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Interactions among water depth, algae, and macrophytes

Hanife ÖZBAY

Biology Department, Faculty of Art and Sciences, Nevşehir Hacı Bektaş Veli University, 50300, Nevşehir, Turkey.

Corresponding author: hanifozbay@gmail.com

Abstract: The simultaneous effect of water depth and algae on the growth rate of submersed macrophytes was investigated in this study. *Ceratophyllum demersum* L. and *Myriophyllum verticillatum* L. were used as submersed macrophytes. A total of 54, 10 cm length, weighted individual shoots of each species were planted in square plastic pots, filled with lake sediment. All planted plastic pots were positioned in 30 liter capacity plastic buckets, with dechlorinated tap water added to depths of 20, 30 or 40 cm (each depth had three replicates) above the soil surface; 36 buckets were used and each bucket held three pots. The experiment was consisted two groups, including Group I (GI) with 18 buckets containing only planted plastic pots of each species, and Group II (GII) with 18 buckets with plant + algal inoculum. *Scenedesmus quadricauda* (Turpin) de Brebisson was used as the added test alga. The RGR of *C. demersum* was found to differ significantly with the depth of the water in both GI and GII. Likewise, significant differences in RGR were found for *M. verticillatum* for all treatments in both groups. For both species, the no algae added group (GI) had a greater growth rate than the algae added group (GII) in all treatments.

Keywords: Algae, Macrophytes, *Scenedesmus quadricauda*, *Ceratophyllum demersum*, *Myriophyllum verticillatum*.

Introduction

Submersed macrophytes are thought to perform a key function in stabilizing shallow freshwater ecosystems (Jeppesen et al., 1998; Scheffer, 1998). Light is considered a key factor in regulating the growth and distribution of submersed freshwater macrophytes, due to its rapid attenuation with depth (Spence, 1967; 1972, 1976). In lakes, eutrophication usually causes a decline in submersed aquatic macrophytes, often caused, apparently, by increased periphytic and filamentous algae, and also shading by phytoplankton blooms (Phillips et al., 1978) under increased nutrient loadings, which reduce the amount of light reaching the macrophyte's photosynthetic tissue.

The relative importance of nutrient and light limitations on algal growth also vary with depth. Algae will be relatively more nutrient limited when they receive the high light intensity available at the top of the water column and growth rates are potentially high, but become relatively lighter limited when in low light regions towards the bottom of a column (Huisman and Weissing, 1995).

However, although interactions between submersed macrophytes and phytoplankton and their seasonal dynamics in shallow lakes have been studied extensively (Scheffer et al., 1994; Van Donk and Gulati, 1995; Jeppesen et al., 1998; 2000; Sayer et al., 2010), the simultaneous effects of water level and algae on the growth characteristics of macrophytes have received relatively little attention. Therefore, the aim of this study was to investigate the simultaneous effect of water depth and algae on the growth rate of submersed macrophytes. Water depth will of course vary in natural ecosystems, where submersed macrophytes and algae are interacting, and it is important to know how the interaction operates at different depths.

Materials and Methods

Ceratophyllum demersum L. and *Myriophyllum verticillatum* L. were used as submersed macrophytes in this study. Both species were collected from Lake Çalı (41°12'N, 43°12'E, Kars, Turkey) in July 2010, sorted

from debris and stored at 15°C in a constant temperature room for four days. A total of 54, 10 cm length, weighted individual shoots of each species were planted in square plastic pots, 4 cm on each side and 5 cm deep, filled with lake sediment. All planted plastic pots were positioned in 30 liter capacity plastic buckets, with dechlorinated tap water added to depths of 20, 30 or 40 cm (each depth had three replicates) above the soil surface; 36 buckets were used and each bucket held three pots. To create various water depths, 20 cm high plastic benches were placed in the 20 cm water level treatment containers, and 10 cm high plastic benches were placed in the 30 cm water level treatment containers. Therefore, the surface of the water was kept at an identical level in each container. Evaporation losses were negligible.

The experiment contained two groups; Group I (GI) with 18 buckets containing only planted plastic pots of each species, and Group II (GII) with 18 buckets with plant + algal inoculum. *Scenedesmus quadricauda* (Turpin) de Brebisson was used as the added test alga. *Scenedesmus quadricauda* is a common, widely distributed tychoplankton of shallow, eutrophic waters. It was obtained from Gazi University's Microalgae Collection Centre (Ankara, Turkey). *Scenedesmus* quadricauda were added to the culture solution in a ratio of 1 ml to 1 L.

The buckets were placed in a growth room with a 12 hour light: dark cycle, at 15°C and irradiance of 60-90 µmol PAR m^{-2} s^{-1}. To create a high nutrient level, supplemental N and P were added in the form of Ca $(NO_3)_2$ $4H_2O$, but since tap water already contains some N and P, hence the actual nutrient levels were as follows:

Tap water level N (μgl^{-1})	Tap water level P (μgl^{-1})	Added N (μgl^{-1})	Added P (μgl^{-1})	Total N (μgl^{-1})	Total P (μgl^{-1})
326	19	4000	200	4326	219

The appropriate nutrient ranges were defined by Jones (1994). At the beginning and end of the experiment, pH, conductivity and dissolved oxygen (DO) levels in the water in each container were measured, using a WTW Oxi 197i oxygen meter, a WTW cond 315i/set meter and a WTW pH meter 315i/set meter, respectively. Because at the beginning the levels of the nutrients were known, and the level of chlorophyll in tap water is negligible, at the end of the experiment, composite water samples for chemical and chlorophyll a analyses were collected from each bucket. NH_4-N, NO_3-N, and Soluble Reactive Phosphorus (SRP) were analysed according to APHA (1999). Chlorophyll *a* was extracted in acetone, and the concentration was calculated from the absorbance reading at 663 nm (Talling and Driver, 1961).

Light levels were measured using a Macam Quantum Radiometer/ Photometer Q101 (Macam Photometers Ltd., Livingston, Scotland). The biomass of each sample was determined from the dry weight as a Relative Growth Rate. The plants were collected from the buckets, sorted from debris, and dried to constant weight in an oven at about 70°C. Their dry weight was then measured. The relative growth rate (RGR) of each species was calculated from the dry weight as follows:

$RGR = \log_e$ final dry wt–$\log_e$ initial dry wt/duration of the experiment (Hunt, 1990).

A functional approach was used to obtain the initial dry weight. The ratio of the wet weight to the dry weight of the shoots of each plant was calculated at the end of the experiment, and the mean values were used to extrapolate the initial dry weight.

The experiment was run for 21 days. All statistical analyses were performed using Minitab 11 (Minitab, 1996).

Results

The pH, conductivity and DO of the growth medium tended to increase with time in both GI and GII for both plant species. In contrast, NO_3-N, NH_4-N and the SRP of the growth medium showed decreases over time in both GI and GII for both plant species. Chlorophyll *a* was also increased at the end of the experiment in both GI and GII for both plant species (Table 1).

The RGR of *C. demersum* was found to differ significantly with the depth of the water in both GI and GII (P=0.038 and P=0.001, respectively). Likewise, significant differences in RGR were found for *M. verticillatum* for all treatments in both groups (P=0.002 and P=0.001, respectively) (Fig. 1). For both species, the no algae added group (GI) had a greater growth rate than the algae added group (GII) in all treatments (Fig. 1).

The growth rates for the 20 and 30 cm (-) algae and (+) algae groups were significantly different for *C. demersum* (P=0.031 and P=0.036, respectively, from t-test) (Fig. 2),

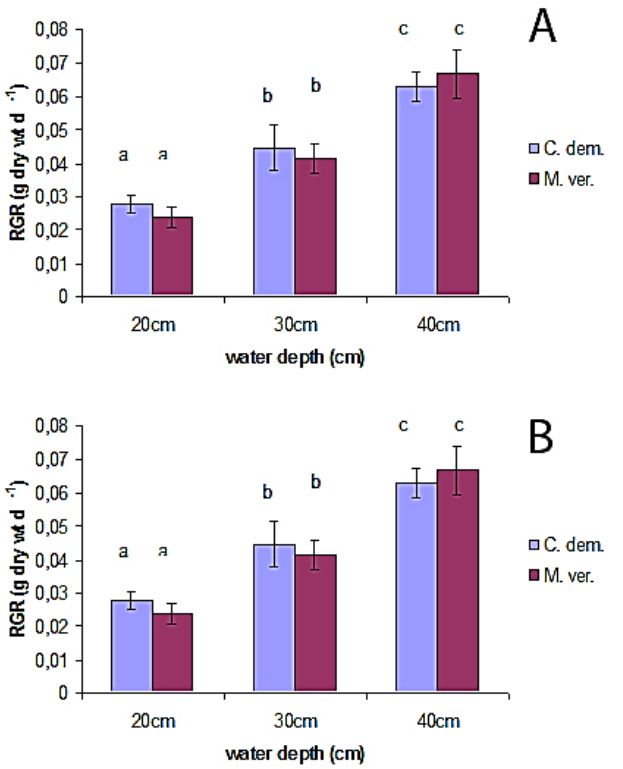

Figure 1. Growth rate of *Ceratophyllum demersum* and *Myriophyllum verticillatum* expressed as RGR for water depth treatments in GI (A) and in GII (B). Error bars are shown as ±SD. Letters indicate the results of the Tukey test.

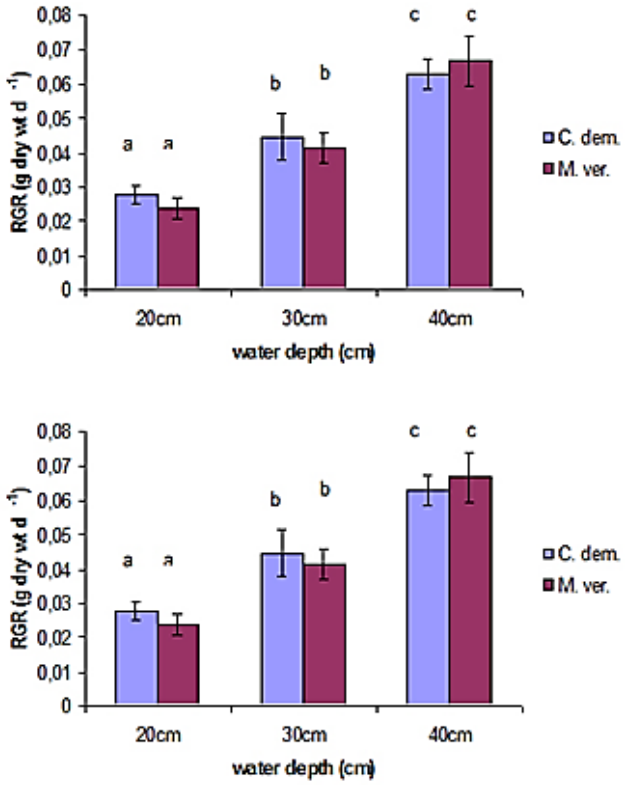

Figure 2. Growth rate of *Ceratophyllum demersum* I (GI) and *Ceratophyllum demersum* II (GII), and *Myriophyllum verticillatum* I (GI) and *Myriophyllum verticillatum* II (GII) expressed as RGR for water depth treatments.

whereas at the 40 cm water level the difference was insignificant (P=0.057 from t-test). Likewise, the differences in growth rate between GI and GII at all water depths were highly significant for *M. verticillatum* (P=0.0007 for 20 cm, P=0.0023 for 30 cm and P=0.021 for 40 cm from t-test).

Discussion

The increase in chlorophyll *a* with time was less marked in both plant species in GI, whereas in GII, the chlorophyll *a* showed a relatively greater increase. Likewise, the RGR of both plants tended to increase more in GI than GII. It is probable that the plants and algae were competing more for nutrients in GII. The pH and DO of the growth medium increased over time, but the increases in pH and DO were relatively larger in GII. It is probable that photosynthesis by both macrophytes and algae was increasing in both GI and GII. This increase was seen for all of the plants tested. The increases observed in the conductivity of the growth medium could have resulted in releasing of ions by plants or sediments over time. However, NO_3-N, NH_4-N and the SRP of the growth medium decreased over time because of nutrient uptake by the growing plants and algae. Furthermore, nutrient uptake from the water by plants and algae was more effective in GII. Although the plants and algae used some nutrients from the water in both experimental groups, the reductions in nutrient levels did not become insufficient for growth, because some nutrients were also released from the sediment to the water. It is probable that this release compensated for nutrient uptake from the water by the plants and algae.

Algal growth, expressed as chlorophyll *a* (µg l^{-1}) was significantly affected by water depth in both GI and GII with both plants. Although no algae was added to GI, by the end of the experiment some algal growth must have occurred with GI, due to the natural algal flora associated with macrophytes. A growth medium which contained relatively high N and P levels was used in this experiment. It is therefore extremely unlikely that algal growth could be limited by nutrient availability. Light is also an important factor for algal growth. In shallow water, the light level will always be relatively high. In this experiment however, different water depths were used,

Table 1. Physical (at the beginning and at the end of the experiment) and chemical (at the end of the experiment) composition of the water determined for both groups (GI and GII). Values shown are means (n=3) with standard deviation in parenthesis.

Week	Species	Water depth (cm)	pH (log unit)	Coductivity (μscm^{-1})	DO ($mg.l^{-1}$)	NO_3-N ($mg.l^{-1}$)	NH_4-N ($\mu g.l^{-1}$)	SRP ($\mu g.l^{-1}$)	Chl *a* ($\mu g.l^{-1}$)
GI									
1	*M. verticillatum*	20	7.5 (0.124)	103 (4.109)	5.5 (0.204)	-	-	-	-
		30	7.5 (0.169)	105 (7.586)	5.6 (0.244)	-	-	-	-
		40	7.3 (0.124)	197 (13.888)	5.4 (0.163)	-	-	-	-
	C. demersum	20	7.4 (0.124)	110 (14.445)	5.7 (0.216)	-	-	-	-
		30	7.8 (0.244)	109 (11.430)	5.9 (0.326)	-	-	-	-
		40	7.6 (0.249)	112 (15.297)	5.7 (0.509)	-	-	-	-
3	*M. verticillatum*	20	8.4 (0.294)	132 (6.164)	7. (0.124)	4.3 (0.03)	23.3 (1.01)	192.6 (2.867)	5.3 (0.43)
		30	8.3 (0.249)	169 (9.392)	8.3 (0.163)	5.7 (0.29)	31.4 (1.26)	206.3 (2.494)	3.6 (0.36)
		40	8.3 (0.163)	180 (8.653)	8.5 (0.339)	3.7 (0.36)	25.7 (1.60)	211.0 (1.632)	0.49 (0.06)
	C. demersum	20	8.4 (0.294)	174 (5.354)	8.9 (0.163)	4.5 (0.33)	27.5 (1.26)	192.0 (1.632)	6.6 (0.71)
		30	8.6 (0.216)	175 (9.977)	8.9 (0.286)	5.6 (0.33)	27.9 (2.12)	197.3 (0.942)	5.5 (0.49)
		40	8.8 (0.339)	192 (8.602)	9.2 (0.244)	4.1 (0.24)	24.8 (1.40)	202.6 (1.247)	0.58 (0.07)
GII									
1	*M. verticillatum*	20	7.5 (0.047)	107 (9.809)	5.8 (0.081)	-	-	-	-
		30	7.6 (0.329)	105 (9.177)	5.8 (0.249)	-	-	-	-
		40	7.3 (0.124)	110 (3.299)	5.7 (0.169)	-	-	-	-
	C. demersum	20	7.4 (0.163)	112 (5.312)	5.7 (0.368)	-	-	-	-
		30	7.6 (0.339)	116 (7.039)	5.9 (0.286)	-	-	-	-
		40	7.4 (0.163)	112 (4.546)	5.8 (0.374)	-	-	-	-
3	*M. verticillatum*	20	8.9 (0.163)	142 (4.988)	8.6 (0.244)	2.86 (0.205)	23.7 (1.732)	113 (15.06)	466 (44.9)
		30	8.3 (0.309)	154 (7.257)	8.1 (0.402)	3.36 (0.124)	26.5 (1.306)	135 (0.198)	260 (50)
		40	8.6 (0.286)	173 (9.177)	8.9 (0.205)	3.73 (0.124)	27.5 (1.890)	183 (10.62)	41 (3.0)
	C. demersum	20	8.5 (0.326)	154 (5.354)	8.5 (0.286)	2.63 (0.124)	25.3 (2.984)	107 (7.039)	543 (67)
		30	8.5 (0.294)	153 (9.392)	8.6 (0.286)	2.63 (0.047)	24.4 (1.309)	134 (10.65)	483 (57)
		40	8.7 (0.355)	184 (5.312)	8.9 (0.244)	2.43 (0.047)	22.4 (1.309)	175 (5.792)	40 (5.2)

and therefore light penetration would also be affected by the water depth; thus algal growth could potentially have been restricted by the reduced light accompanying increasing depth of the container.

On the other hand, some studies have suggested that allelopathic impact may be involved in macrophyte-

microphyte interactions (Phillips et al., 1978; Scheffer et al., 1993). Here in this study, allelopathic interaction could be taking place, especially in GII, the algae added group. *C. demersum* also showed allelopathic activity against phytoplankton (Kogan and Chinnova, 1972; Wium-Andersen et al., 1983; Jasser, 1994, 1995). However, in this experiment, algae grew well, especially in shallow containers (20 and 40 cm) with *C. demersum* in GII. Other works have showed that allelopathic compounds released from *C. demersum* are inhibitory, especially to cyanobacteria (Jasser, 994). Here in the pesent study, *S. quadricauda*, green algae, was added to the containers as a test alga. Therefore, the allelopathic inhibitor release from *C. demersum* probably has less effect on the green algae, and as a result of this greater algal growth was determined in the 20 and 30 cm containers in GII with *C. demersum*.

On the other hand, members of *Myriophyllum* are highly competitive submersed macrophytes (Grace and Wetzel, 1978; Smith and Barko, 1990; Madsen et al., 1991; Weisner et al., 1997). Many studies also report their allelopathic effects on algae and cyanobacteria (Fitzgerald, 1969; Planas et al., 1981; Agami and Waisel, 1985; Saito et al., 1989; Aliotta et al., 1992; Gross et al., 1996; Nakai et al., 2000). However, Planas et al. (1981) also suggested cyanobacteria were not sensitive to allelopathic compounds released from *Myriophyllum* when compared with Chlorophytes *Selenastrum* and *Scenedesmus*. This may explain why the growth rate of algae was less with *M. verticillatum* in 20 and 30 cm depth containers in GII.

However, Scheffer et al. (1992) suggested that the effects of phytoplankton on macrophytes were not very important in shallow lakes because of “escape effects”, in which the submersed macrophytes stretch towards and concentrate their shoot biomass near the water surface. Here, in this experiment, the RGR of submersed macrophytes seems to be largely determined by some other factors. It is well known that aquatic macrophytes often face severe competition for resources such as space, light and nutrients. The RGR for both submersed macrophytes used in this experiment tended to increase with increasing water depth, in both GI and GII. In both experimental groups for both plants, the smallest RGRs were measured at the shallowest (20 cm) water depth, suggesting that space is the major factor in reducing the growth rate of macrophytes.

It is clear that the growth rate of the macrophytes was affected by the presence of algae and by the depth of the water. Water depth also affected the growth rate of the algae. It is also evident that macrophytes may affect the growth rate of algae by allelopathic mechanisms. However, interactions among algae, water depth and macrophytes are clearly complex and require more detailed study than is presented here.

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