

# Photosynthesis vs Irradiance (PI) Experimental Protocol

Written 01 Oct 1999 wkoz; updated 09/08 wkoz

PI incubations are used to estimate Photosynthesis as a function of Irradiance (light intensity). The response curve is hyperbolic and from this curve we estimate three key parameters:  $\alpha$  (alpha) or light-limited photosynthesis,  $P_{max}$  or photosynthesis at light saturation, and  $I_k$ , the irradiance level where the sample reaches light saturation. From these three parameters we can estimate photosynthesis at depth, model integrated photosynthesis for the euphotic zone (the illuminated surface layer of the ocean), establish light adaptation for a given sample and compare photosynthetic efficiency for any type of phytoplankton.

## I. Preparation of the incubator

The PI incubator needs to be ready before collecting any sample as the time between sample collection and incubation should be minimized in order to avoid phytoplankton adapting to non in situ conditions before the experiment.

A. *Maintenance*: Incubator should be cleaned, the irradiance calibrated for each position, the positions labeled, light checked, and water should be circulating at in situ temperature. This procedure should be done at the beginning of a cruise or after the incubator has been moved. It should be repeated as necessary. In a cruise or between experiments leave the water running. Turn on the lights at least 1/2 hour before experiment.

*Cleaning*: To clean the incubator, take black lids off. Take the frame to hold the lids, also off. Pick up carefully the metal block which holds the hoses, and pick up the Plexiglas tray. In between the tray and the metal holder there is a gray PVC sheet with 50 holes which contain the filters to control the irradiance levels. If dirty, pick up PVC sheet (be careful because filters will come loose and fall off) and filters and wash as necessary. Every part should be rinsed with DI water and dried before placing it back. Wipe all surfaces and place parts back, in reverse order. After this procedure, calibrate incubator before next experiment.

*Change of lamps*: If a lamp burns up and needs replacement, disassemble incubator as explained in previous section. Once incubator is open, carefully remove the Plexiglas placed in front of lamps which is filled with blue water. Total burn times on the lamps should be recorded.

B. *Calibration of Incubator*: The incubator has 25 positions for 7 ml scintillation vials. Three of those positions should be used as blanks and kept with a black filter. The other 22 positions should have an approximate range of irradiances from 1 to 400 micromol Quanta  $m^{-2}s^{-1}$  (same as microEinstein  $m^{-2}s^{-1}$ ). These irradiances are reached by 2 means: distance of the position from the light source and the addition of filters, as needed. About 1/2 of the irradiances should be between 1 and 30 micromol  $Q m^{-2} s^{-1}$ , another 25% between 30 and 100 micromol  $Q m^{-2} s^{-1}$ , and the rest between 100 and 400 (to 600) micromol  $Q m^{-2} s^{-1}$ .

To measure irradiance use a cosine PAR collector, from 400 to 700 nm. For the PI Incubators, use a Biospherical Instruments QSL-101 (same as the QSL-100, but with a

smallest size sphere that fits into the 7ml scintillation vials). Using a vial that has the top broken off it, put 2.0ml water in a vial and with a stopper slipped around the wand of the QSL to keep the sensor depth consistent and stray light out, measure irradiance for each position in the incubator and repeat at least once, preferably twice. Darken room as much as possible or measure at night, to decrease stray light. Once finished, plot irradiances to make sure light range and distribution of light treatments are correct. If necessary, change filters to obtain desired irradiances and recalibrate. Store all calibration measurements in \\\ter99\primpro\pi\picalib.xls (see spreadsheet for how to enter / plot data). \*\* Note that pictures below are “old” incubator style – incubators updated during winter 2001.



## II. *Supplies needed* (most in $^{14}\text{C}$ lab and labeled with ‘radioactive’ tape)

- $^{14}\text{C}$  stock as bicarbonate (stored in rad refrigerator in lab 4)
- Eppendorf repeater pipette with 25 or 50 ml tips
- Labeled, dark bottle to store 60 ml sample (labeled and stored in lab 10)
- P-200 Pipeteman with tip, set at 0.1 ml (for NaOH)
- P-200 Pipeteman with tip, set at 0.1 ml (for Sp. Act.)
- P-100 Pipeteman set for dispensing of  $^{14}\text{C}$  stock (volume will vary with stock)
- Dispensette with 20% HCl set at 0.2 ml
- Gloves and Paper towels
- 27 x 7-ml glass scintillation vials labeled sequentially with number tape  
(1-25 for treatments and 2 for  $t_0$ )
- 2 x 7-ml glass scintillation vial with 0.1 ml of NaOH (keep closed) for the Specific Activity.
- Holder for 7 ml scintillation vials
- Universol ES with dispensette set at 5 ml
- Photosynthetron with water bath circulating at in situ temperature

## III. *Before sampling*

- A. Set out 29 vials for each experiment to be done
  1. 2 Specific Activities (SA)
  2. 25 Samples
  3. 2 Time Zeros ( $t_0$ )
- B. Record vial numbers in vial log notebook

- C. Record information about the station and vials in PI log notebook
- D. Immediately upon return from sampling turn on incubators and water baths to allow to equilibrate (they need at least ½ hour to get to temperature)

#### IV. After samples have been collected

- A. Gently mix sample water that has been kept refrigerated until use
- B. Rinse 100ml graduated cylinder and 100ml PI beaker with approx. 10 ml sample water
- C. Dispose of rinse water in Rad Waste
- D. Measure exactly 60.0 ml sample into PI beaker
- E. Pipette 0.060 ml (or 60 µl)\* <sup>14</sup>C concentrated stock into beaker using the P-100
  - \* Want final concentration of <sup>14</sup>C in sample water to be 2µCi/ml or 120µCi for the entire 60ml sample
- F. Rinse pipette twice with sample water – dispose of tip in sharps waste
- G. Swirl to mix sample water and <sup>14</sup>C
- H. Load Sample Vials
  - 1. Using Eppendorf Re-Pipettor and 25ml tip, pipette 2.0 ml (Eppendorf on “4”) into each of the 25 sample vials and the 2 t<sub>0</sub> vials
    - a. Take care to avoid bubbles in the tip when loading Eppendorf – dispense first 2.0ml back into beaker.
    - b. Empty excess sample water back to beaker for use in SA’s
    - c. Can also use 50ml Eppendorf; set pipettor to “2” to dispense 2.0ml
  - 2. Dispose of tip in sharps container
  - 3. Cap each of the 25 sample vials, place rack in carrying tray and put vials in correct incubator in aquarium (or rad van), being sure to put them in the correct order (start at top left with vial #1, next row down starts vial #6 etc)
  - 4. Be sure to note Incubator Number, actual Water Bath Temperature and Incubation Start Time on PI Log Sheet

#### V. Treat Time Zero Vials

- A. In hood, add 0.200ml (200µl) 20% HCl to each of the 2 t<sub>0</sub> vials (use 100 – 500µl dispenser if available)
- B. Swirl to shake and place in rack in hood to offgas for a minimum of 12 hours

#### VI. Treat Specific Activity Vials

- A. In the hood, using P-200, dispense 0.100ml (100µl) 1M NaOH into each of the 2 SA vials
- B. With fresh tip on the P-200, dispense 0.100ml (100µl) sample water into each of the 2 SA vials
- C. Swirl to mix; Add 5.0ml Universol Cocktail, cap and shake well.
- D. Set 2 SA vials in rack to wait for sample vials

#### VII. After 2 hours incubation time

- A. Record total incubation time
- B. Pull 25 sample vials from incubator and carry back to rad lab / van, being careful to maintain correct order of the vials
- C. Uncap, and dispense 0.200ml (200µl) 20% HCl to each vials

D. Allow vials to sit in hood at least 24 hours to offgas remaining inorganic  $^{14}\text{C}$

VIII. **After at least 12 hours offgas time**

- A. Re-Cap all vials, place in LSC racks in correct order \* and read on LSC for 300s  
\* 2 SAs, 25 Samples, 2  $t_0$ s
- B. Be sure ALL information is filled out in PI Log Sheet in Notebook

IX. **Estimation of photosynthetic parameters**

- A. Put PI data into 9900pi.xls
- B. Copy 1L or 1R template sheet onto another blank sheet in 9900pi.xls workbook.
  - 1. If you need to add more blank sheets, goto *insert, worksheet*.
- C. Name new worksheet (double click on sheet tab) by julian day and station/event number.
- D. Correct column one as necessary.
- E. Add julian date and station name to box C3.
- F. Add actual incubation time to box E2.
- G. Check that sample volume and specific activity volumes are correct (E3 and E4)
- H. From LSC file, copy and paste DPM counts to column D, rows 9 - 37, being sure SAs are in rows 9 and 10 and time zeros are in rows 36 and 37.
- I. Add LSC filename into C5.
- J. If chl data available, add chla concentration to E5.\*\*\*see note below
- K. Make rough plot to find outliers that might need to be deleted
  - 1. highlight the two columns titled "Irrad" and "Photos" and run the PIPlot macro (ctrl-e)
    - a. note on macros: be sure that no other excel sheets with this same shortcut are open – you will get an error if there are.
  - 2. be sure to add correct title to plot
  - 3. if you get an error, one thing to check is within the VB editor, check the macro to be sure that you're activating the correct worksheet (at the end)
- L. If there are major outlying points, note them for later changing the text file

\*\* Notes on spreadsheets written for 1999-2000 season; change years as needed for data

X. **Save 9900pi.xls (now with new sheets for each new station added).**

- A. location: lter99\primpro\9900pi\9900pi.xls
  - 1. in 9899 these files became so large that we ended up breaking them up by month – use your discretion for filenames, but remember that matlab can only handle eight characters – you might limit filename length.

XI. For each new station added, **save data** as text file:

- A. select cells A1 through H35.
- B. right mouse, copy
- C. *File, New, Workbook*
- D. select cell A1
- E. right mouse, paste special, values
- F. if there were outliers, change the photos column in those outliers to the number "-999"

F. save as: lter99\primpro\pi\matlab99\juliandatestation].txt (select file type text, don't just type ".txt") (okay that it saves only active sheet)

XII. Close **all** copies of **text** files within Excel

XIII. Goto **Matlab** - calculate curve, Pcalc, alpha, and beta

A. Start Matlab (either through run command or startup menu or explorer)

B. Get into matlab99 directory:

1. type cd \

2. type cd lter99\primpro\pi9900\matlab99

C. Type "piall"

C. Enter text file just created (be sure to include .txt extension)

D. Matlab will create a chart with three different plots of the curve data

1. once done, from figure box, select *Edit, Copy Options* and be sure that Metafile is selected, then *Copy*

E. *Paste* this figure into the excel workseet below the scatter plot of the data

IXX. For each of the different curve fits, either *copy* and *paste* Pcalc, Alpha and Beta, or type them in manually

XX Save again 9900pi.xls

XXI Paste Special - LINK Pcalc, alpha, beta, Ik and Pmax (as calculated by Excel) values to lter99\primpro\pi\9900summ.xls

XXII Save 9900summ.xls

XXIII Be sure to link DPM counts to radlog99.xls for vial activities.

\* Note this not applicable if using RadTrack – you won't be able to link, but will have to type in vial activities \*

\*\*\*\* Most times chl data will have to be added at a later date for calculation of photosynthesis per unit chlorophyll - be sure to note those stations where chl has been accounted for in the notebook, so we don't go back and do this twice.

IXXX. How the spreadsheet is calculating photosynthesis:

$$\frac{\mu\text{g Carbon}}{\text{liter} * \text{hour}} = \frac{\text{DPM Light} - \text{DPM to}}{\text{DPM Sp. Act.} * 20} * 24,000 * 1.05 * \frac{1}{\text{act inc. time (h)}}$$

where:

- DPM Light and Dark measured on 1 ml of sample
- Sp. Act. was measured on 0.1 ml
- actual time of incubation is corrected to one hour
- 1.05 is to account for <sup>14</sup>C/<sup>12</sup>C uptake correction
- 24,000 mg HCO<sub>3</sub> m<sup>3</sup> in seawater
- 20 is to account for the difference in volume between Light and Sp.Act.

For P/chl divide μgC l-1 h-1 by chlorophyll concentration in μg l-1.