Symbiosis

Subject matter: Identify and describe the symbiotic relationships in a coral colony (including polyp interconnections and zooxanthellae.

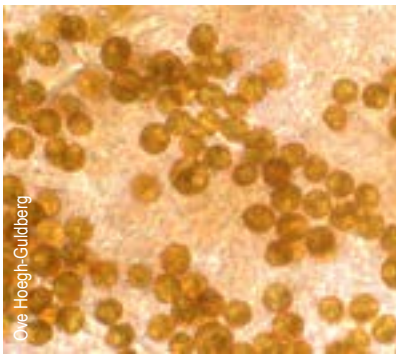
Recommended reading: Coral Reefs and Climate Change - Zooxanthellae (p.89)
View video: Coral Reefs and Climate Change DVD series - Coral Bleaching

Investigate zooxanthellae in corals - Lab

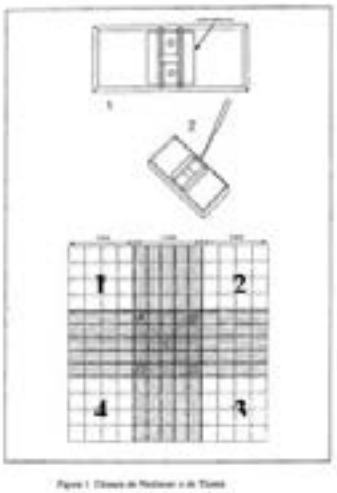
1) Remove small coral fragment or Anemone tentacle. Don't collect from the wild unless you have a permit.
2) Add 5ml of PBS or seawater into zip lock bag
3) Place coral fragment into the zip lock bag
4) Use the airpik/waterpik to clear a surface of the coral (aim for a circle or square so you can estimate the surface area).
5) Make sure you get all of the tissue off the area of coral
6) (If possible complete the following step) Centrifuge the solution to concentrate the algal pellet and remove the excess solution/supernatant from above the pellet. Then resuspend pellet in known volume of seawater /PBS
7) Measure (if step 6 is not completed) and Mix the pellet solution with a pipette to ensure its thoroughly mixed
8) Place a cover glass on the haemocytometer(counting chamber) and pipette a known volume at the edge of the cover slip/haemocytometer and allow surface tension and pressure to drawn solution across the measuring area.
9) View the sample under the microscope and count the cells between the double lines in square of 20 divisions (5 squares). Keep to regular square count between samples. For example use 3 or 5 square in your counting regime. Clean and repeat 3-5 times with fresh subsamples and take the average of the counts as a measure per volume. If there are cells that are on the double line borderline, and more than their half inside then include these in the count. NB: If there are too many cells in your solution or they are clumped together, then remix and dilute further. Like wise if there are very few cells then concentrate the main sample.

Equipment
Small coral fragment/ anemone tissue
1 pipette (1 ml) and disposable tips
Cutters
Airpik or Aaterpik
Airpik tips
Small zip lock
5ml of PBS or filtered seawater
Calipers (to measure coral)
Hemacytometer
Hemacytometer cover slips
Microscope
Pipettes
Tissues for cleaning

Safety precautions
Coral tissue contain nematocyst that may cause respiratory issues when tissue is released, therefore all work should be conducted outside in a well-ventilated area or in a fume hood.



Further activities
Describe the symbiotic relationship between the symbiotic algae and the coral.



The hemocytometer is a counting-chamber device originally designed and usually used for counting blood cells.

Note: Counting zooxanthellae in coral tissue is a bit more difficult than it sounds. You need specialized equipment to get the tissue separated from the coral in a controlled system (fume hood) and after that you need to separate the zooxanthellae from the tissue by centrifuge/vortex, homogenizing, etc. This all needs to happen on ice and you need to know the size and surface area of the coral to calculate how much tissue you have and known very small amounts of homogenizer - this all to later calculate exactly how many zooxanthellae the coral has per cm^2. But before these calculations you need a special tool (haemocytometer) to calculate the number of zooxanthellae in your suspension. An easier solution is to use the tentacle of an anemone. See example next page.

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Photos: CoralWatch

Aiptasia pulchella in aquarium.

Aiptasia pulchella in vial.

Cutting one tentacle.

Seperate tentacle and anemone between coverslips.

Investigate zooxanthellae in anemone - Lab

Explore what zooxanthellae look like and the large amount that is present.

You can use anemone that clownfish use (a variety of species, most common *Heteractis spp* or *Stichodactyla spp*) or another anemone species (*Aiptasia pulchella*). If you have an aquarium you can use the anemone in there or buy one from the shop. Don't collect from the wild unless you have a permit.

Steps to take

1) Depending on species available and amount of people involved in your experiment, decide if you just need one tentacle or several small animals. The photos of this experiment show Aiptasia pulchella species and individual animals were fairly small.
2) To relax the tentacles we used magnesium chloride (FC: 0.36 M).
3) Cut one tentacle and/or placed this together with one small animal in between cover slips.
3) Looking under the microscope zooxanthella and nematocyst should be able to be identified between 10x and 40x magnification.

NB: Makes sure no saltwater /PBS comes into contact with the microscope as this will cause corrosion and damage

Tips when using a tentacle from a bigger anemone

Place the tentacle on top of a coverslip and look under the microscope at the tentacle. If the tissue is clear and thin, you can already see the zooxanthellae. Cut the tentacle in half, look again. Squash tentacle a bit with flat object and add a drop of water, look again. Squash tentacle even further, add more water if needed. You can squash the tentacle more and add a cover slip carefully, try to avoid bubbles underneath it. Check the different amounts of zooxanthellae and the nematocysts (stinging cells) during your experiment.

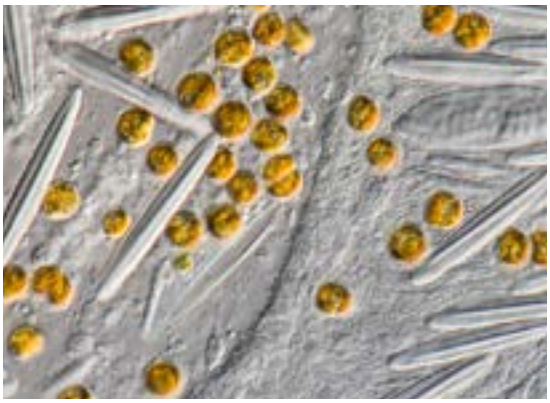
Be careful with using the coverslips, squashing is difficult without breaking the cover slip. Make sure you don't cut yourself.

Further activities

- Describe the symbiotic relationship between the symbiotic algae and the sea anemones.
- Compare the amount of zooxanthellae and nematocysts at the tip of the tentacle with the base of the tentacle - what is the ratio between the two?
- Draw a nematocyst, find out more about their function.

Viewing zooxanthellae with the microscope.

Photo: 2012 Waldo Nell



Symbiodinium are the golden-brown circles. The elongated light grey cells are stinging cells, nematocyst.

Equipment

Tentacle of anemone
1 pipette (1 ml)
Cutters
1 Vial
5ml of PBS or filtered seawater
Magnesium chloride (0.36M)
2 cover slips
Seawater/PBS for rinse before and after MgCL2
Microscope
Tissues for clean up