

# Coral respiration/photosynthesis protocol

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At the Bermudian Mesocosms Facility

3 PreSens Oxy-4 with 4 temperature and oxygen probes each

2 lamps

2 spinning tables for 6 incubation chambers from AIMS

2 battery chargers

12 620mL incubation chambers from AIMS

12 magnetic stirring bars

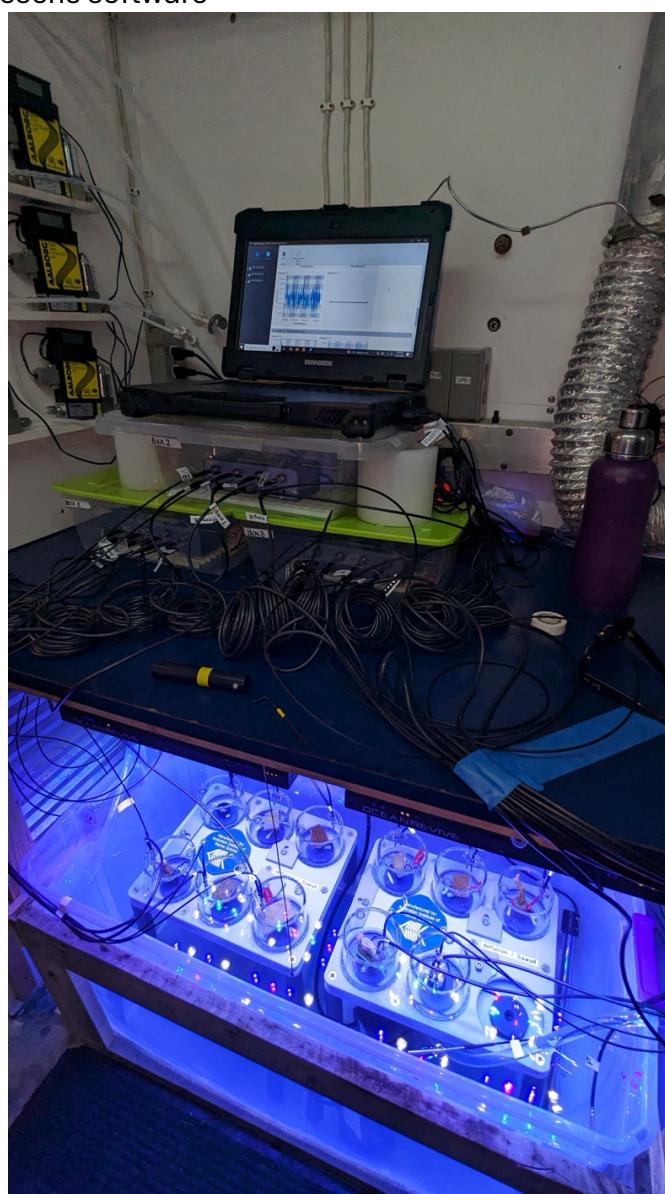
1 tank used as a water bath to control the water temperature in the incubation chambers

1 chiller

1 heater

1 chiller/heater temperature controller

1 computer with Presens software



### Tank preparation (lab teacher)

Before starting the incubation, the water in the tank must have reached the incubation temperature. Fill the tank with seawater until the water reaches almost the top of the incubation chambers and turn on the temperature controller. It will automatically measure temperature and turn on the chiller or heater.

Check the batteries of the spinning tables.

For net photosynthesis measurements turn on the lights. Measure the light intensity with a PAR sensor and adjust the light.

For dark respiration, turn off the lights, cover the tank with a tarp, and wait 5 min.

### Sample preparation (students)

Corals are fixed on plugs with epoxy (or any other organisms that fit in the chambers and allow water to move around). Clean the base of the coral with a toothbrush to take off all organisms that grew on the plug and epoxy (we only want the coral respiration or photosynthesis).

Write down the name of the samples you are going to use on the Presens software and link the name to one of the 12 channels. One channel must be a blank, with only seawater. It permits to know the oxygen production or consumption by the microscopic organisms in the seawater we use for incubation.

As soon as the seawater in the tank has reached temperature, we can prepare the incubation chambers with the corals. Secure the plug of the coral to the base of the incubation chambers. Be careful to choose the incubation number that corresponds to the channel you selected for the samples. Add a magnetic stirrer to each incubation chamber. It will permit to homogenize the water inside the chamber to get a more accurate oxygen reading. Submerge the two parts of the incubation chambers under the water and carefully close the chamber. The chambers SHOULD NOT have ANY air bubbles, which could highly modify the oxygen probe readings.

Turn on the spinning table. Secure each incubation chamber on the spinning table, be careful about the number of the incubation chamber, it needs to be on the same number on the tables. Plug the temperature and oxygen probes into the two holes of the incubation chamber, and choose the probes that have the same number as the incubation chamber.

As soon as all the chambers are on the spinning tables with probes and all of them have a spinning magnetic stirrer, start the run on the Presens software.

Usually, we start with light measurement for 20-30min, then turn off the light and dark measurements for 20-30min. With no pause in between. Write the start and end times for both light and dark runs.



#### Volume of the coral (students)

In order to know the volume of water in the chamber, we need to know the total volume of the chamber (0.62L) and subtract the volume of the coral.

To measure the volume of the coral, we place a container (1) with water filled to the top (all the way to the tension point), inside an empty container (2). We then immerse the coral inside the container (1) with water by holding the coral from the tip of the tag. The overflowed water in container (2) corresponds to the volume of the coral. The water in container (2) is poured into a measuring cylinder to get the volume.

#### Surface area of the coral (students)

The surface area of the coral is the living tissue surface area of the coral, the part that respires and photosynthesizes. The surface area of the coral will help us standardize the respiration and photosynthesis rates between every coral to be able to compare them. Take a picture from the top of the coral with a ruler as a scale. They will be analyzed with the ImageJ software.

#### Data analysis (students)

**Software needed:** excel and ImageJ (Fiji) <https://imagej.net/software/fiji/downloads>

After finishing the run, the .csv files are saved into the computer and sent to the students.

The .csv file has a lot of columns but only a few are interesting.

-“Time”: (hours:minutes:seconds) is the time when the incubation was running, the time should have been written down for start and end of light and dark incubations.

-“delta\_t”: (in minutes) is the number of minutes from the start of the incubation (0 min) to the end.

-“Value”: (in  $\mu\text{mol/L}$ ) the oxygen concentration

- Coral surface area measurement

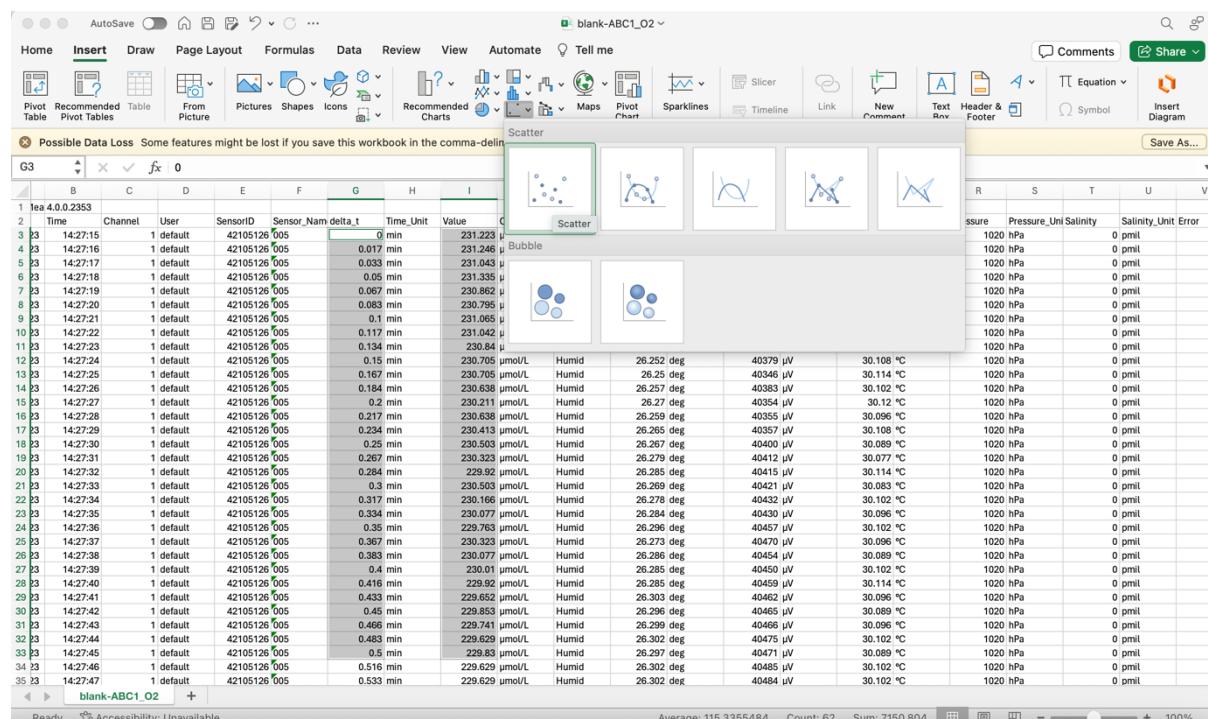
The coral surface area is obtained from the pictures taken from the top of the corals analyzed on the software ImageJ (to be downloaded before the lab).

- Blank analysis

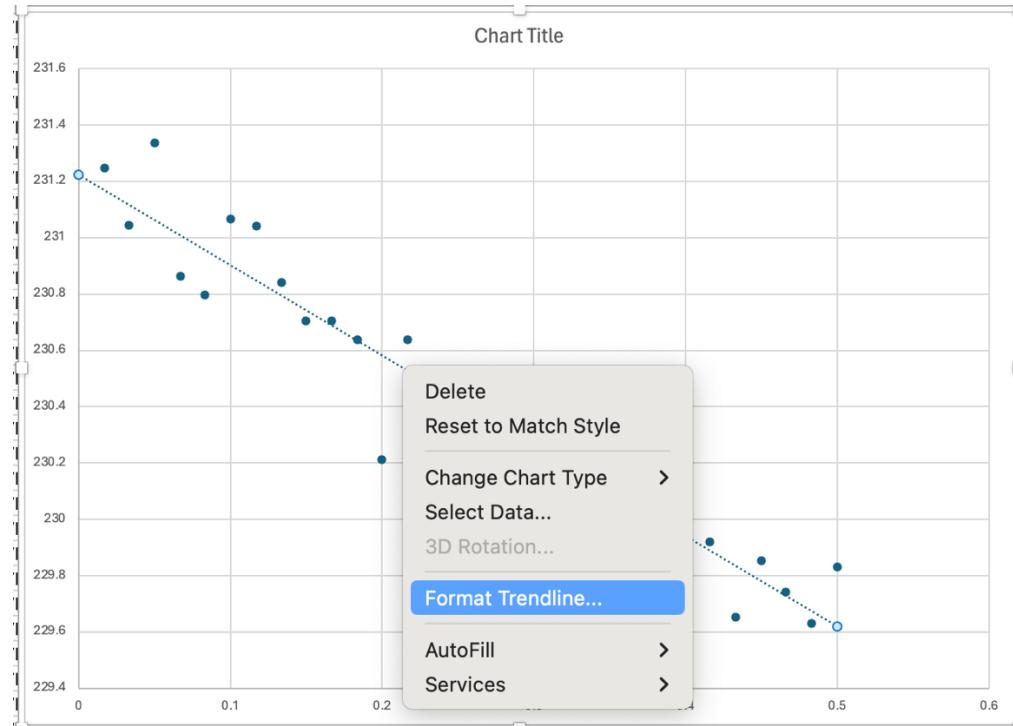
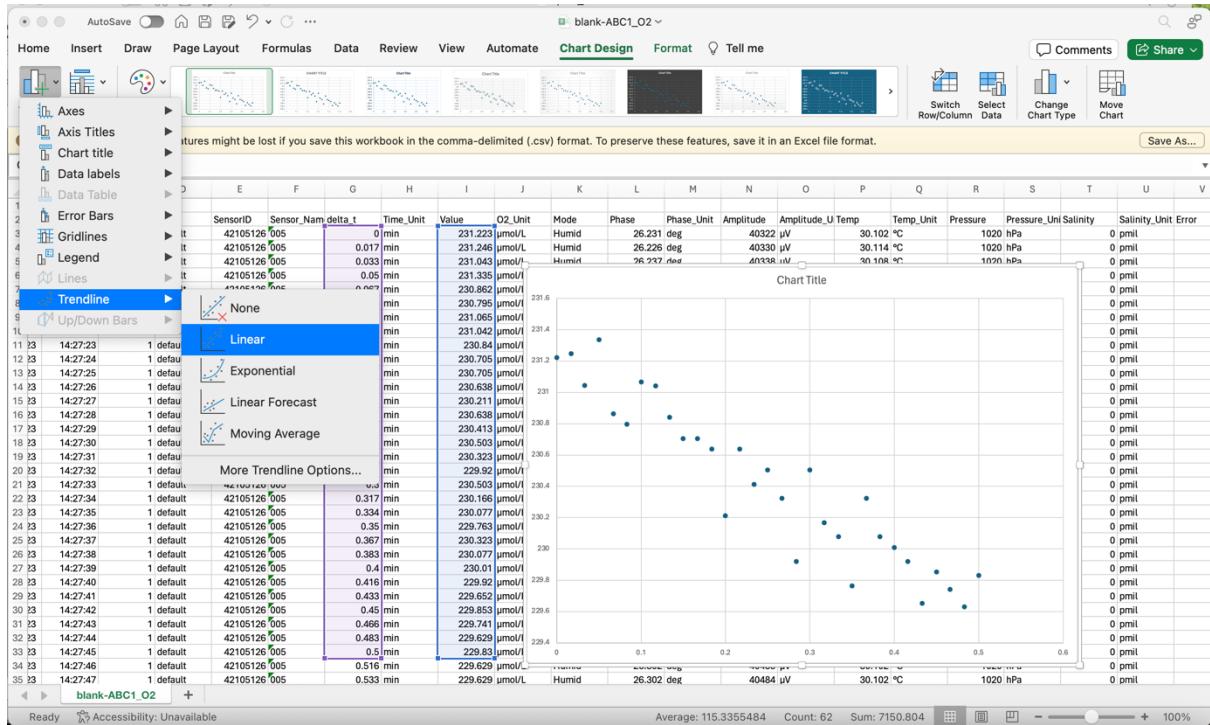
The blank is used to take off any background oxygen from respiration or photosynthesis from microorganisms in the water.

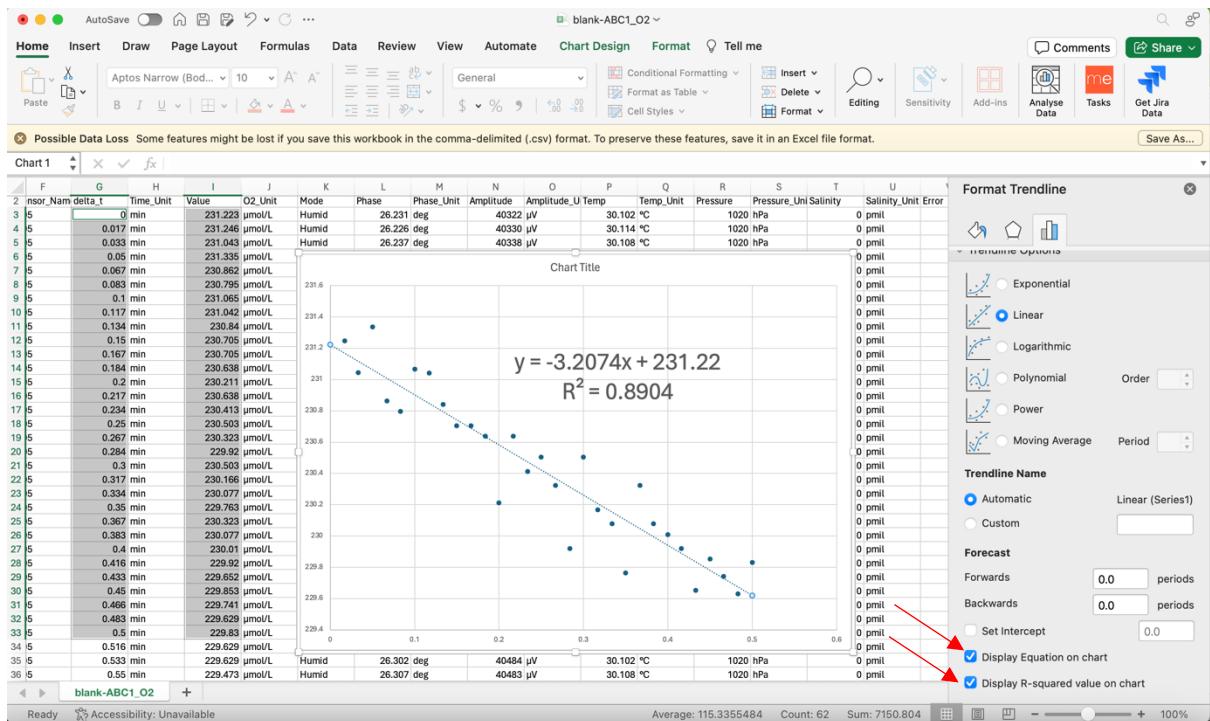
Open the blank .csv file in Excel. You can paste the columns “Time”, “delta\_t” and “Value” to another page for an easier analysis.

Select the data from “Value” and “delta\_t” from the start and end time of light measurement. Insert a graphic with scatter points. “Value” in y and “delta\_t” in x.



Draw a regression line and add the equation of it as shown in the pictures. If you don't see the regression line, you can change the color and size of the dotted line.





The equation format obtained is  $y = Ax + B$

Open the Excel sheet template sent to the students.

Template_oxygen																								
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	
1	date	sample_id	treatment	incubation	volume_chamber	volume_coral	total_volume																	
2	06/05/2024	blank	ambient	light		0.62		L	cm <sup>2</sup>															
3	06/05/2024	blank	ambient	dark		0.62		L	cm <sup>2</sup>															
4																								
5																								
6																								

Fill in the volume of the coral, the surface of the coral, the time\_start, time\_end, delta\_t\_start, delta\_t\_end, and A and B obtained in the equation. (for the blank: the volume is 0, the surface area is 1).

“time\_start”: is the time when we started the light or dark incubation

“time\_end”: is the time we stopped the light or dark incubation

“delta\_t\_start”: is the delta\_t corresponding to the “time\_start” (same line)

“delta\_t\_end”: is the delta\_t corresponding to the “time\_end” (same line)

The “total\_volume” is obtained by “volume\_chamber”-“volume\_coral”. Make sure that your volume\_coral is in L and not mL.

“oxygen\_start” = “A” \* “delta\_t\_start” + “B” : Measures the concentration of oxygen at the start of the incubation thanks to the equation obtained

“oxygen\_end” = “A” \* “delta\_t\_end” + “B” : Measures the concentration of oxygen at the end of the incubation thanks to the equation obtained

“diff\_time” = “delta\_end” – “delta\_start” : the duration of the incubation

“diff\_oxy” = “oxygen\_end” – “oxygen\_start” : the oxygen change for the duration of the incubation. Is negative when the oxygen has been consumed and positive when the oxygen has been produced.

“oxy\_quantity” = “diff\_oxy” \* “total\_volume” : the quantity of oxygen dissolved in the volume inside of the chamber.

“oxy\_per\_hour” = “oxy\_quantity” / “diff\_time” \* 60 : the total amount of oxygen produced/consumed per hour.

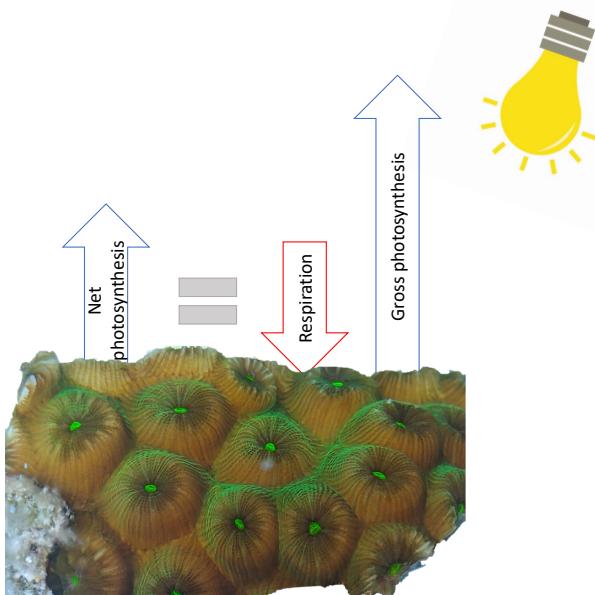
“corr\_oxy\_hour” = “oxy\_hour” of the sample - “oxy\_hour” of the blank for light or dark : **this is only applied to coral samples (not blanks)** to take off the oxygen changes on the background by the microorganisms in the seawater.

“oxy\_hour\_surface” = “corr\_oxy\_hour” / “surface\_coral” : the total amount of oxygen produced/consumed per hour for 1 cm<sup>2</sup> of coral tissue.

⇒ When this has been done for the light incubation, repeat it for the dark incubation, and the coral samples light and dark incubations.

### Net photosynthesis, growth photosynthesis and respiration.

The oxygen data we obtain during light incubation is the result from **respiration** (consumption of oxygen) and **gross photosynthesis** (gross production of oxygen) occurring at the same time, also called **net photosynthesis**. If the coral is bleached, there is a possibility to get a negative net photosynthesis, because respiration is higher than gross photosynthesis.



To obtain the gross photosynthesis:

“gross\_photo” = “oxy\_hour\_surface” of light - “oxy\_hour\_surface” of dark