

# THE ACIDIFICATION AND BUBBLING RADIOCARBON METHOD, USED IN THE ASSESSMENT OF PHYTOPLANKTON PRODUCTION IN ROMANIAN COASTAL LAKES

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## ABSTRACT

One of the most used techniques to assess primary production in lake phytoplankton is the  $^{14}\text{C}$  method that measures the rate of incorporation of a radioisotope tracer ( $^{14}\text{CO}_2$ ) into organic material.

In the frame of the ESTROM project 2005-2007, the  $^{14}\text{C}$  acidification and bubbling method (ABM) has been introduced as a new methodology for the assessment of primary production of Tasaul Lake under Swiss assistance and is a novelty in Romania. This paper describes the use of this method in Tasaul Lake, a shallow Black Sea coastal lake. Primary production of phytoplankton was assessed during an entire year from December 2006 until December 2007. C-assimilation is limited by a strong light absorption within the water column to the uppermost layer of the well mixed lake. Daily production ranged between 273 and 3798 mg C m<sup>-2</sup> d<sup>-1</sup> with an efficiency (assimilation number) of 0.03-0.71 mg C (mg chl *a*)<sup>-1</sup>h<sup>-1</sup>. Chlorophyll *a* concentration varied between 134 and 1025 mg chl *a* m<sup>-2</sup>. Preliminary estimates yield an annual production of 500 to 600 g C m<sup>-2</sup> a<sup>-1</sup>, which correspond to a highly eutrophic status of the lake.

**KEY WORDS :** primary production, C-assimilation, acid bubbling method (ABM), in situ incubation, Tasaul Lake, shallow lake

## INTRODUCTION

Water is the most important environmental factor that contributes to the quality of life. Surface water quality monitoring of rivers and lakes has the aim to protect aquatic ecosystems by maintaining and improving their quality and natural productivity. The collaborative effort between Romanian and Swiss institutions within the frame of the ESTROM Project contributed to a better understanding of the problem of eutrophication and has performed a more effective assessment of nutrients enrichment in Tasaul Lake.

Tasaul Lake, situated 20 km north of the city of Constantza, at the central Romanian Black Sea coast, is an ecosystem classified as Important Bird Area by Birdlife International. In the 1920s, the former lagoon was altered significantly by hydro-technical constructions and turned into a freshwater system. This shallow lake, with a maximum depth of 4 m is threatened by various polluters such as industry, wastewater, solid waste deposits and agriculture.

Excessive algal blooms, observed in Lake Tasaul, are evidence of eutrophication and degraded water quality. They compete with submerged aquatic vegetation by blocking light penetration. Since phytoplankton are at the base of the food chain, they have been monitored from 2005 until 2007 to investigate community composition and primary production.

The assessment of phytoplankton primary production by measuring  $^{14}\text{C}$  radiocarbon uptake (STEEMAN NIELSEN, 1952, modified by GÄCHTER and MARES, 1979) has been introduced into the monitoring program 2006 and 2007 of Lake Tasaul with the assistance of the Swiss Water Institute EAWAG. This method is considered more sensitive and precise, offering several advantages when compared to other known techniques which are used for determination of the photosynthetic activity of phytoplankton (GÄCHTER *et al.*, 1984).

A similar  $^{14}\text{C}$  uptake method was applied in Romania in the Black Sea during 1977 and 1978, and in Sinoe, Mamaia and Bicz lakes during 1978 (BOLOGA and FRANGOPOL, 1979). Afterwards,  $^{14}\text{C}$  uptake methods have not been used any more in Romanian water research until this project in Lake Tasaul.

This short note presents some selected data of primary production in Lake Tasaul in 2007 to give a preliminary estimate of annual primary production.

## MATERIALS AND METHODS

Sampling of phytoplankton, chlorophyll *a* and water quality monitoring were done monthly, during 2006 and 2007 in the water column.

Water samples were taken usually from depths of 0, 0.12, 0.25, 0.37, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 meters.

*In situ* incubations to assess photosynthetic  $^{14}\text{C}$ -assimilation by phytoplankton (BOSSARD *et al.*, 2001) were performed in 100 ml Duran bottles at each specific depth. The profiles were chosen and adapted according to Secchi disk transparency measurements. The incubation took place for three hours, generally at noon, during the interval from 10:00 until 14:00 local time, after addition of 5  $\mu\text{Ci}$   $\text{NaH}^{14}\text{CO}_3$  per bottle. From each of the samples taken between 0 and 1 meters, sub-samples were incubated *in situ* in Duran bottles covered with a UV-screen (transparent hard PVC tube according to BÜHLMANN *et al.*, 1987) to measure the difference in  $^{14}\text{C}$ -assimilation, when UV radiation is removed. After incubation, the  $^{14}\text{C}$  samples were immediately transported to the radioactivity laboratory of the National Institute for Marine Research and Development “Grigore Antipa” Constantza and processed by the acidic bubbling method (ABM, GACHTER and MARES, 1979). Radioactivity was determined by liquid scintillation counting (Tri – Carb 1000 Hewlett Packard, USA), after addition of 10 ml of Instagel (Packard, USA) to 7 ml water sample in glass vials of 20 ml volume.

The Specific Photosynthetic Production (SPP, often defined as  $\text{P}^{\text{B}}$  or Assimilation Number AN) was calculated as C-assimilation per chlorophyll *a* [ $\text{mg C (mg chl } a)^{-1}\text{h}^{-1}$ ].

Chlorophyll *a* was measured at depths of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 m (in  $\mu\text{g/l}$ ). Sample preparation and chlorophyll extraction were performed according to International standard ISO 10260 (SCOR-UNESCO, DEV 1972-1989, as described in FINGER *et al.*, 2007).

Samples were filtered through Millipore 0.45 $\mu\text{m}$ . The filters were put in Sovirel tubes filled with 8 ml of 90 % ethanol. Chlorophyll was extracted by heating the samples (for 10 min) in a water bath at 75°C. Before spectrophotometrical analysis (UV-VIS 205, at 665 nm and 750 nm wavelengths), the chlorophyll extracts were filtered through Millipore Millex FG 0.2  $\mu\text{m}$  membrane filters.

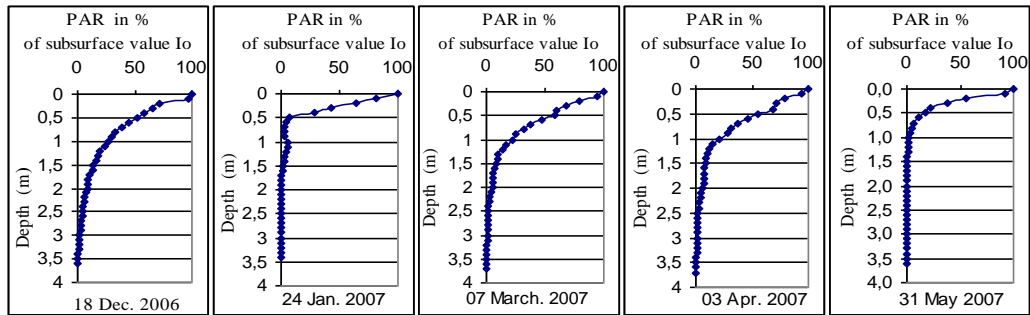
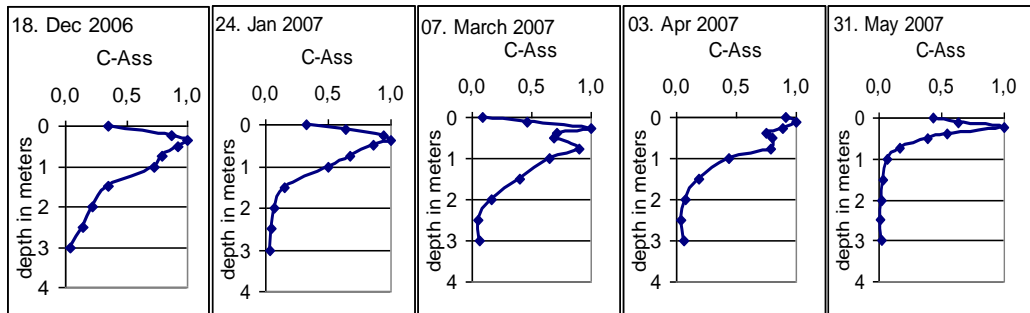
*In situ* photosynthetically active radiation (PAR, denoted as  $\mu\text{E m}^{-2}\text{ s}^{-1}$ ) was measured with a scalar quantum sensor (LI 190 SB) connected to an integrating quantum meter (LI 188) made by LI-COR Inc, USA. Simultaneously a cosine corrected PAR sensor (LI 190) served as a reference, measuring PAR above the water surface.

## RESULTS AND DISCUSSIONS

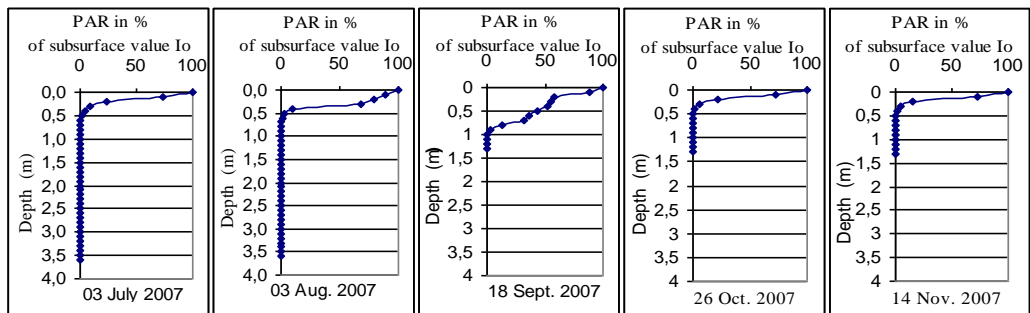
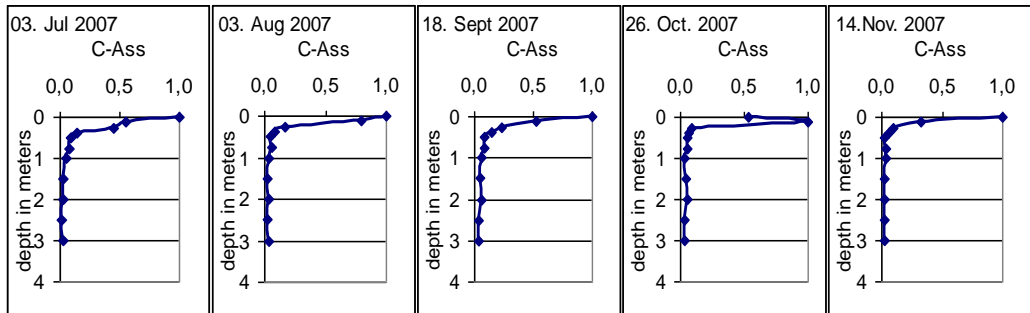
Areal primary production in Tasaul Lake ranged in 2007 between 33 and 271  $\text{mg C m}^{-2} \text{ h}^{-1}$  during incubation at noon. Daily areal production ranged between 273 and 3798  $\text{mg C m}^{-2} \text{ d}^{-1}$ . The efficiency of primary production (SPP, AN, C-ass/chl *a*) was 0.03-0.71  $\text{mg C (mg chl } a)^{-1} \text{ h}^{-1}$ . Chlorophyll *a* standing crop varied between 134 and 1025  $\text{mg chl } a \text{ m}^{-2}$ , while chlorophyll concentrations were homogeneously distributed from the water surface to the bottom of the lake during all times of the year. This suggests that the lake is not stratified during any time of the year, which is also supported by the vertically homogeneous distribution of temperature. Figure 1 shows the vertical distribution of primary production ( $^{14}\text{C}$ -assimilation), relative to the maximum production being equal to 1, and of PAR in % of the incident surface radiation. The profiles were assessed in monthly, respectively, bimonthly intervals from 18 December 2006 until 14 November 2007 and followed the constraints of the corresponding light transparency.

During the winter months near surface inhibition of primary production was detectable. During the summer months no inhibition was detected near the water surface, because, with the applied incubation method, it was technically impossible to differentiate primary production well enough within a vertical distance of only a few centimeters.

During the productive summer period C-assimilation was limited to the uppermost meter or even less, while during the less productive and more transparent winter period C-assimilation was spread over a thicker layer of 2 to 3 meters. Preliminary estimates of total annual primary production are based on daily primary production and yield values of some 500-600  $\text{g C m}^{-2} \text{ a}^{-1}$ . This number suggests that Lake Tasaul is a highly eutrophic shallow lake.



Date	18.12.06	24.01.07	07.03.07	03.04.07	31.05.07
PAR (Io) $\mu\text{E m}^{-2} \text{s}^{-1}$	286	422	657	499	1450
Daily prod. $\text{mg C/m}^2 \text{day}$	475	1150	838	1624	2069



Date	03.07.07	03.08.07	18.09.07	26.10.07	14.11.07
PAR (I <sub>0</sub> ) μE m <sup>-2</sup> s <sup>-1</sup>	1429	1375	1153	489	668
Daily prod. mg C/m <sup>2</sup> day	883	1900	800	3798	273

Fig.1 - Vertical distribution of primary production (<sup>14</sup>C-assimilation), relative to the maximum production being equal to 1, and vertical distribution of PAR in % of the incident surface radiation, in Tasaul Lake during 2007

Monitoring of phytoplankton in enriched coastal waters is an important aspect of implementing the EU Water Framework Directive. The assessment of phytoplankton abundance, community structure and biomass can be supported by measuring primary production (with the <sup>14</sup>C method) and chlorophyll *a* concentration. The aim of these analyses is to assess the algal blooming phenomenon, to understand aquatic ecosystem processes and to take measures for reducing eutrophication.

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