ENVS 2001/2014 ENVIRONMENTAL FIELD AND LAB METHODS
SWIMS FIELD TRIP 2013.11.16

WELCOME TO SWIMS!

Today we plan a variety of activities, focused on showing you how my lab group conducts field and lab work! When we arrive, please feel free to explore the SWIMS area (safely) while we prepare for today's activities.

Today you will experience:

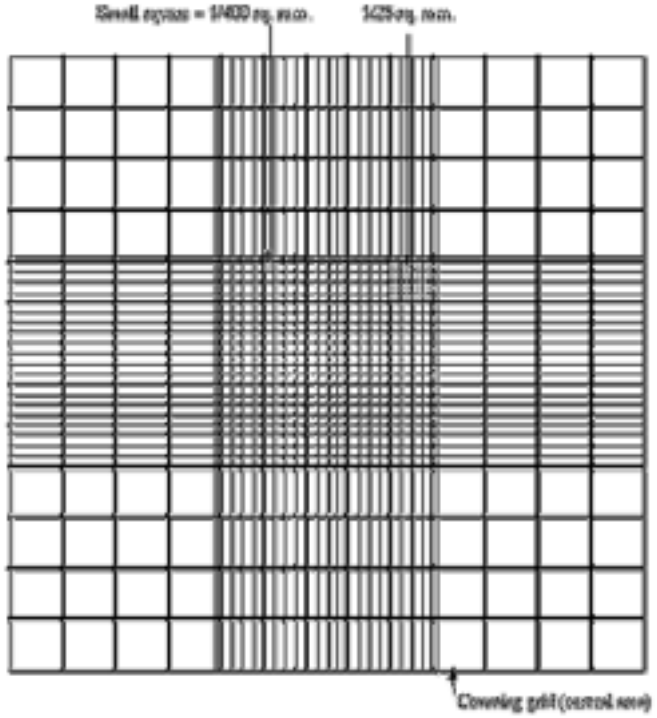
1) coral fragmentation for transplantation and research (with Dr. Duprey)
2) a "real" P:R (productivity) experiment using corals and sponges and a Nexsense Oxygen electrode (with Dr. Freeman)
3) separating coral tissues from the skeleton (with Ms. Chan & Mr. Zhu)
4) cell counting (zooxanthellae) via hemocytometer (with Ms. Chan & Mr. Zhu)
5) skeletal surface area determination using a Next Engine 3D scanner (with Dr. Baker)
6) demonstration of HOBO pendant and conductivity data loggers (with Dr. Baker)

Most of these techniques will be explained to you as you do them. Please pay attention to the station leader and take notes.

Below you will find some information on methodologies we use and some background.

Most important today is to experience science, have fun, & enjoy the sun after a gloomy week!

--DMB



The hemocytometer.

Box 1.1 What happens during coral bleaching?

The productivity of reefs is ultimately attributable to the symbiotic relationship between the coral polyp and its dinoflagellate algae, known as zooxanthellae, which live packed within the coral's tissues. Under normal conditions, the zooxanthellae perform photosynthesis and provide energy-rich compounds to the coral animal. However, under conditions of increased temperature, the algae are unable to process incoming light without releasing harmful oxygen radicals, similar to those involved in aging. When this happens the coral-algal relationship is disrupted and the zooxanthellae either degenerate in the tissue or are released from the tissue. Consequently, the bright white coral skeleton is visible through the unpigmented tissue, making the corals appear 'bleached'.

Under normal conditions microscopic algae, called zooxanthellae, live inside the tissues of the coral animal and provide up to 90 per cent of the coral's energy requirements

From Marshall & Schuttenberg (2006) A Reef Manager's Guide to Coral Bleaching. IUCN Publications, Cambridge, UK.

Healthy coral

Bleached coral

Dead coral

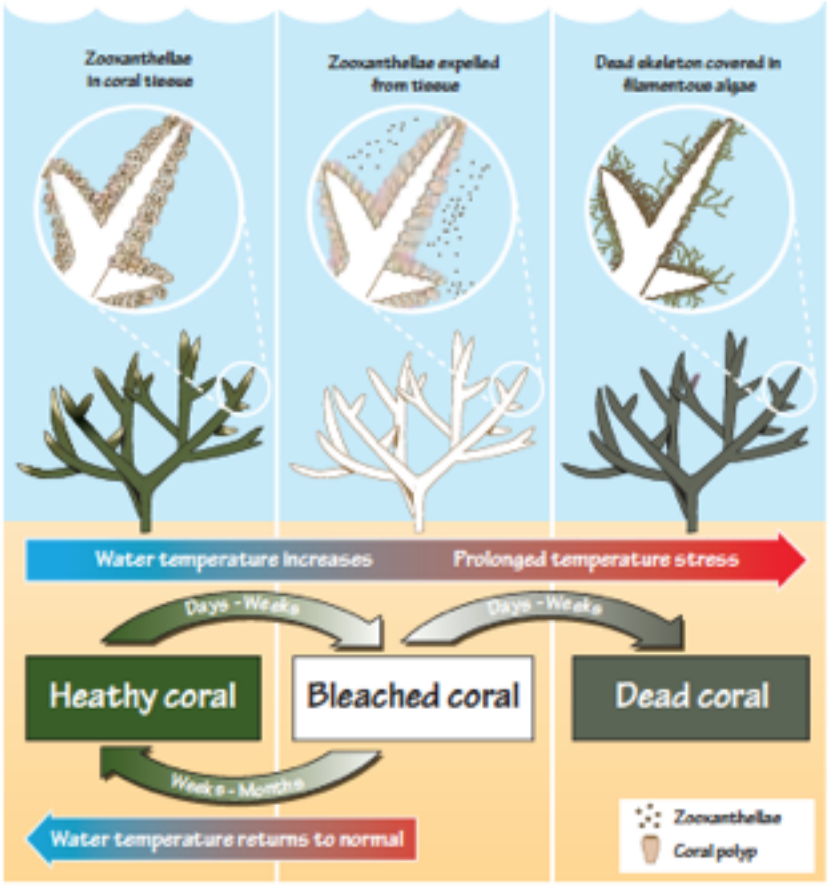


Figure 1.3 Stages in mass coral bleaching

During mass coral bleaching, water temperature increases above a critical threshold, typically over a large area. Under these stressful conditions, corals begin to lose their zooxanthallae, eventually appearing 'bleached'. At this stage, the bleached corals are still living and, if stressful conditions subside soon enough, they can regain their zooxanthallae. In this case, corals can survive, but are likely to suffer sub-lethal impacts, such as reduced rates of growth and reproduction and increased susceptibility to diseases. However, should temperature stress continue, corals are likely to die. Where mass coral bleaching causes high levels of coral mortality, these ecosystems typically take years to decades to recover.

Count zooxanthellae using Improved Neubauer haemocytometer (v. detailed)

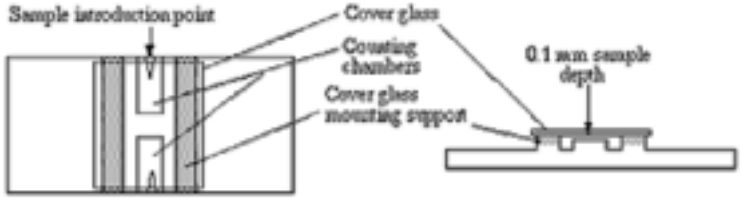
• **Optional:** Add a drop or more of Lugol's reagent (An aqueous solution of iodine and potassium iodide in water) to the homogenate before spinning down pellet. Note: if the algae you are counting are motile (e.g. live cultured algae) you must immobilize them first, Lugol's does this as well. Lugol stains starch granules and makes cells darker and also heavier. They are therefore easier to visualize and sink faster onto your counting chamber surface and plane of focus.

• Add an exact (known) but minimum (<=1 mL) volume of filtered sea water (0.45um filtered; FSW) to the pellet and resuspend well by vigorous shaking or vortexing until no visible clumps remain.

• Take an aliquot of the resuspension to dilute if necessary.

• I recommend using a dilution that will give a concentration of 25 cells/square. I try to count the 4 corner squares, so that in total I count about 100 cells per loading of the chamber. If the sample is very low in cells, count the whole chamber (9 squares). If you count less than 4 squares, always count the same ones, e.g. the top left and bottom right. If you count only one always count the center square. **Keep track of your dilution factor (DF=final vol/initial vol).**

Filling the chamber:



NOTE: CONSISTENCY IN FILLING TECHNIQUE IS PARAMOUNT TO SUCCESSFUL COUNTS!!!!! (Practice first with mock samples and not with your precious samples!)

• This is so critical that <u>normally</u> it is recommended that one person do the counts, or at least does the counts of one type of samples.

• Clean chamber and coverslip with lens paper with a little ethanol to remove any grease.

• **Always** use the correct coverslip (rectangular glass provided with chamber), a regular coverslip will **not** give you accurate values. Make sure you have extra coverslips!

• Use a glass Pasteur pipette to mix your sample well. I recommend that you use the same pipette to load all samples, just have a little beaker with water to rinse the pipette between samples. Do not use plastic pipettes since they can bind significant amounts of debris and algae. The surface of a fresh glass pipette will also bind

some algae but it quickly coats and equilibrates. The initial mixing will help achieve this.

- With the coverslip on the chamber quickly touch the chamber inlet groove with the tip of the pipette making sure there is liquid at the tip. Hold the pipette with one hand and use the upper surface of the index finger of your other hand to guide the tip; the pipette should be held at 45° angle to the chamber. The chamber should fill very quickly (almost instantaneously) with liquid by capillary action. Not much pipette bulb pressure is required. If the is any slow filling or if the liquid front is not even (e.g. when it forms a bubble) start over. If it takes too long to fill (I count three or five seconds) while you are filling, redo.

- Each haemocytometer has two chambers. Remix your sample and draw a new sample for every chamber. i.e. do not fill both chambers with liquid from the same draw.

- The part of the haemocytometer that is filled is just the plateau, polished glass surfaces that has the grid etched onto it, not the whole slide (i.e. not the deep grooves around the plateaus. Quickly draw any excess liquid from the filling port.

- Mount slide onto microscope stage, being careful to have secure mounting. Allow time (about 0.5 -1.0 minute) for the algae to settle. Tip: Have two (or three) haemocytometers so that you can fill them at the same time and while you count one the other will be ready.

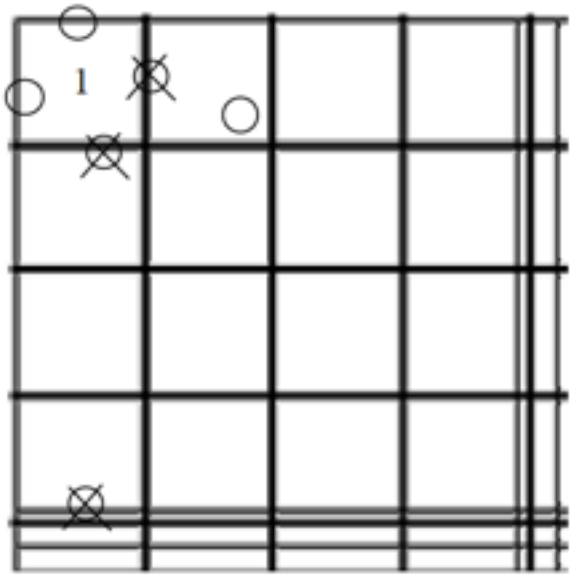
- Count away… (a counting clicker or two helps as well as some techno music)

Counting

- *Visually check to make sure your dilution and mixing was adequate (see sample prep above). You do not want too many overlapping algae and you do not want clumps.*

- Use 450x magnification for zooxanthellae.

- Scan square subdivisions left to right, updown and count algae. Count algae if they are **touching** the left or top line of each square (doesn't matter which ones –it can be bottom and right line but be consistent!) too make sure algae touching lines are not counted twice. In the example shown, do not count cells with an X for subdivision 1. Do not count the cell in the bottom row either.



Decide what a dividing algae looks (versus what a doublet -or two algae in host symbiosome, looks like) like and how you are going to count it. I count a dividing algae as two.

Doublet
Look for cell wall material between cells

Dividing Cells

• What to do when you run into an arbitrary clump…as mentioned if you see too many of these better vortex your sample some more. If you count a clump, count some number of at least 1 but don't count all of them. If you have a "feel" for what the average density of cells is throughout the chamber, use that as an approximation. Whatever you decide to do be consistent within your own technique as well as among people counting.

• Keep track of how many squares you count. The whole chamber has 9 squares (See last figure). The 4 corner squares have 4X4 subdivisions. The center square has 5x5 subdivisions which are further divided into 4x4. Each square is $1mm^2$ and the chamber depth is 0.1mm; therefore the volume overlying each square is $0.1mm^3$ (or 0.0001ml = 0.1µl). Calculate the average number of cells per square (total cells counted/#of squares used) and multiply by 10^4 and the dilution factor to cells per ml. For example, if you resuspended your algal pellet with 250ul of FSW and from this resuspension you took an aliquot of 100ul to dilute with 400ul of FSW (for a total of 500ul), and you counted 100 cells in 4 squares, your sample cell concentration is:

(100 cells /4 squares) X (500/100) X 10000 = 1.25×10^6 cells/ml

To get the total number of cells (your biomass), multiply this measured cell concentration by the original volume. In the example, 250ul = 0.25ml, times your concentration, you get 0.31×10^6 cells. If you are measuring mitotic index (fancy name for % dividing cells), have a separate clicker to count the number of cells dividing and just divide this by the total number of algae counted (x100%).

About replicate counts, this depends on the amount of variability between counts and the worse the technique, the more replicate chamber counts you will have to perform. It also depends on how critical it is to get an accurate count. In my experience it ranges anywhere from 6 to 20 reps!!! It is helpful to enter the numbers directly onto a spreadsheet using a laptop, that way you can enter a formula to keep track of your coefficient of variation (standard deviation/mean expressed as%) and decide what your maximum should be, e.g. less than 10%. Remember to count your samples at random!!!

HAPPY COUNTING!!!!!!!!!!!!!!!!