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5. METHODS OF BIOLOGICAL SAMPLING

5.1 PHYTOPLANKTON – PHOTOSYNTHESIS

(by M.T. Dokulil and I. Holst)

5.1.1 Introduction

Measurements of photosynthetic activity of the phytoplankton in fresh waters are commonly performed by the popular *„in situ"* light- and dark bottle technique. This method requires water samples, collected from various depths, that are siphoned into clear and darkened bottles, resuspended at the depth from which they came and incubated for a certain length of time (Gaardner & Gran, 1927; Steemann-Nielsen, 1952). As the exposure time of the bottles may vary from two hours to half a day, the method is very time consuming and costly in terms of ship time and logistics on large cruises, such as in the sea. Furthermore, the number of stations that can be processed in a day is far too small to provide synoptic information over larger areas.

Alternative techniques – exposing bottles in an incubator at constant temperature on board ship, to either direct sun light (Riley, 1939) or artificial illumination (Steeman- Nielsen & Jensen, 1957) – adapted during marine studies and later applied to fresh waters – are reviewed in Stee-

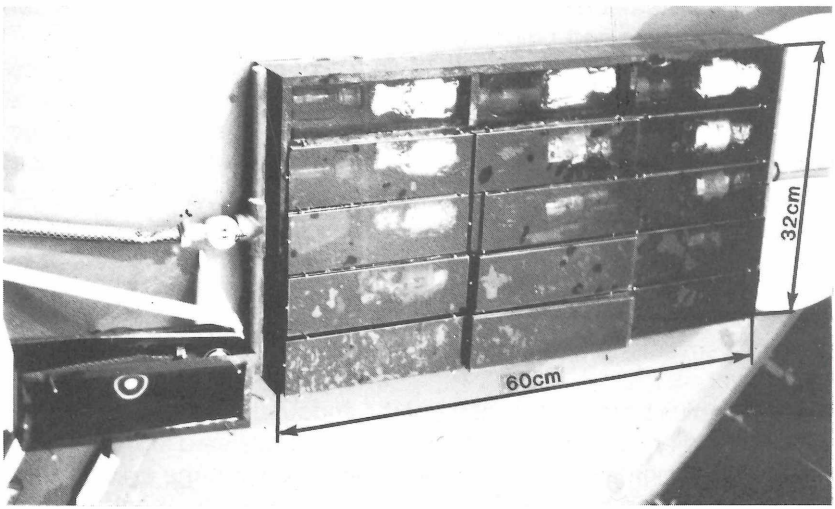

Plate 5.1.1. Deck-incubator for the simulated *in situ* incubation as described in the text.

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mann- Nielsen (1957), Talling (1974) and Gargas (1975). Numerical models for the conversion of potential production measured in tanks to *in situ* productivity have been developed, among others, by Fee (1973 a, b).

5.1.2 Equipment

During the present investigation on a large river impoundment, a simple deck-incubator constructed from black perspex has been tested and used and exposed to full day light (Plate 5.1.1). *In situ* water temperature is provided by continuously pumping surface water through the incubator. Different light levels are obtained by dark-glass filters. No attempt has been made to simulate the spectral composition of underwater light. A separate chamber, housing a flat quantameter sensor (Li-Cor), is used to measure and control photosynthetic available radiation (PAR) within the experimental compartments (Plate 5.1.1). The incubator enables the exposure of a total of 30 bottles, each with a volume of 50 ml. Light-and-dark bottles from three sampling stations can thus be processed simultaneously at five different irradiance levels (Holst, 1987).

5.1.3 Methods of analysis

Photosynthetic rates were estimated from changes in dissolved oxygen concentration detected by the Winkler technique, using back titration and amperometric end-point detection (Talling, 1973).
At a reference station, 100 ml bottles were exposed *in situ* from a vertical rack holding two pairs of light-and dark bottles in a horizontal position at each depth. To compare the oxygen- and the ^{14}C-method, one set was spiked with ^{14}C-bicarbonate (1 ml, 4 μ Curies), the other was analyzed for oxygen as above.
Radio-activity incorporated into algal cells was determined using both filtration (Millipore, 25 mm, 0.2 μm pore size) and acid bubbling (8 ml sample, 100 μl 2N HCl, 30 min bubbled) techniques (Sondergaard, 1985). Samples were processed with Aqualuma and Lumagel cocktail (Baker Chemicals) respectively and counted in a liquid scintillation counter.
Water samples from the top metre of the impoundment were taken with a 50 l Schindler sampler (see chapter 5.2) and transfered into a 10 l container from which all subsamples were siphoned into the experimental bottles. Preceding investigations indicated that the basic conditions for such a sampling procedure – homogeneous distribution of the plankto-

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nic algae and complete vertical mixing – are ideally met in the river impoundment Altenwoerth.
The underwater light field was quantified with a spherical quantum sensor (Li-Cor), estimations of secchi depth and gravimetrical analysis of turbidity (Strickland & Parsons, 1968).

5.1.4 Results

Comparison of *in situ* depth profiles of photosynthetic rates to the corresponding measurements from the deck incubator, converted to depth according to light intensity are exemplified in Figure 5.1.1 Both curves closely resemble each other in depth and distribution ($r = 0.95$, $P \leq 0.001$, $n = 19$). Absolute rates obtained in the incubator are on average 5 % higher than measurements performed *in situ.*

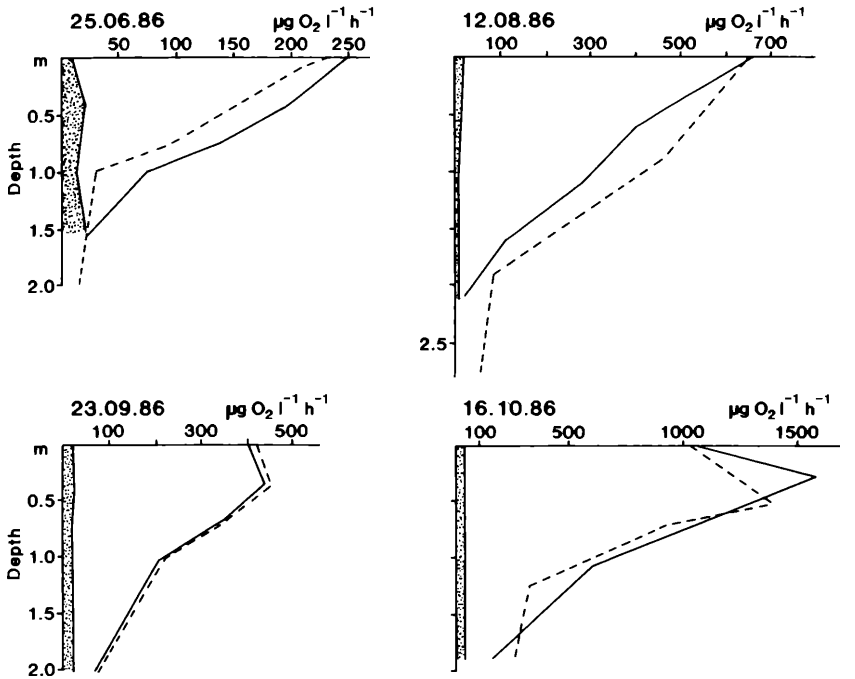

Fig. 5.1.1. Vertical distribution of photosynthetic rates and respiration for four different dates, 25.6., 12.8., 23.9., 16.10.1986, as measured *in situ* and converted from the deck incubator, both measured by the oxygen method. – = *in situ;* --- = on deck.

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Total gross productivities within the euphotic zone (integral rates per square metre) calculated from the two incubation techniques are compared in Figure 5.1.2. Observed data are best fitted by the exponential curve y = exp (0.00169 x) * 158.482 with a correlation coefficient of r = 0.99 ($P \leq 0.001$, n = 10). This non-linear relationship indicates that integral rates calculated from incubator measurements will underestimate *in situ* productivity at low rates and overvalue it at higher rates. Linear regression, however, yielded a correlation coefficient of 0.94 which is still highly significant. Measurements in the deck- incubator therefore adequately simulate photosynthetic rates *in situ.*

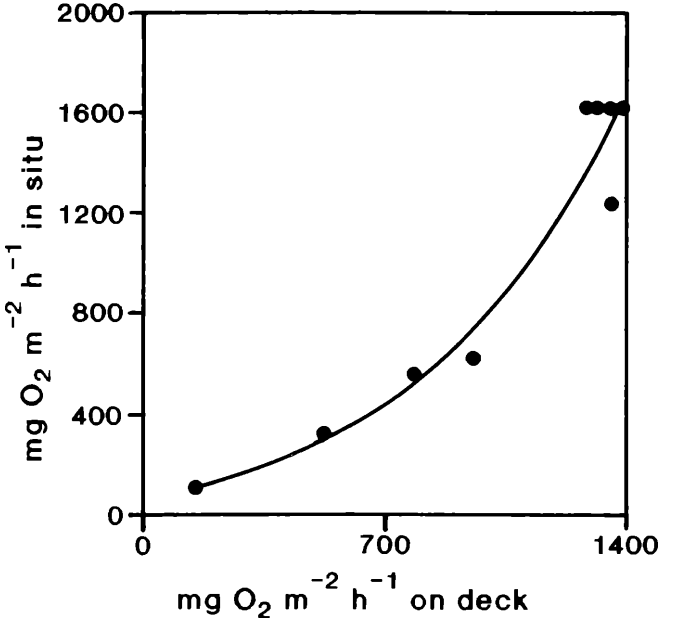

Fig. 5.1.2. Relationship of integral productivity from *in situ* and on deck inkubations, both measured by the oxygen technique.

The relationship between ^{14}C uptake and oxygen evolution is shown in Figures 5.1.3 and 5.1.4 for all *in situ* observations. Close agreement between both techniques was observed. On average, carbon uptake represents 80 % ± 38 % of gross- and 95 % ± 41 % of net oxygen production ($r = 0.97$, $P \leq 0.001$, n = 37). The photosynthetic quotients are 1.18 ± 0.29 and 1.05 ± 0.26 respectively. Incorporation of radioactive carbon may be considered therefore as a measure of net-photosynthesis.

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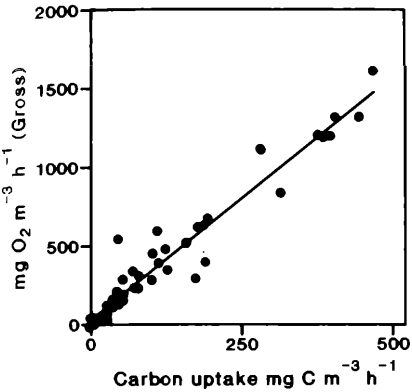

Fig. 5.1.3. Relationship of gross-oxygen evolution to carbon uptake both measured *in situ.*

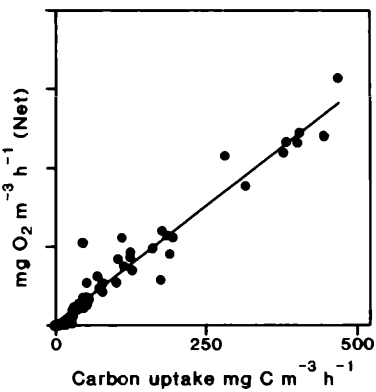

Fig. 5.1.4. Relationship of net-oxygen evolution to carbon uptake both measured *in situ.*

On several occasions, subsamples from ^{14}C-incubation bottles were processed by filtration and the acid-bubbling- method (ABM). Both tech-

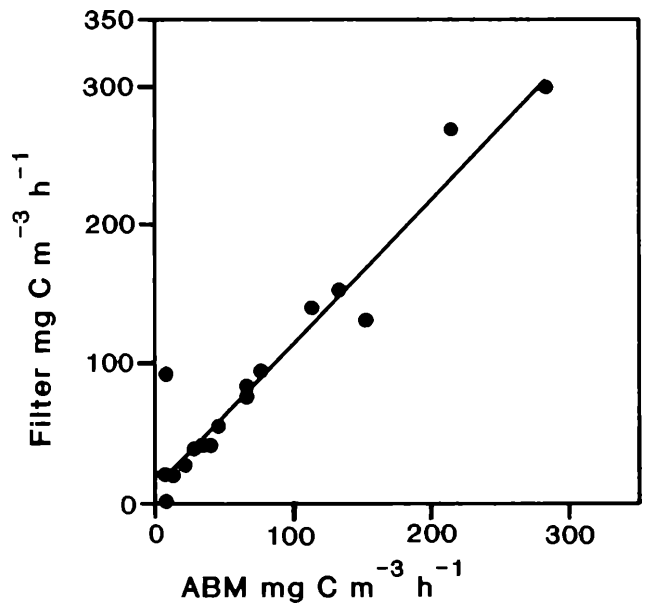

Fig.5.1.5. Relationship between carbon uptake measured by the acid bubbling technique (ABM) and by the filtration method.

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niques gave almost identical results ($r = 0.90$, $P \leq 0.001$, $n = 18$) as depicted in Figure 5.1.5. ABM values were 4 % to 24 % lower than those from the filtration procedure, similar to observations by Sondergaard (1985).

5.1.5 Conclusions and recommendations

a) Simulated *in situ* incubations on deck adequately describe photosynthetic rates of the phytoplankton in large rivers and impoundments and other well mixed systems.

b) No *in situ* exposures are required, saving ship time and complicated exposure racks, which is of special advantage in fast flowing rivers.

c) Synoptic studies are possible because a greater number of stations can be processed within a day.

d) The oxygen technique should preferably be used for analysis whenever possible, since more information is gained (gross and net rates, compensation point); moreover respiration data can be used to estimate net-production over 24 hours.

e) For the ^{14}C-technique the authors strongly recommend that samples should be processed with the acid bubbling technique because less transfer steps are required.

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