



Coral growth rate monitoring in vivo on similarly healthy reinforced steel bar structures with and without mineral accretion device in an algae dominated reef.

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1. Introduction

Very few is known about coral reefs in the average modern society and even interested people learn about them on a daily basis. Indeed, these ecosystems are amongst if not the most diverse in the world and are known to host over 25% of marine species [10]. Moreover, it is known that such ecosystems can act as natural barriers [12] and can prevent coastal erosion besides having a tremendous influence on human economy [11].

The lacking overall understanding of these marine organisms causes anthropogenic threats but naturally occurring phenomenon can also give rise to damaged reefs.

In that aspect, the common acceptance is that two major types of reefs exist: coral dominated reefs and algae dominated reefs. Indeed, when describing a reef, the general idea that comes to mind is of a healthy and colorful coral dominated reef with its entire usual indicator species. The second type of reef comes with much less biodiversity and is the result of too large influence of competitive algae growth in an environment where nutrient waters have reached a too high level. This can occur due to various reasons which either, augment the amount of nutrient in the water or diminish the coral coverage in the reef (human destruction, climate change, acidification of the water ...). Moreover, the issue with these reefs is the low number of predators feeding on the algae that end up overpopulating the area. Therefore, this last type of reef would represent a good environment for marine conservation projects.

As a first basis active measure to restore and rehabilitate reefs, this study was based on Koh Tao island in the Gulf of Thailand. A mineral accretion project inspired by the Biorock device (see later for references and description) was put into place on the 26 of June 2015 in Tien Og Bay as to verify if a solar powered device could prove to function efficiently in a long period of time. Thereafter, as a second step for this device implementation, an algae dominated reef restoration investigation was chosen to be performed based on the following hypothesis: The mineral accretion device would enable a faster growth of chosen coral colonies on the structure of study. Moreover, a few interrogations could come into account such as if a more efficiently branching in a coral colony was synonym to a better health; if the presence of competitive organisms next to the subject would decrease the growth rate or if a different type of growth behavior (branching, fattening, overgrowing obstacles ...) would mean less axial growth. For this study, the Acropora coral genus was chosen as the usual monopolistic colony in Tien Og Bay before its overall bleaching event.

Coral reefs and threats to their expansion

Corals are marine invertebrates that are part of the Anthozoa class within the Cnidaria phylum. Their Calcium carbonate (CaCO₃) exoskeleton gives rise to their overall structure of a population of monoclonal polyp colony. Thanks to a symbiosis behavior with microalgae called zooxanthellae (which also gives them their color); they gain 90% of the necessary energy from photosynthesis in exchange for glucose and essential elements ^[13].

The coral species was chosen to be roughly separated into soft and hard corals. The latter are known to take much longer to grow and expand but will result in more durable entities. This is the main reason why the conservation program will be interested in studying such corals (Acropora being one of these most often branching hard coral colonies).

All these organic cycles are comprised in the ocean chemistry. Indeed, when looking at overall CO_2 consumption, coral reefs represent the major characters ^[14]. The process that occurs involves the reaction of dissolved CO_2 with water in order to form carbonic acid (H_2CO_3) which will itself break down into bicarbonate (HCO_3^-) . The final byproduct of this will be the much needed carbonate ion (CO_3^{-2}) . As a carbon sink, the ocean will shift the equilibrium of this process towards a less carbonate concentrated milieu if CO_2 is uncontrollably dissolved in the water ^[15].

Due to the increase of dissolved CO_2 in the ocean, the pH of the environment has been seen to decrease altering this ocean chemistry [16]. The overall behavior of the reaction goes as follows:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

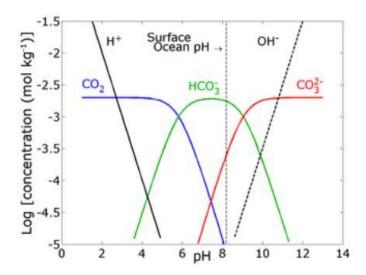


Figure 1: Graph of how the pH-value influence on the concentrations of CO₂, HCO₃ and CO₃. (from eoearth.com).

As one may deduce, the proton formed by the H_2CO_3 break down will react with a carbonate ion and create an even bigger shift to the left of the equilibrium. This will eventually limit the amount of building blocks for the coral exoskeleton and lead to ocean acidification [17]. The saturation state of a mineral in seawater is also useful information to understand the mecanism here:

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{[Ca^{2+}][CO_3^{2-}]_{eq}} = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}}$$

Where [X] are the concentrations of ion X, and K_{sp} the rate constant of the reaction at equilibrium.

Such changes in the environment will forcibly and unnecessarily stress the coral colonies affected by these changes and phenomenon like increased temperature or increased water nutrients. This will then lead to a release of their symbiotic zooxanthellae and bleaching will occur. The white color will then be synonym to lack of ideal energetic availability but not direct death of the colony. Regaining the microalgae is definitely possible but bleaching will eventually kill the corals if not stopped.

These threats have all led to a global decreasing of the coral population and overall, one-third of reefbuilding corals face elevated extinction risk from climate change and local stress factors ^[18].

2. Materials and methods

Shark Bay - algae dominated reef - first trial

Post to literature reviews and lectures on different environments at hand around Koh Tao Island, the primary task was to choose two already existing similarly healthy artificial reef structures in terms of coral growth. Therefore the hardship lay in the actual choice of a location easily reachable for data collection after implantation of the device that also met the research's requirements in terms of finding an interesting enough environment for such growth monitoring. Therefore, monitoring coral growth in an algae dominated reef environment represented an interesting opportunity (see the introduction on coral reefs on the ecological monitoring program manual ^[9]).

Thereafter, the first choice that was exploited was Tien Og bay also known as shark bay, an algae dominated reef with average depth of 5 [m] (see previous data analysis and results for Koh Tao reef status ^[1] and particular case of arbitrary occasional Tien Og bay study ^[8]). In comparison to the EMP surveys done on a regular basis, the occasional ones are performed more in depth and more details are taken into account for a general assessment of the reef's health. For Tien Og bay in particular, the 1998 bleaching that occurred and obliterated most if not all of the growing Acropora corals rendered it even more interesting for a coral growth experiment. The two structures already present were then selected for their apparent overall health in terms of these same coral colonies (see coral taxonomy in ecological monitoring program manual ^[9]).

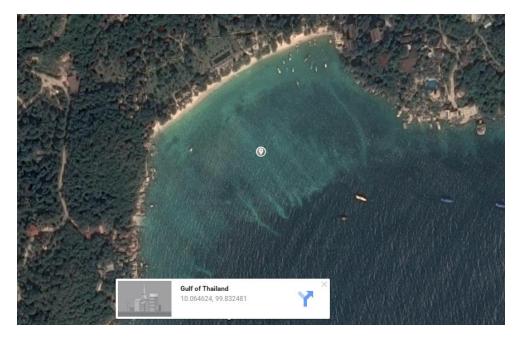


Figure 2: Overview of Tien Og bay and approximate location of device (visible on field with signaling flag).



Figure 3: Picture showing the healthy longtail-like structure in the middle of a rubble filled algae dominated reef (Tien Og bay). The rubble is mostly previously bleached Acropora due to the 1998 temperature rise.

The metal structures used underwater on the island are usually made of concrete (optimal for drilling and coral recruits' larvae deposition), PVC tubes (introduction of branching corals to help their directional growth – also greatly used as support for rope nurseries), glass bottles and reinforced steel (rebar) structures which are of interest in this study. Indeed, this material is used as the basis for various structure models for economic reasons mostly as it is accessible by local entrepreneurs but still appears like a fairly good substrate according to observations over time around the island (see various articles that have proven the overall efficiency of such structures ^{[2],[3],[6]}). Moreover, they have proven to be stress relievers for natural reefs around the island ^[4].

Indeed, concerning the material used for this cathode, G. Terlouw ^[5] states that it may consist of any conductive piece in any shape desired even though non-galvanized (the zinc layer involved in the process wouldn't be welcome in our case) steel mesh (chicken wire) is preferred (see G. Terlouw even though quantitative results still need to be generated for a better proof). The anode was proven to be efficient when using titanium. For the voltage, a direct current ranging from 1 - 24 [V] with a 3 [A/m²] current density (most important factor as higher current densities induce faster precipitations that will cause a more brittle working material) seemingly gave the best results.

As the purpose of this study is a quantitative comparison of a normal structure to another powered with a mineral accretion device, rebar was of course chosen as the only conductive material under low voltage input underwater used on the restoration program; it also has the advantage to be exploitable by local entrepreneurs for its easy access as a construction material and cheap price. All of which was according to the hypothesis that the mineral accretion device enables a growth rate about 5 times greater that of a normal rebar structure in the same conditions.

Indeed, the process of mineral accretion uses electrolysis of seawater to diminish the surrounding pH to a more alkaline state in order to cause ions such as Ca²⁺ and CO₃²⁻ to precipitate on the structure in the much needed calcium carbonate that corals need to synthesize their exoskeleton. The following reactions occur at the ends of the device:

Anode: $2H_2O_{(l)} \rightarrow O_{2(g)} + 4H^+_{(aq)}$

Cathode: $2H_2O_{(l)} \rightarrow H_{2(g)} + 2OH^-_{(aq)}$

This will then increase the pH at the cathode to generate enough ${\rm CO_3}^{2-}$ for spontaneous precipitation of calcium carbonate. The pH reached can go up to 11 and the overall seawater chemistry is not affected as the overall net charge of the device is zero.

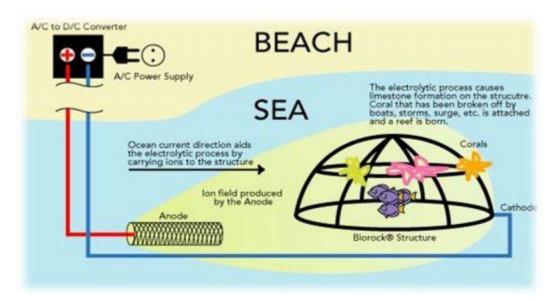


Figure 4: Schematic representation of a Biorock. Alternating electrical current is transported from a power supply on shore, converted to direct current by a convertor. The small anode is placed below or on the sediment. The cathode is constructed of metal or other conducting material and can be built in any shape. (taken from Terlow G. ^[5]).

The technology that was used was inspired by the previously described biorock [5] work and modified as well as improved to become known as the bobrock [7]. The solar panel had the following properties:

- 9 over 4 cells of 6x5 cm² with 1.5 mm flat wires going through the center of the cell on top of it.
- 2 mm length separation for a 4 mm width separation.

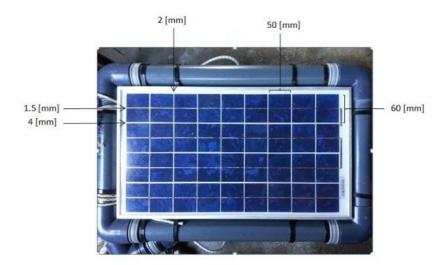


Figure 5: Picture of device's solar panel used to power the solar anode.

This solar panel is the connected to the anode by regular electrical wires coated by water resistant material. Moreover the supply is such that the current automatically copes with the changes in light conditions (Maximum Power Point Tracking - MPPT).

The actual device uses a newly developed Impressed Current Cathodic Protection-technique to enhance coral growth and its resilience. Indeed, the basis is to supply a constant low voltage current to the conducing substrate/structure of choice (rebar in our situation). This open source system apparently constantly adapts to changing circumstances, like water flow, water resistance, temperature and substrate conductivity. For the solar anode ^[9] the first prototype's characteristics are as follows:

- Anode material:
 - o MMO on titanium mesh.
 - PVC: 50mm tubing 10bar-rated Solar panel: 20watts 36 cells Circuitry: CoalAID design
 2015MK1
- All built around Recom DC-DC converters with an output voltage dependent on the amount of light on the solar panel to reach an optimal efficiency range. The lower part is the battery. A charger and water detector are also present so one can see if the tightening of the PVC tubing was done properly.

Titanium was used as the anode material as it can resist brutal environments without being corroded. Indeed, the oxide layer it will form protects the metal up to a voltage of 9 [volts] (maximum input voltage) with surrounding chlorides. However, this protective layer does not conduct current. Coating with mixed metal oxides (MMO) was apparently used to cope with this issue as it has a similar resistance to titanium, better specifications than platinum and are much cheaper.

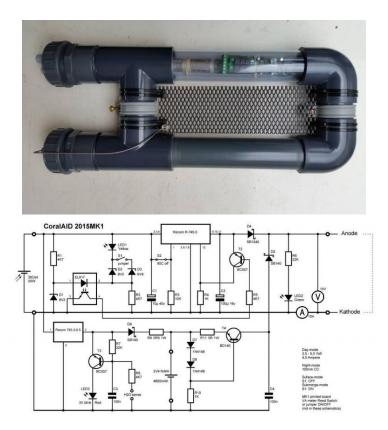


Figure 6: Solar anode and its characteristic schematics (from coral aid website)

The circuitry is then connected to the cathode or conductive structure chosen prior to installation.

The installation protocol was performed in one dive as follows:

- Material preparation on boat:
 - Anode and floating solar panel attached to concrete block for proper stability in bay.
- At a depth of about 5 [m], the cathode of the device was chosen to be a long tail boat like rebar structure with 6 healthy Acropora colonies. Out of the 6 colonies attached to the structure, 4 were chosen to perform the study:
 - o Estimation of average depth variation from one colony to the other: 20 [cm]
 - Colonies dispatched along the 3 [m] long structure had equivalent sunlight exposure and other competitor organisms weren't endangering the colonies' growth.
- Each colony had two branches selected for the study:
 - One horizontal and one vertical branch per colony (approximate angle): 8 branches
 - A zip tie or tyrap was tightened around selected branches at 3 [cm] from the apex growth point of the branches.
- The same steps were applied for a non-electrified structure located at about 200 [cm] from the first one.
- An indication that the device is working is the bubble generation at the cathode end and gradual precipitation of calcium carbonate around the structure.

After installation, waiting was of the essence as such organisms take time to grow. However, the first hindrance came about since one needs to take all aspects of the environment into account in such studies: three days post installation, free diving at the location for a routine check-up resulted in finding the solar panel's cable cut clean and 50 m from the structure most likely due to a long tail boat propeller.

Shark Bay - algae dominated reef - second trial

Therefore, the whole device was taken back and water infiltration caused it to malfunction. Such an obstacle led to questioning if the location should be changed but the algae dominated reef represented a very interesting study. Therefore, a solution for signaling the location of the device to approaching boats had to be thought of and we decided to build a signaling red floating flag to attach to a second identical device.

The flag was composed of a 150 [cm] PVC tube for the pole with a diameter if 3.1 [cm]. The lower end of the pole had to be inserted into a bigger holding PVC ring and loose metal wire had to be attached as well. These two last features had the sole purpose of retaining the counterweight that was to be molded afterwards. Indeed, an 8.5 [cm] diameter over a length of 12 [cm] cylindrical counterweight was created. Sole concrete had to be mixed to rocks and sand for a heavy enough weight. Afterwards, three yellow floating buoys were attached in the center of the flag pole using bowline and clove hitch knots. Finally a red signaling piece of fabric was attached at the end to signal danger or at least attract attention.



Figure 7: Picture showing signaling flag to prevent boats from breaking the device

The second device used here came from a biodome (name of the structure due to its shape) close to shore in the same bay as a proof of it working. Therefore, after verifying its functionality, we took it to the actual experimental location. The anchor point was this time chosen to be two new bottle nurseries (concrete base with glass bottles sunk in as substrate for future growing coral colonies) and a third one already present on the structure. Transportation of the whole system was done by swimming with the help of a floating kayak for the heavy parts and installation was performed while scuba diving.

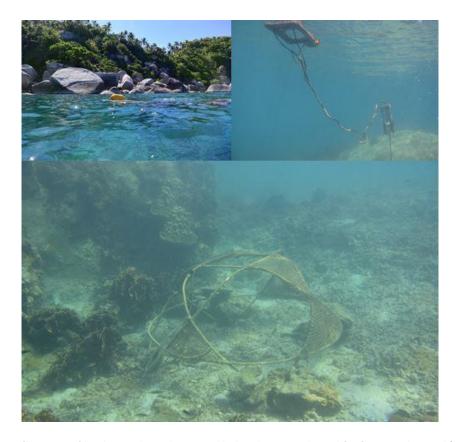


Figure 8: Picture of location of biodome where the second bobrock was implanted for functionality trial (to be moved to previously described location for study at hand)



Figure 9: Pictures of clamp used to connect wires to structure (proof it is functional with the gas bubbles at the end of the clamp)

At that point, even though time had passed without electrifying the structure the growth of the chosen colonies was still taken into consideration. This actually represented an even more thorough basis for comparison of future growth rates. The first set of data was then collected supported by pictures for future visual feedback. The depth of each colony was measured (an approximation has to be made since the tides change as time passes) and a sketch of the two study structures was made.



Figure 10: Picture showing the whole longtail structure with the chosen colonies for the study (red circles are the chosen colonies)

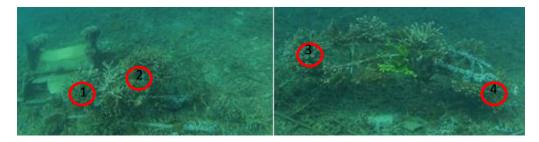


Figure 11: Pictures showing the control structure close to the previous study structure (red circles are the chosen colonies)

Pictures for feedback were gathered as follows in order to have a feedback of a simple measurement and to have data less prone to human error:



Figure 12: Pictures of coral branch measurements

Moreover, a snorkeling and free dive session two days later enabled to assess the whole system's functionality and the whole system was still in place with good calcium carbonate coverage.

Another set of pictures data was taken and in situ measurements were also taken two weeks post to the installing of the device. At this point, it also seemed important to indicate the state of the coral colony (healthy, slightly bleached ...).

Finally, the solar panel required regular maintenance thanks to snorkeling trips in order to remove any filamentous algae coverage that would unable proper supply of power to the underwater device.

3. Results

The data gathered during the various sessions was separated between the colonies for each date and between horizontal and vertical branching within a specific colony.

Table 1: Tables of the growth data collection for the electrified (E) structure and non-electrified one (NE) - a corresponds to the more horizontal branches and b to the vertical ones – voltage input was performed on the 28th day.

Subject structure	17.07.15	14.08.15	28.08.2015	06.09.2015	11.09.2015
time [days]	0	28	42	51	56
E1a [cm]	3	3,3	3,4	4	4,1
E1b [cm]	3	4,1	4,4	4,6	4,9
E2a [cm]	3	3,5	4,3	4,5	4,8
E2b [cm]	3	3,3	4	4,6	4,9
E3a [cm]	3	3,5	3,8	4,6	4,7
E3b [cm]	3	3,1	3,2	3,6	3,7
E4a [cm]	3	4,2	4,8	5,6	5,9
E4b [cm]	3	3,5	3,7	3,9	4,9

Control structure	17.07.15	14.08.15	28.08.2015	06.09.2015	11.09.2015
time [days]	0	28	42	51	56
NE1a [cm]	3	3,6	4	4,4	4,4
NE1b [cm]	3	4	4,2	4,3	4,5
NE2a [cm]	3	5	5,4	5,6	5,7
NE2b [cm]	3	3,8	4,1	4,5	4,6
NE3a [cm]	3	5	6,4	6,5	6,6
NE3b [cm]	3	4,6	5,5	6	6,1
NE4a [cm]	3	3,5	5,5	5,8	6
NE4b [cm]	3	3,5	4,2	5,2	5,6

These two different sets of growth data were analyzed through a descriptive statistics tool on Excel as follow:

Table 2: Example of descriptive statistical analysis with subject structure (the same was performed with the control structure and used for the plotting over time) – green highlighting represents the powering of the subject structure.

Subject structure	0 days	28 days	42 days	51 days	56 days
Mean	3	3,5625	3,95	4,425	4,7375
Standard Error	0	0,1375	0,188982237	0,216093564	0,228299224
Median	3	3,5	3,9	4,55	4,85
Standard Deviation	0	0,38890873	0,534522484	0,611204899	0,645727718
Sample Variance	0	0,15125	0,285714286	0,373571429	0,416964286
Range	0	1,1	1,6	2	2,2
Minimum	3	3,1	3,2	3,6	3,7
Maximum	3	4,2	4,8	5,6	5,9
Sum	24	28,5	31,6	35,4	37,9
Count	8	8	8	8	8

The average Length for each date was plotted with its standard error variation for each structure.

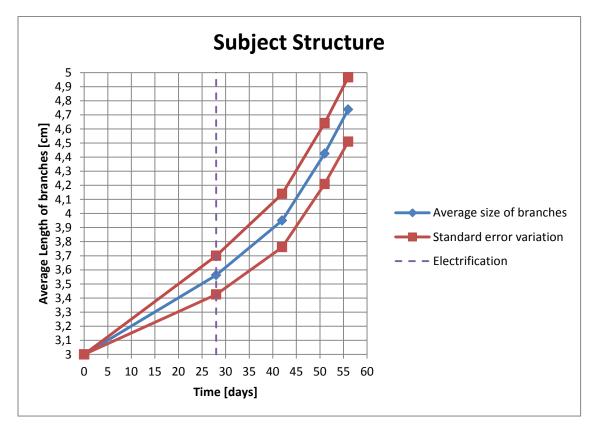


Figure 13: Graph of average Acropora branch growth over time onto a structure equipped with a mineral accretion device

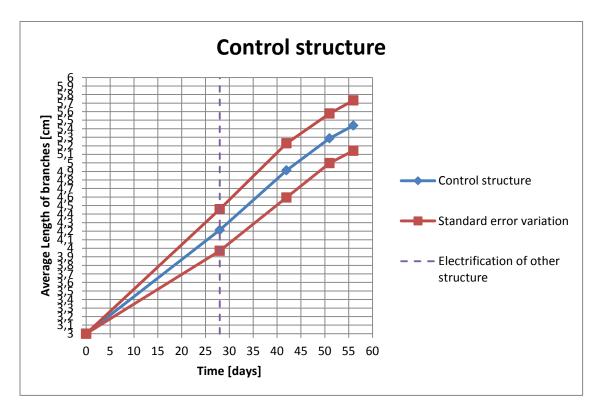


Figure 14: Graph of average Acropora branch growth over time onto a structure without a mineral accretion device

When looking at the overall behavior of the two structures (Table 3), it appears that during the test period without electricity running through the conductive materials, the branches seemed to have a fairly high overall growth. Indeed, even though the scarcity of specimen in the table don't allow a very thorough standard deviation, has had a growth of about 0.022 [cm/day] for the horizontal branches and an average vertical growth rate around 0.017 [cm/day]. The values of 0.054 and 0.035 [cm/day] were obtained for the control structure respectively.

Table 3: Table of growth rates for first growth trial without electricity running through the structures for a 28 days period.

after 28 days	subject structure [cm/day]	control [cm/day]	
average H no electricity	0,022321429	0,054761905	
Stdev H no electricity	0,012209638	0,023570226	
average V no electricity	0,017857143	0,034821429	
Stdev V no electricity	0,013363062	0,014369176	

As extra data, an overall health evaluation of the colony was also performed. The overgrowing of the zip tie was separated from the rest as well as the presence of tunicates around the colonies.

Table 4: Tables of overall health of coral colonies and their branching total.

E structure health	17.07.15	14.08.15	28.08.2015	06.09.2015	06.09.2016
Healthy	4	3	3	3	3
Partially bleached	0	1	1	1	1
Bleached	0	0	0	0	0

NE structure health	17.07.15	14.08.15	28.08.2015	06.09.2015	06.09.2016
Healthy	3	3	3	3	2
Partially bleached	1	1	1	1	2
Bleached	0	0	0	0	0

E structure branching	17.07.15	14.08.15	28.08.2015	06.09.2015	06.09.2016
Branching total	8	9	10	10	10

NE structure branching	17.07.15	14.08.15	28.08.2015	06.09.2015	06.09.2016
Branching total	8	11	19	24	25

Table 5: Overgrowth of the zip ties around the coral's branches (start = coral begins to grow over zip tie; no = no growth over zip tie; yes = zip tie overgrowth)

overgrow zip tie?	28.08.2015	06.09.2015	11.09.2016
E1H	start	start	start
E1V	no	no	yes
E2H	no	no	no
E2V	no	no	no
E3H	no	no	no
E3V	no	no	yes
E4H	start	yes	yes
E4V	yes	yes	yes
NE1H	no	no	start
NE1V	no	no	no
NE2H	no	no	no
NE2V	no	no	yes
NE3H	start	start	start
NE3V	no	no	no
NE4H	yes	yes	yes
NE4V	yes	yes	yes

Table 6: Presence of tunicates on same branch as colony

Tunicates	28.08.2015	06.09.2015	11.09.2016
E1	yes	yes	yes
E2	yes	yes	yes
E3	no	no	No
E4	yes	yes	yes
NE1	no	no	no
NE2	no	no	no
NE3	no	no	no
NE4	yes	yes	yes

4. Discussion and conclusion

The first aspect to be discussed is the growth trial without current that we ended using as a basis for further study. Indeed, when looking at the questions that were asked at the beginning of the protocol, various behaviors have risen. Overall, the growth rate of the colonies on the control structure seems to have been much larger than the one chosen for voltage application (too few subjects to study will although lead to a too high standard deviation). No real conclusion can be drawn from such information but the very rapid growth of some branches (colonies 3 and 4 from control structure) may be consequences of a bias coming from the human factor: wrong zip tie placement which can lead to a movement due to strong currents, wrong measurements (easy tasks above surface may not be as simple underwater especially with a wavy weather). If we use the hypothesis that branching is synonym to health, then the fact that the control structure branched more and grew faster in that period of time may be a suggestion that its colonies are healthier. But then again, too many conditions (depth, temperature, light exposition) influence coral health. The second question addressed here about the placement of the branch on a colony seems to hold on both structures for this trial period. Finally, the higher presence of tunicates on the study structure (longtail form) may also have its influence on growth as they can behave as competitive organisms and "steal" building elements from the colonies. The average plots of the size for the first period of time seem to correlate with this information.

The second set of data taken with time intervals shorter than before displays various interesting features. Firstly, the growing overall standard error shows that a larger population study would have been more thorough (even though the information can still be used to reach some results). Still in terms of standard error, the subject structure seems to have a lower one for each trial which can come from more precise measurement and less fluctuating data resulting in a lower uncertainty.

Now looking at the same information as for the first time period (average growth rates) but for the post electrification time, the first two data collections seem to correlate with the fact that low voltage input has led to an increase in the growth rate of the colonies. Indeed, the curve's shape itself indicates such an information with a gradually increasing curve whereas the control structure exhibits a more constant rate that can even seem to decrease after a short while. The fact that the control curve still has a larger growth rate between the two first measurements after low voltage input can come (as said previously) from a previously more healthy coral population on the structure. It is however taken over by the next time gap where the growth rate of the subject structure surpasses the control. The last data collection proved to go in the same direction and the exponential-like behavior of the curve (which would settle to a constant growth rate at one point of course) is still characteristic of the electrified structure.

Overall health (thanks to the extra data) of the structures can also provide some insight. Under the hypothesis that branching correlates with a healthier colony, the control structure clearly displays a better branching behavior which explains the first parts of the graphs. The fact that one colony from the subject structure bleached a little could also correspond to such an information but represents a too low population variation to give a clear conclusion.

The overgrowing of the zip tie after electrification doesn't really give any insight to the problem since the control structure just seem to have a slight superiority to the subject one. More overtaking could have meant a better health. The tunicate coverage is a possible second explanation of the first trial without electricity since the control seems to have done better during that time than the study structure. However, these tables cannot be used to reach clear conclusions.

In the end, the overall data and conclusions that can be drawn from it seem to start to correlate with the first hypothesis of increased coral growth on a structured equipped with a mineral accretion device; and moreover with this particular one (solar powered coral aid device). Indeed this experimental study can represent a basis to success of the second step trial for the developed biodome in a hostile to coral growth environment such as Tien Og Bay. Moreover, even though this report doesn't prove things in a greater scale, one can say that it has proven that the device at hand can be a very resourceful (and cheap enough) technology for future active coral restoration techniques.

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