Simulated in situ (SIS) Experimental Protocol

Written: 10/94 mvernet; last updated 09/08/08 wkoz

SIS incubations are used to estimate photosynthesis as a function of depth in deck incubations. Although ideally samples should be incubated at the depth in which they are collected, logistic constraints may dictate on-deck incubations. In situ light is simulated by neutral density, nickel plated screens. Incubations last from 4 to 24 hours: during short incubations we estimate gross primary production (total inorganic carbon uptake) while 24 h incubations are closer to net photosynthesis (gross photosynthesis-respiration). Integrated photosynthesis is calculated for the euphotic zone from surface to the depth of 1% or 0.1% of incident radiation. Photosynthesis : chlorophyll *a* ratio (assimilation number) gives an estimate of photosynthetic efficiency which it is function of temperature, light adaptation and taxonomic composition.

I. Preparation of the incubator

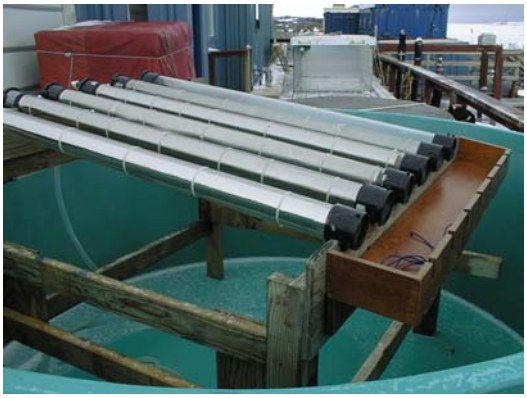
The SIS incubator consists of a series of Plexiglass tubes covered with nickel plated screens which simulate 100% (surface), 50%, 25%, 10%, 5%, and 1% of incident radiation (Io). As for all incubations, the time between sample collection and incubation should be minimized in order to avoid phytoplankton adapting to non in situ conditions before the experiment. Note that there are two different sets of tubes and incubators– one is UV transparent, and one is UV blocking. "Standard" BP-016-O incubations are done in the UV blocking tubes. The UV transparent tubes are used for separate experimental incubations.

A. *Maintenance*

Check that the running seawater is ready (if not, check with an MT), that water flows rapidly (enough that the water will not freeze in the tanks – if it's cold outside you will need to keep the water moving quickly!) through the tubes to be used, that the screens are clean, that the incubator is located away from shade, and that no major leaks are seen (to avoid formation of ice on deck).

B. *Calibration of Incubator Tubes*

The incubator has 6 tubes for 125 ml borosilicate bottles. Irradiance (PAR = Photosynthetic Available Radiation between 400 and 700 nm) can be measured introducing the Biospherical Instruments Inc. Model QSL-100 into the tubes in the location where experiments will be run. If possible introduce 2pi collector into borosilicate bottle (or the bottle in which experiments will be performed) and place the bottle inside appropriate tube. This minimizes variability. Record all calibration numbers in \lterXX\primpro\LTERcalibs.xls



II. Prior to sampling

A. Set out 30 vials for each experiment

1. 1 specific activity (SA)
2. 1 time zero (t_o)
3. 2 light treatments (L1 and L2)
4. 1 dark (D) for each of the six light levels sampled

B. Record vial numbers in Vial Log

C. Be sure that you have 24 clean SIS bottles for each experiment

1. 1 time zero
2. 2 light treatments
3. 1 dark for each of the six light levels sampled

D. Mix up standard stock dilution

1. Using P5000, pipet 2.3 ml (2300µl) DI water into a clean, un-numbered glass 20ml scint vial.
2. Using P200, pipet 0.120 ml (120µl) concentrated ^{14}C stock into water vial.
3. Swirl to mix, and store in refrigerator until used.

** These volumes / concentrations may change based number of station per day you're processing and/or on expected response!! See Karie or Wendy to be sure!

III. With sample water

A. Fill SIS bottles

1. Start with 100% sample – mix gently and rinse 100ml graduated cylinder with approx 10-20ml sample water. Continue with that water to rinse the four SIS bottles for that light level
2. Dispose of rinse water as Radioactive Waste
3. Pour 100.0ml sample water into each of the four SIS bottles for that light level
4. Continue filling all light levels following pattern of 1 – 3.

B. Spike Samples with ^{14}C

1. Using a P-200 Pipet, dispense 100µl (0.005mCi [5µCi]) into each bottle (5µCi x 24 bottles = 120µCi per experiment)
2. Cap each bottle tightly, mix gently

C. Load Incubator

1. Put each of the Dark bottles in the Rad Refrigerator
2. Put each of the two light treatment bottles in the appropriate tubes, closing each securely with screening and a cable tie
3. Record time and any comments on Log Sheets

D. Treat Specific Activities and Time Zeros

1. In each SA vial, pipet 0.100ml (100µl) 1N NaOH – set in separate vial rack in hood (** this can also be done in advance, before filling sample bottles)
2. Load six places in the filtration manifold with GF/F filters
3. With NO vacuum running, pour each to bottle into separate funnel

4. With P-200 Pipet, draw 0.100ml (100µl) from each cup and dispense into appropriate to vial (that already has NaOH in it) for your Specific Activity
5. Swirl to mix and dispense 5.0ml Universol to each
6. Cap, shake well, and set aside
7. Turn on vacuum and filter all remaining sample water – rinse walls of funnel cups with filtered seawater – shut off valves as filters run dry
8. Place each filter in respective t_o vial
9. Acidify filter with 0.200ml (200µl) 20% HCl, place in hood to blow off excess inorganic ^{14}C for a minimum of 12 hours

IV. After 24 hours

A. Remove samples from incubator (taking care to keep them in correct order) and Darks from refrigerator

B. Record time Log Sheets

C. Filter all samples

1. With GF/F filters in place, filter all 100ml of each light treatment bottle and each dark bottle from each light level

** this means three times resetting the manifold (three filters each for six light levels = 18 filters **

2. Place each filter in appropriate vial and when all depths are done, acidify all filters (as done in step II D) and set in hood to blow off excess inorganic ^{14}C

V. After a minimum of 12 hours acidification

A. Add 5.0 ml Universol to each vial, cap, shake well and put in correct order in LSC to count

B. Be sure all information is recorded in SIS Logs

VI. Estimation of photosynthesis [to be expanded upon at some future date – wendy]

Load counts, efficiency and vial numbers into EXCEL, \lter06\primpro\sis\06sispal.xls (for Palmer or \lter07\primpro\sis\07siscr.xls (for Cruise). These file will contain one worksheet for each sample day (may have two to five experiments per worksheet). Copy or enter numbers from the LSC.

Data to enter:

Processing date
Sampling date, Station number, depths
Start and finish of incubation (total hours)
Integrated irradiance
% Light: will be there according to screens
Irradiance = Integrated light for the time of incubation * % Light
DPM counts
Volume filtration for Particulate C
Volume Specific Activity
Volume sampled for Total C

Data to be calculated:

Photosynthesis PC/ volume / time
Photosynthesis PC/ chlorophyll *a/time*
Photosynthesis TC / vol / time
Integrated photosynthesis from surface to 1% surface irradiance (euphotic zone)

$$\text{mgPC/m}^3\text{/d} = \frac{\text{(DPM PC light/vol PC light-DPM PC dark/vol PC dark)}}{\text{(DPM Sp.Act./vol Sp Act)}} * 24000*1.05*(24/\text{h act inc})$$

$$\text{mgTC/m}^3\text{/d} = \frac{\text{(DPM TC light/vol TC light - DPM TC dark/vol TC dark)}}{\text{(DPM Sp. Act./vol Sp Act)}} * 24000*1.05*(24/\text{h act inc})$$

Where:

volumes are in ml
24000 is mg HCO_3 m^3 in seawater
1.05 is to account for $^{14}C/^{12}C$ uptake correction

Save file with name of Julian date.
Copy results of profile and integrated production into summary data file.
Be sure to fill out completely and then file SIS Log Form.