Bermuda Biological Station For Research, Inc.
Bermuda Atlantic Time-series Study

Chapter 18. Primary Production

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1.0 Scope and field of application

1.1 This procedure describes a method for the determination of primary production in seawater, expressed as mg C m^{-3} day^{-1}. The method is suitable for the assay of all levels of primary production found in the ocean.

2.0 Definition

2.1 Primary production is defined as the uptake of inorganic carbon into particulate matter as:

$$\text{Primary production} = \text{mg Carbon } m^{-3}\ day^{-1}$$

3.0 Principle of Analysis

3.1 The rate of carbon fixation by autotrophs in seawater is measured by tracing the uptake of radioactive ^{14}C from the inorganic form to the particulate organic form. Radiocarbon is added at an assumed ratio to the total inorganic carbon content of the seawater sample. The uptake of radiocarbon by the particulate phytoplankton is converted to total carbon uptake by the application of this radiocarbon:total carbon ratio. Inorganic carbon uptake is not measured because samples are acidified before analysis.

4.0 Apparatus

4.1 *Packard Tri-Carb 2000CA Liquid Scintillation Analyzer:* Samples in liquid scintillation cocktail are counted for 4 minutes using the following energy window settings:

Channel A: 0.0 - 156 keV

Channel B: 4.0 - 156 keV

An external gamma source is used to assess quenching of individual filter samples for conversion of counts per minute (CPMs) to disintegrations per minute (DPMs). The Packard analyzer uses a proprietary method to mathematically transform the raw Compton spectrum generated in the scintillation cocktail by the external source. This procedure minimizes distortions due to wall and volume dependent effects which can vary from sample to sample. The quench indicating parameter (Qip) is referred to as the transformed spectral index of the external standard (tSIE). The computer-aided liquid scintillation analyzer computes the DPMs during the counting process and provides both CPM and DPM information for each sample. Quenching of the total radioactivity in the vials is determined by an internal standard.

4.2 *Incubation Bottles:* Polycarbonate 0.25 l bottles are used for productivity incubations. New bottles are soaked for 72 hours in a 5% solution of Micro detergent. Bottles are then rinsed thoroughly with tap water, and subsequently soaked for 72 hours in the acid cleaning solution (5% HCL). The acid is discarded and the bottles rinsed 3 times with Milli-Q water and then soaked in Milli-Q for at least 48 hours. Once a new bottle has been cleaned as described above, then cleaning between cruises consists of soaking in the acid cleaning solution until approximately 6 hours before use. The bottles are then rinsed and filled with Milli-Q until use.

4.3 *250 µl Eppendorf Pipet and Tips*. Before use, tips are rinsed two times in acid cleaning solution and four times in Milli-Q water. Cleaned tips are stored in a polyethylene glove until use.

4.4 *GoFlo Bottles*: for trace-metal clean sampling. They are acid cleaned every three months.

5.0 Reagents

5.1 *Stock ^{14}C sodium bicarbonate* (aqueous, specific activity 2 mCi ml^{-1}): purchased from ICN Pharmaceuticals, Inc (Cat.# 17441H).

5.2 *Working Solution*: A sodium carbonate (anhydrous, Aldrich # 20, 442-0) solution is prepared by dissolving 0.15 g in 500 ml Milli-Q water in a clean 500ml Teflon bottle. A clean 100ml Teflon bottle for the working solution is rinsed and then filled and soaked with this carbonate solution for 6 hours. The ^{14}C stock is diluted in the100ml bottle (1.25 ml stock added to 60 ml of the carbonate solution), giving a final specific

activity of approximately 41 μC ml^{-1}. This working solution is stored refrigerated (5°C).

5.3 *Acid Cleaning Solution* (0.5 N HCl, Baker Instra-Analyzed): prepared using Milli- Q water.

5.4 *Ethanolamine (*Sigma): Used to prevent the radiolabeled inorganic CO_2 from escaping to the atmosphere.

5.5 *Scintillation Cocktail*: Aquasol (Packard Instruments)

5.6 *Preparation of Reagents:* Polyethylene gloves are worn during handling of materials which come into contact with isotope solutions. The 100 ml Teflon bottle to hold the working solution is cleaned as described below.

6.0 Sampling

6.1 *Shipboard sampling*

6.1.1 *Sampling Depths*. A set of 8 standard depths on 20 m intervals from 0 to 140 m are sampled (approximate light levels include 95% – 0.6%).

6.1.2 *Hydrocast*. Two hours before dawn, seawater samples are obtained using 12 l Go-Flo bottles deployed on a Kevlar line. The bottom weight on the line is wrapped in plastic. The line is lowered over a plastic polycarbonate-wrapped sheave, and bottles are triggered with brass messengers.

6.1.3 *Sampling*. Polyethylene gloves are worn during handling of samples. The productivity bottles are filled directly from the Go-Flos under low light conditions. Bottles are rinsed 3 times before filling. Five bottles are filled for each productivity measurement.

6.1.4 *Isotope Inoculation*. Under low light conditions, 250μl of the ^{14}C working solution (10.25 μC) is added to each bottle using a cleaned polypropylene pipet tip.

6.1.5 *Time Zero samples*. A 50 ml aliquot is taken from one bottle from each depth and immediately filtered, as in Section 7.1.

6.1.6 *Dark Bottle*. A dark bottle is made by wrapping one of the remaining 4 inoculated bottles in aluminum foil and placing it in a black cloth bag with a velcro closure.

6.1.7 *Total Radioactivity.* A 250µl aliquot for counting total added ^{14}C activity is removed from each of the time-zero bottles and placed in a 20 ml glass scintillation vial containing 250µl ethanolamine (Sigma). The mixture is held at room temperature until subsequent liquid scintillation analysis on shore. This procedure is repeated for an additional aliquot drawn randomly from one of the 3 light bottles from each depth at the end of the incubation period.

6.2 *In Situ Incubation Procedures*

6.2.1 *Preparation.* The dark bottle and 3 light bottles for each depth are hooked together with a combination of plastic electrical tie wraps and a length of bungi cord. These are kept in dark plastic bags until deployment.

6.2.2 *Deployment.* Approximately 1 hour before sunrise, the productivity array is deployed. The bottom weight, attached to a pre-measured polypropylene line, is lowered first. The bungi cords are then secured to hooks attached to the line at each marked depth. The entire productivity line is suspended from an orange plastic float, which is attached to a spar equipped with strobe flash and VHF radio beacon (Novatech). Time and position of deployment is recorded.

6.2.3 *Recovery.* Approximately 0.5 hours after sunset, the array is recovered. Sample bottles are detached from the line and placed in dark plastic bags until filtration. Time and position of recovery are recorded.

7.0 Procedures

7.1 *Sample analysis*

7.1.1 *Filtration.* Under low light conditions, a 50 ml aliquot is withdrawn from each productivity bottle using a 60 ml plastic syringe. This aliquot is filtered onto a 25 mm Whatman® GF/F filter maintaining vacuum levels of 70 mm Hg or less. Neither the filter nor the 50 ml syringe is rinsed. The filter is placed in a 20 ml glass scintillation vial, covered with 250 µl 0.5 N HCl, capped, and held at room temperature until subsequent processing on shore.

7.1.2 *Filter Processing.* At a shore laboratory, the productivity sample vials are uncapped in a fume hood, and allowed to dry overnight. This procedure ensures complete removal of unfixed inorganic ^{14}C. Ten ml of liquid scintillation cocktail are added to the dried filters and the samples are shaken vigorously.

7.1.3 *Total Radioactivity Sample.* Ten ml of liquid scintillation cocktail plus 2.5ml of Milli-Q water are added to the vials containing the 250 µl sample and 250 µl ethanolamine (see above). The mixture is shaken vigorously.

7.1.4 Samples are held at room temperature for 2 days before counting.

8.0 Calculation and expression of results

8.1 *Rate Calculations.* DPM values are converted to daily productivity rates using the following equation:

$$Production\ (mg\,C\,m^{-3}d^{-1}) = \left(\frac{SDPM}{V}\right)(W)\left(\frac{0.25\times10^{-6}}{TDPM}\right)(1.05)\left(\frac{1}{T}\right)$$

Where:

SDPM	=	DPMs of sample
V	=	volume of sample filtered in liters (usually 0.05 l)
TDPM	=	Total ^{14}C DPMs (250 µl)
W	=	25000 mg C m^{-3} (estimated mass of C in seawater)
T	=	Time in days

This calculation is made for each light bottle, and the triplicate values are averaged. A similar calculation is made for time zero and dark bottle samples. The dark bottle rate is subtracted from the mean rate for the light bottles to correct for non- photoautotrophic carbon fixation or adsorption.

8.2 *Integrated Water Column Production.* The individual depth measurements of daily production are used to calculate water column integrated production (mg C m^{-2} d^{-1}) by trapezoidal integration. The rate nearest the surface is assumed to be constant up to 0 m, and a zero rate is assumed for 200 m.

9.0 Quality Control

The measurement of primary production generally has no independent method for calibration. Intercomparison of techniques is also difficult without explicit activities on the same ship or same station. Data are generally evaluated for "reasonableness" in the

context of other core measurements. The coefficient of variation for replicate samples should be ≤ 10% (Richardson 1991).

10.0 Notes

Precaution should be taken to avoid exposure of productivity samples to high light. This is most important for samples collected from deep in the euphotic zone that are photo-adapted to very low light levels. Short-term exposure to high light can both enhance (provide more light for photosynthesis) or degrade (light shock) the photosynthetic performance of the phytoplankton.

Precautionary measures should also be taken to avoid even trace levels of contamination by metals.

11.0 References

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