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A simple and rapid device for measuring planktonic primary production by *in situ* sampling, and ^{14}C injection and incubation

YVES DANDONNEAU* and AUBERT LE BOUTEILLER†

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Abstract—A system (LET GO) is described that enables measurements of primary production at sea after *in situ* incubations, with ^{14}C being injected at depth immediately after enclosure of the sample. Each incubation cell, about 200 ml, is made of two transparent plexiglass cups facing each other. Mechanical energy to operate the system is provided by the tension of the nylon line between the drifting buoy, which holds the experimental equipment, and a weight at the bottom: when the line is strained, the two cups enclose the water sample, and the ^{14}C is delivered by a syringe. Absence of metallic or rubber parts ensures that toxicity effects are minimized and that reliable results can be expected. Furthermore, *in situ* incubations can start 1 or 2 min after arrival on station, leaving the research vessel and winches available for other tasks. These points make it possible to make *in situ* ^{14}C incubations during most oceanographic cruises and to increase greatly the acquisition rate of primary production data. The LET GO device has been tested in parallel with the conventional technique. Both techniques showed similar vertical patterns. Carbon fixation measured with the LET GO, however, was greater by a factor 1.3.

INTRODUCTION

WHEN KOBLENTZ-MISHKE (1965) published her first large-scale analysis of primary productivity data, obtained by the ^{14}C technique, data from 3000 stations in the Pacific had already been collected. Such a large data set resulted from a considerable international effort following the description of the ^{14}C technique by STEEMANN-NIELSEN (1952). Since then, many sources of errors reported in the ^{14}C technique have been overcome by additional precautions and improved equipment; more time and experienced scientists were then needed to carry out the experiments at sea.

A long controversy was initiated when it was shown that direct ^{14}C measurements of primary production using the ^{14}C technique could account only for a part of the oxygen accumulation below the summer thermocline (SHULENBERGER and REID, 1981). Subsequently, the "clean" ^{14}C technique (FITZWATER *et al.*, 1982) was developed to provide realistic values of photosynthetic carbon fixation, but the technique is so sophisticated that few laboratories have the facilities to carry it out. Clearly, the "clean" method is suitable only when a few, precise results from field experiments are needed, while the capability to map photosynthesis over large areas has generally been left to satellite-borne sea color sensors. The ^{14}C technique thus has evolved in a direction opposite to that recommended

*ORSTOM/LODYC, Tour 14, 2ème étage, 4 Place Jussieu, 75252 Paris cedex 05, France.
†Centre ORSTOM, B.P. A 5, Nouméa, New Caledonia.

by MUNK and FARMER (1988) who believe that the density of biological sampling must be intensified to take advantage of large-scale physical oceanography programs, such as TOGA (Tropical Ocean–Global Atmosphere) or WOCE (World Ocean Circulation Experiment).

The device described here has been named LET GO. It does not enable a truly high density sampling, but it is simple enough to be operated during physical oceanography stations. It has been tested during cruise SURTROPAC 12 (a TOGA cruise carried out by the ORSTOM Center at Nouméa, New Caledonia). Additional time required at each station was no more than 20 minutes. This method has thus the potential to increase the density in space and time of measurements of marine photosynthesis. Furthermore it satisfies most of the requirements necessary to obtain a valid representation of photosynthetic carbon fixation.

AIMS AND CONSTRAINTS

In situ ^{14}C experiments usually require that seawater is first taken at the incubation depths, preferably using Go Flo bottles. Seawater is then transferred into glass or polycarbonate bottles, ^{14}C is added, the bottles fastened to a line, and the line deployed vertically at sea, suspended from a buoy. Because these operations require 1 h of ship-time, physical oceanographers are reluctant to take such measurements during their cruises. "Simulated *in situ*" experiments are therefore generally chosen, during which incubations are made onboard under artificial light and temperature conditions, which do not exactly reproduce the *in situ* environment. Another problem with water sampling is not fully understood: a sea-water sample which is taken near the bottom of the photic zone makes an up and down voyage before it comes back to its original depth for incubation. During this time, phytoplankton cells experience a more intense light, higher temperature and lower pressure, the effects of which are not well known. While oceanographers have conceived of an *in situ* system in which the ^{14}C is injected at sampling depth, only a few technical solutions have been proposed (BROUARDEL and RINCK, 1958; WATT, 1965; GUNDERSEN, 1973; TAYLOR *et al.*, 1983). These devices, however, have been experimental and not used routinely at sea.

In addition to minimizing the light, temperature and pressure stresses, a completely *in situ* technique should allow incubation immediately after the ship has stopped for a station. Our choice was guided by time constraints that enable sufficiently long incubations when the research vessel stops for stations (for instance, deep CTD stations). All the experimental steps (i.e sinking to the desired depth, water sample enclosure and ^{14}C injection) are operated from a single source of mechanical energy, provided by a weight at the bottom of the line. The resulting simplicity of this system facilitates its use at sea, and it renders it reliable and inexpensive. In addition (and perhaps the main advantage over previous systems), our device needs no winch, so that the vessel is free for other operations during incubation time.

DESCRIPTION

The principle of LET GO is based upon the deformation of a pentagon (ABCDE) in which the main nylon line makes two adjacent sides (AB and CD) with an angle at (B) (Fig. 1). When the line is stretched between the buoy at the surface and the weight at the

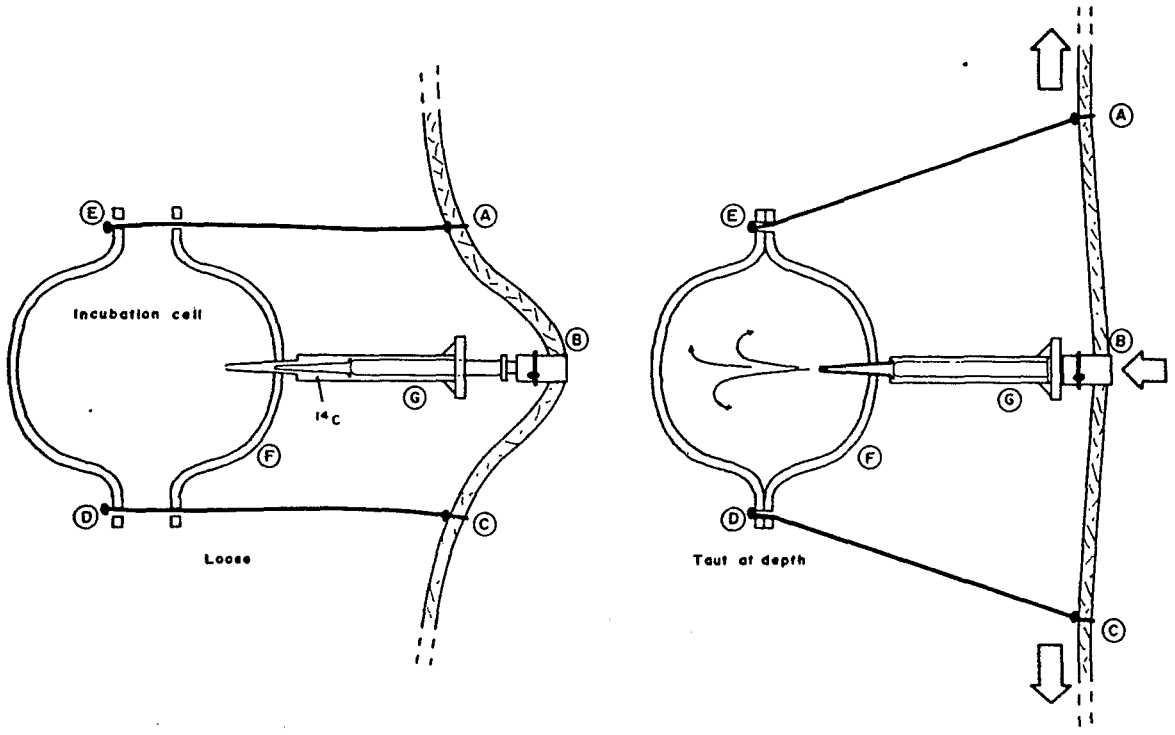

Fig. 1. Configuration of the LET GO incubation cells. Left: incubation cell prior to deployment. Right: when the line (ABC) gets taut, the pentagon ABCDE is deformed; the apex B pushes the cup F towards the opposite side DE, and operates the syringe G.

bottom, these two sides tend towards a straight line, and point B moves toward the opposite side of the pentagon (DE). This motion pushes forwards a rigid compound piece (F + G) which slides loosely along the other two sides (AE and CD). The front part (F) of this compound piece (a plexiglas cup with flat edges) is brought into contact with side DE of the pentagon (a symmetric cup) enclosing the water sample for incubation. The sample volume is about 200 ml. The rear part of the compound piece is a syringe (G) containing the ^{14}C, whose tip is fastened to the plexiglas cup (F), and whose piston is tied to the line, making the angle at point B; when the loosely sliding plexiglass cup is stopped by the fixed symmetric cup, the apex of angle (B) now pushes the tightly sliding piston, injecting the ^{14}C into the incubation cell.

The flat edges of the two cups face each other, and have been polished with sandpaper on a flat surface to tightly enclose the water sample. In fact, one can open the cells only if the tip of the syringe is pulled off, allowing air to enter. Free sliding of the cup (F) along the sides EA and CD of the pentagon ensures that the two cups are closed before the ^{14}C is injected into the incubation cell. The syringe, a 2.5 ml combitip of an Eppendorf multipipette dispenser, offers two advantages: its long and conic tip can be easily fastened into a drill hole at the top of the sliding plexiglass cup (F), and it contains no rubber part that could cause toxicity problems (WILLIAMS and ROBERTSON, 1989). The two sides AE and CD (Figs 1 and 2) are constructed of plastic cable ties commonly used by electricians. These cable ties are tightened to the line in A and C, and the free ends pass through the flat edges of the plexiglass cups; the running knots of other cable ties are threaded onto the free

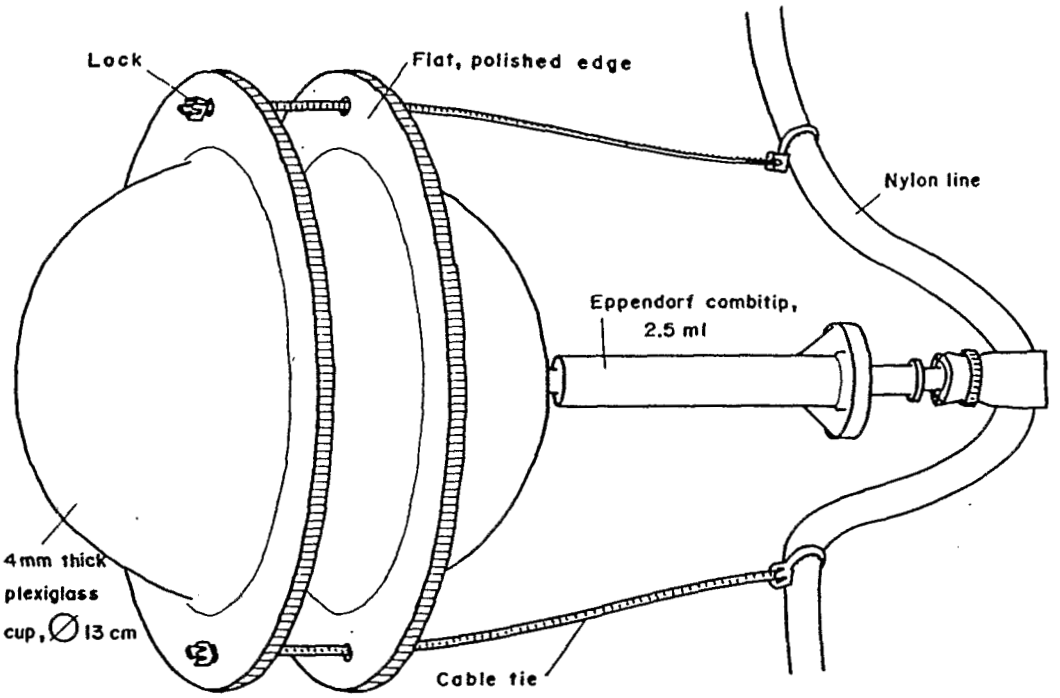

Fig. 2. Incubation cell. The plexiglass cups are formed with hot air blown over a mould at vacuum. The two locks (cut from other cable ties) are adjusted in such a way that the angle at apex B is about 170° when taut (see Fig. 1).

ends, and pushed forward to adjust points D and E so that points A, B and C make an obtuse angle (about 170°) when the system is in its final position.

Since the incubation cells are operated as soon as the line is strained, line must remain loose until the cells arrive at the pre-determined sampling depths. Line and incubation cells must be arranged loosely on a frame so that the whole system can be handled easily onboard and deployed at sea, and that the nylon line is paid as the frame is sinking. The line must not get entangled, and water must flush the incubation cells during sinking. These constraints are met in the following way:

(1) A 50-cm wide, weighted bucket holds and deploys the line and the incubation cells.

(2) The "depth" end of the line is tied to this bucket, and the line is laid down inside. At intervals of about 10 m, the line is made taut between two diametrically opposed points R_1 and R_2 (Fig. 3, left). This precaution prevents the line from floating and becoming tangled. The two points give way easily when pulled.

(3) The cells fastened to the line are hung outside the bucket, with the flat edges of the two cups inserted into two vertical parallel slots in the wall of the bucket, 3 cm apart; a cable tie fastened to the line 50 cm above each cell serves as a pin inside the bucket to attach the cells to the bucket (Fig. 3, right). Up to 12 cells (two rows of six) can be attached to the bucket.

(4) The "surface" end of the line is fastened to a buoy that holds the whole system during incubation.

(5) ^{14}C is sucked into the syringes which are then fastened to the cells hanging outside the bucket.

After these preparations have been carried out (≈20 min), the whole system can be deployed immediately on arrival at station. The bucket and the buoy are carefully dropped

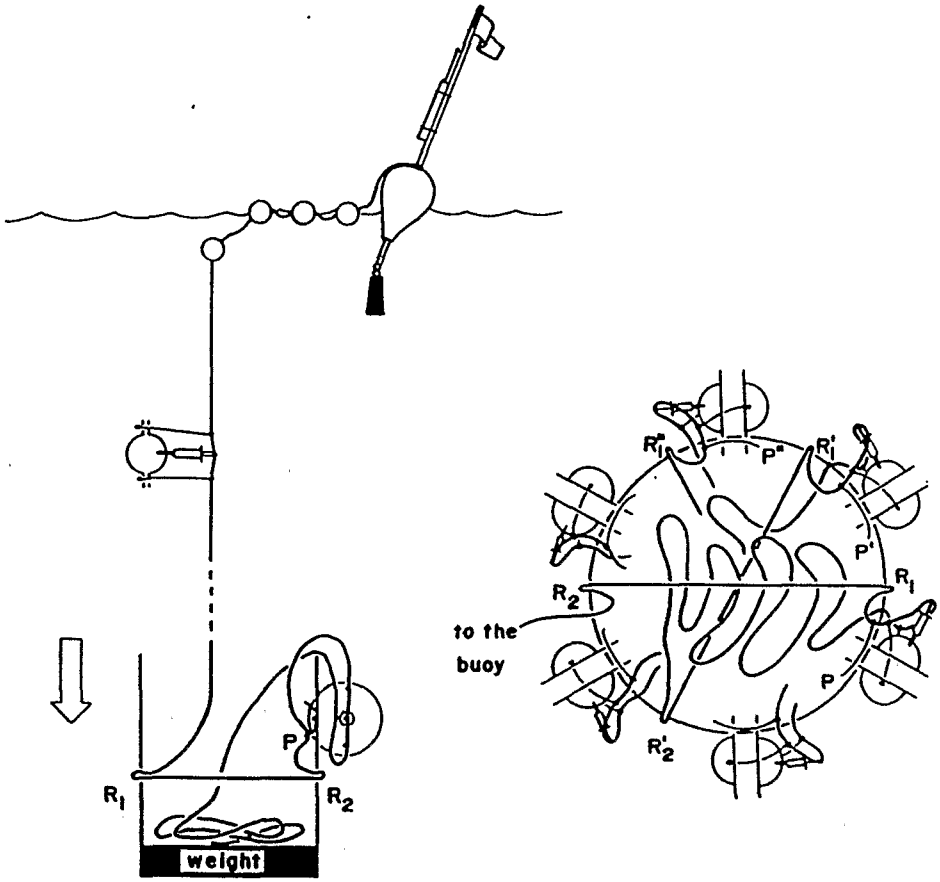

Fig. 3. Deployment of the LET GO setting at sea. Left: the line fastened to a buoy at the surface is delivered from the sinking bucket. R_1 and R_2 give way when the upper portion of the line becomes taut; the pin P is then pulled and the cell liberated. For sake of simplicity, only one cell is presented (the system has 12 cells). Right: top view of the bucket showing the fixation of the first row of cells through the wall of the bucket.

into the water. The buoy remains at the surface, while the weighted bucket begins to sink and pay out the line. During sinking, the cells in open position outside the bucket are flushed through by sea-water. For each incubation cell, the following operations are automatically repeated in sequence: (1) points R_1 and R_2 (Fig. 3, left) give way when the upper portion of the line becomes taut; (2) the pin (P) is pulled out, and the incubation cell is liberated from the bucket; (3) the line for the next incubation cell is paid out from the sinking bucket; (4) the tension of the line just before points R_1 and R_2 give way for the next cell is strong enough to close each cell and inject the ^{14}C.

SAMPLE TREATMENT AND ANALYSIS

When incubation is over, the buoy is recovered and the line is hauled onboard manually. The contents of incubation cells are poured into clean glass bottles. Prior to filtration, a small aliquot (0.5 ml) is pipetted from each bottle and transferred into vials containing 5 ml of liquid scintillation cocktail (Aquasol® or equivalent). The vials are immediately sealed and placed in a deep freezer. In such conditions, no loss of radioactive carbon has been found after 1 month storage. Another way to preserve inorganic ^{14}C in the liquid scintillation cocktail is to add phenethylamine (IVERSON *et al.*, 1976) or ethanolamine.

These 0.5 ml aliquots will be used later to estimate the amount of ^{14}C present into each cell during incubation. This amount cannot be pre-determined, as the volume of ^{14}C solution sucked into each syringe cannot be known with enough precision, and also as a small, variable fraction of the ^{14}C might be lost through the open tip of the syringe during deployment of the LET GO. Knowing V_i, the volume of incubation cell i (in ml) and Q_i, the number of counts per minute (cpm) given by the corresponding 0.5 ml aliquot, we obtain the amount of ^{14}C present in the cell during incubation (expressed as cpm):

$$Q_i = Q'_i \times V_i/0.5. \quad (1)$$

Incubated water samples are then filtered on Whatman GF/F glass fiber filters; at the end of the filtration, the filters are thoroughly rinsed with sea-water to remove any trace of dissolved ^{14}C. Filters are then stored in a deep freezer in vials for liquid scintillation measurements. Prior to counting, the filters are acidified and dried in an oven at 60°C. Scintillation cocktail is subsequently added, and radioactivity retained by the filters (q_i, in cpm) is counted. Carbon fixation for each cell i during the incubation is then:

$$P_i = C \times q_i/Q_i, \quad (2)$$

where Q_i is given by equation (1) and C is the concentration of inorganic carbon in sea-water, which can be measured, calculated or taken from the literature.

INITIAL RESULTS

There exists no method that can serve as an absolute reference to measure photosynthetic carbon fixation at sea. Thus the reliability of a new technique must be judged on whether its results account properly for the effects of light, chlorophyll concentration and nutrient concentration, among other factors.

The LET GO device was deployed successfully for the first time in July 1989. As expected, maximum ^{14}C uptake values were found near the surface, and decreased regularly down to 120 m depth (Fig. 4). Chlorophyll measurements made at the same

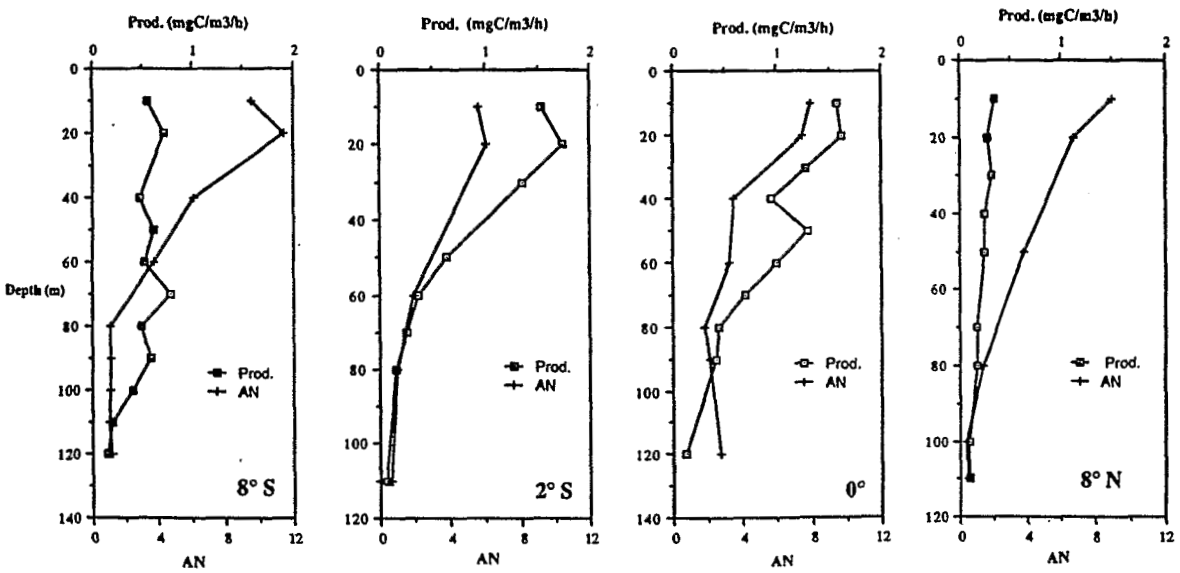

Fig. 4. First results obtained with the LET GO during cruise SURTROPAC 12 along 165°E. Squares: carbon fixation, in mg m^{-3} h^{-1}. Crosses: assimilation number, in mg C (mg Chl h)$^{-1}$, computed when chlorophyll measurements matched with the incubation depths.

stations (PROPPAC program) allow us to compute the assimilation number AN: carbon fixation per unit of chlorophyll per hour (EPPLEY, 1972). The profiles of AN (Fig. 4) decreased regularly from the surface ($5 < AN < 10$) to depth (AN was generally <1 below 100 m). The AN >2 below 100 m at the station at the equator might be caused by the combined effect of measurement error with low values of both chlorophyll and production. Photoinhibition at the surface at 8° and 2°S stations can explain low AN values at 10m: these stations were made on a bright day, whereas light was dimmed by clouds during stations at 8°N and at the equator.

Average AN values found in the equatorial Atlantic Ocean range from 14 mg C (mg Chl h)$^{-1}$ at 10 m to 1.5 at the bottom of the photic layer (LE BOUTEILLER and HERBLAND, 1984). In the North Pacific Central Gyre, LAWS *et al.* (1987) report AN values ranging from 9.5 at the light saturation level to less than 2 at the bottom of the photic layer. In the upper 50 m in the same area, MARRA and HEINEMANN (1987) estimate an average AN of 7.4. In the Costa Rica dome, KING (1986) observed AN = 7.5 between 100 and 25% incident light levels.

AN values obtained with the LET GO setting are thus very similar to those found by other authors dealing with tropical seas. Most encouraging is the vertical structure of both carbon fixation and AN, and especially the low values found at 120 m. These low values at depth, under dim light conditions, indicate that the high carbon fixation rate found at the upper levels must be attributed to photosynthesis, rather than to an artifact (e.g. insufficient rinsing), which also would have produced high values at depth. Horizontal structure (assuming that it can be described with only four stations) also agrees with the equatorial upwelling dynamics: maximum primary production at or near the equator (stations at 0° and 2°S), and very low production in waters with a deep and strongly stratified thermocline (the station at 8°N) confirm previous results obtained in this region (OUDOT and WAUTHY, 1976; BLANCHOT *et al.* 1989).

For another test, 15 March 1990, in the vicinity of New Caledonia (Fig. 5), the LET GO was deployed four times, at 6:00–9:00 h, 9:08–12:07 h, 12:15–15:00 h and 15:10–18:00 h.

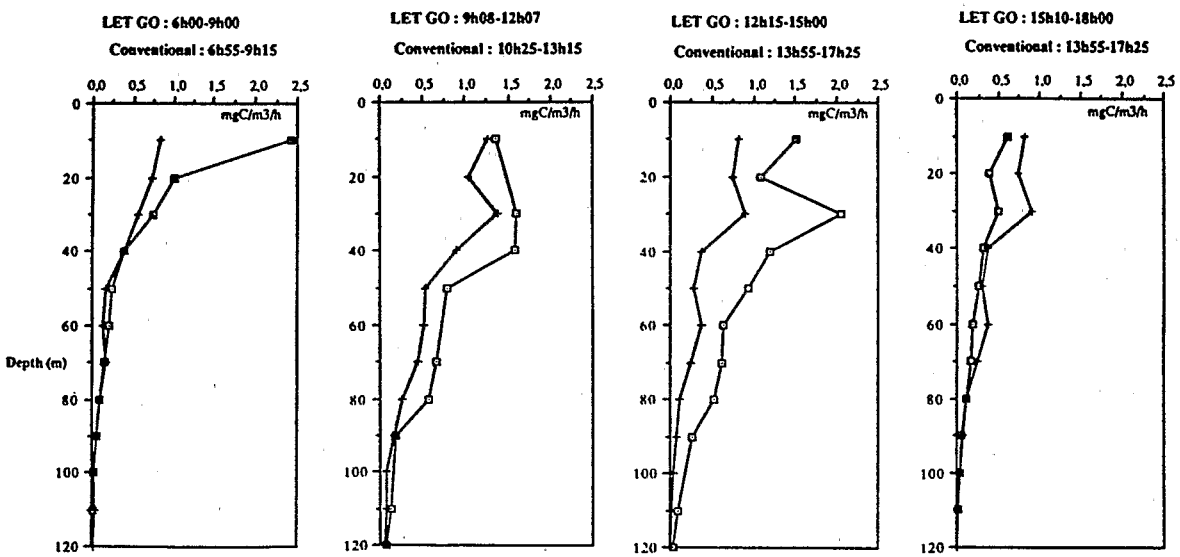

Fig. 5. Carbon fixation measured with the LET GO (squares) and with a conventional *in situ* technique (crosses) during a diurnal cycle (15 March 1990, 22°30'S, 165°13'E).

All deployments were successful, except for a few cells, generally at depth, in which the syringe broke. At the same time, we made three incubations using a more conventional *in situ* technique (sampled with Niskin bottles with new rubbers, clean incubation bottles, ^{14}C injected on deck) at 6:55–9:15 h, 10:25–13:15 h, and 13:55–17:25 h (Fig. 5). Both techniques gave vertical profiles of carbon fixation that show high values at the surface, a maximum at the depth of the the thermocline (30 m), and values that decrease to quasi-null values at 120 m. Shifts in time between deployment of the LET GO and the conventional *in situ* technique prevent a direct comparison of results. However, comparing the LET GO values with those from the conventional *in situ* incubations by linear interpolation shows that results from both techniques are tightly correlated ($r = 0.92$, $n = 36$). The regression slope indicates that LET GO values are greater than the conventional ones (Fig. 5) by a factor of 1.3. The high carbon fixation rates given by the LET GO, however, are plausible: summing the results from the four LET GO deployments gives a daily carbon fixation rate of 884 mg C m^{-2} day^{-1} which compares closely with the 777 mg C m^{-2} day^{-1} found by LAWS *et al.* (1990), at 26°N, in similar tropical conditions. Higher carbon fixation rates given by the LET GO may result from the absence of stress in this "completely *in situ*" technique.

CONCLUDING REMARKS

The LET GO incubation cells are made with transparent plexiglass, and the line, ties and syringes are made of nylon or plastic. There are no rubber or metallic parts, so that toxicity problems should be negligible. Furthermore, with the LET GO system, sampling and ^{14}C injection are made at depth, and incubation starts immediately after sampling, minimizing the stress due to abrupt changes of temperature, light or pressure, as well as intense microscale turbulence during transfer from the sampling bottle to the incubation bottle. Some additional work, however, is required: the amount of ^{14}C injected must be measured after each incubation, doubling the number of radioactivity measurements and corresponding expense; the arrangement of the line prior to deployment at sea is rather delicate, but one can easily prepare the line in 20 min after some training.

The LET GO offers the possibility to carry out *in situ* incubations of ^{14}C at sea and to obtain reliable results, with a minimum loss of ship time. Therefore, using this technique on physical oceanography cruises is quite practical, and it allows large-scale coverage on such international programs as JGOFS, WOCE, or TOGA. Even if one station *in situ* ^{14}C experiment per day is the maximum rate which can be expected during non-specialized cruises, the LET GO makes it possible to greatly increase the number of primary production measurements.

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