# DIPLOMARBEIT 

Titel der Diplomarbeit

# Ecophysiology of filamentous green algae in astatic saline-alkaline ponds 

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Für meine Eltern
Josef und Veronika Fuhrmann
die mich stets unterstützt haben

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## 1. Einleitung

## Der Seewinkel

Der rund $450 \mathrm{~km}^{2}$ große Seewinkel befindet sich in Österreich östlich des Neusiedler Sees an der Grenze zu Ungarn. Das Klima der Region wird als pannonisch-kontinental bezeichnet mit Jahresdurchschnittstemperaturen von ungefähr $10{ }^{\circ} \mathrm{C}$ und einem jährlichen mittleren Niederschlag von rund 640 mm . Mit mehr als 240 Tagen im Jahr über $5{ }^{\circ} \mathrm{C}$ und mehr als 60 Tagen über $25^{\circ} \mathrm{C}$ ist das Gebiet eines der wärmsten in Österreich. Hohe sommerliche Temperaturen und häufig auftretende Winde führen außerdem im niederschlagsarmen Seewinkel zu hohen Verdunstungsraten (Dokulil et al., 2001; Löffler, 1982).

## Die Salzlacken

Die klimatischen Bedingungen spiegeln sich auch im Wasserhaushalt der rund vierzig alkalischen, kleinen und extrem flachen Gewässer, die sich im Seewinkel befinden, wider. Diese sogenannten Salzlacken sind gekennzeichnet durch regelmäßige, zumindest partielle Austrocknung, vor allem im Sommerhalbjahr. Fallen die Lacken trocken, so wird im Boden angereichertes Salz durch den Verdunstungssog in die Lacke gesaugt. Dadurch entstehen richtiggehende Salzkrusten auf dem ausgetrockneten Lackenboden, die sogenannten „Salzausblühungen". Wenn herbstliche Niederschläge die Lacken wieder füllen, lösen sich die Salzablagerungen im Wasser und erzeugen so den typischen Salzlackencharakter. Den Hauptbestandteil des Salzmixes macht ein Salz names „Soda" aus, das sich aus Natrium und Karbonat zusammensetzt. Aus diesem Grund werden die Gewässer auch „Sodalacken" genannt. Das Salz bewirkt den hohen, basischen pH-Wert der Lacken (Dick et al., 1994; Löffler, 1982, 2000; Wolfram et al., 1999).

## Die Algenmatten

Jedes Jahr im Frühjahr bilden sich dichte Matten aus filamentösen Grünalgen in den Salzlacken, die schließlich wie grüngelbliche Teppiche große Teile der Wasseroberfläche bedecken. Dieses Massenauftreten von Grünalgen spielt vermutlich eine wesentliche Rolle im Ökosystem der Sodalacken. Das Geflecht aus Algenfäden bietet Lebensraum, sowie Futter- und Rückzugsplätze für eine Vielzahl verschiedener Wasserlebewesen und erhöht dadurch die Biodiversität in den Gewässern. Außerdem bieten die Matten Fläche für kleinere, aufwachsende Algen, die wiederum von Insektenlarven abgeweidet und als Nahrung verwendet werden (Dodds, 1991; Lauringson and Kotta, 2006; Norkko et al., 2000; Pieczynska et al., 1999).

Massenauftreten von filamentösen Grünalgen können manchmal allerdings auch einen negativen Einfluss auf die Lebensgemeinschaften haben, wenn sich unter extrem dichten Matten sauerstoffarme, lebensfeindliche Bedingungen entwickeln (Berezina and Golubkov, 2008; Hansen and Kristensen, 1997).

Eine besondere Sensation war die Entdeckung, dass Weißbartseeschwalben (Chlidonias hybridus) in einer der Lacken auf den Algenmatten brüten, da dies der erste Brutnachweis für diese Vogelart in Österreich war (Dvorak et al., 2010). Außerdem können Algenmatten einen wesentlichen Anteil an der Photosyntheseleistung in Gewässern beisteuern, der bis zu $90 \%$ der gesamten Primärproduktion betragen kann (Gubelit and Berezina, 2010). Darüber hinaus beeinflussen sie Nährstoffkreisläufe und chemische Prozesse im Sediment (Dalsgaard, 2003; Valiela et al., 1997).

Aufgrund all dieser Faktoren ist davon auszugehen, dass das Massenauftreten filamentöser Grünalgen in den Seewinkellacken einen starken Einfluss auf das ökosystemare Gefüge hat. Allerdings existieren noch kaum Studien zu dieser Thematik.

## Die Studie

In der vorliegenden Studie untersuchten wir die Biomasseentwicklung und Artzusammensetzung der Algenmatten im Laufe einer Saison. Dazu wurden in einer ausgewählten Salzlacke fünf Probeflächen bestimmt, in denen von April bis September 2008 regelmäßig Proben für die Bestimmung von aschefreiem Trockengewicht und Chlorophyll a genommen wurden. Die Flächen waren mit einem grobmaschigen Zaun umgrenzt, um die Wasserzirkulation zu ermöglichen. Außerdem wurden begleitend das Ausmaß der auftreibenden Algenmatten in der gesamten Lacke erhoben und wöchentliche Messungen von wasserchemischen Parametern durchgeführt.

Im Labor führten wir darüber hinaus Messungen zum Austrocknungs- und Regenerationsverhalten der filamentösen Grünalgen durch. Dazu wurden Teile aus den Matten unter Simulation hochsommerlicher Temperaturen getrocknet und anschließend mit Lackenwasser wiedervernässt. Um Informationen über die Vitalität der Algen während dieser Versuche zu bekommen, verwendeten wir die „pulse amplitude modulated (PAM) Fluoreszenz" - Technik, mit deren Hilfe man Aufschluss über die Photosyntheseleistung und Fitness einer Pflanze bekommt (Krause and Weis, 1991; Maxwell and Johnson, 2000).

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Biomass development and seasonal succession of mat forming
filamentous green algae in alkaline saline ponds in the Seewinkel region (Austria)

### 2.1 Abstract

Biomass development and seasonal succession of mat forming filamentous green algae were investigated in one of the ephemeric alkaline-saline ponds located in the semi-arid Seewinkel region (Austria). Nutrients and ions of the pond were analysed on a weekly basis from April to September 2008, when the water body was almost dried out. Moreover, the area of floating algae mats covering the pond surface was estimated repeatedly. $\mathrm{Na}^{+}$and $\mathrm{HCO}_{3}{ }^{-}$were the most dominant ions reaching a maximum of 147.2 mval L- ${ }^{-1}\left(\mathrm{Na}^{+}\right)$and $84.3 \mathrm{mval} \mathrm{L}^{-1}\left(\mathrm{HCO}_{3}^{-}\right)$in the mid of September when the pond was nearly dried out. Phosphorus concentrations ranged around $362 \pm 187 \mu \mathrm{~g} \mathrm{~L}^{-1}$ (SRP, mean $\pm \mathrm{SE}$ ) and indicated hyper-eutrophic conditions. Maximum amounts of floating algae mats were reached in the first week of July with $14 \%$ coverage of the pond surface indicating a massive impact on the ponds ecosystem. From April to the beginning of June Spirogyra $s p$. was prevailing in the mats, then a shift to Cladophora $s p$. and Rhizoclonium $s p$. occurred, which were predominating until the end of the investigation period From late April to the beginning of September, we installed five mesocosms at different sites, from which we took quantitative samples to gain information about algal biomass development expressed as chlorophyll a and organic content. Maximum biomass was reached in the last week of July with $59 \pm 10 \mathrm{~g} \mathrm{~m}^{-2}$ ashfree dry mass and $133 \pm 17 \mathrm{mg} \mathrm{m}^{-2}$ chlorophyll a. The chlorophyll a to ash-free dry mass ratio was significantly higher when Spirogyra was dominating with $4.010^{-3} \pm 0.8$ $10^{-3}$ compared to $2.110^{-3} \pm 0.710^{-3}$ when Cladophora and Rhizoclonium were predominating ( $\mathrm{p}<0.05, \mathrm{n}=4$ for each period). This result could be due to thinner cell walls of Spirogyra and/or allelopathic activity of this genus inhibiting Aufwuchs. The results indicate, that mat forming algae play a key role in these ecosystems and influence nutrient availability and recycling and other chemical processes.

Moreover, their contribution to primary production must not be neglected. Algal mats are refuge for invertebrates and because of covering wide areas, they heavily influence water-air exchange.

Keywords: Spirogyra, Cladophora, Rhizoclonium, desiccation, astatic

### 2.2 Introduction

Mass development of filamentous algae is a widespread phenomenon in both freshwater and in coastal systems especially under conditions with elevated nutrient supply (Berglund et al., 2003; Gubelit and Berezina, 2010; Higgins et al., 2008; McGlathery et al., 2007; Norkko et al., 2000; Pieczynska et al., 1999; Pihl et al., 1999; Valiela et al., 1997). Filamentous green algae commonly form dense mats and have a high impact on the benthic macrofaunal community (Berezina and Golubkov, 2008; Hansen and Kristensen, 1997; Lauringson and Kotta, 2006; Norkko et al., 2000; Osterling and Pihl, 2001; Pieczynska et al., 1999). On the one hand macroinvertebrate communities can be negatively affected due to oxygen depletion below thick layers of filamentous algae (Berezina and Golubkov, 2008; Hansen and Kristensen, 1997). On the other hand, algae mats increase habitat complexity: they provide feeding places and refuges from predators thus leading to an increase of macrofaunal diversity (Lauringson and Kotta, 2006; Pieczynska et al., 1999). Norkko et al. (2000) found a huge abundance of invertebrates of more than 1000 individuals $\mathrm{g}^{-1}$ dry mass (DM) in algae mats surpassing even densities recorded for seagrass communities. Furthermore, filamentous algae act as a substrate for epiphytes, which serve as a food source for epiphyte grazers (Dodds, 1991). Their occurrence is heavily influencing the systems and sometimes might lead to serious eutrophication problems. Even human beings may suffer from gases, which are released into the atmosphere through decomposition processes.

Mass occurrence of algal mats alters nutrient recycling and sediment chemistry. They take up high quantities of nitrogen; carbon derived from these algae moves quickly through microbial and consumers food webs (Dalsgaard, 2003; Valiela et al., 1997). Additionally, filamentous green algae can act as a buffer against a shift to phytoplankton dominance thus maintaining a clear-water state and they account for up
to $90 \%$ of total primary production in the ecosystem (Gubelit and Berezina, 2010; Irfanullah and Moss, 2005). Nozaki et al. (2001) detected filamentous green algae being dominant in the benthic algae community of Lake Biwa, Japan, from May to December, where they accounted for the most part of the daily gross production rates. Although quite a number of studies about mass development of macroalgae in marine, coastal and freshwater habitats can be found, only little is known for saline-alkaline inland water bodies. In the Seewinkel region in eastern Austria several saline-alkaline pans are located, where large mats of filamentous green algae frequently develop during the summer season. These mats are likely to play a key role in the functioning of the ecosystems. In addition to the important effects mentioned above, Chlidonias hybridus was found breeding on these mats (Dvorak et al., 2010). We investigated the seasonal succession of the mat-forming filamentous algae and focused on the taxa composition of the mats, biomass development and considered various chemical parameters and weather data during the investigation period. We expected to measure high amounts of biomass, especially in periods with high temperature and moderate ion concentrations. Moreover, we expected a shift in algal community over the season due to an increase in salinity.

## Study site

The study was conducted in a salt pan called Mittelstinker (Figs. 1, 2) located in the Seewinkel region, which is around $60.000 \mathrm{~m}^{2}$ in size and approximately 25 cm deep. The Seewinkel region is an area of $450 \mathrm{~km}^{2}$ located in the North-Eastern part of Austria near the Hungarian border at $115-130 \mathrm{~m}$ above sea level. It contains around 40 shallow ponds with elevated conductivity (Löffler, 2000). Whereas saline inland waters are common in dry regions like Africa, Australia, Central Asia or in some parts of the United States, they are quite rare for Europe (Hungary, Turkey, Ukraine). The climate
can be classified as pannonian-continental and is characterized by an average temperature of around $9.9 \pm 0.6^{\circ} \mathrm{C}$ and an average yearly rainfall of $639 \pm 96 \mathrm{~mm}$ (Dokulil et al., 2001). With more than 240 days a year having an average temperature more than $5^{\circ} \mathrm{C}$ and more than 61 days over $25^{\circ} \mathrm{C}$, it is one of the warmest regions of Austria. Most of the precipitation is recorded in late spring and early summer, but high summer temperatures and strong winds lead to high evaporation rates so that semiarid conditions can be reached (Dokulil et al., 2001; Löffler, 1982). Therefore the water level is usually reduced during summer and some pans can even dry out. Transpiration leads to a transport of salt from deeper soil layers into the pond creating saline conditions. Sodium and hydrogencarbonate are the ions largely dominating in this saline-alkaline water bodies (Dick et al., 1994; Löffler, 1982; Wolfram et al., 1999). In addition to filamentous green algae, which develop huge mats in the summer season, the ponds are characterized by the occurrence of Chara canescens J.L.A. LoiseleurDeslongschamps in spring an high abundances of Potamogeton pectinatus L., especially during the summer months.

### 2.3 Material \& Methods

Precipitation was measured on a daily basis with an Ombrometer and air temperature was recorded every 15 min with a data logger (Logotronic Gealog). From end of April to the beginning of September 2008, the size of floating algae mats was estimated by weekly field observations and recorded into a map. Afterwards the percentage of coverage was calculated (MicroImages TNT). Water chemistry was analysed weekly (for methods and variables see Tab. 1).

To assess information about biomass of the filamentous algae, five mesocosms $2 \mathrm{~m}^{2}$ in size and fenced with a wire mesh to allow water to circulate were installed (Fig. 2). Between the last week of April to the last week of August, samples were taken eight times. At each sampling date, four cores were obtained from each mesocosm and water temperature was measured (WTW Con.315i). In the laboratory, the supernatant of the core tubes was removed by suction with a vacuum pump. Algae filaments of each core were carefully rinsed with water, then cut into small pieces with scissors, re-dissolved in a defined volume of water and divided into two parts, which were filtered on separate glass fibre filters. One filter with algae material was stored in the freezer at $-20^{\circ} \mathrm{C}$ until pigment analysis, the other filter was put in the cabinet dryer (Heraeus UT 6120), dried at $95^{\circ} \mathrm{C}$ for two days minimum to measure dry mass (DM). Afterwards this filter was put into a muffle furnace (Carbolite ELF $11 / 14$ ) at $450^{\circ} \mathrm{C}$ for two hours to assess ash mass. Then ash-free dry mass (= organic content) was calculated by subtraction of ash mass from dry mass. For pigment analysis, the filter was put into $90 \%$ acetone and ground with a homogenizer (Polytron PT 1600E) to assist pigment extraction. Extraction took place for 10 h under dark conditions at $4^{\circ} \mathrm{C}$. Afterwards samples were centrifuged and the supernatant measured spectrophotometrically (Hitachi U-2001). Chlorophyll $a$ was calculated after Jeffrey and Humphrey (1975).

Algal taxa were identified through a compound microscope (Nikon Optiphot-2).
Statistical analyses were performed using the software package PASW 18. For comparing chlorophyll $a$ per ash-free dry weight ratios, independent student's t-tests were performed.

### 2.4 Results

## Weather data

For 2008, the mean annual air temperature was $11,9^{\circ} \mathrm{C}$; the region received an annual precipitation of 562 mm . Average weekly temperature in the investigation period was lowest in the last week of April with $14.3^{\circ} \mathrm{C}$ and reached maximum values at the end of June $\left(23.8^{\circ} \mathrm{C}\right)$ and the end of July $\left(23.4^{\circ} \mathrm{C}\right)$, respectively (Fig. 3). Weekly rainfall showed high fluctuations and reached a maximum in the first week of June ( 72 mm ). Total precipitation in the investigated period was 353 mm with June and July being the wetter months with monthly precipitation of around 130 mm , and May and August being months with low rainfall (about 40 mm ; Fig. 3).

## Hydrochemistry

Phosphorus contents were lowest at the beginning of the experiment with around 125 $\mu \mathrm{g} \mathrm{L}^{-1}$ of total phosphorus ( $\mathrm{P}_{\mathrm{tot}}$ ) and $70 \mu \mathrm{~g} \mathrm{~L}^{-1}$ SRP. Phosphorus concentrations showed four main peaks with a maximum $\mathrm{P}_{\text {tot }}$ recorded with $874 \mu \mathrm{~g} \mathrm{~L}^{-1}$ in the first week of June; maximum SRP concentrations were analysed in the $4^{\text {th }}$ week of June $\left(752 \mu \mathrm{~g} \mathrm{~L}{ }^{-1}\right)$. $\mathrm{NO}_{3^{-}}$ N was less varying with values ranging between 35 and $86 \mu \mathrm{~g} \mathrm{~L}^{-1}$ (Fig 4). Values of the investigation period were $518 \pm 224 \mu \mathrm{~L}^{-1}$ for $\mathrm{P}_{\text {tot }}($ mean $\pm \mathrm{SE}), 362 \pm 187 \mu \mathrm{~g} \mathrm{~L}^{-1}$ for SRP and $48 \pm 9 \mu \mathrm{~g} \mathrm{~L}^{-1}$ for $\mathrm{N}-\mathrm{NO}_{3}$ Conductivity varied between 3500 and $12550 \mu \mathrm{Scm}^{-}$ ${ }^{1}$ during the investigation period. $\mathrm{Na}^{+}$was the most dominant cation with concentrations ranging from $38.7 \mathrm{mval} \mathrm{L}^{-1}$ at the end of April and to $147.2 \mathrm{mval} \mathrm{L}^{-1}$ in the second week of September, when the pan was nearly dried out (Fig. 5). Values for $\mathrm{Mg}^{2+}\left(7.9 \mathrm{mval} \mathrm{L}^{-1}\right.$ to $14 \mathrm{mval} \mathrm{L}^{-1}$ ) and $\mathrm{Ca}^{2+}$ concentrations ( 0.5 and $6.6 \mathrm{mval} \mathrm{L}^{-1}$ ) were much lower. $\mathrm{HCO}_{3}{ }^{-}$ was the most prominent anion with $22.8 \mathrm{mval} \mathrm{L}^{-1}$ at the beginning of the investigation period reaching $84.3 \mathrm{mval} \mathrm{L}^{-1}$ during the dry period (Fig. 5), followed by $\mathrm{SO}_{4}{ }^{2-}$ and $\mathrm{Cl}^{-}$
(Fig. 5). Water temperature reached a maximum of $29 \pm 0.5^{\circ} \mathrm{C}$ in the fourth week of June (Fig. 6).

## Algal mats

Spirogyra $s p$. was predominating in the mats until the beginning of June, and then a shift to Cladophora $s p$. and Rhizoclonium $s p$. occurred; those two taxa were predominating until the end of the investigation period. Biomass in the five mesocosms showed three major peaks, one at the end of April, one in the last week of May and another one in the last week of July. Ash-free dry mass was $27 \pm 6 \mathrm{~g} \mathrm{~m}^{-2}$ (mean $\pm \mathrm{SE}$ ) at the first peak and $21 \pm 4 \mathrm{~g} \mathrm{~m}^{-2}$ at the second peak (both Spirogyra dominated) and reached a maximum at the third peak (Cladophora and Rhizoclonium dominated community) with $59 \pm 10 \mathrm{~g} \mathrm{~m}^{-2}$. Chlorophyll $a$ amounts were $89 \pm 21 \mathrm{mg} \mathrm{m}^{-2}$ and $107 \pm$ $26 \mathrm{mg} \mathrm{m}^{-2}$ at the Spirogyra dominated peaks and showed only slightly higher values $\left(113 \pm 17 \mathrm{mg} \mathrm{m}^{-2}\right)$ at the third peak (Fig. 7). The chlorophyll $a$ per ash-free dry mass ratio was significantly higher in the Spirogyra dominated phase ranging between $3.310^{-}$ ${ }^{3}$ and $5.010^{-3}$ from the end of April till end of May and from $1.310^{-3}$ to $2.910^{-3}$ in the Cladophora and Rhizoclonium dominated period from June until August (p=0.005, $\mathrm{n}=$ 4; t-test for two independent groups). Areal coverage of floating mats showed four distinct peaks, the first one at the end of April ( $4 \%$ coverage of the pond), the second one at the end of May ( $5 \%$ coverage) followed by a maximum in the first week of July ( $14 \%$ coverage) and a last peak in the first week of August ( $6 \%$ coverage) (Fig. 8).

### 2.5 Discussion

Total precipitation of 2008 is comparable to other years. More than half of the annual precipitation was recorded during the study period thus affirming long term observations that most of the rainfall takes place in late spring and early summer months (Löffler, 1982). The annual average temperature of $11.9^{\circ} \mathrm{C}$ was slightly increased compared to long-term monitorings done in this region ((9.9 $\pm 0.6)$ (Dokulil et al., 2001)). Phosphorus concentrations indicated hyper-eutrophic conditions of the pan, but $\mathrm{NO}_{3}-\mathrm{N}$ amounts were however somehow reduced. This could be explained by denitrification processes, which play a major role especially in shallow eutrophicated water bodies (Nilsson and Jansson, 2002). Neither phosphorus nor $\mathrm{NO}_{3}-\mathrm{N}$ concentrations seem to be related to algal biomass indicating that the mats were not growth-limited by nutrient depletion. We found an ion composition, which is typical for saline-alkaline inland water bodies with $\mathrm{Na}^{+}$and $\mathrm{HCO}_{3}{ }^{-}$dominating. Dry conditions in August and at the beginning of September caused a reduction in water level and led to an accumulation of ions, which is also expressed by a significantly increased conductivity at the end of the investigation period.

Spirogyra sp. was dominating the benthic algal community during spring and developed slimy "clouds", which finally covered some areas of the pan. Spirogyra sp. is a common freshwater genus and is known for its potential allelopathic activity (Mohamed, 2002; Weber and Schagerl, 2007) and for its effectiveness to remove heavy metals from water (Gupta et al., 2001; Singh et al., 2007). Moreover, Spirogyra sp. has a strong tendency to form unialgal mats, which we also observed in our study. Hainz et. al (2009) collected Spirogyra spp. at 133 different sites all over Central Europe and found the taxon colonizing habitats with $\mathrm{P}_{\text {tot }}$ concentrations ranging from $<1$ to $2240 \mu \mathrm{~g} \mathrm{~L}^{-1}$ and specific conductivities of $75-1500 \mu \mathrm{~S} \mathrm{~cm}^{-1}$. Interestingly, Spirogyra sp. was occurring at
much higher conductivity levels during our study ranging between $3500 \mu \mathrm{~S} \mathrm{~cm}^{-1}$ and $5600 \mu \mathrm{~S} \mathrm{~cm}^{-1}$. Spirogyra was finally replaced by large mats consisting of Cladophora sp. and Rhizoclonium sp. in June. Cladophora is a widespread genus and can be found from freshwater to marine biotops. This taxon seems to prefer elevated nutrient levels, where it is likely to occur in high abundances (Higgins et al., 2008; Pihl et al., 1999; Vis et al., 2008). Rhizoclonium is common in brackish water tolerating a wide range of salinity (Chao et al., 2005; Imai et al., 1997; Matsuyama-Serisawa et al., 2004; Taylor et al., 2001). The reason for the observed community shift might have been induced by several factors. First of all, the increase in conductivity due to evaporation processes has to be mentioned. Additionally, other factors like water temperature and irradiance supply come into play. Berry et al. (2000) investigated factors controlling Spirogyra abundance in a freshwater lake in Indiana (USA), where it mainly grew during the spring season and disappeared in summer. They found a negative correlation between Spirogyra occurrence and water temperature and a negative effect of low light supply to cohesiveness of mats. The authors concluded that a combination of elevated water temperature and increased self-shading might be a cause for the breakdown. Water temperature seems also to be a key-factor for the seasonal occurrence of the green alga Ulva sp. mats growing along the Korean coast (Choi et al., 2001). Another potential cause for the community shift could be grazing. Liess and Kahlert (2007) detected a shift from Spirogyra sp. to Chaetophorales and they argued, that Spirogyra $s p$. was more sensitive against grazing pressure. Cladophora sp. might be more resistant against grazing and bacterial degradation because of the high cellulose concentrations in its cell walls (van den Hoek et al., 1995). Maximum biomass values in the mesocosms were reached with $59 \mathrm{~g} \mathrm{~m}^{-2}$ DM at the end of July when Cladophora and Rhizoclonium were dominating indicating that the mats play an essential role in such shallow ecosystems.

Sometimes, even higher biomass developments of Cladophora glomerata mats were found reaching e.g., $100-300 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ in the Laurentian Great Lakes (Higgins et al., 2008). Maximum biomass values of filamentous green algae in marine coastal systems were similar showing values up to $300 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ for Cladophora glomerata mats in the Baltic sea and maximum values of $286 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ along the Swedish coast (Cladophora sp. and Enteromorpha sp.; (Gubelit and Berezina, 2010; Pihl et al., 1999). These values were surpassed by Ulva sp. mass developments reaching $720 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ (Choi et al., 2001) and drifting algae mats mainly composed of brown algae ( $830 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ (Norkko et al., 2000); $1390 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ (Lauringson and Kotta, 2006)). Chlorophyll $a$ values reached $107 \mathrm{mg} \mathrm{m}^{-2}$ when Spirogyra sp. was dominating and $113 \mathrm{mg} \mathrm{m}^{-2}$ when Cladophora sp. and Rhizoclonium sp. were dominating. Comparable results were found by Townsend and Padovan (2009) who measured $73 \mathrm{mg} \mathrm{m}^{-2}$ Chlorophyll $a$ for Spirogyra in an Australian river. The Chlorophyll $a$ per ash-free dry mass content was significantly higher in our experiments for Spirogyra. Besides thinner cell-walls of Spirogyra, this result might reflect also the allelopathic activity of Spirogyra inhibiting Aufwuchs including heterotrophs. From the microscopical studies, Cladophora and Rhizoclonium were overgrown by heterotrophic organisms. However, further studies on this topic are needed for clarifying this phenomenon.

Moreover it would be interesting to get an overview of algae mat abundance and their contribution to primary productivity and respiration in other ponds of the region. In the Mittelstinker at the maximum $14 \%$ of the water surface was covered by algae mats, but we observed around $50 \%$ coverage in other salty ponds of the Seewinkel. So it is likely that in other water bodies even higher amounts of biomass can be reached. Large mats of macroalgae have a high impact on the ecosystem.

They influence, for example, diversity by providing food source and habitat for macroinvertebrates (Lauringson and Kotta, 2006; Norkko et al., 2000; Pieczynska et al., 1999). It is likely that this is also the case for the huge algae mats in the Seewinkel ponds because during field work we detected high abundances of water insects inhabiting the mats. Moreover filamentous green algae act as substrate for epiphytes and food source for epiphyte grazers (Dodds, 1991) and they can even provide breeding place for birds like Chidonias hybridus, which was found breeding on algae mats in the Seewinkel region by Dvorak et al. (2010). Moreover mass development of macroalgae plays an important role in nutrient recycling and chemical processes in the sediment (Dalsgaard, 2003; Valiela et al., 1997) which can also lead to anoxic conditions under thick layers (Berezina and Golubkov, 2008; Hansen and Kristensen, 1997; Salovius and Bonsdorff, 2004). Therefore it would be interesting to gain information about the dimension of algae mat coverage considering all the approximately 40 ponds in the region. This approach could be realised by aerial photography combined with sampling. This method was already used in previous studies to get an overview of algae mat biomass development (Berglund et al., 2003; Pihl et al., 1999). Wolfram et al. (1999) found in their studies dealing with the zoobenthic community of the alkaline-saline ponds of the Seewinkel region high abundances of chironomids and crustaceans when dense macrophyte carpets (Chara canescens Desvaux \& Loiseleur in LoiseleurDeslongchamps and Potamogeton pectinatus Linnaeus) were stabilizing the sediment. We also observed such sediment stabilizing effects due to coverage by large macroalgae mats, so probably macroalgae coverage has a similar impact on invertebrate community as dense growth of macrophytes (Pieczynska et al., 1999) Other ideas for future studies include the causes for the shift in algal communities in the mats and the contribution of the filamentous algae to the metabolism of the water body in relation to phytoplankton.

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### 2.7 Legends

Fig. 1 Arrow indicating the position of the investigated pan called "Mittelstinker" - the saline-alkaline ponds of the Seewinkel region are coloured in black

Tab. 1 Methods for chemical analysis of nutrients and ions

Fig. 2: Map of the Mittelstinker; dots indicating the position of the five mesocosms

Fig. 3 Weekly temperature and weekly rainfall in the investigated period (end of April until middle of September 2008) in the Seewinkel region

Fig. 4 Temporal nutrient concentration from the end of April to the beginning of September 2008 in the "Mittelstinker"

Fig. 5 Temporal ions concentrations from the end of April to the middle of September 2008 in the "Mittelstinker"

Fig. 6 Water temperature of the "Mittelstinker" from the end of April until the beginning of August 2008 (dots $=$ means, error bar $=$ standard error $)$

Fig. 7 Ash-free dry mass and Chlorophyll $a$ content from mesocosm samples form the end of April to end of August (dots = means; error bar = standard error); Dotted bar = Spirogyra sp. dominating in algae mat community; striped bar = Cladophora sp. and Rhizoclonium sp. dominated community

Fig. 8 Percentage of algae mat coverage of the pond surface over the investigated period (end of April until beginning of September); Dotted bar = Spirogyra sp. dominating in algae mat community; striped bar = Cladophora $s p$. and Rhizoclonium $s p$. dominated community

### 2.8 Tables and Figures

Tab. 1 Methods for chemical analysis of nutrients and ions

| parameter | Method used | Equipment used |
| :--- | :--- | :--- |
| $\mathrm{P}_{\text {tot }} /$ SRP | EN ISO 6878-2004 | Spektrophotometer Shimazdu UV 1800 |
| $\mathrm{NO}_{3}{ }^{-} / \mathrm{SO}_{4}{ }^{2-} / \mathrm{Cl}^{-}$ | NORM EN ISO 10304-1: 1995-04 | Dionex Ionchromatograph ICS 1000 |
| $\mathrm{Na}^{+} / \mathrm{Mg}^{2+} / \mathrm{Ca}^{2+}$ | NORM EN ISO 14911: 1999-12 | Dionex Ionchromatograph DX 120 |
| $\mathrm{HCO}_{3}{ }^{-}$ | DIN 38409-7: 2005-12 | Titrator Mettler Toledo DL50 Graphix |



Fig. 1 Arrow indicating the position of the investigated pan called "Mittelstinker" The saline-alkaline ponds of the Seewinkel region are coloured in black.


Fig. 2: Map of the Mitterstinker; dots indicating the position of the five mesocosms


Fig. 3 Weekly temperature and weekly rainfall in the investigated period (end of April until middle of September 2008) in the Seewinkel region


Fig. 4 Temporal nutrient concentration from the end of April to the beginning of September 2008 in the "Mittelstinker"


Fig. 5 Temporal ions concentrations from the end of April to the middle of September 2008 in the "Mittelstinker"


Fig. 6 Water temperature of the "Mittelstinker" from the end of April until the beginning of August 2008 (dots $=$ means, error bar $=$ standard error $)$


Fig. 7 Ash-free dry mass and Chlorophyll $a$ content from mesocosm samples form the end of April to end of August (dots = means; error bar = standard error); Dotted bar = Spirogyra $s p$. dominating in algae mat community; striped bar $=$ Cladophora $s p$. and Rhizoclonium $s p$. dominated community


Fig. 8 Percentage of algae mat coverage of the pond surface over the investigated period (end of April until beginning of September); Dotted bar = Spirogyra sp. dominating in algae mat community; striped bar $=$ Cladophora $s p$. and Rhizoclonium sp. dominated community

# Desiccation and recovery potential of green algae mats in astatic saline-alkaline ponds 

### 3.1 Abstract

We studied desiccation tolerance and recovery potential of mat forming filamentous green algae. The mats originated from two saline - alkaline astatic water bodies located in the Seewinkel region (Austria). Samples were taken in June (maximum occurrence of vital mats covering around $14 \%$ of the surface area) and September 2008 (desiccation period). To gain insight into the overall photosynthetic performance of the algae, the non-invasive pulse amplitude modulated (PAM) fluorescence technique was applied. The initial maximum fluorescence yield ( $\mathrm{Fv} / \mathrm{Fm}$ ) of fresh, vital samples was comparable for both months with average values of 0.69 in June and 0.67 in September. The decrease in yield related to water loss during desiccation showed a significant difference in the two months due to an earlier decrease of $\mathrm{Fv} / \mathrm{Fm}$ values in June. For the recovery experiment we grouped samples according to their desiccation state defined as percentage water content. Samples having 15 to $30 \%$ water content (WC) were treated as slightly desiccated, specimens with 5 to $15 \%$ WC represented intermediate desiccation and samples with less than $5 \% \mathrm{WC}$ were considered as severely desiccated. The recovery potential was surprisingly low, indicating full recovery only for the slightly desiccated samples. Medium and severely desiccated samples were not able to recover indicating irreversible cell damage. Senescent, yellowish mats floating on the water surface showed significantly lower initial $\mathrm{Fv} / \mathrm{Fm}$ values compared to vital, fresh mats growing near the bottom. Furthermore, senescent mats showed a significant different desiccation rate compared to the vital ones, due to a sharper drop of $\mathrm{Fv} / \mathrm{Fm}$ at decreased WCs and an increase in yield when samples were just exposed to air ( $95 \%$ WC ), which might be due to increased $\mathrm{CO}_{2}$ diffusion rates in air.

Keywords: drying, Cladophora, filamentous, fluorescence

### 3.2 Introduction

Water loss is a big stressor for plants especially for algae, who do not possess an impermeable cuticle. Therefore various mechanisms have been developed to prevent severe damage caused by desiccation, which otherwise would lead to the destruction of cell membranes and the denaturation of proteins. Strategies include the production of mucilage envelopes (Berard-Therriault and Cardinal, 1973), reduction of size and surface (Schonbeck and Norton, 1980), the accumulation of compatible solutes to prevent osmotic stress (Bäck et al., 1992; Kirst, 1990) or growth in dense mats (Bewley, 1979; Hunt and Denny, 2008). Numerous investigations have been conducted on algae facing desiccation. These studies, for example, dealt with intertidal algae (Dring and Brown, 1982; Hunt and Denny, 2008; Möstl and Schagerl, unpublished), Codium sp. occurring in estuaries (Kim and Garbary, 2007), or microbial mats on beach rock (Schreiber et al., 2002) and in intertidal zones (Fleming et al., 2007). Furthermore, desiccation tolerance and recovery of algae were studied for algae growing in deserts (Gray et al., 2007), on tree barks (Lüttge and Büdel, 2009) including also symbionts in lichens (Gasulla et al., 2009) and in Antarctic wetlands (Sabacka and Elster, 2006). All this studies showed that algae originating from habitats permanently or periodically exposed to air usually recover quite fast after desiccation even after elongated periods of desiccation (Gray et al., 2007; Lüttge and Büdel, 2009) and high degree of water loss (Kim and Garbary, 2007; Möstl and Schagerl, unpublished). Commonly, desiccation stress is combined with other stressors like excess irradiance, elevated ambient temperature or changes in salt concentration which might have negative impacts on the recovery potential of algae (Fleming et al., 2007; Gray et al., 2007; Kim and Garbary, 2007). However, some studies also indicated that moderate irradiance supply might be
required to enhance regeneration during the recovery phase (Fleming et al., 2007; Gao et al., 1998; Schreiber et al., 2002).

Eutrophicated coastal areas and inland water bodies, especially shallow ones, are sometimes covered by dense mats of macroalgae (Berezina and Golubkov, 2008; Dalsgaard, 2003; Gubelit and Berezina, 2010; Higgins et al., 2008; Norkko et al., 2000; Pieczynska et al., 1999; Pihl et al., 1999; Valiela et al., 1997). They certainly hold a key position in these ecosystems as they influence geochemistry and nutrient fluxes (Dalsgaard, 2003; Valiela et al., 1997) and have a massive influence on macroinvertebrate composition (Berezina and Golubkov, 2008; Hansen and Kristensen, 1997; Lauringson and Kotta, 2006; Norkko et al., 2000; Osterling and Pihl, 2001; Pieczynska et al., 1999). Macrolagae may count up to $90 \%$ of total primary production in an ecosystem; they provide substrate for epiphytes, food source for epiphyte grazers and maintain the clear-water state (Gubelit and Berezina, 2010; Irfanullah and Moss, 2005). However, mass occurrences of some macroalgae taxa like green tides of Ulva sp. on the Brittany coast (Robic et al., 2009) and in the Yellow Sea (Liu et al., 2010) can even lead to a threat for human healthy when decomposing mats on the coastline emit toxic gases. Interestingly, only little is known about ecological and ecophysiological aspects of mats developing in astatic inland water bodies and wetlands. Contrarily to systems with elongated dry periods or habitats with a diurnal pattern of air-exposure, algal mats in astatic water bodies develop under a permanent water cover, before they are finally exposed to air. Depending on the weather conditions, desiccation and rehydration are stochastic events. Here, some questions arise: How do such mats react to desiccation and is a recovery possible? Is there a difference concerning resistance to desiccation depending on the time of the year? Do vital, greenish or senescent,
yellowish algae behave in a similar manner and are both able to recover after desiccation when rehydrated?

To gain more insights into this community we studied filamentous green algae mats of two saline - alkaline ponds located in the Seewinkel region, Austria. Every year, dense mats develop during springtime, which finally cover large areas of the ponds. By time, the shallow pans are drying out due to high evaporation and increased insolation. We hypothesized that algae mats from such biotops have developed some strategies to cope with desiccation showing a decrease of photosynthetic performance only at elevated water loss. Moreover, we expected that yellowish algae that have been floating on the water surface for some time show a higher resistance to desiccation than greenish ones growing near the bottom of the ponds. We assumed some recovery potential for the filamentous algae after rehydration similar to specimens in other habitats with altering water and air exposure (Fleming et al., 2007; Gasulla et al., 2009; Gray et al., 2007; Hunt and Denny, 2008; Kim and Garbary, 2007; Lüttge and Büdel, 2009; Möstl and Schagerl, unpublished).

## Study site

The Seewinkel region covers an area of about $450 \mathrm{~km}^{2}$ and is located in the NorthEastern part of Austria near the Hungarian border at 115 - 130 m above sea level. It contains around 40 shallow ponds with elevated conductivity (Löffler, 2000). Whereas saline inland waters are common in endorheic basins like the East African Rift Valley, Australia, Central Asia or in some parts of the United States, they are quite rare for Europe (Hungary, Turkey, Ukraine). The climate can be classified as pannoniancontinental and is characterized by an average temperature of around $9.9 \pm 0.6^{\circ} \mathrm{C}$ and an average annual rainfall of $639 \pm 96 \mathrm{~mm}$ (Dokulil et al., 2001). With more than 240 days a year having an average temperature more than $5^{\circ} \mathrm{C}$ and more than 61 days over
$25^{\circ} \mathrm{C}$ it is one of the warmest regions of Austria. Most of the precipitation is recorded in late spring and early summer, but high summer temperatures and strong winds lead to high evaporation rates so that semiarid conditions can be reached (Dokulil et al., 2001; Löffler, 1982).

The water level of the ponds is usually reduced during summer and some pans can even dry out. Transpiration leads to a transport of ions, mainly $\mathrm{Na}^{+}$and $\mathrm{HCO}_{3}{ }^{-}$from deeper soil layers rich in salts into the ponds creating alkaline-saline conditions (Dick et al., 1994; Löffler, 1982; Wolfram et al., 1999).

### 3.3 Material \& Methods

## Seasonal comparison

Sampling was conducted in two of the shallow saline-alkaline ponds in the region, the Zicklacke and the Lettengrube in June and September 2008 (Fig. 1). We collected vital, green material near the bottom. On thee sample days in June and five in September, 2 to 6 pieces of algae mats each of about $10 \times 15 \mathrm{~cm}$ were prepared in the laboratory. The mats were repeatedly rinsed with filtrated pond water to get rid of the sediment; animals were removed with forceps. We applied the non-invasive pulse amplitude modulated (PAM) fluorescence technique for information of the overall photosynthetic performance of the algae, (Diving PAM, Walz): as a first step, the minimum fluorescence Fo is measured after at least 10 min dark acclimation, when all photosynthetic reaction centers are open and fully oxidized. This is followed by a saturating pulse of actinic light, which closes all reaction centers and induces the maximum fluorescence Fm . The maximum fluorescence yield $(\mathrm{Fm}-\mathrm{Fo}) / \mathrm{Fm}=\mathrm{Fv} / \mathrm{Fm}$ ( $\mathrm{Fv}=$ variable fluorescence) is an excellent tool to gain information about the photosynthetic performance and is therefore commonly used in plant physiology (Krause and Weis, 1991; Maxwell and Johnson, 2000). Thereafter, mats were carefully transferred to absorbent paper to remove most of the capillary water. Fresh mass (FM) was measured and mats were put into a cabinet dryer at $33^{\circ} \mathrm{C}$ to simulate desiccation at summer temperatures. During the drying process, $\mathrm{Fv} / \mathrm{Fm}$ and algal mass were repeatedly measured at least every hour. Afterwards the pieces were dried at $95^{\circ} \mathrm{C}$ for at least 12 h to determine dry mass (DM). Water content (WC) was calculated according to $($ Bjork et al., 1999 $) . \mathrm{WC} \%=($ desiccated mass- DM $) /(\mathrm{FM}-\mathrm{DM}) \times 100$.

## Recovery experiments

One to three small pieces where cut out of the algae mats at different drying stages during the desiccation experiments in September. Both the small piece and the remaining part were weighted to ensure correct calculation of water loss. After Fv/Fm of the small piece was measured, it was put into filtrated pond water for rehydration. To record recovery, Fv/Fm was re-measured one hour after rewetting. For evaluation of the recovery experiment, vital samples were grouped into 3 groups depending on the state of desiccation before rehydration was started: $\mathrm{V}_{\mathrm{s}}$ was slightly desiccated (WC between 15 and $30 \%$ ), $\mathrm{V}_{\text {med }}$ was medium desiccated (WC between $5 \%$ and $15 \%$ ), and $\mathrm{V}_{\max }$ was severely desiccated with WC lower than $5 \%$.

## Vital - senescent comparison

In September, sub-vital, yellowish mats floating on the water surface were collected on three days. Desiccation and recovery experiments were carried out in the same way as the seasonal comparison. For analysing the recovery of senescent mats, they were put into two groups with $\mathrm{S}_{\mathrm{s}}$ showing a WC between 10 and $40 \%$ and $\mathrm{S}_{\text {max }}$ with WC lower than $10 \%$ before recovery tests started.

## Statistics

Statistical analysis was performed using the software package PASW 18. After checking for normal distribution via Kolmogorov-Smirnov-test, comparison of initial values with student's t-test for two independent variables was performed; for analyzing recovery data, student's t-test for two dependent variables was used. Spearman correlation factor was calculated for the linear regressions and comparison between slopes of regression followed the z-statistics (Clogg et al., 1995).

### 3.4 Results

## Seasonal comparison

During the investigation period, algae mats were well developed and covered large areas of the pans; they mainly consisted of filamentous green algae like Cladophora sp . and Rhizoclonium sp. Initial Fv/Fm of June ( $0.69 \pm 0.05, \mathrm{n}=14$ ) and September $(0.67 \pm$ $0.03, \mathrm{n}=19$, ) showed no significant differences ( $\mathrm{p}=0.074$, t -test). Fv/Fm over a desiccation gradient followed a logarithmic curve in both months (Figs. 3 and 4). Spearman correlation coefficients of $r=0.901$ for June and $r=0.885$ for September indicated a strong relation. Z-statistics used for comparing the slopes of the regression curves (June: $y=46.73 x+7.55$; September: $y=38.78 x+27.93$ ) revealed a $z$-value greater than 1.96 indicating a significant difference $(z-v a l u e=2.90)$.

## Comparison Vital-Senescent

Initial $\mathrm{Fv} / \mathrm{Fm}$ of vital green algae mats $(0.67 \pm 0.03, \mathrm{n}=19)$ differed significantly from senescent, yellowish ones $(0.35 \pm 0.05, \mathrm{n}=9 ; \mathrm{p}=0.000$, t -test; Fig. 5), which shows that senescent samples started from much lower initial yields in the desiccation experiment. Both curves followed a logarithmic function with a decrease of $\mathrm{Fv} / \mathrm{Fm}$ starting at approximately $30 \%$ water content (Figs. 6 and 7). The Spearman correlation factor was $\mathrm{r}=0.885$ for vital mats and $\mathrm{r}=0.779$ for senescent ones showing a strong correlation, slopes of the regression curves differed significantly (vital: $\mathrm{y}=38.775 \mathrm{x}+27.931$; senescent: $\mathrm{y}=54.597 \mathrm{x}+6.295$; z -value $=4.815)$.

## Recovery potential

For evaluation of the recovery, vital samples were classified into 3 groups. $\mathrm{V}_{\mathrm{s}}$ showed an average $\mathrm{Fv} / \mathrm{Fm}$ value of $0.56 \pm 0.07$ before recovery experiments started. A significant recovery was observed with an average value of $0.63 \pm 0.13$ after 1 h of rehydration ( $p=0.033, n=12$, t -test with 2 dependent groups). Initial $\mathrm{Fv} / \mathrm{Fm}$ of $\mathrm{V}_{\mathrm{med}}$
ranged around $0.35 \pm 0.06$ and for $\mathrm{V}_{\max }$, values of $0.15 \pm 0.05$ were measured. Both these two groups showed no recovery after 1 h of rehydration. Surprisingly the yield even further decreased significantly during rehydration in both groups $\left(\mathrm{V}_{\text {med }}=0.24 \pm\right.$ $0.14 ; \mathrm{V}_{\max }=0.08 \pm 0.05$; t-test with 2 dependent groups; Fig. 8).

For analysing recovery data of senescent samples they were put into two groups. $\mathrm{S}_{\mathrm{s}}$ showed initial $\mathrm{Fv} / \mathrm{Fm}$ of $0.22 \pm 0.06$ and of $0.17 \pm 0.11$ after 1 h of rehydration. $\mathrm{S}_{\max }$ started from an average value of $0.04 \pm 0.02$ and had an average $\mathrm{Fv} / \mathrm{Fm}$ of $0.05 \pm 0.03$ after rehydration. Both groups showed no significant differences between values before and after rehydration (t-test with 2 dependent groups).

### 3.5 Discussion

## Seasonal comparison

Both Cladophora sp. and Rhizoclonium sp. are widely distributed genera and both are able to develop huge biomass especially in eutrophicated water bodies (Gubelit and Berezina, 2010; Higgins et al., 2008; Khanum, 1982; Pihl et al., 1999; Taylor et al., 2001; Vis et al., 2008). Interestingly, these generi seem to be quite resistant against elevated ion concentrations. Rhizoclonium is found in fresh and brackish waters and shows a high tolerance against elevated salinity (Chao et al., 2005; Imai et al., 1997; Matsuyama-Serisawa et al., 2004; Taylor et al., 2001), Cladophora comprises both fresh water and marine taxa (Ensminger et al., 2001; Gubelit and Berezina, 2010; Higgins et al., 2008; Rani, 2007; Taylor et al., 2001; Vis et al., 2008).

In our experiments initial $\mathrm{Fv} / \mathrm{Fm}$ values of vital mats composed of Cladophora sp . and Rhizoclonium sp. ranged around 0.70 , which is similar to values obtained by Hiriart Baer et al. (2008), who measured average Fv/Fm values of 0.711 for unstressed healthy Cladophora specimens. The significant $\mathrm{Fv} / \mathrm{Fm}$ difference along the desiccation gradient between June and September is due to a sharper decrease of $\mathrm{Fv} / \mathrm{Fm}$ in June. Both groups had comparable initial values, differences started at around $40 \%$ water content, when September samples still reached $90 \%$ of the initial yield whereas June samples already decreased to around $82 \%$ of the initial yield. At $10 \%$ water content this difference was even more pronounced with September samples still having $67 \%$ of initial $\mathrm{Fv} / \mathrm{Fm}$ in comparison to $54 \%$ for June samples. These results indicate some acclimation processes to avoid severe damage at intermediate desiccation in the course of the season, when the probability of drying up increases.

In comparison to other studies conducted in periodically drying habitats, the filamentous green algae in our experiments resisted quite long against desiccation.

Codium sp. collected from estuaries, for example, showed already a $50 \%$ decrease of Fv/Fm when 40 \% water content was reached (Kim and Garbary, 2007) and the electron transport rate of the intertidal algae Endocladia muricata (Endlichter) J. Agardh was almost zero at $30 \%$ water content (Hunt and Denny, 2008). According to Johnson et al. (1974), most intertidal species show high rates of photosynthesis only until $70 \%-40 \%$ water content. It appears, that effective acclimation mechanisms are linked to an early decrease of $\mathrm{Fv} / \mathrm{Fm}$ already under only slight desiccation.

## Comparison vital - senescent

Compared to vital, green specimens, senescent yellowish mats showed significantly decreased initial $\mathrm{Fv} / \mathrm{Fm}$ indicating a lowered photosynthetic performance. High irradiance and elevated temperatures near the water surface obviously caused some damage, which might be linked to altered pigment concentrations (Fuhrmann and Schagerl, unpublished). Along the desiccation gradient, Fv/Fm of vital and senescent specimens was significantly different and senescent ones showed a sharper drop of $\mathrm{Fv} / \mathrm{Fm}$ at decreased water contents. Contrarily to our expectations, senescent mats occurring near the surface seemed even more sensitive against desiccation than the vital ones growing near the bottom. So it is likely that those specimens are already partly damaged. Interestingly, at slightly desiccated states the $\mathrm{Fv} / \mathrm{Fm}$ values exceeded initial values. This effect might be due to higher $\mathrm{CO}_{2}$ diffusion rates in air than in water, which has already been reported for cyanobacteria, lichens and mosses (Coxson et al., 1983; Fleming et al., 2007; Titus and Wagner, 1984). In their studies on Fucus spiralis L., Madsen and Maberly (1990) found at slight desiccation of about $95 \%$ WC an increase of net photosynthesis up to 110 to $148 \%$ compared to fully hydrated plants. In our experiments senescent samples reached $114 \%$ of initial $\mathrm{Fv} / \mathrm{Fm}$ at the $95 \%$ water content level whereas vital samples reached only $105 \%$.

It has to be considered here, that vital samples are starting from optimum Fv/Fm, so almost no increase is possible, whereas senescent samples showed already reduced initial values.

## Recovery potential

Contrarily to our expectations, algae mats showed only little recovery potential. In detail, only vital specimens with slight desiccation were fully recovering. For medium and severely desiccated filaments, a continuing decrease in $\mathrm{Fv} / \mathrm{Fm}$ even after 1 h of rehydration was detected which points to a severe cell damage due to desiccation. From our results, we conclude, that no effective protection mechanisms against severe desiccation have been developed. Also in senescent mats, which are more exposed to unfavorable conditions than vital ones, no significant recovery could be detected for both categories. This goes along with the results from our desiccation experiments where senescent algae did not resist longer to desiccation compared to the vital ones.

Our results are quite surprising, because algae from intertidal zones commonly show a high recovery potential (Davison and Pearson, 1996; Fleming et al., 2007; Hunt and Denny, 2008; Kim and Garbary, 2007; Möstl and Schagerl, unpublished). Also algae growing in habitats with elongated dry phases like tree barks (Lüttge and Büdel, 2009), deserts (Gray et al., 2007) or phycobionts (Gasulla et al., 2009) show a high recovery potential after desiccation. Often recovery was very fast (Gray et al., 2007; Möstl and Schagerl, unpublished) and recovery was still possible after high water loss and elongated dry periods (Kim and Garbary, 2007; Lüttge and Büdel, 2009; Möstl and Schagerl, unpublished). Contrarily to astatic water bodies, which were investigated in this study, these habitats receive either regular desiccation and rewetting events (intertial) or elongated dry periods (tree barks, desert crusts). Recovery has to be very fast to ensure that available water can efficiently be used for active growth.

Because the ponds of the Seewinkel region are usually filled with water for some months, other strategies have been developed to occupy these habitats: a fast growth rate at optimal living conditions and a development of resting stages at adverse conditions. They obviously do not invest energy in physiological acclimation processes to resist severe desiccation. Gray and Lewis (2007) showed in their study dealing with photosynthetic recovery potential after desiccation that aquatic algae which do not need to cope with desiccation stress have a much lower recovery potential in comparison to close relatives living in periodically desiccating desert habitats. This underlies the point that algae only invest in protection mechanisms if there is really a need for it. We conclude from our investigation, that Cladophora. and Rhizoclonium, which form dense algae mats in the saline-alkaline ponds in spring and early summer, survive slight desiccation stress but they do not have physiological adaptations, which allow them to recover after full desiccation events during the dry season - it is likely that they escape unfavorable conditions by producing resting stages.

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### 3.7 Legends

Fig. 1: Map of the Seewinkel region. The saline-alkaline ponds are colored black (villages are grey). Arrows indicate sampling stations.

Fig. 2: Initial Fv/Fm values of vital algae mats from samples taken in June $(\mathrm{n}=14)$ and September $(\mathrm{n}=19)$ 2008. Boxes are indicating median and $25^{\text {th }}$ and $75^{\text {th }}$ percentile; dots represent outliers.

Fig. 3: Fv/Fm along a gradient of desiccation, samples were taken in June 2008 and September 2008

Fig. 4: Percentage of initial $\mathrm{Fv} / \mathrm{Fm}$ related to the log-percentage of water content. Dotted line $=$ linear regression curve of June data $(y=46,728 x+7,545)$, solid line $=$ linear regression curve of September data ( $y=38,775 x+27,931$ ).

Fig. 5: Initial $\mathrm{Fv} / \mathrm{Fm}$ values of vital $(\mathrm{n}=19)$ and senescent $(\mathrm{n}=9)$ algae mats from samples taken in September 2008. Boxes are indicating median and $25^{\text {th }}$ and $75^{\text {th }}$ percentile; dots represent outliers

Fig. 6: Fv/Fm along a gradient of desiccation for vital and senescent algae mat samples taken in September 2008)

Fig. 7: Percentage of initial $\mathrm{Fv} / \mathrm{Fm}$ related to the log-percentage of water content; comparison between senescent and vital samples. Dotted line = linear regression curve of senescent samples $(y=54,597 x+6,295)$, solid line $=$ linear regression curve of vital samples ( $\mathrm{y}=38,775 \mathrm{x}+27,931$ )

Fig. 8: Recovery of vital algae mats (September 2008); Start $=\mathrm{Fv} / \mathrm{Fm}$ values measured after the desiccation process just before rehydration was started, End $=\mathrm{Fv} / \mathrm{Fm}$ values measured after 1 hour of rehydration; $\mathrm{SD}=$ Slightly desiccated group ( $\mathrm{n}=12$ ), MD = Medium desiccated group ( $\mathrm{n}=12$ ), $\mathrm{SeD}=$ Severely desiccated group ( $\mathrm{n}=18$ ), $\mathrm{WC}=$ Water content; Boxes are indicating median and $25^{\text {th }}$ and $75^{\text {th }}$ percentile; dots represent outliers


Fig. 1: Map of the Seewinkel region. The saline-alkaline ponds are colored black (villages are grey). Arrows indicate sampling stations.


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Fig. 4: Percentage of initial $\mathrm{Fv} / \mathrm{Fm}$ related to the log-percentage of water content. Dotted line $=$ linear regression curve of June data $(y=46,728 x+7,545)$, Solid line $=$ linear regression curve of September data $(y=38,775 x+27,931)$.


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Fig. 6: $\mathrm{Fv} / \mathrm{Fm}$ along a gradient of desiccation for vital and senescent algae mat samples taken in September 2008


Fig. 7: Percentage of initial Fv/Fm related to the log-percentage of water content; comparison between senescent and vital samples. Dotted line = linear regression curve of senescent samples ( $y=54,597 x+6,295$ ), solid line $=$ linear regression curve of vital samples ( $\mathrm{y}=38,775 \mathrm{x}+27,931$ )


Fig. 8: Recovery of vital algae mats (September 2008); Start $=\mathrm{Fv} / \mathrm{Fm}$ values measured after the desiccation process just before rehydration was started, End $=\mathrm{Fv} / \mathrm{Fm}$ values measured after 1 hour of rehydration; SD = Slightly desiccated group ( $\mathrm{n}=12$ ), $\mathrm{MD}=$ Medium desiccated group ( $\mathrm{n}=12$ ), $\mathrm{SeD}=$ Severely desiccated group ( $\mathrm{n}=18$ ), $\mathrm{WC}=$ Water content; Boxes are indicating median and $25^{\text {th }}$ and $75^{\text {th }}$ percentile; dots represent outliers

## 4. Summary

The Seewinkel region located in the East of Austria and characterized by a semi-arid climate hosts around forty shallow saline-alkaline ponds. These small, endorheic water bodies are characterised by high nutrient levels and elevated conductivities. Because of high summer evaporation their water level is usually reduced during that period and some salt pans may even dry out. Every year, dense mats of filamentous green algae develop during springtime, which finally cover large areas of the ponds. In our study we focused on the biomass development and seasonal succession of such algae mats in one of the ponds. The study covered a period between April and September and included also weekly measurements of water chemistry parameters. Moreover we investigated the ability of these algae to cope with desiccation by carrying out desiccation and recovery experiments in the laboratory.
$\mathrm{Na}^{+}$and $\mathrm{HCO}_{3}{ }^{-}$were the most dominant ions reaching a maximum in the mid of September, when the pond was nearly dried out; phosphorus concentrations indicated hyper-eutrophic conditions. Maximum algae biomass was reached in July with $59 \pm 10$ $\mathrm{g} \mathrm{m}^{-2}$ ash-free dry mass and $133 \pm 17 \mathrm{mg} \mathrm{m}^{-2}$ chlorophyll $a$ measured in mesocosms, which were installed in the pond. At this time, floating algae mats covered $14 \%$ of the pond surface. These high biomass values clearly indicated that such mass developments of filamentous green algae play an important role in the functioning of the ecosystems. From April to the beginning of June Spirogyra sp. was prevailing in the mats, followed by a shift to Cladophora sp. and Rhizoclonium sp., which were predominating until the end of the investigation period. Interestingly the chlorophyll $a$ to ash-free dry mass ratio was significantly higher when Spirogyra was dominating, which might be due to thinner cell walls of Spirogyra and/or allelopathic activity of this genus inhibiting Aufwuchs.

Desiccation and recovery experiments were carried out in the laboratory using the noninvasive pulse amplitude modulated (PAM) fluorescence technique, which is an excellent tool for getting information of the overall photosynthetic performance of the algae. Samples taken in September seemed to resist a bit longer during the desiccation process than samples taken in June, showing a significantly different desiccation rate. This might indicate some acclimation processes at intermediate desiccation in the course of the season, when the probability of drying increases. However, recovery experiments carried out in September revealed a very low ability to recover after severe desiccation. Only the slightly desiccated samples could significantly recover when rehydrated.

When comparing vital, algae mats growing on the bottom of the pond with senescent, yellowish ones floating on the surface, we measured significantly lower initial fluorescence values of the senescent mats, indicating a lowered photosynthetic performance. This could be due to some irreversible damages caused by excess irradiance and elevated temperatures near the water surface. Moreover, senescent mats showed a significantly different course of desiccation compared to the vital ones, due to a sharper drop of the yield at decreased water contents and an increase when samples were just exposed to air ( $95 \%$ water content), which might be explained by increased $\mathrm{CO}_{2}$ diffusion rates in air. When rehydrated after desiccation senescent samples did not recover at all.

The low ability to regenerate after desiccation for vital as well as senescent algae is quite surprising because usually specimens occurring in periodically desiccating habitats show quite a high recovery potential. Maybe the reason for this fact is that algae in the Seewinkel ponds do not need to invest in mechanisms to cope with
desiccation because the water bodies are usually filled with water for several months before drying out.

Contrarily to algae growing in intertidal zones, the algae in the ponds do not face a diurnal pattern of desiccation and rewetting or have to deal with very rare events of sufficient water supply, like species in deserts or on tree barks. So it is likely that filamentous algae in the Seewinkel ponds use the optimum growth period in spring for a fast biomass increase. Afterwards, resting stages are produced to survive the unfavorable conditions of drying.

## 5. Zusammenfassung

Im Seewinkel im Osten von Österreich befinden sich rund vierzig flache, alkalische Salzlacken. Charakteristisch für diese kleinen, endorheischen Gewässer sind die hohen Nährstoffwerte und die durch die hohe sommerliche Verdunstung und semi-arides Klima angetriebene, zumindest teilweise Austrocknung im Sommerhalbjahr. Jedes Jahr im Frühjahr entwickeln sich dichte Matten aus filamentösen Grünalgen, die schließlich große Bereiche der Lacken bedecken. In unserer Studie untersuchten wir im Zeitraum von April bis September die Biomasseentwicklung und Sukzession der Algen in einer ausgewählten Lacke; begleitend wurden wöchentlich chemische Parameter erhoben. Außerdem untersuchten wir die Fähigkeit der mattenbildenden Algen Austrocknung zu ertragen, indem wir im Labor Austrocknungs- und Regenerationsversuche durchführten. $\mathrm{Na}^{+}$und $\mathrm{HCO}_{3}{ }^{-}$waren die dominanten Ionen in der Lacke, die Mitte September - als die Lacke kurz vor der Austrocknung stand - ein Maximum erreichten. Die über die Untersuchungsperiode gemessenen Phosphor-Konzentrationen zeigten hyper-eutrophe Bedingungen an. Maximale Biomasse-Werte wurden im Juli mit $59 \pm 10 \mathrm{~g} \mathrm{~m}^{-2}$ aschefreiem Trockengewicht und $133 \pm 17 \mathrm{mg} \mathrm{m}^{-2}$ Chlorophyll $a$ in Mesocosmen gemessen, die in der Lacke installiert waren. Maximale Bedeckungswerte auftreibender Algenmatten wurden ebenfalls im Juli mit 14\% Bedeckung der Lackenoberfläche ermittelt. Diese hohen Biomassewerte lassen vermuten, dass das Massenauftreten filamentöser Grünalgen in den Lacken eine wesentliche Rolle im Ökosystem der Gewässer spielt.

Von April bis Anfang Juni dominierte Spirogyra sp. in den Algenmatten, danach setzten sich diese vor allem aus Cladophora sp. und Rhizoclonium sp. zusammen. Interessanterweise war das Verhältnis von Chlorophyll $a$ zu aschefreiem Trockengewicht signifikant höher, als Spirogyra dominierte. Möglicherweise lässt sich
dies durch die dünneren Zellwände von Spirogyra und/oder durch die Aufwuchs verhindernden, allelopathischen Eigenschaften dieser Gattung erklären.

Austrocknungs- und Regenerationsversuche wurden im Labor unter Verwendung der „pulse amplitude modulated (PAM) Fluoreszenz" - Technik durchgeführt, mit deren Hilfe man Aufschluss über die Photosyntheseleistung bekommt. Proben, die im September genommen wurden, hielten im Vergleich zu den Juni-Proben der Austrocknung etwas länger stand, was sich in einer signifikant unterschiedlichen Trocknungsrate niederschlug. Möglicherweise deutet dies auf Adaptionsmechanismen hin, die im Laufe der Saison erworben werden, um mit leichter Austrocknung besser fertig zu werden. Allerdings, zeigten Regenerationsversuche, dass die Algen sehr sensibel auf stärkere Austrocknung reagieren; lediglich die nur wenig ausgetrockneten Matten erholten sich nach der Wiedervernässung.

Beim Vergleich von frischen, benthisch wachsenden Algenmatten mit älteren, an der Wasseroberfläche treibenden, zeigte sich, dass die auftreibenden Matten einen signifikant niedrigeren Grund-Fluoreszenzwert aufweisen. Dies deutet auf eine verminderte Photosyntheseleistung hin; möglicherweise verursacht durch Schäden, die durch die starke Strahlung und die hohen Temperaturen an der Wasseroberfläche entstanden sind. Außerdem zeigten die älteren Matten eine signifikant unterschiedliche Austrocknungsrate begründet durch einen steileren Abfall der Fluoreszenz-Werte gegen Ende der Austrocknung und einen Anstieg der Werte kurz nach Beginn der Austrocknung (95\% Wassergehalt), was möglicherweise mit der besseren $\mathrm{CO}_{2}-$ Verfügbarkeit an der Luft zusammen hängt. Die Regenerationsversuche ergaben, dass die älteren Matten überhaupt nicht fähig zur Regeneration waren.

Die Tatsache, dass die Algenmatten insgesamt sehr wenig Regenerationsvermögen zeigten, ist überraschend, da Algen, die in periodisch trocken fallenden Habitaten leben,
häufig ein sehr ausgeprägtes Regenerationspotential aufweisen. Möglicherweise liegt das darin begründet, dass die Seewinkellacken vor der (partiellen) Austrocknung monatelang mit Wasser gefüllt sind und daher Anpassungen, wie sie Algen in Gezeitenzonen mit sehr kurzen Abfolgen von Austrocknung und Wiedervernässung oder in Habitaten, mit nur äußerst seltener Wasserverfügbarkeit (in Wüsten, an Baumstämmen) brauchen, nicht von Nöten sind. Die filamentösen, mattenbildenden Grünalgen in den Salzlacken nutzen die optimalen Bedingungen im Frühjahr für schnelles Wachstum und produzieren dann vor der Austrocknung Dauerstadien, anstatt in aufwendige Mechanismen zur Bewältigung der Austrocknung zu investieren.

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## Lebenslauf

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## Ausbildung

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## Berufspraxis

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Ferialpraxis - Amt der Burgenländischen Landesregierung Ferialpraxis bei der Firma Factory Outlet als Verkäuferin Lehrtätigkeit als Tutorin an der TU Wien Ferialpraxis bei der Firma Factory Outlet als Verkäuferin Ferialpraxis - Sozialversicherung der gewerb. Wirtschaft Nachhilfelehrerin - Studienkreis Eisenstadt

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Ökopädagogin für den WWF Österreich
Tätigkeit als selbstständige Naturführerin:

- Durchführung von naturkundlich geführten Kanuexkursionen am Neusiedler See
- Vorträge in der Sonderkrankenanstalt St. Andrä am Zicksee über den Nationalpark
- Naturführungen für den Naturpark Neusiedler See Leithagebirge
- Konzeption von Mikrokosmos - Exkursionen für den Nationalpark Neusiedler See - Seewinkel
- Tätigkeit als Vortragende für den Nationalparkexkursionsleiter - Ausbildungskurs 2010

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2010-2011 Konzeption von ökopädagogischen Konzepten für Schulklassen zum Thema „Wasserlebensräume" für den Nationalpark Neusiedler See - Seewinkel

## Weitere Qualifikationen

Internationale Projekte Mitarbeit bei dem internationalen Forschungs-Projekt „Climate change impacts on the trophodynamic couplings of temporary wetland foodwebs." Acciones Integradas Project ES 16/2008.

Teilnahme an einem Meeresbiologischen Kurs des Hydra - Instituts auf Elba (Italien)

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