**CMORE summer course in Microbial Oceanography-2012**

**Protocol based on JGOFS Core Measurement Protocol Manual**

**Measuring primary production and photosynthesis in aquatic ecosystems**

Goal: Learn two major techniques (primary production, photosynthesis-irradiance curves) for measuring the primary production and photosynthetic efficiency of marine phytoplankton communities. Apply these techniques to characterize how different size classes of phytoplankton contribute differentially to marine ecosystem functioning.

**Principle of Method**

 Seawater samples are inoculated with a known amount of radiolabeled 14C-bicarbonate. The autotrophic plankton assemblages assimilate this inorganic carbon substrate during the incubation period (usually 12 to 24 hours for primary production, and 1-2 hours for photosynthesis-irradiance relationships). At the end of the incubation period, plankton are concentrated onto filters and excess inorganic carbon is converted to CO2 via acidification of the samples. The amount of radiocarbon assimilated by autotrophic plankton is measured by liquid scintillation. Total carbon incorporated by the phytoplankton during the incubation is calculated using the ratio of radioactive to total inorganic carbon available.

Photosynthesis (as defined by the method employed here) is a measure of the rate that inorganic carbon is assimilated into particulate matter:

= mol C L-1 d-1

A vertical profile of photosynthetic rates can be depth-integrated to yield an areal measure of primary production:

=mmol C m-2 d-1

This provides an estimate of the daily rate of carbon fixation via photosynthesis during a day. Note that this method has been widely criticized due to various uncertainties, not least of which relate to whether the method measures net or gross production. For recent discussion of some of these uncertainties refer to Phytoplankton Productivity: Carbon Assimilation in Marine and Freshwater Ecosystems edited by Williams, Thomas, and Reynolds.

**Sample Collection**

 Samples will be subsampled from the CTD rosette into non-toxic, acid-cleaned polycarbonate bottles. During sample collection, sample vessels should be rinsed three times before the final fill.

**Methods:**

Acid Washing of sample bottles

Allow sample vessels to soak for 24 to 96 hours in 10% HCl (trace metal grade). After acid washing, bottles are rinsed six times with ultrapure ddH2O.

**Primary Production**

(1) Polyethylene gloves should be worn and trace metal clean techniques should be practiced whenever possible.

(2) Fill labeled sample containers (500 ml polycarbonate centrifuge bottles) to the rim with seawater. Cap bottles assuring no head space remains.

(3) In radiation control area, inoculate each sample with 250 l of NaH14CO3 stock solution (final activity ~0.1 Ci ml-1) being careful not to drip or otherwise spill any of this solution. Eject tip in 14C waste container.

(4) Hang samples on spreader bars and attach the spreader bars to the *in situ* primary production array.

(5) At the end of the incubation (usually ~12-15 hours; the full daylight period), retrieve samples from the *in situ* array and place them into a darkened cooler. In the radiation control area, subsample 250 l of well-mixed 14C-inoculated seawater from each bottle and place the aliquot into a 20 ml scintillation vial containing 500 l -phenylethlyamine. Add 10 ml scintillation cocktail. These samples are termed “SA” for specific activity-they will be used to determine how much 14C has been added to each sample.

(6) Filter remaining sample onto 25 mm 0.2 m, 2 m, and 10 m polycarbonate or glass fiber filters (Whatman GFF) using low vacuum (<200 mmHg

 (7) Place filters into 20 ml scintillation vial, and in a flow hood using a repeater pipette add ~500 l 1N HCl and leave uncapped in the hood to vent for 24 hours.

(8) After 24 hours, add ~10 ml scintillation cocktail, cap tightly, shake well and count DPM on the liquid scintillation counter. When finished, remove the vials and place in the radioactive disposal drum.

Calculate Primary Production using the following equations:

Photosynthesis (mol C L-1 d-1) =

((DPMsample/V)/(DPMSA/VSA) / (DIC concentration in sample))\*(1.06/T)

where

DPMsample = DPM of filtered sample

V = volume of filtered sample (usually 20 ml)

VSA = volume of specific activity sample (usually 0.25 ml)

DPMSA = DPM of specific activity sample

1.06 = correction for lower uptake of 14C relative to 12C

T = time (usually in days)

Oceanic concentrations of DIC are typically:

5 m – 2004 mol C L-1

25 m – 2005 mol C L-1

45 m – 2001 mol C L-1

75 m – 2009 mol C L-1

100 m – 2017 mol C L-1

125 m - 2035 mol C L-1

Note that DPM (disintegrations per minute) = CPM /counting efficiency

Where counting efficiency = CPM of measured sample/CPM of known material

 CPM = counts per minute (counts that are measured)

 DPM = disintegrations per minute

Recall that 1 Ci=2.22 x 1012 disintegrations minute (DPM)

**NOTES:**

Note that because we are using radioactive, carcinogenic, and toxic substances, it is imperative that you be safety minded including wearing gloves, lab coats, eye protection, and generally staying aware of the hazards of the substances that are being used.

Carbon-14 is an unstable isotope of carbon-12 which decays through the emission of a  particle. It is this decay that allows us to quantify carbon uptake by phytoplankton. In terms of radiation,  particles are fairly innocuous, being stopped by glass, plastics, and even human skin. In addition, the form of carbon that we are going to use, bicarbonate, is an inorganic form which is extremely unlikely to be incorporated in the human body if ingested. However, to be safe always wear gloves and lab coats while handling carbon-14.

 -phenylethylamine is an extremely caustic substance that is used to bind all available carbon so that it will not escape via volatilization. This should be used under the laboratory hood because of strong vapors. In addition, gloves and safety glasses should be worn when using it.

**Photosynthesis-Irradiance Procedures:**

**Principle of Method**

Seawater samples are inoculated with a known amount of radiolabeled 14C-bicarbonate. Samples are incubated at varying light intensities (typically ranging between ~1 and 1500 mol quanta m-2 s-1) and the assimilation of radiolabeled substrate under the varying light regime provides information on the photophysiological response of the autotrophic plankton assemblage. At the end of the incubation period, plankton biomass is concentrated onto filters and excess inorganic carbon is converted to CO2 via acidification of the samples. The amount of radiocarbon assimilated by autotrophic plankton is measured by liquid scintillation. Total carbon incorporated by the phytoplankton during the incubation is calculated using the ratio of radioactive to total inorganic carbon available. A photosynthesis-irradiance (P-E) response curve is constructed and various photophysiological parameters derived.

**Methods:**

**Acid Wash**

Allow sample vessels to soak for 24 to 96 hours in 10% HCl (trace metal grade). After acid washing, sampling vessels are rinsed six times with ultrapure ddH2O.

**Photosynthesis-irradiance relationships:**

(1) Subsample seawater from the CTD rosette into a 1 liter, acid washed polycarbonate bottle. Rinse sampling bottle 3 times with seawater, then fill with ~600 ml of seawater. Place bottle in a cooler to maintain temperature and keep dark.

(2) In the radiation control area, reduce sample volume to 600 ml of seawater. Add 1.5 ml of 14C-bicarbonate stock solution (final activity ~0.1Ci ml-1). Swirl bottle lightly to assure solution is well mixed. Eject tip in 14C waste container.

(3) Using a bottle top dispenser set to 20 ml dispense 20 ml of 14C-labeled seawater into 24 acid-cleaned 20 ml glass scintillation vials.

(4) Fill one additional 20 ml scintillation vial with labeled seawater for determination of the specific activity. Remove 250 l of seawater from this vial and add to a prelabeled 20 ml scintillation vial containing 500 l of -phenylethylamine. Cap this vial and freeze for subsequent determination of specific activity.

(5) Dump remaining sample volume in the 1 liter bottle into the liquid radiation waste; be careful not to spill.

(6) Cap each of the scintillation vials containing the sample, and place each vial into the sample wells of the photosynthetron; each of the 24 wells has a unique light level. The photosynthetron is plumbed to a temperature-controlled water bath to maintain samples at in situ temperatures.

(7) Let incubate at various light levels in photosynthetrons for 2 hrs.

(8) At the end of the incubation (2 hours), filter the samples onto 25 mm polycarbonate or glass fiber filters using a low vacuum (<200 mmHg vacuum) and the filter manifold.

(9) Place the filters into prelabeled 20 ml scintillation vials, and in a flow hood using a repeater pipette add ~500l 1N HCl to each scintillation vial with filter and leave uncapped in the hood overnight.

(10) Measure light-levels (irradiance) of each well in the photosynthetrons using a light meter.

(11) The next day, add ~10 ml scintillation cocktail, cap tightly, shake well and count DPM on the liquid scintillation counter. When finished, remove the vials and place in the radioactive disposal drum.

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Photosynthesis (mol C L-1 d-1) =

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where

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DPMSA = DPM of specific activity sample

1.05 = correction for lower uptake of 14C relative to 12C

T = time (usually in days)

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Note that DPM (disintegrations per minute) =

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 CPM = counts per minute (counts that are measured)

 DPM = disintegrations per minute

Recall that 1 Ci=2.22 x 1012 disintegrations minute (DPM)

Calculations:

Plot photosynthesis versus irradiance (P-E).

Derive the following parameters of photosynthesis:

 = initial slope of the curve (light-utilization index)

Pmax = maximum value of the P-E curve (light saturated photosynthesis)

Ek = Pmax/ (light saturation index)

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