Lim	Woe	
<i>Suriella terricola</i> Lange-Bertalot & E.Alles	-	-	-	-	-	-	-	1	-	-	-	-
<i>Tryblionella</i> cf. <i>angustata</i> W.Smith	-	-	-	-	-	-	-	-	1	-	-	-
<i>Ulnaria ulna</i> (Nitzsch) P.Compère	-	2	-	1	-	4	1	-	1	-	1	-
Chlorophyta & Streptophyta												
<i>Chaetophora elegans</i> (Roth) C.Agardh	-	-	-	-	-	2	-	-	-	-	-	-
<i>Cosmarium</i> sp.	-	-	1	-	-	1	1	-	-	-	-	-
<i>Oocardium stratum</i>	-	2	3	-	2	4	3	4	-	4	4	3
<i>Mougeotia</i> sp.	-	-	-	-	-	2	2	-	3	-	-	-
<i>Spirogyra</i> sp.	-	-	-	-	-	2	2	-	1	-	1	-
<i>Zygnema</i> sp.	-	-	4	-	-	2	3	-	-	1	-	2

Table 5. Summary of the indicator species analysis with abbreviation codes. Only species with a p-value < 0.05 are shown.

Species	Abbreviation	Group	Indicator Value [%]
<i>Nitzschia monachorum</i>	Nitz_mona	1	100.0
<i>Gomphonema micropus</i>	Gomp_micr	1	71.4
<i>Gomphonema angustratum</i>	Gomp_angu	1	66.7
<i>Eunotia arcus</i>	Euno_arcu	2	75.0
<i>Amphipleura pellucida</i>	Amph_pell	2	70.0
<i>Cymbella helvetica</i>	Cymb_helv	2	66.7
<i>Cyclotella cf. cyclopuncta</i>	Cycl_cycl	2	62.5
<i>Meridion circulare</i>	Meri_circ	2	51.9
<i>Denticula tenuis</i>	Dent_tenu	2	43.6
<i>Cymbella excisiformis</i>	Cymb_exci	2	40.0
<i>Cymbopleura subaequalis</i>	Cymb_suba	3	100.0
<i>Diploneis separanda</i>	Dipl_sepa	3	44.4
<i>Diatoma tenuis</i>	Diat_tenu	4	92.3
<i>Brachysira calcicola</i> ssp. pfisteri	Brac_calc	4	81.8
<i>Epithemia adnata</i>	Epit_adna	4	75.0
<i>Encyonopsis microcephala</i>	Ency_micr	4	36.4
<i>Spirogyra</i> sp.	Spir_sp.	5	64.3
<i>Diatoma mesodon</i>	Diat_meso	6	60.4
<i>Caloneis lancettula</i>	Calo_lanc	6	52.2
<i>Fallacia cf. lenzii</i>	Falla_lenz	7	62.5
<i>Encyonema minutum</i>	Ency_minu	7	45.0
<i>Navicula cf. tripunctata</i>	Navi_trip	7	29.1

Table 6. Summary statistics of the CCA.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.401	0.286	0.255	0.202
Species - environment correlations	0.97	0.97	0.96	0.93
Cumulative percentage variance of species - environment relation	12.0	20.6	28.2	34.2
Sum of all eigenvalues	3.346			
Test of significance of the first canonical axis: p-value	0.001			
Test of significance of all canonical axes: p-value	0.001			

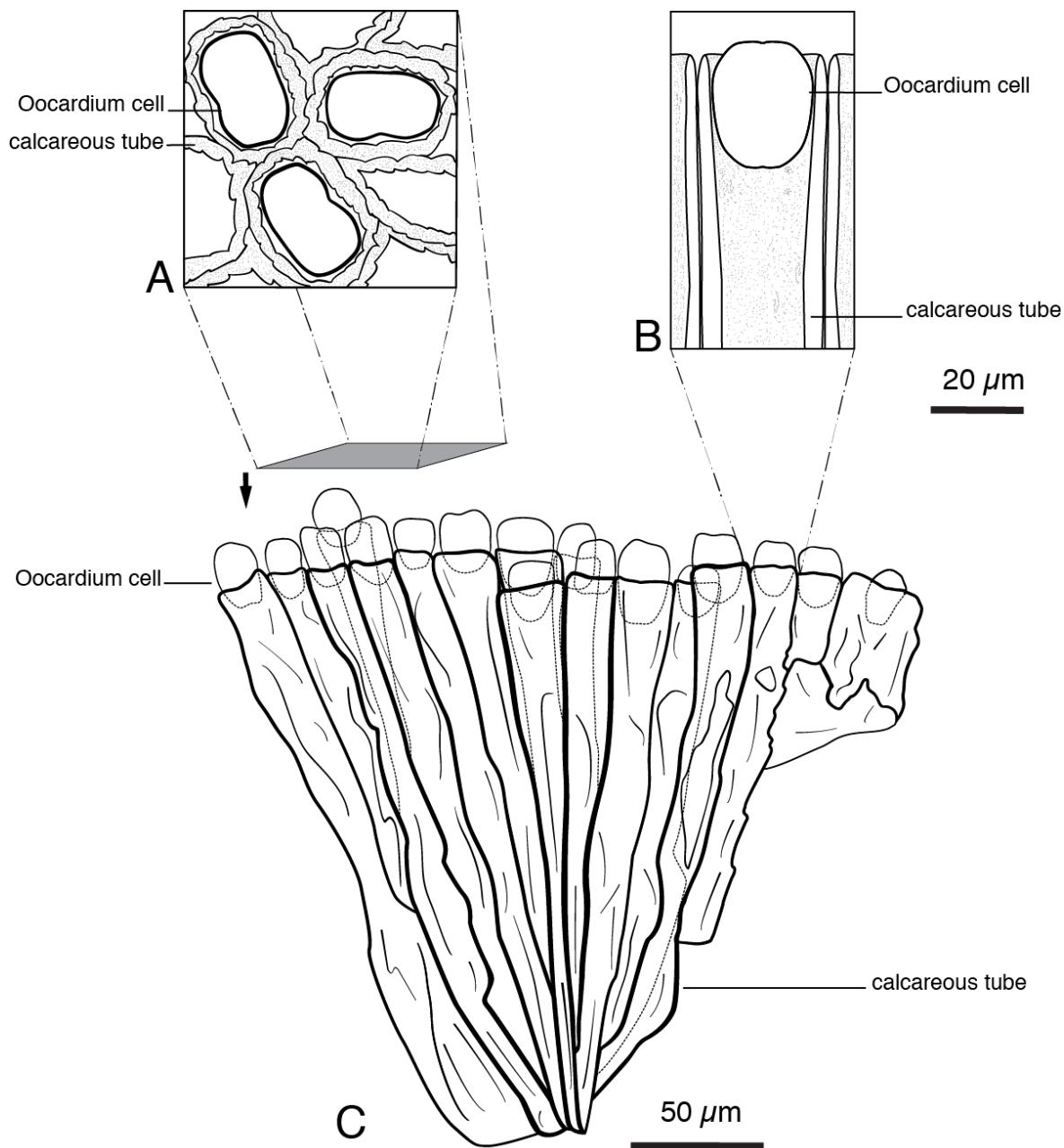


Fig. 1. Overview of *Oocardium stratum*. Apical view (A), side view (B) and cross section of a colony (C).

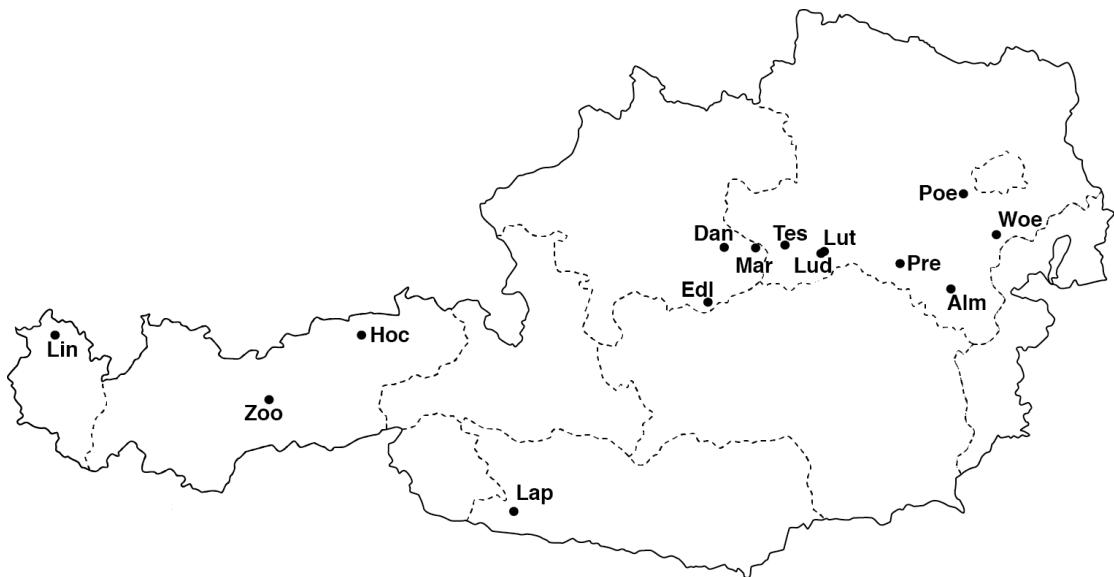


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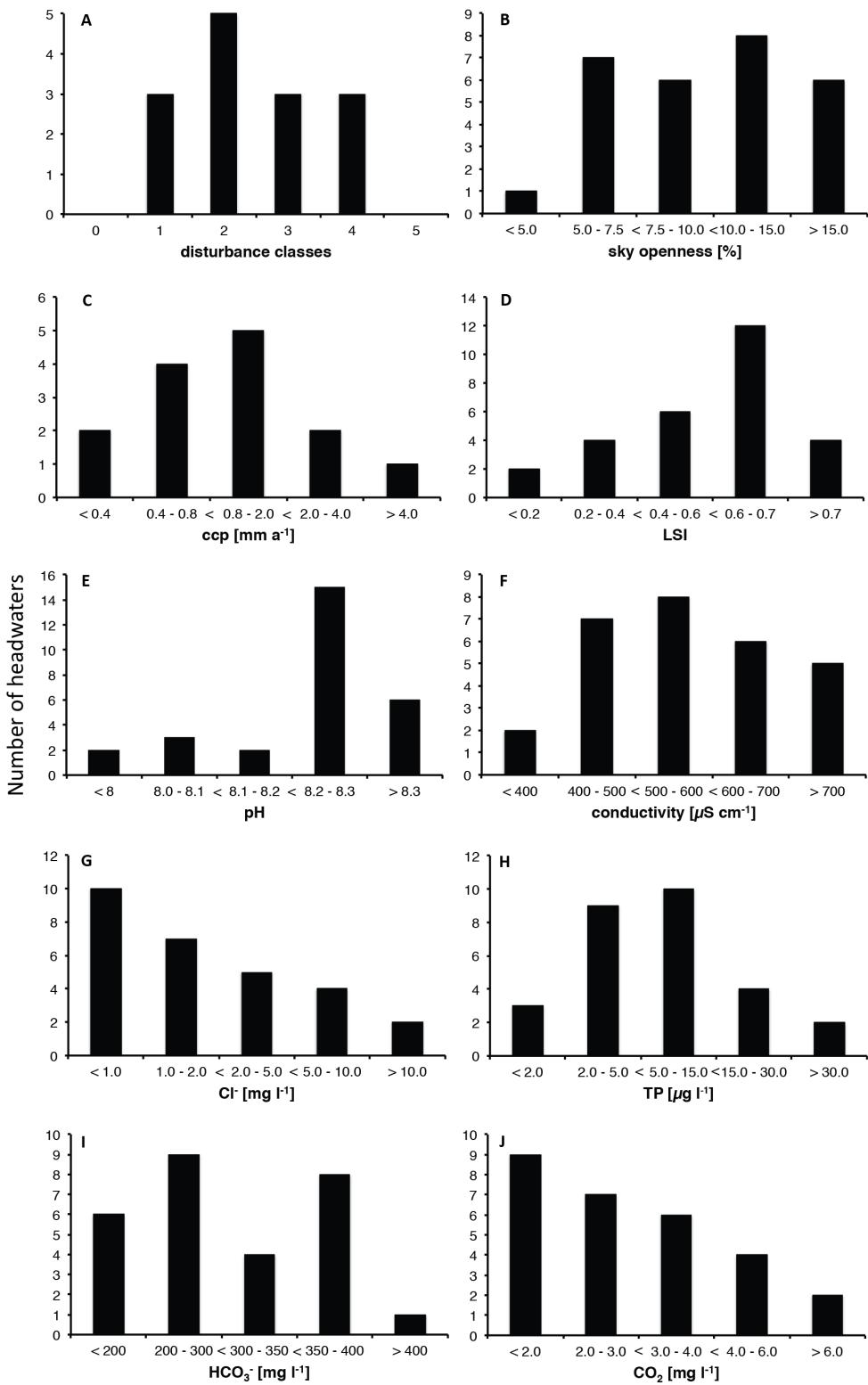


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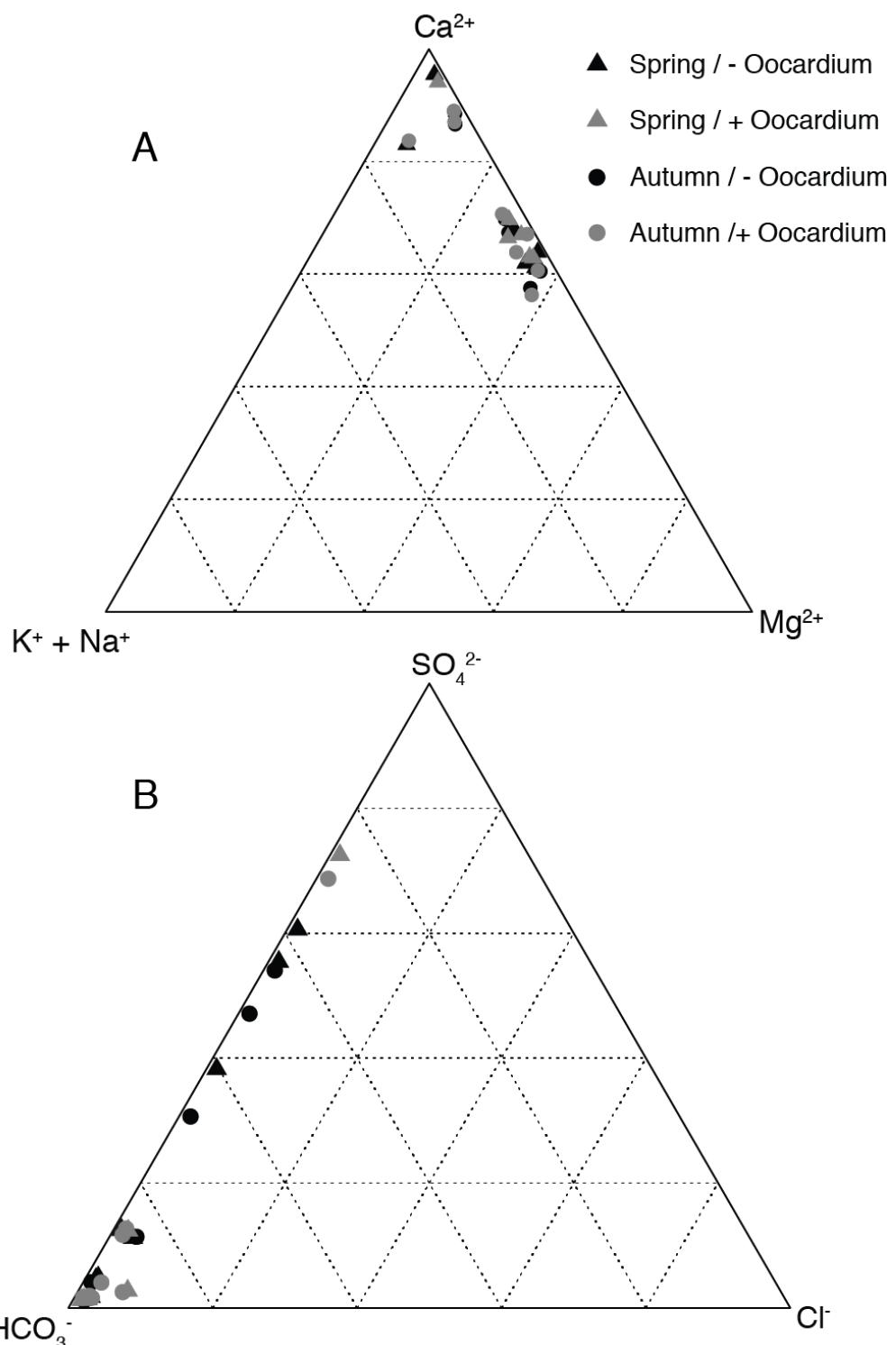


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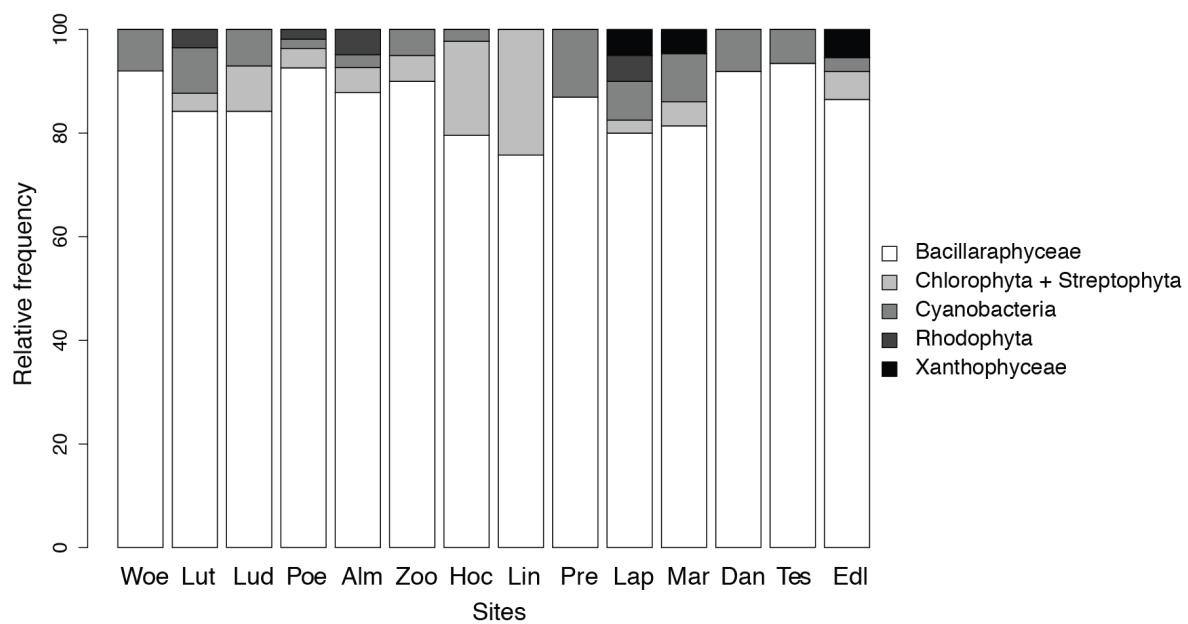


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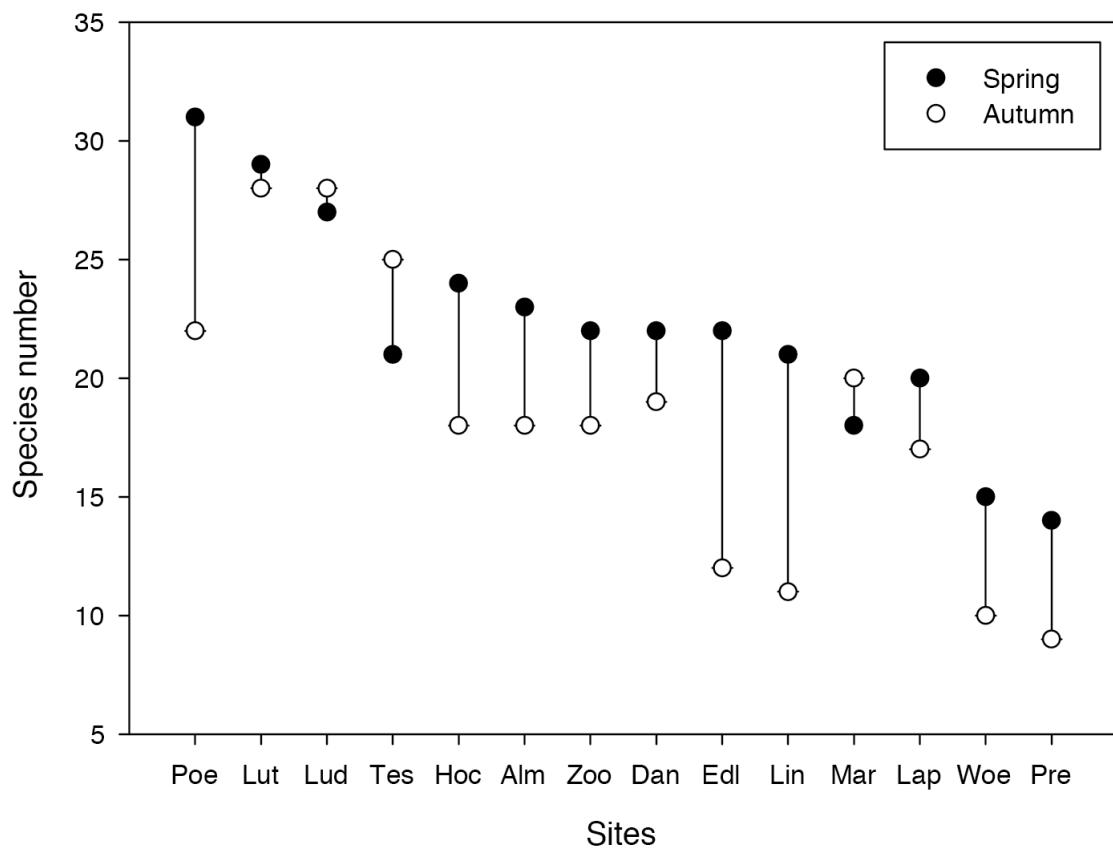


Fig. 6. Species richness of each site differentiated by season. In total 93 species were found. For abbreviations of the sites refer to Table 2.

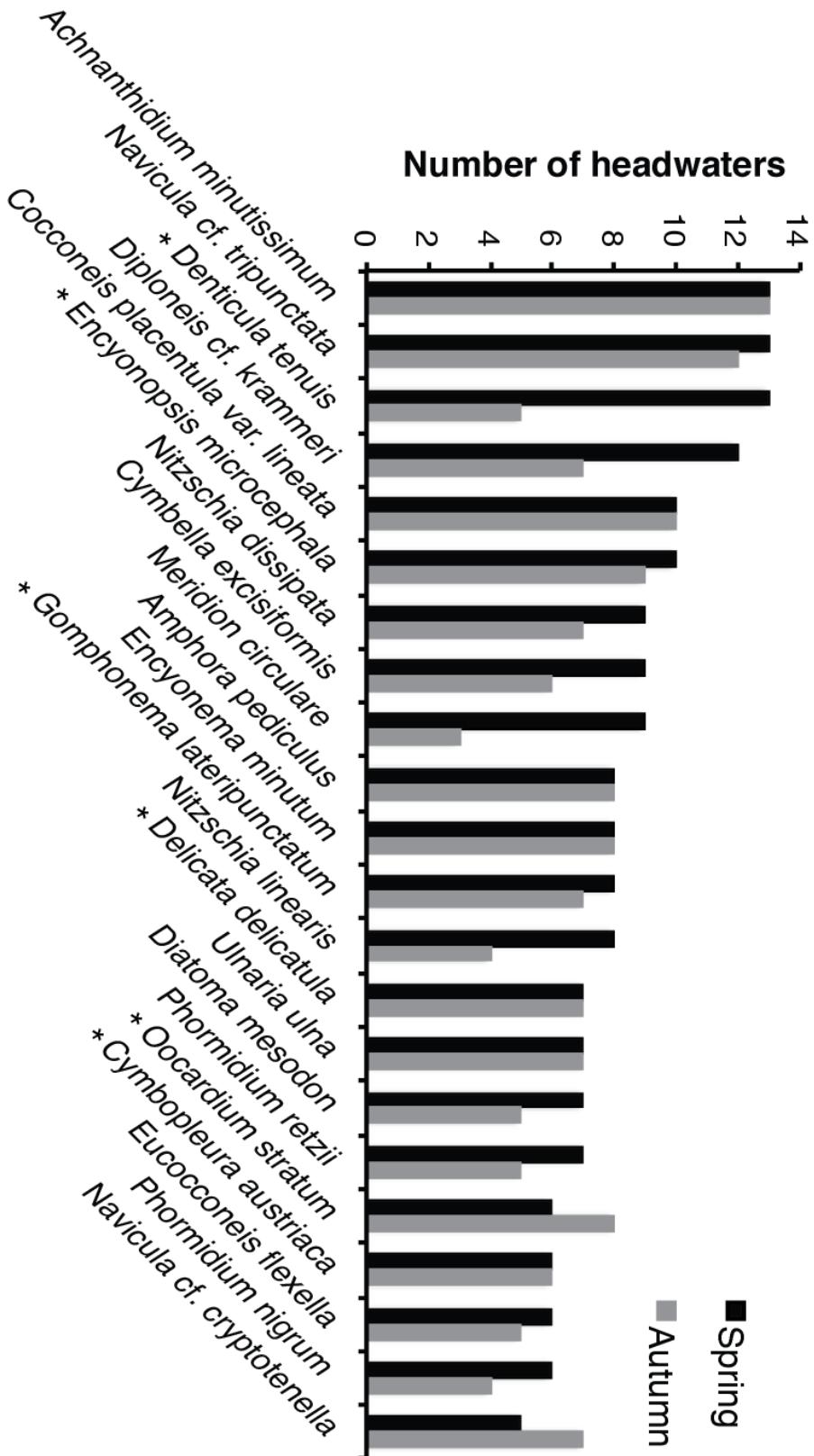


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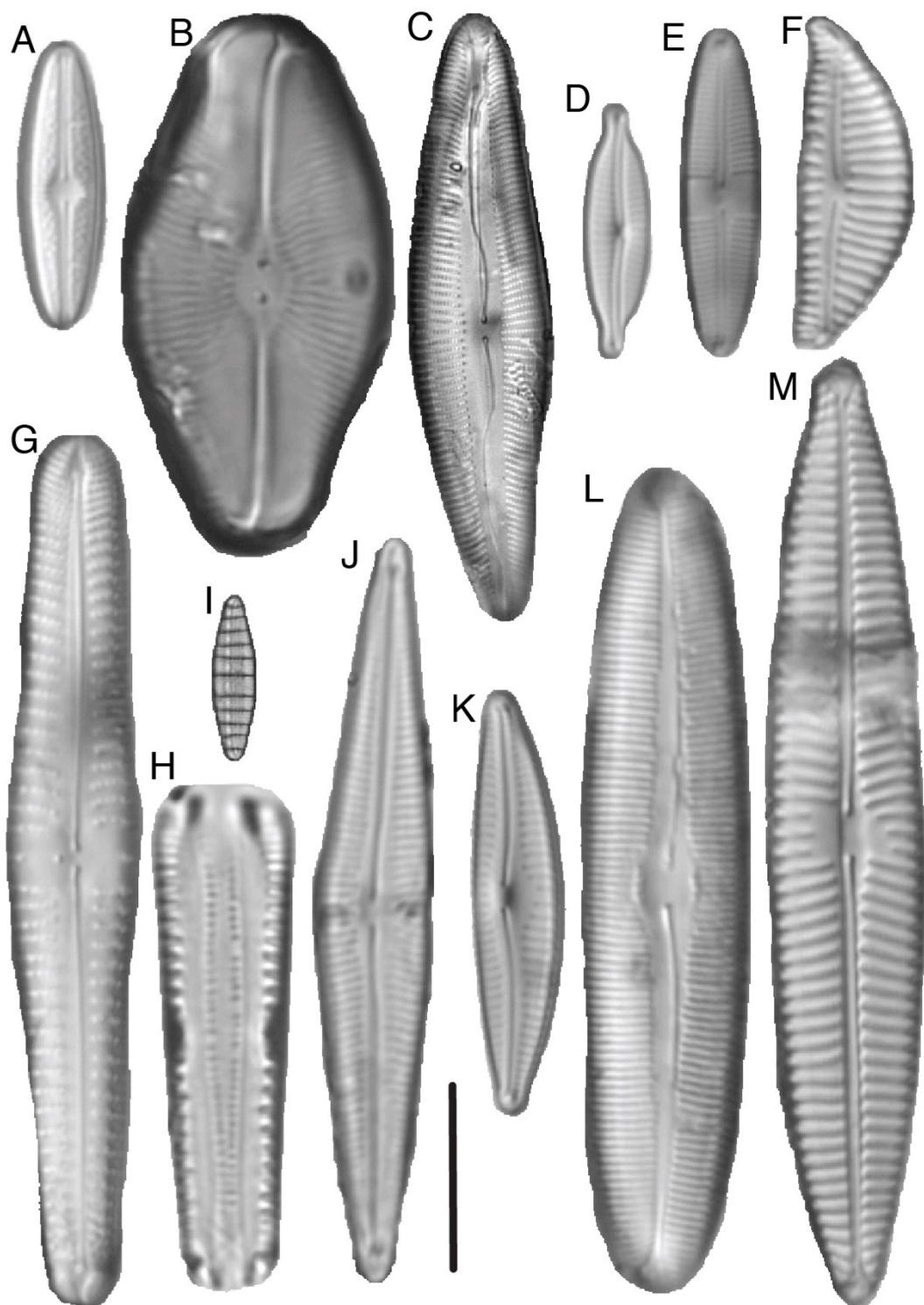


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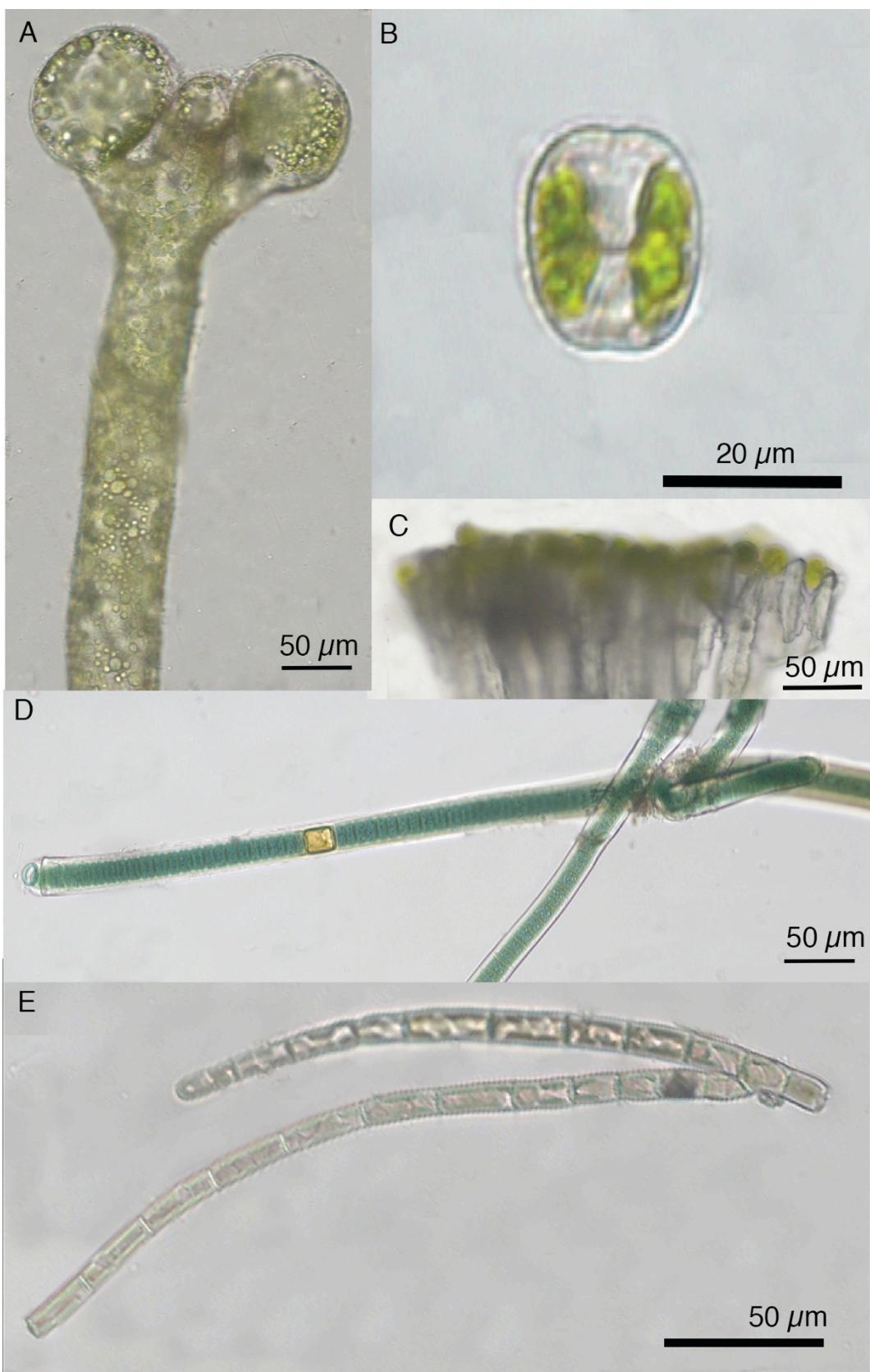


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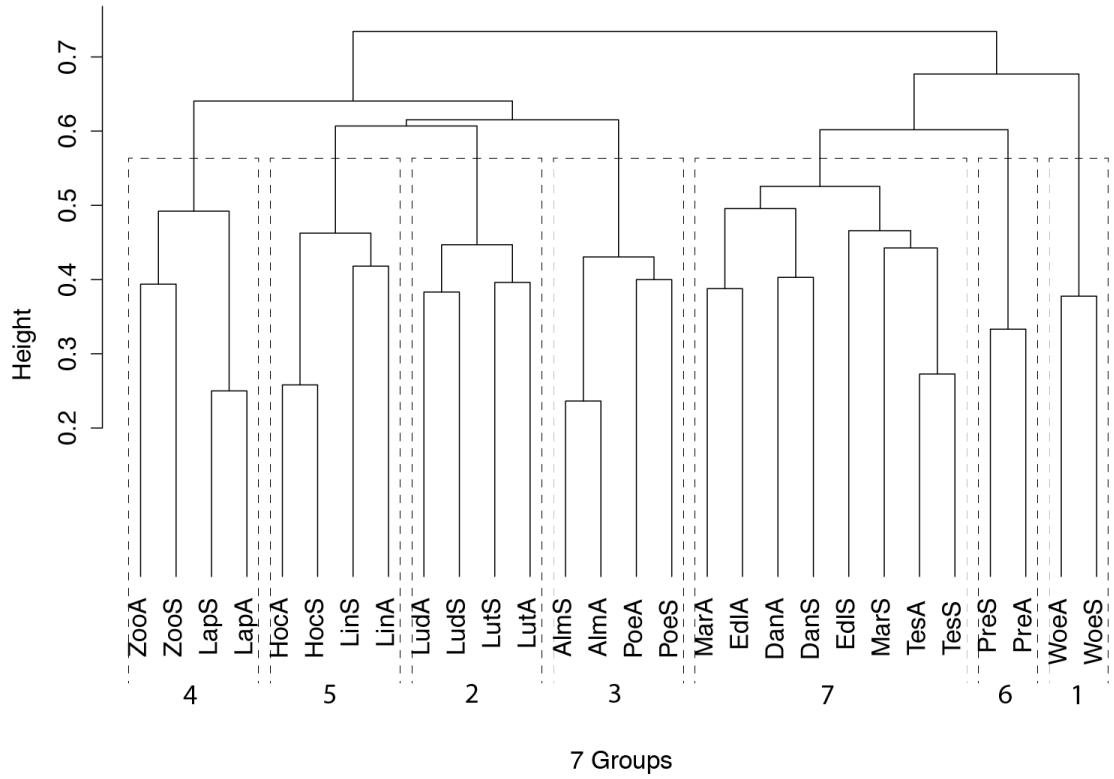
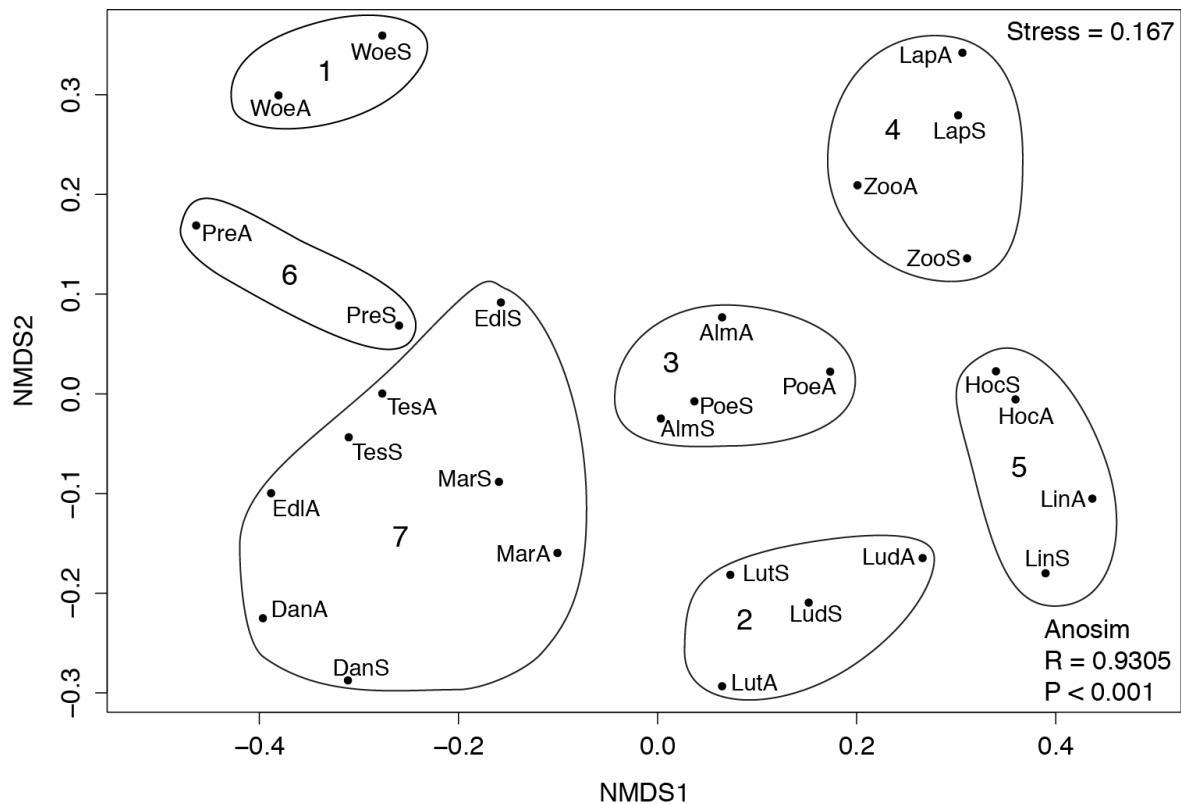


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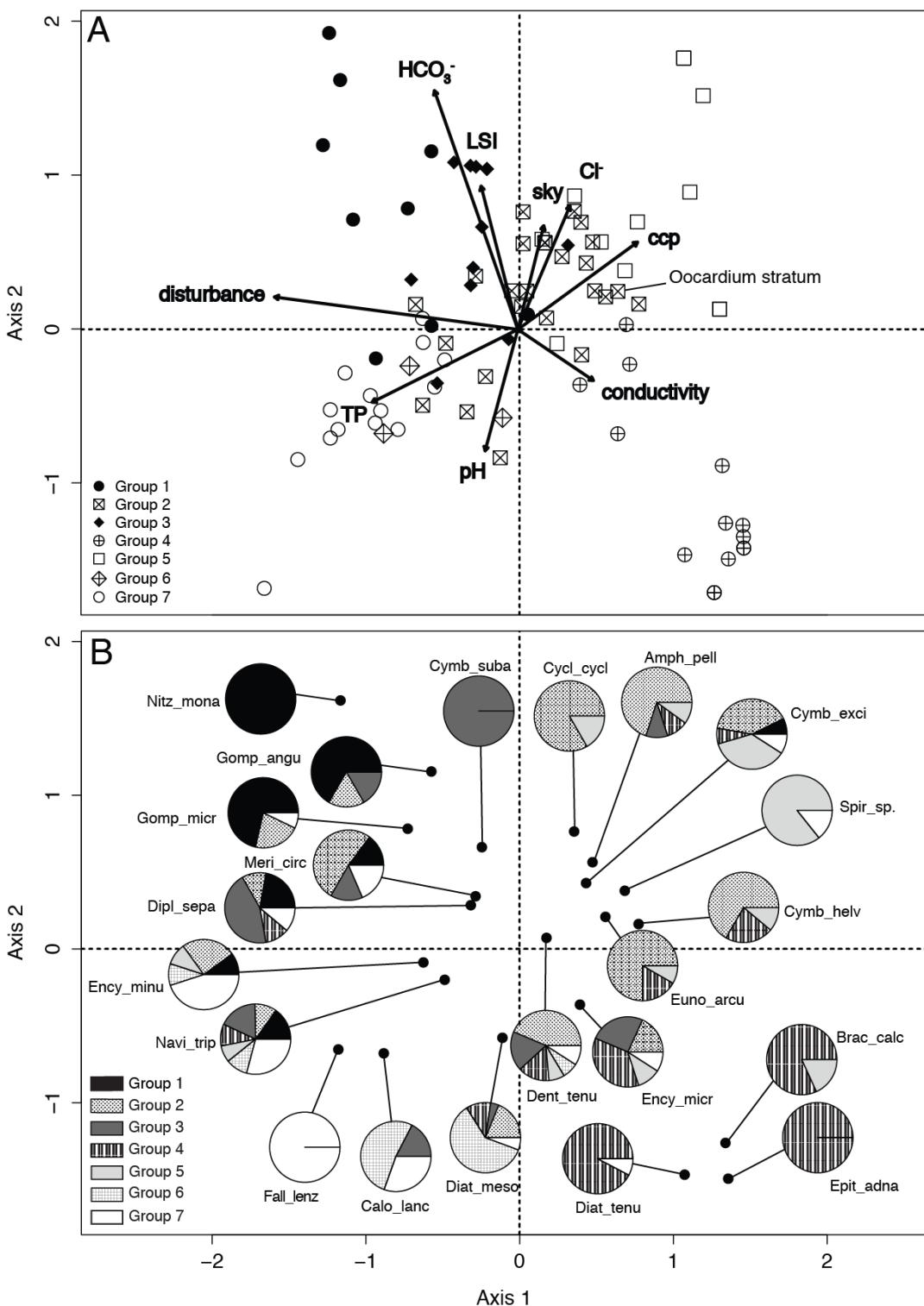


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Appendix

Zusammenfassung

Tuffbäche bieten einzigartige hydro-chemische und geomorphologische Bedingungen, welche auch Organismengemeinschaften strukturieren. Tuff (Travertin) ist das Produkt der Kalkausfällung, welche durch Unterschiede im CO₂-Partialdruck zwischen Wasser und der Atmosphäre hervorgerufen wird. Das Grundwasser ist mit CO₂ angereichert, welches sich mit Wasser zur Kohlensäure verbindet. Diese schwache Säure löst Kalkstein auf. Kommt das Grundwasser an die Oberfläche, beginnt CO₂ in die Atmosphäre auszusagen, wodurch als Nebeneffekt Kalk ausfällt.

In dieser Studie wurden Algengemeinschaften an 14 Tuffbächen in Österreich im Frühling und Herbst 2014 hinsichtlich ihrer Artenzusammensetzung untersucht. Weiteres wurden einige Umweltparameter ermittelt. Typische quell-assoziierte Tuffarten konnten gefunden werden, wie zum Beispiel die Kieselalgen *Delicata delicatula*, *Denticula tenuis*, *Encyonopsis microcephala*, *Cymbopleura austriaca*, *Encyonopsis cesati*, *Gomphonema lateripunctatum*, *Brachysira calcicola* ssp. *pfisteri* und *Oocardium stratum*. *Oocardium stratum* ist eine sehr seltene Zieralge, welche ausschließlich in Tuffbächen anzutreffen ist. Sie bildet Kalkröhren aus, welche durch physiologische Aktivitäten verlängert werden. *Oocardium stratum* wurde an den vier bereits bekannten Standorten in Österreich identifiziert; weiters wurden für Österreich drei neue Standorte entdeckt. Multivariate statistische Analysen zeigten, dass es keine saisonale Variabilität in den Algengemeinschaften gibt. Es konnte jedoch ein geographisches Muster ermittelt werden. Eine Indikatorartanalyse konnte 22 Indikatorarten für sieben Taxagruppen identifizieren. Signifikante Umweltparameter für die Artenverteilung sind vom Menschen verursachte Veränderungen an den Standorten, der Gesamtphosphor, pH, Leitfähigkeit, die Hydrogenkarbonatkonzentration, der Langelier Sättigungsindex, die Lichtversorgung, die Chloridkonzentration und die Kalkfällungsrate. *Oocardium stratum* kommt bei intakten

Standorten mit niedrigen Phosphorwerten und hohen Kalkausfällungsraten vor. Im Großen und Ganzen wurden typische Algengemeinschaften gefunden und wichtige Umweltparameter für die Artenkomposition ermittelt.

Summary

Spring-associated limestone habitats provide unique hydro-chemical and geomorphological conditions with specialized biological communities. Travertine is the product of calcium carbonate precipitation due to a difference in the CO₂ partial pressure of the water and the atmosphere. The groundwater is highly enriched with CO₂, which dissociates to carbonic acid. This weak acid is able to dissolve limestone. If the groundwater reaches the surface, the CO₂ will outgas to the atmosphere and calcium carbonate will precipitate.

In this study algae communities were investigated regarding their taxa composition at 14 headwater streams in spring and autumn 2014 in Austria. Furthermore some environmental parameters were determined. Typical spring-associated limestone taxa were investigated such as the diatoms *Delicata delicatula*, *Denticula tenuis*, *Encyonopsis microcephala*, *Cymbopleura austriaca*, *Encyonopsis cesati*, *Gomphonema lateripunctatum*, *Brachysira calcicola* ssp. *pfisteri* and the green alga *Oocardium stratum*. *Oocardium stratum* is a rare desmid occurring exclusively in travertine depositing headwaters. It builds calcareous tubes and is anchored with gelatinous stalks in these tubes. The tubes are extended through physiological activity. Four headwaters streams were known for the occurrence of *Oocardium stratum* and 3 new locations were discovered. Multivariate statistics did not reveal any seasonal difference between microphytobenthos communities. Instead, a geographical pattern was obtained resulting in 7 groups. An indicator species analysis identified 22 indicator species for these groups. Significant environmental parameters for taxa composition are anthropogenic alteration of the habitat, total phosphorous, pH, conductivity, bicarbonate concentration, Langelier saturation index, sky openness, chloride concentration and carbonate precipitation rate. *Oocardium stratum* occurs exclusively in intact headwaters with low anthropogenic disturbance and decreased total phosphorus amounts. Furthermore it exists at higher calcium carbonate precipitation rates. All in all

typical spring-associated limestone taxa were found and important environmental parameters for taxa composition were investigated.

Pictures



A.1. Woe – Woellersdorf, Lower Austria



A.2. Lut – Lunz (top), Lower Austria



A.3. Lud – Lunz (down), Lower Austria



A.4. Poe – Poellerbach, Lower Austria



A.5. Alm – Almassysschloessl, Lower Austria



A.6. Zoo – Tuffbach, Tyrol



A.7. Hoc – Hochtalalm, Tyrol



A.8. Lin – Lingenauf, Vorarlberg



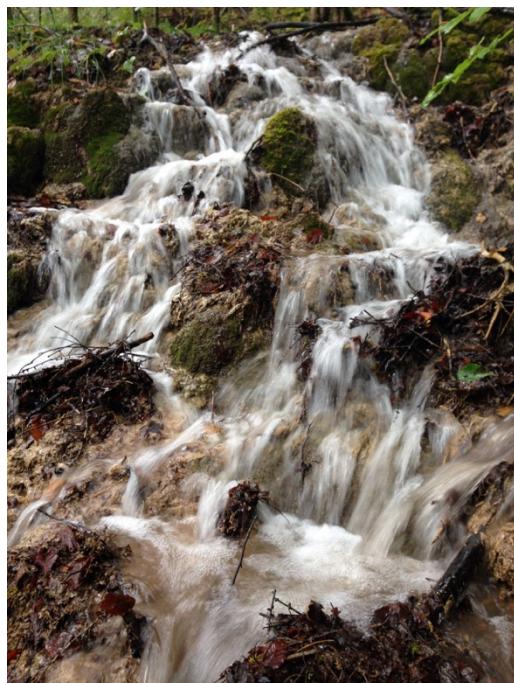
A.9. Pre – Preintal, Lower Austria



A.10. Mar – Maria Neustift, Upper Austria



A. 11. Dan – Dandlgraben, Upper Austria



A.12. Lap – Lappenbach, Carinthia



A.13. Tes – Teschengraben, Lower Austria



A.14. Edl – Edlbach, Upper Austria



A.15. artificial substratum (nail and washer) for calcium carbonate precipitation rate.



A.16. self-made sampling device for algae communities.

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