

## Influence of the Inorganic Carbon Addition on Photosynthesis of Algae and Some Macrophytes

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**Abstract:** A two-step experiment was conducted in order to understand whether natural algal communities (fish-pond water) and selected macrophytes (*Potamogeton pectinatus* L., *Elodea nuttallii* (Planch.) St. John. and *Utricularia* L. sp.) + algae were limited by lack of inorganic carbon (IC). First, 0.6 ml 1 M  $\text{KHCO}_3$  was added to the water of Vajgar and Ratmirovsky ponds, South Bohemia, and incubated for 180 minutes under  $125 \mu\text{E m}^{-2} \text{s}^{-1}$  light. Then light intensity was increased to  $150 \mu\text{E m}^{-2} \text{s}^{-1}$  and 0.6 ml 1 M  $\text{KHCO}_3$  was added to selected macrophytes + Vajgar pond waters and incubated for 50 and 180 minutes. Initial and final pH, alkalinity and dissolved oxygen were measured and oxygen production was calculated. The results show that neither algae nor algae + experimental plants were limited by the lack of inorganic carbon. Adding  $\text{KHCO}_3$  did not cause a considerable increase in oxygen production. As an exception, *Utricularia* L. sp. was the only macrophyte which increased oxygen production after adding  $\text{KHCO}_3$ .

**Key Words:**  $\text{KHCO}_3$  uptake, algal photosynthesis, pH, alkalinity, oxygen production, fish-pond

### Bikarbonat İlavesinin Alg ve Bazı Makrofitlerde Fotosenteze Etkisi

**Özet:** Güney Bohemia da iki balık havuzunun doğal alg komunitesi ve bu balık havuzlarında yetişen makrofitlerden *Potamogeton pectinatus* L., *Elodea nuttallii* (Planch.) St. John ve *Utricularia* L. sp. ve üzerinde iki aşamalı bir çalışma gerçekleştirildi. Birinci aşamada Vajgar ve Ratmirovsky balık havuzlarından alınan sulara (50 ml) 0.6 ml 1 M  $\text{KHCO}_3$  ilave edildi ve  $125 \mu\text{E m}^{-2} \text{s}^{-1}$  ışık şiddetinde inkübasyona bırakıldı. İkinci aşamada ışığın şiddeti  $150 \mu\text{E m}^{-2} \text{s}^{-1}$  'a yükseltilerek üç makrofit ve sadece Vajgar balık havuzundan alınan su örneği üzerine 0.6 ml 1 M  $\text{KHCO}_3$ - eklendi, 50 ve 180 dakika inkübasyona bırakıldı. Deneylerin başlangıç ve bitiminde pH, alkalinite ve oksijen üretimi ölçüldü. Deneyler sonucunda her iki aşamada da inorganik karbon'un sınırlayıcı olmadığı, inorganik karbon ilavesinin *Utricularia* L. sp. dışında oksijen üretimini kayda değer miktarda artırmadığı gözlemlendi.

**Anahtar Sözcükler:**  $\text{KHCO}_3$  kullanımı, Algal fotosentez, pH, Alkalinite, Oksijen salınımı, Balık havuzu

### Introduction

It is widely accepted that inorganic carbon (IC) is an inexhaustible resource as it can be continuously supplied from the atmosphere. However, the strong increase in pH observed in productive lakes indicates that the supply rate may often be outstripped by the demand (Scheffer, 1998).  $\text{HCO}_3^-$  users take up free  $\text{CO}_2$  mainly at pH 5-7 in bicarbonate media, whereas at pH values higher than 8.3-8.8  $\text{HCO}_3^-$  use is dominant (Lucas, 1983; Pokorny et al., 1985) and  $\text{CO}_3^{2-}$  ion is completely excluded from photosynthesis (Raven, 1970).

Algae and macrophytes grow at different pH levels and  $\text{CO}_2$  forms. For instance, blue-green algae are able to photosynthesize at lower free  $\text{CO}_2$  concentrations than most green algae. Therefore, blue-green algae have a competitive advantage under low free  $\text{CO}_2$  conditions while green algae have an advantage under high  $\text{CO}_2$  conditions (King, 1970). One of the submerged macrophytes, *Elodea canadensis* Michx. having been grown in media at high  $\text{HCO}_3^-/\text{CO}_2$  ratios, had a high affinity to  $\text{HCO}_3^-$  and vice versa; in media at very low  $\text{HCO}_3^-/\text{CO}_2$  ratios,  $\text{HCO}_3^-$  use might disappear (Adamec,

1993). Similarly, Vestergaard and Sand-Jensen (2000) reported that Elodeids dominate in vegetation at high alkalinity.

On the other hand, although both  $\text{CO}_2$  and  $\text{HCO}_3^-$  are potential sources of carbon for photosynthesis in microalgae and macroalgae, their acquisition by these plants is different. While  $\text{CO}_2$  can diffuse through the cell membrane,  $\text{HCO}_3^-$  needs specific transport mechanisms to enter the cell (Raven and Johnston, 1991; Beer, 1994).

In this study, we tested whether the photosynthesis of algae and some macrophytes (*Elodae nuttallii* (Planch.) St. John, *Potamogeton pectinatus* L. and *Ulricularia* L. sp.) + algae in eutrophic ponds (Vajgar and Ratmirovsky) is limited by inorganic carbon or not.

## Materials and Methods

Water samples and macrophytes were collected from the fish-ponds Ratmirovsky (A= 78 ha and  $Z_{\text{max}}= 4$  m) and Vajgar (A= 40 ha and  $Z_{\text{max}}= 3.5$  m). Both fish ponds are eutrophic and have been used for fish production intensively (Eiseltova and Pokorny, 1994). During the sampling period, filamentous blue-green algae (cyanobacteria) were dominant in both fish-ponds. *Anabaena flos-aquae* Breb. ex Born. et. Flah. and *Aphanizomenon gracile* Lemm. were the dominant species in Ratmirovsky pond and *Anabaena flos-aquae*, *Aphanizomenon issatschenkoi* (Usacev) Prosk.-Lavr. and *Limnithrix redekei* (Van Goor) Meffert were dominant species in Vajgar pond (Albay and Pokorny, 1999). Selected water quality parameters are given Table 1.

Table 1. Water quality characteristics of the two studied fish-ponds (Albay & Pokorny, 1999).

Parameters	Ratmirovsky Pond	Vajgar Pond
Dis. Oxygen ( $\text{mg l}^{-1}$ )	12.2	12.6
Secchi depth (cm)	18	23
$\text{PO}_4\text{-P}$ ( $\mu\text{g l}^{-1}$ )	207	143
$\text{NH}_4\text{-N}$ ( $\mu\text{g l}^{-1}$ )	7.34	29.8
$\text{NO}_3\text{-N}$ ( $\mu\text{g l}^{-1}$ )	8.62	10.4
Tot. N ( $\text{mg l}^{-1}$ )	7.24	4.38
Tot. P ( $\mu\text{g l}^{-1}$ )	424	245
Tot. N:Tot. P	17.24	17.88

To understand bicarbonate uptake and measure oxygen production, three experiments were conducted under different light intensities and incubation time. All experiments were performed in a water bath at  $22 \pm 2$  °C.

In the first experiment the pond waters, which were dominated by filamentous blue-green algae, were exposed to continuous light at  $125 \mu\text{E m}^{-2} \text{ s}^{-1}$  provided by white fluorescent lamps in eight test-tubes of 50 ml (four were used for control and to the other four 0.6 ml of 1 M  $\text{KHCO}_3$  was added) in a water bath. The lids of the test tubes were not closed tightly. To prevent cells from undergoing sedimentation, the test-tubes were shaken by hand at about 10 minute intervals throughout the experiment. Initial oxygen concentration, temperature and pH were measured. Dissolved oxygen was measured with a Clarke-type sensor and pH was measured with a multimeter (MEM 102, Laboratorni Pristojke, Prague). To measure alkalinity water samples were titrated with 0.1 N HCl. In order to obtain a stable temperature ( $22 \pm 2$  °C), a thermostatic chamber was used as described by Allen and Spence (1981). Final oxygen concentration, pH, temperature and alkalinity were measured after 180 minutes of exposure. At the end of the experiment, oxygen production was calculated in each test-tube and reported as per hour, per litre.

For the second experiment only light intensity was increased and the algae and macrophytes (*Elodae nuttallii* (Planch.) St. John, *Potamogeton pectinatus* L. sp.),  $7.5 \pm 0.2$  g FW each, together were exposed to light  $150 \mu\text{E m}^{-2} \text{ s}^{-1}$  provided by white fluorescent lamps in 14 test-tubes of 50 ml (0.6 ml of 1 M  $\text{KHCO}_3$  was added to seven tubes and the others were used as controls) in a water bath. Epiphytes of the macrophytes were not washed before the treatment since the test-tubes were filled with pond water. After that the same procedure was applied to the test-tubes as mentioned above. Final oxygen concentration, pH and temperature were measured after 180 minutes of exposure. At the end of the experiment, oxygen production was calculated.

The third experiment was performed with the same macrophytes and pond waters as in the second experiment under the same conditions. Only incubation time was shortened and the test-tubes were exposed to continuous light ( $150 \mu\text{E m}^{-2} \text{ s}^{-1}$ ) for 50 minutes instead of 180 minutes.

## Results

### Experiment I

There were no noticeable differences in the effect of  $\text{HCO}_3^-$  addition on oxygen production in the water of Ratmirovsky and Vajgar ponds in the first experiment after 3 hour exposure under constant light ( $125 \mu\text{E m}^{-2} \text{s}^{-1}$ ) and temperature ( $22 \pm 2 \text{ }^\circ\text{C}$ ). In addition, oxygen production was slightly higher in tubes to which  $\text{HCO}_3^-$  was not added (Table 2).

Final pH was slightly low in Ratmirovsky and Vajgar ponds when we added  $\text{HCO}_3^-$ , but alkalinity increased three-fold in Vajgar pond when  $\text{HCO}_3^-$  was added (Table 2).

### Experiment II

There were no great differences in oxygen production between  $\text{HCO}_3^-$  added tubes and the control tubes after 180 minutes under  $150 \mu\text{E m}^{-2} \text{s}^{-1}$  illumination and constant temperature. *E. nuttallii* and *P. pectinatus* had a higher oxygen production when  $\text{HCO}_3^-$  was not added, whereas *Utricularia* L. sp. had a higher oxygen production when  $\text{HCO}_3^-$  was added (Table 3).

When  $\text{HCO}_3^-$  was not added, a large increase in pH was found with *E. nuttallii* and *P. pectinatus*, whereas pH

increased only slightly when no plants were present. The increase in pH was lower in *Utricularia* L. sp. + pond water treatment than in the other plants (Table 3).

Alkalinity was higher in the tubes to which  $\text{HCO}_3^-$  was added with *E. nuttallii*, *P. pectinatus* and *Utricularia* L. sp. than in the controls (Table 3).

### Experiment III

Oxygen production did not increase after  $\text{HCO}_3^-$  addition. Only *P. pectinatus* had a small amount of oxygen production. Natural algal communities (control tubes), *E. nuttallii*, and *Utricularia* sp., had higher oxygen production when  $\text{HCO}_3^-$  was not added (Table 4).

After  $\text{HCO}_3^-$  addition, pH was increased in Vajgar pond water, whereas a considerable increase was recorded with *E. nuttallii* and *P. pectinatus* when we did not add  $\text{HCO}_3^-$ . No noticeable differences in pH were observed between tubes with  $\text{HCO}_3^-$  and tubes without it, with *Utricularia* L. sp. (Table 4).

## Discussion

In eutrophic waters with high amounts of autotrophic biomass pH rises, and it is considered that in such waters there is a lack of inorganic carbon. An other explanation

	Ratmirovsky Pond Water					
	$\text{HCO}_3^-$ added			$\text{HCO}_3^-$ not added		
	pH	Alk. (mmol)		pH	Alk.(mmol)	
Initial	8.83			7.87		
Final	9.11	8.3		9.34	7.2	
Oxygen production ( $\text{mg l}^{-1} \text{h}^{-1}$ )*		2.21 ± 0.1			2.32 ± 0.2	

  

	Vajgar Pond Water					
	$\text{HCO}_3^-$ added			$\text{HCO}_3^-$ not added		
	pH	$\text{O}_2$ (mg/l)	Alk. (mmol)	pH	$\text{O}_2$ (mg/l)	Alk.(mmol)
Initial	9.02			8.42		
Final	9.29		6.66	9.53		2.24
Oxygen production ( $\text{mg l}^{-1} \text{h}^{-1}$ )*		2.94 ± 0.5			2.95 ± 0.3	

Table 2. Effects of  $\text{HCO}_3^-$  addition on natural phytoplankton community of Ratmirovsky and Vajgar ponds after 180 minutes of exposure.

\* Mean of four determinations ± standart deviations

Table 3. Effects of HCO<sub>3</sub><sup>-</sup> addition on oxygen production of natural phytoplankton community and selected macrophytes after 180 minutes of exposure.

Vajgar Pond Water						
	HCO <sub>3</sub> <sup>-</sup> added			HCO <sub>3</sub> <sup>-</sup> not added		
	pH	Alk. (mmol)		pH	Alk.(mmol)	
Initial	7.64			7.21		
Final	8.25	11.6		7.30	4.9	
Oxygen production (mg l <sup>-1</sup> h <sup>-1</sup> )*	7.99 ± 0.6			6.04 ± 0.4		

  

<i>E.nuttalli</i> + Vajgar Pond Water						
	HCO <sub>3</sub> <sup>-</sup> added			HCO <sub>3</sub> <sup>-</sup> not added		
	pH	O <sub>2</sub> (mg/l)	Alk. (mmol)	pH	O <sub>2</sub> (mg/l)	Alk.(mmol)
Initial	7.64			7.30		
Final	8.91	10.45		9.83	1.28	
Oxygen production (mg l <sup>-1</sup> h <sup>-1</sup> )*	10.45 ± 0.91			13 ± 0.93		

  

<i>P.pectinatus</i> + Vajgar Pond Water						
	HCO <sub>3</sub> <sup>-</sup> added			HCO <sub>3</sub> <sup>-</sup> not added		
	pH	O <sub>2</sub> (mg/l)	Alk. (mmol)	pH	O <sub>2</sub> (mg/l)	Alk.(mmol)
Initial	8.54			7.40		
Final	9.54	11.16		10.1	1.26	
Oxygen production (mg l <sup>-1</sup> h <sup>-1</sup> )*	7.99 ± 0.97			14.77 ± 1.51		

  

<i>Utricularia</i> sp. + Vajgar Pond Water						
	HCO <sub>3</sub> <sup>-</sup> added			HCO <sub>3</sub> <sup>-</sup> not added		
	pH	O <sub>2</sub> (mg/l)	Alk. (mmol)	pH	O <sub>2</sub> (mg/l)	Alk.(mmol)
Initial	7.59			7.36		
Final	8.89	12.5		8.66	1.4	
Oxygen production (mg l <sup>-1</sup> h <sup>-1</sup> )*	11.27 ± 1.59			6.52 ± 0.89		

\* Mean of four determinations ± standart deviations.

Table 4. Effects of  $\text{HCO}_3^-$  addition on oxygen production of natural phytoplankton community and some macrophytes after 50 minutes of exposure.

Vajgar Pond Water						
$\text{HCO}_3^-$ added			$\text{HCO}_3^-$ not added			
	pH	Alk. (mmol)	pH	Alk.(mmol)		
Initial	7.37		6.9			
Final	8.49		7.50			
Oxygen production ( $\text{mg l}^{-1} \text{h}^{-1}$ )*	0.34 ± 0.26		5.75 ± 0.61			
<i>E.nuttalli</i> + Vajgar Pond Water						
$\text{HCO}_3^-$ added			$\text{HCO}_3^-$ not added			
	pH	$\text{O}_2$ (mg/l)	Alk. (mmol)	pH	$\text{O}_2$ (mg/l)	Alk.(mmol)
Initial	7.66			6.8		
Final	8.47			9.32		
Oxygen production ( $\text{mg l}^{-1} \text{h}^{-1}$ )*	1.07 ± 0.93			7.75 ± 1.31		
<i>P.pectinatus</i> + Vajgar Pond Water						
$\text{HCO}_3^-$ added			$\text{HCO}_3^-$ not added			
	pH	$\text{O}_2$ (mg/l)	Alk. (mmol)	pH	$\text{O}_2$ (mg/l)	Alk.(mmol)
Initial	7.55			6.86		
Final	9.56			9.74		
Oxygen production ( $\text{mg l}^{-1} \text{h}^{-1}$ )*	13.41 ± 1.74			12.06 ± 1.91		
<i>Utricularia</i> sp. + Vajgar Pond Water						
$\text{HCO}_3^-$ added			$\text{HCO}_3^-$ not added			
	pH	$\text{O}_2$ (mg/l)	Alk. (mmol)	pH	$\text{O}_2$ (mg/l)	Alk.(mmol)
Initial	7.79			6.91		
Final	8.68			7.89		
Oxygen production ( $\text{mg l}^{-1} \text{h}^{-1}$ )*	2.37 ± 1.02			8.02 ± 1.54		

\* Mean of four determinations ± standart deviations.

is that depletion of inorganic carbon limits the growth of aquatic plants. Shapiro (1990) stated that a lack of inorganic carbon develops, being most pronounced in late summer.

In this study, it was tested whether a natural algal community is limited by the lack of inorganic carbon or not. The results, given in Table 2, show that algae from Vajgar and Ratmirovsky ponds, which were dominated by blue-green algae during sampling, are not limited by a lack of inorganic carbon. Adding  $\text{HCO}_3^-$  did not cause any increase in oxygen production. Drapcho and Brune (2000) recorded that when no external carbon was added to the cultures, one of blue-green algae, *Merismopedia*, grew luxuriantly. However when external inorganic carbon was added at rates of 0.6 or 1.2 mmol l<sup>-1</sup> per day, the green algae *Dictyosphaerium* and *Scenedesmus* were the dominant genera. The other point is that dense fish culture (*Cyprinus carpio* L.) has been implemented in these ponds since the 16th century. Therefore, external inorganic carbon additions for algal growth may not be needed in these systems, which receive aquaculture effluent from high density fish culture, due to  $\text{CO}_2$  inputs from fish respiration and bacterial oxidation of organic matter (Drapcho and Brune, 2000).

Aquatic macrophytes responded with difficulty to the adding of  $\text{HCO}_3^-$ . *Utricularia* L. sp. was the only macrophyte which increased oxygen production after adding  $\text{HCO}_3^-$  in the second experiment. Surprisingly, adding  $\text{HCO}_3^-$  resulted in a decrease in photosynthetic oxygen production by the other tested plants (*E. nuttallii* + Vajgar pond water and *P. pectinatus* + Vajgar pond water) in the second experiment (Table 3). No oxygen production was measured after adding  $\text{HCO}_3^-$  to *Utricularia* L. sp. + Vajgar pond water and *E. nuttallii* + Vajgar pond water in the last experiment. *P. pectinatus* showed a small amount of oxygen production (Table 4). The third experiment was performed over only 50 minutes and it seems that *P. pectinatus* must have had priority to use bicarbonate over the other macrophytes.

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In general, adding inorganic carbon in the form of  $\text{HCO}_3^-$  did not increase the photosynthesis of the macrophytes or algae.  $\text{HCO}_3^-$  was used as an inorganic carbon supply and pH was not decreased in order to prevent a rise in the concentration of free  $\text{CO}_2$ . Vestergaard and Sand-Jensen (2000) stated that the strong relationship between species distribution and  $\text{HCO}_3^-$  concentration suggests a direct influence of  $\text{HCO}_3^-$  on the photosynthesis, growth and long-term survival of aquatic plants. This also depends on the absolute concentrations and concentration ratios of  $\text{HCO}_3^-$  to  $\text{CO}_2$  in the water as well as on the carbon requirements of the plants (Sand-Jensen and Gordon, 1986).

## Conclusion

- The second and third (last) experiments were performed at 24 hour intervals under similar conditions and with the same pond waters. Only the incubation time was shortened and the test-tubes were exposed to continuous light ( $150 \mu\text{E m}^{-2} \text{s}^{-1}$ ) for 50 minutes instead of 180 minutes in the last experiment. After adding 0.6 ml 1 M  $\text{KHCO}_3$  to natural algal communities, oxygen production had decreased compared with the control tubes at the end of 50 minutes (Table 4). It was thought that adding bicarbonate showed a shock effect; therefore, different concentrations of  $\text{HCO}_3^-$  and incubation times should be tested to avoid possible shock in future studies.
- The effect of different pH ( $\text{CO}_2$  /  $\text{HCO}_3^-$  ratio) should be tested.
- Natural algal communities were dominated by cyanobacteria 90% and 85% in Ratmirovsky and Vajgar fish ponds respectively during the sampling. Shapiro (1990) stated that cyanobacteria have lower light-energy requirements than do greens and diatoms. Therefore, different light intensity series should also be tested.

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