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TESTING THE SUITABILITY OF THREE LOCATIONS FOR OUT-PLANTING STAGHORN CORAL ON SABA

Saba Conservation Foundation

van hall larenstein university of applied sciences

Testing the suitability of three locations for out-planting *Acropora cervicornis* regarding fragment health, survival, coral growth, sedimentation and turbidity

Bachelor Thesis research project

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Abstract

The previously dominant, reef-building *Acropora cervicornis* abundance decreased drastically across the grater Caribbean area since the 1970s. This is mainly due to the white band disease (WBD) and coral bleaching events. They have been considered a critically endangered species by the IUCN Red List since 2008 and restoration efforts are paramount to assist recovery of this species population. The exceptionally high growth rate and possibility of fragmenting this species, make it very suitable for a restoration technique called coral gardening. This technique grows coral colonies from small fragments in ex- or in-situ coral nurseries before out-planting them into the natural reef. On Saba, a small island in the Dutch Caribbean, such a coral nursery is present. To test which locations are best suited for focusing future out-planting efforts, all previous out-planting trials have been analyzed to select three promising locations. In this research, two rebar frames have been installed at each of these locations. Per location, one frame was equipped with 20 fragments of the genotype 'HiC' and the other one with 20 fragments of the genotype 'LL'. For the following 70 days fragment health, survival, growth rates, sediment settlement and turbidity have been measured biweekly. No fragments were lost or experienced any partial or complete mortality. A significant effect of the combination of genotype and location on growth rate could be found. The growth rates at location 'Nursery' and 'Big Rock Market' did not differ, however, at location 'Hole in the Corner' the genotype HiC had a significantly higher growth rate compared to the other locations and genotype. Turbidity at the Nursery had higher variation than at the other locations, however no statistical significance could be found. The same trend could be seen in the sedimentation, the Nursery had much higher variation and significantly more sediment settlement than the other two locations. The location Big Rock Market experienced significantly less sedimentation than the location Hole in the Corner but both locations experienced more stable conditions than the Nursery and are considered suitable. The high amount and variation of turbidity and sedimentation at the Nursery make this location unsuitable for out-planting efforts. Sedimentation at Big Rock Market was exceptionally low and consistent, which could benefit out-planting efforts, but faster growth rates at Hole in the Corner will yield more coral. Because the locations are approximately 1.5km apart and both considered suitable, it is recommended to outplant at and in between both locations instead of focusing out-planting efforts on one location, in order to increase resilience of the out-planting efforts against natural stressors such as new outbreaks of the WBD.

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1. Introduction

Coral reefs are amongst the most productive and diverse ecosystems on this earth (Bellwood & Morias, 2020). Covering only 255.000 km² of marine waters (Spalding & Grenfell, 1997), they inhabit approximately 9% of the global fish stock by providing habitat and important nursery grounds for many species (Sorokin, 1995). Humans benefit from coral reefs not only from the jobs and economic value they generate through fisheries and the tourism industry, but also ecosystem functions such as shoreline protection (Ferse, 2008), creating a global annual net benefit of approx. $29.8 billion in economic activities and resources (Pilnick A. , O'Neil, Patterson, & Moe , 2021).

Coral reefs consist mainly of two different types of corals. Reef building corals or so-called stony corals and the non-reef building corals, known as soft corals (Castro & Huber, 2005). Reef building corals build up a calcium skeleton which creates a three-dimensional structure in the reef which will maintain even when the coral is dead, providing habitat for invertebrates, algae and fish species to live and grow on (Ferse, 2008) (Castro & Huber, 2005). One of the most important reef-building corals in the tropical western Atlantic is *Acropora cervicornis*, known as Staghorn coral. This fast-growing coral species creates dense thickets in forereef, backreef and patch-reef environments in depths of up to 20m (Ware, et al., 2020). *A. cervicornis* and *A. palmata* were the two main habitat building coral species in the Caribbean, commonly covering more than 30-50% of the total coral cover in reefs <20m depth (Bellwood, Hughes, Folke, & Nystroem, 2004).

However, a series of events caused the abundance of this species to significantly decline across the tropical western Atlantic. The first recorded outbreak of the "white-band-disease" killed over 80% of the populations of *A. palmata* and *A. cervicornis* in the 1970s to 1980s (Weil, Coral Reef Diseases in the Wider Caribbean, 2004). The following mass mortality of the keystone herbivore urchin *Diadema antillarum* removed approx. 95% of the population in 1983-1984 (Lessios, 1995). In combination with overexploitation of most herbivorous fish species due to overfishing (Homes, 2021), the mass mortality of the *Diadema antillarum* resulted in a phase shift from coral dominated reefs to algae dominated reefs (Hughes, 1994). Higher algae coverage directly restricts coral settlement, because the settlers need bare substrate to settle (Highsmith R. C., 1979). These events were followed by local and regional outbreaks of coral bleaching in the 1980s and 1990s, leading to further replacement of corals by macroalgae (Jackson, Donovan, Cramer, & Lam, 2014) (Hughes, 1994). An additional stressor on Caribbean coral reefs and especially *Acropoids*, because of their 3-dimensional appearance, are hurricanes, which are projected to increase in frequency and intensity (Eakin, et al., 2010). The mechanical damage hurricanes cause on coral reefs reduces the average coral coverage by 17% in the year following the hurricane (Gardner, Cote, Gill, Grant, & Watkinson, 2005). The combination of these stressors resulted in a dramatic decline of coral coverage and the abundance of *Acropoids* in the greater Caribbean area (Eakin, et al., 2010). This decline, specifically of *A. cervicornis* and *A. palmata* resulted in a loss of habitat complexity, biodiversity and compromises reef functioning (Ware, et al., 2020). *A. cervicornis* is considered a threatened and protected species by ESA, SPAW and CITES (NOAA Fisheries, 2022). Natural recovery of this species to pre- 1980's levels is unlikely without assistance, due to stressors remaining high and potentially increasing in the context of climate change. Therefore, restoration efforts are paramount for this species to achieve population recovery.

On Saba, a small volcanic island in the Dutch Caribbean (Figure 1), the last wild populations of *A. cervicornis* that remained after the most recent outbreak of the white-band-disease, did not survive the category 5 hurricanes Irma and Maria in 2017 (Homes, 2021). However, fragments of two wild mother colonies remain in the coral nursery, which was set up in 2017 before the hurricanes hit the island. This nursery was set up in the context of a three-year study for Restoration of Ecosystem Services and Coral Reef Quality (RESCQ) on Saba, St Maarten, St Eustatius and the Turks and Caicos Islands, to study and develop coral gardening methodologies (RESCQ, n.d.).

Figure 1: Map showing Saba in the Caribbean Sea (Island Life Caribbean, 2023)

Coral gardening is a restoration technique established in 1995 which has crystalized itself as a key restoration tool (Epstein, Bak, & Rinkevich, 2003). Adapted from terrestrial reforestation, this technique grows coral colonies from small fragments in either ex- or in-situ coral nurseries, before out-planting them into natural reefs once they reach the desired size for out-planting purposes (Epstein, Bak, & Rinkevich, 2003). Compared to direct fragment transplantation or larval enhancement, coral gardening stands out by mass-producing coral fragments with low-tech methodologies independently from successful coral settlement or availability of wild donor colonies (Bowden-Kerby, 2001) (Herlan & Lirman, 2008). For restoring populations of *A. cervicornis*, this restoration technique promises an effective approach, considering success and resources of gardening practices (Young, Schopmeyer, & Lirman, 2012). This is mainly because of this species remarkably high growth rate of 10-30 cm annually (Weil, Hammerman, Becicka, & Cruz-Motta, 2020) (Reef Renewal Bonaire, 2022) and fragmentation mimicking the species natural asexual reproduction typically caused by storms (Bowden-Kerby, 2001) (Meester, Boomstra, Hurtado-Lopez, Montbrun, & Virdis, 2015) (NOAA Fisheries, 2022). *A. cervicornis* is known to experience an increase in growth rate after approx. 3-4 weeks recovery from the fragmentation. Axial corallites start to form and develop new branches which causes the fragment to have an increased growth rate for the following 3-4 months (Lirman, et al., 2010). This suggests a shift in resources within the coral to promoting formation of new branches, which will be referred to as a ‘growth shock’ reaction. The combination of fast growth and fragmentation probability make this species very suitable for coral gardening and rearing in a coral nursery.

The nursery on Saba was maintained by the Saba Conservation Foundation (SCF) even after the end of the RESCQ-project in 2020 with the intention to use it for a continuous out-planting program. Multiple out-planting trials have been conducted to find a suitable location to focus out-planting efforts on. These trials only out-planted low numbers of fragments and paid no attention to the difference between genotypes, making the results not very accurate, because different genotypes might differ in their general and location specific performance. This research is conducted to gain more insight into the suitability and performance of potential out-planting locations. Therefore, the results of these trials have been analyzed to exclude locations with high mortality before consulting with the SCF about which three potentially suitable locations to test in a final out-planting trial.

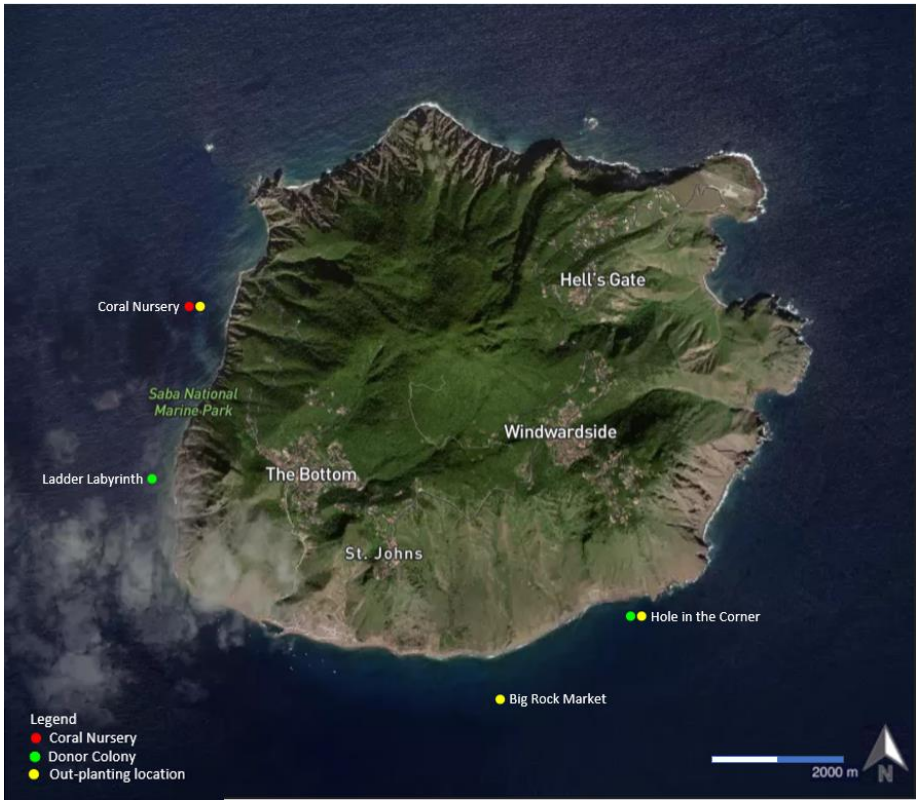

Figure 2: *Map of Saba showing out-planting locations at the Coral Nursery ((17°38.235 N, 63°15.376 W)., Big Rock Market (17°36.740'N, 63°14.249'W) and Hole in the Corner (17°37.009'N, 63°13.683'W), as well as locations of the donor colonies in Ladder Labyrinth (LL) and Hole in the Corner (HiC)*

The selected locations are the 'Nursery', 'Big Rock Market' and 'Hole in the Corner' (Figure 2). This research will test suitability of these locations with regards to the two genotypes that were taken from the last wild colonies at Hole in the Corner and Ladder Labyrinth (Figure 2) in 2017. For a location to be suitable for large scale out-planting efforts, fragment survival, fragment health and growth rates need to yield the best possible results. According to benchmarks for *A. cervicornis* restoration established by Shopmeyer, et. Al. 2017, a fragment survival of >77% and live tissue of >75% are a reasonably high result (Schopmeyer, et al., 2017). Thus, a performance of the tested locations comparable to these benchmarks is considered suitable. Besides these requirements, lower sediment settlement or turbidity would make a location more suitable than another, since these factors are known to be limit coral health, growth and survival (Roy & Smith, n.d.) (Rogers, 1990). Especially on Saba these factors seem to be predominant limitations, because of the island's topography (Mulder, 2017). Saba, being of volcanic origin, has many steep and loose cliffs that often collapse into the sea and with heavy rain create large amounts of wash off. This results in high sediment settlement at some locations and frequently turbid waters.

Sedimentation has killed coral reefs through geological time before any aspects of anthropogenic stressors have. Sedimentation causes a diversion of energy by the corals to remove sediment particles from coral tissue (Rogers, 1990), thereby limiting available energy for coral growth. Sediment-clearing rates decline after ≤ 2h making *A. cervicornis* resilient to occasional sedimentation events, but extended periods of high sediment settlement negatively affect colony growth, cause tissue loss and reduce densities and degenerative zooxanthellae (Hodel & Vargas-Angel, 2007) (Risk, 2014) (Rogers, 1990). Depending on the composition of sediment, partial tissue loss or colony mortality can occur within 24h (Weber, de Beer, & Fabricius, 2012). Finer particles that stay suspended in the water column for longer can create a dense layer of turbid water which limits light availability and thereby the photosynthetic activity in the reef. This is limiting calcification rates and colony growth, because of their dependency on light intensity (Enochs, et al., 2014). Turbidity and sedimentation correlate negatively with fecundity, thereby greatly limiting the long-term success of out-planting purposes to restore populations of *A. cervicornis* (Kojis & Quinn, 1984).

1.1. Research questions

The main objective of this research is:

> What differences in suitability of the selected locations can be found within 3 months monitoring after out-planting *Acropora cervicornis* on Saba?

To answer this, the following sub questions needed to be answered:

> 1. What are the differences between locations regarding survival and growth rate of the out-planted fragments within 3 months?
> 2. What differences in sediment settlement and turbidity of the tested locations can be found within 3 months?

2. Methodology

2.1. Deployment of frames

The frames used to deploy the fragments on are 95cm x 95cm x 95cm cubes built from 3/4` rebar (Figure 3). On the bottom side of the frame is a double rebar bracing to increase the strength of the structure. The frames were cleaned meticulously before deployment using a variety of metal brushes, with emphasis put on the top of the frames where the fragments will be attached. The locations of deployment in the reef have been scouted beforehand and sand patches that could fit 2 frames at 15m ±1m depth have been marked for deployment, using a surface marker buoy. All frames have been placed approx. 2m distance to the closest reef and approx. 4-5m apart from each other. To deploy the frames, 8 rebar pieces of 50-60cm are hammered into the ground (Figure 3). To be able to tell the frames apart and trace back which genotype is attached, a steel label has been attached to each frame.

Figure 3: *Deployed frames next to reef at BRM*

2.2. *Acropora cervicornis* fragments and fragment deployment

2.2.1. Genotype availability

The nursery is located towards the east, on the leeward side of the island (Figure 2), between Ladder Bay and Wells Bay. It contains 13 'PVC trees' (picture in Appendix I.) at which the 380 *A. cervicornis* fragments grow in suspension at a depth of approx. 15 m. Of these 380 fragments, 50 can be traced back to be the genotype originating from the donor colony in 'Hole in the Corner' (HiC) and 161 fragments can be traced back to be the genotype originating from the mother colony in 'Ladder Labyrinth' (LL). The other fragments in the nursery could not be traced back to their origin because of lacking documentation and therefore have been excluded for donating fragments for this out-planting project.

2.2.2. Fragment availability

The growth of *A. cervicornis* colonies increases with increasing colony size, but colony productivity declines exponentially (Lirman, et al., 2014). Survival of the out-plants cannot be guaranteed, thus out-planting large fragments to increase total tissue generation also risks the loss of larger fragments. With the limitation of available fragments of the genotype HiC the risk of losing a substantial part of the nursery was mitigated by choosing colony productivity over total growth. Productivity describes the ratio of colony growth to initial colony size, which declines the larger the initial colony size colony is. The highest productivity of *A. cervicornis* colonies was found for a fragments size of <15cm (Lohr, Bejarano, Diego, Stephanie, & Carrie , 2015). An out-planting trial on Saba found fragments in a 15-30cm size class to experience the highest growth and fragment health (Fontijn, 2018). For this project a 15-20cm fragment size was chosen, to keep productivity high without mitigating fragment health.

2.2.3. Fragmentation, transportation and deployment

After fragments suitable for fragmentation have been located in the nursery, a team of divers descend to collect the corals. The corals have been measured and 15-20 cm fragments have been taken according to coral gardening practices developed by Lirman, et. Al. 2010, to minimize the impact of fragmentation (Lirman, et al., 2010). The fragments have been stored in genotype specific buckets which have been equipped with a thermostat. Once the buckets have reached the surface and have been stored on the boat, the temperature was checked in 5-minute intervals. As soon as the temperature increased by 1° C the water was partly replaced with fresh sea water. If the temperature remained constant, the water was changed after 20 minutes. Arriving at the out-planting location a team of divers descend with each bucket and started deploying the fragments at the frames. The most common methods of deploying fragments when out-planting are using epoxy or zip-ties (Hollarsmith, Griffin, & Moore, 2012). For this project the inexpensive and less labor-intensive method of securing the fragments using zip-ties will be used. At each side of the frame five 4mm zip ties have been deployed evenly distributed across the length of the frame. Before fastening the 4mm zip ties, a 2mm zip tie has been laid in between the zip tie in the frame, lining up alongside the bar of the frame. This 2mm zip tie is used to attach the fragment in the middle, causing the fragment to be slightly elevated above the frame with as little contact with the frame as possible. A picture of this can be found in Appendix 1 for better understanding. Only fragments with 100% live tissue have been used and deployed in this project. All divers involved have received specific instructions on how to handle and deploy the fragments in a meeting. The fragmentation of coral was only conducted by experienced personnel.

2.3. Monitoring and data collection

Monitoring and data collection occurred biweekly using the Saba Marine Park boat 'Lady Rebecca'. Protocols for size measurements, fragment health assessment, sediment and turbidity measurements can be found in Appendix II.

2.3.1. Fragment size

To determine the growth rate of the fragments and compare it between the different locations, a size measurement of each fragment was taken. The measurements have been taken biweekly during SCUBA diving, starting at the day of deployment. The pictures of each fragment have been taken ensuring to capture the fragment at an angle where its curvature can be measured. Afterwards, pictures capturing each secondary branch in an angle that allowed accurate measurements to be taken. The pictures have been analyzed using the open-source software ImageJ. The length of the primary branch was measured first, followed by measurements of each secondary branch exceeding 1cm in length. The combination of all measurements will be noted as Total Linear Extension (TLE) and will be used to determine the growth rate.

To check for possible measurement errors, a reliability-test was conducted, by double measuring the fragments on one of the frames and comparing the TLE measurements. Additionally, the frame was measured and recorded measurements were compared to known dimensions of the frame.

2.3.2. Fragment health status

The fragment health was assessed by measuring the percentage of healthy tissue and dead or algae overgrown tissue on the pictures taken for the size measurement. The fragments health is classified in 6 health classes as used by Fairhurst 2018 (Table 1). Examples of tissue loss, bleaching, predation and algae overgrowth can be found in the protocols in Appendix II.

Health class	Criteria
1	The fragment shows no signs of tissue mortality, predation, algae overgrowth or broken tips
2	>25% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
3	25%-50% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
4	50%-75% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
5	>75% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
6	fragment is missing

***Table 1**: Health classes of out-planted A. cervicornis by* (Fairhurst, 2018)

2.3.3. Turbidity

To measure the turbidity a Secchi disk was used. Personal observations around Saba show that the surface water is often more turbid than the deeper water layers. Therefore, a vertical Secchi disk was chosen to measure turbidity. In the case of such low turbidity that the Secchi disk can still be seen on the sea floor at the out-planting location (16m depth), the measurement was taken in the same general area, but in deeper water (approx. 30m-40m depth). The turbidity measurements have been taken both before and after the dive at each location.

2.3.4. Sedimentation

The difference in sedimentation between the locations was measured using sediment traps. A sediment trap was placed on the landward side, next to each frame. Resulting in a total of 6 samples being collected and replaced every 2 weeks. The sediment captured in the traps was dried according to the protocol (Appendix II.) and the dry weight was measured and compared between locations.

2.3.5. Statistical analysis

Statistical analysis was performed using IBM SPSS (statistics 26). Growth rate and sedimentation data was found to be normally distributed. Turbidity data was not normally distributed.
To test the differences in growth rate per genotype per location a Two-Way ANOVA was used. To compare sedimentation between locations a Two-Way-ANOVA was used. To test changes of growth rate over time per genotype and location, a General Linear Model was used. Because turbidity data was nonparametric, a Kruskal-Wallis test was performed to compare observed turbidity between locations. All tests have been performed with a 95% confidence interval ($\alpha = 0.05$).

3. Results

3.1 Fragment health

In the 3 months monitoring, none of the 120 out-planted fragments experienced any partial or complete tissue loss. Although coralivorous fish have been observed in the vicinity of the outplants, no signs of predation could be found and no fragments have been lost. One fragment got broken in half at the Nursery location two weeks before the end of the monitoring period. This happened most likely by an accidental fin kick of a diver and thus is not appropriate for any interpretation of suitability of the tested locations. Besides this one fragment, all fragments at each location have been in health class 1 (0% tissue loss) for the duration of this monitoring.

3.2 Fragment growth

The two-way analysis of variance revealed no significant effect of location (df=2, F=0.667, P=0.515) and genotype (df=1, F=2.970, P=0.088) on the growth rate of the out-planted fragment. At the Nursery the average growth rate was 1.9 ±0.7cm /14 days (9.6 ±3.6cm total growth, Productivity of 2.7cm/year), slightly more than at Big Rock Market, where it was 1.8 ±0.6cm /14 days (9.2 ±3.2cm total growth, productivity of 2.6cm/year), but smaller than at Hole in the Corner was 2 ±0.8cm /14 days (9.8 ±3.8cm total growth, productivity of 2.9cm/year). A significant effect of the combination of location and genotype (df=2, F=4.504, P=0.013) could be found as shown in Figure 4. Post hoc comparisons using the Tukey test indicated that the mean growth rate at location Hole in the Corner was significantly higher (P=0.006) for genotype HiC with 2.4 ±0.4cm (11.5 ±4.1cm total growth, productivity of 3.5cm/year), than for genotype LL with 1.6 ±0.4cm (8.2 ±2.6cm total growth, productivity of 2.3cm/year). For this reason, further analysis was conducted differentiating between the different genotypes.

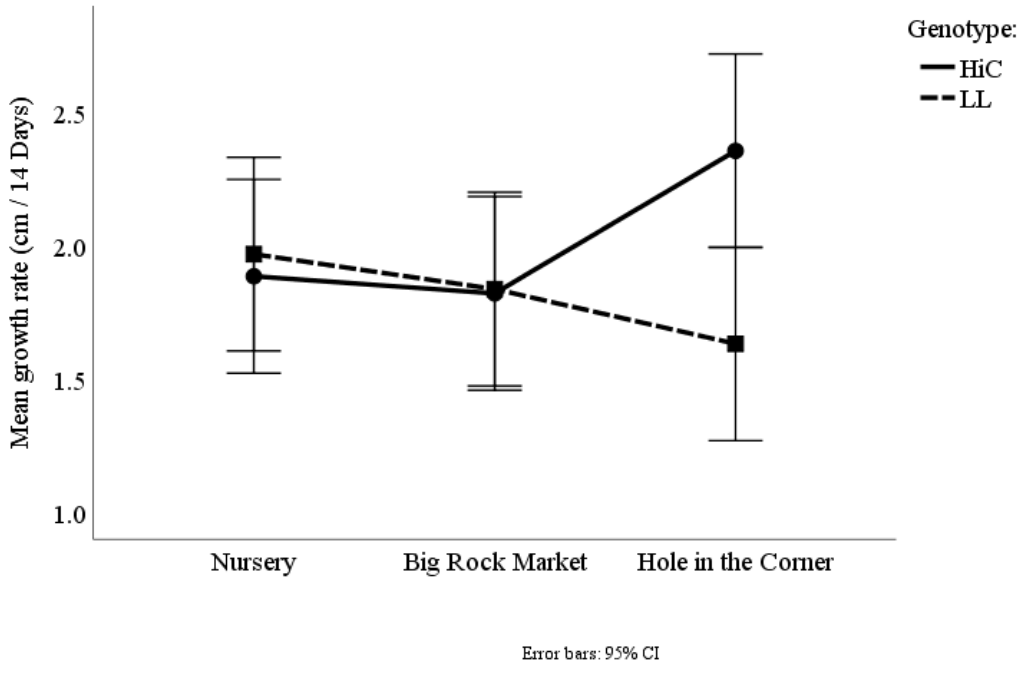

***Figure 4**: Mean growth rate (cm/14 Days) per location and genotype within the 70 days monitoring period.*

A general linear model was used to gain insight into how the growth rate changed over time. Results show that time has a significant effect on the mean growth rate ($F=191.103$, $P<0.001$) across all locations and genotypes as shown in Figure 5. The mean growth rate at day 28 (0.9 ±0.5cm) is significantly lower ($P<0.001$) than at day 42 (2.3 ±1.5cm) and the mean growth rate at day 70 (3.1 ±2.5cm) is significantly higher than growth rates before day 56. The growth rates per location and genotype did not differ statistically significant until day 56. At this point the genotype HiC had a significantly higher ($F=4.041$, $P=0.04$) growth rate with 2.8 ±1.8cm than genotype LL with 2.4 ±1.4cm. This is due to genotype LL experiencing a decline in growth rate at location Nursery and Hole in the Corner from day 42 to day 56, due to branching. No statistically significant different growth rate between genotypes or locations can be found at day 70, indicating that growth rates stabilize after day 70.

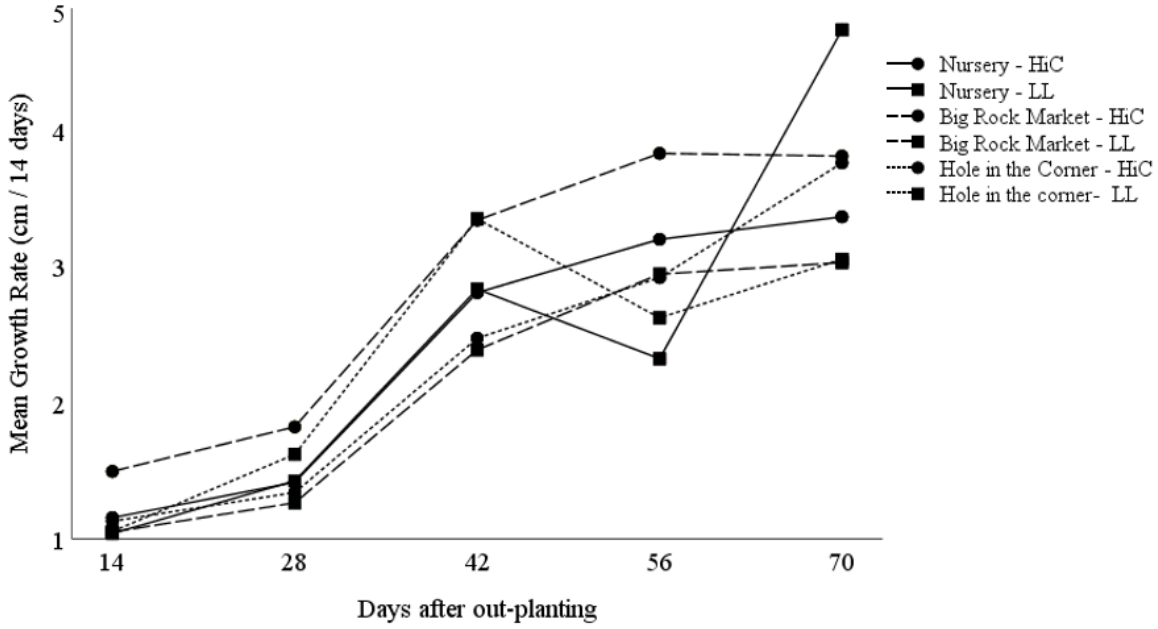

Figure 5*: Mean growth rate (cm/14 days) per genotype and location over time.*

3.3 Turbidity

The turbidity at the Nursery experienced the highest variation with 17.5 ±4.9m. The lowest mean turbidity and least variation was measured at Big Rock Market with 20.3 ±2.5m. The location Hole in the Corner had a mean turbidity of 19.8 ±3.2m. The results of the two-way analysis of variance showed no statistical significance for the differences of turbidity data between locations. However, the range of measurements indicated greater variation in turbidity at the Nursery, ranging from 10m up to 25m, compared to Big Rock Market (17m-24m) and Hole in the Corner (16m-25m) as shown in Figure 6.

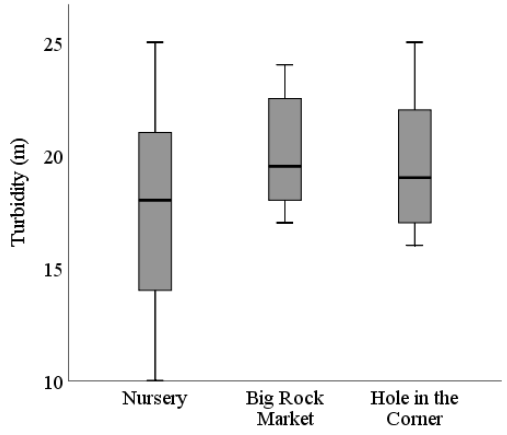

***Figure 6**: Mean turbidity per location within the 70 monitoring period*

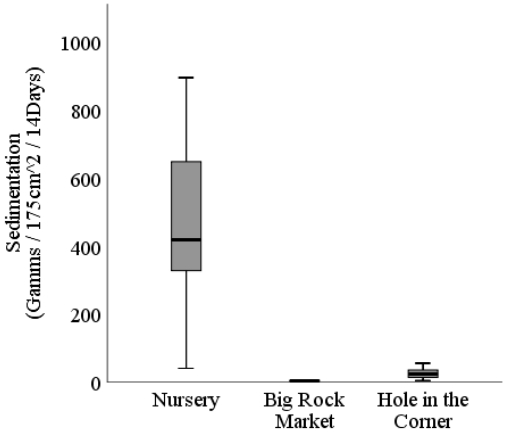

***Figure 7**: Sedimentation (g/175 cm^2/14 days Days) per location within the 70 days monitoring period*

3.4 Sedimentation

Sedimentation data was not normally distributed. The Kruskal-Wallis comparison showed significant differences between the sediment collected, as shown in Figure 7. Mean sedimentation per 14 days in the Nursery was 447.3 ±267.7g, this is significantly more than at Hole in the Corner (P=0.006), with a mean sedimentation of 27.2 ±20.3g. The mean sedimentation measured at Big Rock Market was 3.4 ±1.4g, which is significantly lower than the sedimentation at Hole in the Corner (P=0.025). The variation of collected sediment is very high at the Nursery, ranging from 40g to 895g, with variation below 380g appearing to be the exception. The locations Big Rock Market and Hole in the Corner also experience variation, however the range in which these occur is much smaller, with sedimentation at Big Rock Market ranging from 2-6g and at Hole in the Corner from 5-72g.

4. Discussion and Conclusion

This research monitored out-planted *A. cervicornis* fragments at three different locations around Saba for 70 days. To assess the suitability of these locations for future out-planting projects, fragment survival, health, growth rate, turbidity and sediment settlement have been monitored biweekly. High survival rates are essential for a location to be considered suitable. A direct mortality response to the fragmentation and out planting process is not uncommon. Mervado-Molina, Ruiz- Diaz & Sabat, 2014 reported a survival rate of 90% within the first 4 weeks after out-planting and Herlan & Lirman, 2008 reported a survival of 82.7% % after 8 weeks (Mercado-Molina, Ruiz-Diaz, & Sabat, 2014) (Herlan & Lirman, 2008). The locations tested in this study experienced 100% fragment survival and 0% tissue loss within the first 70 days after out planting. Other out-planting efforts on Saba reported lower survival and health (Fairhurst, 2018), indicating a suitability of these locations considering the direct mortality response. Benchmarks for assessing the success of out-planting efforts suggest a location to be unsuitable when survival is less than 77% within the first year (Schopmeyer, et al., 2017). To gain further insight into the suitability of the tested locations regarding long-term survival, it is advised to revisit the out-planting locations again in December 2023 to assess survival rates one year after out-planting. Notice that *A. cervicronis* is known to experience a decreasing survival rate over time, regardless of the suitability of a location. A location with survival of 85% after the first year can result in 50-60% survival 3-4 years after out-planting (Ware, et al., 2020). Therefore, monitoring decreasing survival at the locations tested in this study is a natural occurrence and does not necessarily indicate unsuitability of the location. Growth rates of out-planted *A. cervicornis* can be genotype specific and are dependent on fragment size and out-planting depth, which needs to be taken into consideration when comparing growth rates. A previous out-planting trial on Saba using a fragment size of >15cm and an out-planting depth of ~18m, reported an average growth rate of 5cm/year (Fairhurst, 2018). The observed average growth rate in this study was 45.6cm/year. This growth rate was expected to be higher than in the previous trial, due to a shallower out-planting depth and a larger fragment size. Still, the large difference between these two trials might also indicate a better suitability of the locations tested in this study than the previous locations. Wild colonies at >10m in Jamaica and Barbados grew 12-26cm/year although the shallower depth suggest higher growth due to more light availability (Weil, Hammerman, Becicka, & Cruz-Motta, 2020). As well as observations from out-planting projects in the Florida Keys reporting an average growth rate of 10cm/year during the first two years (Ware, et al., 2020). The exceptionally high growth rate monitored in this study compared to other observations can be explained by the growth shock, which is the fragments response to the fragmentation. After recovery from mechanical damage to the colony, recourses are shifted internally toward the formation of new branches, resulting in an increased growth rate for a duration of 3-4 months post fragmentation (Lirman, et al., 2010). This growth shock reaction can be seen in the results of the general linear model analysis, which shows the significance of time on growth rate. Before day 28, the fragments were recovering from the fragmentation process. The steep increase of growth rate from day 28-56 shows the beginning of the growth shock reaction. The increase of growth rate seems to stabilize after day 56 reaching a peak growth rate for approx. 2-3 more months (Lirman, et al., 2010). This trend can be seen across locations and genotypes. Genotype LL at location Nursery and Hole in the Corner experience a decrease of growth rate at day 56, followed by a steep increase on day 70. The reason for this is the methodology chosen to measure growth and branching of the fragments. Since only branches reaching a size of ≥1cm have been included in the TLE measurements, fragments can invest a lot of energy into growing branches, before this will show in the data. For example, a fragment growing 4 branches that are <1cm and thus are not included in the TLE measurements at day 56, will appear to have a lower growth rate. When these branches have reached a size of ≥1cm at day 70 they will be included in the TLE measurements, causing the growth rate to appear much higher than it was, because the growth of the branches that was not included in the previous data collection will show as an increase between day 56 and 70.

This happened for genotype LL with an increase in ≥1cm branches from day 56 to day 70 of 28% at location Hole in the Corner and an increase of 35% at the Nursery. The monitoring period of 70 days captured the beginning of the growth shock reaction but stopped at the point where a stabilization of growth rate could be seen. This makes comparison to literature difficult, because growth rates are often reported annually, which relativizes the effect of the growth shock, because post growth shock growth rates are included. This explains the in comparison very high growth rates monitored in this study. Nevertheless, the great differences in growth rates between the previous trial and the current suggests that all three tested locations are more suitable than the locations tested in the out-planting trial. Comparison of the different locations showed that growth rates did not differ significantly from another, but a significant combined effect of location and genotype could be found. The growth rate of genotype HiC at location Hole in the Corner was 57.6cm/year, which was significantly faster than the growth rate of genotype LL with 38.4cm/year. Showing that genotype HiC is adapted and thriving in the specific conditions in which the donor colony grew in Hole in the Corner prior to 2017. A longer monitoring period would have provided further insight into post growth shock growth rates, enabling direct comparison to literature and possibly revealing long term differences between tested locations. The results of turbidity and sedimentation measurements show clearer differences between the tested locations. Turbidity at the Nursery is much more variable than at the other two locations. The Nursery experiences much more unstable conditions than the other locations, including light limiting conditions with a visibility of 10m. This inconsistency and the occurrence of such high turbidity make this location the least suitable of the three locations. The other two locations have been observed to have more stable conditions and less variability. Personal observations confirm the documented variation at the Nursery compared to relatively stable conditions at Big Rock Market and Hole in the Corner. Especially rain resulting in wash off can cause large parts of coastal water to become extremely turbid within hours. These turbid waters often linger in the leeward part (south-west) of the island, whereas the windward side usually has currents that transport such wash off away. This results in more turbidity and sedimentation on this side of the island, which can be seen in the sedimentation. The Nursery experienced significantly higher sedimentation than the other two tested locations. The average sediment settlement at the Nursery was 15 times higher than at Hole in the Corner. The fragments capability of removing sediment covering coral tissue declines after ≤ 2h and depending on the composition of sediment, can cause tissue mortality within 24h (Weber, de Beer, & Fabricius, 2012). Sediment settlement at the Nursery would potentially bury fragments directly out-planted to the reef. The out-plants of this study and the coral growing in the nursery grows on set-ups suspended from the ground, eliminating the chance of getting buried. The conditions at the Nursery are unsuitable for out-planting at this location. The sediment settlement at Big Rock Market was significantly lower than sedimentation at Hole in the Corner with an average of 3.4 g/175cm^2/14 days. This exceptionally low average sedimentation at Big Rock Market makes this location suitable for further out-plants. Although sedimentation at Hole in the Corner was significantly higher than at Big Rock Market, it is still considered suitable, because an average of 27.2 g/175cm^2/14 days sediment settlement within 14 days is less than 0.1g of sediment settlement per day per cm^2. Considering the high sedimentation and large variation in turbidity at the Nursery, make this location unsuitable to focus out-planting efforts on. The more stable conditions at Big Rock Market and Hole in the Corner make these locations suitable to focus out-planting efforts on. The higher growth rate of genotype HiC at Hole in the Corner can be favorable for the success of such efforts but the exceptionally low and stable sedimentation at Big Rock Market might be beneficial in the long run. However, both locations are considered suitable and out-planting can occur at either of them.

5. Recommendations

It is recommended to revisit the out-planting locations approx. 1 year after out-planting (December 2023) to assess fragment health and survival. This could provide further insight into the suitability of the locations and if the performance of the fragments differs over time. Considering stressors that caused the decline of *A. cervicornis* populations such as the WBD or hurricanes, it is suggested to not only outplant in one location. Spreading out-planting efforts across multiple locations reduces the risk of a total fatality due to such natural disasters. The finding of this study shows that genotype HiC grows significantly faster at Hole in the Corner, but sediment settlement is lowest at Big Rock market. These two locations are approx. 1.5km apart, indicating a threshold of lowest sedimentation in combination with highest possible growth of genotype HiC somewhere in between. It is not necessary to locate this threshold if not desired, but the results of this research suggest suitability for out-planting on this 1.5 km stretch. When out-planting efforts occur, a similar deployment method as the one used in this study is suggested. Using rebar frames is cheaper and fragment deployment is more time efficient compared to other out-planting methods. Further, the zip-ties used to deploy the fragment were overgrown by the fragments within 8 weeks and did not limit the fragment in any way. The frames provide 3D structure which was less due to the decline of *A. cervicornis* populations. During the data collection it was noticed that this structure was used as shelter by various fish species. To further improve this method a few alterations are suggested. The frames can be cut in half, which would lower the height of the frames to 45cm but increase their stability and double the number of available frames because both halves can be used. Also, it is suggested to add cross sections onto the frame to allow more space for fragments to deploy. This would cause fragments to be deployed relatively closely to another which can promote fusion and interbranching of the fragment if only one genotype is used per frame. This way the frames might get overgrown completely and form one large colony after a couple of years. If this is the case, the frames can be used modularly to restock larger areas within a reef, greatly improving shelter availability in the reef. The potential of this method is emphasized and further studies to improve on its efficiency are recommended.

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Appendix 1 - Example Pictures

Looped zip-ties

Picture showing how fragments have been attached to the frame using zip-ties:

- attach a 2mm zip-tie to the frame using a 4mm zip tie (ensure the 2mm zip-tie is attached so that it can be closed above the frame

- pull the 4mm zip-tie as tight as possible

- use 2mm zip-tie to attach the fragment

- fasten the 2mm zip-tie enough that the fragment has no play, but not that much that it gets damaged

- cut of left over pieces of zip-ties as close as possible to the closing mechanism

PVC trees in coral nurseries

A structure consisting elevating coral fragments to enhance coral growth and limit the effects of sedimentation. PVC is used because it is available across the world and is a cheap and long-lasting building material. The structure is anchored to the ground with cinder blocks and held upright by a positively buoyant attachment at the top.

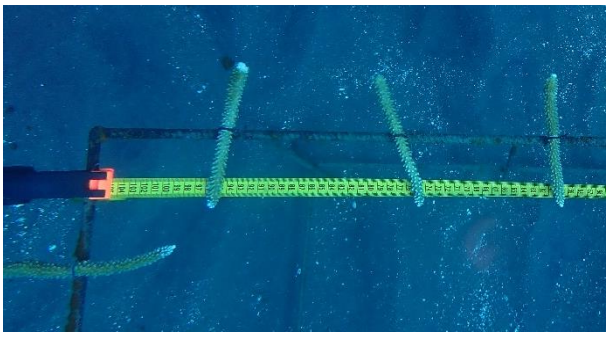
Appendix II – Protocols
Size measurements of outplanted *Acropora cervicornis*

Materials:

- Underwater camera (OLYMPUS tough TG-6)
- Charging cable of camera (mini-USB to USB)
- Measuring tape (ca 1.5m length)
- Spring clamp
- ImageJ (program)
- Computer

Method for pictures:

1. Attach measuring tape to the side of the frame using the spring clamp. Ensure that the measuring tape is as close as possible, but beneath all fragments.



2. Take a picture of the tag of the frame.

3. Take a picture of each fragment (example on page 24) following a clockwise rotation around the frame, starting at the corner with the tag. Ensure to take the picture in an angle that the extension of the fragment (curvature and branching) is orthogonal to the camera and that the measuring tape is clearly visible. Depending on the complexity of the fragment, multiple pictures of one fragment are needed to capture the extension in a measurable way.

4. After pictures of all fragments on one side of the frame (5 fragments per side) are taken, a picture of the divers' hand is taken, indicating which side of the frame is the next, by showing 2, 3 or 4 fingers.

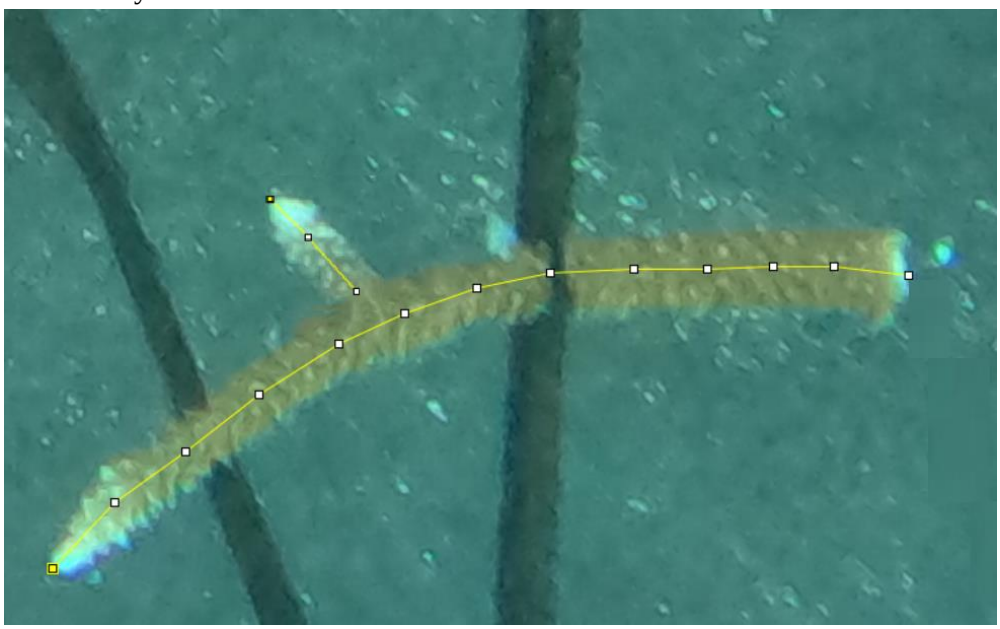
Following these steps for each frame, will result in a series of pictures which will first show the tag of the frame, then pictures of the first five fragments on that frame, then a hand showing a '2', indicating the second side of the frame will be shown next. Then pictures of fragment 6-10, followed by a hand, showing a '3', indicating the 3rd side of the frame is shown next. The pictures of the fragments 11-15 are shown next, followed by a hand showing a '4', indicating the last side of the frame is shown next, holding fragments number 16-20.

Transferring and saving pictures:

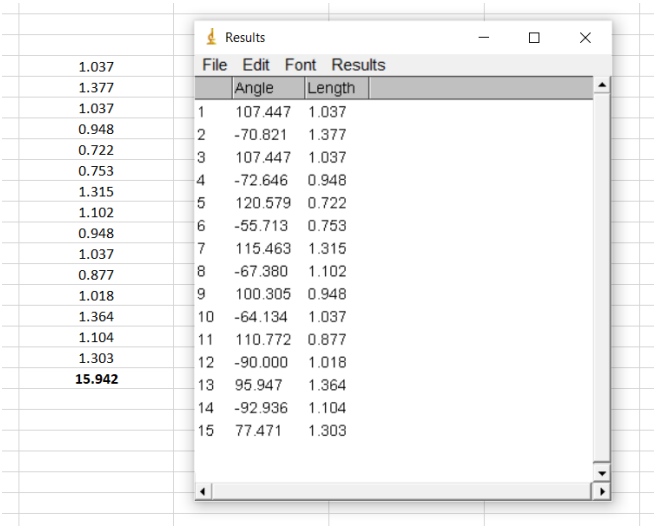
1. Connect the OLYMPUS TG-6 to a computer using the charging cable.
2. Select ‘storage’ on the camera.
3. Select the pictures of each frame individually to transfer them to the computer
4. Save the pictures following this structure:
   Location > Genotype > Sampling date

Measuring fragment length:

1. Open the image file in ImageJ (Ctrl + O)
2. Zoom in on the measuring tape and set the measuring scale to 1cm by selecting the *straight* tool and measure 1 cm.
3. Go to ‘Analyze’ > ‘set scale…’ > “Known distance” and fill in ‘1’
4. After the scale has been set, start measuring the fragment using the *straight* tool. Follow the contour of the fragment in the middle of the fragment, measuring (Ctrl + m) step by step in 0.5-1.5cm steps (as shown in example). Secondary branches of >1cm will be included in the TLE measurements.



5. The resulting measurements can be added up in excel and the resulting TLE will be noted in the excel sheet.



Following and repeating these steps, size measurements of each fragment, per frame and location can be taken. This data will be saved in an excel sheet following this structure:
Location > Genotype (frame) > fragment Nr. > date

Assessing fragment health

Materials:

- Fragment pictures from size measurements
- ImageJ
- Computer

Assessing fragment health:

1. When measuring the fragment size, check if the fragment shows any signs of tissue mortality, predation, algae overgrowth or broken tips
2. Assign which health class the individual fragment is in
3. Note health class per fragment, per time in the data set

The different health classes and the criteria for each can be found in the table below.

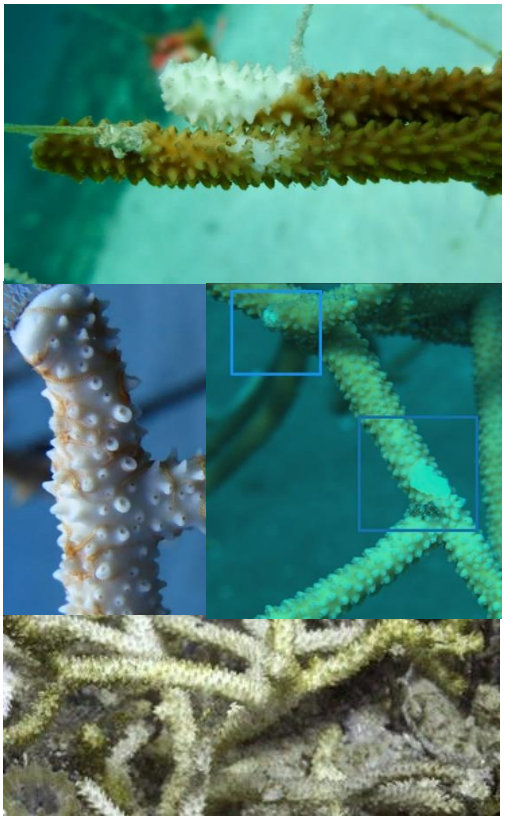
Health class	Criteria
1	The fragment shows no signs of tissue mortality, predation, algae overgrowth or broken tips
2	>25% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
3	25%-50% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
4	50%-75% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
5	>75% of the fragment shows signs of tissue mortality, predation, algae overgrowth or broken tips
6	fragment is missing

Examples of tissue mortality, predation and algae overgrowth:

Tissue mortality / partial bleaching

Predation (left - brittle star, right - parrot fish)

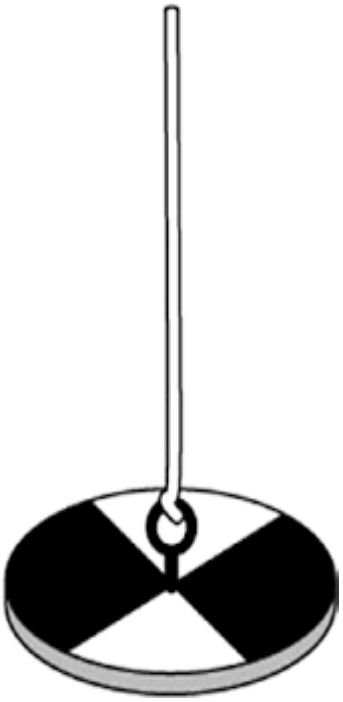
Algae overgrowth



Turbidity measurements

Materials:

- 20cm- diameter Secchi disk with an eye in the middle, divided into black and white quarters
- Ca. 200g weight
- >50m strong fishing line (>80lb)
- Diving slate with pencil



Assembly:

1. Mark the fishing line with a permanent marker at every meter
2. Attach the weight to the lower side of the Secchi disk
3. Attach the fishing line to the eye on top of the disk

Measuring turbidity:

1. Lower the Secchi disk into the water on the leeward side of the boat
2. Record the depth at which the disk is not visible anymore
3. Slowly raise the disk until it just becomes visible again and record that depth
4. Calculate the average of these two measurements and note it on the slate
5. Repeat this process to increase accuracy of the measurement

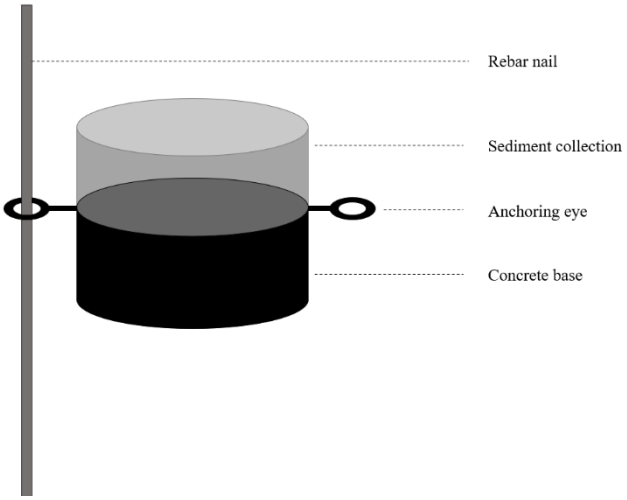
Consideration:

1. The quality of the depth data is user-dependent (personal function of vision)
2. The depth visibility of the Secchi disk is dependent on external factors such as sun light intensity, wave intensity and wind conditions.

Sediment traps

Materials:

- 75cm rebar
- Hammer
- Sediment trap
- Silicone lid with hose clamp
- Flat head screwdriver
- Class containers
- Rice cooker
- Scale



Installation:

1. Choose a spot between the reef and the frames and drive the piece of rebar about 50cm deep into the sand, by hammering it in like a nail
2. Place one of the sediment traps on the rebar. One of the eyes on the trap will is placed over the rebar, ensuring the trap to stay upright and in place for the deployment period
3. Repeat this process per frame and location (in total 6 times)

Collection:

1. After 14 days a diver will decent to the sediment traps and carefully place the silicone lid on it
2. When the lid is in place, it will be sealed by screwing the hose clamp tight
3. After the sediment trap is secured and collected, a new trap will be placed onto the rebar nail to collect sediment for the next 14 days

Dry weight:

1. Weigh an Erlenmeyer or glass jar and note the weight on it
2. Place the content of one sediment trap in this vessel and label which sample it is on the vessel
3. If there is still sediment left in the trap, flush it out with fresh water. This is done by filling up the trap with fresh water and closing it with the silicone lid, then shake the trap and remove the water. Repeat this until all sediment is removed from the trap
4. Wait approx. 2-3h till the free floating particles have settled and the water is clear
5. Carefully pour out the clear water; Caution, that only water is poured, and no sediment is lost
6. Repeat step 4 and 5, till most of the water is gone
7. Dry the sample at 60°C for at least 24h
8. When the sample is completely dry, measure the weight. To ensure the sample is completely dry, measure the weight of the sample after 24h and 26h, if the weight is constant, all water has evaporated. If not, keep measuring in ca. 2h intervals, until the weight is constant
9. Subtract the weight of the vessel and note the dry weight of the sediment sample in the data sheet