5. Recommended Procedure for the Measurement of Primary Production Using the C^{14} Method

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It is assumed that the carbon-fourteen light-dark bottle technique for determining the rate of primary production is the only technique that need be considered as useful on ocean surveys. The conditions under which work must be done include daily measurements from a vessel which is: a) underway at about 15 knots; b) on hydrographic stations at various times of the day; and c) stopped for up to one hour at standard times of day for this and other biological work. Furthermore, it is recommended that the measurements made should be the absolute (as opposed to relative) rate of productivity of the populations sampled. It is in the light of these practical and certain technical considerations that the following particular methodology has been recommended at this time.

The following types of experiments have been recognized and the terminology presented below is used to avoid confusion:

A - "In situ" ---

1. Water samples incubated at the depths from which they originally came.

2. Subsamples of a surface water sample incubated at the different depths.

B - Incubator ---

1. With ambient natural light (i.e., sunlight)
   a) Without light filters
   b) With spectrally neutral light filters
   c) With spectrally selective light filters

2. With constant light (i.e., fluorescent, incandescent, etc. light)
   a) Without light filters
   b) With spectrally neutral light filters
   c) With spectrally selective light filters

In the above list, it should be noted that the term "simulated" does not appear. This term is used very differently by different people and its use here has been purposely avoided.

The manner in which sample depths are selected vary. These include:

A. Surface water only;
B. Water obtained at standard depths below the surface; and
C. Water obtained from depths with reference to the light intensity relative to that incident upon or penetrating the sea surface.

Finally the incubation periods utilized by different laboratory groups may be as short as two hours or as long as 24 hours, and incubation may be initiated at any time of day or at particular times of day.

These differences are the principal variations in technique employed at present and which affect the comparability of results. The purpose of this recommended procedure is to permit the obtaining of values which are comparable regardless of the laboratory group obtaining them.

It is not practical to rely upon the "in situ" method for routine measurement; though this method is believe to reflect the natural events best. Thus, an incubator yielding results most comparable to the "in situ" method should be used (see below). However, it is recommended that the incubator method (see below) and the "in situ" method be used simultaneously as often as possible with subsamples of the same water being used with both techniques.

Sample time should be after 10:00 and as shortly before local apparent noon as practical. Experiments (in situ and incubator) should run from noon to sunset.

Samplers and other equipment coming into contact with the water samples should be constructed of material which has been demonstrated to be non-toxic to phytoplankton and the photosynthetic process. A few plastics and most metallic materials are known to be toxic.

Sample depths. At least five optical depths (light percent depths) through the euphotic zone (i.e., above the depth to which about 1 percent of the blue-green light penetrating the sea surface reaches). The optical depths to be sampled should be determined at 480 (±30-40) mμ and corrected for surface reflection so as to match the light existing in the bottles during incubation with the intensities at the sample depths. The equipment and techniques of light measurements should conform to those given in the "Recommended Procedure for the measurement of solar radiation in the sea".

While sampling through the euphotic zone must be done as often as possible, this should not deter individuals from obtaining measurements from the surface waters. Such surface sampling is especially useful between standard sample times or between the stations where complete sampling is done. Such sampling is especially to be encouraged at dawn and at local apparent noon. If sampling can only be done from a ship underway, surface sampling should be employed; otherwise, it should only be used as a supplement to the complete sets of samples recommended above.

Water used should be subsampled from the same samples used to provide samples for other measurements, such as of pigments, inorganic substances, seston, and phytoplankton.

Sample bottles should be of pyrex or similar non-toxic glass and production determinations should be made with duplicate light bottles and a dark bottle at each depth sampled.

The incubator used for the determination of primary production should be exposed to ambient solar radiation and located on the vessel so as to minimize possible shading. The incubator should be cooled by circulating water (or other means) and the samples maintained at sea surface temperature.

The reduction of ambient flux in the bottles should be controlled by the use of spectrally neutral screens (nylon netting, metal screens, etc.). The transmission of the screens should be determined carefully and the sample depths chosen to correspond to these transparencies (see Sample Depths, page 2).

Carbon-fourteen bearing solutions must be kept sterile (e.g., in ampoules autoclaved after sealing).

Membrane filters (or equivalent) which retain the phytoplankton materials on their surfaces should be used for the planchets. The membrane filters and the pressures to be used should conform to that specified in the "Recommended Procedure for the Measurement of Phytoplankton Pigments". The planchets should be dried and stored in desiccators over silica gel. Sometime prior to radioactive assay the membrane filters (i.e. planchets) should be fumed over concentrated HCl and again redried in a desiccator over silica gel.

Observations reported should include the raw data and include: total count for each bottle, minutes counted (these two permit estimation of such correction factors as coincidence and counting error as well as counting rate), background count per minute, counts per minute of carbon-fourteen added, sampling time, "time in" and "time out", depth of sample both in percent of light and in meters and the method employed to calculate total CO_2 in the samples. Location and other identification data must be reported such as latitude and longitude, date, cruise, station and scientist responsible.

Recommendations for Additional Studies

1. It is recommended that intercalibration meetings be planned and carried out whereby the above procedure can be used in a parallel fashion by different investigators with sufficient replication to gain insight into the magnitude at differences encountered using a single technique. In addition, the effect of any possible changes in the recommended procedure should be examined before being adopted.

2. Additional attention must be given to the selection of what measurements should be made with reference to the different types of sampling opportunity.

3. Additional attention must be given to apparatus cleaning and radiation safety requirements.

4. Adequate standard calibration methods for measuring light in incubators and for determining the transmission of neutral and selective filters should be developed and specified.