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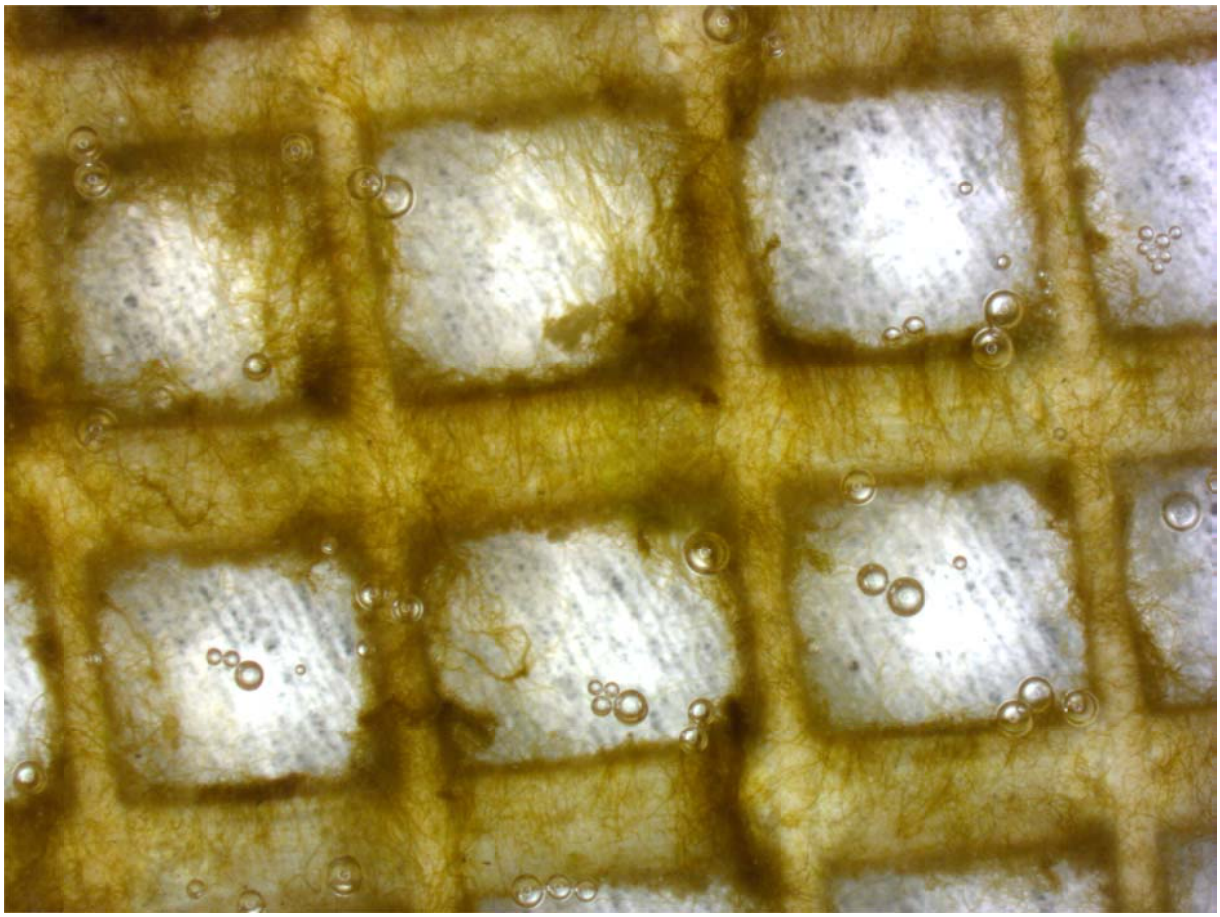

Algal community on artificial substrate

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1 Allgemeine Einleitung

Unter dem Begriff „Algen“ versteht man eine heterogene Gruppe von Photosynthese betreibenden Organismen, die durch funktionelle Merkmale definiert wird und sowohl Prokaryoten als auch Eukaryoten umfasst. Von höheren Pflanzen unterscheiden sich Algen lediglich durch ein Fehlen des typischen Kormusbaues und Leitgeweben (Graham *et al.*, 2009). Algen leisten mit ungefähr 45% an der Gesamtproduktion einen enormen Beitrag zur Fixierung von Kohlenstoff auf der Erde, die globale jährliche Produktion beträgt alleine in den Ozeanen zwischen 36,5 und 50 Gt C pro Jahr (Antoine *et al.*, 1996, Longhurst *et al.*, 1995). Außerdem spielten Algen als photoautotrophe (sich „von Licht ernährenden“) Organismen in der Erdgeschichte eine entscheidende Rolle, denn sie trugen zur Entstehung der Erdatmosphäre, wie wir sie heute kennen, maßgeblich bei (Catling & Claire, 2005). Neben diesen wichtigen Prozessen sind Algen für den Menschen auch wegen ihrer vielfältigen Verwertbarkeit von Bedeutung. Algenbiomasse wird als Nahrung und Nahrungsergänzungsmittel eingesetzt, Bestandteile von Algen als Eindickungsmittel (Agar, Carrageen und Carragenan) und als Farbstoffe in kosmetischen Produkten verwendet (Carole A. Lembi, 1988, Milledge, 2011). Die Schalen abgestorbener Kieselalgen finden sogar in Form von Kieselgur als natürliches Insektizid Anwendung (Korunic, 1998). Auch bei der Produktion von Biotreibstoffen könnte Algen in Zukunft eine größere Bedeutung zukommen (Chisti, 2007). Weiters stellt der Einsatz von Algen zur Reinigung von Abwässern eine sinnvolle Alternative zu chemischen Reinigungsprozessen dar (Hoffmann, 1998).

Die positiven Eigenschaften von Algen können schnell an Bedeutung verlieren, wenn sie z.B. durch unerwünschte massenhafte Vermehrung (Algenblüten) in Erscheinung treten. Vor allem das Massenvorkommen der häufig als „Blaualgen“ bezeichneten Cyanobakterien stellt ein Gesundheitsrisiko dar, da sie für Wirbeltiere giftige Toxine produzieren können (Falconer & Humpage, 2005). Die Gründe für das Auftreten von Cyanobakterien über lange Zeiträume sind bis heute nicht vollständig geklärt, dürften aber in einer Multiplikation verschiedener Faktoren zu suchen sein (Dokulil & Teubner, 2000). Die Ursachen für die Eutrophierung natürlicher Gewässer sind vielfältig, meist sind sie aber auf zu hohe Nährstoffeinträge, vor allem von Phosphor, zurückzuführen. Erhöhte Nährstoffeinträge und die Akkumulation von Nährstoffen kommen häufig durch Eingriffe des Menschen in natürliche Systeme zustande, etwa durch Verschmutzung von Oberflächengewässern sowie des Grundwassers. In eutrophierten Gewässern kann es auf Grund der Sauerstoffzehrung im Zuge des Abbaus

abgestorbener Algenbiomasse zu Fischsterben kommen (Stengel *et al.*, 2011). Des Weiteren führt die erhöhte Produktivität der Algen zu einem Anstieg des pH-Wertes, was in Gewässern mit bereits erhöhter Ammoniumkonzentration zu einer Umwandlung von Ammonium zum toxischen Ammoniak führen kann (Lampert & Sommer, 1999). Die Eutrophierung von Gewässern stellt durch ihre negativen Auswirkungen ein ernsthaftes Problem dar und sollte durch gezielte Maßnahmen möglichst unterbunden werden.

Das Heustadelwasser ist ein ehemaliger Altarm der Donau und liegt im grünen Prater in Wien. Im Zuge der Regulierung der Donau 1875 wurde das Gewässer vom Hauptstrom abgeschnitten und blieb nur mehr über das Grundwasser mit diesem verbunden. Der Kraftwerkbau Freudenau und die damit einhergehenden baulichen Veränderungen führten schließlich dazu, dass das Heustadelwasser völlig vom Grundwasserstrom abgeschnitten wurde (StadtWien, 2011). Die ehemals vorhandene Dynamik der ursprünglichen Aulandschaft ging verloren und die Akkumulation von Nährstoffen sowie Sedimenten würde ohne weitere menschliche Eingriffe früher oder später zur Verlandung des Gewässers führen. Eutrophierungserscheinungen einschließlich Fischsterben konnten im Unteren Heustadelwasser in der Vergangenheit bereits beobachtet werden, weshalb eine Sanierung des Gewässers in Angriff genommen wurde. Der zu diesem Zweck installierte Kiesfilter mit anschließender chemischer Phosphor-Fällung (Neptunanlage©) sollte eine Retention von Nährstoffen bewirken. Eine erste Nährstoffreduktion konnte im Jahr 2010 verzeichnet werden (Donabaum *et al.*, 2011), eine weitere Senkung des Trophiegrades war jedoch erwünscht.

Zur weiteren Reduktion von Nährstoffen ohne chemische Hilfsmittel bietet sich die Anwendung des Algal Turf Scrubber (ATS) an - einer Technologie, die erstmals von Adey and Loveland (1991) beschrieben wurde. Dabei handelt es sich um Fließrinnen, auf denen Algenrasen kultiviert werden. Ihr Funktionsprinzip ähnelt dem Selbstreinigungsprozess fließender Gewässer, in denen Biofilme höchst effizient organische und anorganische Bestandteile aus dem Wasser entfernen (Sabater *et al.*, 2002). Benthische Algen finden auf den ATS aus mehreren Gründen optimierte Wachstumsvoraussetzungen vor. Zum einen werden die Algen durch das vorbeiströmende Wasser ständig mit neuen Nährstoffen versorgt und zum anderen zeichnen sich die seichten Miniaturbäche durch optimale Lichtverfügbarkeit aus. Ein weiterer Vorteil ergibt sich durch die schubweise

Wasserversorgung mittels Kippschalen, da so um die Algen befindliche Grenzschichten, welche üblicherweise den Zu- und Abtransport von Nährstoffen und Stoffwechselprodukten limitieren, minimiert werden (Adey & Loveland, 1991). Eine weitere Reduktion von Nährstoffen kann dadurch erreicht werden, dass Phosphor in der Gegenwart von Photosynthese betreibenden Algen durch ausfallenden Kalzit gebunden wird. Zusätzlich kann die Nährstoffentfernung durch den sogenannten „Luxury uptake", bei dem Algen mehr Phosphor aufnehmen als sie benötigen, erhöht werden (Powell *et al.*, 2009).

Die erfolgreiche Anwendung von ATS-Systemen konnte schon mehrfach belegt werden. Sie eignen sich sowohl zur Tertiärreinigung von Abwässern (Craggs et al., 1996), als auch zur Reinigung von industriell verunreinigtem Grundwasser (Adey et al., 1996) und zur Gewässersanierung (Mulbry et al., 2010). Auch zur Nährstoffentfernung in Aquarien werden ATSs eingesetzt.

Zusätzlich zur Reinigungsleistung können die auf den ATS kultivierten, periodisch abgeernteten Algenrasen genützt und die gewonnene Biomasse für industrielle Zwecke weiterverwendet werden. Wie Mulbry *et al.* (2005) zeigen konnten, eignet sich die gewonnene Biomasse zum Beispiel als Langzeitdünger und ist mit der Effektivität von kommerziellen Düngern durchaus vergleichbar. Weiters wäre die Algenbiomasse als Futter für Aquakulturen oder zur Elektrizität- und Wärmegeneration einsetzbar. Auch die Verwertung der Biomasse zur Produktion von Biotreibstoffen ist denkbar, da manche Algengruppen effizienter Lipide produzieren als Kulturpflanzen (Chisti, 2007). Allerdings gibt es hinsichtlich Lipidgehaltes und Produktivität gravierende Unterschiede zwischen den Algenarten (Mata *et al.*, 2010).

Die ATSs wurden in der vorliegenden Arbeit durch natürliche Besiedelung kolonisiert, wodurch das Anwachsen verschiedenster Algenarten auf dem angebotenen Substrat ermöglicht wurde. Algenkulturen höherer Diversität sind z.B. in der Lage, bis zu 4,5-mal schneller Nitrat zu fixieren als Monokulturen (Cardinale *et al.*, 2011), weshalb sich eine verbesserte Reinigungsleistung ergibt.

Die natürliche Besiedelung des Substrates mit verschiedensten lokal vorkommenden Arten ermöglicht eine zeitliche Abfolge der Algengesellschaft – die Sukzession. Diese findet statt, wenn sich die Gemeinschaft weiterentwickelt, unterschiedliches Wachstum von Arten Änderungen derselben hervorruft und sich die Umweltbedingungen sowohl autogen als auch allogen ändern (Stevenson *et al.*, 1996). Um die Verwertbarkeit der Algenbiomasse zu beurteilen, sowie die stattfindenden Prozesse auf den ATS verstehen zu können, ist es

notwendig, die vorliegende Artengemeinschaft zu kennen. McCormick and Stevenson (1991) konnten zeigen, dass sich die in Bächen vorkommende Artengemeinschaft in frühe und späte Sukzessionsarten einteilen lassen. Diese Änderung der Artenzusammensetzung kann bei der Bestimmung des Erntezeitpunktes relevant sein, um die Zusammensetzung der erhaltenen Biomasse zu beeinflussen. Die ist auch insofern wichtig, weil es bei Überschreiten einer gewissen Komplexität des Biofilmes zum Abreißen der Algenmatten und damit zum Verlust von Nährstoffen kommt. Durch das periodische Abernten der Biomasse zum richtigen Zeitpunkt kann dieser Verlust reduziert werden. Die Entfernung der Biomasse bedeutet eine massive Störung der Artengemeinschaft und die ATS müssen neu besiedelt werden. Nach der Störung einer Artengemeinschaft erfolgt die ideale Sukzession der Wachstumsformen von fest anhaftenden Algen, die trotz Störung am Substrat haften blieben, zu schnell wachsenden apikal anhaftenden Taxa, die schließlich von gestielten, filamentösen oder beweglichen benthischen Algen abgelöst werden (Stevenson et al., 1996).

Um herauszufinden, wie sich die Algengemeinschaft auf den ATS zusammensetzt und im Laufe der Versuchsperiode ändert, wurden in der vorliegenden Diplomarbeit folgende Fragestellungen untersucht: (1) Wie rasch werden die angebotenen ATS-Flächen besiedelt, (2) welche Algentaxa dominieren die Algengemeinschaft und (3) wie verändert sich die Diversität der Algen während dieser Zeit?

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2 Algal community structure on algal turf scrubbers

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2.1 Abstract

In the present study, the algal community structure growing on artificial stream mesocosms was investigated. The so-called algal turf scrubbers (ATSs were connected to a water purification system of a highly eutrophicated backwater in Vienna. The main objective of this study was to identify the succession rate and composition of the algal biomass.

Sampling was done on a weekly basis with a modified Douglas sampler from June to August 2011 and comprised 3 runs. Estimation of the coverage and photographic documentation for micromapping was done in the field and microscopic analyses of both living and fixed material were carried out in the laboratory. Additionally, pigment analyses were performed to get insight into contribution of algal groups to total biomass.

The microscopic analyses revealed a highly diverse community. About 200 taxa were identified mainly belonging to Chlorophyta (64 species), Bacillariophyceae (63 species) and Cyanoprocaryota (34 species). Already one week after exposition, 20 – 30 different taxa were detected on the ATSs, suggesting a rapid colonization of the offered substrate. Additionally the results obtained by nonmetric multidimensional scaling (final stress of 8.58) implicate that not only seasonal but also successional processes had an impact on the species composition on the ATSs. The first axis (r^2 = 0.387) separated the algal community found at earlier stages from the dates towards the end of each run and the second axis (r^2 = 0.459) split the first run clearly from the third run.

Keywords: Periphyton, eutrophication, succession, ecological engineering, biofilm

2.2 Introduction

Restoration of natural waters plays a key role in water management, because high nutrient inputs favour eutrophication processes. Highly eutrophicated waters feature algal blooms and comparably low species diversity (Graham *et al.*, 2009). Especially the mass development of cyanobacteria, also known as blue-green algae, can cause severe problems. Some cyanobacteria are known to produce several kinds of toxins (Falconer & Humpage, 2005), which are a threat to human health. Moreover, algal blooms are often followed by fish kills due to oxygen depletion (Ruuhijarvi *et al.*, 2010), when algal biomass is degraded. Furthermore, high algal productivity leads to an elevation of the pH thus causing a shift from ammonium to toxic ammonia. This may cause serious problems in water bodies with already elevated ammonium contents, such as fish ponds (Lampert & Sommer, 1999). Another negative effect of algal blooms is a reduced recreation value for humans because of high turbidity and scums drifting on the water surface.

The reasons for enhanced nutrient inputs or accumulation are highly divers. Amongst others, disconnection of side arms from the main channel can favour eutrophication due to the biomass aggregation and siltation. This is also the case at the Heustadelwasser, a former backwater body of the river Danube. Structural measures, such as the regulation of the main river and the construction of hydropower plant Freudenau, led to a disconnection from the main river and to eutrophication. Therefore, the city of Vienna conducted a first set of restoration measures to reduce nutrient levels and to prevent fish kills (Donabaum *et al.*, 2011). In the course of the restoration measures, the so-called Neptunanlage was built. This construction comprises a gravel filter facilitated with a chemical phosphorus precipitation. These measures showed already first positive effects, but a further reduction of nutrients is desired.

One approach to reduce nutrient levels in water bodies is the application of so-called algal turf scrubbers (ATSs), a technology first described by Adey and Loveland (1991). The underlying process is comparable to the self-purification process in natural streams, where biofilms are highly efficient in removing both inorganic and organic compounds (Sabater *et al.*, 2002). The photoautotrophic biofilm developing on the ATSs incorporates nutrients of the concerned water and can be harvested afterwards. In comparison to suspended algae there are several advantages of the periphyton growing on the ATS (Hoffmann, 1998). The prevailing optimized conditions lead to an enhanced nutrient uptake and allow the benthic

community to outcompete the planktic species occurring in the Heustadelwasser. A further reduction of P can be reached due to co-precipitation of inorganic phosphate on calcite in the presence of photosynthesizing algae, as it was observed by Hartley *et al.* (1997). Additionally the application of the ATS technology enables the so-called “luxury uptake” of phosphorus (Powell *et al.*, 2009), which increases P removal. Moreover the use of wave surge buckets ensures improved growth conditions because boundary layers, which otherwise would limit nutrient and metabolite exchange, get reduced (Adey & Loveland, 1991)

ATS-systems have a big potential to improve water quality in several areas of application. In some of them, such as aquaria this technology is already well established and nutrient removal is quite efficient. Additionally, ATSs have already been tested successfully as tertiary treatment of secondary sewage (Craggs *et al.*, 1996), for purification of industrially contaminated groundwater (Adey *et al.*, 1996) or in the course of restoration measures of big streams (Mulbry *et al.*, 2010).

Compared to other water purification systems, the ATS technology is an economical and environmental friendly way to improve the water quality, because the use of chemicals is not necessary. Additionally, algal biomass and the retained nutrients can be harvested periodically and utilized for industrial purposes. For example, its application as slow-release organic fertilizer turned out to be as effective as commercial produced fertilizers (Mulbry *et al.*, 2005). Furthermore the algal biomass could be used as feedstock for the production of renewable biofuel, because many algal species produce fatty acids more efficiently than crop plants (Chisti, 2007). Algae taxa vary in lipid contents and productivity (Mata *et al.*, 2010), therefore it is advantageous to know species dominating the community growing on ATSs.

As a result of the varying productivity, the cleaning efficiency of the biofilm strongly depends on the algal assemblage, which in turn results from the prevailing conditions on the ATS. In our case the algal community on the ATS-systems consisted of a natural selection of local taxa occurring in and around the Heustadelwasser. This approach of natural settlement was already applied by Adey *et al.* (1993), who carried out a study on the phosphorus removal from natural waters using controlled algal production.

For nutrient removal efficiency of ATSs, it is necessary to know the taxa composition of the communities and how they change within the season. Such studies help understanding the on-going processes of benthic algal succession and enable a classification of early and late

successional species, as McCormick and Stevenson (1991) did. This information is also important for defining the best time for harvesting the photoautotrophic biofilms. We expected this natural algal assemblage to change throughout the study period: abiotic factors as irradiance and nutrient supply and temperature change during the year; biotic processes on the ATS force a shift of the algal community leading to an altered nutrient uptake or even a nutrient release when the senescent biofilm detaches from the ATS surface.

The objective of this study was to investigate the algal community structure over time occurring on the ATSs. The following study questions were raised: (1) how fast do the ATS get colonized after exposition; (2) which algal taxa dominate the community on the ATS; and finally (3) how does the species diversity change over time? We analysed the algal community on the ATS with the help of light microscopy and pigment analyses, as well as through visual inspection and digital imaging documentation. The results obtained in this pilot study certainly helps to improve water cleaning systems based on algae cultivation and thus support environmentally friendly solutions for nutrient recycling.

2.3 Material and methods

Study site

The Lower Heustadelwasser, a former backwater body of the Danube is located in the recreation area "Prater" in Vienna, Austria: Longitude 16°25'59'', Latitude: 48°11'56''. The area is 1.8 ha (=18,000 m^2) and the mean depth 1.5 m (154.96 m.a.A). The mean conductivity from June to September 2011 was 548 ± 217 µS cm^{-1}, total nitrogen ranged between 0.64 and 1.23 mg L^{-1} and the total phosphorus ranged between 36 and 67 µg L^{-1} (Table 2), (Donabaum et al., 2011).

Algal turf scrubber (ATS)

We constructed several vertical and horizontal ATS prototypes to find out which type of ATS would meet the requirements given at the study site. Four replicates (A, B, C, D) of a horizontal type were finally used and connected to the existing irrigation system on top of the gravel filter which is part of the Neptunanlage. The system consisted of a horizontal wooden flow lane covered with pond liner and equipped with a tipping bucket at the inflow of the ATS. To facilitate growth of periphyton, a 5 mm mesh size polyethylene net was attached at the flow lane surface, which had a slope of about 1 %. As the irrigation of the gravel filter was not continuous, a 2 cm high bar was fixed at the outflow of each ATS in order to prevent the biofilm from drying out. The system was equipped with 30 cm high splash protection behind the tipping bucket to reduce water loss. The amount of water remaining on the ATS between two tipping events was about 15 l and comparable to the volume of the tipping bucket (~ 10 l). The tipping buckets were made out of 2 mm thick stainless steel facilitated with disassembled bicycle hubs which were used as ball bearings. Due to the special shape of the buckets and the position of the rotation points, centres of gravity were changing while charging and discharging of the buckets. Therefore the tipping buckets worked without additional energy supply. The tipping frequency of the buckets was 2.71 ± 0.77 times min^{-1} and the amount of water running over one ATS was 1,529 ± 443 l h^{-1}.

Altogether 3 runs were conducted. Run 1 lasted from 3.6.2011 until 12.7.2011, run 2 from 12.7.2011 to 9.8.2011. Run 3 was carried out from 16.8.2011 to 20.9.2011. Sampling was done weekly with a modified Douglas sampler (Douglas, 1958). The algal biomass was scrubbed of with a brush (~ 3 mm long bristles) and transferred to the collecting bottle with

the help of a vacuum flask and a suction unit. Random numbers from 1 – 64 were generated with the help of the statistic software R 2.12.1 and a grid with 64 fields was constructed for ensuring arbitrary sampling positions on the net (Figure 9, centre). Depending on the thickness of the biofilm mixed samples from 1 – 4 fields were taken to get enough biomass for further analysis. For qualitative microscopical analysis additional samples of filamentous algal colonies were taken. The whole biomass growing on the ATSs was harvested every 28 – 39 d with the help of a window cleaner. Afterwards, the substrate was cleaned with brushes before starting the next run.

Macroscopic estimation of biofilm

Total coverage of the ATS, the green and the brown fraction were estimated in percentage of the area within the used grid. Estimation was done at every sampling date before taking quantitative samples.

Micromapping

Digital images of the biofilm surface on the four ATSs were taken with a single lens reflex camera (Nikon D90 equipped with a circular polarization filter) at every sampling date. Later on the photos were rectified with the software ShiftN 3.6 and the software package ImageJ 1.46 was used to split the RGB-channels with the task „Image - Colour - Split RGB“. Labelling of the respective coloured area (green algae, diatoms and uncovered area) was done with the function „Image – Adjust – Threshold“ and with „Analyse – Analyse Particles“ the percentage of every colour fraction was calculated separately. Pictures taken from the ATS before exposition (without biofilm) served as zero values.

Chlorophyll-a

The chlorophyll-a (chl-a) amount of the biofilm growing on the ATS was measured spectrophotometrically. A defined volume of each sample was homogenized, filtered (GF/C filters) and stored at minus 20 °C. For the extraction of the pigments, the filters were crushed with a Polytron homogenizer (Polytron PT 1600 E), covered with 9 ml of 90% acetone and stored for 12 h in the fridge at 5 °C. After centrifugation, the supernatant was measured with the spectrophotometer U – 2001 (Hitachi) at 663 nm (Lorenzen, 1967). Chl-a of the supplying

water was analysed as threshold value.

High Performance Liquid Chromatography (HPLC)

HPLC was used for quantification of algal pigments. Samples were homogenized through shaking and a defined volume transferred on a GF/C filter. The further procedure was done according to chl-a. Pigments were analysed by means of a VWR Hitachi LA CHROM Elite®-System (composed of a L-2130 pump, a L-2200 Auto Sampler, a Column Thermostat L-2300 with temperature of 35 °C, a L-2455 Diode Array Detector and a L-2485 FL-Detector; Column Superspher RP – 18, adjusted with a Precolumn LIChrocart rP-18; gradient program after (Wright *et al.*, 1991). Pigments were quantified at 440 nm and identified by comparison of the retention times with authentic standards (DHI, Denmark)and spectral data within the VIS range (Jeffrey, 1997). For the calculation of algal groups based on total chl-a, we used the software package Chemtax 1.95 (Mackey *et al.*, 1996)

Microscopy

Both living material and fixed samples were identified using the compound microscopes (Zeiss Axio Imager M1, Axio Cam MRc5, Axio Vision Release 4.7.2; Reichert Polyvar, Olympus soft imaging solutions FireWire, Cell^{F}). Additionally, intact biofilm was analysed with the stereo microscope Zeiss Stereo Lumar.V12 (Axio Cam ERc5s, Axio Vision Release 4.8.2) for investigating its 3-dimensional structure. Relative frequency estimation and classification on a semi-quantitative scale from 1 to 5 (1 = occasional, 2 = rare, 3 = common, 4 = frequent, 5 = dominant) was done with living material. For identification and quantification of the diatoms, permanent slides from run 1 and 3 were prepared and 500 frustules identified (Pfister & Pipp, 2010): samples were wet combusted with HCl, HNO_3 + H_2SO_4 and embedded in Naphrax. Identification followed: Bacillariophyceae: Krammer and Lange-Bertalot (1988, 2004, 1991, 1986); Chlorophyta: Ettl (1983), Ettl and Gärtner (1988), Kadłubowska (1984), Mrozińska (1985), Pascher A. (1930); Chrysophyceae: Starmach (1985); Conjugatophyceae: Lenzenweger (1996, 1997, 1999); Cyanoprokaryota: Geitler (1930), Komárek and Anagnostidis (1999, 2005); Dinophyta: Popovský and Pfiester (2008); Xanthophyceae: ,Ettl (1978), Rieth (1980). The faunal community was not identified in detail.

For characterizing algal diversity on the ATSs, species richness (= number of taxa S; alpha diversity) over time was analysed for all taxa. The Shannon Index H_s was calculated for the

diatom communities of run 1 and 3 following the formula $H_s = - \Sigma\ p_i \cdot \ln p_i$, where p_i is the proportion of the number of individuals of taxon i to the total of individuals (Smith & Smith, 2009). Run 2 was excluded because of a malfunction of the Neptunanlage. Additionally, the Shannon-Wiener Evenness E_s was calculated with the formula $E_s = H_s / H_{max}$, where $H_{max} = \ln S$ (Smith & Smith, 2009, Krebs, 1986).

Succession of run 1 and run 3 were quantified with the help of species turnover rates. Abundance data of the diatoms and estimated abundance of the fresh samples were transformed to presence / absence data and for the whole algal community the turnover rate (TO) was calculated according to Smith and Smith (2009) using the formula $TO = (I + E) / (S_t + S_{t+1})$, where TO = turnover between the timespan t and t + 1, I = Invasion (number of species present at t+1 but absent at t), E = Extinction (number of species present at t but absent at t+1), S_t = Society at t (number of species present at t), S_{t+1} = Society at t+1 (number of species present at t+1). TO ranges between 0.0 (= no gain or loss of species) and 1.0 (= complete change of species).

Statistical Analysis

Nonmetric multidimensional scaling (NMS)

To find community patterns, nonmetric multidimensional scaling (NMS) was performed (PC-ORD 5), (McCune & Mefford, 2006). Data of the fixed samples (diatoms) consisting of absolute frequencies. An additional NMS run was performed with the total algal community by merging the relative frequencies of the fresh samples with diatom frequencies (percentage contribution of diatom taxa was transformed to relative frequencies according to < 0.4 % = 1; 0.4 – 2 % = 2; 2 – 10 % = 3; 10 – 50 % = 4; > 50 % = 5). The pattern obtained with the diatom community was comparable with the results received from the whole dataset. For the final solution exclusively absolute diatom data were used because the results showed a higher final stability and lower stress. Before performing the NMS, the matrix was checked for failures (in the PC-Ord menu: “Advisor – show current profile”). Furthermore, the two species *Cocconeis pediculus* and *Fragilaria capucina var vaucheriae* were excluded because they only occurred in run 2. NMS was finally performed with 63 taxa. First, NMS was performed three times with the autopilot. The resulting scree plots were checked for the number of dimensions necessary to explain the underlying pattern of the diatom community. Except of this the plot of stress versus iteration number was examined for a constant low

stress to ensure stability of the solution. Sorensen (Bray-Curtis) coefficient was used as distance measure and three dimensions were chosen for the final solution to guarantee sufficient stress reduction and still produce an interpretable result. With the real data, 250 runs were carried out with a maximum number of 500 iterations for the final solution. Random numbers were used as starting configuration by choosing time of day for the random number seeds. A Monte Carlo test was run (1000 times) and the resulting p-value examined to see if a similar final stress could have been obtained by chance. The final NMS was performed six times to check for stability of the result and the graph chosen which explained the underlying pattern best.

Further statistical analyses were performed using R 2.12.1, SigmaPlot 11.0, and Microsoft Excel 2010.

2.4 Results

Estimation of biofilm

After the initial phase of 6 d, around 70 – 90 % of the ATS area was covered with algal biomass. Just at the end of run 2, the total coverage was lowered because a defect of the Neptunanlage resulted in deficient water supply. Within the first run the biofilm covered about 90 % of the area after one week and didn't increase further. At the beginning of run 1 the brown fraction was dominating, indicating a high abundance of Bacillariophyceae, followed by a shift to green in the next weeks. These findings were supported by the micromapping results. Run 2 differed from the first run in several ways. First of all, the estimated coverage was slightly higher (up to 100 %), second a raised green area – compared to run 1 – was observed already at the beginning of the second run, which was replaced by the brown fraction until the end of the run. In general the brown fraction seemed to dominate in the 2nd run. The third run was characterized by a strong decline of biofilm at the end of the run, where just 60 % of the area was colonized. Furthermore diatoms were clearly dominating, whereas green algae covered less than 20 % of the area (Figure 1).

Micromapping

Analyses of the digital images showed comparable patterns to microscopic results. Within a few days after exposition, the area corresponding to the blue channel (= colonized area) decreased indicating a rapid colonization of the ATS. The fraction of the blue channel was lowest between the third and fifth week of every run. The green and red channel (green algae and diatoms) showed a high variation between the three runs. In run 1, diatoms dominated and accounted for approximately 60% in the first half of the run. Thereafter, they decreased and were partly replaced by green algae. At the beginning of the second run green algae were nearly as abundant as diatoms, and both groups showed a further increase in the next weeks. Especially, green algae showed a high standard deviation indicating a varying development of the four replicates. In run 3, unicellular diatoms developed faster than green algae but were overgrown by green filamentous algae in the second week. In the third and fourth week colony forming and epiphytic diatoms got more abundant and diatoms dominated again. After this, the senescent diatom colonies detached and facilitated a recovery of green algae, but the uncolonized area increased up to 20 % (Figure 2). The visual

inspection of the digital images (Figure 3) showed the following changes: within a few days after exposition the ATSs were covered with a thin brown layer mainly consisting of diatoms. Two weeks later fast growing diatoms had formed filamentous arrangements which resulted in an enhanced patchiness of the biofilm. Additionally the first attached green algae appeared, especially close to the tipping buckets, where the flow velocity was highest. Between the second and third week after exposition, enhanced growth of filamentous algae was observed and the thickness of the biofilm increased. Within the third week the first floating algal mats occurred. Maturing of the biofilm finally led to detachment of the algal mats between fourth and fifth week, which resulted in a decreased coverage of the ATSs.

Chlorophyll-a

At the beginning of run 1, algal growth started somehow delayed (Figure 4, bottom), followed by a rapid accession after 3 weeks. A first chl-a maximum was reached with 310 ± 90 mg m^{-2} after 4 weeks and a second one was recognized in the sixth week, where 355 ± 30 mg m^{-2} chl-a were measured. Run 2 showed a much faster chl-a increase and the highest values were obtained after 3 weeks, when chl-a amounted to 700 ± 120 mg m^{-2}; this was the highest value occurring for the whole experiment. The third run's chl-a peak was measured between third and fourth week. The chl-a content of the water pumped to the ATS showed just small deviations and ranged between 21.1 ± 1.5 and 69.1 ± 1.9 µg L^{-1} chl-a.

High performance liquid chromatography

With the help of HPLC analysis, the four major algal groups Bacillariophyceae, Chlorophyta s.l., Chrysophyceae and Cyanoprokaryota were identified (Figure 4, top). Bacillariophyceae always accounted for more than 50 % of total biomass. Within the third run diatoms made up to around 90 %. The second largest group was the Chlorophyta (maximum of 41 % at the end of run 1). Chrysophyceae were the third smallest algal group contributing up to 12 % of Chl-a at the beginning of run 1; thereafter, they declined until the end of run 1 and remained low for the remaining period. The smallest group comprised Cyanoprocaryota with less than 3 % throughout the investigation period.

Microscopy

Over the whole sampling period we identified 196 algae taxa belonging to 9 different phyla (Table 1). The Chlorophyta were represented by 64 taxa and also the Bacillariophyceae were highly abundant (63 taxa). The third largest group were the Cyanoprocaryotes, where 34 taxa were identified. Just one single species belonged to the Rhodophyta and one to the Haptophyta (Figure 5). As expected, we found mainly benthic species, however some planktic taxa were also observed. *Mougeotia* sp., *Coelastrum astroideum* De Notaris*, Pediastrum duplex* Meyen*, Phacotus lenticularis* (Ehrenberg) Stein*, Coelosphaerium aerugineum* Lemmermann were occurring during the whole sampling period, the latter four are planktic. Microscopy of the living samples showed that within the Chlorophytes the filamentous genera *Mougeotia*, *Cladophora*, *Spirogyra* and *Oedogonium* were very common. The highest abundance of these genera was detected in June and July together with chl-a – maxima. After the malfunction of the Neptunanlage in August, the filamentous greens were less frequent until the end of the observation period, except of *Cladophora*. Planktic algae belonging to the genera *Scenedesmus* (8 species), *Pediastrum* (6 species), *Coelasturm* (6 species), *Tetraedron* (5 species) and *Chlamydomonas* sp. were present over the whole sampling period, but not in high numbers. Although low in frequency, the green flagellate *Phacotus lenticularis* was present at all runs. The prokaryotic taxa *Coelosphaerium aerugineum* and *Microcystis* sp. were found constantly over the whole sampling period, but also in low number. Furthermore, *Chroodactylon ornatum* (C.Agardh) Basson (Rhodophyta) and *Hymenomonas roseola* Stein (Haptophyta) were found on the ATSs. The most common diatom species was *Fragilaria capucina* Desmaziers, which formed huge ribbon-shaped colonies and made up to 88% of the fixed samples. Other highly abundant species were *Diatoma tenuis* C.Agardh, *Achnanthidium minutissima* (Kützing) Czarnecki, *Cymbella caespitosa* (Kützing) Brünn and the planktic species *Cyclotella ocellata* Pantocsek. *Diatoma vulgaris* Bory was also quite common, but did not occur before the end of run 1. The genus *Cymbella* was represented by 13 species, but just two of them – *C. microcephala* Grunow and *C. caespitosa* – were found in both runs (1 and 3) and *C. affinis* Kützing was highly abundant in run 3. Furthermore taxa like *Gomphonema*, *Nitzschia* and *Navicula* were also present, but in low number. Rare species were the centric diatom *Stephanodiscus hantzschii* Grunow and *Amphipleura pellucida* (Kützing) Kützing. Other planktonic genera were found occasionally such as the centric diatoms *Cyclotella* (5 species), *Melosira* and *Stephanodiscus*; they were

probably transferred from the Heustadelwasser to the ATSs. *Cyclotella ocellata*, *C. distinguenda* Hustedt and *C. radiosa* (Grunow) Lemmermann were found at all sampling dates. According the fauna on the ATS, we observed only few rotifers, nematodes, Vorticellidae and other ciliates. Additionally, the intact biofilm revealed the presence of a *Hydra* sp.; during sampling, a few leeches were noticed on the ATSs.

The species richness of fixed taxa ranged from 27 to 53 (Figure 6). The richness of run 1 was lower compared to run 3 where the Bacillariophyceae (fixed samples) were the most diverse group, Also within the living samples, a slight increase of the species number was detected over time. At the end of each run a decreasing amount of species was found on the ATS (Figure 6).

The calculated Shannon Index of the diatom community on the ATS ranged from 1.44 ± 0.33 to 2.45 ± 0.16 and was generally higher in run 3 compared to run 1 (Figure 7, top). Within the first run H_s increased up to 2.32 ± 0.10 on the 5th of July but dropped just before this sampling date. Run 3 revealed a similar pattern with the highest value (2.45 ± 0.16) occurring on the 13th of September (Figure 7, top). E_s calculated for the first run ranged between 0.48 ± 0.08 and 0.71 ± 0.02; it was lowest on the first sampling date. The third run's E_s showed a smaller variation and ranged between 0.63 ± 0.03 and 0.72 ± 0.06 (Figure 7, centre). Generally, E_s showed nearly the same pattern as H_s.

The highest TO of the whole sampling period occurred in run 1 one week after installation of the ATSs and amounted to 0.53 ± 0.04 (Figure 7, bottom). The third run showed a low TO (0.32 ± 0.03) at the beginning than the first run, peaked in the third week and decreased again to a minimum of 0.28 ± 0.02.

Statistical analyses

The best solution of NMS with 3 dimensions had a final instability of < 10^{-5} and a final stress of 8.58 (p-value 0.0010). NMS results indicated that the first run was more homogenous compared to the third one, which is more scattered (Figure 8). The first axis (r^2 = 0.387) separated the algal community found at earlier stages (left side) from the dates towards the end of each run (right side). Axis 2 (r^2 = 0.459) splits the first run clearly from the third run. The third axis (r^2 = 0.105) was less important and separated one group (replicate C from run 3) from the remaining data. This group showed a much higher abundance of *Diatoma vulgaris* and *Achnanthidium minutissima*, compared to the second group.

2.5 Discussion

The high degree of coverage (70 – 90 %) within the first week after installation of the ATS clearly pointed at the fast colonization of the offered substrate. Already after 5 d after exposition, we found between 22 and 33 taxa on the 4 replicates; more than the half belonged to the Bacillariophyceae. The colonization was apparently accelerated due to the pulsed water supply which was ensured by the tipping buckets. The use of tipping buckets has several advantages, which were already pointed out by Adey and Loveland (1991). First, particles and algal cells can settle at the flow lanes between two tipping events. Second, the flushes of water lead to an optimized nutrient supply by minimizing the boundary layers around algal cells, which otherwise would limit nutrient and metabolite exchange (Adey & Loveland, 1991). Another positive effect of the pulsed water supply is the improved irradiance supply: particles entering the system via the water supply are periodically washed away. Above this, the 2 cm high barrier at the outlet of the ATSs prevented the biofilm from drying out, due to the inhibition of water runoff during the irrigation breaks of the Neptunanlage.

The results suggest that diatoms dominated the algal community; regardless of the sampling date, the algal biomass was composed of more than 50 % Bacillariophyceae. Also in the Heustadelwasser, diatoms reached their first maximum in June and July, which possibly promoted the high abundance of diatoms on the ATSs. The proportions of diatoms and green algae varied between the three runs, which might be attributed to a strong impact of the season. At the start of run 1, diatoms dominated the community, but they were partly overgrown by filamentous green algae in the following weeks. The slight decrease of green algae at the end of the first run could be attributed to the recovery of diatoms. This pattern was however not observed in runs 2 and 3. The second run most likely differed because of faster re-colonization of the ATSs by green algae from the beginning. This could be due to remaining basal algal elements, attachments or holdfasts from the first run as it was also the case in a study carried out by Adey et al. (1993). Green filamentous algae were also responsible for maximum chl-a values in the experiments. A chl-a loss was detectable after the third to fifth week, and could be traced back to the detachment of the senescent biofilm. The results of the HPLC analyses were supported by the results of area estimation, micromapping and microscopy but the high pigment-fraction accounting for Chrysophyceae was not sustained by the microscopic results. With the help of microscopic analyses there

was just one genus detected, which belonged to the class Chrysophyceae. This could be an overestimation caused by Chemtax, or the chrysophytes were destroyed for the most part during sampling (by scraping off and homogenization).

At the onset of each run high numbers of single-celled algal taxa were recorded, but with increasing age of the biofilm, slowly growing filamentous green algae and other colony forming algae became more abundant. In the course of succession, a change in vertical community structure from low to high physical stature occurs, suggesting a competition for substrate surface (Hoagland *et al.*, 1982). The enhanced 3-dimensional structure provided by filamentous algae like *Cladophora glomerata* (L.) Kützing can act as substrate for other microalgae (Malkin *et al.*, 2009). The raised structural complexity of diatom and green algal colonies was well documented by the photos taken for micromapping and later on responsible for a reduced resistance of the algae, leading to the detachment of the biofilm from the substrate at the end of each run (Peterson and Stevenson (1992). The detachment of algal mats was most likely based on a process called autogenous succession (Stevenson *et al.*, 1996), which occurs when interspecific concurrence between benthic algae or algal colonies causes changes in their environment, leading to favourable or inhibiting conditions for other algal species. According to McCormick and Stevenson (1991), autogenic factors outweigh seasonal effects in short time processes leading to the assumption that autogenic succession on the ATSs was very important within a single run. In contrast, transplantation experiments carried out by Krammer and Lange-Bertalot (1991) showed that bacterial community structures were initially controlled by allogeneic factors and then followed a succession pattern dominated by autogenic factors. Larson and Passy (2012) suggest that succession in algal biofilms is largely influenced by environmental or abiotic factors. The recorded change of the species composition on the ATS showed that seasonal succession had a strong impact, because the algal community differed to a large extent between the three runs. Conditions on the ATSs were mostly the same throughout the experimental period (Table 2) but as the three runs differed from each other in respect to coverage or species composition and abundance, other external factors like irradiance supply or temperature probably had a strong impact on the successional process. According to the NMS results, mainly two processes influenced the community development on the ATSs. First of all run 1 and 3 are separated, which means that the season played a key role for the community composition (allogeneic factors). Second, earlier dates are separated from later ones

indicating that the algal community changed because of the on-going succession (autogenic succession).

Except of the changing species assemblage through time, we also recognized spatial differences of the biofilm along the flow lanes. The unprocessed photos taken for micromapping analyses showed an enhanced growth of green filamentous algae close to the tipping buckets near the inflow. The association of filamentous green algae around the buckets could be a hint for their resistance to higher current velocities. As mentioned by Catling and Claire (2005), *Cladophora* growth is usually related to the highest standing crops of benthic algae in moderate to fast currents. The area close to the tipping buckets was not included in the analysis because of too heterogeneous conditions compared to the remaining areas. In general, varying conditions on the ATS probably leads to increased biodiversity, which promotes enhanced nutrient removal (Cardinale, 2011). The high biodiversity recorded is also a result of the biofilm's filtering effect. Planktic taxa originating from the Heustadelwasser were transferred through the pipes onto the biofilm and finally retained. This observation is underlined by pictures taken with the help of the stereo microscope, showing colonies of *Microcystis* sp. trapped in filamentous colonies of diatoms (Figure 9, bottom).

For a comparison between runs, The Shannon index H_s was calculated for diatoms. Additionally to the species number, this index considers the number of individuals and offers therefore more specific information compared to the species richness. H_s rises at increasing species number and uniform distribution (indicated by the Evenness E_s) of species. The analysed community of run 3 was more diverse and more evenly distributed in relation to run 1. These findings are in accordance to increased species richness (Figure 6). The calculated evenness E_s for run 1 suggests a stronger dominance of single species compared to run 3, where a more uniform distribution existed. The decline of H_s at the third week of both runs can be explained by a change in algal composition induced by the amplified growth of filamentous green algae. Furthermore the species richness explained the change of diversity very well.

In contrast to the H_s and species richness, TO was generally decreasing until the end of the experiments, indicating that stability and balance of the algal community increased over time towards a climax stage. This implements that the gain or loss of new species declines, as it was the case in other studies, as reviewed by Anderson (2007). These findings implicate a

negative relation to species richness over time. One explanation is the increased complexity and heterogeneity within the developing biofilm and a diversified species pool (high rate of natural seeding through water supply from the Heustadelwasser), which makes community dynamics becoming a composite of differential responses (Pandit & Kolasa, 2012).
According the fauna on the ATS, our observations implicate that the algal community on the ATS was not highly affected by grazing and therefore not top-down controlled. Similar results are documented by Adey et al. (1993), who described a more divers macro-invertebrate community but who found no significant effect on the algal productivity by herbivorous forms.

The present study suggests that the algal community on ATSs strongly depends on the season. For an extended usage of the system in temperate regions like Austria, further investigations throughout the growing season from spring to autumn would be helpful to see if this is practicable in Austria. In more southern regions the ATS-technology can be also used in winter months (Adey et al., 1993). Our results show that harvesting intervals between 3 to 4 weeks are advantageous to prevent detachment and loss of algal biomass in summer. Moreover the harvesting date is important for the composition of the algal biomass, because the species abundance changes over time due to succession. If required for further applications, the biomass on the ATSs could be modified by altering growth conditions. A higher flow velocity for example could enhance the quantity of green filamentous algae. Furthermore a different slope of the flow lanes or other kinds of substrate will change the algal community. We were able to show that natural seeding of the ATS is a good way to ensure high species diversity, which probably accelerates the cleaning efficiency of the ATS. Additionally the filtering effect of the algal mats promoted particle retention. In addition to this study nutrient analyses of the algal biofilm were carried out by (Mayr, 2012) and showed, the ATS-technology has a great potential to reduce nutrients and produce algal biomass in a cost effective and environmental friendly way. For further improvements we suggest the application of serial arrangements of ATSs to get higher cleaning efficiencies. Vertical systems, which need a permanent water supply, could provide a space-saving alternative.

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2.8 List of tables and figures

2.9 Tables and figures

Table 1: Species list

Charophyta - Zygnematophyceae

Closterium aciculare T. West
Closterium ehrenbergii Meneghini ex Ralfs
Closterium sp.
Cosmarium cf punctulatum Brebisson
Cosmarium regnelli Wille
Cosmarium sp.
Mougeotia cf parvula Hassal
Mougeotia sp.
Spirogyra sp.
Staurastrum sp.
Staurastrum tetracerum Ralfs ex Ralfs
Staurodesmus cuspidatus (Brébisson) Teiling
Pleurotaenium sp.

Chlorophyta - Chlorophyceae

Ankistrodesmus bibraianus (Reinsch) Korshikov
Ankistrodesmus fusiformis Corda ex Korshikov
Chaetophora elegans (Roth) C.Agardh
Chlamydomonas sp.1
Chlamydomonas sp.2 (palmella)
Coelastrum astroideum De Notaris
Coelastrum microporum Nägeli
Coelastrum cf polychordum (Korshikov) Hindák
Coelastrum pseudomicroporum Korshikov
Coelastrum reticulatum (P.A.Dangeard) Senn
Coelastrum sp.
Coenocystis sp.
Eudorina sp.
Kirchneriella contorta (Schmidle) Bohlin
Kirchneriella cf irregularis (G.M.Smith) Korshikov
Kirchneriella lunaris (Kirchner) K.Möbius
Kirchneriella obesa (West) West & G.S.West
Kirchneriella sp.
Oedogonium sp.
Pandorina sp.
Pediastrum biradiatum Meyen
Pediastrum boryanum (Turpin) Meneghini
Pediastrum duplex Meyen
Pediastrum simplex Meyen
Pediastrum tetras (Ehrenberg) Ralfs
Pediastrum sp.
Phacotus lenticularis
Phacotus sp.
Scenedesmus gutwinskii Chodat
Scenedesmus aculeolatus Reinsch
Scenedesmus acuminatus (Lagerheim) Chodat
Scenedesmus obtusus Meyen
Scenedesmus quadricauda (Turpin) Brébisson
Scenedesmus sempervirens Chodat
Scenedesmus serratus (Corda) Bohlin
Scenedesmus sp.
Stigeoclonium sp.
Tetraedron caudatum (Corda) Hansgirg
Tetraedron minimum (A.Braun) Hansgirg
Tetraedron regulare Kützing
Tetraedron sp.
Tetraedron triangulare Korshikov
Tetraspora sp.
Uronema confervicolum Lagerheim

Chlorophyta - Siphonocladophyceae

Cladophora sp.

Chlorophyta - Trebouxiophyceae

Botryococcus cf. braunii Kützing
Crucigenia tetrapedia (Kirchner) Kuntze
Crucigeniella apiculata (Lemmermann) Komárek
Crucigeniella sp.
Crucigeniella cf rectangularis (Nägeli) Komárek
Dictyosphaerium cf ehrenbergianum Nägeli
Dictyosphaerium pulchellum H.C.Wood
Dictyosphaerium sp.
Dictyosphaerium tetrachotomum Printz
Lagerheimia ciliate (Lagerheim) Chodat
Micractinium sp.
cf Neglectella sp.
Oocystis cf parva West & G.S.West
Oocystis sp.
Selenastrum sp.

Chlorophyta - Ulvophyceae

Geminella interrupta Turpin
Geminella sp.
Ulothrix sp.
Ulothrix tenerrima (Kützing) Kützing

Cryptophyta - Cryptophyceae

Cryptomonas sp.
Rhodomonas sp.

Dinoflagellata - Dinophyceae

Gymnodinium sp.
Peridinium sp.

Euglenophyta - Euglenophyceae

Euglena sp.
Phacus cf curvicauda Svirenko
Phacus cf pleuronectes (O.F.Müller) Nitzsch ex Dujardin
Phacus sp.
Phacus sp.
Trachelomonas sp.

Haptophyceae - Prymnesiophyceae

Hymenomonas roseola Stein

Ochrophyta - Bacillariophyceae

Achnanthes clevei Grunow
Achnanthes cf conspicua A. Mayer
Achnanthes curtissima J. R. Carter
Achnanthes lanceolata (Brébisson ex Kützing) Grunow
Achnanthidium minutissima (Kützing) Czarnecki
Achnanthes sp.

Amphipleura pellucida (Kützing) Kützing
Amphora lybica Ehrenberg
Amphora pediculus (Kützing) Grunow
Asterionella cf. formosa Hassall
Cocconeis placentula Ehrenberg
Cocconeis sp.
Cyclotella distinguenda Hustedt
Cyclotella meneghiniana Kützing
Cyclotella ocellata Pantocsek
Cyclotella pseudostelligera Hustedt
Cyclotella radiosa (Grunow) Lemmermann
Cymbella affinis Kützing
Cymbella caespitosa (Kützing) Brünn
Cymbella cistula (Ehrenberg) Kirchner
Cymbella cymbiformis C.Agardh
Cymbella lanceolata (C.Agardh) Kirchner
Cymbella cf leptocerus Hustedt
Cymbella microcephala Grunow
Cymbella minuta Hilse
Cymbella prostrate (Berkeley) Cleve
Cymbella cf silesiaca Bleisch
Cymbella sp.
Cymbella tumida (Brébisson) van Heurck
Diatoma sp.
Diatoma tenuis C.Agardh
Diatoma vulgaris Bory
Epithemia sp.
Fragillaria brevistriata Grunow
Fragilaria capucina Desmaziers
Fragillaria crotonensis Kitton
Fragillaria cf pinnata Ehrenberg
Fragilaria sp.
Fragilaria ulna var. acus (Kützing) Lange-Bertalot
Gomphonema acumiatum Ehrenberg
Gomphonema parvulum Kützing
Gomphonema truncatum Ehrenberg
Melosira granulate (Meneghini) Zanardini
Melosira cf.italica (Ehrenberg) Kützing
Melosira sp.
Navicula capitatoradiata Germain
Navicula cryptotenella Lange-Bertalot
Navicula oblonga (Kützing) Kützing
Navicula sp.
Navicula radiosa Kützing
Nitzschia amphibian Grunow
Nitzschia cf bacillum Hustedt
Nitzschia dissipata (Kützing) Grunow
Nitzschia fonticola (Grunow) Grunow
Nitzschia graciliformis Lange-Bertalot & Simonsen
Nitzschia microcephala Grunow
Nitzschia cf.palea (Kützing) W.Smith
Nitzschia paleaceae
Nitzschia radicula
Nitzschia sp.
Nitzschia subacicularis
Nitzschia tubicola
Rhoicosphenia abbreviate (Agardh) Lange-Bertalot
Stephanodiscus hantzschii Grunow

Ochrophyta - Chrysophyceae

Dinobryon sp.

Ochrophyta - Synurophyceae

Mallomonas sp.

Ochrophyta - Xanthopyceae

Goniochloris smithii (Bourrelly) Fott
Goniochloris spinosa Pascher
Tribonema affinis (Kützing) G.S.West
Tribonema regulare Pascher
Tribonema sp.
Tetraplektron cf laevis (Bourrelly) Ettl
Ophiocytium capitatum Wolle
Ophiocytium sp.

Rhodophyta - Stylonematophyceae

Chroodactylon ornatum (C.Agardh) Basson

Cyanoprocaryota - Cyanophyceae

Aphanocapsa cf conferta (West & G.S.West) Komárková-Legnerová & Cronberg
Aphanothece cf smithii J.Komárková-Legnerová & G.Cronberg
Aphanothece sp.
Calothrix sp.
Chroococcus cf minutus (Kützing) Nägeli
Chroococcus cf limneticus Lemmermann
Chroococcus sp.
Coelosphaerium aerugineum Lemmermann
Coelosphaerium cf dubium Grunow
Coelosphaerium sp.
Eucapsis carpatica J.Komárek & F.Hindák
Geitlerinema sp.
Gomphosphaeria sp.
Leptolyngbya cf bijugata (Kongisser) Anagnostidis & Komárek
Leptolynbya sp.
Limnothrix sp.
Lyngbya sp.
Merismopedia sp.
Merismopedia tenuissima Lemmermann
Merismopedia punctata Meyen
Microcystis cf aeruginosa (Kützing) Kützing
Microcystis sp.
Microcystis viridis (A.Braun) Lemmermann
Microcystis cf wesenbergii (Komárek) Komárek in Komárek & Ettl
Oscillatoria sp.
Oscillatoria cf simplicissima Gomont
Oscillatoria tenuis C.Agardh
Planktolyngbya cf limnetica (Lemmermann) J.Komárková-Legnerová & G.Cronberg
Pseudanabaena cf limnetica (Lemmermann) Komárek
Snowella lacustris (Chodat) Komárek & Hindák
Snowella litoralis (Häyrén) Komárek & Hindák
cf Spirulina sp.
Woronichinia cf naegeliana (Unger) Elenkin
Woronchinia sp.

Table 2: Characteristics of the water supply for the ATSs (mean values with standard deviations and number of samples) from the Lower Heustadelwasser (June – September 2011).

Parameter	Mean	SD	n
Temperature [°C]	20.74	3.10	16
pH [-logH^+]	8.31	0.18	16
Conductivity [µS cm^{-1}]	548.19	216.98	16
Total phosphorus [µg L^{-1}]	50.75	9.74	12
Total nitrogen [mg L^{-1}]	1.04	0.16	16
Water supply of the ATSs [l h^{-1}]	1529	443	100
Tipping frequency of the buckets [tipping events h^{-1}]	109	85	100

Table 3: Maxima of the biotic parameters on the ATSs (mean values with standard deviations, n=4)

Parameter	Run 1	Run 2	Run 3
Total phosphorus [mg m^{-2}]	261.5 ± 49.9	441.8 ±69.6	224.0 ± 18.0
Areal removal rate of total phosphorus [mg m^{-2} d^{-2}]	9.7 ± 2.6	19.1 ± 2.5	10.6 ± 0.3
Dry mass [g m^{-2}]	251.9 ± 44.4	221.0 ± 23.5	249.7 ± 42.7
Ash free dry weight [g m^{-2}]	52.0 ± 10.7	68.9 ± 7.0	62.6 ± 5.12
Chl-a [g m^{-2}]	355.1 ± 35.2	700.5 ± 141.1	399.2 ± 132.9

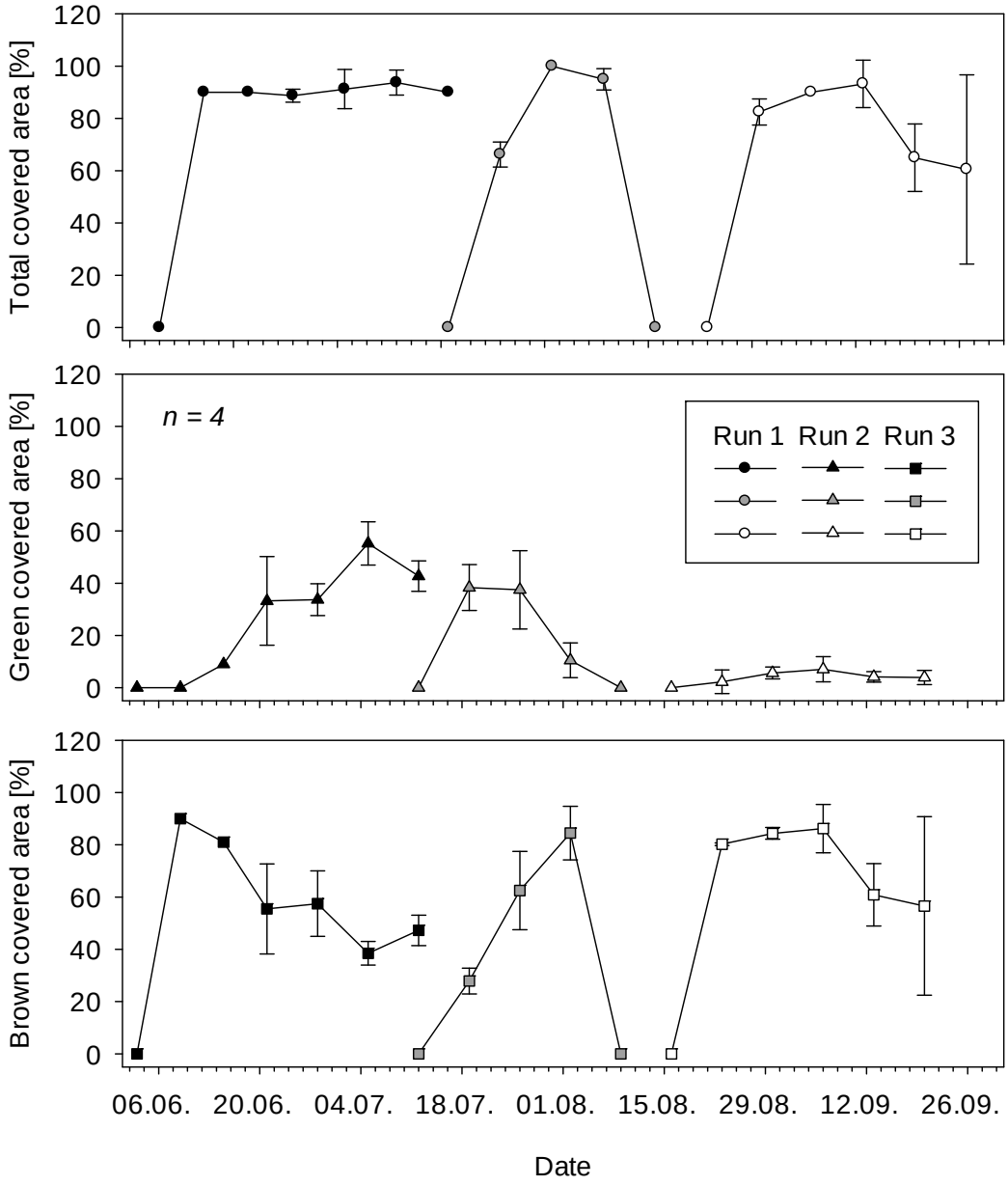

Figure 1: Estimation of coverage in percent of ATS area (arithmetic means with standard deviations)

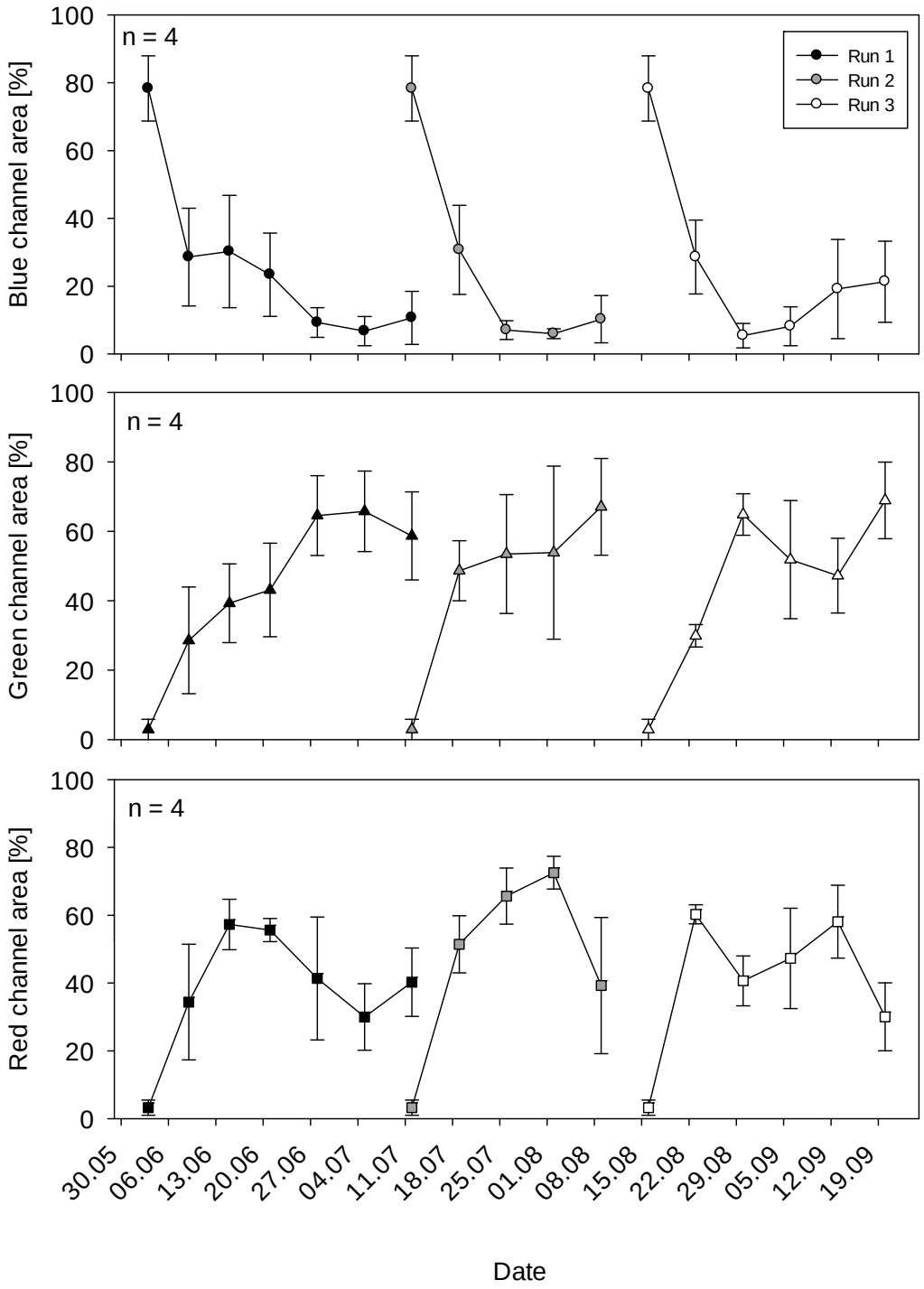

Figure 2: Area of blue channel (= uncolonized area), green channel (= green algae) and red channel (= diatoms) calculated with pixelcounts of the RGB-channels of digital pictures.

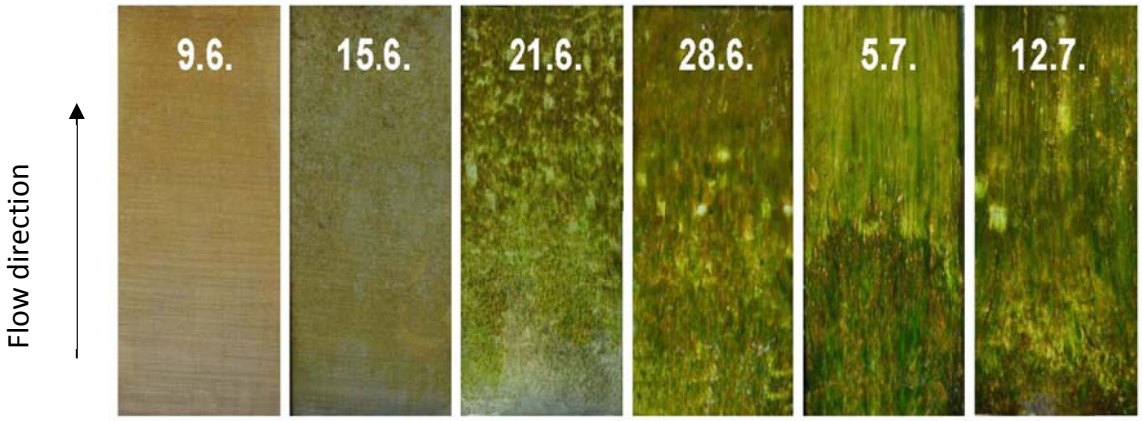

Figure 3: Pictures of the developing algal biofilm over one experimental period (run 1). RGB-Channels were used for area estimation of algal groups

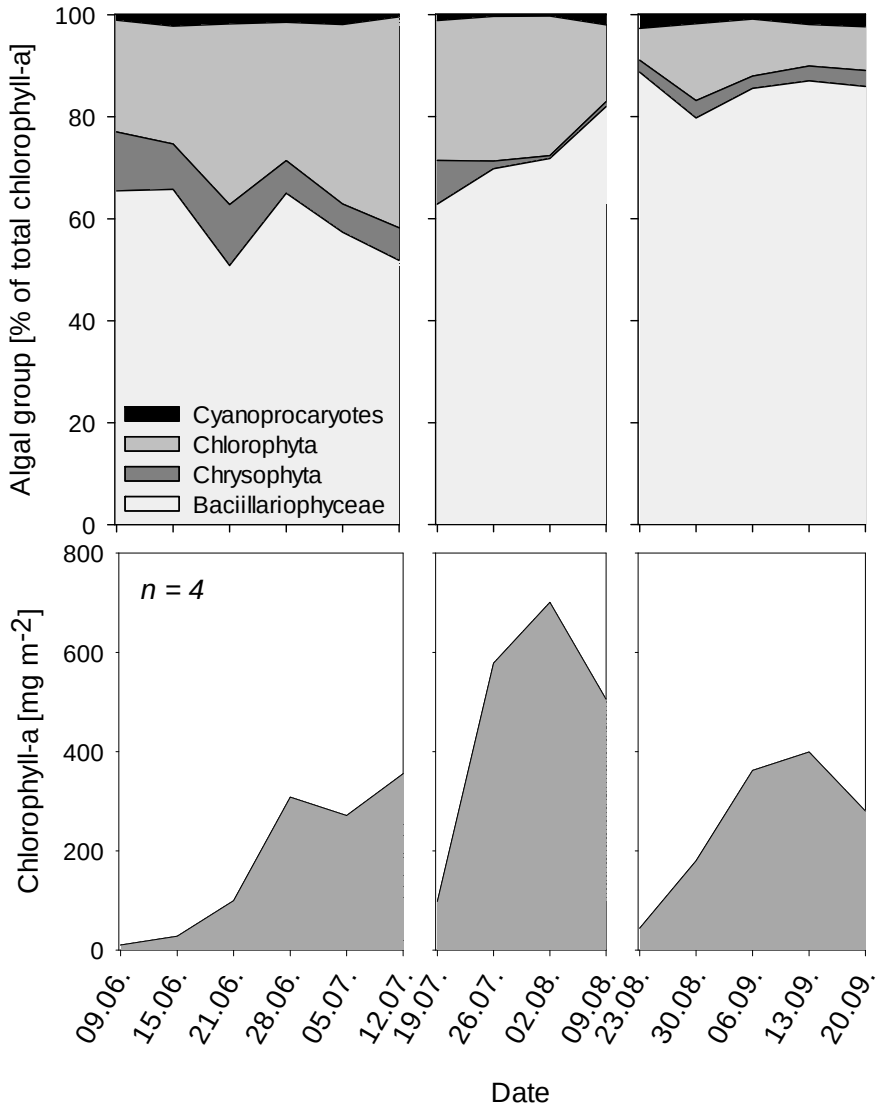

Figure 4: Dominating algal groups calculated from HPLC analyses: run 1 – 3 (upper row). Chlorophyll-a measured spectrophotometrically run 1– 3 (lower row).

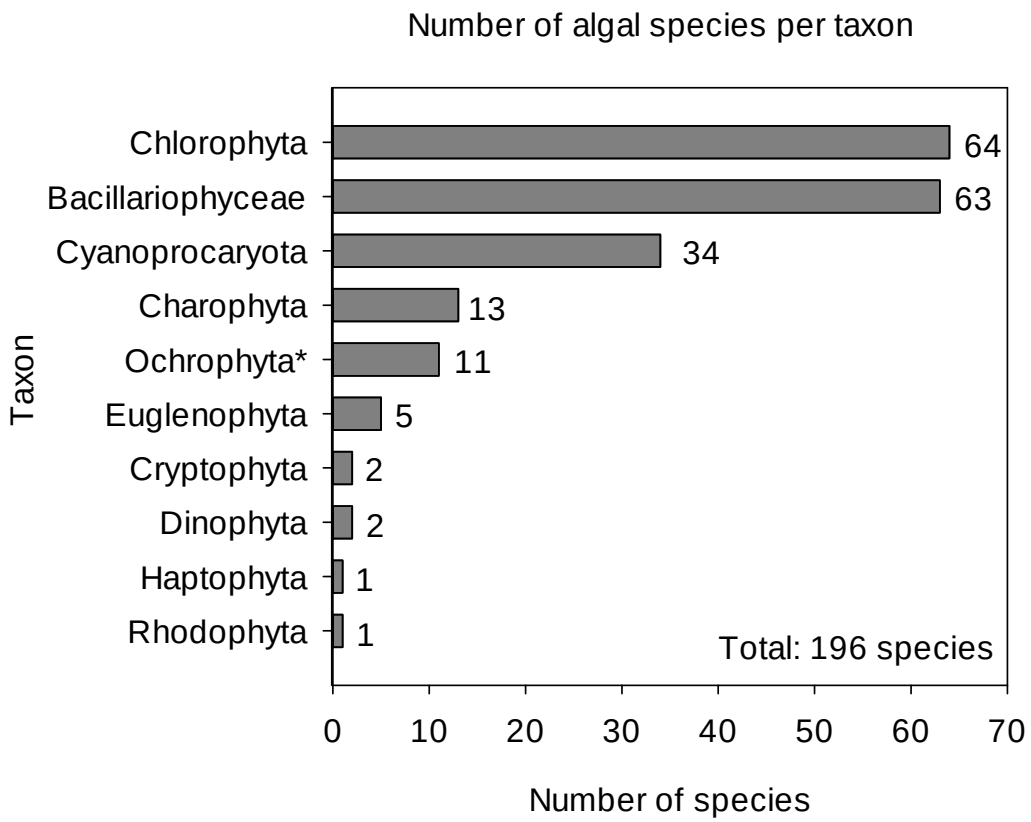

Figure 5: Number of identified algal species per taxon, over the whole sampling period (* = Ochrophyta without Bacillariophyceae).

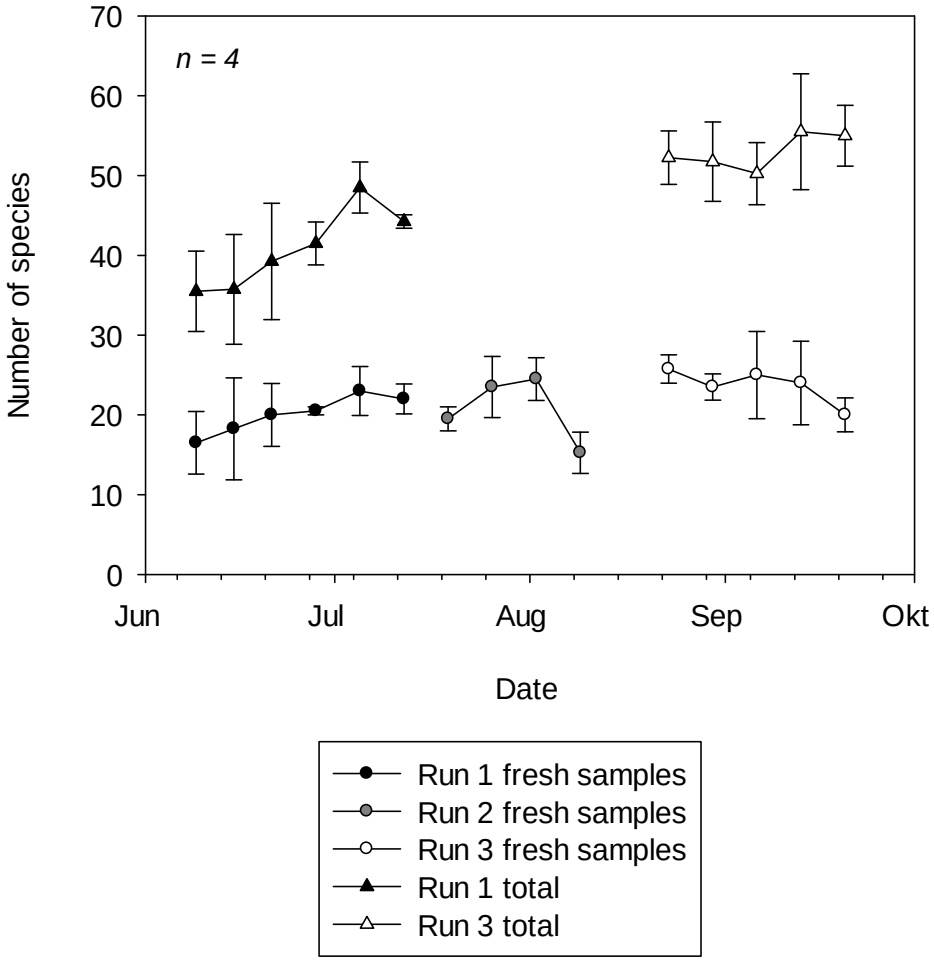

Figure 6: Number of algal species over time (total = fresh samples plus fixed samples; arithmetic means with standard deviations).

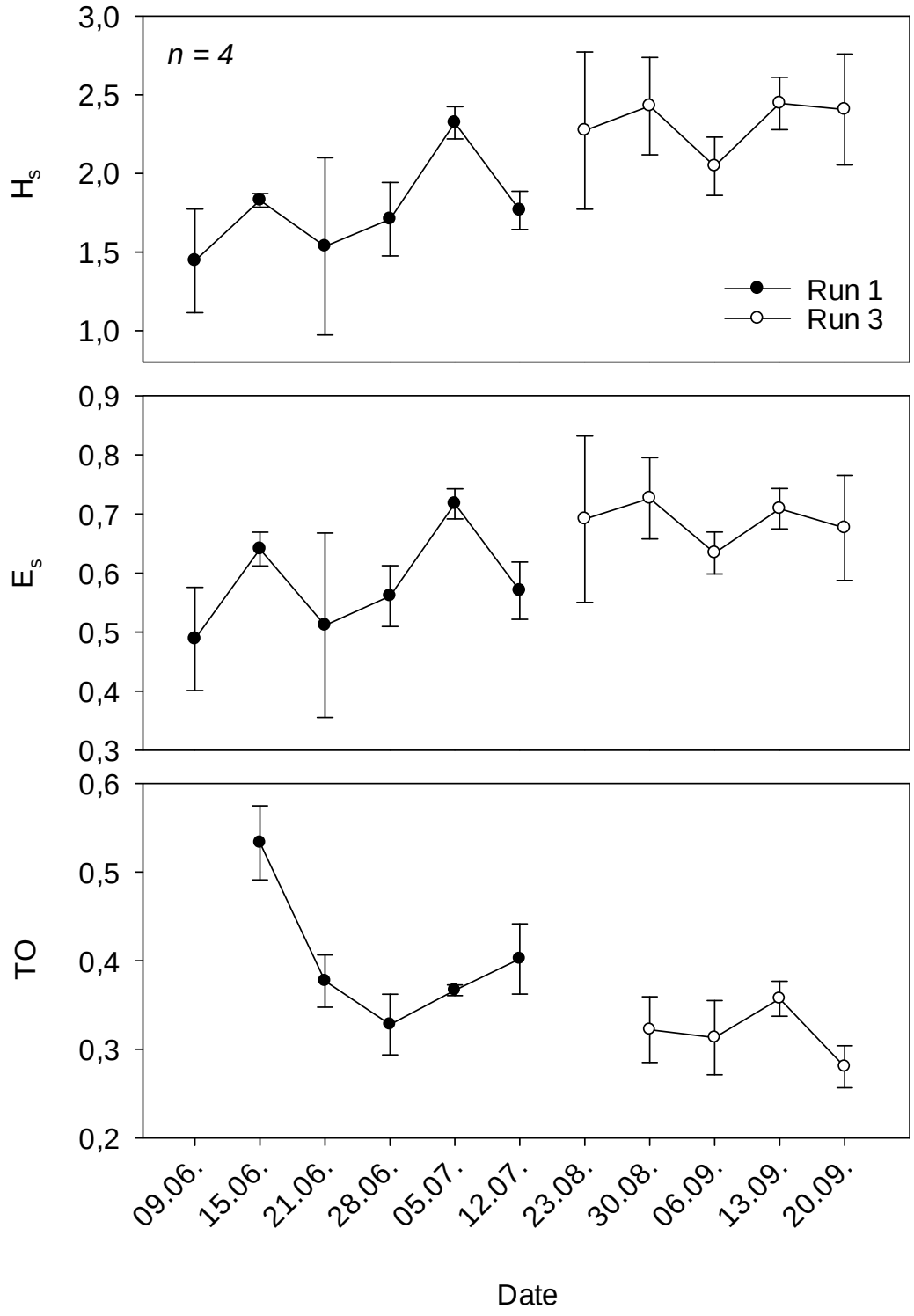

Figure 7: Shannon Index H_s (above), Evenness E_s (middle) and turnover TO (below) of the diatom community on the ATSs (arithmetic means with standard deviations)

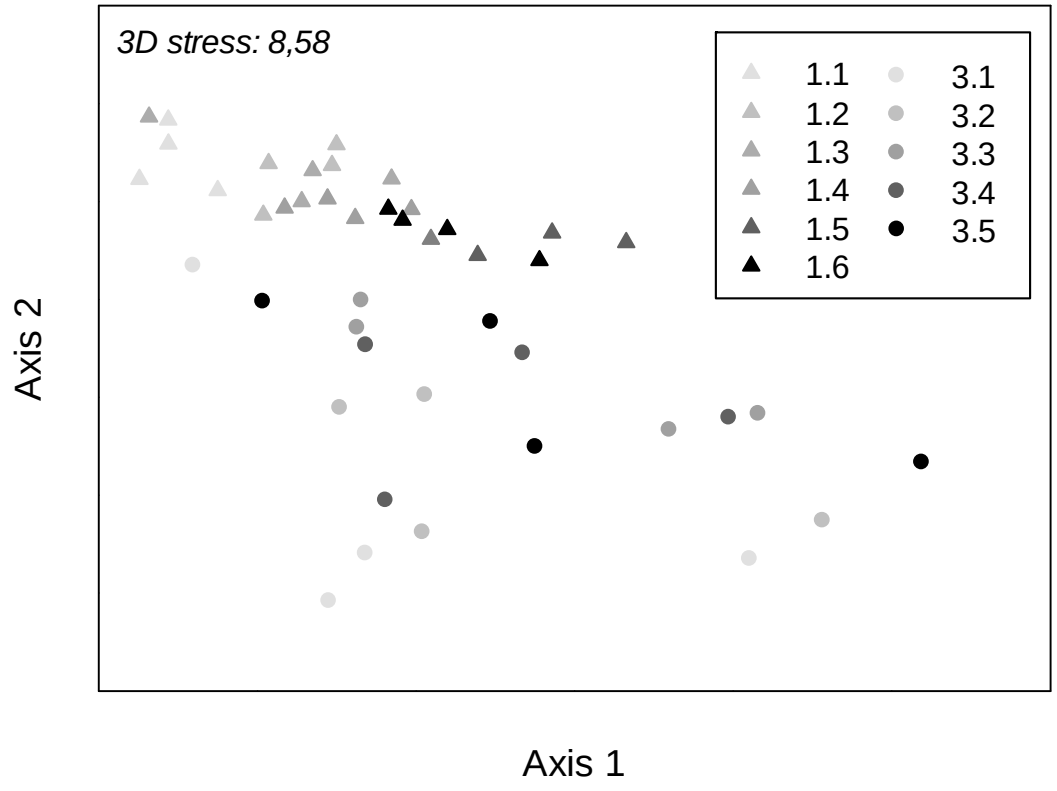

Figure 8: NMS plot showing ordination of the diatom community, in terms of absolute values of runs 1(triangles) and 3(circles); earlier dates are represented by bright colours, later ones by dark colours.

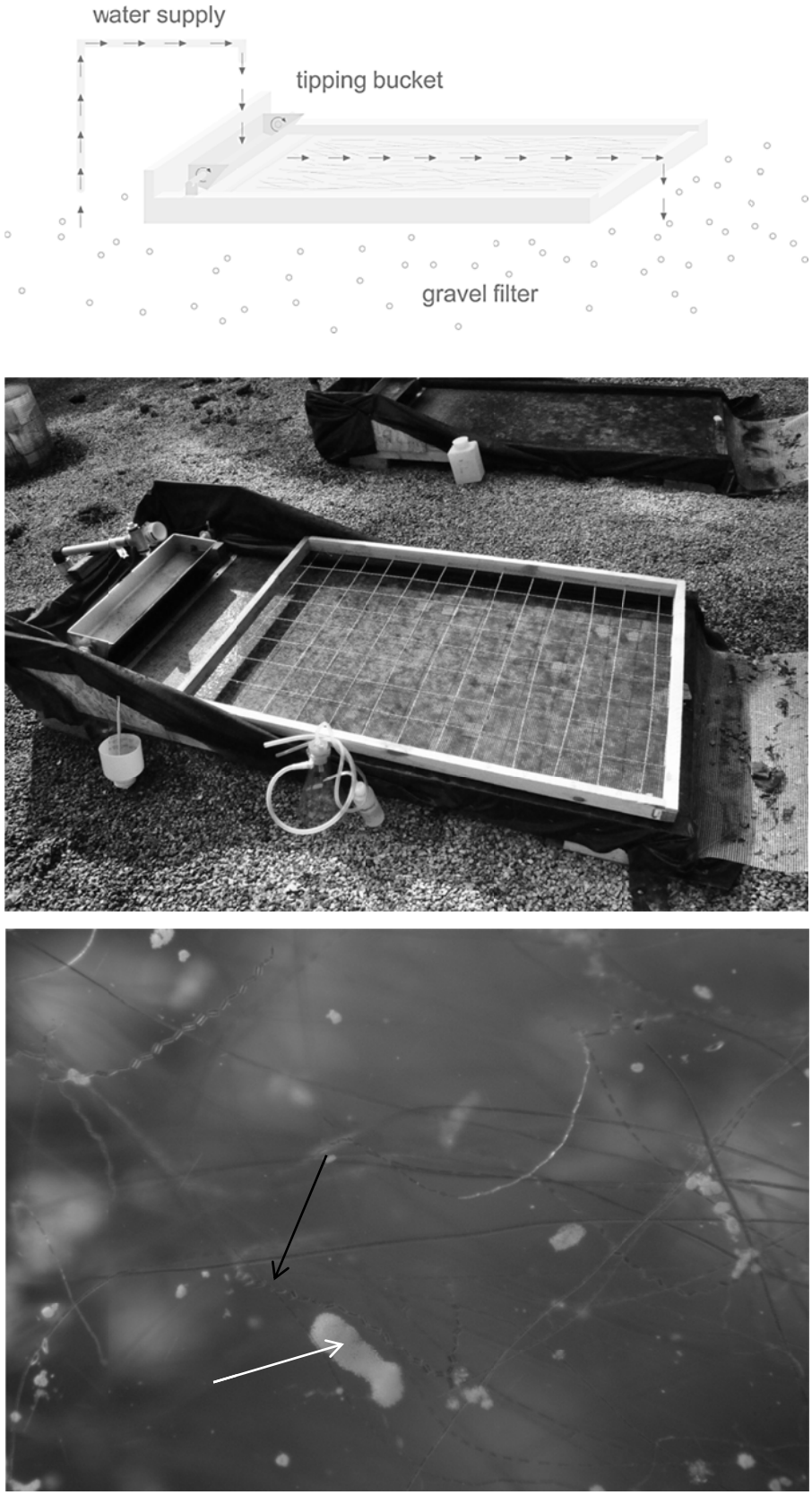

Figure 9: Scheme of the ATS (above), photography of the ATS with the constructed grid (centre), stereomicroscopic picture of the biofilm, showing colonies of *Microcystis* sp. (white arrow) trapped in filamentous colonies of *Fragilaria* sp. (black arrow), (below).

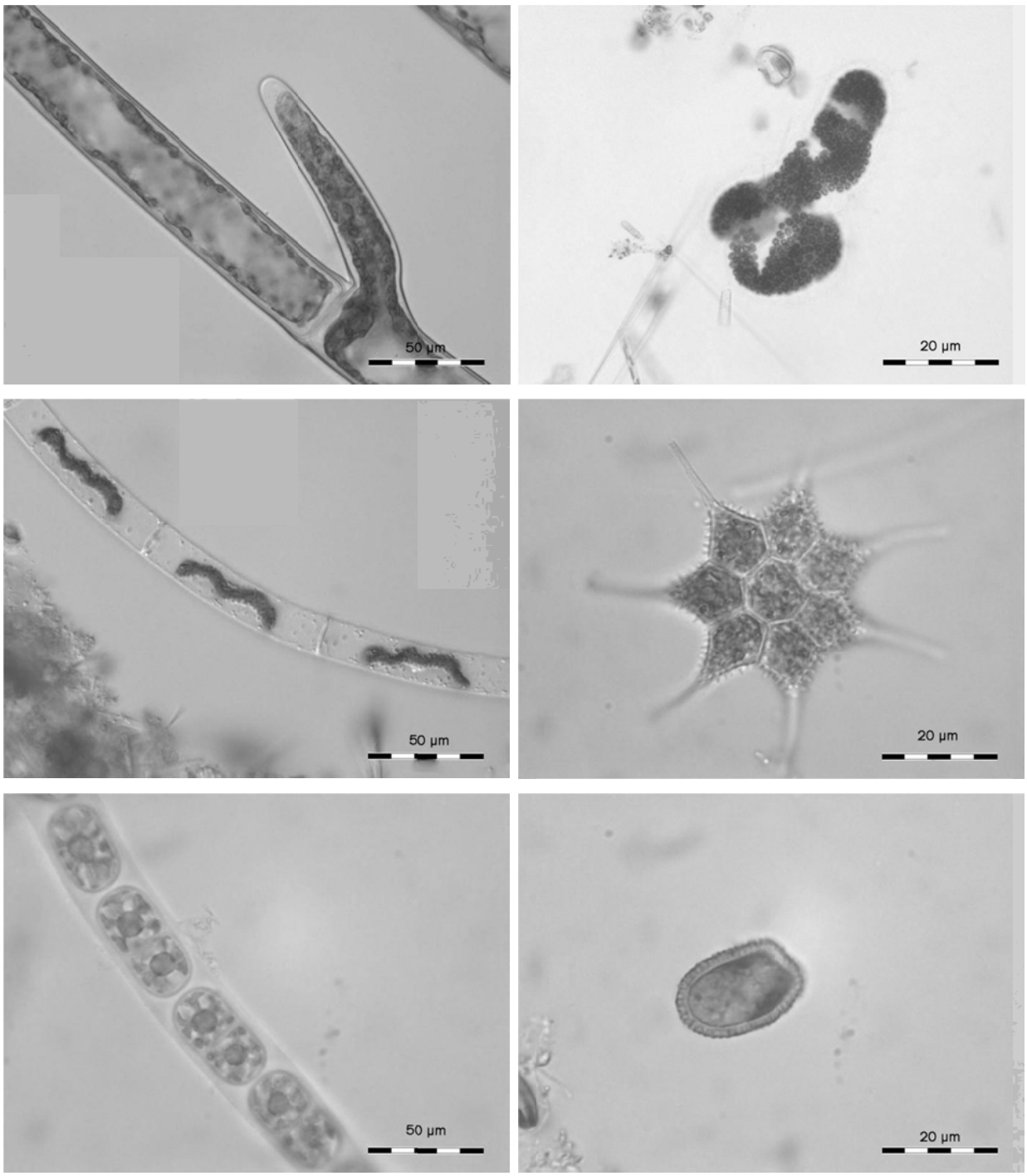

Figure 10: From the top to bottom, left: *Cladophora* sp., *Mougeotia* sp., *Chroodactylon ornatum* (Magnification: 500 times); right: *Microcystis wesenbergii*, *Pediastrum simplex*, *Hymenomonas roseola* (magnification: 1000 times).

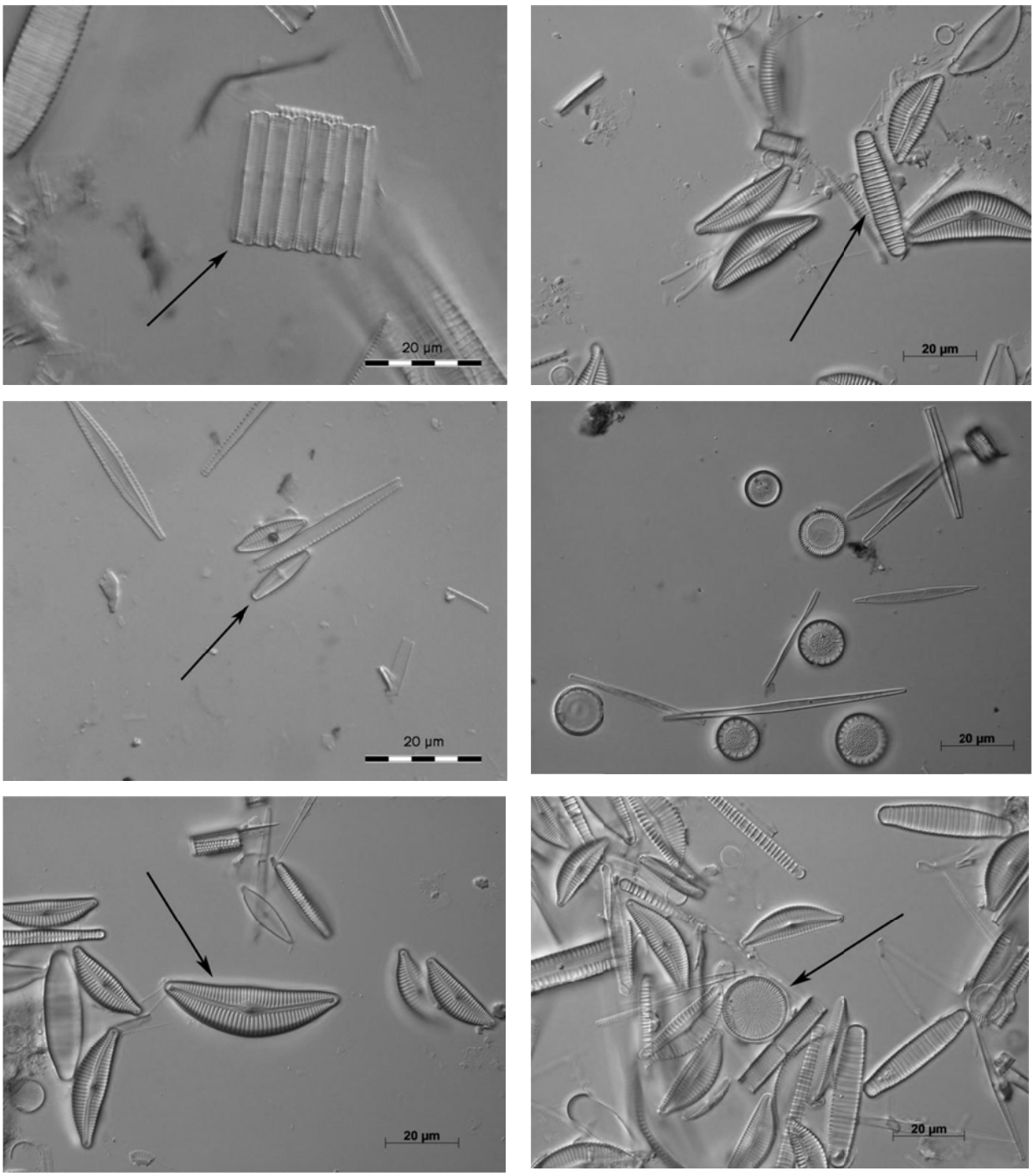

Figure 11: From upper left to lower right: *Fragilaria capucina*, *Diatoma vulgare*, *Achnanthes minutissima*, *Cyclotella* sp., *Cymbella affinis*, *Stephanodiscus hantzschii* (magnification: 1000 times).

3 Summary

This pilot study was conducted at the Heustadelwasser, a backwater of the Danube in Vienna, which is nowadays highly eutrophicated. In order to reduce the nutrient content of the water body and to minimize negative consequences of the eutrophication, a purification system called Neptunanlage had been installed at the Heustadelwasser in 2007. To further improve the cleaning efficiency, the so-called algal turf scrubber (ATS) technology was applied in this survey. The ATSs were connected to the Neptunanlage and supplied with water from the Heustadelwasser to reach a further reduction of nutrients. This was realized because the developing biofilm, which consisted to large extent of photoautotrophic organisms, incorporated nutrients, was harvested afterwards. The main objective of this study was to identify the composition of the algal biomass on the ATSs, to find out the speed of colonization and to define the optimal time for harvest. Furthermore, the turnover rate was studied and the diversity changes through time were documented. The investigation period lasted from June to August 2011 and comprised 3 runs. We used a modified Douglas sampler for weekly sampling. Estimation of the coverage and photographic documentation of the biofilm was done in the field. Microscopic analyses of living samples and fixed material and pigment analyses were carried out in the laboratory to quantify the algal biomass. The obtained results showed that the offered substrate was colonized very fast, as we found 20 – 30 different taxa already in the first week after exposition. The algal community was highly diverse, all together nearly 200 taxa were detected, which mainly belonged to the Chlorophyta, Bacillariophyceae and Cyanoprocaryota. Species richness generally increased over time and statistical analyses of the diatom community showed, that the algal community composition was mainly influenced by the season and succession.

4 Zusammenfassung

In der vorliegenden Studie wurde die Algengemeinschaft auf künstlichen Fließrinnen, sogenannten Algal Turf Scrubbers (ATSs) untersucht. Die Studie wurde am Heustadelwasser, einem ehemaligen Donaualtarm, der sich im Prater in Wien befindet, durchgeführt. Die starke Eutrophierung des Gewässers erforderte die Errichtung einer Gewässerreinigungsanlage (Neptunanlage), welche den hohen Nährstoffgehalt des Gewässers und die damit einhergehenden, negativen Folgen reduzieren sollte. Die ATSs wurden an das bestehende Reinigungssystem angeschlossen um eine weitere Retention der Nährstoffe zu erzielen. Die Funktionsweise eines ATS ähnelt der Selbstreinigungsleistung von natürlichen Gewässern, da im Wasser befindliche Nährstoffe vom Biofilm aufgenommen und in die Biomasse eingebaut werden. Die auf den ATSs wachsende Algenbiomasse wurde regelmäßig abgeerntet und analysiert. Ziel der vorliegenden Studie war es, die Zusammensetzung der Algengemeinschaft zu charakterisieren, sowie die stattfindende Sukzession zu beschreiben. Weiters wurde untersucht, wie schnell die ATSs besiedelt werden und wie sich die Diversität der Algen im Laufe der Zeit ändert. Der Untersuchungszeitraum reichte von Juni bis August 2011 und wurde in 3 Durchgänge unterteilt. Die Fließrinnen wurden wöchentlich mithilfe eines modifizierten Douglas-Samplers beprobt. Des Weiteren wurde die Oberfläche des Biofilmes fotografiert, graphisch analysiert und die Bedeckung der ATSs im Freiland geschätzt. Die mikroskopische Auswertung und Pigmentanalysen erfolgten im Labor. Die Ergebnisse der Studie zeigen, dass das angebotene Substrat innerhalb kürzester Zeit kolonisiert wurde. Bereits eine Woche nach Exposition der ATSs konnten 20 – 30 verschieden Arten bestimmt werden. Weiters zeichnete sich die Algengemeinschaft durch hohe Diversität aus, denn während des gesamten Untersuchungszeitraumes wurden an die 200 verschiede Algentaxa registriert. Diese gehörten hauptsächlich zu den Chlorophyta, Bacillariophyceae und Cyanoprocaryota. Generell stieg die Zahl der vorkommenden Arten im Laufe der Versuchsperiode an und statistische Auswertungen der Kieselalgengemeinschaft zeigten, dass die Zusammensetzung der Arten hauptsächlich von saisonalen Faktoren und der Sukzession beeinflusst wird.

5 Curriculum vitae

Personal data

Date of birth	03.09.1986, Klagenfurt, Austria
Nationality	Austria
Family status	Single
Adress	1120 Wien, Pachmüllergasse 10/9
Telephone	0650 / 620 82 87
E-mail	jacqueline.jerney@gmx.at

Education

1993 – 1997	Elementary school Pädak, Klagenfurt
1997 – 2001	Grammar School BG/BRG Viktring, Klagenfurt
2001 – 2006	Secondary School for Agriculture and Nutrition Pitzelstätten
2006 – 2012	Study of Biology - Department of Limnology at the University of Vienna

Practical trainings

2003 – 2004	Several practical trainings in the fields of gastronomy and agriculture
July 2005	Municipal Theater Klagenfurt
Sep. – Okt. 2007 and 2008	Civil engineering and consulting office ATC, Klagenfurt
July – Okt. 2009	Mapping of *Myotis daubentonii* at Arge NATURSCHUTZ, Klagenfurt
July 2010	Carinthian Institute for Lake research KIS, Klagenfurt

Scientific work experience

March 2011 – Dec. 2011	Project employee at the University of Vienna (Pilot study AlgenKULT)
June 2012 – June 2013	Project employee at the University of Vienna (SAM – Synergie aus Mikroalgenkultivierung und Abwasserreinigung)

Other work experience

Oct.2010 – Oct. 2011	Caritas emergency phone
March 2011 – July 2011	Tutor at the University of Vienna (Course „Kenntnis mitteleuropäischer Lebensgemeinschaften“)
Nov. 2011	Association Volksbgehren Bildungsinitiative
Oct. 2011 – Jan. 2012	Tutor at the University of Vienna (Course “Diverstität und Systematik niederer Pflanzen”)

Further qualifications

Language skills	German: First language English: Business, fluent (Business English Certificate) Italian: Basic knowledge Slovenian: Basic knowledge
Computer literacy	MS-Office, several statistical and graphic programs, GIS
Microscopy and photography	Technical knowledge of light microscopy, REM, TEM, and SLR cameras

Personal interests

Voluntary work	Environmental organisation Greenpeace, non-profit organization lobby.16, political engagement in several working groups
Hobbies	Artistic activities, photography, regatta sailing, hiking
Miscellaneous	Driving licences A, B and E to B, diving licence (OWD), sailing licence (A)

Vienna, 15 November 2012