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Influences of a submerged macrophyte on colony formation and growth of a green alga

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ABSTRACT: The effects of the submerged macrophyte *Ceratophyllum demersum* on the growth and morphology of the green alga *Scenedesmus obliquus* were assessed by conducting co-culture laboratory experiments (15 and 25°C, presence/absence of 3 g fresh wt of *C. demersum* per litre of water in *S. obliquus* culture). Growth rate, photosynthetic pigment-based growth, photosynthetic activity and colony formation of *S. obliquus* increased in the presence of *C. demersum*. The proportion of 4-celled colonies of *S. obliquus* increased from 36 to 76% at 25°C, and from 51 to 71% at 15°C in the presence of *C. demersum*. The induced morphological shift from unicells to colonies in *S. obliquus* promoted sedimentation to the bottom-water region, thereby providing a competitive advantage for the submerged macrophyte.

KEY WORDS: *Scenedesmus obliquus* · *Ceratophyllum demersum* · Growth · Colony formation · Sedimentation

INTRODUCTION

Numerous clear-water lakes dominated by macrophytes worldwide are shifting to turbid lakes dominated by phytoplankton because of eutrophication (Blindow 1992, Moss et al. 1996, Jeppesen et al. 1997). Submerged plants have important functions in preserving the clear-water state of water ecosystems. One method employed by submerged plants to eliminate competitors (i.e. phytoplankton) is the release of inhibiting compounds. This process is known as allelopathy (Phillips et al. 1978). A great number of allelopathic interactions between macrophytes and phytoplankton have been described (Gross 2003). Macrophytes produce active substances that inhibit phytoplankton photosynthesis (Anthoni et al. 1980) or suppress algal growth, consequently lowering phytoplankton biomass. Plant species that can allelopathically inhibit the growth of cyanobacteria include *Myriophyllum spicatum* (Gross et al. 1996), *Ceratophyllum demersum* (Körner & Nicklisch 2002), *Chara* spp. (Mulderij et al. 2003) and *Elodea* spp. (Erhard & Gross 2006).

Except in the case of nuisance cyanobacterial blooms, green algae, specifically *Scenedesmus* spp., pose a major threat to ecosystems, and particularly to eutrophic freshwater ecosystems. However, Mulderij et al. (2003) indicated that *Chara*-filtered water has no allelopathic effects on *Scenedesmus obliquus*. Jasser (1994) indicated that cyanobacteria are more sensitive to allelochemicals from *Ceratophyllum demersum* than are green algae, which are unaffected by these allelochemicals. Wu et al. (2011) found that the growth of *S. obliquus* co-cultured with *Ceratophyllum demersum* was inhibited. Chen (1999) indicated that a *Ceratophyllum demersum* culture filtrate with low biomass promotes the growth of *S. obliquus*, whereas that with a high concentration inhibits the growth of the algae.

Scenedesmus spp. is commonly found in freshwater ecosystems with diversified ecomorphs (Trainor & Egan 1991). Previous studies have indicated that high

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total salts (Shubert & Trainor 1974), low temperature (Siver & Trainor 1981, Trainor 1992, 1993) and grazers such as *Daphnia magna* or their filtrates promote *Scenedesmus* colony formation (Hessen & Van Donk 1993, Lampert et al. 1994, Lürling & Van Donk 1996, Lürling 1999, 2003, Von Elert & Franck 1999, Ha et al. 2004, Verschoor et al. 2004, Yang & Li 2007). However, to date, only a limited number of studies have investigated the colony formation of *Scenedesmus* induced by macrophytes. It has been reported that *Chara*, *Elodea*, *Myriophyllum*, and *Potamogeton malaianus* had no morphological effect on *Scenedesmus obliquus* (Lürling et al. 2006, Wu et al. 2007), whereas the colony formation of *Scenedesmus obliquus* has been shown to be induced by submerged *Stratiotes aloides* (Mulderij et al. 2005b).

The present study attempts to understand the influences of *Ceratophyllum demersum* on the growth and colony formation of *Scenedesmus obliquus*.

MATERIALS AND METHODS

Macrophytes and algae culture

Scenedesmus obliquus was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan, China. Prior to experimentation, algae were batch-cultured in 2000 ml Erlenmeyer flasks with 1600 ml of BG11 medium (Rippka et al. 1979) at room temperature (25°C) under a 12 h light:12 h dark cycle (25 µmol photons s^{-1} m^{-2}).

Ceratophyllum demersum samples were collected from Dianchi Lake in Yunnan Province, China. Plants were carefully rinsed with tap water to remove adhering epiphytes and zooplankton (Mulderij et al. 2005a, Wu et al. 2007). The plants were then acclimated in laboratory conditions for 3 mo prior to experimentation.

Experimental design

The growth and development of *Ceratophyllum demersum* in natural conditions occurs at temperatures exceeding 15°C. A temperature of 25°C is considered suitable for the plants. Consequently, these 2 temperatures were selected for testing the response of *Scenedesmus obliquus* to *C. demersum*.

When it reached an exponential growth phase, *Scenedesmus obliquus* was cultivated in 250 ml glass flasks filled with 150 ml of fresh BG11 medium under a 12 h light:12 h dark cycle (25 µmol photons s^{-1} m^{-2}) at 15 and 25°C in the absence (control) or presence of 3 g fresh weight (FW) of *Ceratophyllum demersum* per litre of water. The cultivation flasks were covered with parafilm, and each treatment had 3 replicates. Cultures were shaken manually twice a day to maintain cell suspension. Experimentation lasted for 15 d, and regular sampling was conducted every 3 d on the benchtop to prevent contamination of other cultures.

Growth rate of *Scenedesmus obliquus*

Growth rate (µ) was calculated by the equation

$$\mu = (\ln x_2 - \ln x_1)/(t_2 - t_1) \quad (1)$$

where x_1 and x_2 represent optical density (absorbance at 680 nm) at time t_1 and t_2, respectively.

Photosynthetic pigments

The centrifuged cells (12 000 rpm, 10 min, 4°C) were extracted with 95% ethanol for 24 h at 4°C in the dark. An Ultrospec 3000 ultraviolet/visible spectrophotometer was used to obtain absorbance readings at 665, 649 and 470 nm (A_{665}, A_{649} and A_{470} respectively) (Lichtenthaler & Buschmann 2001). Concentrations of chlorophyll *a* (chl *a*) and carotenoid (both in mg l^{-1}) were calculated as follows:

$$\text{Chl } a \text{ concentration} = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (2)$$

$$\text{Carotenoid concentration} = (1000 \times A_{470} - 2.05 \times \text{chl } a \text{ concentration})/245 \quad (3)$$

Chlorophyll fluorescence

Chlorophyll fluorescence parameters, such as maximal Photosystem II (PSII) quantum yield (F_v/F_m, i.e. variable fluorescence divided by maximum fluorescence) and maximum electron transport rate of photosynthesis (ETR_{max}), were measured by Phyto-PAM (Walz). This device can work effectively with very low biomass densities because of its sensitive fluorometric measurements (Körner & Nicklisch 2002).

Morphology of *Scenedesmus obliquus* population

For each replicate, 2 ml samples were fixed with Lugol's fixative and viewed under an inverted microscope at 400× magnification to count the number of cells per colony (Lürling & Van Donk 1999).

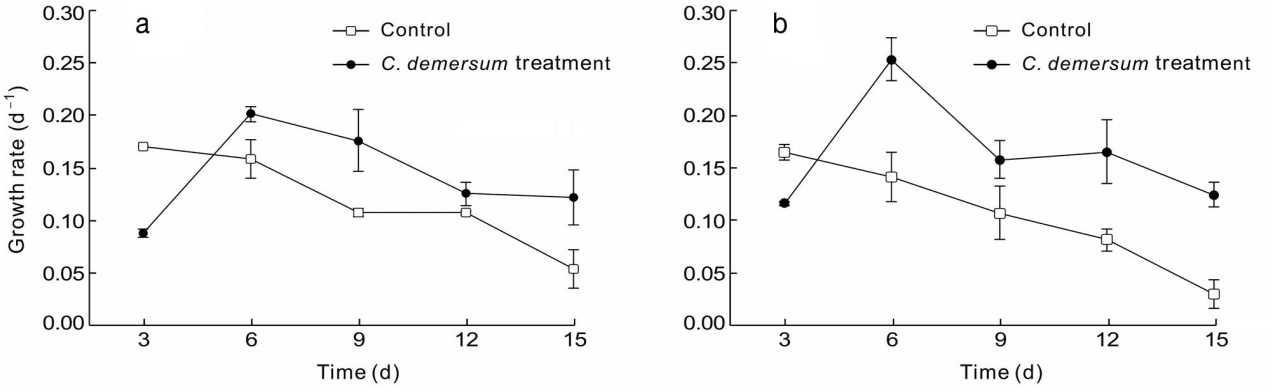

Fig. 1. Growth rate of *Scenedesmus obliquus* in the absence (control) and presence of *Ceratophyllum demersum* at (a) 15°C and (b) 25°C

Sedimentation experiments

At the end of the experiments, 25 ml of algae were sampled from each treatment and placed in a glass column (25 × 2 cm) in the dark to initiate sedimentation (Lürling & Van Donk 2000). Each treatment had 4 replicates. Prior to sedimentation, algae were thoroughly mixed, and the initial algae biomass (C_0) was calculated. After 1 h, 2 ml of algae was sampled from the column, and its biomass (C_1) was measured. The sedimentation rate (r) of the algae from the column can be defined according to the SETCOL procedure (Bienfang 1981):

$$r = (C_1 - C_0)/C_1 \quad (4)$$

Data analysis

Mean values and standard deviations were calculated for the different replicates (n = 3). At the end of the experiments, statistical analysis of the data between the control and *Ceratophyllum demersum* treatment at 15 and 25°C was performed using SPSS 18.0. Two-way ANOVA was used to compare the final growth rate, pigment-based growth, photosynthetic activities, population proportion (unicells and 4-celled coenobia) and sedimentation rate between the control and the *C. demersum* treatment (Lürling & Van Donk 1999). A value of $p < 0.05$ was considered significant in all analyses.

RESULTS

Growth of *Scenedesmus obliquus*

During the first 3 d, which was considered the lag period, *Scenedesmus obliquus* in the *Ceratophyllum demersum* treatment exhibited a slower growth rate compared with the control. However, during the subsequent period, *S. obliquus* in the *C. demersum* treatment exhibited a higher growth rate compared with the control. In the present study, the presence of *C. demersum* promoted the growth of *S. obliquus* (Fig. 1). Two-way ANOVA revealed that *C. demersum* ($p < 0.01$), temperature ($p < 0.05$) and interaction ($p < 0.01$) had significant effects on the growth rate μ of *S. obliquus* (Table 1).

Photosynthetic pigment-based growth

Chl *a* and carotenoid of *Scenedesmus obliquus* were higher in the *Ceratophyllum demersum* treatment at both 15 and 25°C than in the control (Fig. 2). At 15°C, chl *a* concentration was 6.3 mg l^{-1} in the control and 10.3 mg l^{-1} in the *C. demersum* treatment, and at 25°C, it was 6.4 and 12.7 mg l^{-1} respectively. For carotenoid, the concentration was 2.6 mg l^{-1} in the control and 3.8 mg l^{-1} in the *C. demersum* treatment at 15°C, and at 25°C, it was 2.5 and 4.5 mg l^{-1} respectively. Two-way ANOVA revealed that *C.*

Table 1. Between-subject effects on the dependent variable, *Scenedesmus obliquus* growth rate (μ). *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	0.693[a]	3	0.231	104.667	0.000
	6.208	1	6.208	2812.958	0.000
Temperature	0.024	1	0.024	11.011	0.011
C. demersum	0.553	1	0.553	250.767	0.000
Temperature × *C. demersum*	0.115	1	0.115	52.222	0.000
Error	0.018	8	0.002		
Total	6.918	12			
Corrected total	0.711	11			

[a]$R^2 = 0.975$ (adjusted $R^2 = 0.966$)

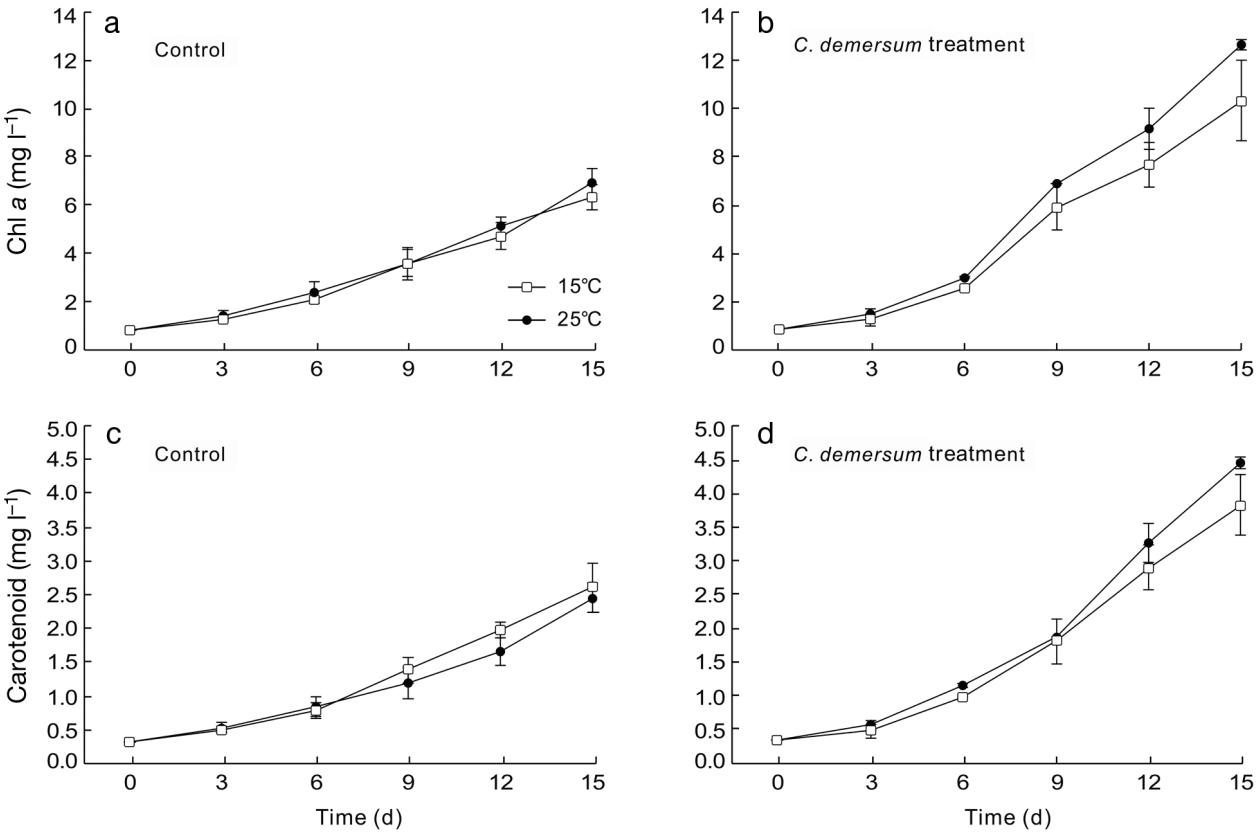

Fig. 2. (a,b) Chl *a* and (c,d) carotenoid concentrations in *Scenedesmus obliquus* in the absence (control) and presence of *Ceratophyllum demersum* at 15 and 25°C

Table 2. Between-subject effects on the dependent variable, chl *a* concentration in *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	87.636[a]	3	29.212	64.040	0.000
	959.076	1	959.076	2102.540	0.000
Temperature	4.346	1	4.346	9.528	0.015
C. demersum	79.496	1	79.496	174.275	0.000
Temperature × *C. demersum*	3.794	1	3.794	8.317	0.020
Error	3.649	8	0.456		
Total	1050.361	12			
Corrected total	91.285	11			

[a]R^2 = 0.960 (adjusted R^2 = 0.945)

Table 3. Between-subject effects on the dependent variable, carotenoid concentration in *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	8.380[a]	3	2.793	56.932	0.000
	133.519	1	133.519	2721.395	0.000
Temperature	0.162	1	0.162	3.299	0.107
C. demersum	7.761	1	7.761	158.194	0.000
Temperature × *C. demersum*	0.456	1	0.456	9.304	0.016
Error	0.393	8	0.049		
Total	142.291	12			
Corrected total	8.772	11			

[a]R^2 =0.955 (adjusted R^2 = 0.938)

demersum presence ($p < 0.01$), temperature ($p < 0.05$), and their interaction ($p < 0.05$) exerted significant effects on the chl *a* concentration of *S. obliquus* (Table 2). However, the effect of temperature on the carotenoid concentration was not significant ($p > 0.05$) (Table 3).

Chlorophyll fluorescence

F_v/F_m and ETR_{max} are important indicators of photosynthetic efficiency and activities of PSII. ETR_{max} is closely associated with carbon assimilation activity in photosynthesis.

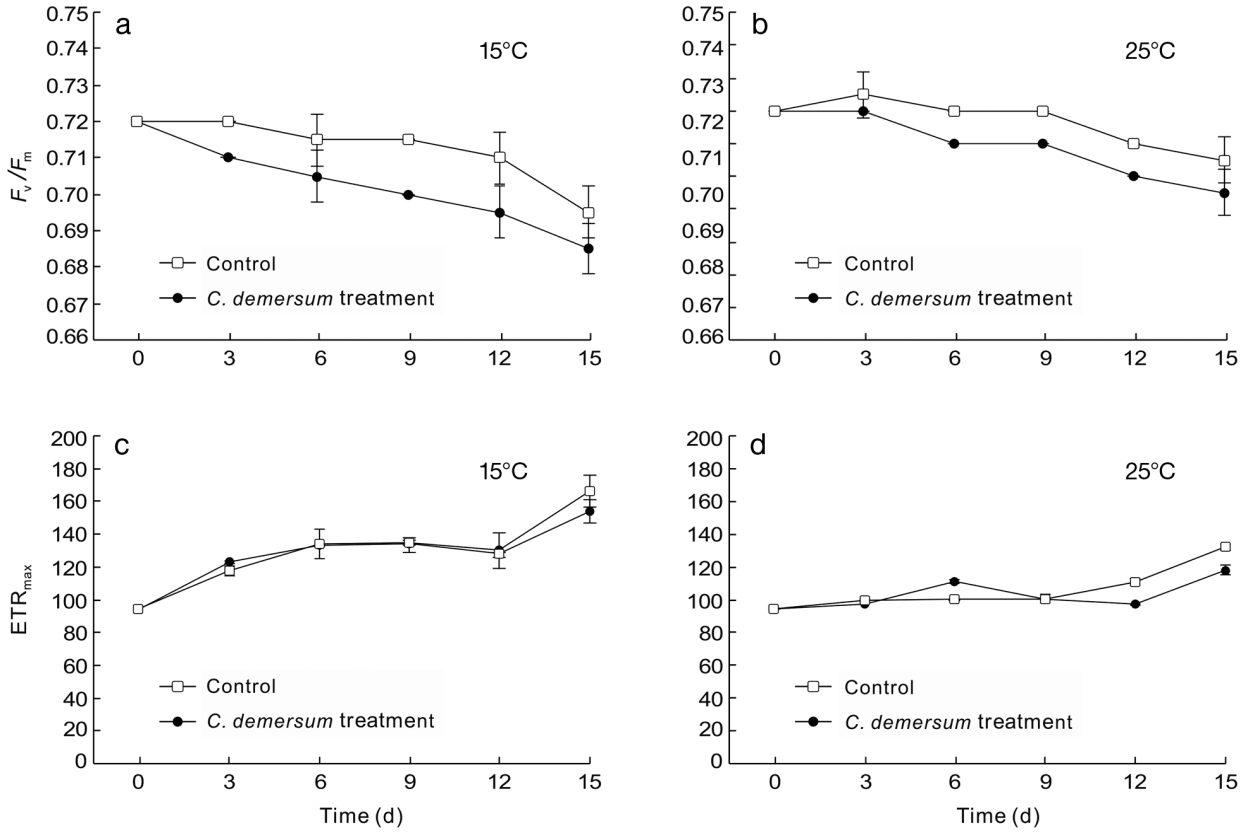

Fig. 3. Photosynthetic activities measured as (a,b) ratio of variable fluorescence to maximum fluorescence (F_v/F_m) and (c,d) maximum electron transport rate (ETR_{max}) of *Scenedesmus obliquus* in the absence (control) and presence of *Ceratophyllum demersum* at 15 and 25°C

The F_v/F_m of *Scenedesmus obliquus* in the control and *Ceratophyllum demersum* treatment ranged approximately from 0.67 to 0.73 (Fig. 3). The presence of *C. demersum* distinctly enhanced the F_v/F_m and ETR_{max} of *S. obliquus* at both 15 and 25°C relative to the control (Fig. 3).

Two-way ANOVA revealed that *Ceratophyllum demersum* presence ($p < 0.01$) and temperature ($p < 0.01$) exerted significant effects on the photosynthetic activities of *Scenedesmus obliquus*. However, the effect of the interaction of these 2 factors was not significant ($p > 0.05$) (Tables 4 & 5).

Table 4. Between-subject effects on the dependent variable, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) of *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	0.001[a]	3	0.000	8.000	0.009
	5.796	1	5.796	231852.000	0.000
Temperature	0.000	1	0.000	12.000	0.009
C. demersum	0.000	1	0.000	12.000	0.009
Temperature × *C. demersum*	0.000	1	0.000	0.000	1.000
Error	0.000	8	2.500×10^{-5}		
Total	5.797	12			
Corrected total	0.001	11			

[a]$R^2 = 0.750$ (adjusted $R^2 = 0.656$)

Proportion of *Scenedesmus obliquus* unicells and 4-celled coenobia

All cultures demonstrated obvious inconsistent fluctuations between the ratio of 4-celled coenobia and unicells: as the proportion of one decreased, the other increased (Fig. 4).

The morphology of *Scenedesmus obliquus* changed drastically in the control populations at both 15 and 25°C. During the first 3 d, the number of 4-celled coenobia decreased and unicells developed, followed by a short-time recovery of colony abundance. From the ninth day onwards, the 4-celled colony gradually decreased and unicells increased. At the

Table 5. Between-subject effects on the dependent variable, maximum electron transport rate (ETR_{max}) of *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	4130.871[a]	3	1376.957	71.190	0.000
	243974.342	1	243974.342	12613.790	0.000
Temperature	3627.902	1	3627.902	187.567	0.000
C. demersum	501.167	1	501.167	25.911	0.001
Temperature × *C. demersum*	1.802	1	1.802	0.093	0.768
Error	154.735	8	19.342		
Total	248259.948	12			
Corrected total	4285.606	11			

[a]R^2 = 0.964 (adjusted R^2 = 0.950)

end of the experiments, the proportion of 4-celled coenobia decreased to 51 and 36% at 15 and 25°C, respectively. Meanwhile, the proportion of unicells increased to 19 and 26% at 15 and 25°C, respectively (Fig. 4).

However, a rapidly induced formation of 4-celled coenobia in the treatment populations at 15 and 25°C (up to 71 and 76%, respectively) was observed after the short-term lag phase during the first 3 d. In contrast to the 4-celled coenobia, the ratio of unicells decreased (down to 7 and 5% at 15 and 25°C, respectively) from the third day onwards. However, the population composition seemed to stabilise during the subsequent cultivation periods (Fig. 4).

Two-way ANOVA was performed on the mean ratio per ecomorph (single cell and 4-celled coenobia) at the end of the experiments to compare the populations. Results showed that *Ceratophyllum demersum* presence ($p < 0.01$) and temperature ($p < 0.01$), as well as the interaction between the 2 factors ($p < 0.01$), exerted significant effects on the proportion of unicells and 4-celled coenobia (Tables 6 & 7).

Sedimentation

The sedimentation rate from the water column of *Scenedesmus obliquus* in the control and the *Cerato-*

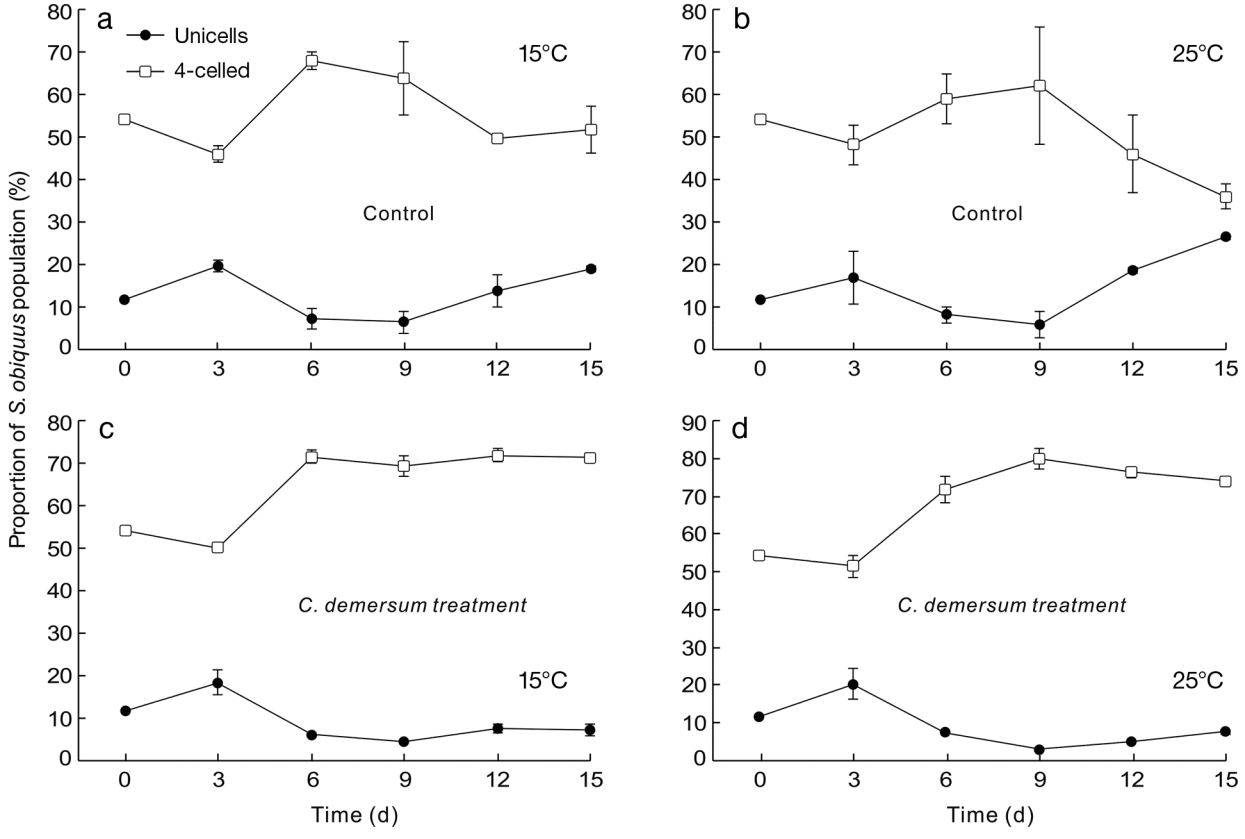

Fig. 4. Different ecomorph proportions of *Scenedesmus obliquus* in the (a,b) absence or (c,d) presence of *Ceratophyllum demersum* at 15 and 25°C

Table 6. Between-subject effects on the dependent variable, proportion of unicells in *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	890.149[a]	3	296.716	184.888	0.000
	2488.759	1	2488.759	1550.778	0.000
Temperature	41.232	1	41.232	25.692	0.001
C. demersum	796.380	1	796.380	496.235	0.000
Temperature × *C. demersum*	52.537	1	52.537	32.737	0.000
Error	12.839	8	1.605		
Total	3391.747	12			
Corrected total	902.988	11			

[a]R^2 = 0.986 (adjusted R^2 = 0.980)

Table 7. Between-subject effects on the dependent variable, proportion of 4-celled coenobia in *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	3315.539[a]	3	1105.180	308.823	0.000
	40805.104	1	40805.104	11402.271	0.000
Temperature	57.235	1	57.235	15.993	0.004
C. demersum	2986.800	1	2986.800	834.609	0.000
Temperature × *C. demersum*	271.504	1	271.504	75.867	0.000
Error	28.629	8	3.579		
Total	44149.272	12			
Corrected total	3344.168	11			

[a]R^2 = 0.991 (adjusted R^2 = 0.988)

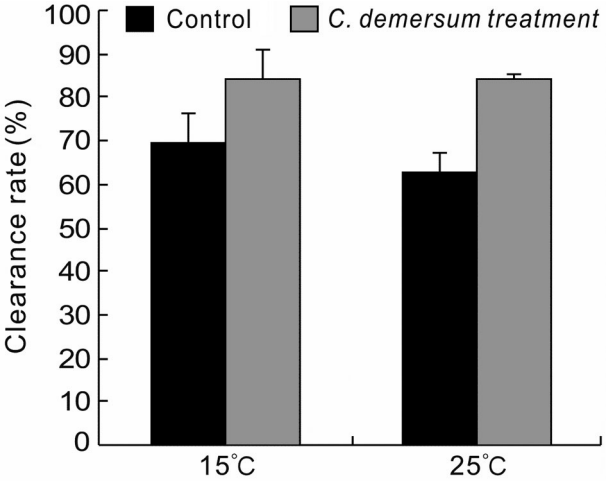

Fig. 5. Sedimentation rate of *Scenedesmus obliquus* biomass in the absence (control) or presence of *Ceratophyllum demersum* at 15 and 25°C

phyllum demersum treatment was calculated and analysed at the end of the experiments (Fig. 5). Results showed that the sedimentation rate was significantly increased by the *C. demersum* treatment ($p < 0.01$) at both 15 and 25°C (Fig. 5, Table 8).

DISCUSSION

Scenedesmus spp. are extremely phenotypically plastic under changing environmental conditions. Algae can be either unicellular or colonial with 2-, 4- or 8-celled coenobia depending on environmental conditions (Trainor & Egan 1991). Having different ecomorphs is considered one of the adaptation strategies of *Scenedesmus* spp. to diversified environments. Hessen & Van Donk (1993) discovered that the coenobia of *Scenedesmus subspicatus* are induced by chemical cues released by zooplankton. Their study is the first report on the colony induction of *Scenedesmus* by biological interaction although there have been numerous studies on grazer-induced colony formation (Lampert et al. 1994, Lürling & Van Donk 1996, Lürling 1999, 2003, Von Elert & Franck 1999, Ha et al. 2004, Verschoor et al. 2004, Yang & Li 2007). It is more difficult for grazers to predate on colonial than on single cell *Scenedesmus*, but it is easier for the colonial formations to sediment in bottom waters under natural conditions (Lürling et al. 1997).

The present study demonstrated another type of colony induction caused by a biological interaction (between phototrophic organisms). A larger number of 4-celled coenobia were observed in the presence of submerged *Ceratophyllum demersum*. This finding is in agreement with Mulderij et al. (2005b) and Leflaive et al. (2008), who made similar observations for *Stratiotes aloides* and *Uronema confervicolum* (Ulotrichales). The present study has provided additional information on the colony induction of *Scenedesmus* spp. by phototrophic organisms. Similar to colony induction by grazers (Lürling & Van Donk 2000, Verschoor et al. 2004), the present study shows that colony induction by submerged plants (macrophyte–phytoplankton interaction) also increases the algae's sedimentation rates to bottom waters (Fig. 5). Insufficient light in the bottom waters

Table 8. Tests of between-subject effects on the dependent variable, sedimentation rate (clearance proportion) of *Scenedesmus obliquus*. *C. demersum* = presence of *Ceratophyllum demersum*

Source	SS (Type III)	df	MS	*F*	p
Corrected model	1412.382[a]	3	470.794	16.308	0.000
	90499.152	1	90499.152	3134.787	0.000
Temperature	52.474	1	52.474	1.818	0.202
C. demersum	1313.375	1	1313.375	45.494	0.000
Temperature × *C. demersum*	46.533	1	46.533	1.612	0.228
Error	346.432	12	28.869		
Total	92257.967	16			
Corrected total	1758.814	15			

[a]R^2 = 0.803 (adjusted R^2 = 0.754)

suppresses algal growth (Lürling & Van Donk 2000), thereby liberating macrophytes from competition for light and/or nutrients with algae. This mechanism provides competitive advantages for submerged plants.

In the present study, *Ceratophyllum demersum* treatment (3 g FW l^{-1}) increased the growth rate, pigments and photosynthetic activities of *Scenedesmus obliquus*. This result is in agreement with the study by Chen (1999), which indicated that a low concentration (<4 g FW l^{-1}) of *C. demersum* promotes the growth of *S. obliquus*, but a high concentration (>4 g FW l^{-1}) of the plant inhibits algal growth. However, our result is not consistent with the study by Wu et al. (2011), which indicated that the growth of *S. obliquus* co-cultured with *C. demersum* is inhibited. The contradicting results may be due to the different *C. demersum* concentrations used. The allelopathic effects of submerged plants on algae depend on the initial biomass of the submerged plant and initial algal density (Körner & Nicklisch 2002, Wu et al. 2007).

The mechanism of the colony induction of *Scenedesmus* spp. by submerged plants is also of interest. The colony induction by *Stratiotes aloides* (Mulderij et al. 2005b) is considered an allelopathic effect. In the present study, the *Ceratophyllum demersum* culture filtrates experiment was also conducted according to Wu et al. (2007) to eliminate the effects of nutrients and light in the co-culture experiment. *C. demersum* (3 g FW l^{-1}) was cultured for 10 d for the experiments, and the initial optical density of *Scenedesmus obliquus* was set at 0.1 (OD_{680} = 0.1), which is the same as in the co-culture experiments. The effects of *C. demersum* culture filtrates on the photosynthetic activities (F_v/F_m; ETR_{max}), photosynthetic pigments (chl *a*; carotenoid) and the growth rate of *Scenedesmus obliquus* were similar to those of the co-culture (the results are not presented in this paper). However, colony induction was not obvious in the *C. demersum* culture filtrates, which may be due to the low concentration of the colony-inducing compounds in the culture filtrates (Leflaive et al. 2008). Similar to *Uronema confervicolum*, the colony-inducing and active effects of *C. demersum* filtrates may be linked to different compounds (Leflaive et al. 2008). Another hypothesis is that the active compounds that induce colony formation are only secreted when *Scenedesmus obliquus* is co-cultured with *C. demersum*. To date, the active compounds released by submerged plants on the ecomorphs of *Scenedesmus* spp. have not been studied. However, kairomones, which are aliphatic sulphates released by *Daphnia*, were found to cause colony formation of *Scenedesmus* (Yasumoto et al. 2005). Thus, further study must be conducted to determine whether the aliphatic sulphates are similar to the active compounds released by *C. demersum*, which caused a morphological shift in *Scenedesmus obliquus*.

The co-culture experiments also indicated that the effect of *Ceratophyllum demersum* on *Scenedesmus obliquus* is related to cultivation temperature. Metabolic processes have been reported to be profoundly affected by temperature (Rhee & Gotham 1981, Davidson 1991). Low temperature can increase the amount of carbohydrates per cell, but decrease the amounts of protein and lipids (Aaronson 1973). In the present study, *C. demersum* treatment at 25°C exerted greater influence on 4-celled colony formation, the proportion of which increased from 36 to 76%, whereas the proportion of 4-celled colony formation only increased from 51 to 71% at 15°C (Fig. 4). The relatively weaker response at the lower temperature may be due to the relatively fewer active compounds released.

The present study provides insight into allelopathic-induced colony formation of *Scenedesmus* spp. by submerged plants. The aliphatic sulphate-like substances released by the plants may be the active compounds that caused the morphological shift in *S. obliquus*. In addition, temperature possibly contributes to the substance release, thereby affecting the morphological shift in *S. obliquus*. However, this is only a hypothesis. Further investigation of the chemical compounds that caused the morphological shift in *S. obliquus* is urgently needed.

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