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Optimum fragment size of A. cervicornis for out planting and in-situ coral nurseries, Saba – Dutch Caribbean

January 2018 – June 2018

RESCQ

WAGENINGEN UNIVERSITY & RESEARCH

SABA CONSERVATION FOUNDATION

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Picture frontpage: Acropora cervicornis coral on the reef (source: scubadiverlife.com)

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Research report
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and the Saba Conservation Foundation

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Summary

Over millions of people around the globe depend on coral reefs for their livelihood and food. Coral reefs also play an important role in coastal protection against strong wave activities and coastal erosion. Globally coral reefs have been declining in the past few decades, due to human-induced climate change and anthropogenic disturbances e.g. coastal development, pollution, overfishing, anchoring etc. The worldwide decline in coral abundance along with losses in key ecosystem services, has prompted multiple efforts to mitigate further losses and restore reef function. One of thos efforts is the use of coral nurseries. . The nursery methodology involves three stages: 1. collection of a limited amount of coral fragments from wild populations; 2. growth of the fragments in a nursery setting; 3. out planting of the grown fragments to damaged reefs. In the Caribbean, the RESCQ-project has been organised to help restoring coral reefs and their ecosystem services damaged by mostly white-band disease, with the use of a coral nursery. The goal of this research is to find the optimum fragment size with the highest growth rate of *A. cervicornis.* Therefore, 50 *A. cervicornis* fragments were measured over a period of 55 days. During this study, the average growth rate of the fragments in the nursery of Saba was 5,7 cm in total length a month, with a survival rate of 80% of the fragments. Depth shows no significant influence on the growth rate of the fragments. Initial fragment size however did have a significant influence on the growth rate.

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1 Introduction

Over millions of people around the globe depend on coral reefs for their livelihood and food, whether by the tourism that they attract or through harvestable marine resources that they provide (Edwards & Gomez, 2007; Kuffner & Toth, 2016). Coral reefs also play an important role in coastal protection against strong wave activities and coastal erosion (Latypov, 2006; Hai Xin et al., 2014). Globally coral reefs have been declining in the past few decades, due to human-induced climate change and anthropogenic disturbances e.g. coastal development, pollution, overfishing, anchoring etc. (Hai Xin et al., 2014). The worldwide decline in coral abundance along with losses in key ecosystem services, has prompted multiple efforts to mitigate further losses and restore reef function (Edwards & Clark, 1998; Edwards & Gomez, 2007).

Elkhorn (*Acropora palmata*) and Staghorn (*Acropora cervicornis*) are branching hermatypic corals and used to be the most abundant reef-structure building species, because of their rapid growth and high calcification rates (Gilmore & Hall, 1976; Tunnicliffe, 1983; Hai Xin et al., 2014). Due to their branching morphologies (Figure 1.1&1.2), these corals provide a suitable habitat for many reef species (Precht et al., 2002; Bellwood et al., 2004; Quinn & Kojis, 2006; Young et al., 2012). Unfortunately, *A. palmata* and *A. cervicornis* populations have declined in the past few decades and their role in the ecosystem have not been taken over by another species (Acropora Biological Review Team, 2005). The last decades these coral species have shown to have low tolerance for extreme temperatures and to be vulnerable for disease and pests (Kuffner & Toth, 2016). During the late 1970's over 95% of the abundance was lost primarily caused by white-band disease and hurricane damage (Rogers, 1985; Acropora Biological Review Team, 2005; Kuffner & Toth, 2016). The constant reappearance of white-band disease and warm-water bleaching made recovery of *A. palmata* and *A. cervicornis* difficult (Kuffner & Toth, 2016). White-band disease completely removes the zooxanthellae-bearing coral tissue from the skeleton, leaving it vulnerable for algal succession (Gladfelter, 1982). Zooxanthellae are the symbiotic algae that live within the coral and provide it with food. Because of this dramatic decline in coral reefs, there has been an increased need of coral restoration methods (Forrester, et al., 2014).

Figure 1.1. Elkhorn coral (source: reefbuilders.com).

Figure 1.2 Staghorn coral (source: reefbuilders.com).

The use of coral nurseries as described by Rinkevich (1995) for active coral restoration is gaining acceptance (Lirman et al., 2010). The nursery methodology involves three stages: 1. collection of a limited amount of coral fragments from wild populations; 2. growth of the fragments in a nursery setting; 3. out planting of the grown fragments to damaged reefs (Shafir et al., 2001; Epstein et al., 2003; Rinkevich, 2006). Coral nurseries play an important role in the recovery of the threatened *A. palmata* and *A. cervicornis* in the Caribbean (Schopmeyer et al., 2017). This method has been ranked as most effective for *Acropora* species (Young et al., 2012), which could be explained by fragmentation being an important life history trait of *Acropora* species (Meesters, 2015).

In the Caribbean, the RESCQ-project has been organised to help restoring coral reefs and their ecosystem services damaged by mostly white-band disease. As part of this project, this research will provide information useful for a higher efficiency of the nursery on one of the islands covered under RESCQ:

Saba. Around Saba, almost all *A. cervicornis* went extinct due to White-band disease. The goal of this research is to find the optimum fragment size with the highest growth rate of *A. cervicornis*, both in the nursery as on the reef.. This way, the number of fragments produced in the nurseries can be increased. Various fragments of *A. cervicornis* will be planted out from the nursery and will be monitored for a certain period of time, to find the optimum start size of Staghorn fragments. Both in the nursery and when planted out. It is expected that the bigger fragments have a higher growth rate, because there is more surface available for the zooxanthellae's to conduct photosynthesis and thus growth.

2 Methods

2.1 Nursery setup

There are two nurseries around Saba. One of them is partially part of the project called RESCQ. The main nursery consists of five *ladder*-like structures and five *tree*-like structures, referred to as ''ladders'' and ''trees'' (Figure 2.1 and 2.2), at approximately 15 meters of depth and is located between Ladder Bay and Well's Bay (Appendix I). The small nursery is located at Rays n' Anchors (Appendix I), and only has one tree. Both nurseries are placed on the west side of Saba, as there is less erosion then other sides of the island (Mulder, 2017). The ladders have a total capacity of 25 coral fragments and are made with two plastic tubes as a top and bottom row and several bamboo sticks as middle rows (Figure 2.3). All rows are held together by rope. The ladder is kept in position with two cinderblock's at the bottom and buoys on top to keep it upright. The trees have a total capacity of 60 coral fragments and are made of several PVC pipes of which the rows are crossed (Figure 2.4). The tree is also kept in position with two cinderblock's at the bottom and buoys on top to keep it upright. Coral fragments are secured to these structures with fishing line and metal crimps, where they grow until big enough to plant out. All ladders and trees are named with letters from A to J. The codes of the fragments are determined as following: structure name (A to J) + row number (starting with 1 from the top down) + place of the fragment on the row (starting with 1 from left to right). For example: the third fragment on the second row on ladder H would be H23. As it was difficult to see wich fragment was the first in the row, a new tagging system with zipties was applied to the ladders (Appendix II). For this research, only ladder, G, I & J with *A. cervicornis* will be measured. Tree A, B, C, D & E belong to the Sanford University and will not be measured. A map of the main nursery is included in appendix III.

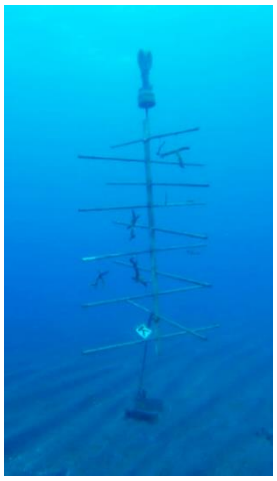

Figure 2.1 Tree-like structure

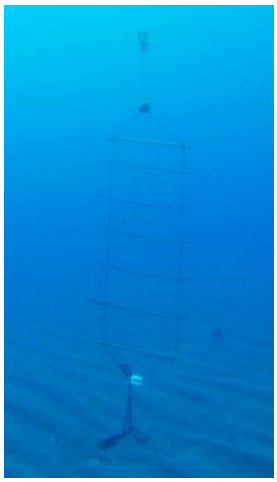

Figure 2.2 Ladder-like structure

Figure 2.3 Close-up of ladder structure

Figure 2.4 Close-up of tree structure

2.2 Mother Colonies

All structures contain fragments from three different mother colonies of *A. palmata* and *A. cervicornis* (Table 2.1). All colonies are found around the island of Saba, Dutch Caribbean. A map with locations of the mother colonies can be found in appendix I. All colonies are named after the closest dive site.

Table 2.1 Overview of mother colonies with species, location and structures.

Name	Genotype	Species	Location	Structures
Ladder Labyrinth	LL	*A. cervicornis*	17°37.636 N, 63°15.591 W	A,C, T
Hole in the Corner	HiC	*A. cervicornis*	17°37.002 N, 63°13.648 W	I, J, G
Green Island	GI	*A. palmata*	17°38.919 N, 63°13.842 W	G

2.3 Data collection

The measurements were done in the nursery and at the out planting site. The out planting site is part of a project by Ginger Fairhurst. As this project is about competition and different treatments, only the control group is used for this research project. This group contains six fragments. 5 spliced fragments remained in the nursery. The measurements were done once every three weeks. One dive per ladder was necessary to do all the measurements. Jelle van der Velde, Saba Marine park ranger, assisted with nursery related activities.

2.4 Measuring A. cervicornis

The growth of *A. cervicornis* fragments is measured in centimetres with a flexible sewing measuring tape. A team of two persons was needed to do the measurements. One person measured the length of the coral fragments, as the other person read the number and wrote it down on a datasheet printed on waterproof paper (Appendix IV). First, the primary branch length was measured. This branch is mostly easy to recognize as the thickest branch. Then, secondary, tertiary and quaternary branches were measured if present (Figure 2.5). The total size of the fragment was calculated by summing up the lengths of the main and al side branches.

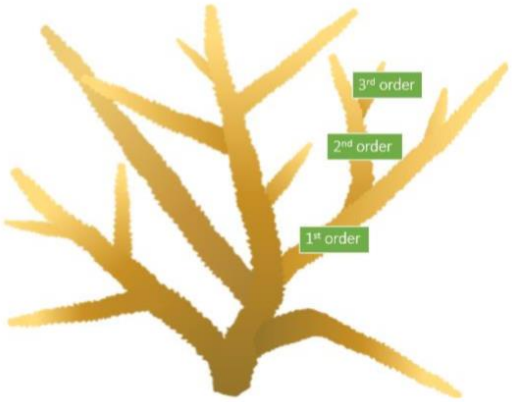

Figure 2.5 Schematic drawing of A. cervicornis with branch orders (source: Daniela Simal)

2.5 Data Analysis

Out of 50 *A. cervicornis* fragments, 49 were used for growth rate analysis. Fragments that showed a negative growth rate, were left out of further analysis as they would not give a proper image. For calculating the survival rate, all 50 fragments were included.

The average growth rate, which was used for the statistical analysis, was derived from true measurements of the fragments. For all statistical test, the alpha value was set to α=0,05 (Appendix V). The influence of depth on growth was analysed with a linear regression test. A Repeated Measures test was applied to analyse the effect of splicing on coral fragments. As there were a few fragments left after the swell on 5th march 2018, only five fragments were used in this analysis. For finding the ideal initial fragment size, a one-way ANOVA test was used. The fragments were placed into classes for this test. These classes were 0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm. For the statistical analysis, the software IBM SPSS Statistics 23 was used. For visualization of data, Microsoft Excel was used.

3 Results

3.1 Survival rate and growth rate

During this study, a total of 10 fragments got (partially) overgrown with algae. However, instead of removing the fragments, they were continued to be monitored. The overgrown fragments cannot be used anymore, making the survival rate 80% by the end of the study (Table 3.1). The average growth rate of 39 healthy and 10 overgrown fragments was derived from the true measurements of their maximum length in centimetres (Figure 3.1). The average measured growth per healthy fragment is (10,66/55) = 0,19 cm day (5,7 cm month / 68,4 cm year). The average measured growth rate per overgrown fragment is (10,11/55) = 0,18 cm day (5,4 cm month / 64,8 cm year). The results of the Repeated Measures tests (Appendix V) show that there is no significant difference in average growth rates between healthy fragments and overgrown fragments (P=0,509). The average number of tips of 39 healthy and 10 overgrown fragments was derived from counting's of their number of tips (Figure 3.2). The results of one-way ANOVA test (Appendix V) show that there is no significant difference in number of tips between healthy fragments and overgrown fragments (P=0,783).

Table 3.1 Survival rate during the study in percentages

Measurement	1	2	3
Days in nursery	19	34	55
Survival rate (%)	92	80	80
Total healthy fragments	46	40	40
Observations	4 fragments show signs of algae overgrowth	6 more fragments show signs of algae overgrowth	-

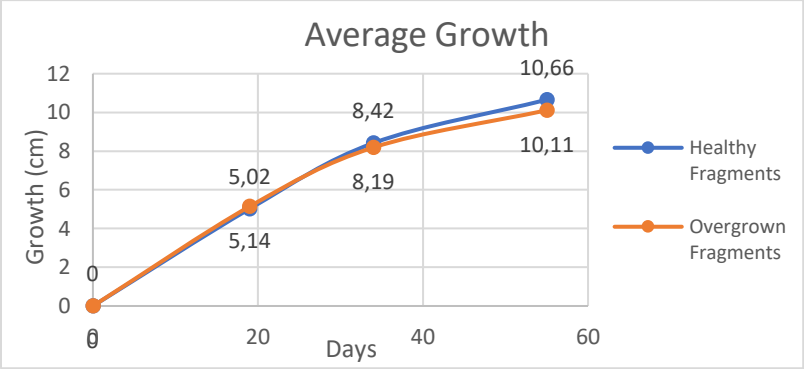

Figure 3.1 Cumulative average growth of the healthy and overgrown fragments over a period of 55 days. The dots represent each measurement.

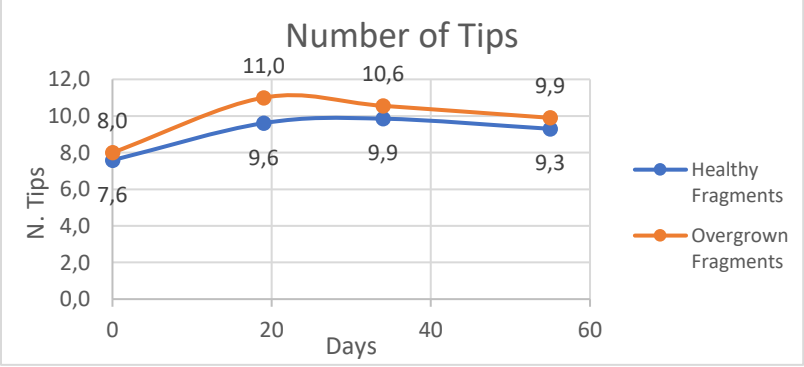

Figure 3.2 Average number of tips of the healthy and overgrown fragments over a period of 55 days. The dots represent each counting.

3.2 Influence of depth on growth

The results of the linear regression tests (Appendix V) show that there is no significant difference in growth between different depths (P=0,273). A different linear regression test (Appendix V) shows that there is also no significant difference in tip counting's between different depths (P=0,331). As pictured in figure 3.3 and 3.4, there is no linear pattern visible.

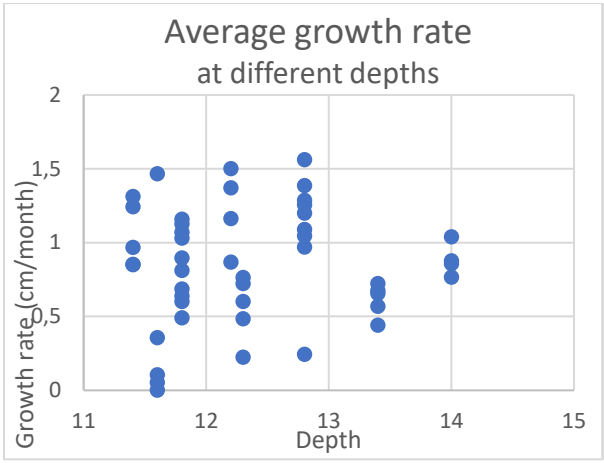

Figure 3.3 Average growth rate at different depths. Each dot represents a measurement.

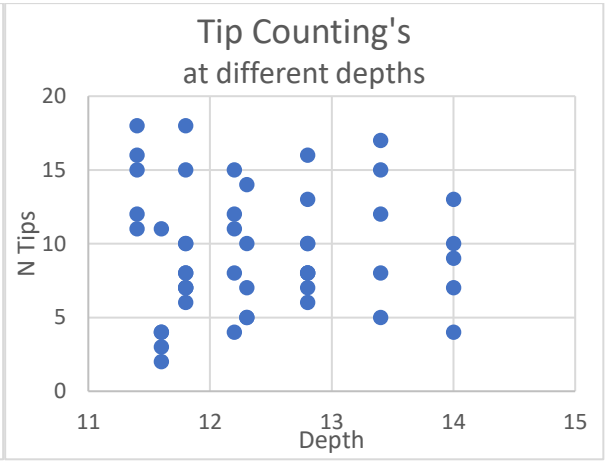

Figure 3.4 Tip counting's at different depths. Each dot represents a measurement.

3.3 Influence of splicing on growth

The results of the Repeated Measures test (Appendix V) show that there is no significant difference in growth between recently spliced fragments and other fragments (P=0,905). Despite that no significant difference was found, it does appear that spliced fragments continue to grow at an higher growth rate for a longer period of time compared with un-spliced fragments (Figure 3.5). An one-way ANOVA test (Appendix V) shows that there is no significant difference in the number of tips between the recently spliced fragments and the other fragments (P=0,987).

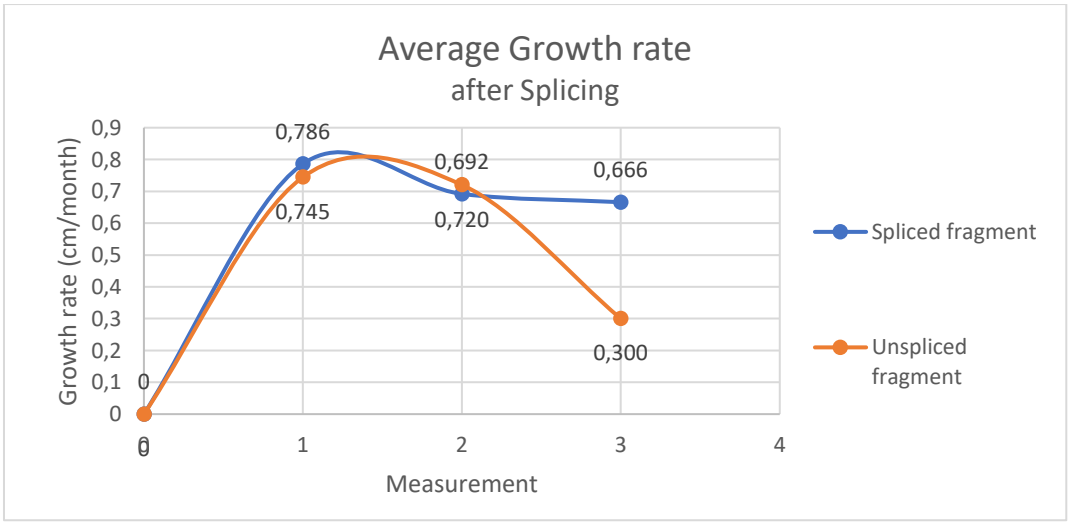

Figure 3.5 Average growth rate after splicing over a period of 55 days. The dots represent each measurement.

3.4 Influence of initial fragments size on growth

The results of the One-way ANOVA test (Appendix V) show that there is a significant difference between at least two groups (P=0,042). To find out where de difference is, a LSD test (Appendix V) was executed. The results of this test show that there is a significant difference between the group 0-15 cm and groups 15-30 cm (P=0,005) and 30-45 cm (P=0,020). The growth rate of an initial size of 0-15 cm is notably lower then the growth rates of bigger initial sizes (Figure 3.6).

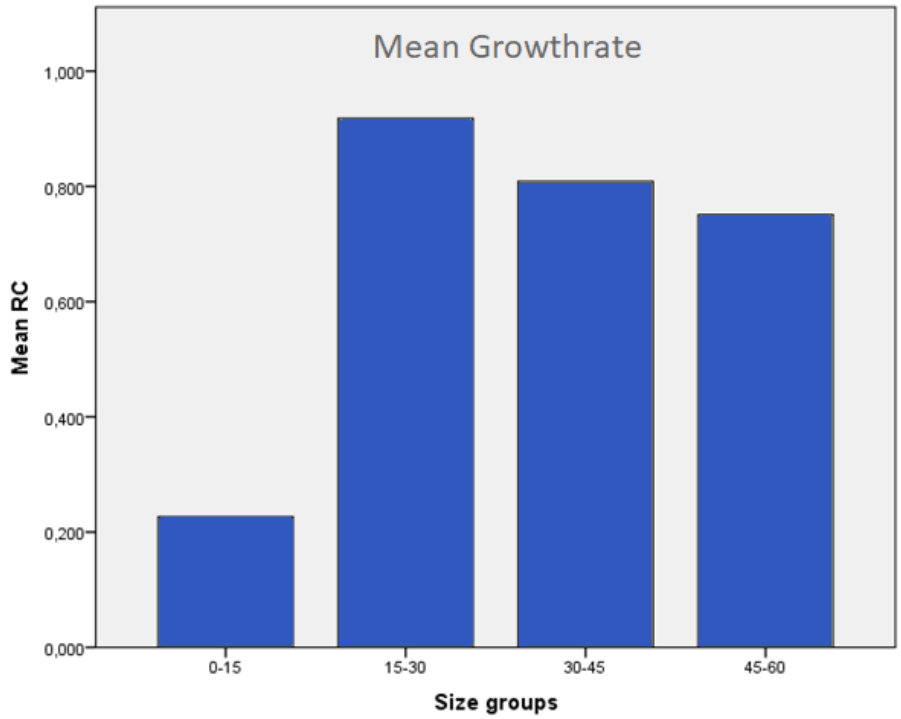

Figure 3.6 Mean Growth rate (cm/month). Each bar represents an initial fragment size group, Ascending from 0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm.

3.5 Comparison between Reef and Nursery

The results of the one-way ANOVA test (Appendix V) show that there is no significant difference in growth between the Reef and the Nursery (P=0,099). Despite that no significant difference was found, it does appear that out planted fragments stagnate in growth in the first period (Figure 3.7). Another one-way ANOVA test (Appendix V) shows that there is also no significant difference in the number of tips between fragments placed on the Reef and fragments placed in the Nursery (P=0,072)

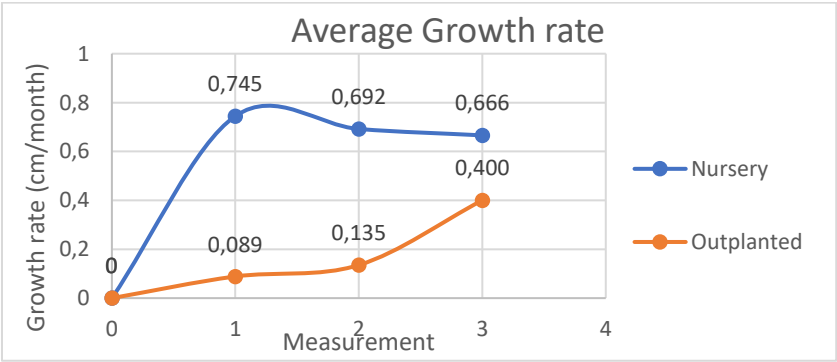

Figure 3.7 Average Growth rate of fragments placed in the nursery and on the Reef. The dots represent each measurement..

4 Discussion

The survival rate of the *A. cervicornis* fragments was 80 percent by the end of the study, with most of the mortality being caused by algae completely overgrowing fragments. Fragments that were only partially overgrown with algae remained alive. As there were still fragments in the nursery which are partially overgrown with algae, it is likely that the survival rate will decline even more (Lirman, 2000). Despite being partially overgrown by algae, the fragments still showed a decent growth rate which is contradictory compared to previous studies (Lirman, 2000; Okubo, et al., 2007). This can be a coincidence as the sample size is relatively small (n = 10) due to a big swell on the 5th of march 2018 (St. Maarten Nature Foundation, 2018). Approximately half of all fragments from the coral ladders were lost during the swell. This caused a gap in data as most fragments that were recovered, had lost their tag. To prevent this from happening again, a new baseline with a new tagging method with coloured zip ties was applied.

The growth of the fragments was not significantly affected by depth according to this study (P=0,273). This is an interesting result, because according to a study done by Enochs et. al. the light intensity has a strong effect on calcification rates of *A. cervicornis* and thus growth. The high water clarity of the Saban water can be an explanation for this result as light can reach to greater depths (Enochs, et al., 2014).

In contrast to the depth, initial fragment size does have a significant influence on the growth rate of the fragments (P=0,042). The growth rate was highest at an initial fragment size between 15-30 cm in total length. Other studies have found that growth rate increases exponentially as fragment size increases (Lirman, 2000).This could be explained by the fact that a larger fragment has a greater surface area, which would add up to a greater amount of energy and thus growth. The same effect was not visible in this study. Fragments bigger then 30 cm slowly decline in growth rate.

Results show that out planted fragments show no significant difference in growth rate with nursery fragments(P=0,099). However, it does appear that the growth of out planted fragments stagnates in the first period. A possible explanation for this is that the fragments first spent energy to attach to the reef before growing in length (Lohr, et al., 2017).

During the research period it was observed that algae proliferated more successful on with bamboo constructed coral ladders, instead of with PVC-pipes constructed coral tree's, which were mainly affected by encrusting fire coral. However, less algae growth was observed on fragments in the ladders then fragments in tree's. A study about the influence of nursery structure is needed as these were only observations.

In order to do the measurements, coral had to be touched at least twice a month. This could have caused damage to the tissue of the fragment. The damage done would be regenerated but slows down growth (Meesters, et al., 1994). This could have affected the measured growth rate in this study.

5 Conclusion & Recommendations

During this study, the average growth rate of the *Acropora cervicornis* fragments in the nursery of Saba was 5,7 cm in total length a month. Additionally, fragments showed a higher growth rate on long-term after splicing. However, this was not significantly proven. A survival rate of 80% of the fragments in the nursery is acceptable. Assuming the growth rate and survival rate continue as measured up to this point, growing coral fragments in a nursery setting in Saba is a viable solution for the declining *Acropora* abundancy. According to this study, depth does not influence the growth rate of the fragments. As this contradicts other studies, more research should be done on the matter. Fragment size did have a significant influence on the growth rate according to this study; fragments between 15-30 cm in total length show the highest growth rate. As an addition to these results, a study on survival rate of different fragment sizes can be useful long-term. This study shows that out planted fragments show no significant difference in growth rate with nursery fragments. Out planting fragments around Saba can be considered successful. However, continued monitoring will be needed to determine survival rate in the long run. As coral nurseries still are a relatively young method to support rehabilitation of coral reefs, it is important to continue studying the nurseries and the out planted fragments in order to make the RESCQ project on Saba and other islands as effective and efficient as possible.

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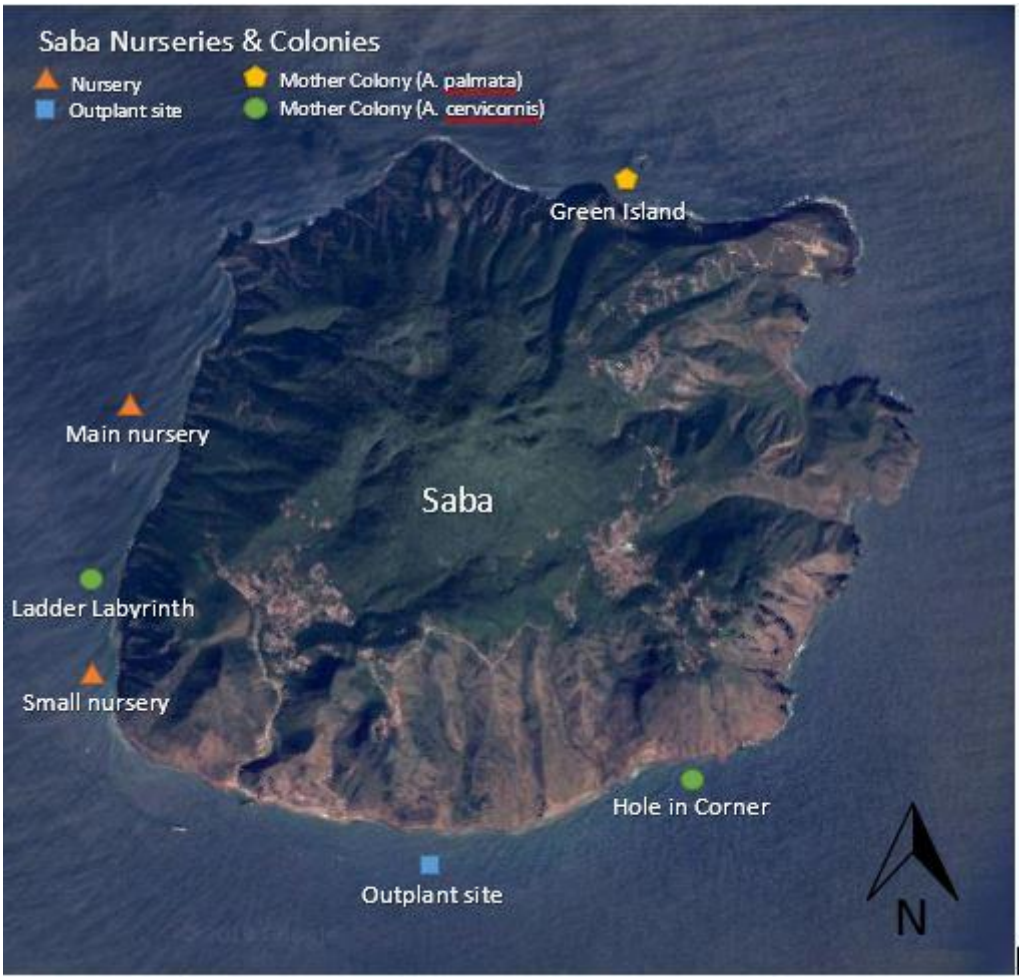
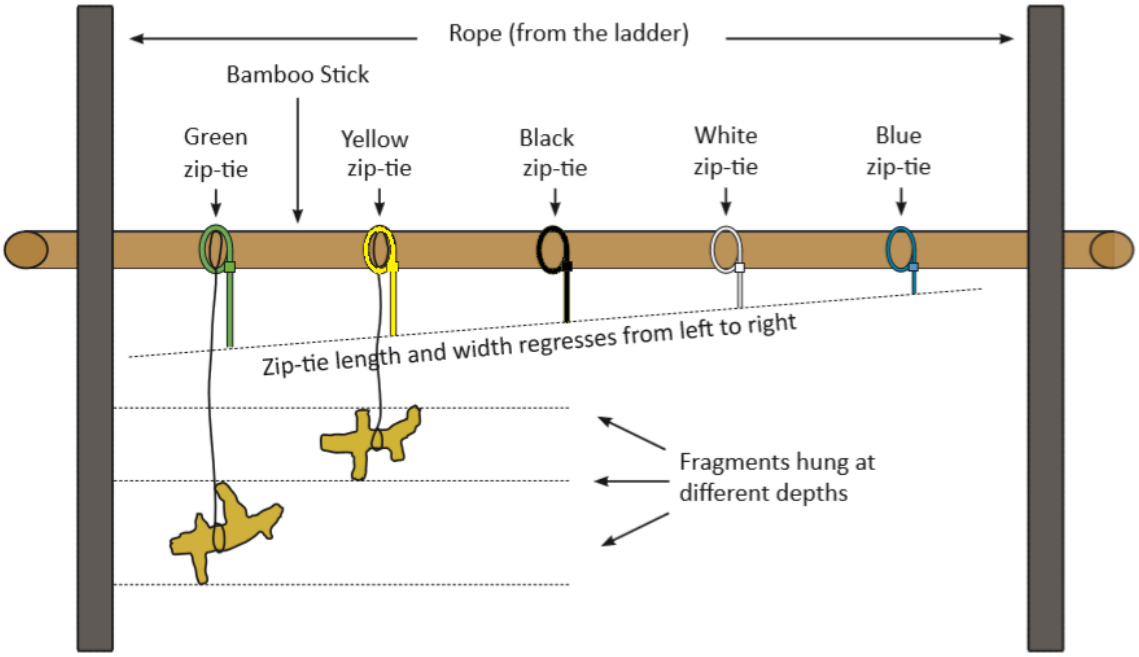
Appendix I

Map of Saba with nurseries and mother colony's marked



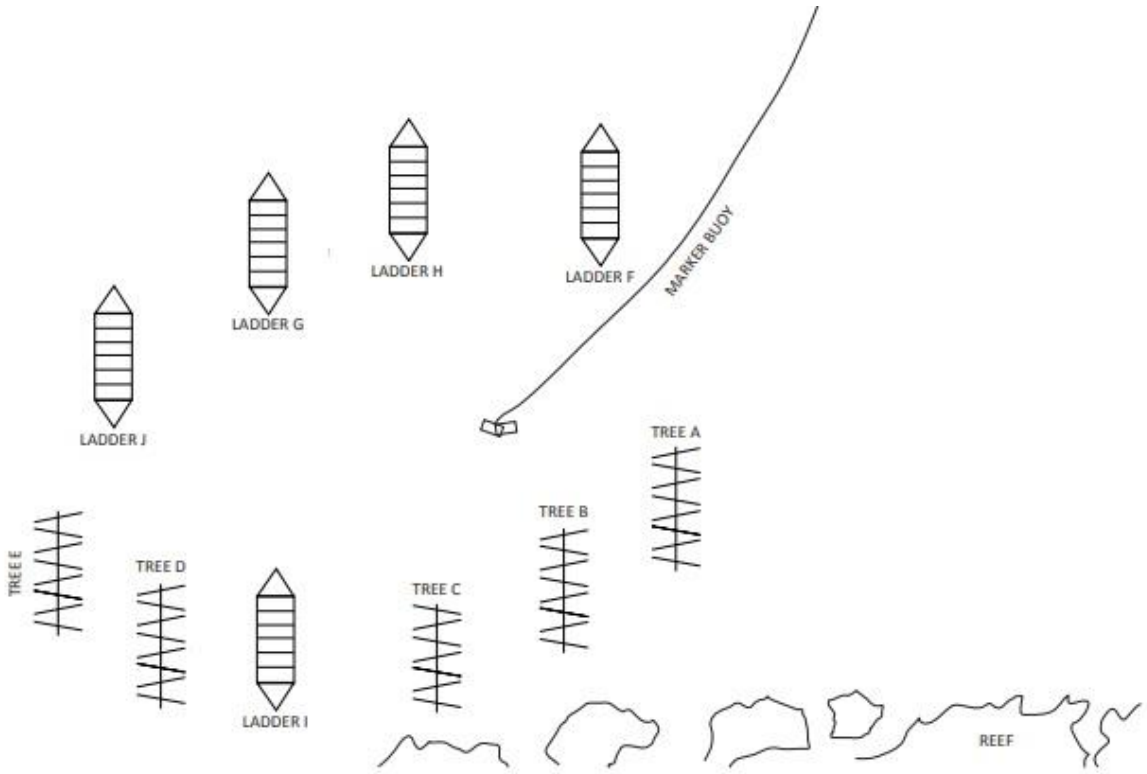
Appendix II

Overview of the new tagging system used on the ladders



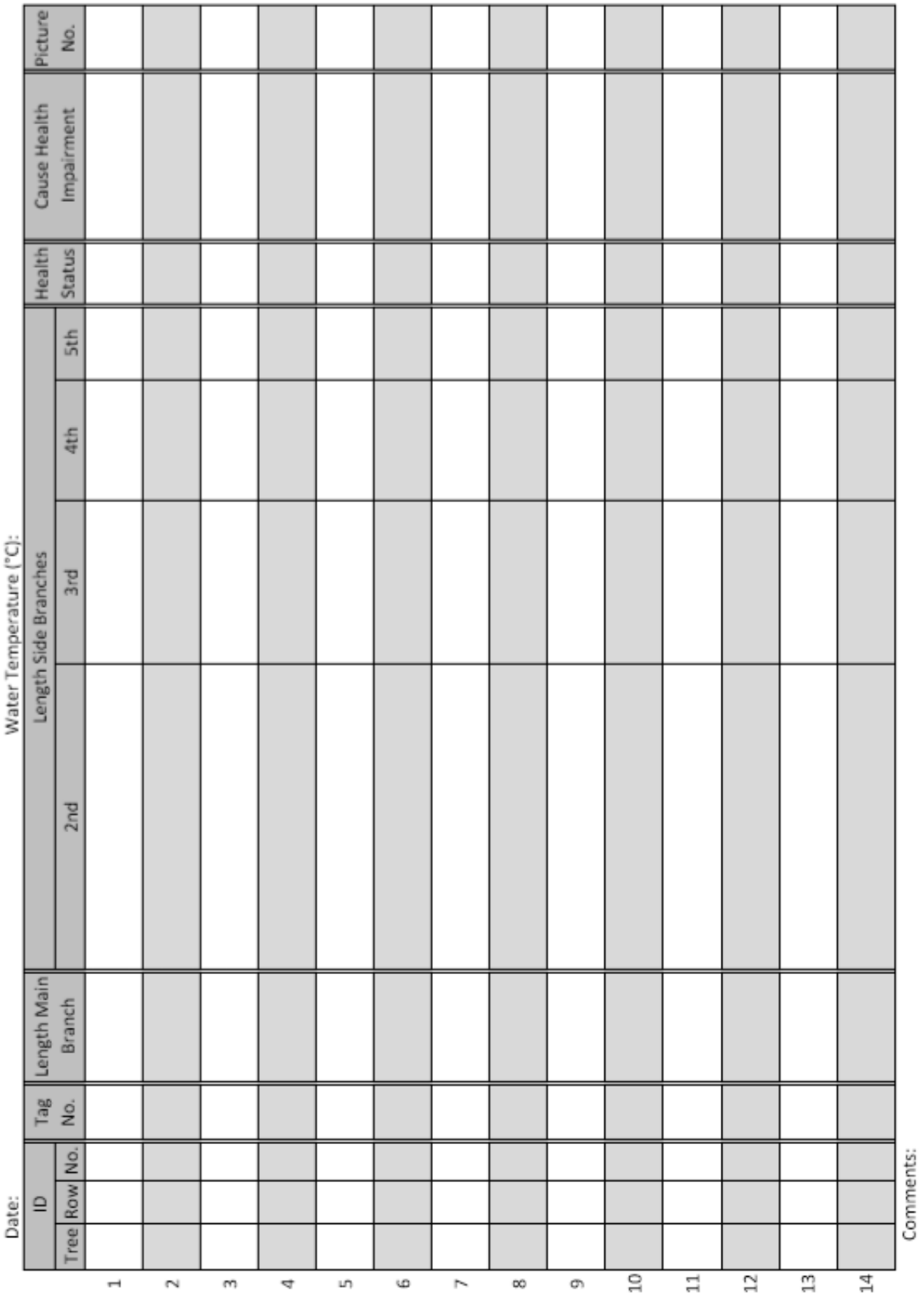
Appendix III

Map of the nursery



Appendix IV

Underwater datasheet for fragment measurements



Date:

Water Temperature (°C):

	ID			Tag No.	Length Main Branch	Length Side Branches					Health Status	Cause Health Impairment	Picture No.
	Tree	Row	No.			2nd	3rd	4th	5th				
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													

Comments:

Appendix V

Results of statistical analysis

Table V.1 Results of Repeated Measures test in SPSS applied on algae presence and growth measurements.

Between-Subjects Factors

		Value Label	N
Algae	0	No	39
	1	Yes	10

Multivariate Tests [a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Growth_rate	Pillai's Trace	,084	2,121 [b]	2,000	46,000	,132
	Wilks' Lambda	,916	2,121 [b]	2,000	46,000	,132
	Hotelling's Trace	,092	2,121 [b]	2,000	46,000	,132
	Roy's Largest Root	,092	2,121 [b]	2,000	46,000	,132
Growth_rate * Algae	Pillai's Trace	,029	,684 [b]	2,000	46,000	,509
	Wilks' Lambda	,971	,684 [b]	2,000	46,000	,509
	Hotelling's Trace	,030	,684 [b]	2,000	46,000	,509
	Roy's Largest Root	,030	,684 [b]	2,000	46,000	,509

a. Design: Intercept + Algae
Within Subjects Design: Growth_rate

b. Exact statistic

Table V.2 Results of one-way ANOVA test in SPSS applied on algae presence and tip counting's.

ANOVA

Total number of tips

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,356	1	1,356	,076	,783
Within Groups	834,644	47	17,758		
Total	836,000	48			

Table V.3 Results of a linear regression test in SPSS applied on depth and growth measurements.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,140 [a]	,020	-,001	,591980

a. Predictors: (Constant), Fragment Depth

ANOVA [a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	,337	1	,337	,962	,331 [b]
	Residual	16,821	48	,350		
	Total	17,158	49			

a. Dependent Variable: RC

b. Predictors: (Constant), Fragment Depth

Table V.4 Results of a linear regression test in SPSS applied on depth and tip counting's.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,055 [a]	,003	-,018	4,211

a. Predictors: (Constant), Fragment Depth

ANOVA [a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2,532	1	2,532	,143	,707 [b]
	Residual	833,468	47	17,733		
	Total	836,000	48			

a. Dependent Variable: Total number of tips

b. Predictors: (Constant), Fragment Depth

Table V.5 Results of the Repeated Measures test in SPSS applied on recently spliced and growth measurements.

Between-Subjects Factors

		Value Label	N
Spliced	0	No	45
	1	Yes	5

Multivariate Tests [a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Growth_rate	Pillai's Trace	,008	,191 [b]	2,000	47,000	,827
	Wilks' Lambda	,992	,191 [b]	2,000	47,000	,827
	Hotelling's Trace	,008	,191 [b]	2,000	47,000	,827
	Roy's Largest Root	,008	,191 [b]	2,000	47,000	,827
Growth_rate * Spliced	Pillai's Trace	,004	,101 [b]	2,000	47,000	,905
	Wilks' Lambda	,996	,101 [b]	2,000	47,000	,905
	Hotelling's Trace	,004	,101 [b]	2,000	47,000	,905
	Roy's Largest Root	,004	,101 [b]	2,000	47,000	,905

a. Design: Intercept + Spliced
Within Subjects Design: Growth_rate

b. Exact statistic

Table V.6 Results of one-way ANOVA test in SPSS applied on recently spliced and tip counting's.

ANOVA

Total number of tips

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,005	1	,005	,000	,987
Within Groups	835,995	47	17,787		
Total	836,000	48			

Table V.7 Results of One-way ANOVA test in SPSS applied on initial fragment size and growth rate

ANOVA

RC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,780	3	,927	2,965	,042
Within Groups	14,379	46	,313		
Total	17,158	49			

Multiple Comparisons

Dependent Variable: RC

LSD

(I) Size groups	(J) Size groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0-15	15-30	-,691039*	,235634	,005	-1,16535	-,21673
	30-45	-,581750*	,242091	,020	-1,06905	-,09445
	45-60	-,523964	,289354	,077	-1,10640	,05848
15-30	0-15	,691039*	,235634	,005	,21673	1,16535
	30-45	,109289	,189703	,567	-,27256	,49114
	45-60	,167075	,247195	,502	-,33050	,66465
30-45	0-15	,581750*	,242091	,020	,09445	1,06905
	15-30	-,109289	,189703	,567	-,49114	,27256
	45-60	,057786	,253357	,821	-,45220	,56777
45-60	0-15	,523964	,289354	,077	-,05848	1,10640
	15-30	-,167075	,247195	,502	-,66465	,33050
	30-45	-,057786	,253357	,821	-,56777	,45220

*. The mean difference is significant at the 0.05 level.

Table V.8 Results of one-way ANOVA test in SPSS applied on location and growth measurements.

ANOVA

RC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12,473	1	12,473	2,812	,099
Within Groups	239,547	54	4,436		
Total	252,020	55			

Table V.9 Results of one-way ANOVA test in SPSS applied on location and tip counting's.

ANOVA

Total number of tips

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56,048	1	56,048	3,378	,072
Within Groups	879,333	53	16,591		
Total	935,382	54			