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Primary Research Paper

Importance of nutrient competition and allelopathic effects in suppression of the green alga *Scenedesmus obliquus* by the macrophytes *Chara*, *Elodea* and *Myriophyllum*

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Abstract

Possible allelopathic effects of substances released from the macrophytes *Chara globularis, Elodea canadensis, Myriophyllum spicatum* on the common green alga *Scenedesmus obliquus* were tested in the laboratory with plastic plants and untreated medium as controls. A two-phase approach was used in which first the effects of physical presence of plants was studied (phase I) followed by the effects of plant culture filtrates (phase II). In the presence of plastic plants growth was reduced only marginally, but strong growth inhibition of *Scenedesmus* occurred in the physical presence of all macrophytes. In contrast, filtrates from *Chara* had no growth inhibitory effect on *Scenedesmus*. *Myriophyllum* filtrate reduced particle-based growth rate by 7% compared to filtration controls, while *Elodea* culture filtrate reduced volume-based growth by 12%, chlorophyll-based growth by 28% and particle-based growth by 15%. Photosystem II-efficiency of *Scenedesmus* was reduced in all three macrophyte treatments in phase I, but not in filtrates from macrophyte cultures (phase II). Thus, while enzyme activity or other physiological aspects may have been affected, the current study yielded no proof for allelopathically active compounds being directed at photosynthesis. Mean particle volume (MPV) of *Scenedesmus* was not influenced by macrophyte exudates and cultures remained dominated by unicells. The strong growth inhibitory effects found for *Scenedesmus* in the physical presence of macrophytes, but not in plastic controls, and no or weaker response in nutrient-enriched filtrates, suggest nutrient competition was a more powerful driving factor than allelochemicals. However, the experimental design does not exclude disappearance of allelochemicals during the filtration process.

Introduction

In shallow lakes, submerged macrophytes exert a strong positive effect on water transparency and show inverse relations with phytoplankton (Scheffer, 1998). Several mechanisms are involved in the antagonistic relationship between phytoplankton and macrophytes. Low water turbulence in macrophyte stands, poor under water light climate and reduced nutrient concentrations, refuge for grazers on algae causing increased predation and the excretion of chemicals that inhibit phytoplankton growth may all contribute to maintenance of low phytoplankton biomass in macrophyte beds (Scheffer, 1998; Søndergaard & Moss, 1998).

Numerous studies have been devoted to the stimulatory and inhibitory effects on primary

producers and microorganisms evoked by extracellular metabolites of primary producers, which is termed allelopathy (e.g. Gross, 2003). Both macrophytes in freshwater systems and seaweed in marine environments may produce compounds with algicidal activity (e.g. Wium-Andersen, 1987; Gross et al., 1996; Jeong et al., 2000; Gross, 2003). Several species of submerged macrophytes may contain allelochemicals that suppress algal growth. Sulphuric compounds isolated from the green macroalga *Chara globularis* may inhibit phytoplankton photosynthesis (Anthoni et al., 1980). Planas et al. (1981) found growth inhibition by phenolic compounds isolated from *Myriophyllum spicatum*, some of which are also released into the surrounding water (Gross et al., 1996; Nakai et al., 1996, 1999; Gross, 2003). Extracts from *Elodea canadensis* have been shown to suppress photosynthesis in the diatom *Nitzschia palea* (Wium-Andersen, 1987).

In addition to effects on phytoplankton photosynthesis and biomass, macrophytes may also have a clear effect on the algal community structure and the size of members forming the community (Jasser, 1995; Søndergaard & Moss, 1998). For example, *Chlamydomonas* was significantly smaller inside macrophyte beds, whereas *Cryptomonas* was significantly larger inside macrophyte stands than outside (Søndergaard & Moss, 1998). Flagellates, such as *Cryptomonas* and *Chlamydomonas*, dominated in the presence of macrophytes, but were replaced by diatoms and green algae, such as *Scenedesmus*, in the absence of plants (Schriver et al., 1995; Van de Berg et al., 1998). Also Hasler & Jones (1949) found *Scenedesmus* among the most abundant algae in the absence of plants, but virtually lacking in the presence of *Elodea canadensis*. In enclosures without *Chara* blooms of *Scenedesmus* developed, while in presence of *Chara* a diverse algal community with low biomass was found (Van Donk & Van de Bund, 2002). Since flagellated species had replaced algae such as *Scenedesmus* (Schriver et al., 1995), reduced resuspension, and thus higher net sedimentation of non-motile algae, in plant beds is assumed the major steering factor (Scheffer, 1998; Søndergaard & Moss, 1998). Several studies have failed to detect allelopathic effects of macrophytes on *Scenedesmus* (e.g. Jasser, 1995; Körner & Nicklisch, 2002; Van Donk & Van de Bund, 2002; Mulderij et al., 2003), which seems to support stagnant water around macrophytes as a major cause for the disappearance of *Scenedesmus* from the water column.

Nonetheless, allelopathic effects on growth and morphology of *Scenedesmus* cannot be excluded. For example, Blindow & Hootsmans (1991) found 10% growth reduction of *Desmodesmus communis* (formerly known as *Scenedesmus communis*) in water from cultures of *Chara globularis*. *Scenedesmus falcatus* was inhibited by tellimagrandin II, the main inhibitory substance in *Myriophyllum*, albeit less than cyanobacteria were (Gross et al., 1996). Moreover, *Scenedesmus* is one of the commonest genera of freshwater algae, which can be found in freshwater bodies all around the world, and even in the soil (Trainor, 1998). *Scenedesmus* species are characterized by a high degree of phenotypic plasticity and a high tolerance to a wide range of environmental conditions (Trainor, 1998). The easy attachment of *Scenedesmus* to substrates, by which it may leave the pelagic habitat and switch from plankton to periphyton (Otten & Willemse, 1988), might confront macrophytes with a potent epiphytic competitor. Hence, one could expect macrophyte exudates to be directed against such common and widespread competitors.

In the present study, potential allelopathic effects on *Scenedesmus* were evaluated of the macrophytes *Chara globularis, Elodea canadensis* and *Myriophyllum spicatum*, of artificial plants, and of medium in which the plants had been grown. In addition to standard endpoints as growth and photosynthesis, effects on mean particle volumes (MPV) were examined. Changes in particle volumes in the presence of plants could reflect an influence in cell or colony size and thereby on sinking properties as *Scenedesmus* colonies do have higher sinking rates than unicells (Lürling, 2003).

Materials & methods

Organisms

The green alga *Scenedesmus obliquus* SAG 276/3a was obtained from the culture collection of the University of Göttingen (Germany). Algal stock

cultures were maintained in 300 ml cellulose plug stoppered Erlenmeyer flasks on a modified WC (Woods Hole modified CHU10)-medium (Lürling & Beekman, 1999) in a climate controlled room at 20 °C in continuous light of 60-μmol quanta m^{-2} s^{-1} (PAR).

Chara globularis, and shoots of *Elodea canadensis* and *Myriophyllum spicatum* were collected from experimental ditches in the vicinity of Wageningen (The Netherlands). Macrophytes were carefully rinsed in the laboratory with ultrapure water and were transferred to 1-l jars with 50% WC-medium and placed for two days at 20 °C in light of 80-μmol quanta m^{-2} s^{-1} (PAR) light in a 16:8 h light:dark rhythm.

Effects of Chara, Elodea and Myriophyllum on Scenedesmus

A two-phase experiment was conducted to examine the effect of macrophytes on *Scenedesmus* growth. In the first phase (I) the effect of physical presence of the macrophytes was investigated, whereas the second phase (II) was comprised of testing the macrophyte culture filtrate (Fig. 1).

Physical presence of macrophytes

Inoculate of exponentially growing *S. obliquus* were transferred to 50 ml fresh WC-medium in 100 ml Erlenmeyer flasks stoppered with cellulose plugs at equal concentrations of 1.4×10^4 particles ml^{-1}, i.e. 2×10^6 μm^3 ml^{-1}. Four replicate flasks were used for controls and contained only medium and *Scenedesmus*. To four other flasks plastic *Myriophyllum*-like plants were added, four flasks received 1 g wet weight *Chara globularis*, to four other flasks 1 g wet weight *Myriophyllum spicatum* was added, and the final treatment consisted of four replicate flasks with 1 g wet weight *Elodea canadensis*. The wet-mass of 1 g per 50 ml corresponds to concentrations of which culture filtrates have been reported to be inhibitory to green algae and cyanobacteria (Nakai et al., 1996, 1999). The flasks were incubated for 3 days at 20 °C in continuous light of 100-μmol quanta m^{-2} s^{-1} on a rotating shaking device (60 rpm). Initially and further daily algal size distributions and densities were measured in the range of 3–30 μm equivalent spherical diameter (ESD) using a Coulter® Multisizer II particle counter (capillary 100 μm orifice

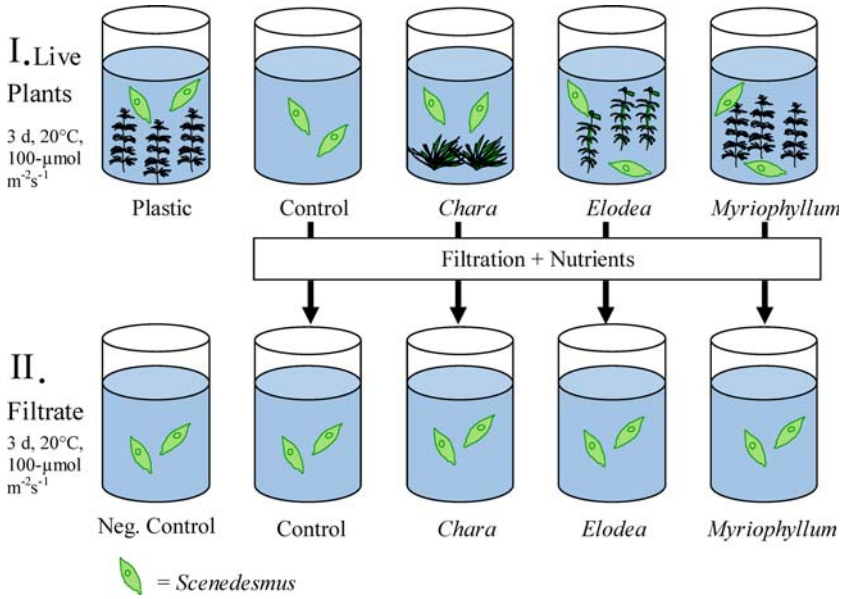

Figure 1. Schematic representation of the two-phase experiment in which in the first phase (I. Live plants) the effects of physical presence of macrophytes (*Chara, Elodea, Myriophyllum*) and artificial plants (Plastic) on the green alga *Scenedesmus* were examined, while in the second phase (II. Filtrate) the culture filtrates of the first phase were examined. Neg. Control represents additional controls with fresh medium.

width, Coulter Electronics Limited, Luton, England). The Chl *a* content ($\mu g\ l^{-1}$) and the efficiency of photosystem II (Φ_{PSII}) were determined using the PHYTO-PAM phytoplankton analyser (Walz, Germany; Lürling & Verschoor, 2003). Since Lampert et al. (1994) have shown that the MPV were highly correlated with the mean number of cells per colony, for statistical comparison of colony size the MPV were used. The MPV that were determined from the electronic particle counter were statistically compared using repeated measures ANOVA followed by Bonferroni *post-hoc* comparison test to distinguish the mean differences that are significantly different at $p = 0.05$ using the statistical tool pack SPSS version 10.1.0. (SPSS, 2000). Growth rates were calculated from the increase in algal biovolume, the number of particles and the Chl *a* concentrations applying linear regressions on natural logarithm transformed data. Growth rates were statistically compared using one-way ANOVA followed by a Bonferroni *post-hoc* comparison test. The Φ_{PSII} after 24, 48 and 72 h were tested over time by means of repeated measure ANOVA.

Macrophytes culture filtrates

After 3 days, 40 ml of each replicate from live macrophyte treatments and controls of the first phase of the experiment (outlined above) was filtered through a glass-fibre filter (GF52, Schleicher & Schuell, Germany). Filtrates were replenished with nutrients that were added as in full WC-medium and filled up with 10 ml fresh WC to a final volume of 50 ml. Four replicate flasks with fresh WC-medium served as additional negative controls that were needed in addition to the procedural controls for filtration (Fig. 1). The resulting treatments were *Scenedesmus* in negative controls, in filtered controls and in flasks with filtrate from *Chara globularis*, *Myriophyllum spicatum* and *Elodea canadensis* (Fig. 1). All resulting treatments were run in quadruplicate with an initial *S. obliquus* inoculate of 1.4×10^4 particles ml^{-1}, i.e. 2×10^6 μm^3 ml^{-1}. The experiment was run as outlined above. Because filtrates from macrophyte treatments received nutrients as in full WC-medium the resulting nutrient concentrations in filtrates could be higher than in full WC-medium. Therefore, prior to the experiment the effect of different concentrations WC-medium on growth of *Scenedesmus* was examined. WC-medium was tested in triplicate at nutrient concentrations of 1, 10, 25, 100% (=standard medium), 250 and 500%. Hereto exponentially growing *S. obliquus* was inoculated at $2\ 10^6$ μm^3 ml^{-1} in 50 ml WC-medium in 100 ml Erlenmeyer flasks that were placed for 3 days at 20 °C in continuous light of 100-μmol quanta m^{-2} s^{-1} on a rotating shaking device (60 rpm). Biovolume-based growth rates were statistically compared by one-way ANOVA followed by a Bonferroni *post-hoc* comparison test.

Comparison between phase I and II

Phase I and II were compared statistically to distinguish between effects caused by the physical presence of macrophytes and allelochemically exerted effects. Growth rates were analysed by two-way ANOVAs with phase (I and II) and treatments (control, *Chara*, *Myriophyllum*, *Elodea*) as fixed factors. Similar incubations (phase I controls and phase II negative controls) were compared by a *t*-test. MPV and Φ_{PSII} were compared by means of repeated measures ANOVA. To pinpoint differences between comparable phase I and II incubations each phase I control or plant treatments and subsequent phase II filtrates were subjected to separate repeated measure ANOVAs.

Results

Physical presence of macrophytes

Biovolume-, Chl *a* and particle-based growth of *Scenedesmus* in the presence of live plants was significantly reduced compared to growth in control medium and treatments with plastic plants (Fig. 2a). One-way ANOVAs on biovolume-based growth rates ($F_{4,15} = 25.7$; $p < 0.001$), Chl *a* based growth rates ($F_{4,15} = 67.6$; $p < 0.001$) and particle-based growth rates ($F_{4,15} = 37.1$; $p < 0.001$) indicated significant differences. Bonferroni's *post-hoc* tests on volume-and Chl *a* based growth showed that controls and plastic plants formed one homogeneous group (treatments which are not significantly different at the 95% confidence level), whereas the three live plant treatments comprised

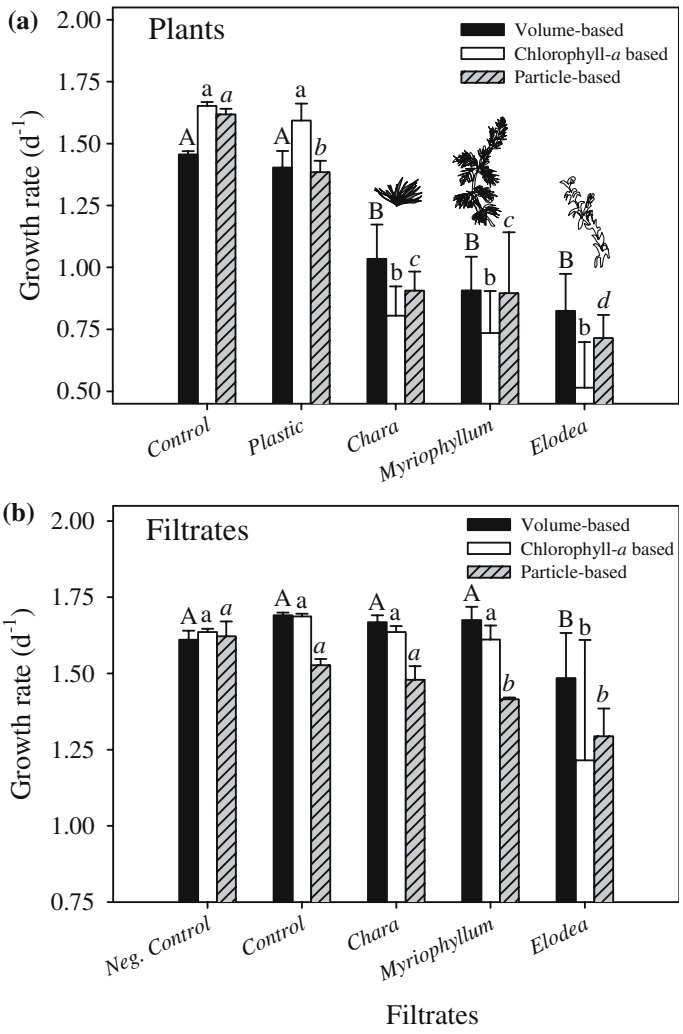

Figure 2. Growth rate of *Scenedesmus obliquus* based on the increase in total algal biovolume (filled bars), Chl *a* (open bars), and particles (shaded bars) over a three days period in the absence (Control) and presence of artificial (Plastic) or real plants (*Chara, Elodea* and *Myriophyllum*; upper panel A), and in their culture filtrates (lower panel B). Neg. Control represents an additional control with fresh medium. Error bars represent one standard deviation ($n = 4$). Similar symbols above the bars (A, B; a, b; *a, b, c*) indicate homogeneous groups that are not significantly different at the 95%-level (Bonferroni's *post-hoc* test).

another homogeneous group (Fig. 2a). For particle-based growth rates Bonferroni's *post-hoc* test revealed four homogenous groups: (1) controls, (2) plastic plants, (3) *Chara* and *Myriophyllum* treatments, and (4) *Myriophyllum* and *Elodea* treatments (Fig. 2a). Particle-based growth for *Scenedesmus* in presence of plastic plants was 14.5% lower than of those in plant free controls, but in presence of live plants particle-based growth was 35–48% lower compared to plastic plant treatments (Fig. 2a).

The average efficiencies of Photosystem II (Φ_{PSII}) of *Scenedesmus* were highest in controls and plastic plant treatments and considerably lower in the presence of live plants (Fig. 3a). A repeated measure ANOVA indicated a significant time (i.e. Φ_{PSII} after 24, 48 and 72 h) effect ($F_{2,30} = 47.8$; $p < 0.001$), a significant treatment and time interaction ($F_{8,30} = 3.87$; $p = 0.003$), and a significant treatment effect ($F_{4,15} = 18.0$; $p < 0.001$). Bonferroni's *post-hoc* test showed that overall Φ_{PSII} in controls and plastic plant treatments

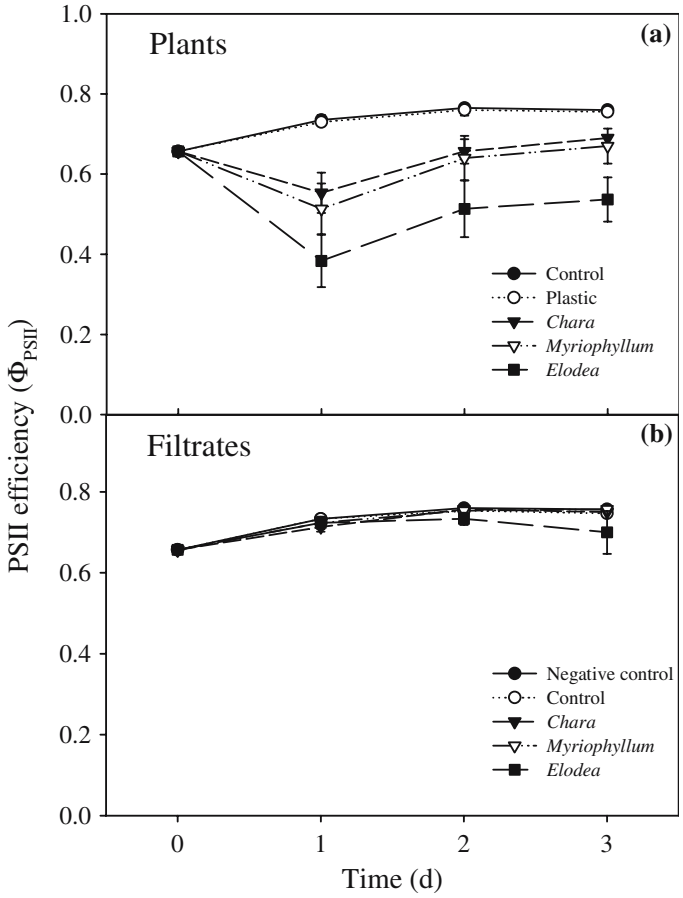

Figure 3. The Photosystem II efficiency of *Scenedesmus obliquus* over a 3 days period in the absence (Control) and presence of artificial (Plastic) or real plants (*Chara, Elodea* and *Myriophyllum*; upper panel a), and in their culture filtrates (lower panel b). Neg. Control represents additional controls with fresh medium. Error bars represent one standard deviation ($n = 4$).

formed one homogeneous group, whereas the three live plant treatments comprised another homogeneous group. The repeated measures ANOVA on the MPV indicated a significant time effect ($F_{3,45} = 12.8$; $p < 0.001$) and a significant treatment × time interaction ($F_{12,45} = 8.11$; $p < 0.001$), but no significant treatment effect ($F_{4,15} = 1.64$; $p = 0.216$). The significant treatment × time interaction is visualised in the course of the MPV of *Scenedesmus* in controls (Fig. 4a). After 1 and 2 days, MPV in controls is significantly larger than in plant treatments, and plastic- and *Myriophyllum* treatments, respectively, while after three days MPV was significantly smaller than in all other treatments (Fig. 4a). MPV did not exceed 300 μm^3 and qualitative microscopic analysis revealed that the cultures were dominated by unicells.

Macrophytes culture filtrates

Prior to the experiment, the effect of different concentrations WC-medium on growth of *S. obliquus* was examined. Biovolume-based growth rates were significantly different for *S. obliquus* grown for three days in WC-medium varying in composition ($F_{5,12} = 180.2$; $p < 0.001$), but Bonferroni *post hoc* comparison revealed that growth was similar in 25%, 100% (= full medium) and 250% WC-medium with rates of 1.32 (± 0.01) d^{-1}, 1.35 (± 0.03) d^{-1} and 1.35 (± 0.03) d^{-1}, respectively. Growth in 1% (0.74 ± 0.01 d^{-1}), 10% (1.21 ± 0.06 d^{-1}) and 500% medium (1.08 ± 0.01 d^{-1}) all were significantly different from each other and the 25%, 100% and 250% medium treatments.

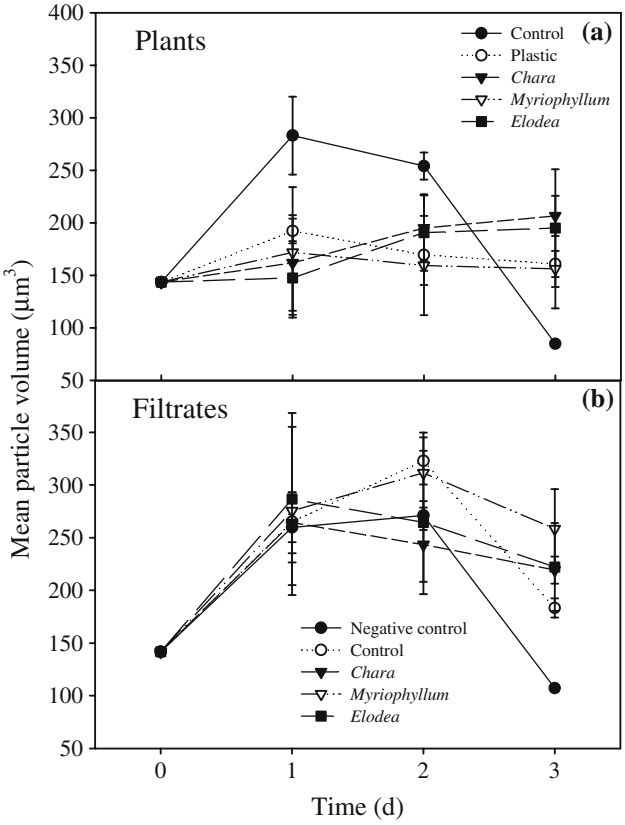

Figure 4. The mean particle volume of *Scenedesmus obliquus* over a 3 days period in the absence (Control) and presence of artificial (Plastic) or real plants (*Chara, Elodea* and *Myriophyllum*; upper panel a), and in their culture filtrates (lower panel b). Neg. Control represents additional controls with fresh medium. Error bars represent one standard deviation ($n = 4$).

When *Scenedesmus* was grown in filtrate from cultures of live plants that was replenished with nutrients both the one-way ANOVA on biovolume-based growth rates ($F_{4,15} = 5.67$; $p = 0.006$) and on Chl *a* based growth rates ($F_{4,15} = 4.71$; $p = 0.012$) indicated significant differences among treatments (Fig. 2b). Bonferroni's *post-hoc* test showed that growth of *Scenedesmus* in filtrate from *Elodea* cultures was significantly lower than in other treatments (Fig. 2b). The reduction compared with filtrate controls was 12 % and 28% for biovolume-based and Chl *a* based growth rates, respectively. When growth rates were calculated from the increase in number of particles, again the one-way ANOVA indicated significant differences ($F_{4,15} = 23.1$; $p < 0.001$). Bonferroni *post-hoc* test revealed that *Scenedesmus* growth rate in filtrate from *Elodea* and *Myriophyllum* cultures was significantly lower than in both controls (Fig. 2b). The reduction compared with filtrate controls was 7% and 15% for *Myriophyllum* and *Elodea* treatments, respectively.

Photosystem II efficiencies (Φ_{PSII}) of *Scenedesmus* in filtrates from macrophyte cultures were considerably higher than in the presence of live plants (Fig. 3b). A repeated measure ANOVA on Φ_{PSII} of *Scenedesmus* in filtrates from macrophyte cultures indicated a significant time effect ($F_{2,30} = 19.2$; $p < 0.001$), no treatment and time interaction ($F_{8,30} = 2.11$; $P = 0.067$), and a significant treatment effect ($F_{4,15} = 3.13$; $p = 0.046$). Bonferroni's *post-hoc* test yielded no significantly different Φ_{PSII} among treatments, but the less conservative Tukey *post-hoc* test revealed that only controls and *Elodea* treatments were significantly different from each other. However, the difference in overall Φ_{PSII} between *Elodea* treatments and controls or filtrate controls was only 3% or 2%, respectively and was only significant in the overall analysis because of small within group variation

(Fig. 3b). One-way ANOVAs on Φ_{PSII} of *Scenedesmus* after one- ($F_{4,15}=2.40$; $p=0.096$), two- ($F_{4,15}=2.65$; $p=0.075$) and three-days ($F_{4,15}=2.61$; $p=0.078$) in phase II showed similar Φ_{PSII} in controls and treatments.

The repeated measures ANOVA on MPV indicated a significant time effect ($F_{3,45}=97.5$; $p<0.001$) and a significant treatment × time interaction ($F_{12,45}=4.60$; $p<0.001$), but no significant treatment effect ($F_{4,15}=2.15$; $p=0.125$). The significant treatment × time interaction is visualized in the course of the MPV of *Scenedesmus* in controls that was similar with other treatments after one and two days, but MPV became significantly lower after three days (Fig. 4b). Mean particle volumes were below 350 μm^3 and microscopy revealed the cultures were dominated by unicells.

Comparison between phase I and II

A two-way ANOVA indicated a significant difference in volume-based growth of *S. obliquus* in phase I and II ($F_{1,24}=193.7$; $p<0.001$), a significant treatment effect ($F_{3,24}=22.9$; $p<0.001$) and a significant phase × treatment interaction ($F_{3,24}=10.3$; $p<0.001$). The significant interaction term is caused by the significantly reduced growth in presence of *Chara* and *Myriophyllum* (Fig. 2a), but disappearance of this effect in their culture filtrates (Fig. 2b). Similar observations were made for chlorophyll-based growth with significant phase ($F_{1,24}=101.6$; $p<0.001$) and treatment effects ($F_{3,24}=29.9$; $p<0.001$) and a significant phase × treatment interaction ($F_{3,24}=10.4$; $p<0.001$). Also for particle-based growth a significant difference between the two phases could be detected ($F_{1,24}=171.3$; $p<0.001$), a significant treatment effect was found ($F_{3,24}=50.6$; $p<0.001$), and a significant phase × treatment interaction ($F_{3,24}=14.0$; $p=0.001$). Comparable incubations in phase II and I were the controls (I) and negative controls (II) that showed a ~5% and significantly lower (*t*-test: $t=4.5$; $p=0.004$) volume-based growth in phase I (1.46 ± 0.01 d^{-1}) than in phase II (1.54 ± 0.03 d^{-1}). However, chlorophyll-based ($t=1.7$; $p=0.147$) and particle-based growth rates ($t=0.2$; $p=0.871$) were similar for these populations.

Comparison of Φ_{PSII} of *S. obliquus* in phase I and II indicated significant differences between the two phases ($F_{1,24}=72.0$; $p<0.001$), a significant treatment effect ($F_{3,24}=15.0$; $p<0.001$) and a significant phase × treatment interaction ($F_{3,24}=11.1$; $p<0.001$). The significant interaction term is caused by the significantly reduced Φ_{PSII} of *S. obliquus* in presence of *Chara*, *Myriophyllum* and *Elodea* (Fig. 3a), but disappearance of this effect in their culture filtrates (Fig. 3b).

Comparison of MPV in phase I controls and real plant treatments with their phase II filtrates (Fig. 4a & b) by repeated measure ANOVA revealed a significant difference between both phases ($F_{1,24}=33.2$; $p<0.001$), a significant phase × time interaction ($F_{3,24}=3.93$; $p=0.021$), but no treatment effect ($F_{3,24}=0.32$; $p=0.814$). Significant differences between the two phases were found for *Chara* ($F_{1,6}=23.1$; $p=0.003$) and *Myriophyllum* treatments ($F_{1,6}=20.1$; $p=0.004$), for controls ($F_{1,6}=80.1$; $p<0.001$) but not for *Elodea* ($F_{1,6}=5.29$; $p=0.061$). Comparison of phase I controls and comparable phase II replicates (negative controls) revealed no differences ($F_{1,6}=1.92$; $p=0.215$) (Fig. 4 a & b).

Discussion

Growth of *Scenedesmus* was reduced significantly in the presence of the three live macrophytes tested. This finding is in agreement with the expectations on the antagonistic effect of macrophytes on phytoplankton biomass (e.g. Scheffer, 1998; Søndergaard & Moss, 1998), which has been found earlier in studies of the effect of *Elodea* and *Chara* on *Scenedesmus* (Hasler & Jones, 1949; Pokorný et al., 1984; Van Donk & Van de Bund, 2002). Several mechanisms can be involved in the antagonistic relationship between phytoplankton and macrophytes (Scheffer, 1998; Søndergaard & Moss, 1998; Van Donk & Van de Bund, 2002), but sorting out the role of these mechanisms in practise remains a major challenge.

In our experiment several potential mechanisms may be ruled out. Increased sedimentation within macrophytes stands may be excluded as a

causal factor as flasks were placed on a shaking device. Increased grazing pressure associated with the refuge macrophytes provide to grazers could be excluded too, because zooplankton was absent. Similarly shading can be ruled out of having a significant effect on volume- and chlorophyll-based growth and on Φ_{PSII} of *Scenedesmus*, as these endpoints were unaffected in the presence of plastic plants compared to plant-free controls. Although particle-based growth was slightly lower in presence of plastic plants compared to plant-free controls, growth rates in plastic plant treatments were still much higher than in presence of real macrophytes. Hence, the strong reduction of growth of *Scenedesmus* by macrophytes seems likely to be the result of allelopathy and/or nutrient competition (Søndergaard & Moss, 1998; Van Donk & Van de Bund, 2002).

Phase II of the experiment was designed to distinguish between allelopathy and nutrient competition as growth inhibitory mechanisms. Nutrients were added to filtrates from phase I plant-free controls and macrophyte treatments to prevent nutrient limitation for *Scenedesmus* in phase II, as *Scenedesmus*, and *Chara*, *Myriophyllum* and *Elodea* shoots in phase I could have depleted nutrients (Nichols & Keeney, 1976; Van Donk et al., 1993; Kufel & Kufel, 2002). Since *Scenedesmus* growth and morphology was unaffected in standard WC-medium or medium that contained 2.5 times more nutrients, masking of a potential allelochemically exerted growth inhibition by nutrient enhanced growth seems unlikely.

Scenedesmus expressed similar growth and morphological variability in two consecutive trials under comparable conditions in phase I (controls) and phase II (negative controls). *Scenedesmus* showed identical courses in MPV and Φ_{PSII}, identical chlorophyll-based and particle-based growth, but a significantly different volume-based growth. The latter was mainly caused by small within group variances and the 5.5% lower volume-based growth measured in phase I was smaller than the phase II within experiment difference of 12% between filtered medium and *Elodea* filtrate treatments. Hence, it may be concluded that differences between the presence of plants and their culture filtrates are due to treatment effects and not the result of between experiment variation.

Comparing the results of physical presence of macrophytes and their culture filtrate yielded significant differences for *Chara* and *Myriophyllum* treatments on almost all endpoints. In general, the effects that *Chara* and *Myriophyllum* had on *Scenedesmus* in phase I disappeared in phase II, where *Scenedesmus* was grown in culture filtrate of these macrophytes. A similar observation has been made for *C. aspera*, which inhibited strongly *S. obliquus* when *Chara* and *S. obliquus* were kept together, but the negative impact was lost when *Chara* had been removed prior to the experiment (Van Donk & Van de Bund, 2002). Van Donk & Van de Bund (2002) explained this by immediate degradation/disappearance of allelopathic substances, because the allelopathic substances from *Chara* are volatile (Anthoni et al., 1980) and therefore may be lost rapidly (Blindow & Hootsmans, 1991; Van Donk & Van de Bund, 2002). In the current study, a rapid removal of volatile sulphuric compounds might have occurred during the filtration step, which was included in the experimental design between phase I and II. In addition to rapid dilution and disappearance, Mulderij et al. (2003) have listed several other potential causes for the absence of an allelopatic effect of *Chara* on *S. obliquus*. The absence of an allelopatic effect on *S. obliquus* might be due to differences in allelopathic activity among strains of *C. globularis*, which might also depend on growth phase. Blindow and Hootsmans (1991) reported 10% growth reduction of *Scenedesmus communis* (*Desmodesmus* communis) in water from cultures of *C. globularis*, but they also found considerable variation between replicate experiments and between different strains of *C. globularis*.

Differential sensitivity among *Scenedesmus* species might be another possibility (Mulderij et al., 2003). However, a very limited number of studies have been performed on allelopathic effects of *Chara* species with *Scenedesmus* as target organisms. Recently, based on 18S- and ITS2-rDNA-sequence analysis the old genus *Scenedesmus* has been divided into two new genera: *Scenedesmus* Meyen for non-spiny organisms and a genus *Desmodesmus* (Chodat) An, Friedl et Hegewald for those which could bear spines (An et al., 1999; Hegewald, 2000; Van Hannen et al., 2002). Consequently, several intensive studies on allelopathic effects of *Chara* species have been

conducted with a *Desmodesmus* species (e.g. Blindow & Hootsmans, 1991; Hootsmans, 1991). Since no study has compared the sensitivity of various *Scenedesmus* species to allelopathic substances from *C. globularis* differential sensitivity remains unclear. Moreover, based on the limited studies that have been performed, sensitivity of *S. obliquus* to allelopathic substances from *C. globularis* cannot be ruled out and more studies under various environmental conditions are needed. Furthermore, a continuous flow design would be needed to achieve continuous addition of volatile allelopathic compounds to receiving test algae.

In the current study, only filtrates from *Elodea* caused growth inhibition in *Scenedesmus* with a reduction of 12 and 28% for biovolume-based and Chl *a* based growth rates, respectively, compared to filter controls. In addition, particle-based growth rates were significantly reduced in both *Myriophyllum* and *Elodea* culture filtrates, with a reduction of 7 and 15%, respectively (*vide* Fig. 2b). This magnitude of growth inhibition is similar to values of 10% found by Blindow and Hootsmans (1991) for actively growing *Chara* in the laboratory and 20% for decaying *Chara* in the field (Hootsmans, 1991).

Scenedesmus growth rates were reduced with 26–68% in the presence of live plants compared to plastic plant controls. Since this reduction is much higher than the 12–28% found in nutrient-enriched culture filtrates, the difference may be caused by rapid nutrient removal in the presence of the plants (Nichols & Keeney, 1976; Van Donk et al., 1993; Kufel & Kufel, 2002). The remaining growth inhibitory effect in filtrates from *Elodea* is then most likely a result of allelochemicals.

Allelopathically active compounds are often directed at photosynthesis (Gross, 2003) and have been extracted from *Elodea canadensis* (Wium-Andersen, 1987), *Chara globularis* (Anthoni et al., 1980; Wium-Andersen et al., 1982), and *Myriophyllum spicatum* (Gross & Sütfeld, 1994). In those studies ^{14}C-radiotracer techniques were applied to demonstrate the allelopathy, while in the current study photosynthetic performance of *Scenedesmus* was distilled from Φ_{PSII} measurements. There exist, however, good correlations between Φ_{PSII} and the rate of C-fixation in algae and algal growth rate (Hofstraat et al., 1994; Geel, 1997). Furthermore, the hydrolyzable polyphenols released by *Myriophyllum* might also inhibit Φ_{PSII} (Körner & Nicklisch, 2002; Leu et al., 2002). However, the results of the current study could not confirm this. Only a marginal inhibition (3%) of Φ_{PSII} of *Scenedesmus* occurred in the *Elodea* culture filtrates compared to negative controls and a non-significant 2% inhibition was found compared to filter controls. The latter reduction was much less than the 12–28% reduction in growth rates. Therefore, in the *Elodea-Scenedesmus* interaction allelochemicals were not directed at photosynthesis, but probably at other physiological processes, such as enzyme activity (Reigosa et al., 1999; Gross, 2003).

Because the cultures were not completely free of bacteria, bacterial degradation of allelochemicals cannot be excluded as has been found for *Myriophyllum* exudates (Gross et al., 1996; Nakai et al., 1996, 1999). However, allelopathic compounds from *Myriophyllum* still had considerable activity after 1 day in a bacterial suspension (63–100% activity), but activity vanished after 3–4 days (Nakai et al., 1996). Thus, the absence of an effect of *Myriophyllum* exudates on Φ_{PSII} of *Scenedesmus* is probably not due to bacterial degradation, but could be the result of adsorption to filters or to *Scenedesmus* in phase I, or of evaporation during the filtration.

The test alga used, *Scenedesmus*, is notoriously phenotypically plastic and can be found as unicells and colonies up to 16 cells linearly arranged along their long axes (Trainor, 1998). In the current study, cell size of *Scenedesmus* expressed as MPV was not affected in the presence of macrophytes or filtrates and no colonies were formed. The MPV varied over time in controls which was the results of normal growth and subsequent carbon-limitation in batch cultures (Lürling, 2003). *Scenedesmus* expressed identical morphological variability in two consecutive trials under comparable conditions, i.e. phase I controls and comparable phase II replicates (negative controls). This means that the slight differences in MPV between phase I controls and plant treatments and phase II filtrates points to a treatment related effect either from the filtration or algal conspecifics. Nonetheless, MPV values were all within the range of populations dominated by unicells and bicells (Lürling, 2003). Furthermore, *Scenedesmus* showed no signs of clumping and/or becoming sticky. Thus, in the current study

macrophyte exudates had no morphogenetic effect on *Scenedesmus*.

In conclusion, allelopathic acitvity of *Elodea canadensis* on *S. obliquus* growth could be demonstrated. *Myriophyllum* reduced only particle-based growth, and no growth inhibition by exudates from *Chara* was found under the conditions employed in the current study. No effect of macrophytes on the morphological appearance of *Scenedesmus* was observed. However, the experimental design cannot rule out disappearance of allelochemicals during the filtration process.

Scenedesmus growth inhibition was more severe in the physical presence of macrophytes compared to plastic plants or macrophyte exudates, which suggests nutrient competition was a more powerful driving factor than allelochemicals.

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