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Instrumentation for the measurement of phytoplankton production[1]

Abstract—Automated instrumentation is described that performs time-course incubation experiments directly in situ where natural conditions of temperature, light, hydrostatic pressure, etc. can be maintained. The sampler incubation device (SID) takes a 1-liter sample from the water and simultaneously introduces an appropriate radiotracer. During subsequent in situ incubation, 50-ml subsamples are withdrawn from the main sample at equally spaced intervals and preserved for laboratory analysis. Representative experiments revealed nonlinear carbon uptake within 0.5–1.0 h, emphasizing that even brief end-point analyses can lead to large errors in estimating phytoplankton production rates. Studies of the rapid fluctuation in phytoplankton activity resulting from cloud-induced variations in light intensity and the application of cellular fractionation methods for measuring the intracellular distribution of newly fixed carbon illustrated the utility of instrumental time-course techniques for studying phytoplankton physiology and community metabolism in situ.

The usual methods for directly quantifying phytoplankton activities are based on the classic technique (Steemann Nielsen 1952) whereby samples from the environment are incubated in enclosed vessels for a given period and an end-point determination is made of the change in a specified tracer compound. It is becoming generally accepted however, that this method as commonly used is subject to various limitations and may not accurately reflect in situ activity (e.g. Goldman 1980; Harris 1980; Li and Goldman 1981; Malone 1982; Glibert et al. 1982). Entrainment of a water sample within an experimental vessel creates an essentially closed system with properties potentially very different from the open environment from which it was derived. The activity and viability of the enclosed phytoplankton will become modified relative to those in situ, increasing the potential for erroneous uptake kinetics. Depending on the environment from which the samples are taken, such confinement effects may become manifest in a remarkably short period (Gieskes et al. 1979), reflecting phenomena such as nutrient depletion (Goldman et al. 1981; Glibert and Goldman 1981), light-related artifacts (Harris and Piccinin 1977; Marra 1978*a*,*b*), chemical toxicity (Carpenter and Lively 1980; Fitzwater et al. 1982; Ortner et al. pers. comm.), phytoplankton growth (Gieskes and van Bennekom 1973), alteration in species composition (Venrick et al. 1977), etc.

Because different samples have different properties, the time of incubation most appropriate for acceptably determining in situ phytoplankton activity is not a priori definable (Marra 1980). Continued incubation of a sample beyond this initial undefined period does not quantify in situ activity, but rather is a measure of the response of the phytoplankton to confinement. To minimize such confinement effects, sample incubations should be kept as brief as possible. The assessment of when experimental biases become significant requires high-resolution, semicontinuous monitoring of the measured activity in time.

For incubations that are to continue for a maximum of a few hours, manipulating the sample before adding tracer may modify subsequent phytoplankton activity unless precautions are taken to maintain in situ conditions at all times. For example, failing to simulate natural light conditions or keeping a sample in a holding vessel for significant periods before its distribution into the incubation vessels may greatly bias the experimental re-

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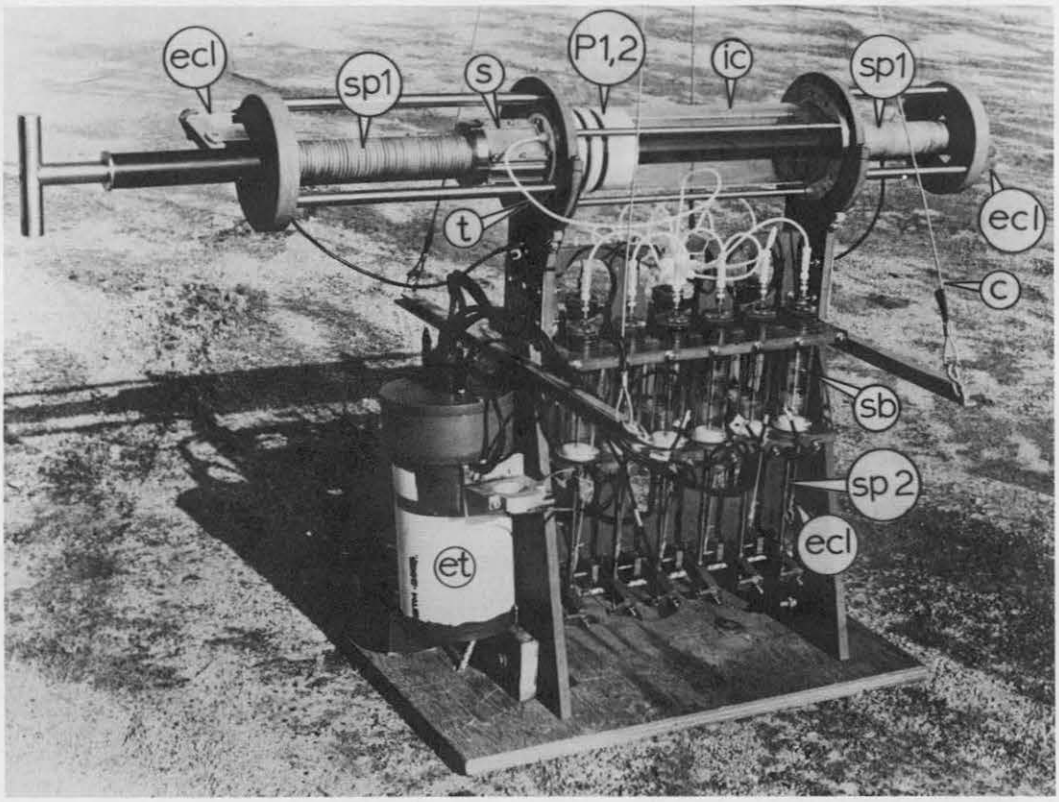


Fig. 1. The sampler incubation device (SID) consists of three functional units: a 1-liter, horizontally mounted glass incubation chamber (ic) equipped with two spring-powered (sp 1) ceramic pistons (P1, 2), a subsampling array of 12 50-ml spring-powered (sp 2) glass subsampling syringes (sb) connected to the main incubation chamber via a Teflon manifold and associated tubing (t), and an electronic timer (et) that initiates mechanical operation through electrocorrosion of stainless steel restraining links (ecl). Screen (s) can be used to remove large particulate material. The SID is suspended in the water by four cables (c) attached to a swivel, mooring line, and float.

Sample is taken into the incubation chamber by activating piston P2 on the right. Tracer substances within the dead spaces of a check valve in piston P1 on the left are swept into the incubation chamber simultaneously with the sample. After incubation chamber is filled piston P1 is activated, generating about 51 kPa (0.5 atm) of internal pressure for transfer of subsamples into each subsampling syringe upon timed release.

sults. One way to avoid potential biases of this kind is to conduct all operations of sample procurement, manipulation, and incubation in situ, so that the experiment begins immediately after sample entrainment.

With the above considerations in mind, we have developed an instrument, designated the sampler incubation device (SID), that performs time-course biological rate measurements directly in situ where natural conditions of the environment are continuously maintained. We appreciate the engineering assistance of K. W. Doherty and acknowledge the workmanship of M. C. Woodward in constructing the component items of the SID. The technical assistance of H. B. Kaplan, D. H. Wiebe, and J. M. Peterson is acknowledged. We thank J. C. Goldman for his critique and the Salt Pond Areas Bird Sanctuaries, Inc., for permission to work in the study area.

The instrument, shown in Fig. 1 and

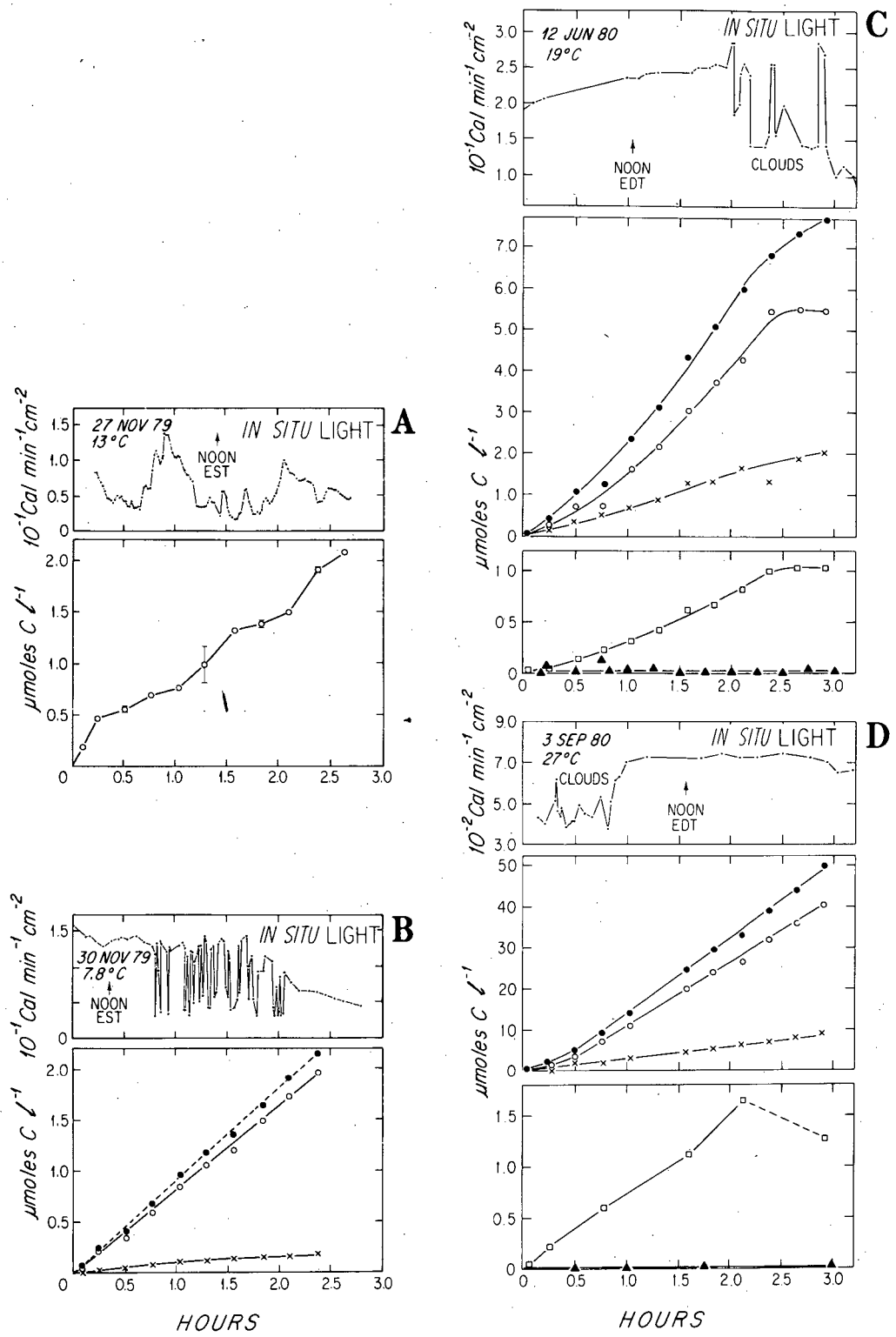
described in detail elsewhere (Taylor et al. in prep.), functions briefly as follows. The device is lowered into the water and suspended at the desired depth from surface floats. Upon activation by an electronic circuit, a sample is taken into a 1-liter, syringelike incubation chamber and automatically mixed with an appropriate radiotracer; incubation begins immediately. In a timed sequence, subsamples are withdrawn consecutively from the main incubation chamber into 50-ml subsampling syringes containing chemical fixatives to end the biological reactions, thus providing a time-course measure of the activity within the incubation chamber.

Figure 2 shows the results of experiments in which the SID was used to measure the incorporation of radiolabeled carbon dioxide into the phytoplankton of Salt Pond, a marine basin in Falmouth, Massachusetts (1971 Annu. Rep.: Salt Pond Areas Bird Sanctuaries, Inc.). The time-course data (Fig. 2C) show a nonlinear incorporation of radiocarbon into phytoplankton cells within 0.5–1.0 h which would not be detectable by short term end-point measurements (3–6 h), and computed rates of in situ carbon incorporation would be in error. For example, if the pattern of carbon uptake observed for the first 2 h of incubation were to continue unimpeded by the influence of cloud cover, an apparent rate of 3.6 μmol $C \cdot liter^{-1} \cdot h^{-1}$ would be obtained from a 3-h end-point measurement. This value is twice the initial rate (1.85 ± 0.12 μmol $C \cdot liter^{-1} \cdot h^{-1}$) obtained by using time-course subsampling techniques, which is probably closer to the activity in the environment at the instant of sample confinement. The exponential character of the data in Fig. 2C suggests that phytoplankton biomass may have increased within the SID chamber. This was not an artifact of incubation within the SID, per se, as parallel shore-based time-course experiments (J. C. Goldman unpubl.) in gently stirred 1-liter chemostat vessels (apparatus described by Goldman et al. 1981) showed a similar upward curving pattern of carbon uptake. A growth response was not always observed in SID-based experiments, indicating that the phenomenon was a characteristic of the resident population rather than an artifact of the mode of incubation.

In situ, time-course measurements are also valuable for investigating short term temporal variations in phytoplankton activity in response to environmental changes such as light intensity. The experiment shown in Fig. 2A was done on an overcast day when average twofold–threefold variations in light intensity resulted in rapid changes of similar magnitude in the rates of carbon incorporation into acid-precipitable polymers. Similar responses to significant changes in light intensity were observed in summer and fall (Fig. 2C,D). Periods of rapid carbon incorporation were generally correlated with periods of relatively high light intensity, although in many instances a distinct lag in the response to light was observed. Brief, intermittent periods of shading on a time scale of 1–5 min, however, did not result in observable changes in the kinetics of carbon incorporation (Fig. 2B).

The probable diel variations in photosynthetic rates (Harding et al. 1982) coupled with the potential for significant deviation from initial rates in a short period suggest that estimates of gross phytoplankton production rates over extended periods (8–12 h) might best be made by the numerical summation of the initial production values obtained from a series of very short consecutive time-course experiments.

Recent phytoplankton production studies have included analytical procedures for fractionating cells into their constituent low molecular weight and polymeric components (Morris et al. 1974; Smith and Morris 1980*a*,*b*; Li et al. 1980; Mague et al. 1980; Hitchcock pers. comm.). When used in conjunction with time-course incubation, these approaches considerably enhance the versatility of the ^{14}C technique by permitting the investigator to study not only the dynamics of total carbon uptake but also the intracellular distribution of newly fixed carbon. From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such data the general physiological state of the phytoplankton population can be deduced and the influence of environmental perturbations on community metabolism can be interpreted. We have used the SID to extend such physiological measurements directly into the field (preliminary studies shown in Fig. 2B–D). Filtration of acid-fixed samples resolves fixed carbon into two determinable fractions: acid-releasable low molecular weight intracellular metabolites plus potential excretion products, and acid-precipitable cell polymers. Summation of these permits total carbon fixation to be expressed in terms identical to that provided by the method of Schindler and Holmgren (1971).

In the experiment shown in Fig. 2B, 86–91% of the newly fixed carbon was incorporated into the acid-precipitable fraction during the incubation. A small and time-varying proportion of fixed carbon was incorporated into the low molecular weight pools (values ranged from 14 to 9%). The plateauing of label in the latter fraction suggests that it is in a relatively rapid state of turnover, ultimately appearing in the polymer fraction. The distribution of label into low molecular weight and potential excretion products was significantly higher on other occasions, however. During the unshaded periods of the incubations shown in Fig. 2C and D, 30 ± 4% and 19 ± 1% of the fixed carbon appeared in this fraction.

The occlusion of light by clouds in the experiment shown influenced carbon fixation primarily through reduction in the rate of incorporation into acid-precipitable polymers. Furthermore, fractionation of this component into protein illustrated that in spring the 1.6-fold–2-fold reduction in light was enough to nearly stop incorporation of label into this fraction. In fall, however, the 60% increase in light influenced the synthesis of polymers other than protein. Comparison of Fig. 2C and D revealed an order-of-magnitude difference in the rates of total carbon fixation (initial rates of 1.9 and 18.5 μmol $C \cdot liter^{-1} \cdot h^{-1}$ in spring and fall) which primarily reflects the large differences in temperature and algal biomass. In addition, there was a significant difference in community metabolism. For example, in the spring population 14 ± 1% of the newly fixed carbon was incorporated into

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Fig. 2. Measurement of incorporation of [^{14}C]bicarbonate into microbiota of Salt Pond with SID. Instrument was kept at a depth of 1.5 m by suspension from the center of a 2.4-m aluminum rod held afloat in a manner preventing occlusion of light. Normal wave action provided gentle mixing. [^{14}C]bicarbonate (300–500 μCi) contained within the check valve assembly was automatically mixed with 1-liter samples, providing specific activities of 636, 137, 365, and 307 $dpm \cdot \mu mol^{-1}$ for experiments A–D; dissolved inorganic carbon concentrations were 1.64, 1.70, 1.70, and 1.64 mM. Subsamples were fixed with aqueous sulfuric acid to a final concentration of 0.35 N within the subsampling syringes.

In the laboratory, 10–25-ml aliquots of the acid-fixed subsamples were filtered through Whatman GF/F glass-fiber filters as described by Cuhel et al. (1981). The filters were rinsed twice with 2 ml of 0.35 N H_2SO_4 and counted in 3 ml of Aquasol. Filtrates were collected, sparged with air ($200 ml \cdot min^{-1}$) for 10 min to remove $^{14}CO_2$, and 3 ml counted in a scintillation cocktail consisting of 11 ml of Aquasol and 4 ml of water.

In specified instances the filters were stored at −20°C, and within 4 days fractionated (procedures of Roberts et al. 1963) to provide a measure of the incorporation of carbon into hot TCA-insoluble protein. Recovery of label as the sum of the obtained fractions was 90–99% of that originally present on the filters.

Light intensity (visible and IR) was measured continuously via a probe from a radiometer attached to the SID at the exact level of the incubation chamber.

Symbols: ○—incorporation of radiocarbon into sulfuric-acid-precipitable cell polymers; ×—radiocarbon contained in filtrate (acid-releasable low molecular weight cytoplasmic pools and excretion products); ●—sum of radiocarbon contained in acid-precipitable polymers and filtrates; □—hot TCA-insoluble protein; ▲—total carbon fixed in 1-liter dark control samples incubated on shore at in situ temperature (at the laboratory of J. C. Goldman). Except where shown, analytical error of duplicate measurements was within confines of the symbols. Data in panels C and D are presented as results of single analyses. Last point in experiment D showing carbon incorporation into protein (dashed line) is probably in error and is not representative of sample response.

protein, as compared to the low levels (5 ± 1%) in the slowly growing fall population. Phytoplankton production in fall was thus disproportionately weighted toward the synthesis of polymers other than protein (e.g. carbohydrate and lipid).

Phytoplankton are intimately coupled to their physical environment, which they both respond to and affect through their metabolism. Thus it is at the level of physiology and metabolism that the most revealing information concerning the interaction of phytoplankton with their environment will be acquired. The in situ sample procurement and time-course incubation capabilities of the SID, coupled with biochemical techniques of subcellular fractionation can provide a high resolution quantitative description of total carbon uptake and a detailed analysis of the metabolic patterns underlying fixation of inorganic carbon into cellular organic matter.

Although we have used measurements of phytoplankton production to illustrate the functioning of the SID, the instrument is universal in its application and can be used for the quantitative investigation of many other microbial rate processes in the aquatic environment.

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