

**Using an Electrical Network Model to Simulate Gas Flux in Perforate
and Imperforate Corals: Calculating the Time Constant of Mixing in
Gastrovascular Fluid Compartments**

A thesis submitted in partial fulfillment of the requirement
for the degree of Bachelor of Science in Physics from
The College of William and Mary

by

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Abstract

The physiological role of the gastrovascular system in scleractinians has been understudied; in particular the implications of perforate vs. imperforate coral colony morphology are largely unknown but may be important to understanding coral response to increasing temperature and acidification in the ocean. This project uses concepts from electronics and fluid dynamics to determine the scale of the time constant of mixing in the gastrovascular system of two imperforate coral species *Montastraea cavernosa*, and *Duncanopsammia axifuga*. These time constants will be applied in an electrical network model that will give insight into how perforate vs. imperforate coral species handle environmental stresses. Six different conductivity probe designs were built to measure the time constant of mixing inside of the two coral species. I determined an average time constant of mixing for *Duncanopsammia axifuga* polyps under a ~ 0.1 m/s flow condition to be 1.45 ± 0.28 seconds and an average time constant of mixing under no flow to be 6.11 ± 2.82 seconds. There is a significant difference between the mixing times under the two flow conditions (p-value of 0.00026). I developed a differential equation model, closely related to the discharging of a capacitor, to be used in accordance with oxygen profiles during a light/dark shift inside a coral polyp as an alternative method to determine the time constant of mixing. I calculated the time constant of mixing to be c. 2 min 10 sec for a *Montastraea cavernosa* polyp by using the differential equation model. This project is an essential step in better understanding ventilation of the gastrovascular system in imperforate and perforate corals.

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1 Introduction

Changing environmental conditions like ocean acidification and ocean warming are predicted to have dire consequences for coral reefs (Hoegh-Guldberg et al. 2007). It is thus important to understand the physiological responses of corals to these changing ocean conditions. Preliminary research has found that the residence time of water in a coral colony's "internal plumbing" will have an effect on the supply of ions for calcification and photosynthesis and will be a critical point of limitation for these processes. This "internal plumbing" is called the gastrovascular system and functions for digestion and material distribution. It consists of a mouth, tubular pharynx (throat), coelenteron (central cavity, like a stomach), and canals that connect polyps in the colony to one another. Sheets of tissues termed mesenteries partition the coelenteron and are covered in cilia (flagellated cells) that drive the mixing of fluids in the system and provide surface area for secreting digestive enzymes and absorbing nutrients. The mixing of fluids in the scleractinian gastrovascular system (in the species *Acropora cervicornis*) was first studied by Gladfelter (1983) by observing fluorescein dye injected into a lateral polyp and using scanning electron microscopy and petrographic thin sections. She found that flagellated endoderm cells line the entire system, determined different flow directions for different sections of the coral branch, and found that light does not affect flow speed but that flow rates do follow a diel cycle (Gladfelter, 1983). The gastrovascular system comes in two different architectures, perforate and imperforate; perforate corals have a complex intra-skeletal canal system connecting every single polyp in the colony to one another and imperforate corals only have canals that connect polyps to their nearest neighbors.

Understanding gastrovascular system mixing in corals is key to predicting how corals will be affected by changing ocean conditions. The physiological role of the gastrovascular system in scleractinians has been understudied; in particular the implications of perforate vs. imperforate coral colony morphology are largely unknown but may be important to understanding coral response to increasing temperature and acidification in the ocean. Metabolic activities that alter water chemistry inside the polyp's gastrovascular system require periodic turnovers of the enclosed fluid, but this phenomenon has yet to be measured in corals. No one has measured the time needed for corals to mix fluid inside of their central cavity. This project uses concepts from electronics and fluid dynamics to develop a method of measuring mixing time and determine this time constant of mixing in the gastrovascular system of two species of imperforate coral, *Montastraea cavernosa* and *Duncanopsammia axifuga*. Once the methods of measuring mixing time have been fully developed, the measured time constants will be applied in an electrical network model that will give insight into how perforate vs. imperforate coral species handle environmental stresses.

1.1 Climate Change and Coral Reefs

There is growing concern that reefs as we currently know them may not survive. Prior to the industrial era (late 1700s) the atmospheric carbon dioxide concentration was approximately 280 ppm, the concentration has steadily increased to 391 ppm in 2011 (IPCC). This is a 40% increase over this time period. The rising atmospheric carbon dioxide is moderated by ocean uptake, which accounts for almost a third of anthropogenic carbon added to the atmosphere (Doney et al., 2009). The increase in $p\text{CO}_2$ in the ocean surface waters causes pH to lower, alters fundamental chemical

balances, and lowers the calcium carbonate saturation levels. Since the 1980s, average pH measurements have decreased approximately 0.02 units per decade (Hoegh-Guldberg and Bruno, 2010). Since pre-industrial times, the average ocean surface water pH has fallen from 8.21 to 8.1 and it is expected to fall further by 0.3 to 0.4 pH units if the CO₂ concentrations reach the projected end-century levels (Doney et al., 2009). Global temperatures have increased by about 0.2 degrees Celsius per decade over the last 30 years, and the oceans absorb most of this added energy (Hoegh-Guldberg and Bruno, 2010). The heat content of the upper levels of the oceans has increased by 14×10^{22} J since 1975 (Hoegh-Guldberg and Bruno, 2010). Figure 1 shows environmental trends and some of their consequences over time (Doney et al., 2012).

Increasing pCO₂ levels and surface water temperatures in the oceans have numerous and widespread consequences for coral reefs. Corals' ability to create the massive skeletal structure that one fourth of all marine species depend upon is very sensitive to relatively small changes in temperature and pH (Doney et al., 2012). Coral reefs are particularly vulnerable because of the losses that they have already suffered; there have been reductions of coral cover of 80% in the Caribbean and 50% in the Pacific (Jackson, 2010). Analyses of cores from the Great Barrier Reef show that calcification rates have declined 21% between 1988 and 2003 (Cooper et al., 2008). Fine and Tchernov (2007) grew two species of hard coral in highly acidified water and found that the corals lost all of their skeleton but regrew it when placed back in normal conditions. The naked polyps remained healthy, but fitness of the coral colony overall decreased because of the loss of its protective skeleton (Fine and Tchernov, 2007). Coral reefs may become more susceptible to storm damage because of compromised skeleton strength due to ocean

acidification, and warmer oceans drive more intense storms.

Warming of just 1 degree Celsius causes coral bleaching, the colorless state due to the expulsion of their zooxanthellae (the photosynthesizing dinoflagellates in the genus *Symbiodinium* that live inside coral tissue and fuel the growth of their coral hosts), that in turn causes more stress and reduced growth and reproductive rates, and makes the coral more sensitive to disease (Doney et al., 2012). Temperature has a fundamental affect on biological processes. It affects the molecular kinetic energy and determines rate of processes such as enzyme reaction, diffusion, and membrane transport. Organisms acclimate to local averages and can only work within a certain temperature range (Hoegh-Guldberg and Bruno, 2010). In the long run, low growth rates caused by a combination of changing ocean conditions may make it difficult for coral reefs to keep up with rising sea levels, losses due to storm damage, bio-erosion, and losses due to disease. Coral reef existence depends on their net positive accretion. If we exceed 2 degrees Celsius above pre-industrial temperatures, and if we reach an atmospheric CO₂ level above 450 ppm, we will drive an unsustainable frequency of mass coral bleaching and mortality events and push coral reefs into a negative carbonate balance worldwide (Hoegh-Guldberg and Bruno, 2010).

1.2 The Coral Gastrovascular System

Hard corals, order Scleractinia, belong to the subclass Hexacorallia, class Anthozoa, in the phylum Cnidaria. Individual, genetically identical, polyps group together to form a colony united over a calcium carbonate skeleton. The gastrovascular system functions for digestion and distributes materials to other parts of the organism; see Figure 2 for the internal anatomy of a coral polyp and Figure 3 for an external view of coral polyps. The

polyp's mouth opens into a tubular pharynx. In some coral species, siphonoglyphs, ciliated grooves, pull in water from the external environment. Mesenteries partition the central cavity, the coelenteron, which increases the surface area available for secreting digestive enzymes and absorbing nutrients. Mesenteries are ciliated, and can be armed with cnidocytes (stinging cells), and have thick longitudinal muscles. It has been estimated that every mesentery endoderm cell has a flagellated cilia. This is an immense number of cilia driving the flow in the coelenteron. The coelenteron serves as a sort of circulatory system by moving nutrients through the coral body, absorbing metabolic wastes from the gastrodermis, and eventually expelling waste through the mouth. Since gas exchange occurs across internal and external body surfaces, corals keep diffusion distances to a minimum by having thin body walls with large internal surface areas. Water is driven through the gastrovascular system by ciliary action and expelled by muscular contraction. Flow in the system is laminar.

Corals can have two types of gastrovascular systems, perforate or imperforate. Perforate corals have an “internal plumbing system” that connects all polyps to the entire colony; imperforate corals do not. Perforate species' internal plumbing system gives them an extended fluid volume for buffering chemical changes or increasing the rapidity with which they exchange fluid with the environment or other polyps. Imperforate corals do not possess the ability of fluid exchange at the whole colony level, since individual polyps are only connected to their nearest neighbors, and only when the polyps are expanded.

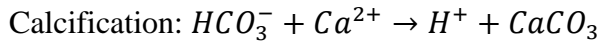
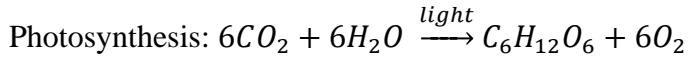
Dr. Elizabeth Gladfelter has done very detailed work on describing the gastrovascular system of *Acropora cervicornis*, an example of a perforate coral (Gladfelter, 1983).

Taylor (1977) studied the gastrovascular system in *Acropora cervicornis* and *Montastraea annularis* and found that it can transport materials over considerable distances and the direction of transport is toward zones of maximum growth and calcification (Taylor, 1977). However, the study of the structure of the gastrovascular system of *Montastraea cavernosa*, an imperforate coral, is not represented in the literature. The digestion system of actinians, sea anemones, close neighbors to scleractinians, has been described. Jones et al. (1977) found a respiratory rhythm in sea anemones that corresponds to a periodic expulsion of coelenteric fluid. Nicol (1959) determined digestion times in a species of sea anemone to be on the order of 8-24 hours. But surprisingly the gastrovascular system in scleractinian coral has been largely ignored in investigations of scleractinian physiology.

1.3 Key Metabolic Processes Affected by the Coral Gastrovascular System

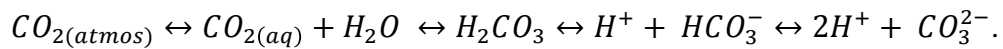
Ventilation of the gastrovascular system affects oxygen and other metabolite dynamics in the coral colony. Oxygen is produced by photosynthesis of the symbiotic algae and consumed by respiration by both coral host and symbionts. Oxygen diffuses through tissues and the environment in a bidirectional fashion, depending on the time of day. Previous research using microelectrodes have found strong gradients in oxygen concentration around individual polyps during the day and night (Shashar et al., 1993; Patterson, 1992). Gladfelter et al. (1989) demonstrated metabolic gradients in a branch of growing *Acropora palmata*. The redox state of tissues may be affected by ventilation and this has implications for colony growth and morphology. Calcifying organisms exert a variable degree of control over biomineralization, which generally involves passive and active ion movement in and out of a calcification compartment isolates in ambient

seawater (Weiner and Dove 2003). Photosynthesis and calcification process happening inside the coral gastrovascular system likely compete for available inorganic carbon.



Paul Jokiel (2011) has done extensive work on better understanding the competition between calcification and photosynthesis inside the coral gastrovascular system. His model presents a unified theory of coral metabolism and provides explanations for many aspects of coral biology (Figure 4) (Jokiel, 2011).

The ocean carbonate system also plays a role in coral reef calcification. Seawater carbonate chemistry is set by the following series of chemical reactions:



At the air-sea interface, gas exchange equilibrates surface water CO_2 to atmospheric levels, so when CO_2 levels are driven up in the atmosphere due to climate change, the levels increase in the ocean as well. Dissolved CO_2 then reacts with water to form carbonic acid (H_2CO_3), which can then dissociate into hydrogen ions, bicarbonate ions (HCO_3^-), and carbonate ions (CO_3^{2-}). At a surface ocean water pH of ~8.1, approximately 90% of inorganic carbon is bicarbonate ion, 9% is carbonate ion, and only 1% is dissolved carbon dioxide. With increasing carbon dioxide in the atmosphere, CO_2 levels in the ocean will increase, driving an increase in bicarbonate and hydrogen ion concentrations. Higher hydrogen ion concentrations correspond to lower pH values

because $pH = -\log_{10}[H^+]$. Carbonate ions will decrease because of the increasing hydrogen ion concentrations.

1.4 An Electrical Network Model of Gas Exchange in Corals

Electrical network modeling has been used to simulate many physiological processes in several kinds of organisms. An electrical network model is constructed from circuit components such as resistors and capacitors that represent a physiological feature. Current is analogous to a flux of interest and voltage is analogous to a driving force for the system. Nobel and Jordan (1983) developed an electrical network model to analyze transpiration rates and water potentials in three very morphologically different desert plants. Other studies using electrical network modeling developed models for the vertebrate respiratory (Campbell and Brown, 1963) and circulatory systems (Dawson et al., 1982), excretory systems (Goldstein and Rypins, 1992), and passive suspension feeding in lower invertebrates (Patterson, 1991).

Drs. Mark Patterson, Elizabeth Gladfelter, and Lawrence Carpenter have developed an electrical network model for gas flux for perforate (Figure 5) and imperforate corals (Figure 6) that makes testable predictions of dissolved oxygen concentration and flux rate at specific locations in the gastrovascular system. The driving force (voltage) is the oxygen concentration and the flux of interest (current) is the rate at which oxygen passes through a compartment in the system. The physical interpretation of resistance is the time needed to pass oxygen through a model component on a per volume basis. There are resistances associated with the coral tissues, boundary layers, and fluid compartments. The gastrovascular system is not expected to have instantaneous changes in gas concentrations, so capacitors are included in the model. In electronics, a capacitor is a

frequency-dependent resistor that can store and release charge. In the model, capacitance is the storage capacity of part of the system for oxygen and is associated with the volume of the fluid compartments and the calicoblastic space located under the calicoblastic endoderm. The capacitances were chosen using geometrical measurements of polyps and gastrovascular spaces reported in Gladfelter (1983), Patterson (1992), and Sakai (1998). Model simulations are done using a 5spice program with AC analysis.

1.5 Mixing Time

Most of the model parameters have already been determined by using point measurements of oxygen concentration as measured by microelectrodes and scanning electron microscopy by Drs. Mark Patterson, Elizabeth Gladfelter, and Lawrence Carpenter. Ciliary action, polyp flexing, and cycles of polyp expansion and contraction mix the fluids in the coelenteron, so that the resistances of the coelentera and gastrovascular canals cannot be determined in the same way as the other parameters. These resistance of the coelenteron (R_c), and the associated capacitance (C_c) can be computed by measuring the time constant of mixing in the system. This “mixing time” may have important ramifications for calcification, photosynthesis, respiration, and other metabolic processes in the coral.

Put simply, the “mixing time” is the time needed for the gastrovascular system to disperse an amount of fluid in the coelenteron. In the electrical network model this time constant of mixing is described by $\tau = R_c C_c$. This equation comes from the modeling of a resistor-capacitor (RC) circuit in electronics. The time constant is equal to the time needed for the capacitor to charge or discharge through the resistor by approximately 63.2%. When a capacitor charges and discharges in an RC circuit, the voltage vs. time

relations follow exponential curves, respectively $V = V_0(1 - e^{-\frac{t}{\tau}})$ and $V = V_0e^{-\frac{t}{\tau}}$. When a coral undergoes a transition period from light to dark, the zooxanthellae endosymbionts stop producing oxygen via photosynthesis and the concentration of oxygen decreases in the coelenteron. This process can be equated to the discharging of a capacitor in an RC circuit, as the system is modeled in the electrical network model. A dark to light transition would be equivalent to the charging of the coelenteron capacitor through the coelenteron resistor. By plotting the change in concentration of oxygen during light dark shifts we can determine the time constant of mixing.

1.6 Objectives

This project attempted to answer the following two questions:

- 1) **How does the imperforate vs. perforate gastrovascular system affect mixing time?**
- 2) **What is the timescale of gastrovascular mixing?**

by attempting the following objectives:

- 1) **Develop a way to measure the time constant of mixing in *Montastraea cavernosa* and *Acropora cervicornis*.**
- 2) **Determine an order of magnitude for mixing time.**

I used various concepts from electronics and fluid dynamics to accomplish these objectives. Six different probe designs were built to measure the time constant of mixing inside of the two coral species. I developed a differential equation model, closely related to the discharging of a capacitor, to be used in accordance with oxygen profiles during a light/dark shift inside the polyp as an alternative method to determine the time constant of mixing. The development of a microelectrode probe to determine mixing time is a novel

way to make gastrovascular measurements inside of a coral polyp. Until now, the timescale of gastrovascular mixing has not been measured. Dr. Patterson's initial electrical network simulations used a time constant of c. 21 minutes. This project is an essential step in better understanding ventilation of the gastrovascular system in imperforate and perforate corals. The results will be applied to determining how coral reefs will survive and shift under changing ocean conditions.

2 Methods

2.1 Challenges of Making Measurements in the Coral Gastrovascular System

In response to being threatened, introduced to a changing environment, or to being disturbed, a coral polyp will completely contract. It will then take hours to fully expand again. This presents a challenge to researchers intending to insert a sensor to measure mixing inside the coral's gastrovascular system. A protocol has to be developed to circumvent these behavioral responses. Some sort of structure needs to be built to support the sensor and for the sensor to be introduced with minimal disturbance. The sensor must be able to be left in a permanent livable space for the coral that does not have changing water conditions. Ideally, the sensor should be sturdy enough to be left *in situ* for a few days. Researchers may have to introduce the sensor into the organism, let it respond, leave the sensor inside the coral for a day or two, and then start making measurements. When considering the size of the sensor, careful measurements of the coral must be made. The mouth of the coral can look larger than it really is because when closed it appears as a line. This "line" is not actually the diameter of the mouth as one might think. Careful observations must be made to fully account for the small size of the organism. Any experiments studying the gastrovascular system of corals need to be thoroughly

planned in advance and take time to fully consider all possible impacts and difficulties for the organism to accommodate a sensor inside the polyp.

2.2 Measuring the Time Constant of Mixing Using a Conductivity Probe with a Pulse of Freshwater

The resistances of tissue layers and the external boundary layers have already been computed by using point measurements of oxygen concentration as measured by microelectrodes. The resistances in the coelentera and gastrovascular canals cannot be obtained this way because they are mixed by ciliary action, polyp flexing, and cycles of polyp expansion and contraction. The resistance of the coelenteron (R_c in the model), and the associated capacitance C_c can be computed by measuring the time constant of mixing in the system. This “mixing time” may have important ramifications for calcification, photosynthesis, respiration, and other metabolic processes in the coral. The time constant is $\tau = R_c C_c$.

By measuring τ , once the related volume of the space (capacitance) is measured, the resistance can be calculated. A microsensor and associated circuit were designed and built to measure the change in current (conductivity) within the polyp as a small volume of different salinity water is introduced and mixed (Figure 7). This change is shown on an oscilloscope, which is then timed from the time of introduction to when, the system is thoroughly mixed back to the original salinity. The state of thorough mixing is defined as the point in time when the signal levels out to a new steady state value after a step change in salinity is introduced into the polyp. The time constant is then defined as the time needed to reach $(1 - 1/e)$, about 63.2%, of the new steady state value.

2.2.1 Circuit Design

The circuit design (Figure 7) used was optimized to deliver a minimum polarization voltage to the probe, so that the organism is not adversely affected, to prevent the electro deposition of salts, and the electrolysis of water into hydrogen and oxygen. A 0.5-0.7 VDC voltage source is used to drive the probe. This polarization voltage was generated by a 9 VDC alkaline battery connected to a potentiometer. The potentiometer sub-circuit (located inside a pico-ammeter used in other work), had to be rebuilt with a new potentiometer and voltage regulator (LM317T) in order to produce the required voltage. The probe was connected to an inverting amplifier (LT1001), wired as a current to voltage converter. Water inside the polyp's coelenteron acts completes the circuit. The transimpedance resistor was set to 689 kOhm after some trial and error.

Changes in the conductivity of the water inside the polyp's coelenteron from the output of the transimpedance amplifier are displayed by an oscilloscope. When a small volume of freshwater is added to the polyp, a drop in voltage is seen as the current is temporarily reduced in the vicinity of the microprobe. As mixing takes place, the voltage rises back to a new value. If the volume of the water injected \ll volume of the coelenteron, the new value of salinity (and hence current converted to voltage) will be very close to the starting value.

The full electronic apparatus can be seen in Figure 8. Numerous tests were done during the design process to account for possible problems. In preliminary tests, using a Keithley pico-ammeter connected to the probe, currents of 0.8 μA and 5 μA were measured in freshwater and seawater, respectively. Using Ohm's law ($V = IR$), these currents were then used to compute the predicted resistances that would be seen in the

different salinity waters (seawater = 140 kOhm and freshwater = 875 kOhm) for the approximate spacing of 250 μm and approximate wire diameter of c. 100 μm , which then allowed an estimate of the change in voltage that would be seen on the scope (0.1 V), if the current-to-voltage converter resistor was set to $\sim 689 \text{ kOhm}$.

2.2.2 Evolution of the Probe

To measure the change in current, a probe small enough to enter the coral's coelenteron had to be designed and built. It has to allow water to be inserted into the polyp at the same time it is in place in the coelenteron, or it has to also be able to inject water into the coral itself. I tried multiple different designs with different types of wire, insulating material, superglue, water delivery methods, and tubing. I built six different probes as part of the research and development process.

Probe A (Figure 9):

The first probe was finished in October. Since it was my first attempt at building a microelectrode, it was more of a proof-of-concept rather than final product. This probe never made it into a coral. The thin wires at the tip were made from 0.004 in (0.1 mm) outer diameter aluminum wire, and they were soldered to 22 gauge insulated wire. The aluminum wire broke very easily, and the soldering connections had to be redone multiple times. I used nail polish as an insulator to cover the aluminum wire up to a point where only 1 mm of wire was exposed. To keep the ends of the wire from touching, I used a small piece of sticky putty. The major problems with this design arose from the type of wire, the nail polish, and the sticky putty. Nail polish may provide insulation, but it is not suitable for such fine wires. The polish used clumped too much. The sticky putty

worked, but was hard to break off into small enough pieces that would still serve as spacers and was thus deemed unreliable.

During this development phase, I built a model of three different-sized polyps from small diameter plastic cylinders (one end closed) found in a hardware store. These models (Figure 10) were used to test if the probe could sense a gradual change from fresh to salt water in a small volume cylinder. Even with all of its problems, this probe did serve its purpose as a proof-of-concept. I was able to hook it up to the circuit and see a difference in voltage when the probe was moved between salt and fresh water.

Probe B (Figure 11):

The second probe consisted of two wires (0.2 mm diameter; 32 mm long) spaced 0.25 mm apart, with 4.35 mm at the tips of the wires exposed to the seawater, with the remaining length insulated. The wire was a type of magnet wire, so that it did not have to be re-insulated using nail polish. The magnet wire was also thinner than the aluminum wire and did not break easily. I used sand paper and a razor blade to scrape off the insulation on the ends. It was easiest to do this by first removing a large portion of the covering and then cutting the wire back to the correct length. The microelectrode ends were then fine-tuned with a razor blade under a dissecting microscope or by using magnifying glasses. I used a cyanoacrylate glue (Loctite Super Glue) as an insulating spacer to keep the microelectrodes from touching each other. Throughout the year, I found that this type of glue worked best as a thin, non-clumping spacer. This probe also did not make it into a coral. However it was tested in a large sea anemone (*Condylactis spp.*), provided by Dr. Jon Allen from the William and Mary Invertebrate Zoology Lab.

During the test in the sea anemone, I found that it was extremely difficult to keep the probe from moving too much and inject fresh water from a pipet into the main gastrovascular cavity at the same time. It was nearly impossible to keep both the pipet and the probe steady. After attempting a measurement for a few hours with difficulty keeping the anemone fully expanded and cooperating, the signal dropped off the oscilloscope and could not be retrieved. I removed the probe and found that I had punctured a gonad. I was not sure how much the inside volume was changing as the anemone expanded and contracted which could affect the mixing time. I learned that I had to let the organism adjust to having the probe and pipet inside before trying to take a measurement. This second design used a much better wire system; however it needed a built in way of injecting fresh water into the organism. I continued to use the magnet wire in all later designs.

Probe C (Figure 12):

The third design took the last design and attached it to the end of a syringe needle. The needle was flattened with a file. The probe tips had to be carefully glued on the end of the needle so that the metal needle did not act as a conductor, which would then short out the sensor. The probe tips were cut down in order to not stick too far out from the opening of the needle. The spacing between the wires was 0.47 mm and the conducting section of the wires was 0.78 mm long. I was able to test this probe in *Montastraea cavernosa*. I only saw very quick signal changes on the oscilloscope when the salinity was perturbed by an injection of freshwater. The measurements corresponded to a mixing time of less than a second, which was too short to be the actual mixing time. These short mixing times could be a function of not all of the fresh water getting into the polyp or

possibly the probe was not in the right place. It was very difficult to see where the end of the pipet was in relation to the probe tips. The glue used to attach the probe to the needle was too clumpy and hindered view of the end of the probe. This design was able to inject fresh water and accommodate the microelectrode tips on one vessel, however it was not streamlined enough to work with small polyps.

Probe D (Figure 13):

In the fourth iteration of the microprobe, I decided to use microtubing connected to a syringe to inject the fresh water. I researched multiple types of tubing and syringe combinations and decided on using Zeus PEEK (polyether ether ketone) tubing with an inner diameter of $1168 \mu\text{m} \pm 25 \mu\text{m}$ and an outer diameter of $1575 \mu\text{m} \pm 25 \mu\text{m}$. The tubing was too wide to fit inside the polyps of *Montastraea cavernosa* and *Acropora cervicornis*, however it works very well for the larger polyp coral, *Duncanopsammia axifuga*. (When ordering the tubing, I measured what I thought to be the mouth in *Montastraea cavernosa*; however, I believe I overestimated its width by including the outer diameter of the oral disk, instead of the more inner diameter of the actual mouth. The tubing was cut to a length of 32 cm and then I ran the magnet wire to the two microelectrode ends through the tubing. The ends of the wires emerged out 0.42 mm from the end of the tubing and were separated by 0.26 mm. At the other end of the tube, the wires come out, and a second piece of different plastic tubing was used to connect the PEEK tubing to the syringe. Cyanoacrylate glue was used to seal the crack between the two tubes in order to have the best seal possible for the syringe to work properly. This probe was able to make a measurement in the *Duncanopsammia axifuga*, incorporate the freshwater injection with the microelectrode, and place the syringe farther from the

Narishige micromanipulators (model M-3333) used to position everything so that it would not jostle the probe when being used. However, it was not small enough to make a measurement in the other two species and the wires were very difficult to thread down the tube.

Probe E (Figure 14):

The fifth probe attempted to solve the size issue of the previous probe by using a pipet with a thin pipet tip to inject the fresh water. I used the Loctite Super glue to attach the two magnet wires to the end of the pipet tip. The space between the wires was 0.37 mm and the conducting part of each wire was 0.54 mm long. This probe was able to fit inside the mouths of *Montastraea cavernosa* polyps. A pipet allows me to inject an exact amount of fresh water in the polyp, however it can only be used once. It was difficult to stabilize the pipet well enough to keep the probe from moving when injecting the fresh water.

Probe F (Figure 15):

The final probe combined the best elements from probes D and E. I cut the end of the pipet tip so that it fit inside the PEEK tubing. The thin tip fits inside of both *Duncanopsammia axifuga* and *Montastraea cavernosa*. The tubing combined with the syringe allows for multiple runs in the same polyp and better stability. I tried the combination of pipet tip and tubing a few different ways, but stuck with the same tip used for probe E. The space between the wires was 0.48 mm and the length of the conducting wire section was 0.5 mm. The magnet wires run the length of the tube on the outside.

Combined with flawless micromanipulator operation, steady hands for using the syringe

plunger, and patience, this probe is the best probe I have developed so far for measuring mixing time.

2.2.3 Protocol

Polyp volumes were calculated using image analysis, approximating the polyps as cylinders. 10% of the polyp's volume of fresh water was expected to be introduced into the polyp's coelenteron. However, since the syringe I used only had increments of 10 microliters, I used 10 μ l for the small polyps and 20-40 μ l for the larger polyps (*Duncanopsammia axifuga*). Since 10-40 μ l is a small volume, no significant organismal shock was expected. The probes are positioned using a Narishige M-3333 micromanipulator with a positioning resolution of 10 μ m in the z-axis and 100 μ m in the x- and y-axes. When the fresh water is introduced into the polyp, the current between the two elements of the probe decreases. As the gastrovascular space is mixed, this current increases as saltier water reenters the zone around the probe tips. The signal derived from this current, viewed on the oscilloscope, drops because there is less current in freshwater than in salt water. A video camera was used to capture the time it takes for the voltage to equilibrate to a new value. The time error associated with the video is $\pm 1/15$ sec (twice the frame rate of the video camera, which is 30 frames/sec. Measurements were taken under flow and no-flow conditions. The procedure for measurements done with the probes is listed in the Appendix.

2.3 Measuring the Time Constant of Mixing Using a Microsensor to Measure Dissolved Oxygen Changes During Light/Dark Transitions

When a capacitor discharges in an RC circuit, the voltage vs. time relation follows an exponential curve, $V = V_0 e^{-\frac{t}{\tau}}$. The time constant, $\tau = RC$, is the time when the voltage reaches $(1/e) \cdot V_0$. In the electrical network model, the coelenteron is comprised of a resistor and capacitor in parallel. “Discharging” of the coelenteron is equivalent to a light to dark shift for the coral. The coral zooxanthellae-endosymbionts stop producing oxygen via photosynthesis the instant light is stopped, and the coral uses up most of the available oxygen very quickly bringing the oxygen concentration to a new lower level inside the coelenteron. In the electrical network mode, oxygen concentration corresponds to a voltage, so I rewrote the RC circuit discharge equation to be $[O_2] = [O_2]_0 e^{-\frac{t}{\tau}}$ for a light to dark transition in the coelenteron of a coral. I can then find the time constant of mixing by multiplying the initial concentration of oxygen by $1/e$ to get the concentration of oxygen at the time constant.

The concentration of oxygen within the coral’s coelenteron can be measured by using a Unisense dissolved oxygen microelectrodes (25 μm tip diameter) connected to a Unisense UWM-4 underwater amplifier/data logger. This sensor is a type of Clark-type amperometric sensor that measures oxygen partial pressures and transmits them as a change in current to the amplifier/data logger. The oxygen microsensor is based on diffusion of oxygen through a silicone membrane to an oxygen-reducing cathode, which is polarized against an internal anode. The net oxygen electro-reduction reaction for the microsensor in water is $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$. Microsensors allow for high spatial resolution, low analyte consumption, and have low sensitivity to stirring (minimal artifact

due to turbulence/diffusivity gradients). A diagram of the sensor is shown in Figure 16. Before use, the microsensor has to go through a two-point calibration, at air saturation and at zero oxygen (Figure 17a). The microsensor is positioned using the Narishige M-3333 micromanipulator. Once the microsensor is in place and the oxygen reading has stabilized, the lights are turned off and data are recorded until the oxygen readings stabilize to a new lower value (Figure 17).

3 Results

3.1 Coral Behavior

The coral polyps exhibited a range of behaviors in response to having the microelectrode probe inserted into their mouths. This made it difficult to take consistent measurements because the active volume of the polyp and the degree of mouth openness could have varying affects on the mixing time inside the coelenteron. If too much fresh water was injected into the polyp, it would often provoke the whole colony into either entirely contracting, or the polyp of interest would open its mouth very wide, presumably to flush out the fresh water. The coral's tentacles would often get in the way of the probe, wrap around the probe, and even act as a "guard" by placing themselves in front of the mouth to sense an incoming probe. Even though coral do not have a central nervous system, they possess a nerve net distributed throughout the colony, and a colony's response time was very fast, on the order of milliseconds. Another interesting aspect of coral behavior was observed when the probe had been left inside a polyp for a long length of time (at least 5-10 minutes). The signal from the probe would seem to get noisy and when pulled out, the probe out would be covered in mucus. The coral could be wrapping the probe tip in mucus as a defense or as a way to try to digest the probe. Examples of the

described coral behavior are shown in Figure 18.

3.2 Mixing Time

3.2.1 Conductivity Probe Measurements

The probe was tested various ways to check if it measured the right quantity and was not just the consequence of some rogue electrical signal. The probe was tested in the model polyps (Figure 10). The expected signal from injecting fresh water into salt water-filled model polyps was expected to drop, but not come back up because there was no mixing in the model polyps. This expected signal did occur in all models of the probe. When the probe was placed in the flume and a small amount (~40 μ l) of fresh water was injected into the water around the probe, no visible drop was seen on the oscilloscope, as expected, because the flume is such a large volume and the flow is unidirectional.

I was able to complete 22 measurements inside the coelenteron of *Duncanopsammia axifuga* using the microelectrode probe that I designed and built. One of these measurements was completed a week before the rest and had a much longer time constant of mixing. This measurement was taken with flow of ~0.1 m/s in the flume, and the polyp was completely expanded with a tightly sealed mouth around the probe. It took 19 min 22.00 s for the signal on the oscilloscope to drop and then rise back up to the original level. This corresponded to a mixing time of 12 min 14.38 s. I estimate the error in this measurement to be ± 2 min. I decided on this error because the signal became noisy towards the end of the measurement, when reaching the equilibrium, due to mucus wrapping around the probe tip and because the camera I was using ran out of memory at multiple points and I had to use a stopwatch while the memory card was changed over.

The rest of the measurements are recorded in Table 1. I took 10 measurements with a flow speed of ~0.1 m/s and 11 measurements with no flow. I first created a box plot of the values to compare the results of with flow and without flow, and determined that the measurement of 15.69 s for a time constant was an outlier for the “with flow” data. The measurement was taken in a mostly expanded polyp with a closed mouth, while the majority of the other measurements were taken in polyps that had open mouths. Figure 19 is the boxplot of the data excluding the outlier. I determined an average time constant of mixing for the polyps under the flow condition to be 1.45 ± 0.28 seconds and an average time constant of mixing under no flow to be 6.11 ± 2.82 seconds. To compare the two flow conditions I used a t-test and determined a t-test statistic of -5.45 and a p-value of 0.00026. There is a significant difference between the mixing times under the two flow conditions. The statistics for these measurements can be found in Table 2.

3.2.2 Oxygen Microsensor Measurements

I was only able to complete one run of the light-to-dark shift oxygen microsensor measurements in *Montastraea cavernosa* because the probe broke when we attempted to move to a different polyp. Water conditions were normal the day that the measurement took place, the temperature was 28.9 °C, salinity was 35 PSU, and the pH was 8.1. The oxygen concentration vs. time curve is shown in Figure 20. The error in the measurements of oxygen concentration is ± 0.001 ppm. To determine the mixing time, the data had to be shifted so that the concentration went to zero, like the charge on a capacitor goes to zero when completely discharged. I subtracted the end value from all values to shift the data. This adjusted curve is shown in Figure 21. I calculated the time constant of mixing to be c. 2 min 10 sec for this *Montastraea cavernosa* polyp.

4 Conclusions

The goal of this project was to measure the mixing time inside the coelenteron of a *Montastraea cavernosa* polyp and *Acropora cervicornis* polyp. These preliminary data will be used in electrical network simulations of colonies of different sizes. A lot was accomplished in improving the process of measuring the time constant of mixing in the coelenteron and better understanding mixing in the gastrovascular system. This project successfully designed and built a microelectrode probe to measure the mixing time inside the main gastrovascular cavity of a coral with larger polyps (*Duncanopsammia axifuga*) and developed a differential equation model for oxygen dynamics inside the coelenteron that predicts the trajectory and time constant of oxygen changes during light/dark transitions.

Time constants of mixing varied with *Duncanopsammia axifuga* polyp conditions. In one measurement, the polyp was fully expanded with its mouth tightly sealed around the probe. This measurement determined a mixing time of 12 min 14.38 sec. All other determined mixing times in this coral species were under half a minute. However, they were mostly taken when the coral had varying degrees of its mouth open and polyp expansion. One possible explanation for the one very long time constant measured could be that the coral was trying to digest food particles while the probe was also in the cavity. Digestion in corals has not been studied as well as in other organisms; however, Nicol (1959) measured digestion to take from 8 to 14 hours in a species of sea anemone. It is possible that mixing inside the coelenteron could slow down to allow the mucus to digest the food particles without being disturbed by movement. Jones et al. (1977) described a respiratory rhythm in sea anemones that appears as a release of coelenteric fluid from the

mouth in various time intervals from 43 min to 24 hr. Perhaps corals have a similar mechanism at smaller time intervals because of their smaller body size. This could possibly account for some of the variation in mixing times. Gastrovascular mixing in corals may be more complex than originally predicted and changes in the electrical network model will be made to account for these complexities.

Flow is known to affect many aspects of coral physiology, so it is no surprise that it has an effect on the mixing time when the gastrovascular space is exposed to external flow. The two measured flow conditions have significantly different time constants of mixing. The mixing time inside *Duncanopsammia axifuga* is approximately 4 times longer in a “no flow” environment than in an environment with flow speeds ~ 0.1 m/s. It is possible that when the flow is on, water flowing by the open mouth of the polyp is able to flush out the fresh water inside the coral more quickly. The coral could be taking advantage of some sort of induced flow produced by the external moving fluid. Current induced flow was first observed *in situ* in sponges by Steven Vogel (1977). There could also be a shear force that helps to speed up the internal mixing under flow conditions. Varying the flow speeds and taking more measurements of the mixing time could determine a relation between external flow speed and mixing time.

The differential equation model for describing oxygen dynamics inside the coelenteron during a light dark shift gave a mixing time of 2 min 10 sec inside a *Montastraea cavernosa* polyp. This measurement has no repetition because the glass microsensor broke after the measurement. More measurements must be taken to achieve statistical significance for this value of the mixing time. However, this measurement does give us some valuable insight into gastrovascular mixing. Initial estimates of mixing time

for an imperforate polyp made by Dr. Patterson using literature-derived values were around 21 min. Components of the electrical network model were based on this estimate and will be greatly affected if the mixing time is really on the order of the time determined from this differential equation model and my initial measurements. It is tempting to compare the times measured with the oxygen microelectrode probe and the conductivity probe, and the time constants for the two species tested with these different technologies were similar within a factor of 2-4 (cf, Fig. 19). The probe that I designed is much larger than the glass oxygen microsensor; presumably this puts it closer to the ciliated covered mesenteries that drive the mixing inside the gastrovascular space. When the light/dark transition occurs and the zooxanthellae stop producing oxygen, the remaining oxygen has to diffuse through the tissues and coelenteric fluid before it reaches the microsensor. A relation between the two methods of measuring mixing time could possibly be determined with further research.

This project has made great strides in making gastrovascular mixing measurements inside coral polyps. The microelectrode probe is a novel way to measure gastrovascular mixing using a conductivity sensor connected to a transimpedance amplifier that converts small current flows between the microelectrodes into a voltage that can be monitored during the injection of water with an altered salinity level. Future research and development will be done to further improve this probe design to work well in smaller sized polyps. Once more oxygen microsensors arrive in lab, we will take more measurements inside the coelenteron to further test the differential equation model for oxygen dynamics during a light/dark transition. This method will then be applied to *Montastraea cavernosa* and *Acropora cavernosa*, the original imperforate and perforate

species of interest. Once measurements of the time constant of mixing are made in these species, more conclusions can be made about the differences of gastrovascular mixing in imperforate and perforate coral species and how the two types will respond to changing ocean conditions. Previously, very little research has been done on the gastrovascular system in scleractinian coral. Before now, the time scale of gastrovascular mixing time was unknown. I determined that the mixing time is on the order of seconds to minutes. This results shows that corals may be able to respond faster than the environment can change. Oxygen concentrations over a coral reef change on a diurnal time scale and also during internal wave events. These changes both occur on longer time scales than that of the mixing times that I measured. This project has enhanced our view of ventilation and mixing in the main cavity of the gastrovascular system. By understanding the gastrovascular system in imperforate and perforate corals, we can better predict physiological reactions of corals to environmental stresses brought about by climate change.

5 Future Directions

5.1 Inferring an Allometric Size Limit for Polyp Size in Anthozoans

My first measurement of the large-polyp species, *Duncanopsammia axifuga*, under perfect conditions (probe inserted inside, the mouth forming a seemingly tight seal) determined a mixing time of around 12 min. Although this measurement has not been repeated, this finding could have significant impacts in understanding polyp size limits. As the volume of the coelenteron increases, the surface area of the ciliated mesenteries increases as the second power of height or radius. These ciliated mesenteries are the primary mixing drivers in the gastrovascular system. We expect the time constant of

mixing for a smaller sized polyp, *Montastraea cavernosa*, to be on the order of 0-3 minutes, based on the differential equation model for oxygen dynamics during a light/dark shift. There could exist an inherent limit to polyp size due to the ability of the coelenteron to mix fluid that can be supported in scleractinians and their close relatives the actinians (sea anemones). Dr. Patterson (1992) predicted polyp geometric dimensions to be diffusively similar, whereby polyps should change their diameter to height ratio in such a way as to keep the normalized diffusive flux into the polyp the same, but his theory has never been fully tested. More data on mixing times in a wide range of polyp sizes will be collected to test this possible upper limit to size, and we may be able to infer an allometric limit to size based on the mixing time constraints.

5.2 Modeling the Effects of Mesenteries: A Mass Transfer Approach using a Wind Tunnel

It is possible that the orientation of the mesenteries may have an affect on the mixing time within the coelenteron. The mesenteries might rearrange themselves into different compartments that could break up the mixing. Since it would be difficult to see what is going on inside the polyp when the probe is taking measurements, a new method might be needed if a lot of variation is seen in multiple trials of determining the time constant. A physical model of the coral polyp could be built to study how the changing compartment sizes might affect the flow in the gastrovascular system.

When there are two geometrically similar vessels in different mediums with the same boundary conditions and Reynolds numbers, dynamic similarity states that the flows will be identical. Reynolds number, Re , is a dimensionless value that is the central

scaling parameter for biological fluid mechanics and gives the ratio of inertial forces to viscous forces.

$$Re = \frac{UL}{\nu}$$

U is the speed of the fluid, L is the length of interest, and ν is the kinematic viscosity of the fluid. If the Reynolds number of an object in water is equal to the Reynolds number of a similar object in air, then the two flow patterns are qualitatively the same. Helmuth et al. (1997) used the concepts of Reynolds number and dynamic similarity to build a physical model to study mass flux of coral branches. The researchers measured water loss rates from scale models in air as proxies for gas flux from corals in water. Helmuth et al. (1997) lined the “coral branches” with soaked filter paper and measured the weight change of the branch after being put through a wind tunnel.

A physical model of a coral polyp could be built with movable mesenteries. The physical model of the coral polyp would be scaled so that its Reynolds number is equal to that of a polyp in water of velocity 10 cm/s, a typical flow speed over a coral reef. Preliminary geometric analysis has determined the Reynolds number of an extended *Montastraea cavernosa* polyp to range from ~309 to ~1049 with differing levels of polyp expansion. Small fans could be placed at the inside base of the polyp to simulate mixing by cilia, and the model would be placed in a wind tunnel for external flow. The walls of the coelenteron could be lined with filter paper soaked in water. Before and after the trial, the model would be weighed to measure the amount of water that evaporated during the trial. Placement of mesenteries could be changed inside the model to see how their configuration affects the time constant. This physical model could determine a time

constant of mixing for the gastrovascular system if there is too much variation in the electrode measurements due to variable mesentery configurations inside the polyp.

5.3 Effects of other Physiological Conditions and Environmental Factors on Mixing Time

Coral disease and bleaching is a topic of great interest to me. My main work in this field of study has been on using spatial epidemiology to describe Caribbean yellow band disease dynamics in two species of reef building coral around the island of St. Croix, USVI, with Dr. Erinn Muller from Mote Marine Laboratory. Various research has supported that disease and bleaching affect different physiological processes in coral such as reproduction and growth rates. I would like to use my method of measuring mixing time to determine if different coral health conditions have different affects on the coral gastrovascular system. I would measure the mixing time in polyps as a function of radial distance from the center of a diseased area. I expect to either find decreased times in the diseased area due to the disease killing off the coral or to find increased times because the coral could be attempting to shift more resources to the damaged area to heal itself. This method could also be applied to coral tissue injury due to fish, boats, or humans.

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8 Appendix

8.1 Figures and Tables

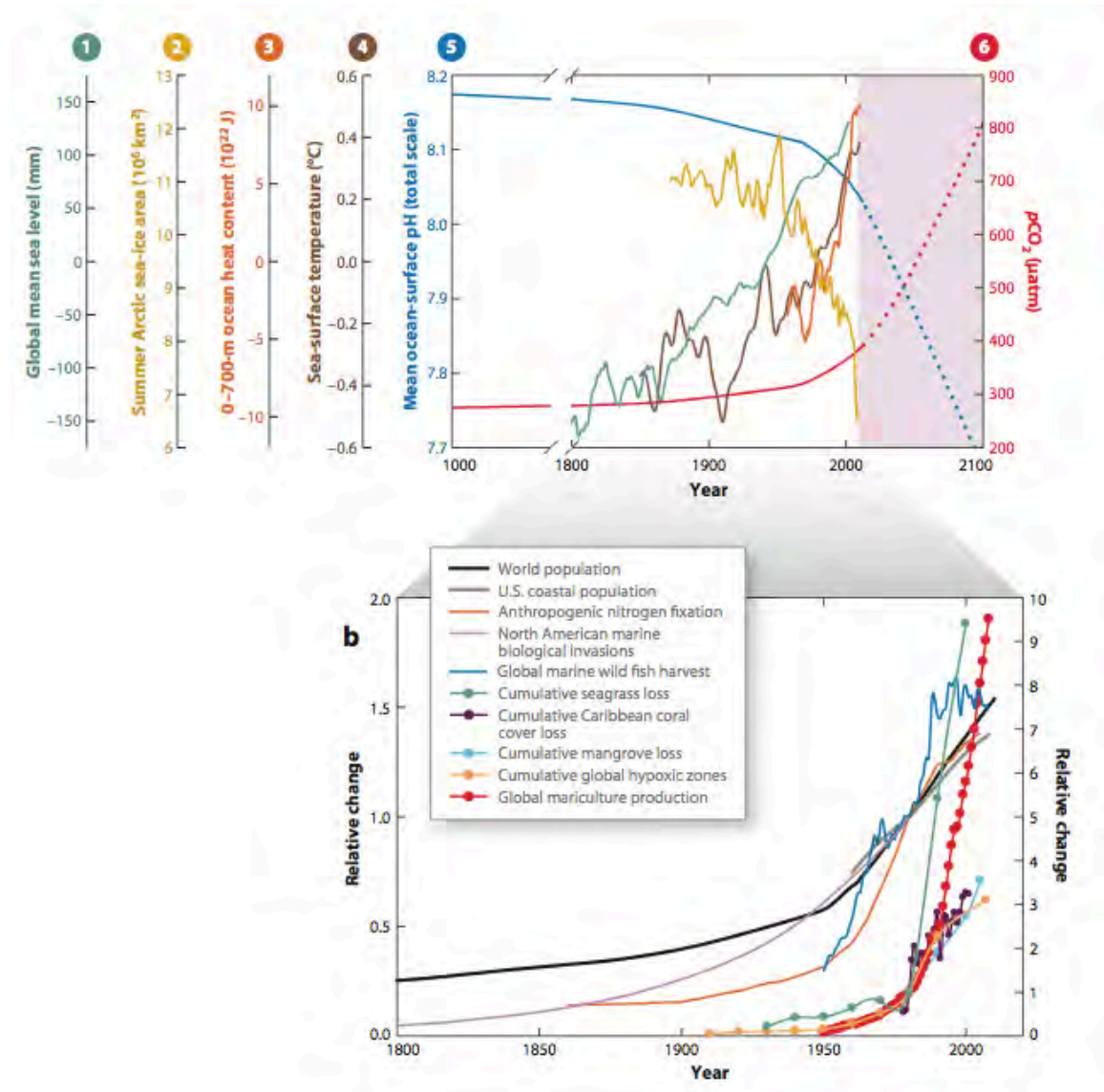


Figure 1: Global environmental trends and their consequences over time (Doney et al., 2012)

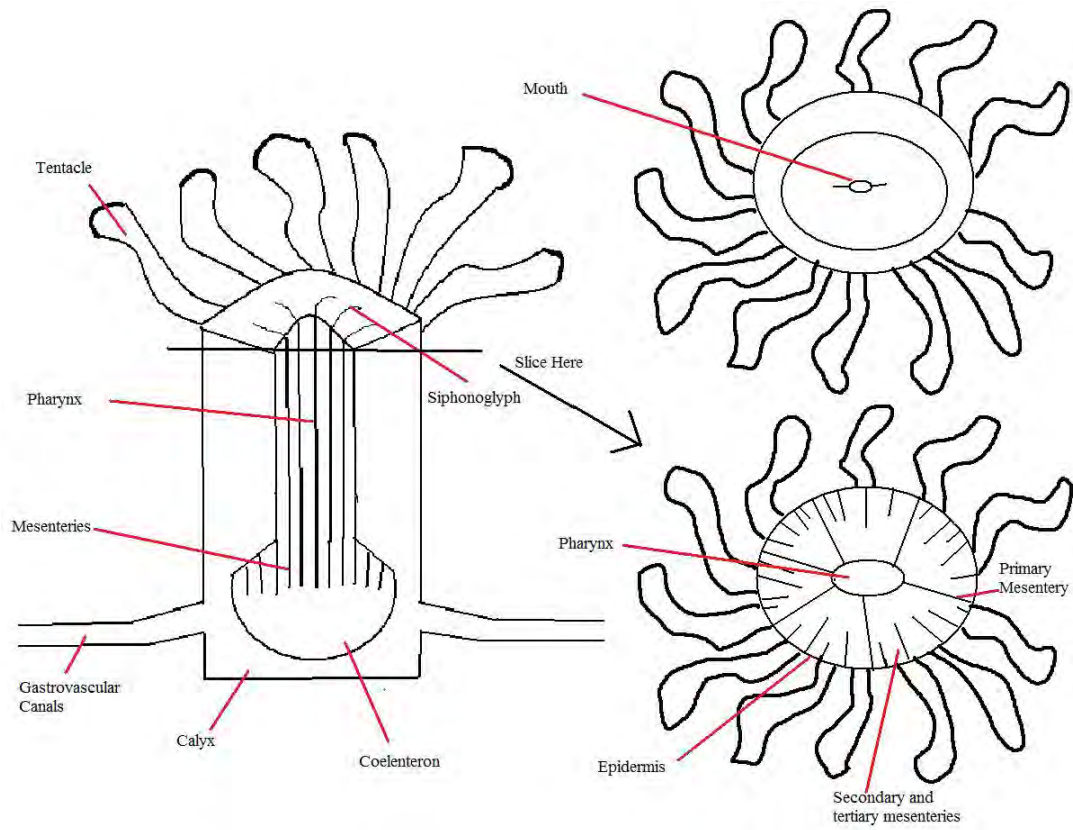


Figure 2: Anatomy of a Coral Polyp



Figure 3: a) *Montastraea cavernosa* polyp

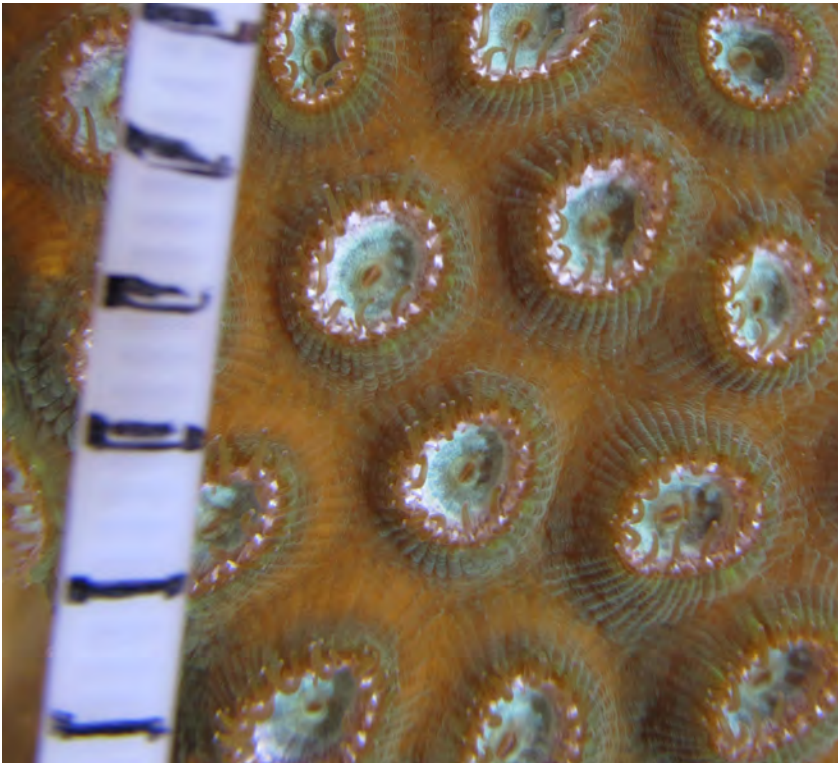


Figure 3: b) Section of a *Montastraea cavernosa* colony, distance between black scale lines is 5 mm.

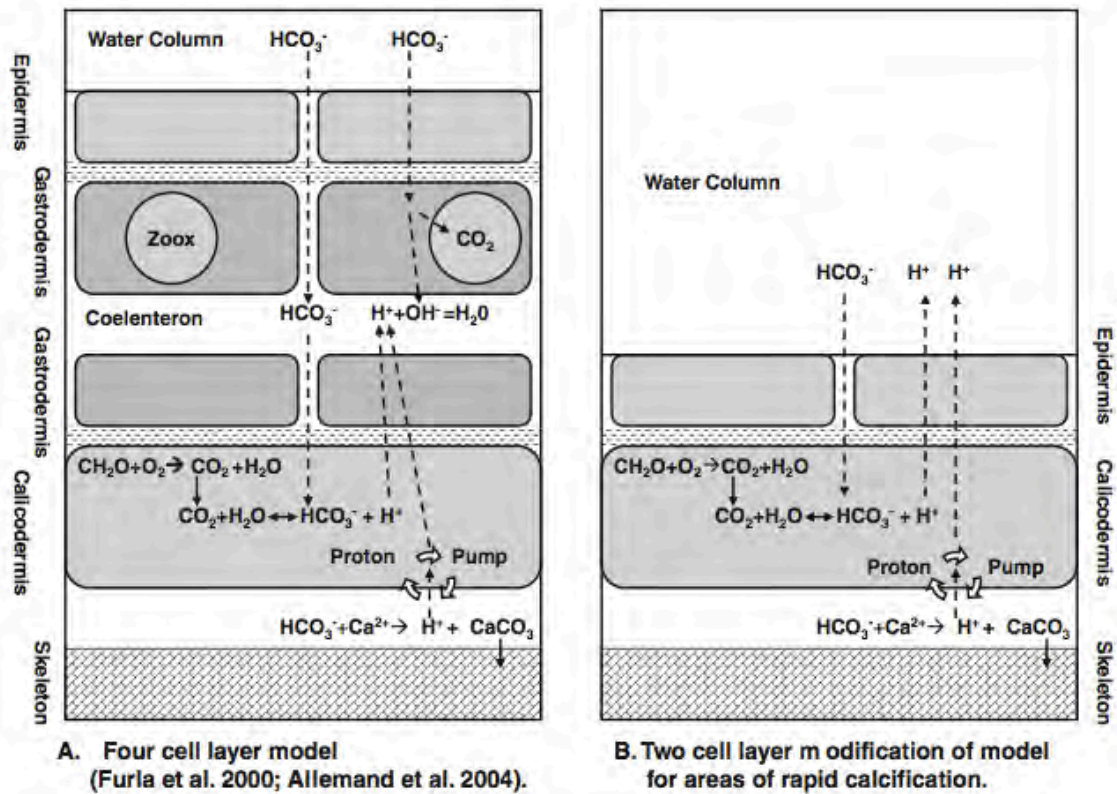


Figure 4: A) Classic four cell layer model of calcification (Allemand et al., 2004; Furla et al., 2000a) B) Model modified to fit a two cell layer structure of rapidly calcifying areas of the corallum as described by Brown et al. (1983), Gladfelter (1982), and Tambutte et al. (2007). (Both figures from Jokiel, 2011)

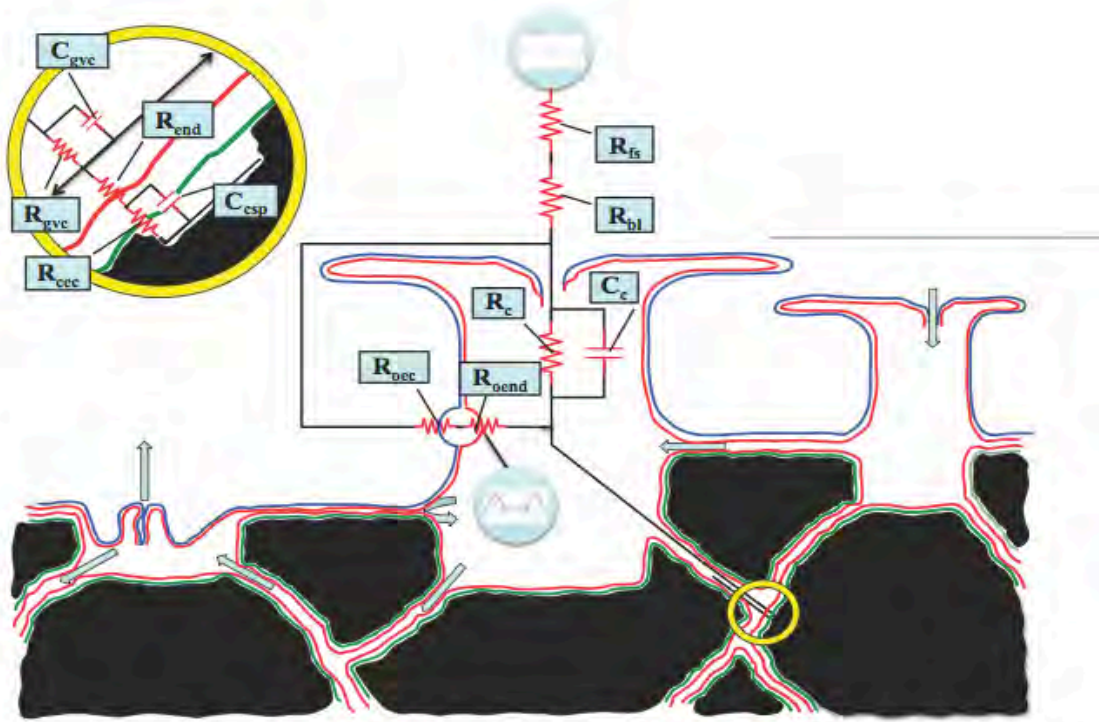


Figure 5: Electrical network model of a perforate coral, two expanded and one contracted polyps shown. Blue arrows indicate fluid movement. Yellow circle inside coral skeleton is shown in magnified view in upper left. Circle exterior to the system (sine wave inside) is a signal generator representing diel fluctuations in oxygen concentration. Circle intercepting R_{oend} is a signal generator (half sine wave inside) representing diurnal generation of oxygen by the zooxanthellae located in this layer. R_{fs} = resistance of freestream (geophysical) boundary layer, R_{bl} = resistance of colony boundary layer, R_c = resistance of polyp coelenteron, R_{gvc} = resistance of gastrovascular tunnels between polyps R_{oec} = resistance of oral ectoderm, R_{end} = resistance of oral endoderm, R_{eeec} = resistance of calicoblastic ectoderm, C_{csp} = capacitance of the calicoblastic space, C_c = capacitance of the polyp coelenteron, C_{gvc} = capacitance of the gastrovascular tunnels between polyps. Figure credited to Drs. Mark Patterson, Elizabeth Gladfelter, and Lawrence Carpenter.

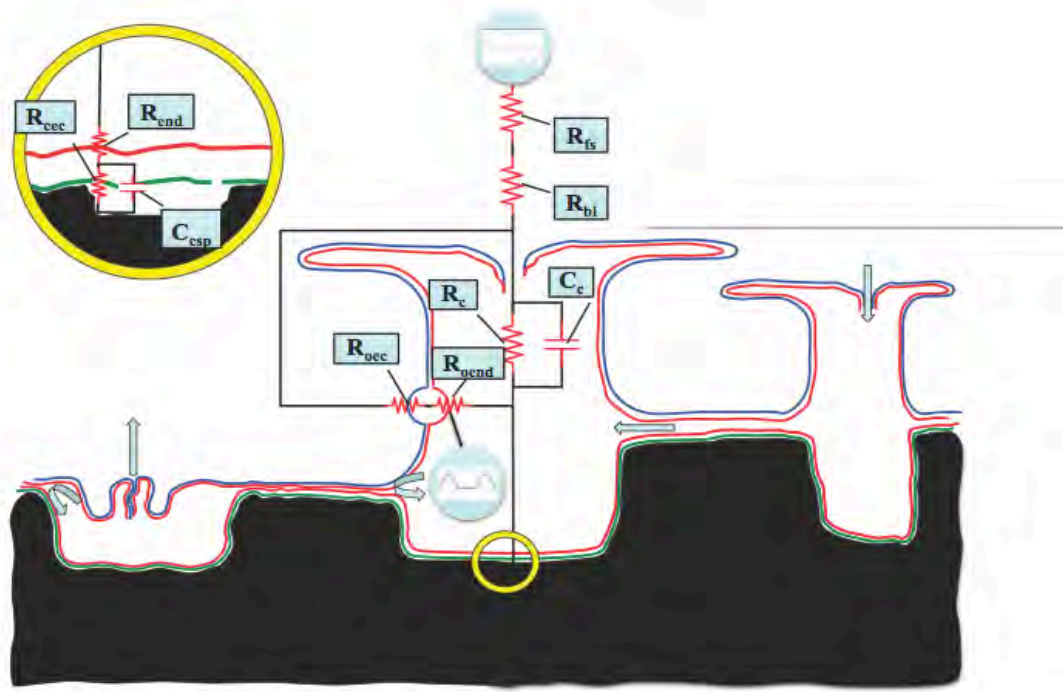


Figure 6: Electrical network model of an imperforate coral, two expanded and one contracted polyp shown. Blue arrows indicate fluid movement. Yellow circle at base of coelenteron is shown in magnified view in upper left. Circle exterior to the system (sine wave inside) is a signal generator representing diel fluctuations in oxygen concentration. Circle intercepting R_{oend} is a signal generator (half sine wave inside) representing diurnal generation of oxygen by the zooxanthellae located in this layer. R_{fs} = resistance of freestream (geophysical) boundary layer, R_{bl} = resistance of colony boundary layer, R_c = resistance of polyp coelenteron, R_{gvc} = resistance of gastrovascular space between polyps (when polyps are expanded), R_{oec} = resistance of oral ectoderm, R_{end} = resistance of oral endoderm, R_{cec} = resistance of calicoblastic ectoderm, C_{csp} = capacitance of the calicoblastic space, C_c = capacitance of the coelenteron. Figure credited to Drs. Mark Patterson, Elizabeth Gladfelter, and Lawrence Carpenter

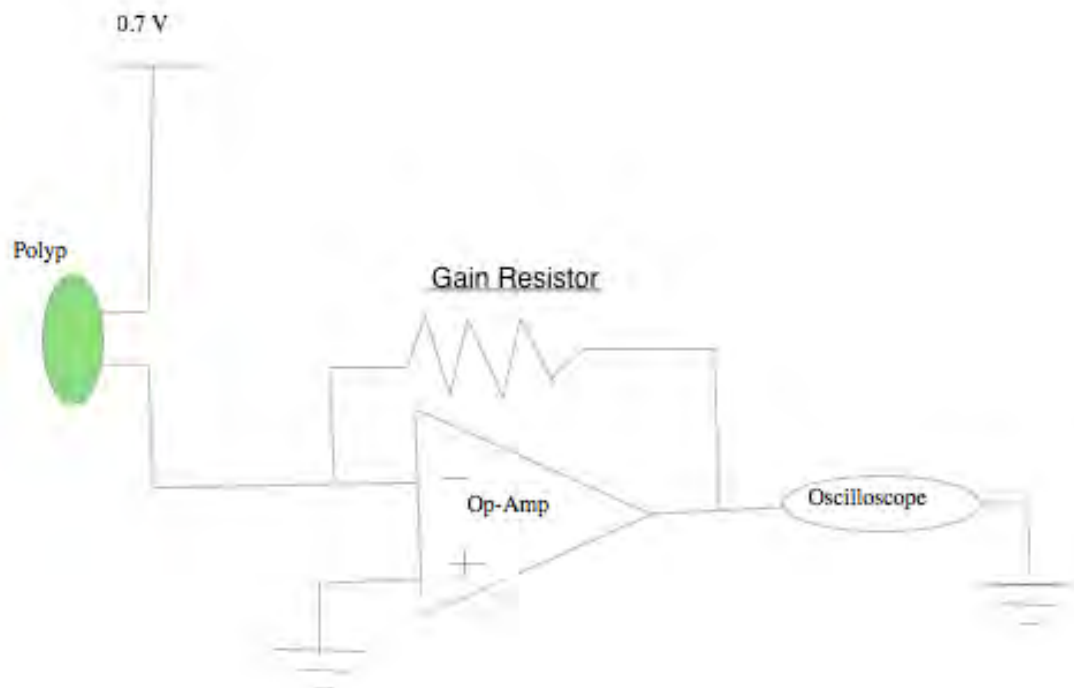


Figure 7: Transimpedance op amp circuit designed to convert current flowing between sensor electrodes (the two wires entering the polyp above) into a voltage change observable on an oscilloscope. The system allowed measurement of mixing time when perturbations to salinity were introduced into the polyp using microcatheter tubing. The op amp was a Linear Technology 1001 precision op amp. The gain resistor was set to 689 kOhm. The sensor polarization of 0.7 VDC was set by a potentiometer circuit.

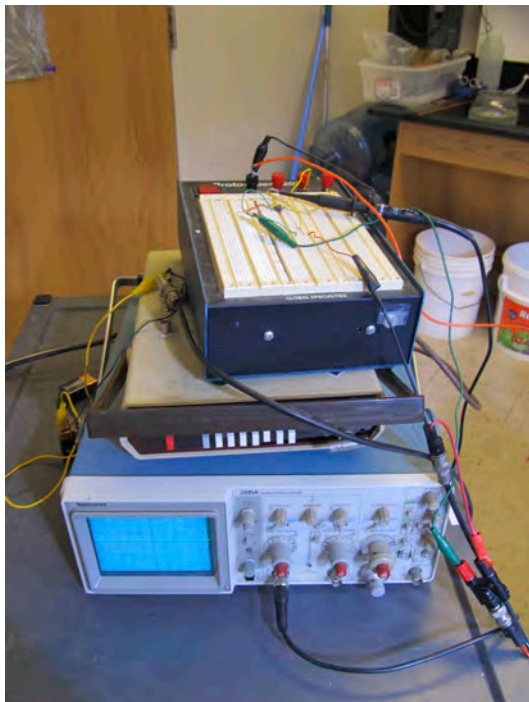


Figure 8: Electronics used to measure mixing time. From the bottom up: oscilloscope, pico-ammeter containing the potentiometer (sensor polarization) sub-circuit, and breadboard with the transimpedance amplifier circuitry.

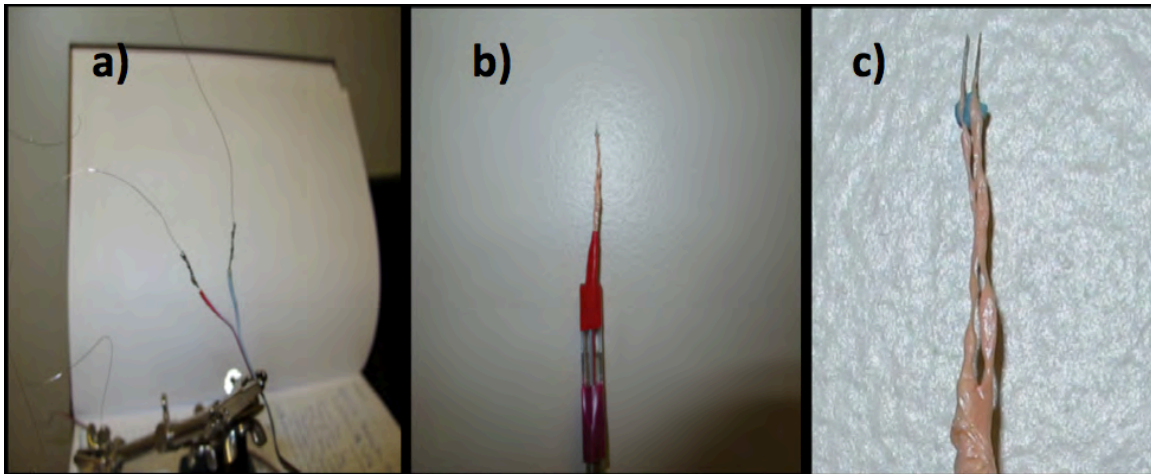


Figure 9: Conductivity probe: first generation. Probe A. a) Aluminum wires soldered to 22 gauge insulated wires b) Probe from a distance c) Close up of conductivity microelectrode tip. Gap c. 0.5 mm. Probe A was a proof-of-concept design and was not used to make any actual measurements.

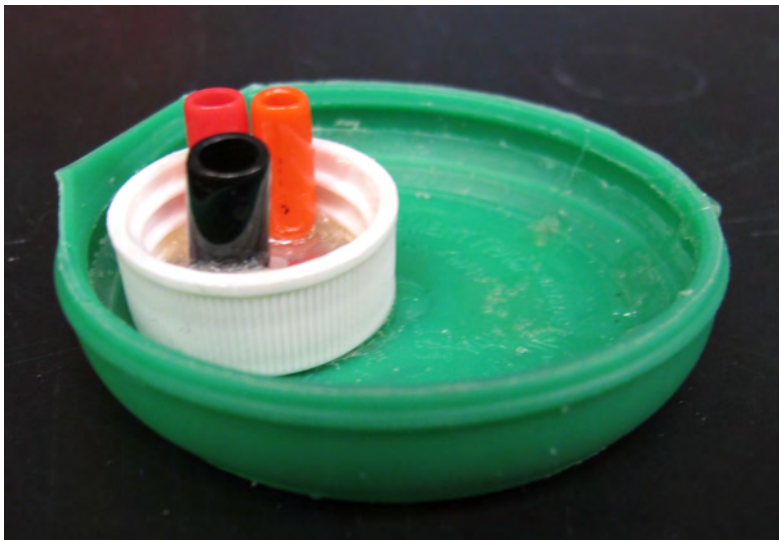


Figure 10: Model polyps used to test the conductivity microprobe. Models were filled with salt water and then the probe measured a change in conductivity when fresh water was added to the models.

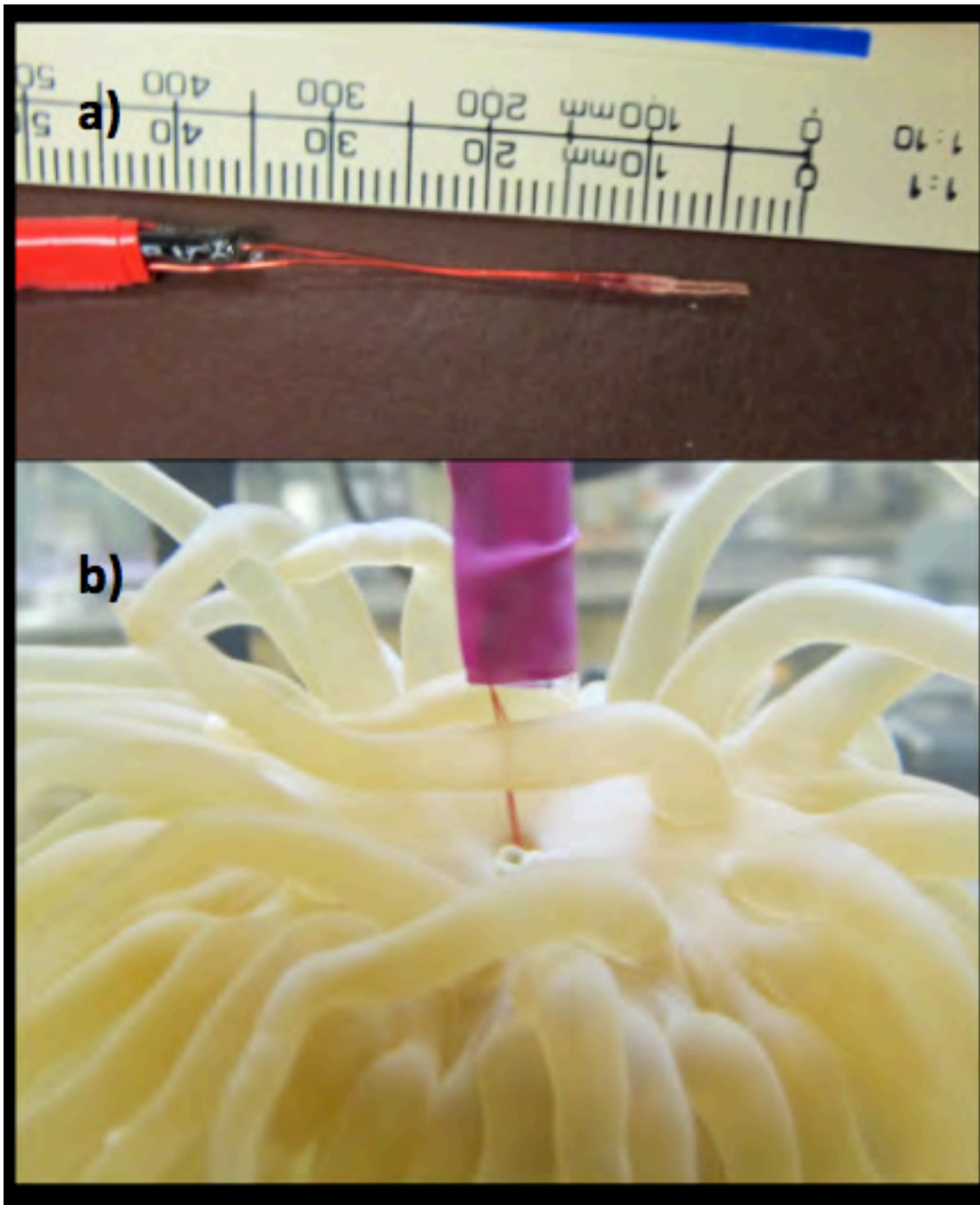


Figure 11: Probe B, second generation. a) Microelectrode end b) Probe B inserted into a large sea anemone, *Condylactis spp.*, during initial testing and protocol development. The second probe consisted of two wires (0.2 mm diameter; 32 mm long) spaced 0.25 mm apart, with 4.35 mm at the tips of the wires exposed to the seawater, with the remaining length insulated. Probe B was the first probe design to use magnet wire, which got rid of the need to use nail polish as an insulator. It also used cyanoacrylate glue as a spacer between the wires.

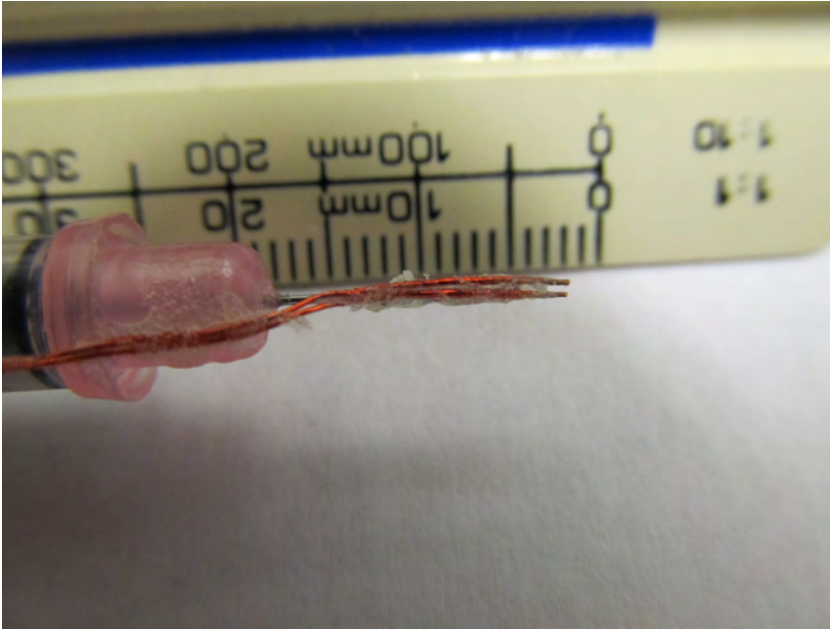


Figure 12 a): Tip of Probe C.

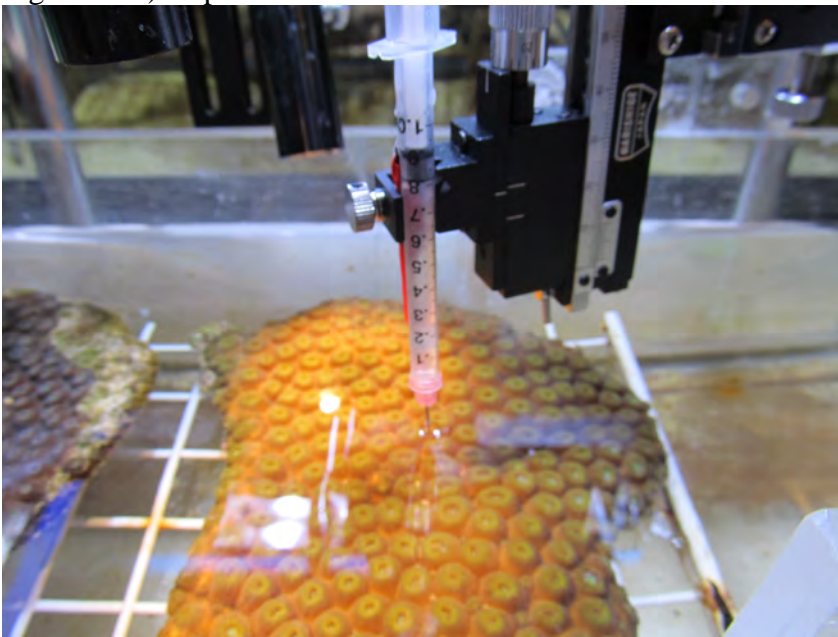


Figure 12 b): Probe C being positioned by micromanipulators above a colony of *Montastraea cavernosa*.



Figure 12 c): Probe C inside of a *Montastraea cavernosa* polyp. The spacing between the wires was 0.47 mm and the conducting section of the wires was 0.78 mm long. Probe C was combined a syringe with the wiring from Probe B to be able to inject fresh water and measure the change with the same probe tip. However it was not streamlined enough to work properly.

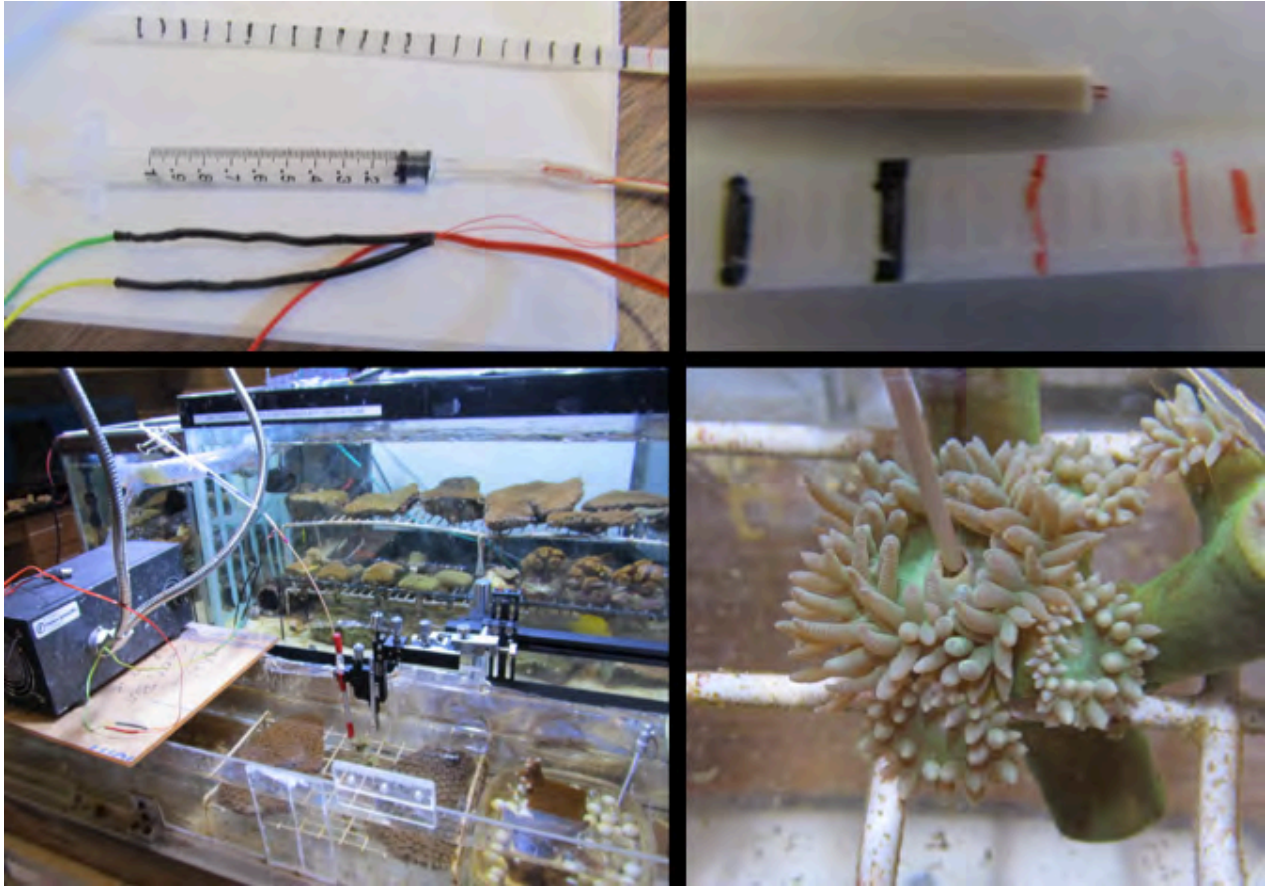


Figure 13: Probe D, fourth generation conductivity probe. Top left shows the syringe to PEEK tubing connection. Top right shows the probe end. The ends of the wires emerged out 0.42 mm from the end of the tubing and were separated by 0.26 mm. Bottom left shows the entire experimental set up with probe and micromanipulators over the flume. Bottom right shows the probe inside of the mouth of a *Duncanopsammia axifuga* polyp. This probe worked very well for the larger polyp species, however it does not fit inside *Montastraea cavernosa* or *Acropora cervicornis*.

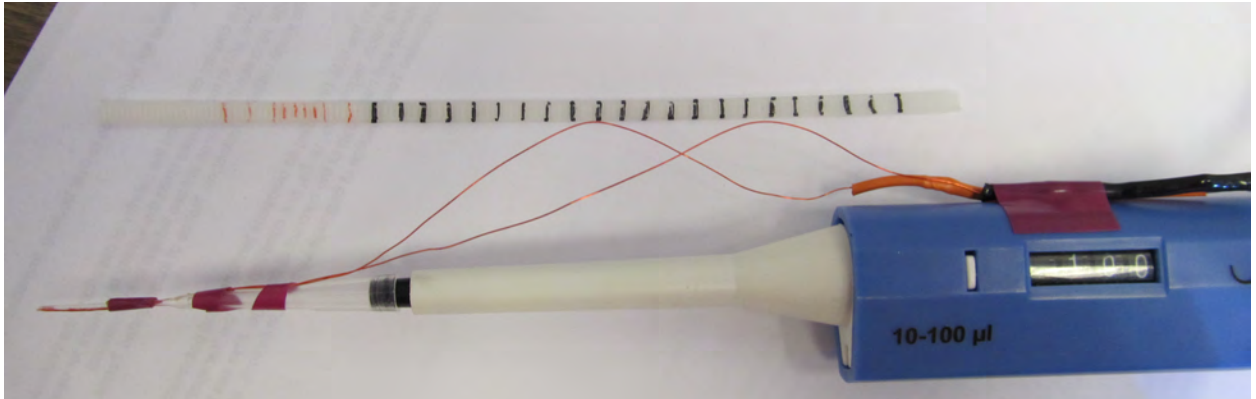


Figure 14 a): Probe E, fifth generation. I used the same wire configuration from Probe C and attached it to the end of a pipet tip. The space between the wires was 0.37 mm and the conducting part of each wire was 0.54 mm long.

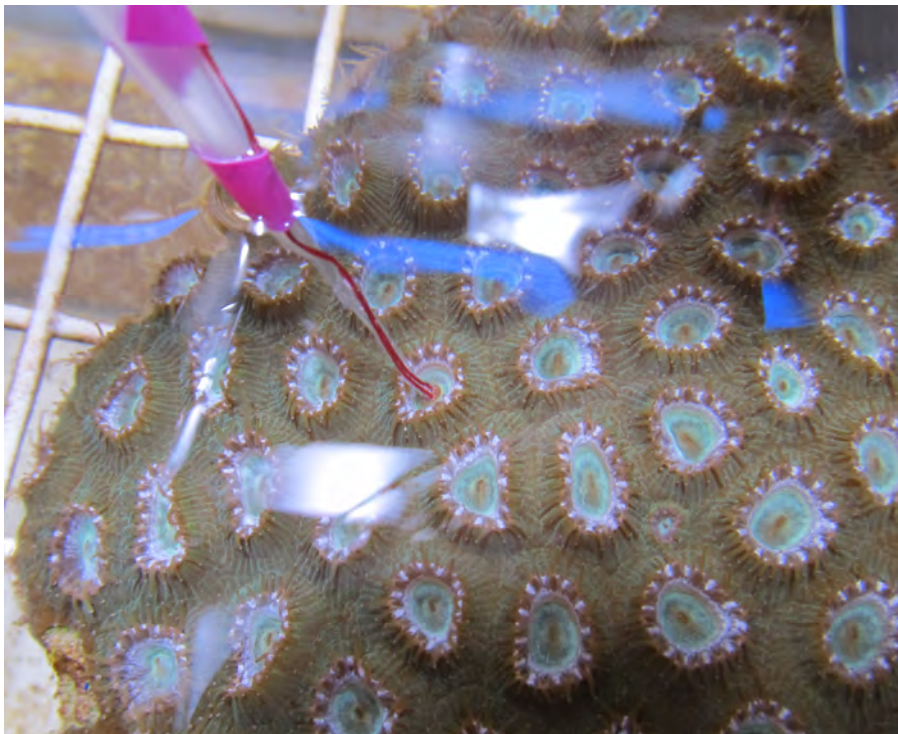


Figure 14 b): Probe E, fifth generation, inside of *Montastraea cavernosa*.

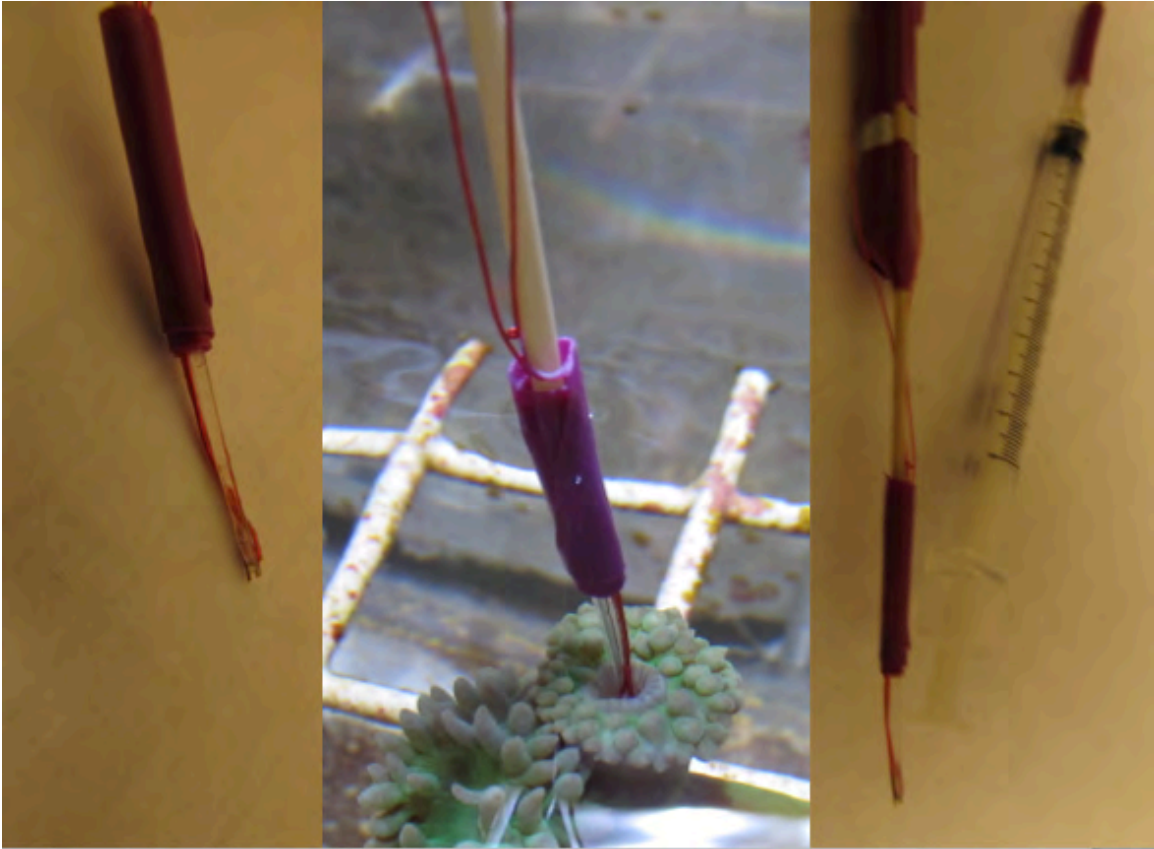


Figure 15: Probe F, sixth generation. The thin tip fits inside of both *Duncanopsammia axifuga* and *Montastraea cavernosa*. The tubing combined with the syringe allows for multiple runs in the same polyp and better stability. The space between the wires was 0.48 mm and the length of the conducting wire section was 0.50 mm. The magnet wires run the length of the tube on the outside

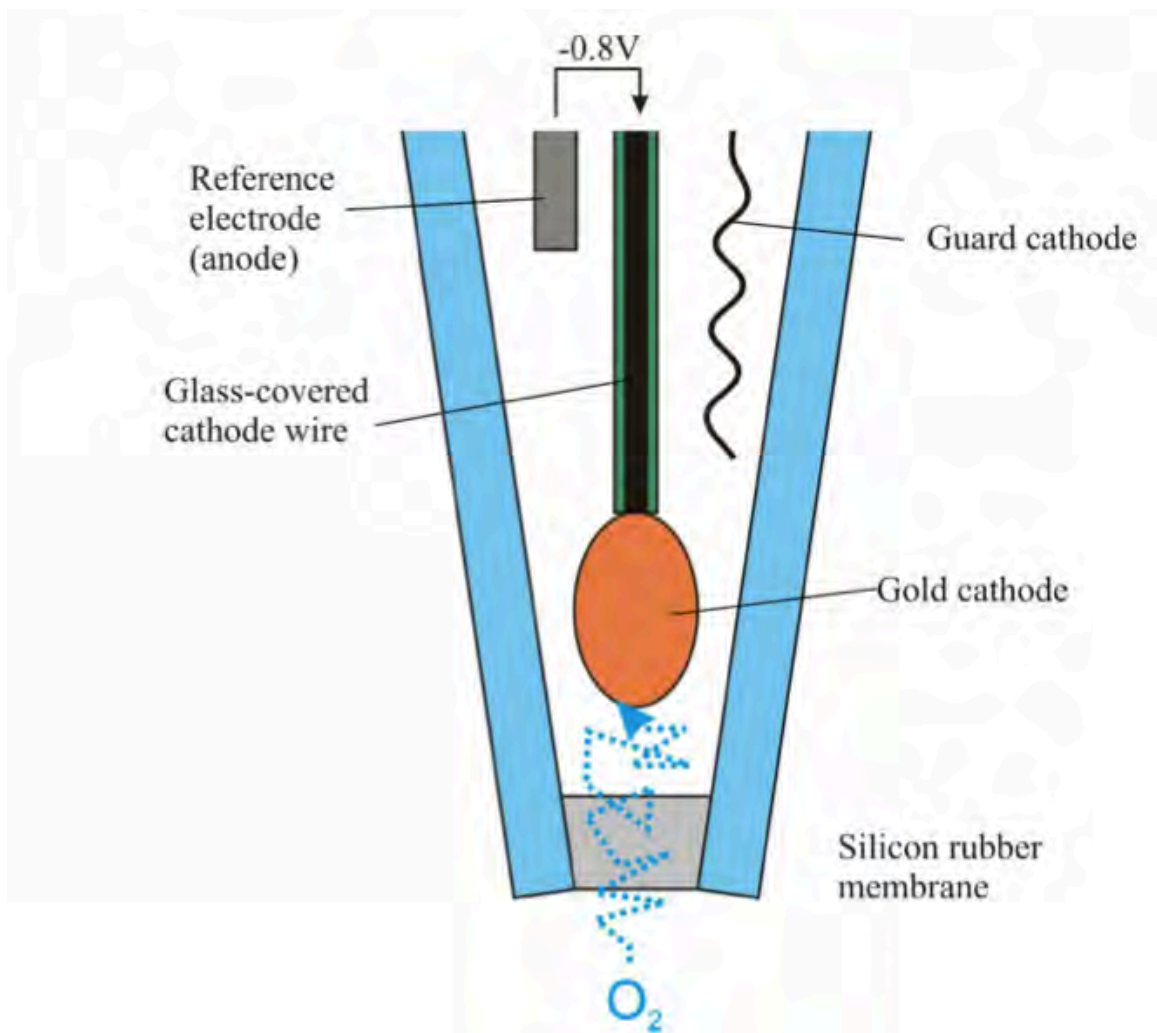


Figure 16: Unisense dissolved oxygen microsensor diagram. Entrance to microsensor is $25\ \mu\text{m}$ in diameter. Response time is c. 0.2-0.4 sec. Oxygen is consumed at cathode. Guard cathode keeps electrolyte reservoir beyond sensing cathode close to zero concentration of dissolved oxygen.



Figure 17 a): Oxygen microsensor inside *Montastraea cavernosa*.



Figure 17 b): Unisense underwater data logger and electrode amplifiers.

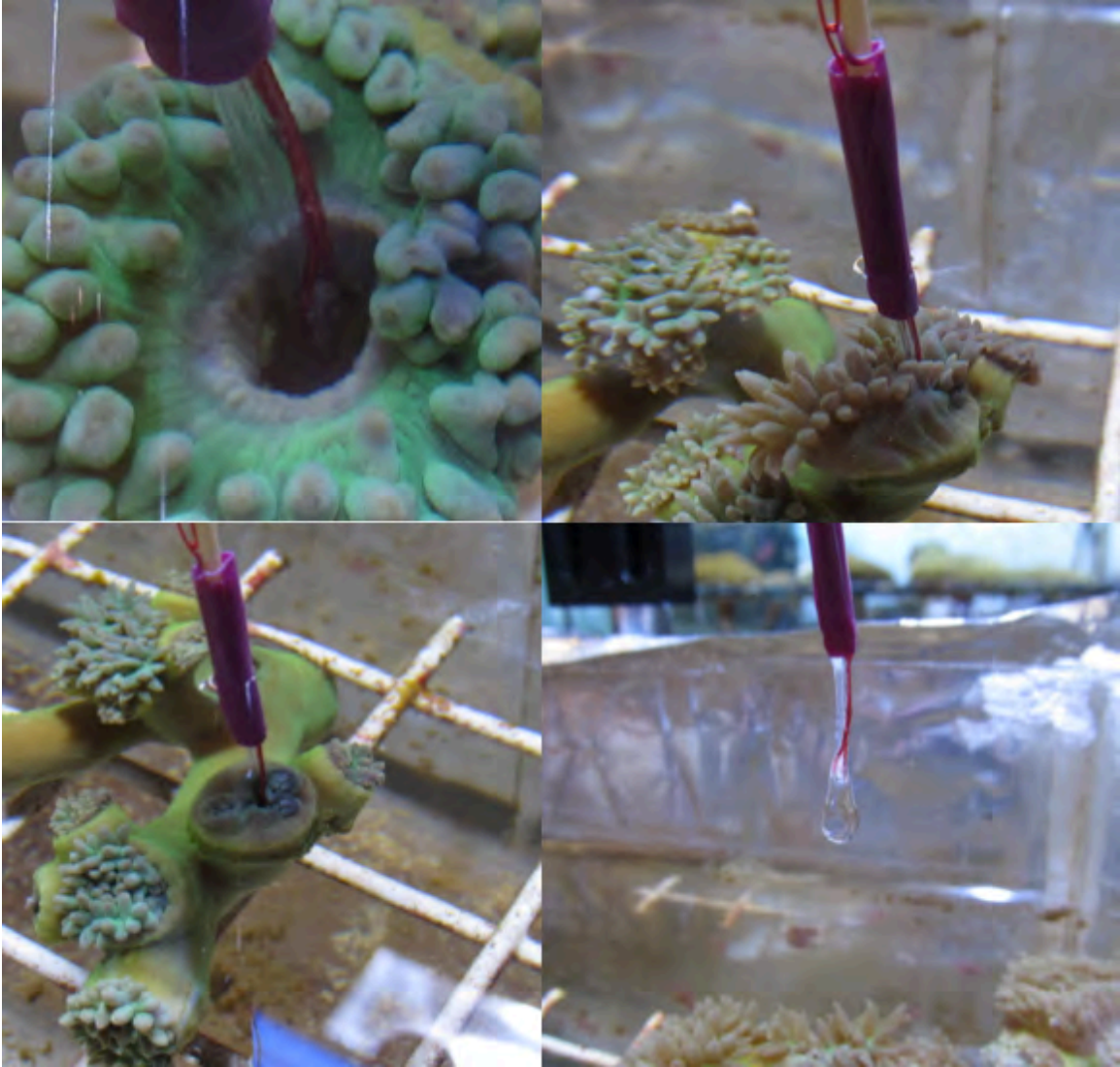


Figure 18: *Duncanopsammia axifuga* with conductivity Probe F. Top left: mouth open. Top right: polyp squished. Bottom left: contracted polyp. Bottom right: coral mucus covering probe after an extended measurement time.

Flow Condition	Total time of observation (s)	Time Constant (s)	Polyp Condition
No Flow	5.7	3.58	Mouth not open, very contracted
No Flow	4.7	2.97	Mouth slightly open, mostly expanded polyp
No Flow	7.8	4.93	Could not tell if mouth open, tentacle in the way, polyp expanded
No Flow	12.8	8.07	Mouth slightly open
No Flow	4.7	2.97	Mouth slightly open
No Flow	10	6.32	Mouth slightly open, mostly expanded polyp
No Flow	5.8	3.69	Mouth slightly open, mostly expanded polyp
No Flow	15.0	9.48	Tentacle hiding mouth, polyp more contracted
No Flow	8.3	5.25	Mouth open, Polyp is very squished
No Flow	16.4	10.36	Mouth is open
No Flow	15.1	9.54	Mouth is open
Flow (~0.1 m/s)	2.8	1.79	Mouth opened after FW injection, polyp expanded
Flow (~0.1 m/s)	1.9	1.18	Mouth open slightly, opened larger after injection, polyp expanded
Flow (~0.1 m/s)	2.2	1.39	Mouth is sunken and contracted, but still open
Flow (~0.1 m/s)	2.1	1.33	Mouth open slightly, polyp expanded
Flow (~0.1 m/s)	2.3	1.45	Mouth open slightly, polyp expanded
Flow (~0.1 m/s)	2.3	1.47	Mouth open slightly, polyp expanded
Flow (~0.1 m/s)	2.9	1.83	Mouth open very wide, polyp mostly expanded
Flow (~0.1 m/s)	2.6	1.64	Mouth slightly open, tentacles obscuring view, polyp expanded
Flow (~0.1 m/s)	1.5	0.95	Mouth open slightly, polyp closed
Flow (~0.1 m/s)	24.8	15.69	Mouth closed, but opened towards end, polyp mostly expanded

Table 1. Table of measurements performed using polyps of *Duncanopsammia axifuga*.

Flow Condition	Average time constant (s)	Standard deviation (s)	t-test statistic	p-value
Flow (~0.1 m/s)	1.449	0.284	-5.45	0.00026
No Flow	6.106	2.815		

Table 2: Statistics for mixing time in *Duncanopsammia axifuga*.

Distribution of mixing times for different flow conditions

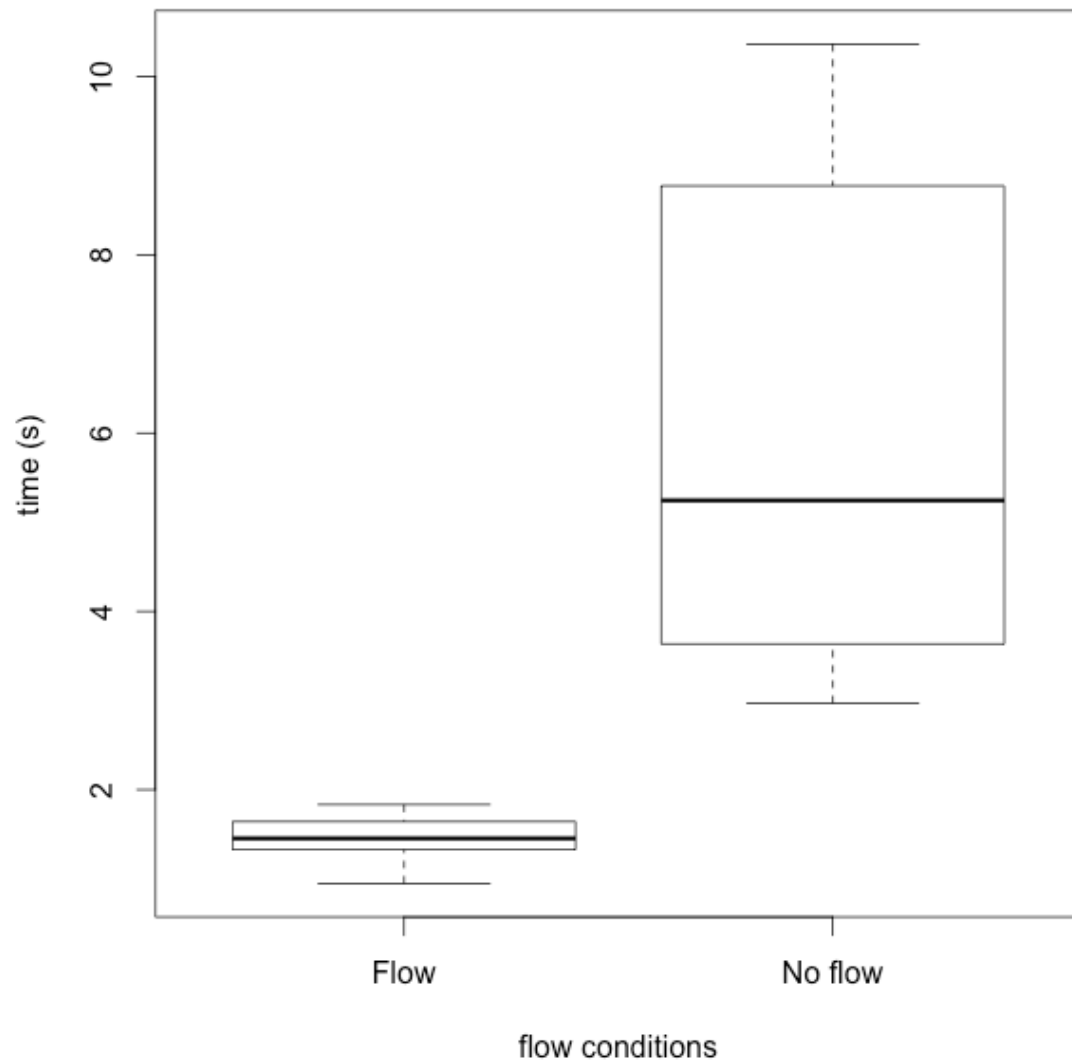


Figure 19: Boxplot distribution of mixing times under two different flow conditions. The average time constant of mixing for the polyps under the flow condition was 1.45 ± 0.28 seconds and the average time constant of mixing under no flow was 6.11 ± 2.82 seconds.

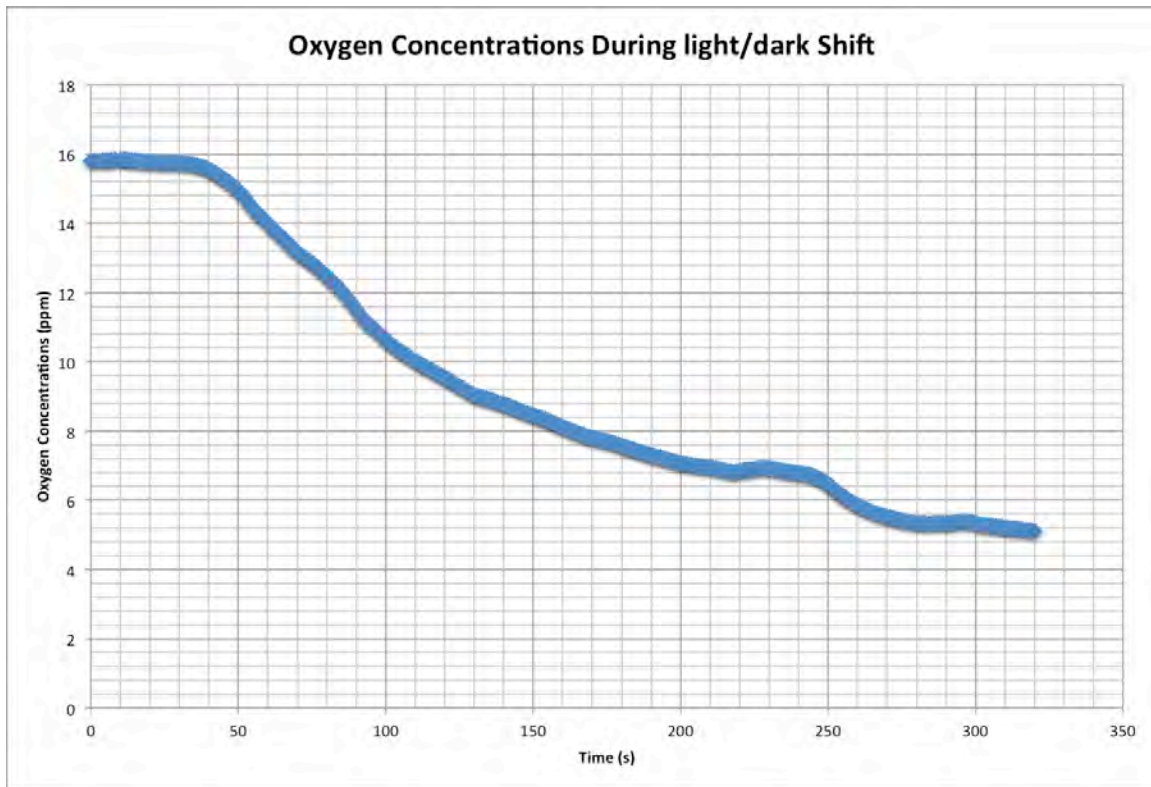


Figure 20: Oxygen concentrations during light/dark transition in a *Montastraea cavernosa* polyp. Error in O₂ concentration measurements is 0.001 ppm. Light is extinguished at c. 12 sec.

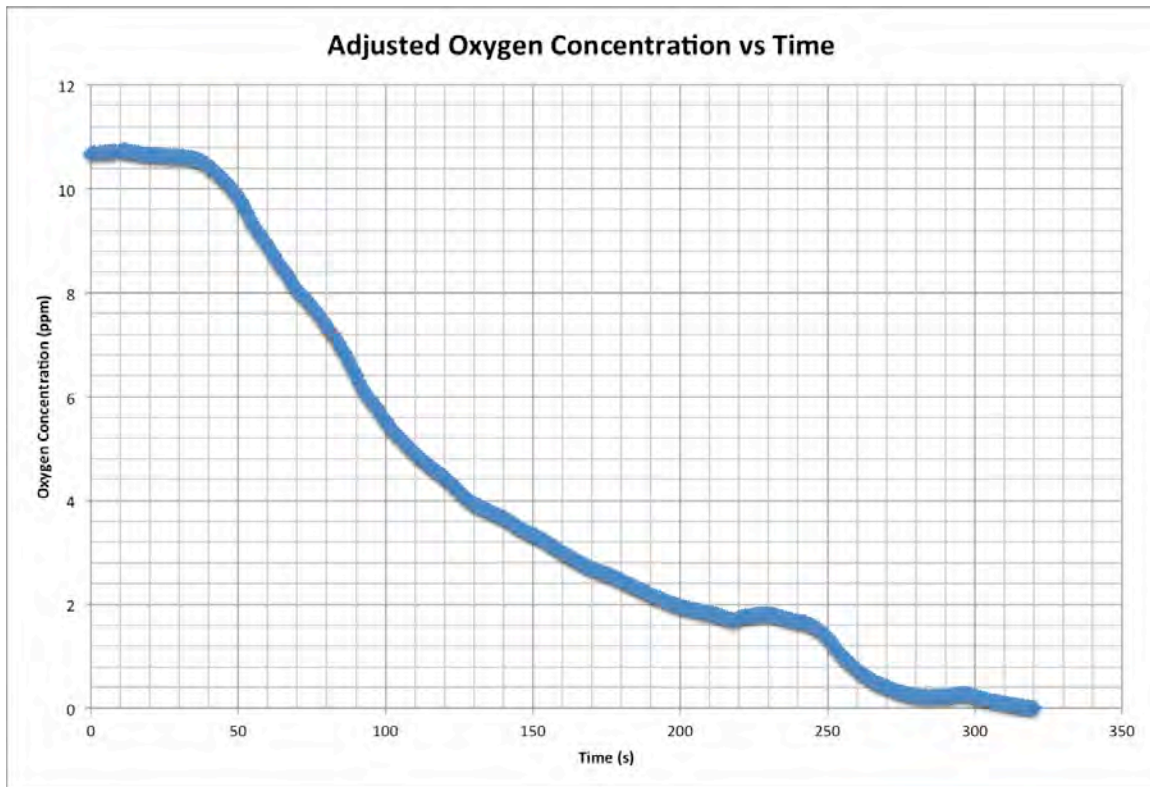


Figure 21: Adjusted oxygen concentration curve used to determine the time constant of mixing using oxygen microsensor measurements. The curve was adjusted by subtracting the lowest oxygen value from every measurement in order to relate it to a discharge of a capacitor from full charge to no charge.

8.2 Mixing Time Measurement Procedure with Probe

1. Get the corals to expand their polyps; Try the following:
 - a. Turn off all overhead lighting.
 - b. Turn off flume pump.
 - c. Give a few drops of food. Not too much, because if they eat, then they will close up again.
 - d. Be patient.
2. Set up micromanipulators and probe
 - a. Load syringe with fresh water.
 - b. Double check tightness of all micromanipulator knobs.
 - c. Check height so that when lowering probe, the manipulators do not end up in water.
 - i. If manipulators do touch salt water, rinse them with DI water
3. Lower probe into mouth of coral; Tips:
 - a. GO SLOW.
 - b. Hover just above mouth, then make X,Y adjustments before going any farther down.
 - c. Use magnifying head gear.
 - d. Do NOT go too far in. Just go in far enough so that it is fully covered.
4. Get rest of equipment set up while waiting for polyp to adjust
 - a. Test 9V battery voltage. It should be between 8-9V. Connect battery to Voltage Regulator Circuit.
 - b. Test voltage coming out of regulator. It should be 0.7 VDC.
 - c. Connect probe to BNC cable.
 - d. Plug in oscilloscope, and transimpedance amplifier on the breadboard.
 - e. Check connections before turning breadboard and scope on at same time.
 - f. Set Scope to 0.5V/div and somewhere in the μ s region for most stable signal.
 - g. Zero signal line.
5. Set up video equipment
 - a. Use a camera with good battery life and large memory storage, or just use computer camera.
6. Check probe position
7. Start video
8. Start freshwater injection
 - a. Say “1,2,3, Injecting fresh water” for audio track of video recording
 - b. Insert 10-20 microliters
9. Watch video
10. Wait one minute after dip to check for others, then stop recording.
11. Analyze in Apple iMovie
 - a. Make sure time-code is visible (turned on) in iMovie
 - b. Total time is time from zero-to bottom of drop then to back to zero