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Measurement of primary production in aquatic systems by the radiocarbon method

1 INTRODUCTION

During the workshop "Measurement of primary production in aquatic systems - comparison and assessment of available methods", several different versions of the radiocarbon method were used. While the procedure of filtering and measuring the fixed radiocarbon was almost the same, there were differences in the incubation methods employed. The methods were: classic in-situ static incubations at defined depths, the simulation of vertical mixing by the use of lifts and the measurement of a photosynthesis-light curve in a laboratory incubator. Additionally, different incubation bottles used by the participants of the workshop were compared in order to find possible bottle effects.

2 MATERIAL AND METHODS

The incubations were performed on 8.10.1997 between 10:45 and 14:45. All incubations were based on the same sample from the Bodden near Zingst. The Bodden water was filled into a mesocosm (ca. 1 m^3) one day before the beginning of the experiments. A subsample from the mesocosm was taken and approximately 0.02 µC ^{14}C ml^{-1} were added. Four different types of incubation were carried out:

- measurement of a photosynthesis-irradiance (P/I) - curve after one hour of incubation in the photosynthetron at defined light intensities. The photosynthetron (Tilzer et al. 1993) is a laboratory incubator which facilitates the measurement of primary production under defined

light conditions. Measurements with the radiocarbon method and fluorescence measurement were conducted at the same time. Results of the fluorescence measurements will be given by Hartig (this issue).

- dynamic incubation in the mesocosm with a frequency of 2 minutes and an amplitude of 0.75 m (surface to bottom of mesocosm) in 500 ml and 25 ml bottles. Detailed description of the bottle lift is given by Behrendt (1989). The Incubation time was 1, 2 and 4 hours, respectively, for light and for dark bottles.
- static incubation in the mesocosm in four different depths (0.02 m, 0.27 m, 0.52 m and 0.77 m) in 25 ml vials. Incubation time was 1, 2 and 4 hours for light and dark bottles.

After the incubation, all samples were processed in the same way. Total ^{14}C (T^{14}C) was determined in a 2 ml subsample, 2 drops of 1 M NaOH and 3 ml of a scintillation cocktail (Ultima Gold, Packard) were added. The particulate organic fixed ^{14}C(PO^{14}C) was determined by filtration of 3 ml of the sample on a 0.2 µm membrane filter (Costar, polycarbonate filter) with subsequent storing on filters soaked with 0.01 M HCl for 30 minutes. After drying, the filters were dissolved in 0.5 ml Soluene-100 (Packard), 2.5 ml scintillation cocktail (Hionic Fluor, Packard) was added. All samples were measured within 6 hours after the processing in a liquid scintillation counter (LSC). The measurement was repeated two days later in the LSC Packard Tri-Carb 2100TR with internal quench correction.

Light doses were recorded by means of both underwater and surface quantum radiometers (Eldonet / Licor). Additional measurements with a spectroradiometer were conducted for the calculation of light attenuation (Schubert, this volume).

Primary production rates were calculated according to equation (1).

$$C_{ass} = DIC \bullet \frac{T^{14}C}{PO^{14}C} \bullet 1{,}06 \qquad\qquad (1)$$

(C_{ass} = rate of carbon assimilation; DIC = concentration of dissolved inorganic carbon [mg l^{-1}]; DO^{14}C = ^{14}C retained on the filter; T^{14}C= ^{14}C concentration in the sample; 1,06 = factor taking into account the discrimination between ^{12}C and ^{14}C by phytoplankton)

The parameters of the photosynthesis-irradiance curve were calculated according to equation (2).

$$P = P_{max} \bullet \left(1 - e^{-\alpha \bullet PFD/P_{max}}\right) + \left(\beta \bullet PFD\right) \qquad (2)$$

(P = rate of primary production [μg C l^{-1} h^{-1}]; α = initial slope of the P/l-curve; β = photoinhibition term; PFD = photon flux density [quanta m^{-2} s^{-1}])

Fitting of data to the model was done with the Solver function of the spreadsheet program Microsoft Excel. For the calculation of integral carbon fixation, dark fixation values were subtracted from the results of the respective light incubated samples.

3 RESULTS

The samples obtained from the different incubation methods were measured repeatedly. Although NaOH was added to the samples for the determination of T^{14}C to give a pH >10, a dramatic loss of radioactivity over the time was observed (Fig. 1).
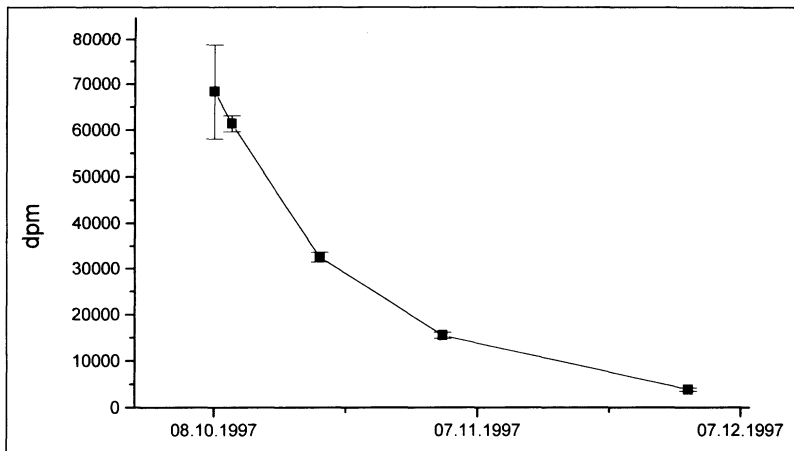

Fig. 1 Loss of radioactivity from the T^{14}C-samples during a two month period of storing. pH of sample rose to 10 before addition of the scintillation cocktail, but was then reduced to about 7 because of the high buffering capacity of the scintillation cocktail.

Further investigations showed that the alkalisation of the **mixture** of the sample and the scintillation cocktail to pH > 10 is necessary to prevent the loss of radioactivity. This finding is important for field work because frequently a longer period of time passes by between the processing of the samples in the field and the measurement in the laboratory.

The samples for the determination of $PO^{14}C$ lost only trace amounts of radioactivity, the results were almost stable over the time.

For the calculation of chlorophyll-specific primary production rates a chlorophyll a-concentration of 50 µg l^{-1} was assumed according to first measurements during the workshop. The DIC-concentration was 23.23 mg l^{-1}. The specific primary production rates are shown in Tab. 1.

Tab. 1 Specific primary production rates [µg C (µg Chl a)$^{-1}$ h^{-1}] of dynamic, static and laboratory incubation.

Incubation	1 h	2 h	4 h
dynamic, small bottles	2,2	1,9	1,9
dynamic, big bottles	2,5	2,2	1,9
static, 2 cm depth	1,7	1,0	0,8
static, 27 cm depth	2,5	2,4	2,1
static, 52 cm depth	2,2	2,6	2,3
static, 77 cm depth	1,7	1,4	1,9
Photosynthetron	0,56-3,42	-	-

Highest specific primary production rates were observed in static incubated bottles at 27 and 52 cm, followed by the dynamic incubated bottles, both small and big bottles. Lowest specific primary production rates, probably due to strong photoinhibition, were found in the near-surface (2 cm) static incubated bottles.

Dark fixation rates were almost the same when small and big bottles were compared. Most of the absolute carbon fixation in the dark bottles took place within the first hour of incubation (Tab.2).The highest dark fixation rate of carbon was measured in the photosynthetron, maybe due to insufficient temperature control.

The photon dose-dependent rates of carbon fixation were linear for incubations for a duration of 1 to 4 hours, with no indication of time-dependent changes in photosynthesis, and showed a strong dependency on the irradiance (Fig. 2).

Tab. 2 Dark fixation rates measured with different incubation types.

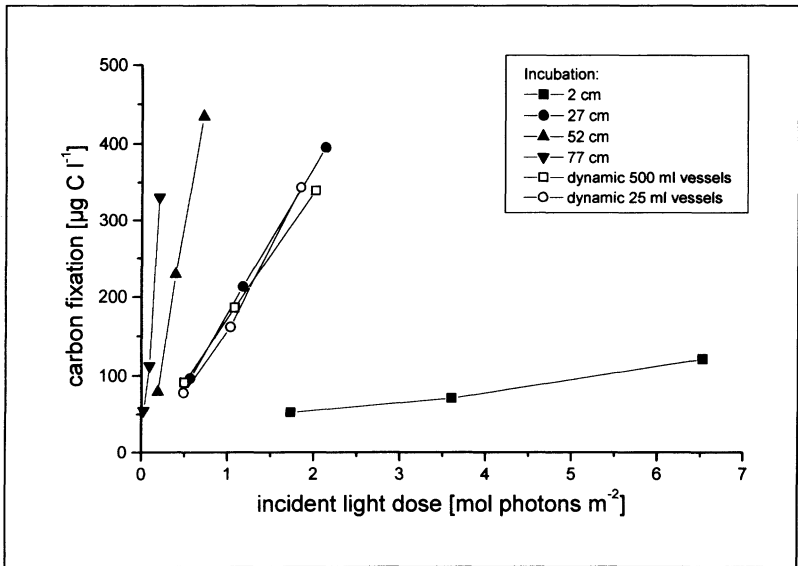| Incubation	Dark fixation[µgC(µg Chl a)$^{-1}$h^{-1}]
static, 1 h, small bottle	0,62
static, 2 h, small bottle	0,31
static, 4 h, small bottle	0,16
static, 4 h, big bottle	0,17
Photosynthetron, 1 h	0,88

Fig. 2 Carbon fixation rate versus light dose plot for the different ^{14}C-incubations. The figure shows the carbon fixation rates of the static in-situ incubations as well as the rates of the dynamic incubations using vessels of different size for incubation times of 1, 2 and 4 hours, representing different light doses received.

Highest light-specific carbon fixation rates occurred in the static incubated samples at the depths of 52 and 77 cm. Carbon fixation in the dynamic bottles was comparable to those of the static bottle in 27 cm depth.

Plotting the ^{14}C-fixation rates versus irradiance for all incubations (including the laboratory incubations in the photosynthetron) showed

similar rates in the light-limited region but a lower P_{max} for the static incubated samples (Fig. 3).
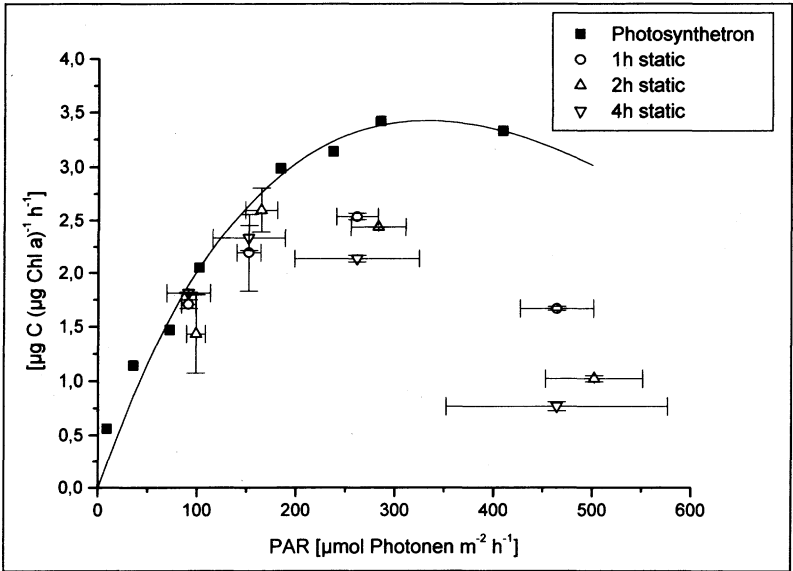

Fig. 3 Photosynthesis-light dependency. The line represents the result of fitting the data obtained with the photosynthetron to equation 2. Error bars represent irradiance amplitude (horizontal) and standard deviation of carbon fixation (vertical).

The lowest rates of photosynthesis were determined in the surface incubated bottles, lower than the rate of the sample exposed in the photosynthetron to comparable light intensities for the same exposure time.

Calculation of integral carbon fixation rates of the mesocosmos for 1, 2 and 4 hours revealed similar results for all incubation methods (Fig.4).
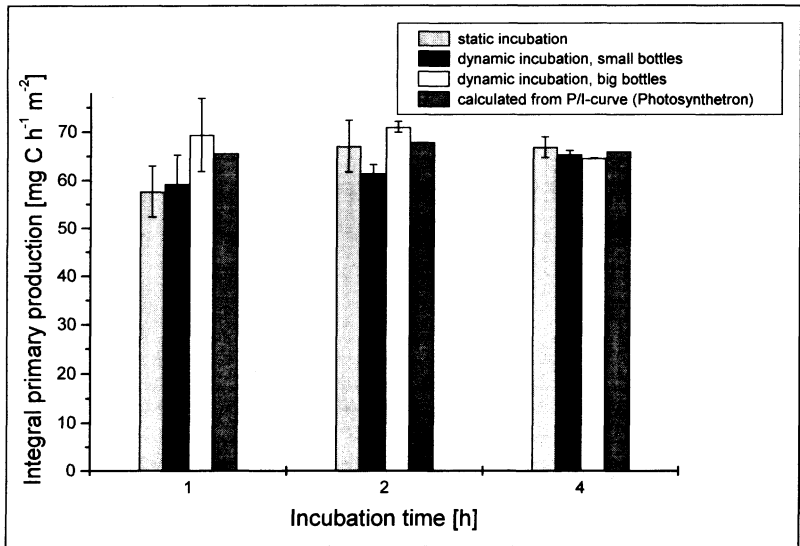
Fig. 4 Integral primary production in the mesocosm calculated from static incubations, dynamic incubations in small and big bottles and from the PI-curve obtained with the photosynthetron

DISCUSSION

Some authors have reported discrepancies between static and dynamic incubations (e.g. Gervais 1997, Lizon & Lagadeuc 1998, Nixdorf 1990). Higher as well as comparable carbon fixation rates for dynamic incubation have been described. In this study we found fairly high agreement between the results of the different incubation methods. The higher carbon fixation rates obtained with the laboratory incubator are probable due to insufficient temperature control. The agreement of the calculated integrated production resulting from the laboratory incubation with the production calculated from the in-situ incubations is a result of the equally elevated dark fixation rates, which were subtracted in each case.

Differences between incubation methods could be explained by photoadaptation occurring in the static incubated bottles but not in dynamic incubated bottles. These differences should increase with longer incubation times. Photoadaptation as well as photoinhibition

occurring in static incubation bottles could enhance or underestimate primary production calculations in comparison to dynamic incubated samples. Whether results from laboratory or in-situ incubations match the „true" values, depends on the possibility to determine the extent of mixing in the field. Therefore, a methodology for the estimation of the extent of mixing is needed. This would also be helpful for the adjustment of frequency and amplitude of dynamic incubation methods to near-natural conditions.

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