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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA  
SANTA CRUZ

**Electrolysis, halogen oxidizing agents and reef restoration**

A thesis submitted in partial satisfaction  
of the requirements for the degree of

MASTER OF SCIENCE

in

OCEAN SCIENCES

by

**John W. Koster**

September 2017

The thesis of John Koster  
is approved:

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2017

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## Abstract

John W. Koster

Electrolysis, halogen oxidizing agents and reef restoration

Applications for electrolysis of seawater include preventing fouling in piping systems, conditioning water for aquaculture and reef restoration. Electrolysis creates a variety of chlorine-produced oxidants that attack essential proteins of living tissues and react with metals, other compounds (e.g., ammonia, nitrites) and organic materials (e.g., amines). The Biorock® process developed by Dr. T.J. Goreau and Dr. W. Hilbertz uses electrolysis for restoring reefs and enhancing growth and survival of corals. It is believed to act by elevating pH and alkalinity at the cathode and/or by reducing enzymatic costs for pumping cations and anions across cell membranes by providing an appropriate electrical gradient (Goreau, 2013). I hypothesize that a third mechanism for enhancing organisms may also be involved: inhibition of microorganisms by significant amounts of chlorine-produced oxidants arising from the anode. Applying Faraday's laws of electrolysis for a system at 8.0 amperes and 90% efficiency gives an estimated ~230 grams of diatomic chlorine per day (equivalent to ~70 liters of gas at STP). In nature (i.e., an open system), diffuse follow-on reaction products (including hypochlorous acid, hypochlorite, hypobromous acid and hypobromite ion) may benefit macrobiota via inactivation of microbial pathogens and competitors, or by other improvements to water quality, as long as concentrations are too low to harm larger, ecotoxicologically less vulnerable organisms.



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## 1. Introduction and Background

### 1.1 Electrolysis

Electrolysis of salt water was first demonstrated in 1800 (Zoulias et al. 2002). From the Latin *electrum* and *-lysis*, a loosening, electrolysis refers to non-spontaneous chemical reactions, especially decomposition, that are driven by passing direct electric current (DC) through liquids or solutions containing conductive free ions (i.e., an electrolyte). Pure water (H<sub>2</sub>O) cannot be electrolyzed since only a tiny fraction of water molecules exist as free hydrogen ions/protons (H<sup>+</sup>) and hydroxyl (OH<sup>-</sup>) ions. Seawater is susceptible to electrolysis because it contains many positively charged ions, the greatest in abundance being sodium ions (Na<sup>+</sup>) at concentrations of ~10.556 mg L<sup>-1</sup>; and negatively charged ions, the most prevalent being chloride ions (Cl<sup>-</sup>) at ~18.980 mg L<sup>-1</sup>.

In metals, the drift of free electrons provides for electrical conductance at or near the speed of light; whereas in liquids, electrical circuits will be completed at much lower speeds via ionic migration (Semat & Katz 1958). In seawater, for example, application of 12 volts (V) of electromotive force to a pair of electrodes spaced 2 meters apart results in a calculated average ionic drift velocity of  $2.5 \times 10^{-5} \text{ cm s}^{-1}$  (<http://web.mit.edu/viz/EM/visualizations/coursenotes/modules/guide06.pdf>). The permittivity (a material property affecting the Coulomb force between two point charges in the material) of water also introduces a capacitance effect (i.e., the ability to store an electric charge).

When DC is provided at appropriate electric potentials, the water molecules are split in different ways at the surfaces of the metallic electrodes: the positive (+) anode and negative (-) cathode. Various competing side reactions can also result, including deposition of minerals on the cathode (see 1.4 Electrochemistry below). Weak DC electrolysis of seawater has recently been developed as a means of creating construction materials *in situ* via precipitation of predominately calcium carbonate ( $\text{CaCO}_3$ ) onto metal frameworks (Hilbertz 1979), as well as being promoted as a reef restoration technology (Hilbertz & Goreau 1996).

At generally higher power levels, additional well-established commercial applications of salt water electrolysis include on-site generation of hypochlorite ( $\text{ClO}^-$ ), which readily combines with sodium ions to form sodium hypochlorite ( $\text{NaClO}$ ), commonly known as liquid bleach. At appropriate levels, injections of this strong disinfectant are used to prevent fouling in marine industrial piping systems, and to condition water for mariculture.

My research project initially focused on the effects of weak DC electrolysis in seawater on biology, particularly that driven by an impressed current (i.e., powered by an electrical device), and later, on consideration of the fate of chlorine-produced and other halogenic oxidants (i.e., reactive nonmetallic elements that occupy Group 17 of the periodic table and form strongly acidic compounds with hydrogen).

## 1.2 Cathodic Protection

The marine and electrical engineering practice of cathodic protection shares many principles with electrolytic reef restoration. Electrochemical principles are applied to reduce corrosion of metallic structures (e.g., steel ships' hulls, offshore platforms, pipelines, etc.) in two general ways (Tezdogan & Demirel 2014):

- Galvanic cathodic protection is a passive process whereby small piece(s) of a metal that is less noble (i.e., less resistant to corrosion and oxidation in moist air) serves as an anode(s) that is/are attached to a larger, more noble, metallic structure, which is to be preserved (the cathode). Galvanic cells formed by the dissimilar metals protect the cathodes via a resulting preferential oxidation/corrosion of such *sacrificial* anodes [see Figure 1: Galvanic or Electropotential Series in seawater].
- Impressed current or active cathodic protection generally employs a metallic *non-sacrificial* or non-consumable anode material that ranks quite noble (e.g., titanium), and which uses a DC power supply to drive the protective electrochemical reaction of the larger, less noble cathodic structure.

## 1.3 Power for electrolysis

In impressed current electrolytic reef restoration, electric power is consumed through a variety of processes that are difficult to quantify and/or allocate. The electrical power available for electrolytic reactions at the surfaces of the anode and the cathode is affected by many factors including (Mazloomi & Sulaiman 2012; Dr. Alexander Kraft; personal communication, and personal insights):

- a. Temperature of the seawater electrolyte.
- b. Electrical resistance (ohmic voltage drop) of metallic conductors.
- c. Electrical resistance of the seawater electrolyte between the anode and cathode.
- d. Activation energy, ionic concentration and mobility (diffusion and migration per Nernst-Einstein equation), surface hindrance including bubble formation (causes electrode area blockage), junction potential (electric potential in the junction between the metal surface and water), and entropy - all of which require greater applied electric potential.
- e. Loss as heat through overpotential (the voltage between a half-reaction's thermodynamically determined reduction potential and the potential at which the reduction/oxidation event is experimentally observed).
- f. Attenuation of the dipole (a pair of equal and oppositely charged poles separated by a distance) electric field with increasing distance.
- g. Water movements including wind, waves and tides (e.g., possible turbulent distortion of the electron-carrying chain of ions in a large fluid system).
- h. The total surface area(s) of the cathode and/or anode.

These many parameters demonstrate that the electrical regime is complex.

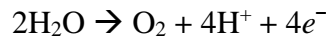
## 1.4. Electrochemistry

### 1.4.1. Anode

Chemical reactions at the anode create a strongly oxidative, acidic, local environment

(Goreau 2012). The anodes of electrolytic installations will corrode rapidly unless they are composed of a metal or alloy ranking highly in the galvanic series.

Above a threshold potential of 1.23 V, electrons from the DC current dissociate water molecules into oxygen ions that combine immediately into gaseous O<sub>2</sub> and free hydrogen ions. The balanced half-reaction (the oxidation or reduction component of a reaction that involves a transfer of electrons between two species) is (Goreau 2012, UC Davis CHEMWiki):



Above 1.36 V, a competing side reaction causes chlorine ions (Cl<sup>-</sup>) in the seawater to combine into diatomic chlorine (Cl<sub>2</sub>):



This side reaction increases as voltage is increased and, between 1.8 and 2.2 V (for most anode materials), the evolution of Cl<sub>2</sub> replaces that of O<sub>2</sub> (Abdel-Aal et al. 2010).

Absorption of chlorine by water is extremely fast (Macdonald & Wong 1977), and the chlorine evolved at the anode undergoes immediate hydrolysis to form the strong oxidant hypochlorous acid (HClO) plus hydrogen and chloride ions:



Above pH 7.4 (which includes typical oceanic pH ranges of  $\geq 8.14$ ), the hypochlorous acid increasingly disassociates into hydrogen ions and hypochlorite ions ( $\text{ClO}^-$ )

(<http://www.atsdr.cdc.gov/ToxProfiles/tp172-c4.pdf>):

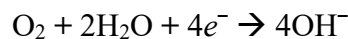


#### 1.4.2. Cathode

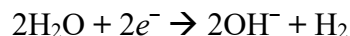
Weak DC electrolysis of seawater forms a reducing chemical environment at the surface of the cathode, and the metal of cathodes does not corrode (oxidize) as long as power is maintained.

Above 1.23 V, two electrolytic reactions occur at the surface of the cathode (Yan et al 1993):

- Oxygen and water are reduced to form hydroxide ions:



- Water is split into hydroxide ions and hydrogen gas ( $\text{H}_2$ ), which bubbles off at a rate of  $\sim 0.4 \text{ L amp}^{-1} \text{ h}^{-1}$  (Kraft 2008):



The side reactions of physicochemical mineral deposition on the cathode are treated



below.

### 1.4.3. Mineralogy

#### 1.4.3.1. Seawater carbonate chemistry

Total carbon dioxide (TCO<sub>2</sub>) is defined as the sum of the concentrations of bicarbonate ions [HCO<sub>3</sub><sup>-</sup>] + carbonate ions [CO<sub>3</sub><sup>2-</sup>] + carbonic acid in aqueous chemical equilibrium [H<sub>2</sub>CO<sub>3</sub><sup>\*</sup>], which are present in oceanic waters at relative concentrations of 90-95%, 2-10% and 0.5-3% respectively (Dickson 2010).

The reactions of carbon dioxide (CO<sub>2</sub>) in seawater are:

1.  $[\text{CO}_2] + [\text{H}_2\text{O}] \leftrightarrow [\text{H}_2\text{CO}_3^*]$
2.  $[\text{H}_2\text{CO}_3^*] \leftrightarrow [\text{HCO}_3^-] + [\text{H}^+]$
3.  $[\text{CO}_3^{2-}] + [\text{H}^+] \leftrightarrow [\text{HCO}_3^-]$

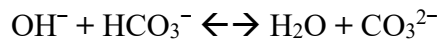
Their resultant is the combined (buffering) reaction:



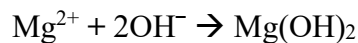
Ocean surface waters are supersaturated with CaCO<sub>3</sub>, which exists in equilibrium with calcium (Ca<sup>2+</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) ions in accordance with a concentration-based solubility product equilibrium constant (K'<sub>sp</sub>) that may be used to calculate the calcium carbonate saturation state (Ω).

#### 1.4.3.2. Cathode deposits

Yan et al's (1993) modeling of cathodically protected steel in seawater implies a steep pH gradient in the diffusion layer. However, the solution at the surface of the cathode does not become very alkaline due to buffering by its reaction with the  $\text{HCO}_3^-$  present in seawater:



The resulting ~8X increase in the concentration of  $\text{CO}_3^{2-}$  on the cathode's surface drives the formation of calcium carbonate (Yan et al 1993). Magnesium hydroxide, or brucite ( $\text{Mg}(\text{OH})_2$ ), is actually kinetically favored to precipitate:



However, calcium carbonate predominates because its saturation level is much higher (Yan et al 1993). Crystals nucleate directly on the surface of the cathode and this mineral layer thickens over time [Figure 2]. These mainly calcareous deposits are porous (Yan et al 1993) and the tiny channels provide a pathway for the seawater electrolyte to remain in electrical continuity with the surface of the cathode, and thus electrolysis is uninterrupted.

#### 1.5. Biomineralization

Calcium carbonate has two isomorphs with the same chemical composition but different crystal structures, solubilities and saturation states ( $\Omega$ ). True corals (Scleractinia) form skeletons by precipitating  $\text{CaCO}_3$  as aragonite. Many other organisms (e.g., foraminifera, coralline algae) deposit  $\text{CaCO}_3$  as calcite. Calcification

occurs in micron-scale spaces between the outer cell membranes (calicoblastic ectoderm) and the existing skeletal surface. Corals actively enhance calcification by modifying the chemical composition of water in these calcifying spaces via raising the alkalinity and aragonite saturation state ( $\Omega_{\text{arag}}$ ) of the fluid. This requires metabolic energy (i.e., ATP) to pump ions across cell membranes, including protons ( $\text{H}^+$ ) out of the space and calcium ( $\text{Ca}^{2+}$ ) into the space (Cohen & McConnaughey 2003). The precise mechanism of coral calcification is not fully known. Cohen & McConnaughey (2003) proposed a “Calcium ATPase and  $\text{CO}_2$  based calcification” scheme, in which the calcium/proton pump is most important for removing protons, thereby converting  $\text{HCO}_3^-$  to  $\text{CO}_3^{2-}$ , and resulting in very high alkalinity favoring high aragonite saturation. Proton removal also lowers the partial pressure of  $\text{CO}_2$  in the calcifying space, leading to net inward diffusion. In the calcifying space,  $\text{CO}_2$  reacts with  $\text{H}_2\text{O}$  and  $\text{OH}^-$  to produce even more bicarbonate and carbonate. A proton gradient of 1 pH unit across the cell membrane can cause a 100X increase in the concentration of  $\text{CO}_3^{2-}$  on its alkaline, calcifying side.

#### 1.6. Biorock®

Because human activities on many scales are adversely impacting tropical coral reefs, there is great interest in reef restoration technologies. One method, called Biorock®, was developed during the late 1980s by Dr. Wolf Hilbertz and Dr. Thomas J. Goreau. It applies relatively low voltage and amperage DC to establish electrolytic cells that deposit  $\text{CaCO}_3$ , mainly as aragonite, on metal frameworks to create reef-like structures (<http://www.biorock.net/>, <http://www.globalcoral.org/>). When fragments of

live hermatypic coral colonies are mounted on such structures in nearshore marine environments, their skeletons readily combine with the thickening inorganic mineral matrix [Figure 3].

#### 1.6.1. History

The late Dr. Wolf H. Hilbertz originated what later came to be named Biorock on the Louisiana coast of the Gulf of Mexico during the 1970s. He sought to develop a new marine construction technique (initially called ‘Seacrete’ or ‘Seament’) by passing weak DC electric currents through seawater from a positively charged non-sacrificial anode to a much larger (mass and surface area) metal cathode, on which predominately  $\text{CaCO}_3$  precipitated (Hilbertz 1979). His first project showed “..spectacular growth of oysters, which settled spontaneously on structures and grew to adult size in months” (Goreau 2013). From 1987, in partnership with Dr. Thomas J. Goreau, the technique was adapted to coral reef restoration, for which a U.S. patent was granted (Hilbertz & Goreau 1996).

#### 1.6.2. Installations

Biorock installations have been deployed for over 25 years at >100 sites in >20 countries (<https://en.wikipedia.org/wiki/Biorock>).

Although other materials may be used, a roll of 1 mm thick mixed-metal oxide-coated titanium expanded metal mesh is commonly used for the anodes of electrolytic reef restoration installations (Borell et al 2010, Zamani et al. 2010, Romatzki 2014). A

type of Dimensionally Stable Anode (DSA), they are chemically prepared, titanium-based, modified electrodes that take advantage of the electrocatalytic properties of rutherfordium and iridium metal oxides. The first such DSAs were created for the chlor-alkali industry in the 1960s (Devilliers & Mahé 2007). Expanded metal meshes are created through a slit & stretch process that results in a flexible screen of ~5 x 2.5 cm diamond shapes. Anodes are usually anchored to the seafloor, from ~1-5 m away from the cathodic structure(s) with which they are paired.

Typical cathodes are open framework structures, often fabricated from small-diameter (e.g., 9 or 12 mm) steel reinforcing bar. While they may be of any shape or size, installations are commonly about 10 m long x 5 m wide x 2 m high, and placed in depths of 2-10 m. Living pieces of coral colonies are attached to the cathode structures with flexible iron wire, plastic ties or monofilament line (Goreau & Hilbertz 2013).

Many types of power sources can be used, including alternating current AC-to-DC rectifying power supplies located at the beachfront, and photovoltaic modules (solar panels). One source may power several structures via paired (+ and -) insulated cables. A Yokohama Type 8750 Integrated Circuit voltage regulated DC power supply, with an output capacity of 9-15 V and 30 amperes (A), is typically used at Gili Trawangan, Indonesia [Figure 4]. The average output of 17 such DC power sources is about 10 V and 11 A, each feeding up to 6 structures of various sizes (Delphine Robbe, Gili Eco Trust Coordinator & Project Manager, personal communication).

### 1.6.3. Benefits

Biorock technology has been reported to enhance the well-being of reef-building tropical corals on or near the cathode. Reports include increased: growth (2-6X), healing after breakage (2-20X), survival after bleaching (16-50X), and settlement and reproduction (100-1000X) (Goreau & Hilbertz 2008, 2013).

### 1.6.4. Biological modes of action

Several mechanisms have been proposed to explain stimulated growth in corals associated with Biorock installations:

1. Higher pH and alkalinity on the cathode reduces metabolic energy required for increasing alkalinity and  $[\text{CO}_3^{2-}]$  at sites of calcification (Goreau et al 2004, Goreau & Hilbertz 2005).
2. The electric field in the surrounding seawater enhances the voltage gradient across external cell membranes, reduces metabolic energy demands for ionic regulation, and makes more energy available for biological activity (i.e., growth, reproduction, healing, and resisting environmental stresses) (Goreau 2013).
3. Magnetic fields may also play a role (Goreau 2013).

### 1.6.5. Design criteria

The energy metabolism and energy processes of corals are poorly understood and much work remains to determine the mechanisms by which weak DC currents and electrolysis affect marine organisms (Goreau 2012). This is reflected in the absence

of numerical formulae relating electrical requirements with dimensions and geometry of electrolytic reef restoration structures. Empirical guidelines for Biorock deployments include:

- a. DC voltages between 3 and 15 V, preferably 6-12 V (Hilbertz & Goreau 1996).
- b. Electric currents between 0.1 and 30.0 A m<sup>-2</sup> of cathode surface area (Hilbertz & Goreau 1996).
- c. Too high currents inhibit mineral deposition and biological responses (Goreau 2013).
- d. Mineral accretion at rates of 1-2 cm per year maximizes structural strength and growth of associated corals (Goreau 2012).
- e. An accretion rate on the cathodes of about 1 kg per kilowatt hour is considered to be an optimal yield of electrolytically deposited CaCO<sub>3</sub> (Goreau 2012).

As seawater is more conductive than a swimmer's body, virtually no sense of an electric current will be felt in the vicinity of an installation, even if one touches both positive and negative electrical elements at the same time (Goreau 2012).

#### 1.6. The scientific literature

Uncertainties remain about the efficacy of Biorock® electrolytic technologies for enhancing biological performance, and the few well-controlled experimental studies have reached mixed conclusions: some report enhanced benefits (e.g., Schumacher & Schillak 1994, van Treek & Schumaker 1997, Sabater & Yap 2002, Kihara et al. 2013), while others report little or no response (e.g., Sabater & Yap 2004, Piazza et al.

2009, Borell et al 2010, Benedetti et al. 2011, Romatzki 2014). Although many factors can affect electrolytic systems, inappropriate electrical currents (especially too much power), may explain the negative responses (Goreau 2013).

### 1.7. Aims and initial hypothesis

My goals were to explore effects of electrolytic processes on marine calcifying organisms under laboratory conditions, and to investigate how weak DC electrolysis influences their growth. These matters depend on downsizing field installation techniques to micro- (aquaria) and meso-scale (outdoor tanks) systems. A more specific goal was to test the hypothesis that weak electric currents increase growth and calcification rates of tropical reef-building corals.

## 2. Experimental systems

A series of experiments were conducted using 4 different systems:

- i. Micro-scale. The first experiments were started indoors in small aquaria without organisms, and they were designed to establish the thresholds of currents needed to achieve electrolysis in seawater, the tradeoff between voltage and amperage, and to gain a sense of how they influence rates and limits of mineral accretion.
  
- ii. Indoor meso-scale. A growth experiment with red abalone (*Haliotis rufescens*) was undertaken in indoor tanks at American Abalone Farms (AAF) commercial mariculture facility in Davenport, California.



iii. Outdoor meso-scale. A set of experiments were conducted in open-air tanks at UCSC's Long Marine Lab (LML), some of which included live temperate climate organisms. The primary focus was to determine differences in the behaviors of impressed current DC electric fields in seawater caused by geometric scaling factors (i.e., upsized from micro-scale) and by the influences of varying cathode characteristics (e.g., surface area and mass), and to map the electric field in three-dimensions.

iv. Waikiki Aquarium. A meso-scale growth experiment with tropical corals was conducted in outdoor tanks at the University of Hawaii's public aquarium in Honolulu, Hawai'i. Aquarium biologist Mark Dimzon was my host-facility counterpart.

## 2.1 Materials and methods

i. Micro-scale (2013): Scoping trials started on a garage test bench at ambient indoor temperature (~18° C) in 9.5 L capacity glass aquaria (30 cm x 15 cm x 20 cm deep) filled with sand-filtered Monterey Bay seawater from LML's supply system (the sand bed is 19.5" deep, consisting of #20 standard silica sand, 0.45 - 0.55 mm grain size). Anodes were 38 x 100 mm pieces of coated titanium expanded metal mesh purchased from the Gili Eco Trust ([www.giliecotrust.com](http://www.giliecotrust.com)). The cathodes were 9 cm squares of 304 stainless steel, welded wire (.81 mm diameter) 1/4" (6.35 mm) mesh hardware cloth. The anodes and cathodes were positioned a few cm away from the end walls at opposite ends of the aquaria and

suspended by their power supply wires (20 AWG insulated multi-strand copper). The power source was a 12 V DC, 7 amp-hour lead-acid battery continuously recharged via a 110 V 60 hz solid state power pack. It was connected to a Voltage Control Module (VCM) that was designed in consultation with electrical engineer John Salisbury to provide very smooth and finely adjustable DC from 0-3.00 volts and 0-100 milliamps [Figures 5, 6]. The VCM was based on Ohm's Law  $V/R = I$  (voltage across a conductor, divided by its resistance in ohms, equals the current in amperes), whereby the current across a 1 ohm resistor is read as volts. The rate of electrolytic reaction was varied by adjusting voltage via a potentiometer. A rotary selector switch and digital multimeter (DMM) were used to display real time data for Volts and Milliamps sensed at the dual outlet jack plugs for separately powering paired electrodes in 2 different tanks at the same time.

ii. Indoor meso-scale (19 September 2013 to 10 January 2014): A meso-scale electrolytic experiment was conducted at AAF in two pairs of 244 x 51 x 41 cm deep, 510 L indoor, covered fiberglass aquaculture tanks (i.e., 2 Powered and 2 Unpowered). Coarsely filtered temperate seawater from their ocean supply system was provided at an inflow rate of  $\sim 30 \text{ L min}^{-1}$ , plus strong aeration by compressed air. Outflow was via standpipes. The paired tanks contained either 250 individual abalone being grown for market (about 2 years old), or  $\geq 112$  larger broodstock. They were fed weekly by "stuffing" the tanks with fresh kelp. The anodes were 10 x 15 cm pieces of coated titanium expanded metal mesh, suspended vertically near the overflow tubes. The cathodes were 1mm thick 25 x 40 cm stainless steel

plates. The electrodes were connected by paired 14 AWG insulated multi-strand copper wires to a 12 V battery with solid-state recharger and a VCM. The electrical inputs were 2.5 V and 50.0 mA, equating to about  $0.25 \text{ A m}^{-2}$  of cathode surface area. Growth was measured by batch-weighing representative of random samples of 50 individuals (at the start, and at Days 64 and 107).

iii. Outdoor meso-scale (2014): Three experiments were conducted in circular 2 m diameter x 1 m deep, 3000 L outdoor polypropylene tanks at LML. [Figure 7] Objectives included various trials to determine differences from the micro-scale system, and influences on the circuits of cathodes having different surface areas and masses. These experiments were:

a.) The tanks were supplied with  $\sim 30 \text{ L h}^{-1}$  ambient filtered seawater ( $\sim 13^\circ \text{ C}$ ) that circulated counterclockwise and exited at the same rate through an overflow tube. The anodes were 10 x 15 cm pieces of coated titanium expanded metal mesh, oriented vertically near the overflow tubes, with their centers at mid-depth (0.5 m) level. Wooden brackets clipped onto the top edges of the tanks were used to suspend the anodes 10 cm from the tank walls. There were 2 cathode configurations: A.) 303 g flat 60 cm squares of 304 stainless steel welded wire mesh (0.81 mm diameter) 1/4" (6.35 mm) hardware cloth, suspended from the wall opposite to the anodes; and B.) 3140 g (48 x 76 x 7 cm) folded carbon steel bedsprings set on top of a concrete building block on the bottom of the tank. The electrodes were connected by paired 14 AWG insulated multi-strand copper wires from a Digimess

Instruments Ltd. PN 300 Programmable Power Supply (0-30.00 V, 0-2.30 A) with digital readouts of output voltage and amperage, used in Constant Current mode.

b.) A growth experiment with live organisms started in 2 tanks on 10 July 2014: one tank was a Powered treatment, and the other identical, but Unpowered.

Initial water inflow/outflow was 30 L hr<sup>-1</sup> of 15.5° C coarsely filtered seawater from the LML supply. Anodes were 10 x 15 cm pieces of coated titanium expanded metal mesh and cathodes were flat 60 cm squares of 304 stainless steel welded wire (0.81 mm diameter) 1/4" (6.35 mm) mesh hardware cloth. The electrodes were connected to the DC programmable power supply by paired 14 AWG insulated multi-strand copper wires. The organisms were subjected to a ~3.25 V, 0.6 A current of (equating to 2.0 A m<sup>-2</sup> of cathode surface area).

The experimental subjects were temperate crustose coralline algae (CCA) and non-zooxanthellate solitary corals *Balanophyllia elegans*. Both came originally from Monterey Bay, and had been maintained indoors under diurnal artificial lighting on water tables. A rock saw was used to cut roughly 2 x 8 cm slices from cobbles covered with mixed communities of CCA on their upper surfaces. At the start, a stand-mounted digital single-lens reflex (SLR)

camera with macro lens was used to image all specimens individually. Ten replicates for each treatment were numbered with a paint pen and evenly spaced along 69 x 17 x 2 cm thick pieces of fluorescent lighting diffuser plastic (12.7 mm grid) placed on top of concrete building blocks positioned in front of the lower edges of the cathodes.

The corals were 15-25 mm tall, with 10 replicates for each treatment. These were placed inside 43 x 13 x 3 cm hinged holders fashioned from the plastic diffuser grid, which kept them in their original order for identification purposes. These holders were hooked onto the interior faces of the cathode screens. Daily environmental measures included V, mA and temperature.

Tanks were exposed to bright summer sunlight, and shade cloth was rigged on the 2<sup>nd</sup> day of the experiment after the coralline alga showed signs of bleaching and temperatures in the tanks had increased to 16.5° and 17.3° C. Both types of organisms continued to show indications of declining health. On 21 July, seawater inlet temperatures was 16.4° but in-tank was 17.4° C. Inlet valves were readjusted to increase flow to 240 L h<sup>-1</sup>. On 22 July, The shade cloth was removed and semicircular plywood covers were installed to cover the halves of the tanks containing the cathodes and the organisms. On 1 August, with seawater inlet temperature reaching 16.9° and within both tanks 19.1° C, seawater inflow was doubled to 480 L h<sup>-1</sup>. The experiment was terminated on 4 August due to inability to maintain appropriate environmental

conditions.

c.) From 21-27 August 2014, I mapped the electric field in one of the 1.0 m deep circular tanks. After consulting two experts in the field of corrosion engineering (Dr. Andrew Drach at University of Texas at Austin and Dr. Frank Ansuini, Technical Director of Electrochemical Devices Inc.), I set up a 1.64 V, 25.0 mA circuit, using an anode of 10 x 15 cm piece of coated titanium expanded metal mesh, and a cathode of a flat 60 cm square of 304 stainless steel welded wire (0.81 mm diameter) 1/4" (6.35 mm) mesh hardware cloth. They were suspended vertically at mid-depth (0.5 m) on opposite sides of the tank. With seawater circulation halted, 2 EDI Seawater Reference Electrode Model IP dry silver/silver chloride elements were plugged into the mV and common jacks of a Fluke 287 True-RMS DMM, with mode selector set to mV DC. The head of one reference electrode was strapped to the center of the side of the anode that faced into the tank. The other reference electrode was repositioned at the intersections of a 3-D grid on 5 minute intervals that allowed for stabilization. A total of 324 readings were taken at 10 cm spaced horizontal intervals for 9 levels of increasing depth (i.e., from 10-90 cm depth at 10 cm vertical intervals). MATLAB computing environment was used to graphically portray the data.

iv. Waikiki Aquarium (26 February to 1 May 2015). Two adjacent 1400 L rectangular (1.93 m long x 1.22 wide x 0.6 m deep) outdoor fiberglass tanks were

set up to be as near identical as was practical. One was Powered and the other Unpowered [Figure 8]. Water from the Aquarium's central saltwater well entered at one end of the tanks via horizontal perforated PCV pipes at mid-depth. Water flowed out over dams at the opposite ends. The inflow rate for both tanks was initially set to about  $\sim 90 \text{ L h}^{-1}$ . The rate of water turnover was estimated to be about one and a half water changes per day (i.e., 24 hours' inflow divided by tank volume).

The anodes were 10 x 15 cm pieces of coated titanium expanded metal mesh. They were suspended from their wires at mid-depth (i.e., centered on 30 cm deep), 10 cm in front of the overflow dams.

The cathodes were flat 50 cm squares of 304 stainless steel welded wire (0.81 mm diameter) 1/4" (6.35 mm) mesh hardware cloth, hung vertically from crossbars, 5 cm from the inflow end walls of the tanks.

To provide substrate for mounting the experimental corals in the saltwater electric field, but not in direct contact with metal, non-conductive plastic screens were installed 10 cm farther into the tanks from the cathodes. These consisted of 2 mm thick high density polyethylene black plastic 8 mm mesh (50 x 50 cm) that were hung vertically from crossbars [Figure 9].

Paired 18 AWG insulated multi-strand copper wires transmitted the current to the

electrodes from a Tektronix PS 280 DC power supply (output capacity 0-30 V, 2 A) via a VCM. The 2.50 V and 0.04 A electrical input to the Powered tank equated to 0.2 A m<sup>-2</sup> of cathode surface area.

Two species of branching Indo-Pacific corals in the Aquarium's collection were used: *Acropora aspera* and *Acropora latistella* [Figure 10]. Forty fragments (~3-5 cm long) were cut from one colony of each species. The two species were handled sequentially. Fragments were weighed with a digital balance after removal of excess water by gently rolling on pieces of paper towel, and laid in numerical order in a shallow tray filled with seawater. Corresponding numbered tags were randomly drawn from a container and used to assign positions for mounting 10 fragments of each species on each of the 2 cathode and 2 plastic screens (i.e., 80 total replicates), using small nylon cable ties.

A close-up photograph of each mounted coral fragment was taken by digital SLR camera with macro lens. Each photograph included a plastic identifying tag and a millimeter rule. The initial photographs were taken in air (i.e., out of the water). Subsequent photographs were taken by laying the screens on a tabletop a few cm below the water's surface and using a "bridge" to support the camera just above the water. This provided vertical downward photographs of each coral at a constant distance, while reducing handling stress. [Figure 11] To document growth, additional photographs of the corals were taken 21 and 78 days into the experiment. Images were analyzed for axial tip growth by the National Institute of



Health's ImageJ image processing program, and JMP Pro 11 statistical analysis software was used.

Supplementary feeding was not done. Data were collected daily using a digital multimeter, Oakton Instruments CON 610 handheld waterproof Temperature/pH/Conductivity/TDS/Salinity/Resistivity meter with probes, and Onset Computer Corporation HOBO 64K Pendant Temperature/Light loggers. Figure 12 depicts environmental conditions in the tanks over the course of the experiment.

## 2.2. Results

i. Micro-scale: In the small aquaria with  $\sim 13^{\circ}$  C seawater, voltage was sequentially increased in steps of  $\sim 1$  minute duration over the power range of the VCM to provide precise 50 mA increasing increments [Figure 13]. An LED flashlight was used to detect the start of bubble formation (i.e., electrolysis) on the surface of the cathode. In repeated tests, the visible threshold was 2.24 V and 5.0 mA, which equated to  $0.83 \text{ A m}^{-2}$  of cathode surface area. In repeated overnight tests, there was consistent accretion of hard, white mineral at 2.6 V and 30.0 mA ( $5.0 \text{ A m}^{-2}$  of cathode surface area). Increasing the current further caused “fizzing” of the evolved gas, flaking off of the accreted mineral, and changes in the appearance of the mineral from grainy opaque white to smoother textured translucent white. This deposit was also softer.

ii. Indoor meso-scale (AAF). The experiment's 2 pairs of tanks at the commercial

aquaculture facility were all housed in the same building, with near-identical conditions and operation (abalone are grown in the dark). All of the abalone appeared to be in good health, with very similar size frequency distributions in the paired tanks (i.e., ~2 year olds being grown for market, and larger brood stock).

Table 1 (below) presents the data from batch-weighed samples of 50 abalone on Days 1, 64, and 107 (when the experiment was stopped).

Table 1. Growth rates of abalones in Powered and Unpowered tanks at the American Abalone Farm

Treatment	Abalone	Day	Total weight in grams	Weight change g day <sup>-1</sup>
Powered	250 Small	1	3679	
		64	4835	0.362
		107	5806	0.442
	110 Large	1	12301	
		64	13998	0.531
		107	15513	0.689
Unpowered	250 Small	1	4309	
		64	5407	0.358
		107	6668	0.587
	144 Large	1	10963	
		64	12564	0.501
		107	14034	0.669

iii. Outdoor meso-scale (LML):

a.) Data for the visible threshold of electrolysis and start of mineral accretion were collected in similar fashion to that for the micro-scale (described above in paragraph 2.2.i.). Figure 13 compares the relationships between V and mA in micro- and meso-scale. Temperatures of the seawater in the tanks were 16.6-17.9° C. In repeated tests, the visually determined threshold of electrolysis for a flat 0.6 m square stainless steel screen cathode was 2.51 V and 100 mA (equating to 0.34 A m of cathode surface area), and the threshold for mineral accretion was 2.69 V and 147 mA (0.50 A m<sup>-2</sup> of cathode surface area). For the much more massive carbon steel (folded bed springs) cathode, bubbles appeared at 2.91 V and 167 mA, and mineral accretion at 2.59 V and 181 mA (calculations of A m<sup>-2</sup> of cathode surface area were not possible due its asymmetrical shape).

b.) The growth experiment with Monterey Bay CCA and *B. elegans* ended after 25 days because temperature in the tanks (i.e., 19.1° C) exceeded maximum temperatures for Monterey Bay and the health of the organisms continued to worsen. All the CCA bleached and 100% of the corals died.

c.) Figure 14 is a 3-D map of the dipole electrical field (in mV DC), looking from the anode (right foreground) towards the cathode. The cylindrical overlay of grey lines indicate the walls of the tank. For clarity, only 3 of the 9 total depths at which data were recorded are portrayed (i.e., top, middle and bottom).

The map's shading depicts a pattern of  $-10$  mV electric potential at the anode (dark blue), which decays exponentially (light blue to red) and then plateaus at  $-3$  mV (maroon) for the remainder to the distance to the cathode. The electric field filled the entire volume of the tank.

iv. Waikiki Aquarium:

a.) Fouling: The extent of fouling by algae, benthic diatoms and/or cyanobacteria was a factor that influenced the results of this experiment. By Day 4 of the experiment, light fouling (filamentous green growths) was present on the tanks' surfaces and on the metal and plastic screens on which the coral fragments were mounted. On Day 13, to reduce fouling, the screens were hand cleaned with a toothbrush. Despite great care, the axial tips of several fragments (mounted lengthwise, directly on the screens) broke off when brushing around them. Therefore, screens were not cleaned again for the rest of the experiment. The screens in the Unpowered tank become more heavily fouled than the Powered tank [Figures 15, 16]. Approaching the Day 40 of the experiment, the colors and textures of fouling growths in the two tanks were markedly different [Figure 17]. The fouling was yellow-green and fluffy in the Powered tank, and dark green and filamentous in the Unpowered tank. The live films in the Powered tank also sloughed the walls, forming piles on the bottom. Figure 18 shows the junction of the southeastern wall and bottom of the Powered tank. The two gray patches, where the wall meets the bottom of the tank, had a very sharp, highly unpleasant smell and appeared to be

relatively large microbial mats. To rid the Powered tank of this apparent infection, I then siphoned approximately 90% of the fouling growths and detritus from the walls and bottoms of both tanks (but not the screens).

b.) Corals: On Day 5 of the experiment, two fragments of *A. latistella* in the Unpowered tank had excreted large amounts of mucus and appeared to be bleaching. Both coral species in that tank appeared increasingly unhealthy, with tentacles less extended than in the Powered tank, and pale coloration or bleached.

Conditions in the tanks started out as near to identical as possible, but their water flows were changed in an attempt to keep the coral fragments alive. On Day 15, the water inlet valve of the Unpowered tank was opened fully (~10X increased inflow) and a submersible pump installed to increase circulation. However, corals in the Unpowered tank did not recover.

On Day 21, growth measurement photographs were taken of every fragment. Up to about 30 days into the experiment, corals in the Powered tank looked vibrant and appeared to be thriving better than those in the Unpowered tank. However, by Day 40, they also started to appear faded. Conditions in both tanks continued to deteriorate, and by Day 64, all fragments in both tanks were dead [Figures 19, 20]. A final set of growth measurement photographs was taken on Day 78, and the experiment ended.

Growth increments were compared in two sample t-tests between Powered and Unpowered treatments, the results of which are presented in Figure 21.

Negative growth figures were due to loss of polyp tissue during death (i.e., bare skeleton lengths). Overall, the Powered corals grew significantly more than the Unpowered corals ( $p < .05$ ).

Over the first period (Day 0 to 21), corals in both tanks had positive growth, with those in the Powered tank ( $N = 36$ ) growing  $\sim 4X$  more than in the Unpowered tank ( $N = 37$ ):  $0.42 \pm 0.10$  vs.  $0.11 \pm 0.10$  mm ( $t_{71} = -2.06$ ,  $p = .04$ ).

During the second period (Day 21 to 78), mean growth in both tanks was negative, with Unpowered control corals ( $N = 31$ ) shrinking  $\sim 5X$  as much as the Powered corals ( $N = 35$ ):  $-0.15 \pm 0.16$  vs.  $-0.71 \pm 0.17$  mm ( $t_{64} = -2.40$ ,  $p = .02$ )

Overall growth for the entire experiment (Day 0 to 78) was positive ( $.023 \pm 0.16$  mm) in the Powered tank ( $N = 37$ ), and negative ( $-0.61 \pm 0.17$  mm) in the Unpowered tank ( $N = 32$ ) ( $t_{67} = -3.67$ ,  $p = .0005$ ).

### 2.3 Discussion

More current was required in the meso-scale than the micro-scale trials, but

differences between their set-ups (e.g., sizes of electrodes, tank geometry) precluded direct comparisons of the results, and I was unable to establish electrical relationships for various geometric configurations and electrical elements that could be used as design criteria.

In the indoor meso-scale experiment at the American Abalone Farms, there were no differences in the weights of abalone between the Powered and Unpowered treatments after 107 days under electrolysis. Complicating issues were the abalone's defensive behavior of clamping to the substrate, and the disturbance and possible injury if they are removed. They are very sensitive to handling and it can take 2 weeks to recover (Tom Ebert, AAF Founder, personal communication). The high turbulence in the aquaculture tanks (i.e., rapid seawater inflow, strong aeration) and a heavy accumulation of detritus (feces and rotting, unconsumed kelp) may have limited crystal nucleation and mineral accretion on the cathodes by fouling their surfaces. Relatively cool seawater temperature is another factor. The high turbulence may also have affected the electric field and/or the current used may have been too low. I ended this experiment because I was unable to resolve these issues.

Failure of the outdoor meso-scale growth experiment with CCA and *B. elegans* can probably be attributed to a combination of inadequate acclimatization from cool, low light indoors to warmer high light outdoor conditions, to limitations of the experimental set-up and, perhaps, to an excessive electric current. Littler & Littler (2013) cite "slow growth" of crustose (nongeniculate) corallines, and the average



growth rate of the oral diameter of *B. elegans* is slow ( $\leq 1$  mm per year) (Fadlallah 1983). Thus, at least a year under cultivation would be necessary to document growth in these temperate organisms.

The map of the dipole electric field in a 2 m diameter tank is consistent with those for unbounded environments reported by Viitasaari (2013), who assessed the growth of corals living within the electric field, but not directly attached to the cathode of a Biorock installation at Gili Trawangan, Indonesia (fragments of *Acropora microphthalma* were suspended on insulating rubber straps). The electrical power in his experiment was 12.0 V DC and 8.0 A, which I estimate equated to approximately  $3.0 \text{ A m}^{-2}$  of cathode surface area. He mapped the electric field by connecting a wire from the negative terminal of a voltmeter directly to the cathode and a wire from the positive terminal to an Ag/AgCl half-cell reference electrode that was serially repositioned along radial transects centered on the cathode. Readings were taken at 1 m below the surface, and each reading took 5 seconds at each of ~115 total stations. He found that the voltage was ~2.5 to 3.0 V at the surface of the anode and ~1.4 V at the center of the cathode. The field declined in strength away from the anode and extended outwards ~15 meters. In this system, the transplanted fragments located within the electric field had greater survival and mean growth rates.

For the experiment with tropical corals at the Waikiki Aquarium, mean growth was significantly greater in the Powered tank than the Unpowered control tank, but the short duration of the experiment, due to the onset of unhealthy condition of the coral

fragments, means these results cannot be interpreted with any confidence. The low water flow in both tanks was intended to create laminar flow that would avoid countercurrents from the anode/outflow end and a possibly difficult-to-account-for distortion of the electric field. While this initial low water flow rate was probably a major contributing factor to the corals' decline, it created conditions that suggested another possible mode of action of electric fields with the potential to benefit organisms. This hypothesis is presented below.

### 2.3.1. Review

Two widely used applications for electrolysis of seawater are preventing fouling of large piping systems and conditioning water for aquaculture through the purposeful generation of chlorine, which reacts with seawater to form chlorine-produced oxidants (e.g., hypochlorous acid, hypochlorite). These oxidants are effective disinfectants when produced at biocidal concentrations. In fish hatcheries, electrolytically produced chlorine can eliminate fungi, protozoa, viruses and bacteria (Taparhudee et al. 2008).

My Waikiki growth experiment was conducted in a partially enclosed meso-scale environment (trough-shaped tanks with flow-through). Because they were identical for the two treatments, differences in temperature or light can probably be ruled out as cause(s) of the bleached appearance and sloughing of its macroalgae from the walls of the Powered tank. I hypothesize that, despite design provisions intended to prevent

accumulation of the chlorine-produced oxidants evolved at the anode, they were still sufficient to affect the microbial films. To evaluate this possibility, I calculated the chlorine production for the Waikiki system, and then for a full-scale electrolytic reef restoration installation in the shallow nearshore environment.

### 2.3.2 Estimate of chlorine evolved at the anode

On the advice of several experts in the fields of electrochemistry and industrial hypochlorite generation (Dr. Alexander Kraft, Gesimat GmbH; Dr. Andrew Boal, MIOX Corporation; and Dr. Shaowei Chen, UCSC), I applied Faraday's Laws of electrolysis to estimate chlorine production.

#### 2.3.2.1. Faraday's Laws of Electrolysis (UC Davis CHEMWiki)

- 1st law of electrolysis: The weights of substances formed at an electrode during electrolysis are directly proportional to the quantity of electricity that passes through the electrolyte.
- 2nd law of electrolysis: The weights of different substances formed by the passage of the same quantity of electricity are proportional to the equivalent weight of each substance.

Using a timescale of 1 day, the mass of a substance liberated at an electrode can be calculated as:

$$m = (It/F)(M/z) \quad \text{where}$$

**m** = mass (grams) of the substance liberated {in this case: g Cl<sub>2</sub>}

**I** = electric current in amperes (= 1 coulomb sec<sup>-1</sup>)

**t** = total time in seconds that a constant current is applied (86,400 seconds day<sup>-1</sup>)

**F** = the Faraday Constant (= 96,485 coulombs mole<sup>-1</sup>)

**M** = molar mass of the substance in grams {for Cl<sub>2</sub> = 71.0 g mole<sup>-1</sup>}

**z** = moles of e<sup>-</sup> required to produce 1 mole of the substance in accordance with its balanced half reaction: 2 Cl<sup>-</sup> → Cl<sub>2</sub> (g) + 2 e<sup>-</sup> {for Cl<sub>2</sub> = 2}

#### 2.3.2.2. Estimates of Cl<sub>2</sub> produced

My calculation of the chlorine produced by the Waikiki meso-scale experiment, which used 0.04 amperes of current, was ~1.14 g day<sup>-1</sup>.

To estimate the amount of chlorine produced by a full-scale system in the shallow nearshore environment, I chose the Biorock installation in Indonesia described by Viitasaari (2013). It operated at 12.0 volts and 8.0 amps, and its anode was a roll of coated titanium expanded metal mesh with ~0.1 m<sup>2</sup> total surface area (which equates ~80 A m<sup>-2</sup> of *anode* surface area). Applying a current efficiency of 90% (Dr. Alexander Kraft, personal communication), daily chlorine production is ~230 grams (equaling 3.24 moles of Cl<sub>2</sub> day<sup>-1</sup>).

Using the Ideal Gas Equation, I calculated the equivalent volume of Cl<sub>2</sub> gas at Standard Temperature and Pressure (STP) (UC Davis CHEMWiki):

$$V = nRT/P \quad \text{where}$$

**V** = volume in L

**n** = number of moles of the substance {**in this case: 3.24 moles Cl<sub>2</sub>**}

**R** = the gas constant (.08206 L atm mol<sup>-1</sup> K<sup>-1</sup>)

**T** = temperature (273.15° K)

**P** = pressure (1.0 atmospheres)

The result is equivalent to ~72.6 L day<sup>-1</sup> of chlorine gas at STP. As chlorine is produced at the anode, it reacts almost instantaneously with many constituents of the ambient seawater, into which the reaction products and any remaining gas rapidly diffuse.

### 2.3.3. Biocidal effects of chlorine reaction products

Above ~1.5 V, electrolysis of seawater results in a variety of chlorine-produced oxidants, including the strong disinfectants hypochlorous acid and hypochlorite ion. 'Free' or 'active' chlorine is the sum of hypochlorous acid (HOCl) and hypochlorite ion (ClO<sup>-</sup>) concentrations. The disinfecting effect of free chlorine is based on the release of atomic oxygen according to the following pair of equations:



During disinfection, chloride ions which have been consumed by electrochemical free chlorine production are reformed. Thus, there is no overall change in the chemical composition of the water during electrochemical water disinfection (Kraft 2008). These oxidants undergo rapid oxidation reactions with organic materials including essential proteins of living tissues, also with metals and other compounds such as ammonia and nitrites. They act as disinfectants by modifying cell membrane permeability and affecting gaseous and ionic exchanges (Abarnou & Miossec 1992). Hypochlorite and high temperatures affect proteins in very similar ways. They both cause proteins to lose their three-dimensional molecular structures, and to clump together forming large, insoluble aggregates (Winter et al. 2008). Many proteins attacked by hypochlorite are essential for bacterial growth, so inactivating those proteins kills the bacteria.

#### 2.3.4. Environmental toxicity

Kudela et al. (2017) reported that the effluent plume from a 22 day, 11M m<sup>3</sup> continuous release of municipal wastewater containing 1743 µM ammonium (NH<sub>4</sub><sup>+</sup>), which had previously been subjected to 2 months of enhanced chlorination disinfection (5-6 mg L<sup>-1</sup> sodium hypochlorite NaClO, followed by dechlorination with sodium bisulfite NaHSO<sub>3</sub> to <1.0 mg L<sup>-1</sup> chlorine), was able to inhibit phytoplankton photophysiology and growth, and “...*the perhaps fortuitous unintended consequence of enhanced chlorination was the production of inhibitory compounds that suppressed the potential phytoplankton response over a large swath of the*

*continental shelf...*”

Chlorine in effluents from wastewater treatment plants form “a bewildering array of products (Jolley 1973 *in* Kudela et al. 2017). At pH 7-9, hypochlorous acid can oxidize bromide ions ( $\text{Br}^-$ ) to form hypobromous acid and, at typical seawater pH, virtually all chlorine added as a biocide reacts rapidly with the bromide ( $\sim 50\text{-}80 \text{ mg L}^{-1}$ ). What are usually reported as chlorine residuals are more likely a mixture of hypochlorite with various bromine species, especially hypobromous acid ( $\text{HBrO}$ ) and hypobromite ion ( $\text{BrO}^-$ ), both of which are even stronger oxidizing agents than the analogous chlorine species. For example, the oxidation of glucose to gluconic acid by hypobromite is 1300 times faster than by hypochlorite (although bromo-organic compounds are generally more unstable than those of chlorine, and degrade easily to innocuous inorganic compounds) (Black & Veatch Corporation 2010). Therefore, it is more appropriate to term the succession of disinfectants arising from added chlorine as *halogen oxidizing agents*.

Residual chlorine is the portion of dissolved chlorine gas that is not bonded to any other reactants (e.g., organic materials, metals, etc.) in the water. For natural seawater with a residual chlorine concentration of  $5 \text{ mg L}^{-1}$ , the rate of removal (known as chlorine demand) is about  $3 \text{ mg L}^{-1} \text{ h}^{-1}$ , and redox processes complete its removal in about 24 hours (Macdonald & Wong 1977).

#### 2.3.4.1. Ecotoxicological benchmark data

Information regarding chlorine's effect on aquatic organisms is not extensive.

Relevant examples include:

- The level of electrolytically-created hypochlorite required to kill or inactivate virtually all fish pathogenic bacteria and viruses in hatcheries is 0.1-0.58 mg L<sup>-1</sup> at flow rates of 2.0-3.0 m<sup>3</sup> h<sup>-1</sup> (Kasai et al. 2000).
- The State of Hawaii's limit for point source discharges from ships is 0.1 mg L<sup>-1</sup> chlorine as total residual oxidant for 2 hours per day (Lucas et al 1996).
- The chronic toxicity threshold of chlorine in the marine environment is ~0.02 mg L<sup>-1</sup> (Macdonald & Wong 1977).
- The half maximal effective concentration (EC<sub>50</sub>) of a toxicant is a measure of its potency. It refers to the concentration that induces a response halfway between the baseline and maximum after a specified exposure time. In terms of photosynthetic inhibition, the 2-3 hour EC<sub>50</sub> for mixed phytoplankton communities is 0.009-0.1 mg L<sup>-1</sup> (Singleton 1989).

Lethal Concentration<sub>50</sub> (LC<sub>50</sub>) is a standard measure of median toxicity whereby half the population of a specific test-animal in a specified period die from exposure (Black's Law Dictionary). Examples for chlorine include those shown below in Table 2 (Singleton 1989).



Table 2. Residual chlorine's aquatic ecotoxicity in mg L<sup>-1</sup>

Organism	Measure	Exposure	Concentration
Mixed phytoplankton	Photosynthetic inhibition EC <sub>50</sub>	2-3 hours	0.009-0.1
Rotifer <i>Brachionus plicatis</i>	Lethal Concentration 50 (LC <sub>50</sub> )	48 hours	0.001
Mollusca larvae <i>Crassostrea virginica</i>	LC <sub>50</sub>	48 hours	0.029
Crustacean eggs <i>Daphnia magna</i>	LC <sub>50</sub>	1 hour	0.063

With regards to hard corals:

- *Stylophora pistillata*: LC<sub>50</sub> 2.3 mg L<sup>-1</sup> (no period specified) (Wang et al. 2008)
- Planulae of 3 species of Hawaiian corals tolerated 0.49 mg L<sup>-1</sup> for up to 7 hours (Davis 1971 *in* Johannes 1975).

One inference from these examples is that the toxic effects of residuals from added chlorine are greater for smaller organisms, especially algae.

The relatively thin cell membrane and high surface-to-volume ratios of many microorganisms may make them especially vulnerable to oxidation by halogen oxidizing agents. This deduction is consistent with characterization of the effects of chlorine generated by electrolytic reef restoration installations as “..no problem at all for life in the ocean, with fish and corals growing well no farther than a millimeter or two from the anode” (Goreau 2012).

### 3. Conclusion

My original goal was to test the hypothesis that weak electric currents increase growth and calcification rates of tropical reef-building corals. However, complications that occurred during an electrolytic experiment in meso-scale tanks at

Waikiki Aquarium led to estimating the production of halogen oxidizing agents at anodes which, in addition to the more direct effects listed above, may be capable of enhancing coral growth by inhibiting harmful microorganisms.

As discussed above, diatomic chlorine streams from the anode(s) of electrolytic reef restoration installations, where it rapidly reacts with, and mixes into, unconstrained seawater by a combination of largely turbulent, but also molecular processes ([http://www.marbef.org/wiki/Transport\\_and\\_dispersion\\_of\\_pollutants\\_nutrients\\_tracers\\_in\\_mixed\\_nearshore\\_water](http://www.marbef.org/wiki/Transport_and_dispersion_of_pollutants_nutrients_tracers_in_mixed_nearshore_water)). Parameters of dynamic wind and waves, the tidal regime, bottom contour and local currents all add a high degree of entropy and complexity ([http://www2.warwick.ac.uk/fac/sci/eng/research/civil/water/events/britcouncildyetracing/4\\_nearshore\\_wave\\_mixing\\_processes.pdf](http://www2.warwick.ac.uk/fac/sci/eng/research/civil/water/events/britcouncildyetracing/4_nearshore_wave_mixing_processes.pdf)).

Significant amounts of diffuse yet potent halogen oxidizing agents are evolved at the anodes of full-scale electrolytic reef restoration installations (e.g., ~230 grams or 3.24 moles of  $\text{Cl}_2$  day<sup>-1</sup>, which are equivalent to 70 L or more of chlorine gas per day).

The ecotoxicological impacts of these oxidizing agents suggest the possibility that prophylactic chemical disinfection occurs in the field (i.e., in addition to the proposed more direct electrical effects listed in paragraph 1.6.4.). Such microbicidal activity involves factors not considered by earlier researchers, and could account for some of the highly variable results seen among different weak DC electrolytic reef restoration installations. The work of Smith et al. (2006) lends additional support to the concept that prophylactic chlorine produced by electrolytic reef restoration installations

benefits corals by limiting macroalgal growths and the dissolved organic carbon they exude (sugars and polysaccharides), which may be used by pathogens that may inhibit, or even kill corals through creation of hypoxic conditions or by other means. Additionally, Saragosti et al. discovered in 2010 that some corals (including *Stylophora pistillata*) produce an antioxidant enzyme called extracellular superoxide dismutase that is capable of detoxifying reactive oxygen species in their surroundings. This represents an additional means by which corals would enjoy a distinct biogeochemical advantage where oxidizing agents, such as added-chlorine reaction products, are present in minute concentrations.

Using aluminum alloy *sacrificial* anodes to power extremely weak electrolysis galvanically (i.e., with electric currents powered by chemical action) in a two year experiment in Okinawa, Kihara et al. (2016) reported 4X increased growth of *Acropora tenuis* fragments at a calculated power of only 5 mA m<sup>-2</sup> of cathode surface area, versus lower rates of corallum size increase for unpowered controls. Their work suggests that the most beneficial direct electrophysiological effects (in contrast to the prophylactic ecotoxicological mode of action that I've proposed) could exist at voltages below those at which chlorine starts to be evolved at the anode (i.e., <~1.5 V).

### 3.1. New hypothesis

The evidence described above has caused me to formulate an alternate hypothesis for effects of electrolysis on corals and other organisms:

*In open systems in nature, diffuse follow-on reaction products of seawater electrolysis (i.e., halogen oxidizing agents) benefit macrobiota indirectly via inactivation of microscopic pathogens and competitors, or by other improvements to water quality, at concentrations too low to harm larger, ecotoxicologically less vulnerable organisms.*

This mechanism is in addition to mechanisms previously advanced – it does not displace them. It is consistent with the conclusion that electrolytic reef restoration technology offers something more than a simple substrate.

#### 4. Recommendations for further research

My new hypothesis provides a strong rationale for the additional testing that will be required to explore this process. The relative impacts of halogen oxidizing agents on spores of turf algae, benthic diatoms and cyanobacteria, etc., and settlement and recruitment of other “weedy” species will be of particular interest.

##### 4.1. At an existing electrolytic reef restoration site:

- a). Spatially map oxidizing potential to reveal the plume of halogen oxidizing agents (using an Oxidation Reduction Potential probe such as the Eureka Manta+).
- b). Use molecular methods to describe and compare microbial communities (e.g., metagenomic sequencing and metabolomics).

c). Use RNA screening to detect differential gene expression of microbes and corals across the field.

4.2. Aquarium prophylaxis. Test periodic applications of tiny amounts of well-mixed amounts of chlorine (electrolytically produced or liquid) to aquarium exhibits and coral nursery tanks to determine whether highly diffused halogen oxidizing agents are capable of safely reducing requirements for recurrent manual cleansing of fouling growths.

4.3. An improved method for mapping an electric field in seawater would be to fix the two reference electrodes on either end of a beam that can be moved around the grid pattern to measure potential at each location (Dr. Frank Ansuini, personal communication). These results will be proportionate only to electrical current flow in the water volume between the references, thereby avoiding an unknown fraction of the applied current entering one reference, going through the measuring circuit and discharging through the other reference.

5. Figures

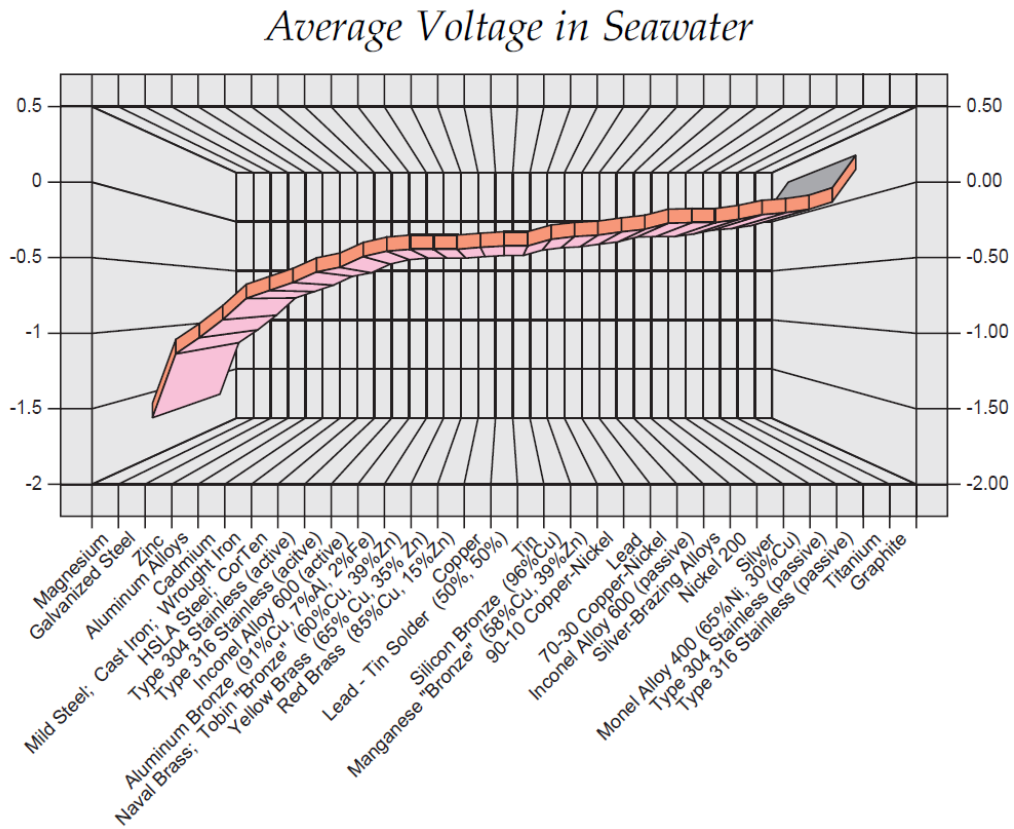


Figure 1. Galvanic or Electropotential Series in seawater with average voltages. Note the relative positions of titanium and steel.  
<http://www.kastenmarine.com/metalparts.htm>

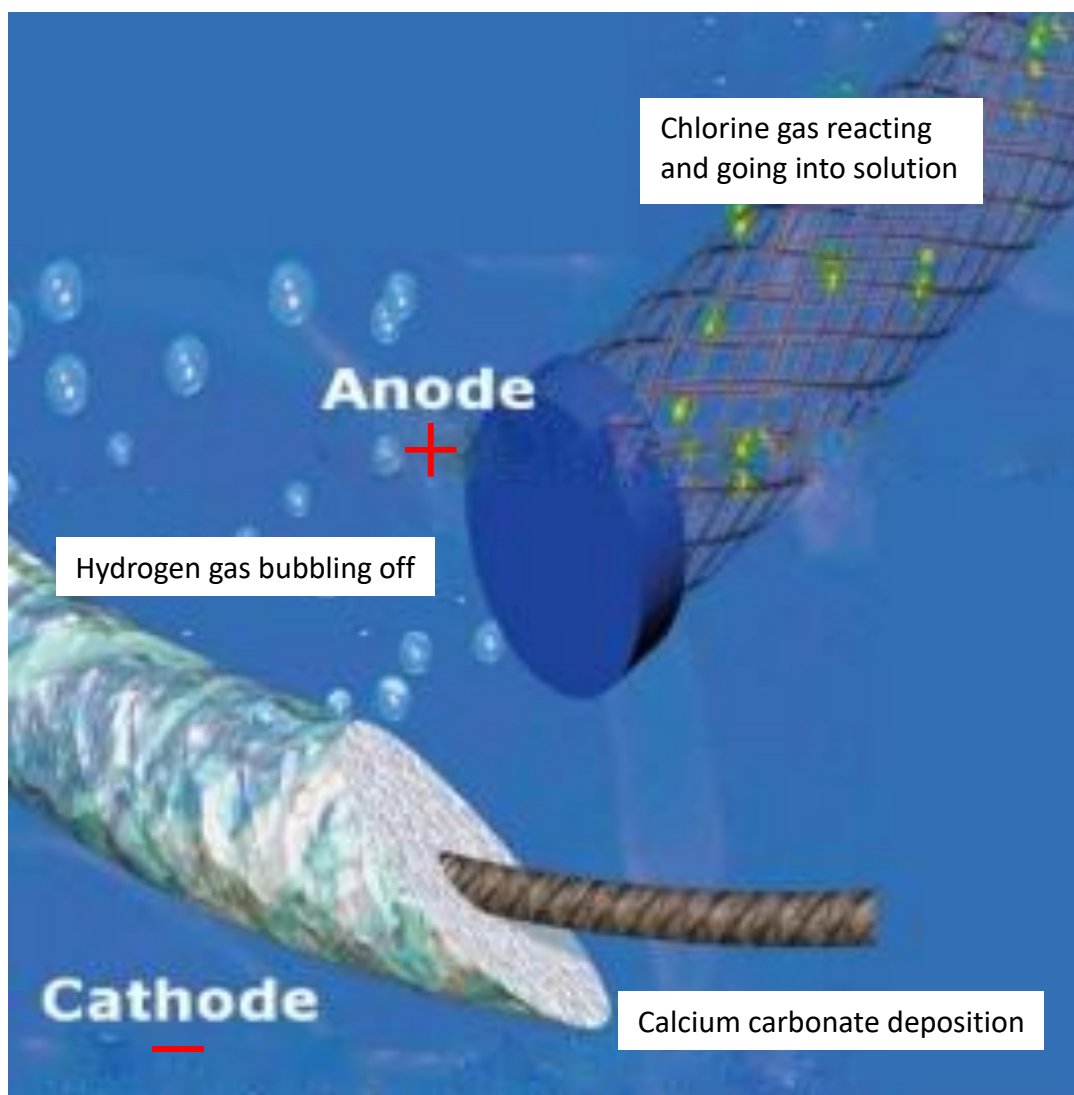


Figure 2. Artist's conception of the electrochemical processes in weak DC electrolysis of seawater. Note accreted minerals on the cathode. (Modified from [http://www.oceancaraibes.com/biorack\\_artificial\\_coral\\_reefs.htm](http://www.oceancaraibes.com/biorack_artificial_coral_reefs.htm))





Figure 3. Portion of a Biorock® cathodic structure at Gili Trawangan, Lombok Indonesia. (photograph courtesy of Karl B. Fellenius)



Figure 4. DC power supply with output cables leading to a Biorock installation in Indonesia. Note current and voltage output meters.



Figure 5. One of the dual-output Voltage Control Modules (VCMs) built for the project.

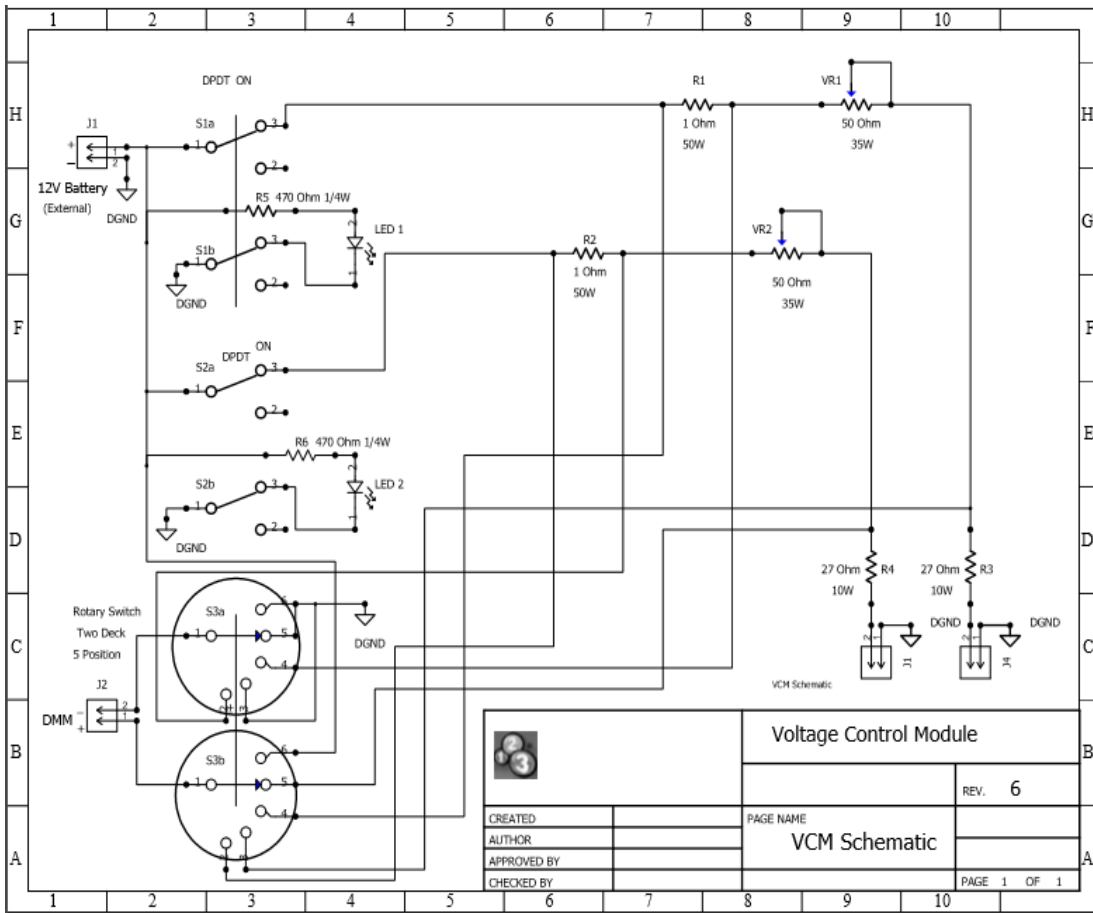


Figure 6. Voltage Control Module schematic diagram (standard electrical symbology).

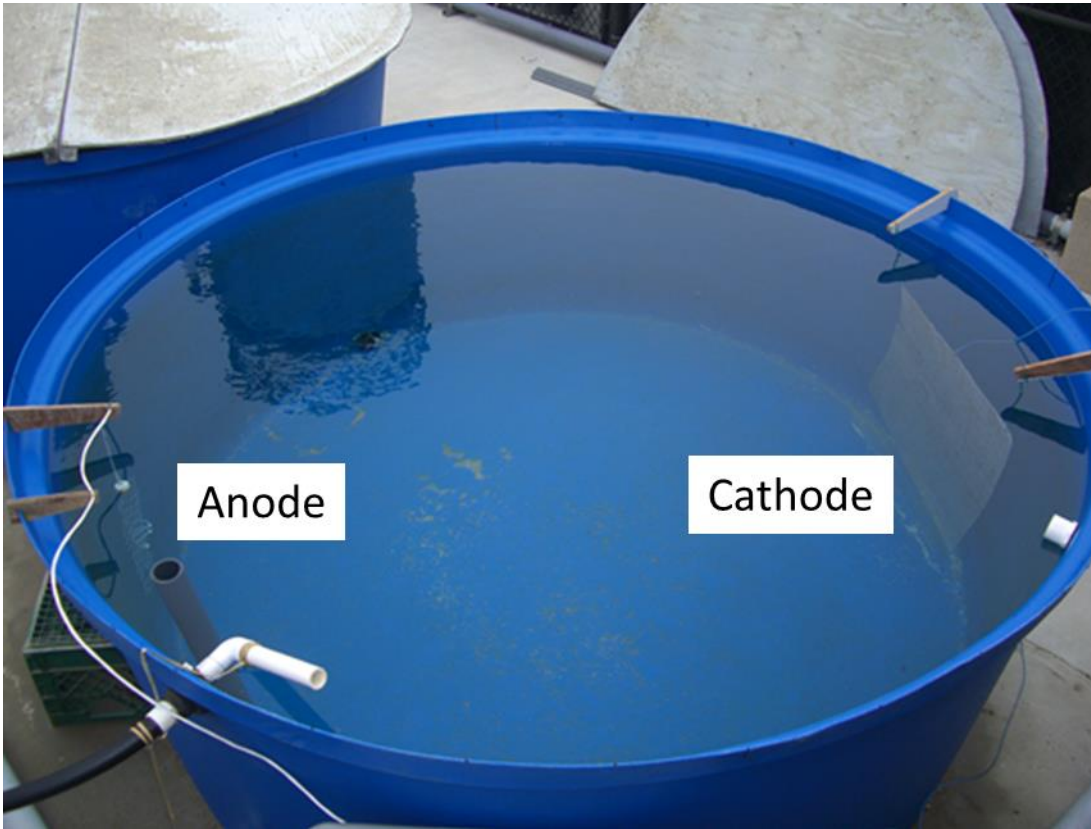


Figure 7. 3000 L experimental tank at UCSC Long Marine Lab. The anode is hanging from the tank wall on the left, and the cathode to the right.



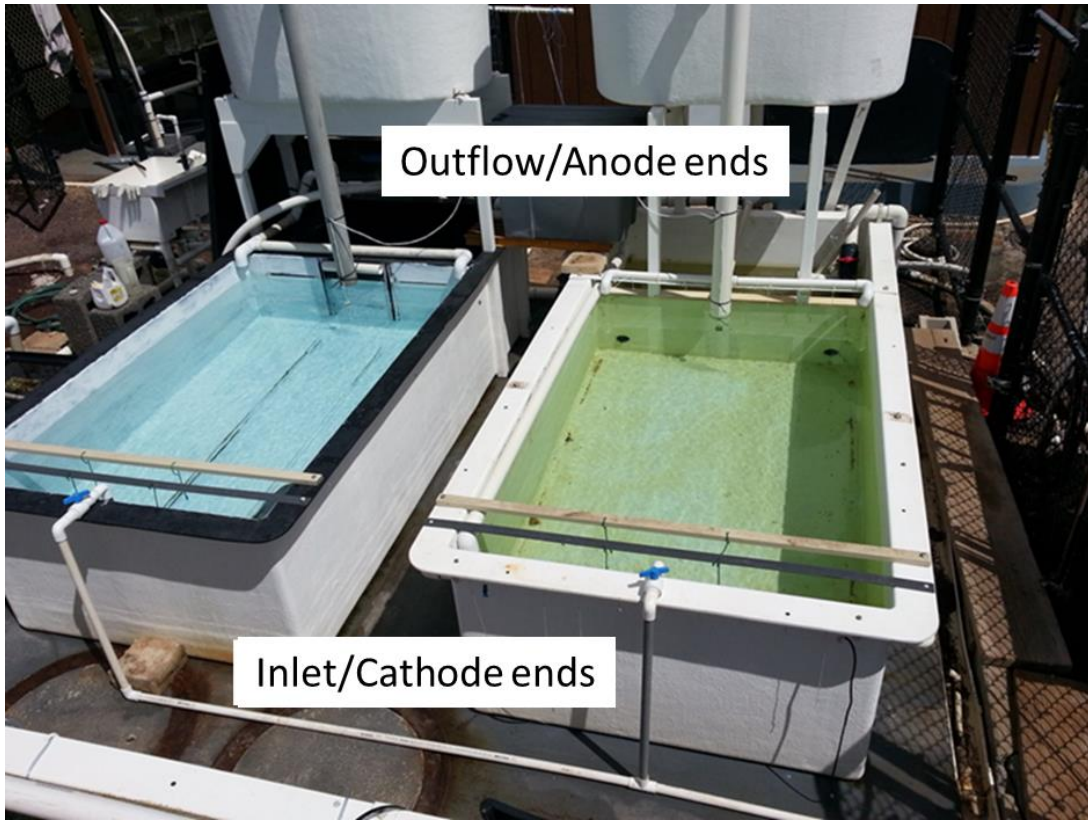


Figure 8. Start of experiment at Waikiki Aquarium with Unpowered (left) and Powered (right) 1400 L tanks. Their anodes and overflow dams are to the rear.

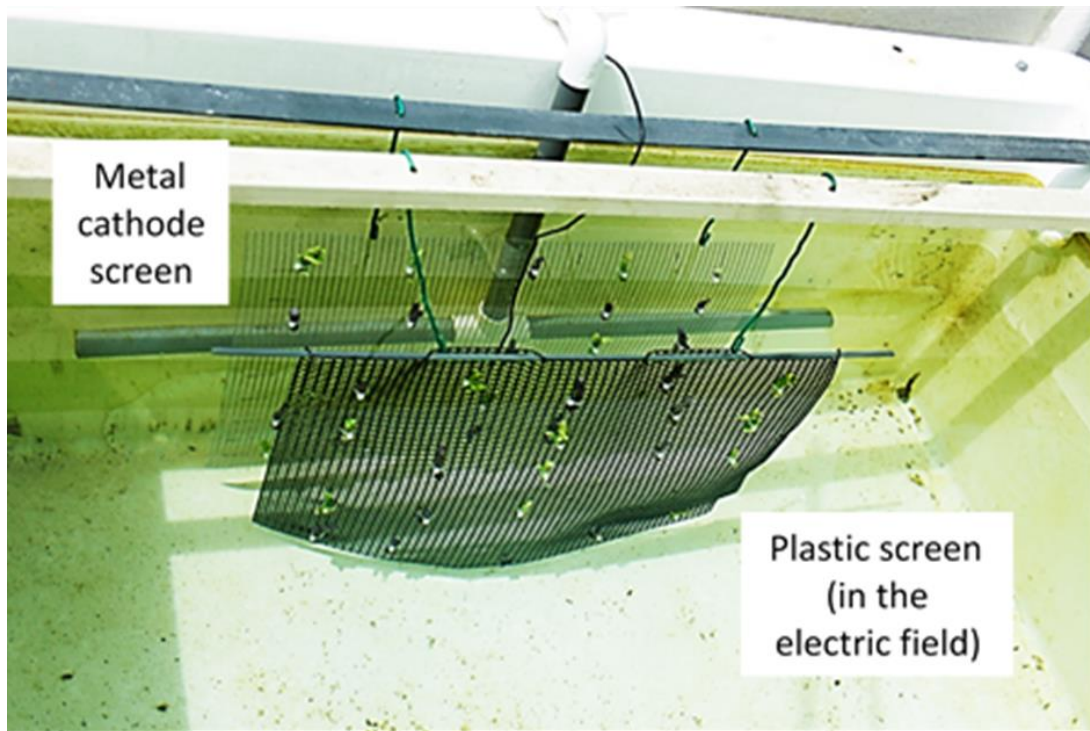


Figure 9. Metal and plastic screens in the Powered tank at the start of the experiment. The metallic cathode screen is behind the non-conductive black plastic screen. Note water input piping and the black power wire leading to connection with the metal screen at its center.

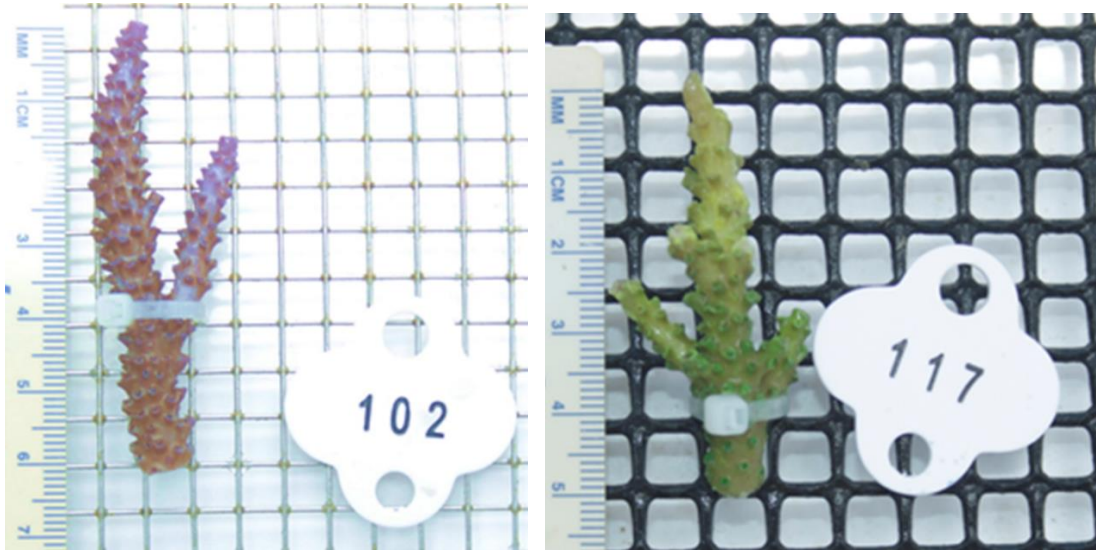


Figure 10. *Acropora aspera* *Acropora latistella*  
Examples of coral fragments mounted on the metal and plastic screens with nylon cable-ties.



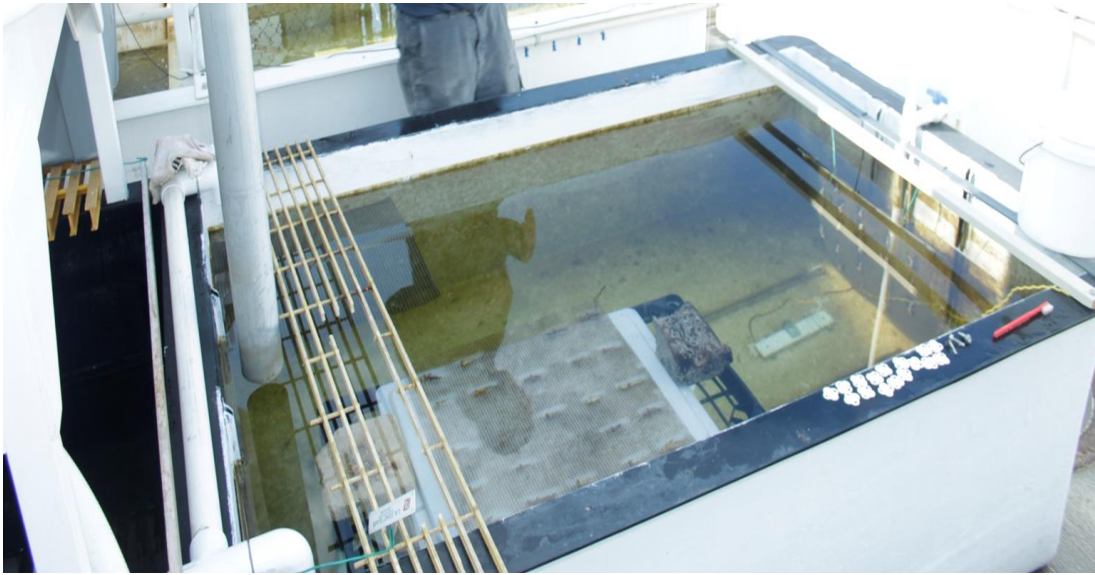


Figure 11. Metal screen with mounted coral fragments on submerged table used for in-water photography. Note movable bridge camera support.

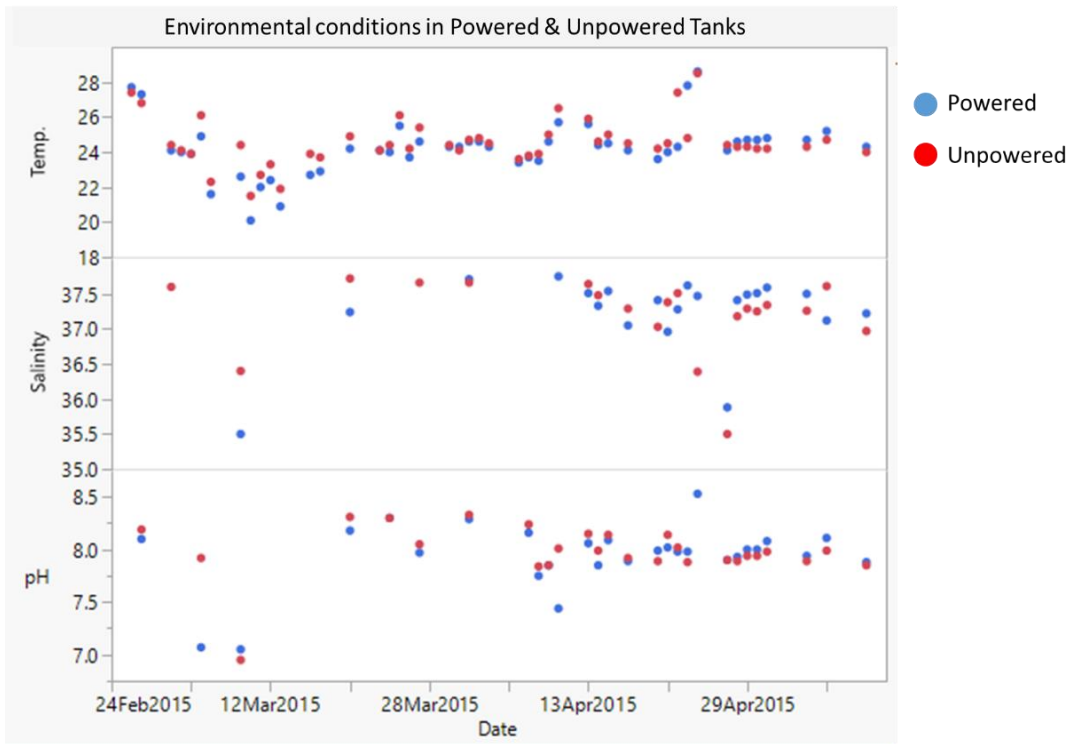


Figure 12. Environmental conditions in Powered (red line) and Unpowered (Control) (blue line) tanks over the course of the Waikiki meso-scale experiment.

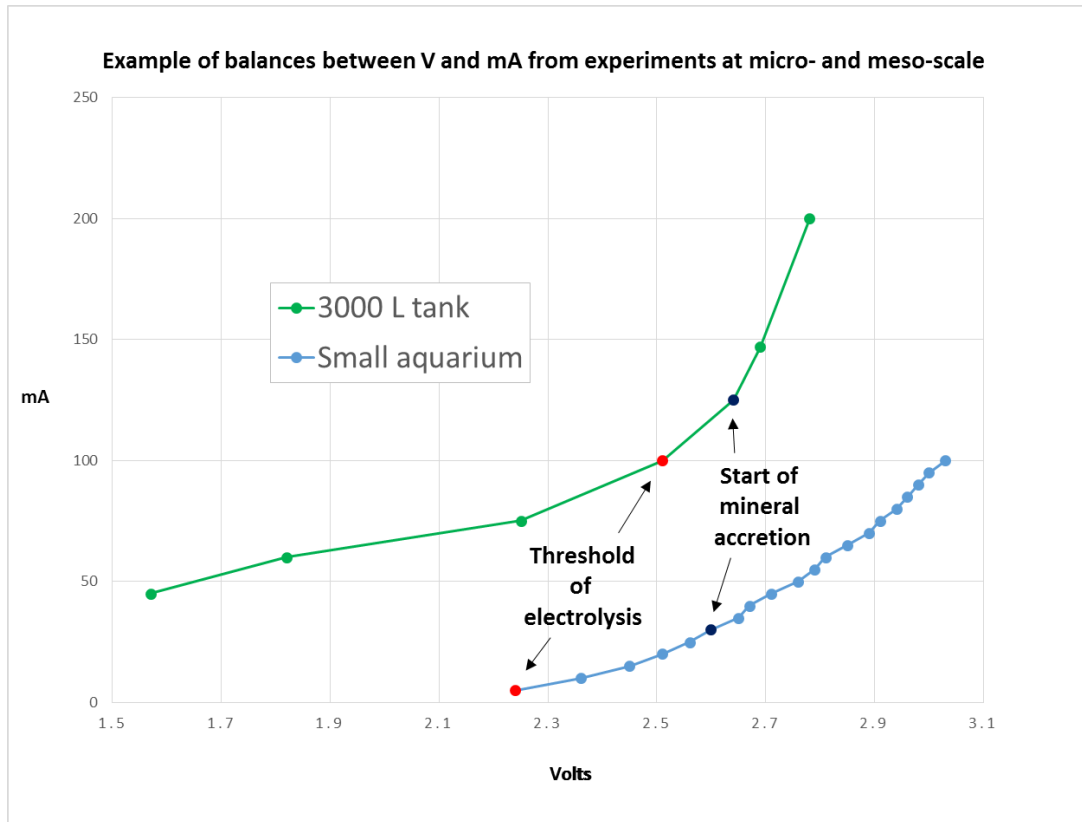


Figure 13. Changes in amperage in response to increasing voltages at micro-scale and meso-scale. The visible threshold of electrolysis at micro-scale (small rectangular aquarium) was 2.24 V and 5 mA, and mineral accretion began around 2.60 V and 30 mA. Comparable points in meso-scale (3000 L circular tank) were 2.42 V and 102 mA, and 2.69 V and 147 mA respectively.

### 3-D representation of electric field in a tank

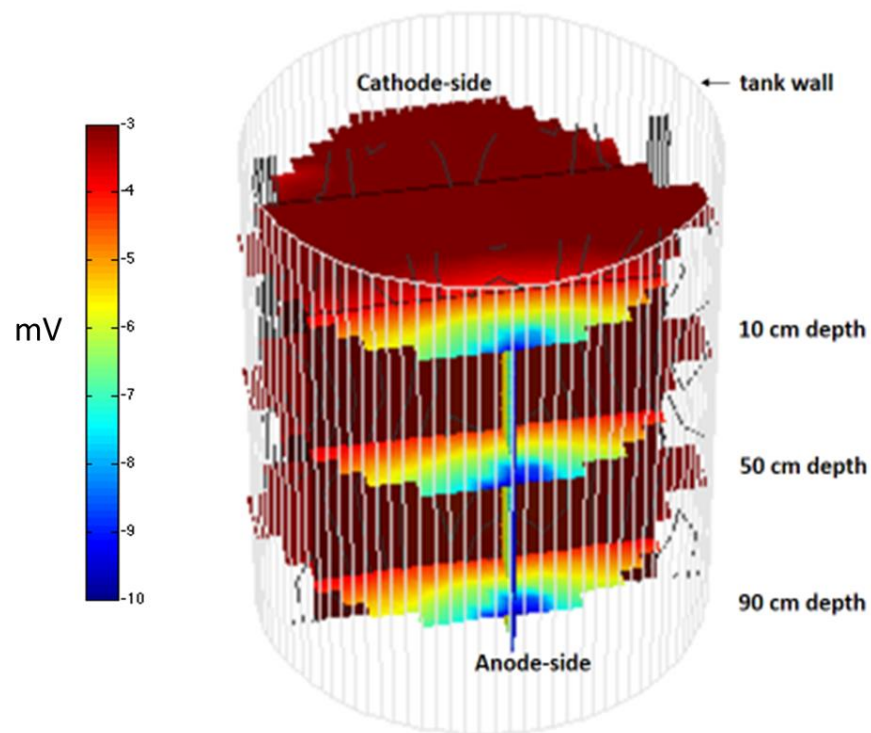


Figure 14. 3-D representation of dipole electric field in millivolts in a circular 2 m diameter x 1 m deep 3000 L plastic tank, looking from slightly above across the tank from anode (right foreground) towards cathode. Data from 3 levels are displayed: 10 cm, 50 cm and 90 cm depth. The electric field fills the entire volume of the tank.

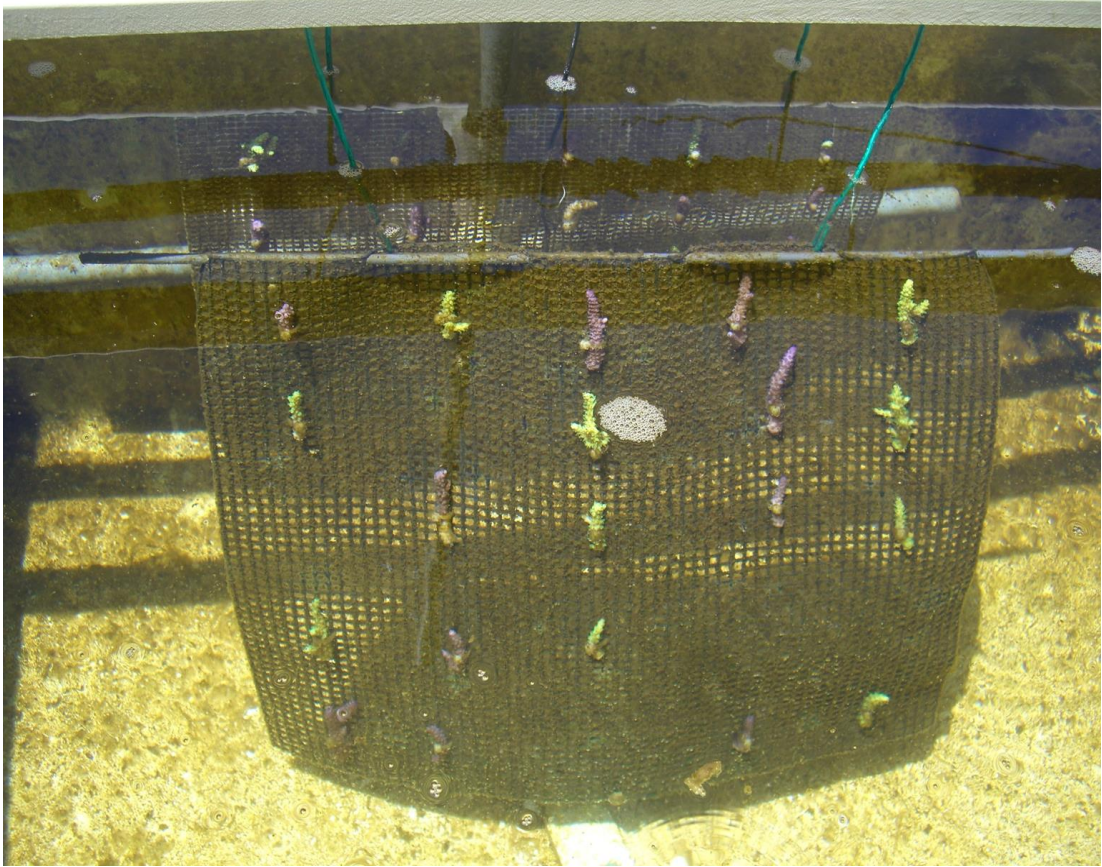


Figure 15. Plastic screen in the Powered tank on Day 21, with healthy corals and a light covering of fouling organisms and minimal  $\text{CaCO}_3$  deposits on the metal cathode (the top of the metal cathode screen is visible 10 cm behind the plastic screen).



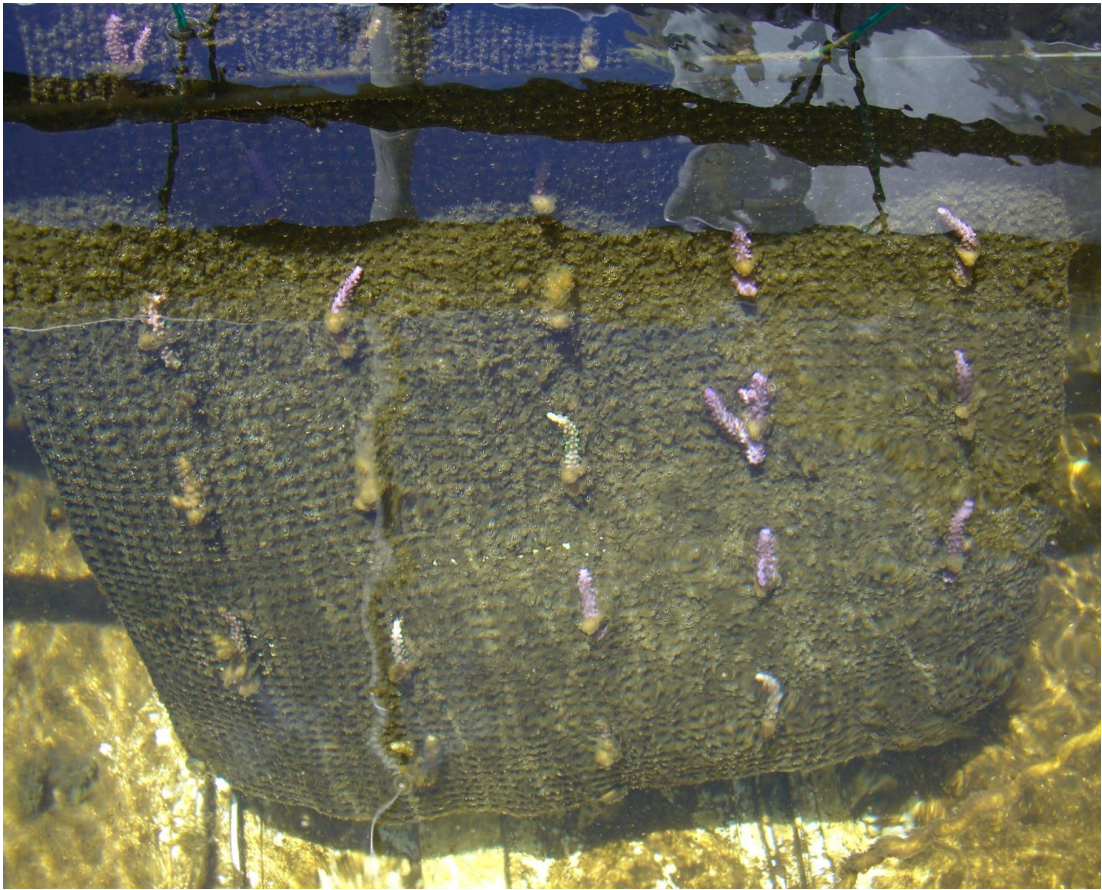


Figure 16. Plastic screen in the Unpowered tank on Day 21 with corals partially covered by a thick layer of fouling organisms.

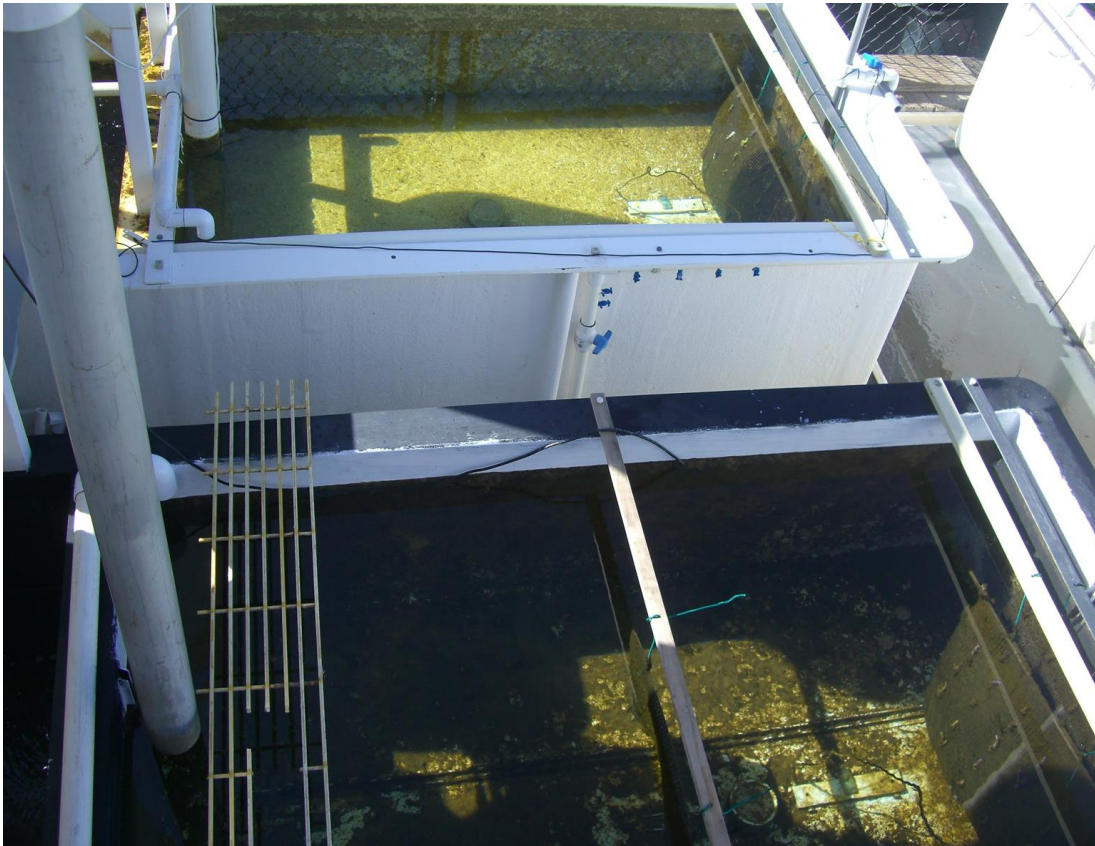


Figure 17. Visible differences in fouling growths at Day 28 with (upper: Powered tank) and without (lower: Unpowered Control tank) weak DC electrolysis.

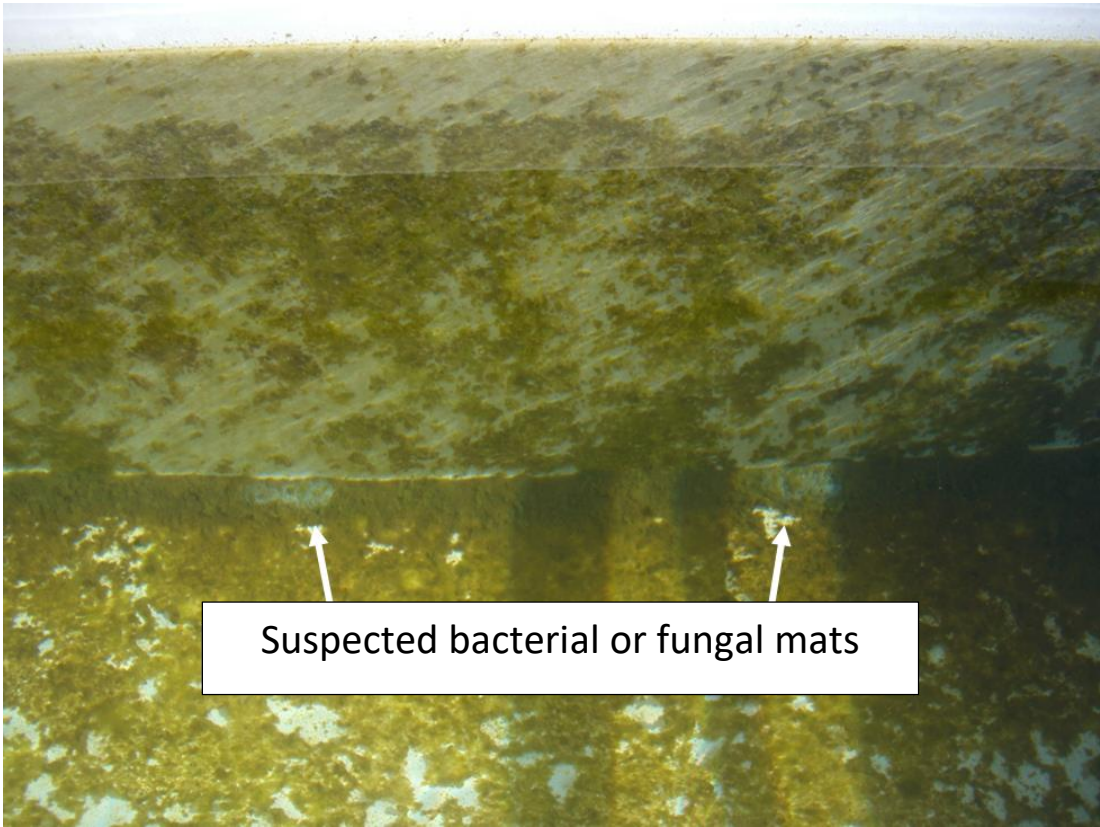


Figure 18. Junction of southeastern wall and bottom of Powered tank showing sloughed fouling matter lying on the bottom. Note the two gray patches where the wall meets the bottom of the tank.





Figure 19. Plastic screen from Powered tank on Day 56 with dead coral fragments.



Figure 20. Plastic screen from Unpowered tank on Day 56 with smothered corals.

Comparisons of mean growth in Powered vs Unpowered tanks by periods

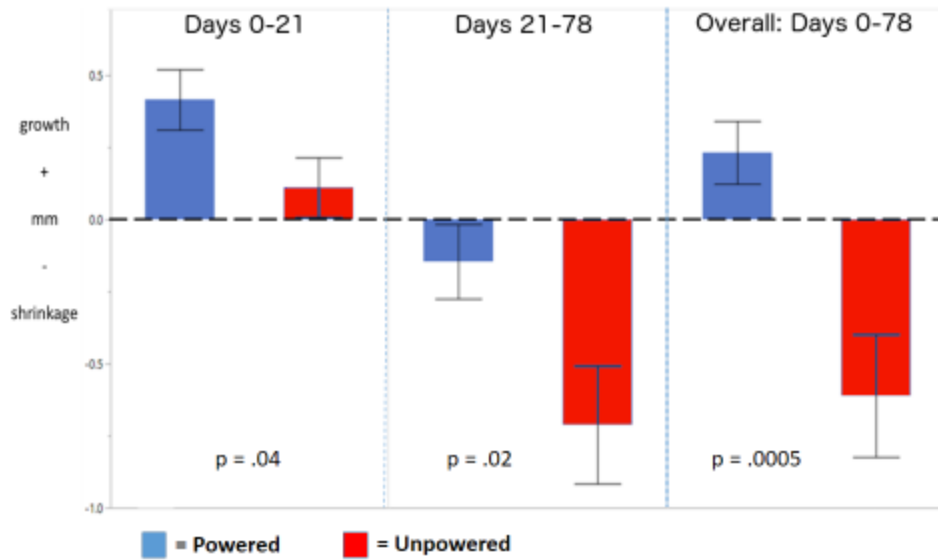


Figure 21. Axial tip growth increments over three different periods of the experiment: Days 0-21, Days 21-78, and overall Days 0-78. Negative growth is due to loss of polyp tissue (i.e., bare skeleton lengths).

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