

# **Coral restoration Bonaire. An evaluation of growth, regeneration and survival.**

Report number C152/15



*Acropora cervicornis* field in Jamaica in 1973 (picture Phillip Dustan)  
in Reef Reminiscences (Sale and Szmant, 2012)

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Picture front: *Acropora cervicornis* field in Jamaica (picture Phillip Dustan)  
in Reef Reminiscences, Sale and Szmant (2012)

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## Abstract

The Coral restoration of Staghorn (*Acropora cervicornis*) and Elkhorn (*A. palmata*) as practiced by the Coral Restoration Foundation Bonaire (CRFB) is shown to be highly successful in terms of growth and survival of new colonies, in both nurseries and transplant locations. Coral restoration is expected to contribute to ecosystem services and increase coastal protection, biodiversity, fish biomass, and tourism.

Staghorn fields once covered the bottom of the shallow reefs of Bonaire for up to 70%. Most of Bonaire's Staghorn and Elkhorn populations have been decimated by white band disease (WBD), leaving the shallow terraces around this popular diving destination as biologically barren sandy plains. The CRFB restoration project attempts to restore these populations by actively (with help from volunteers) rebuilding the once so attractive Staghorn fields. The coral restoration project of CRFB Bonaire needed scientific assessment of important aspects of the restoration methodology such as growth, regeneration and survival of CRFB's corals recruits.

Fragments are created from mother colonies by cutting and these cuts are short-term wounds that need to be regenerated by the living tissue on both the 'parent' and the fragment. Results of a series of field experiments in the CRFB coral nurseries indicated that recovery of cut fragments was 99.6% (n=234). Time until recovery of tissue was extremely fast within approximately 1 week. Full pigmentation and apical polyp formation was generally achieved within two weeks and depended on the origin of parental colonies and the current growing location.

Over 200 colonies were transplanted from the nurseries on to attachment structures at different locations on the reef to determine growth rates and potential effects of location, attachment structure, and parental origin (genetic identity). Transplant sites (several kms apart) differing in environmental conditions and type of attachment structure were not found to impact transplant growth which was exceptionally high (almost 14 cm per year per branch tip and). An average fragment of 25 gram will grow to approximately 12 times its weight within one year. Nursery grown Staghorn fragments developed more side branches compared to their wild counterparts. While the exact mechanism behind side branch formation is not yet understood, several possible explanations are provided. The results of this research generate many follow-up suggestions and exciting ideas for future coral restoration practices.

Results show clearly that current restoration practices by CRFB of transplanting Staghorn colonies to different locations is likely to be an excellent way to restore the Staghorn fields of Bonaire. Parental origin of the transplanted fragments significantly affected damage regeneration and growth rate, opening up the possibilities for active selection of specific genotypes to increase transplantation success. However, genetic diversity of the population should be studied and safeguarded. The measured survival, regeneration, and growth rates indicate that current restoration practices of CRFB are highly sustainable and may create viable clusters of Staghorn colonies which may initiate the regrow of Staghorn corals into thick fields. Coral restoration through fragmentation can create a very large number of viable fragments in a very short time (one fragment can be cut into two fragments within 6 months without influencing survival rate).

## **Acknowledgements**

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# 1 Introduction

The Scleractinian coral *Acropora cervicornis*, known as Staghorn coral, used to be wide spread in the coastal zones of the Caribbean until the white band disease caused a tremendous decline in abundance of this species throughout the region (Hughes, 1994). This is also true for Staghorn corals in Bonaire, which at one point in time used to cover up to 70% of the shallow reef bottom (Duyl 1985, see Fig. 1).

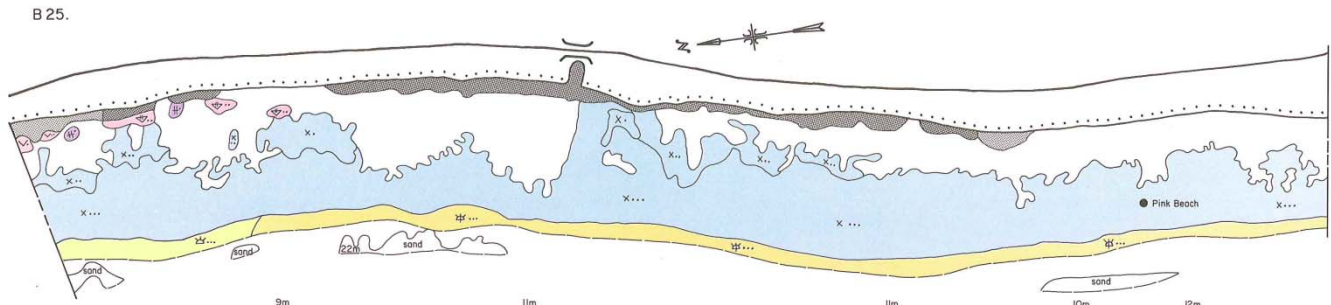


Figure 1. Detail of bottom cover of Staghorn corals around 1984 (Duyl 1985), just before the White Band Disease hit. 'X' in the figure denotes Staghorn fields with more than 40% bottom coverage. The area is north of Pink Beach. Quantitative surveys in 2014-2015 found that the area now consists of 70% sand and rubble (unpub. data IMARES).

Restoration of the Staghorn coral and its congener Elkhorn coral (*A. palmata*) may be a feasible option under certain conditions (Meesters et al, 2014). Coral Restoration Foundation Bonaire (CRFB) attempts to restore these two species on the islands Bonaire and Klein Bonaire (Leeward Antilles, Caribbean, see map below). The employed techniques for growing *A. cervicornis* fragments in the water column appear to result in excellent growth rates (CRFB, 2014).

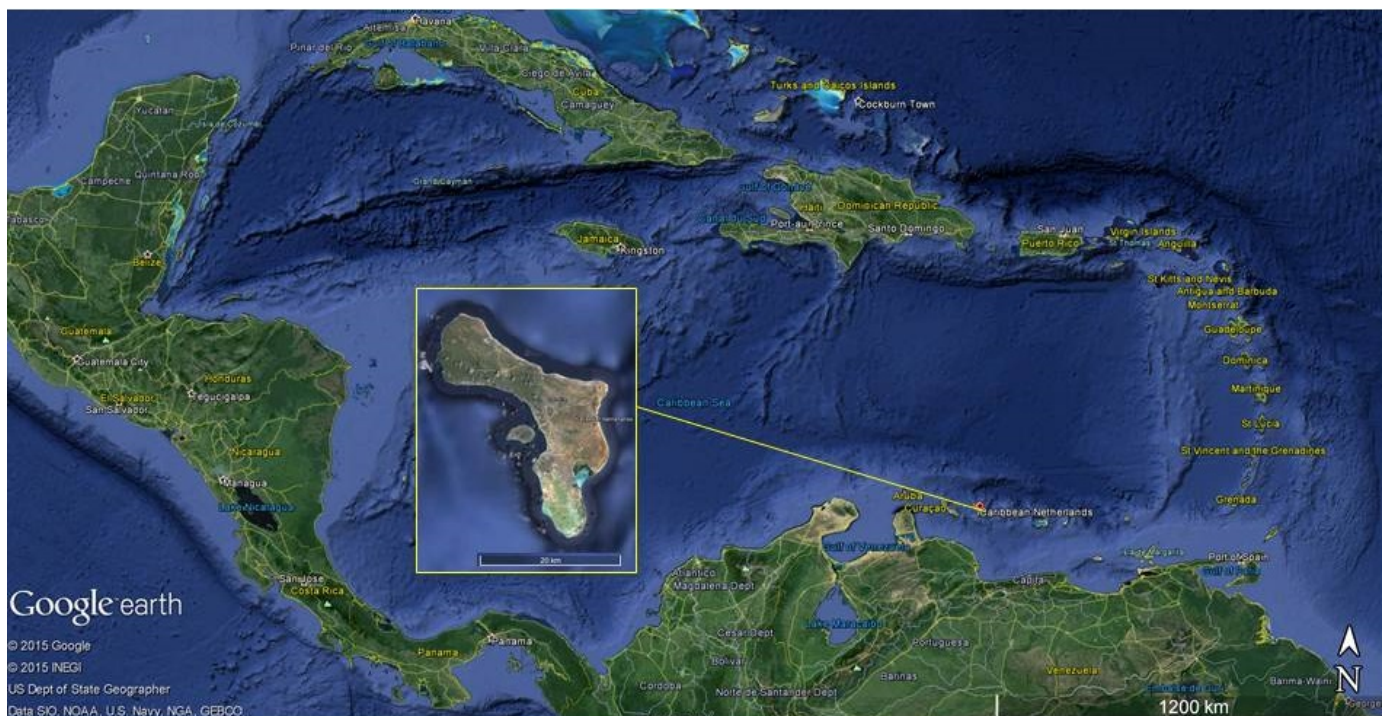


Figure 2. Bonaire and Klein Bonaire in the Caribbean (Google Maps, 2014). The inset shows Bonaire and Klein Bonaire on the left of the island.

At CRFB both coral species are grown in mid-water nurseries (Fig. 3). Fragments of these species are suspended on custom made PVC and fiberglass structures, resembling christmas trees. The coral stock is multiplied through fragmentation, a propagation method often used in active restoration practices worldwide. Under natural conditions fragmentation is considered the main reproduction method used by Staghorn corals (Tunncliffe 1981, Epstein & Rinkevich 2001) as the authors hardly found any small juvenile colonies in the field. This is in agreement with the findings of Bak & Engel (1979), who described similar observations for *A. palmata*. Meesters et al. (1992) showed that this fits perfectly with the general life history strategies of these two species that include high regeneration potential of mechanical damage (breakage) and fast growth rates. Disturbance of the *Acropora* colonies in the CRF nurseries is minimal. Maintenance is limited to removal of fouling and repair of the support structures, removal of fragments with dead parts (often white band disease or predation) and “pruning” to increase the number of coral fragments in the nurseries.





*Figure 3. Picture of mid-water nurseries at Buddy Dive Resort's house reef.*

When fragments of either species have reached a desired size, they are transplanted to support structures on the shallow reef terraces of Bonaire or Klein Bonaire. There they are allowed to grow without further maintenance. For Staghorn corals this size is approximately 30cm. Two methods are used to stabilize fragments on transplant sites: "gluing" fragments to rocky substrate using marine epoxy or attaching fragments to horizontal steel rebar structures some 40-50cm from the bottom with tie-wraps. Both methods are shown in Fig. 4 below.



*Figure 4. Current transplant methods employed by CRF Bonaire. Fragment glued to hard substrate, a small lump of epoxy is visible on a side branch (left). Fragments has been predated by fireworms. Fragment tie-wrapped to a support structure (right). Note how the coral tissue has already overgrown the tie-wraps.*

The use of a supporting structure to attach Staghorn transplants on is gaining popularity at CRFB since it is less labor intensive, faster and appears to be more successful in terms of survival than attaching fragments directly to the reef bottom. Presumably, not being directly in contact with the bottom renders the fragments less sensitive to predation by bottom dwelling organisms such as fireworms. The Staghorn fragments on rebars continue to grow without needing much maintenance and have a tendency to attach themselves to the bars by enveloping the bar with tissue. While fragments are initially small and relatively far from the bottom, they are expected to reach the bottom in a few years' time. The expectation is that at that moment they are also large enough so that predation will not lead to whole colony mortality. Since these coral species are colonial it is perfectly natural if small parts of the colony experience mortality at some point in time. Small

colonies however are especially sensitive to whole colony mortality and it is therefore important to research how survival of offspring and parents can be maximized. A number of important research topics have been identified (Meesters et al 2014). The creation of fragments will create damaged branches of both fragment and source colony. It is important to gain insight into the regeneration of damage and the growth on both fragment and parental colony, the survival of source colonies and fragments, the survival of fragments after transfer to the transplant site, and genetic variation of source colonies.

In this research the above questions were addressed in four field experiments using the facilities generously provided by CRF Bonaire and their main sponsor Buddy Dive Resort Bonaire.

The experiments were used to:

- Calculate regeneration of damage and survival of Staghorn fragments and mother colonies;
- Determine the impact of pruning and fragmenting of Staghorn fragments;
- Measure growth and survival of new fragments in mid-water nurseries;
- Measure growth and survival of experimental fragments at transplant sites;
- Determine the presence of differences in above variables between different genotypes.

Additionally, some extra experiments were carried out to address questions that emerged during the research period:

- We wanted to know whether regeneration of damage could be restarted as some observations by CRFB volunteers suggested (e.g. Miller et al 2014). It is well known that there is a strong redirection of energy towards regeneration directly following the infliction of damage (e.g. Bak et al 1977), but this response quickly abates (Meesters et al. 1994).
- Staghorn colonies grown in CRFB's nurseries appeared to generate side branches more frequently than their "wild" counterparts, but this observation needs to be supported by data. More branches means more growing tips, faster growth, and possibly higher survival chances.

*Table 1. Source colony's location, nursery location and number of the A. cervicornis fragments used to determine tissue recovery rates.*

Latitude	Longitude	(tree number) Local name	Nursery	n
12° 5.868' N	68° 13.643' W	(1) Lac Bay	Bonaire	16
12° 5.725' N	68° 13.776' W	(2) Lac Bay	Bonaire	16
12° 1.479' N	68° 15.030' W	(3) Red Slave	Bonaire	16
12° 1.895' N	68° 15.488' W	(4) Sweet Dreams	Bonaire	16
12° 2.277' N	68° 15.897' W	(5) Vista Blue	Bonaire	16
12° 8.061' N	68° 16.847' W	(6) North Belnem	Bonaire	16
12° 12.519' N	68° 19.173' W	(7) 1000 steps	Bonaire	16
12° 13.088' N	68° 21.012' W	(8) Karpata	Bonaire	16
12° 9.264' N	68° 17.578' W	(9) Nearest point	Bonaire	16
12° 8.944' N	68° 18.781' W	(10) Rock pile	Bonaire	16
12° 5.868' N	68° 13.643' W	(1) Lac Bay	Klein Bonaire	16
12° 1.479' N	68° 15.030' W	(3) Red Slave	Klein Bonaire	16
12° 2.277' N	68° 15.897' W	(5) Vista Blue	Klein Bonaire	16
12° 12.519' N	68° 19.173' W	(7) 1000 steps	Klein Bonaire	10
12° 9.264' N	68° 17.578' W	(9) Nearest point	Klein Bonaire	16



## 2 Materials and methods

### 2.1 Experiment 1: Genetic differences in damage regeneration capability

The main goal of this experiment was to determine the existence of differences in recovery after breakage or cutting of *A. cervicornis* fragments in CRF Bonaire's nurseries, related to the origin of the source colony of the fragments (called F0 or "genotype" by CRFB, both will be used throughout this report even though it is not sure that they are really different genotypes) and nursery location in which the fragment is grown. The experiment involved the regular assessment of the amount of surface area of the cut surface that was covered by new tissue, the appearance of zooxanthellae (start of coloration), and the onset of polyp creation. The fieldwork for this experiment was performed from the beginning of April 2014 until the end of June 2014.

Two nurseries formed the setting of this experiment. One is located on a shallow reef terrace on the south side of Klein Bonaire. The second nursery is located on a shallow terrace on the west side of Bonaire. A nursery consists of a collection of 20-30 submerged floating "trees" used to grow *Acropora* species by hanging fragments on tree branches and letting them grow in situ. Trees were made out of a 2m vertical PVC rod with ten horizontal 1.5m fiberglass rods piercing this rod alternately perpendicular at approximately 20cm intervals. Horizontal rods contained 10 or 16 small drilled holes which were used to attach monofilament lines using aluminum crimp sleeves. An *Acropora* fragment is connected to each monofilament line using another aluminum crimp sleeve. The vertical PVC rod is anchored to the substrate with a duckbill anchor attached to approximately 1m of rope. Multiple 2-4L Styrofoam balls and/or air-filled plastic bottles are attached with 0.5m rope to the other end of the vertical rod, allowing the rod to maintain its vertical position (Fig. 3). A single tree contains *Acropora* fragments from only one source colony (called the F0 generation) and was filled for app. 75% during the entire experiment. The centers of the trees were spaced approximately 3 meters apart. The nursery on the terrace of Klein Bonaire consisted of 3 rows of trees (respectively 11, 11, and 8 trees), with rows parallel to the steep slopes of the adjacent drop-off. The horizontal branches are in between 4.5 and 6.5m water depth. The nursery on the terrace of Bonaire consisted of 4 clusters of 5 trees each app. 20-150m apart. Here the horizontal branches are at an average depth between 3.5-5.5m. The substrate around the nurseries consists mainly of sand (Klein Bonaire) or a mixture of sand and coral rubble (Bonaire).

The used nurseries had been previously built by CRFB staff and volunteers, and had been in use for 2 years before the start of this experiment. Original nursery stocks of *Acropora* were collected according to local regulations and permits after building. A total of ten different origin locations (Table 1) were used to collect the stock. Each nursery trees contains the descendants of one of the original stock colonies collected at different locations and assumed to be genetically different. At this point in time it is still unknown whether the F0 colonies are really different genotypes, but for ease of reading we will call them here genotypes. Some new input of wild *Acropora* has occurred since the first stocking in 2012 when boats have accidentally broken *Acropora* colonies. Presently, there are 12 Staghorn and 11 Elkhorn genotypes (i.e. coming from different parental colonies).

#### *Preparation of Staghorn fragments*

10 or 16 fragments of approximately 10-30cm length and 1-2cm thick were collected from each of 15 trees in this experiment, totaling 234 fragments (Table 1). These 234 fragments (F1) originated from 117 larger, randomly selected fragments (F0) of mentioned size, and were prepared for this experiment by moving them to a depth of 4.6-4.8m (measured with a Suunto Vyper dive computer) in their original tree two weeks prior to the start of the experiment. A 15-20cm piece of 0.5mm diameter monofilament line was looped finger tight around the middle of the thickest branch of an F1 fragment, the loop was secured with a fitting aluminum crimp sleeve using commercial diagonal cutting pliers. The other end was stuck through a hole in the horizontal rods at the appropriate depth and also secured with a similar sleeve. Used horizontal branches were marked with tie-wraps for easy identification of the experimental fragments (Fig. 5). Two weeks after moving the 117 F0 fragments to the experimental depth, the thickest branch of each F1 fragment was cut in half to start the experiment. Cutting through a Staghorn branch resulted in a circular area of directly exposed inner skeleton devoid of living tissue (Fig. 6 right). The F1 fragments taken from the tree to the reef bottom close by and were cut in a single movement using a stainless steel coral cutter (Aquaholland Heavy Duty), allowing symmetrical cuts, creating damage on the parental and offspring branch. The two new (F2) fragments were unequal in length, and are further called F2.max ("MF2") (largest part, 4.0-33.9cm) or F2.min (smallest part, 3.0-8.5cm).

Directly after cutting, the lengths of all branches of the F2 fragments were measured (in mm) using a 1 m fiberglass tape measure. Measurements were performed by measuring the length of a branch along its curve from the cut (or node in case of side branches) to the tip of the apical polyp.

A distinction was made between primary, secondary, tertiary, etc. branches. The primary branch was defined as the thickest branch of a F2 fragment, or the branch with the thickest segment. Secondary branches are segments grown from the primary branch, but with their own distinct apical polyp. Tertiary etc. branches are connected to the secondary branch via additional nodes and also have distinct apical polyps. Apical polyps were defined as polyps with a cylindrical skeleton wall, as opposed to "normal" polyps which, in *Acropora*, have skeleton walls looking like diagonally cut cylinders. In apical polyps the rim of the walls are often white, indicating a lack of zooxanthellae. Apical polyps could be identified at the minimum size of 4mm (pers. obs.). The cuts were photographed using an Olympus  $\mu$  TOUGH-8010 camera in built-in "super macro- mode" (no flash, other settings automatic/default from 5-10cm distance). A clear 2mm thick acrylic plate with etched-in grid (cell size 1.9\*2.4mm) was placed between the camera and the cut, as close to the cut as possible without touching it.

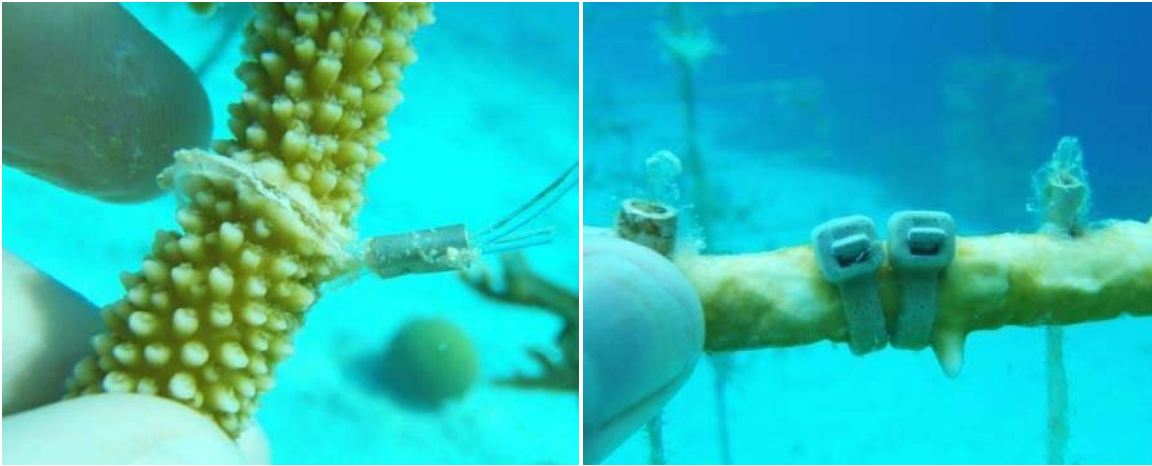


Figure 5. A Staghorn fragment from a nursery after 8 days already showing overgrowth by new tissue (left). The other end of monofilament line was secured to the horizontal branches. Tie-wraps were used to mark experimental fragments, overgrowth of the fiberglass rod with firecoral (*Millepora* spp.) is visible in the right picture.

The camera and plate were held perpendicular to the cut surface and the etched grid facing the cut to minimize distortion in the photographs. To separate photographs of different F2 fragments in a computer's file manager, a picture of a unique number identifying the next F2 fragment was taken. We used the numbers printed on a tape measure. It was assumed that newly grown tissue could be identified on these pictures by the showing of a thin white front emerging from the cut tissue's edge, indicating calcification happening under new tissue. Observation of this front and covered surface area with the naked eye was found to be impossible in the field due to constant movement of the observer and small size of the cuts. For an impression of the difficulty in observing the regenerating translucent tissue over the white skeleton, see Figure 6 below.

Finally, the F2 fragments were returned to the tree at the same depth. Two F2 fragments originating from one F1 fragment were placed next to each other. F2 fragments were no longer moved for the duration of this experiment. The general direction the cut was facing (upward, sideward, downward) was noted for each F2 fragment. Approximately 30 F2 fragments could be prepared, measured and returned to the tree by two researchers in a single 90 minutes dive.



Figure 6. Six apical polyps are visible on an uncut fragment (left). Example of picture taken of the cuts. The cut is clearly visible through the grid (cell size 1.9\*2.4mm) (right).

#### Treatment and measurements

After preparation and returning F2 fragments to their original tree, they were allowed to recover in "normal" nursery conditions, i.e. minimal handling and regular removal of fouling and predators (algae, cyanobacteria, *Millepora* spp., *Hermodice carunculata*) with brushes. To determine surface area cover, photographs of the cuts were taken at intervals (Table 2). Photographs were later analyzed on a computer. Lengths and numbers of (side)branches were also determined at set intervals. F2 fragments were not removed from the tree for these photographs and measurements. At the same intervals fouling and predators were removed. Frequency of photographing and measurements was dependent on the nursery location as Klein Bonaire can only be reached by boat. Photographing was stopped after 47 days because it became impossible to distinguish the cut ends of branches from non-cut ends. A decreasing photographing frequency (with high initial frequency) was chosen because of the expected exponentially decreasing recovery rate (Meesters et al 1997).

Measurements of lengths were done at approximately 2 and 9 weeks after cutting the F1 fragments. Figure 6 below shows some of the measuring and photographing in progress.

Table 2. Time schedule for photographing cuts in F2 fragments and general maintenance of the nursery.

Nursery	Measurement	Days after cutting F1 fragments														
Bonaire	Surface area cut	0	1	2	3	4	6	8	10	13	16	19	22	25	32	38
Klein	Surface area cut	0	1	2	3	4	6	8	10	13	16	19	25	32	40	47

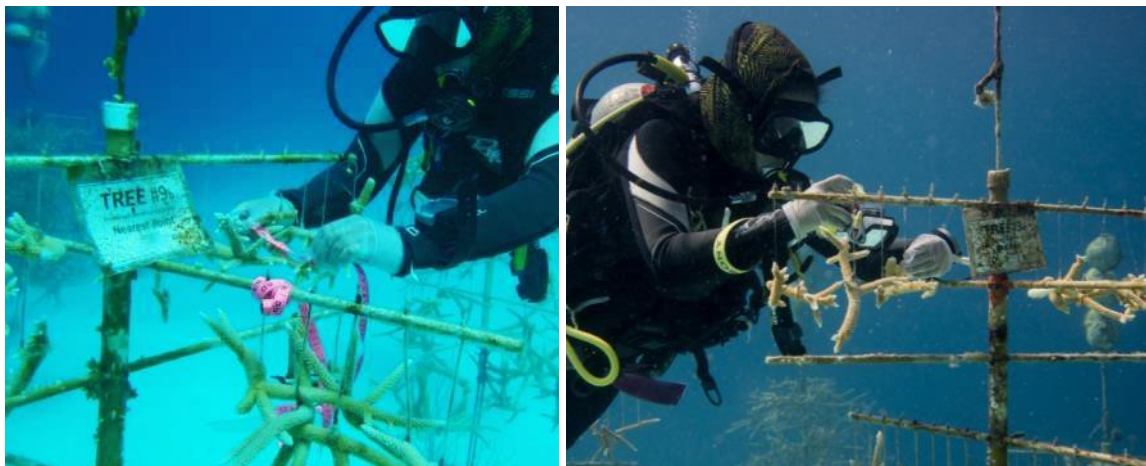


Figure 7. One of the researchers measuring (left) and photographing (right) the F2 fragments.

Results were stored in a database including standardized photographs of the cut, and length and side branch frequency data. A tissue wall distinguishable by a white smooth surface was expected to be visible (Meesters and Bak 1995), however, growth of the tissue edge over the cuts was not visible on most photographs. Adjusting a picture's brightness, contrast and colors did not improve the visibility of the new tissue layer. Therefore it was decided to determine the time (day) of full recovery of the cuts (defined as the first observation moment where underlying skeletal details are obscured from view by an opaque film covering the entire cut), the first observation (day) of first visible zooxanthallae development (indicated by an orange-brown hue emerging in fully recovered cuts), the first observation (day) of an apical polyp visibly developing on the cut and the number of emerging apical polyps on the cut after 35-40 days.

Measured branch lengths (after 2 and 9 weeks) were used to determine the number, total and average length of all categories of branches (primary, secondary, tertiary etc.), the total length of all branches and the total length of the primary and secondary branches in each F2 fragment.

## 2.2 Experiment 2: Growth and survival in nurseries and field

In this experiment growth rates of Staghorn corals in terms of length and buoyant weight were determined in the transplant locations and in the nurseries in relation to the supporting grow-out structure, location and genotype. Fragments were relocated to a transplant site following the CRFB protocol. Transplant locations were close to the transplant/dive sites Jeff Davis (n=44), Tori's reef (45) and Buddy's Reef (45), and as a control the Bonaire nursery (46) as a control.

### Preparation of fragments

180 Staghorn fragments were used in this experiment, originating from six trees (i.e. three presumably different genotypes, 60 fragments per FO origin). The genotypes were selected based on an initial evaluation of their performance in experiment 1 (regeneration of cuts), slow, intermediate and fast recovery. Genotype origin locations were dive sites Red Slave (no. 3), Karpata (no. 8), and North Belnem (no. 6) respectively (Table 1). Two weeks prior to transplantation, 60 healthy fragments of each genotype were relocated in Bonaire's nursery to three trees (organized by FO's origin) at a depth of approximately 3.8m. This depth was similar to the depth at the transplant locations. During these two weeks the fragments were prepared for the experiment. Preparation included weighing, measuring and attaching markers to individual fragments. Each fragment went through this process in a single day. Fragments were removed from the trees during this process. Additionally, the maximum dimensions of a fragment on two of its perpendicular axes (i.e. the minimum necessary length and width of an imaginary rectangular box placed over the fragment to fully enclose it) and the maximum diameter of the fragment's primary branch were determined. This allows for the calculation of 'ecological volumes' (e.g. Rinkevich & Loya 1983), the amount of space taken up by the coral colony.





Figure 8. Weighing of *A. cervicornis* fragments with the underwater balance. Both photographs © S. Gilchrist.

### Buoyant weighing

To determine the buoyant weight of the fragments, the method (and apparatus) described in Herler and Dirnwöber (2011) was used. The weighing apparatus was stabilized and levelled on sandy substrate at app. 4-5m depth, using app. 20kg of lead weights to compensate for the apparatus' strong positive buoyancy. Temperature ( $\pm 0.2^{\circ}\text{C}$ ) and salinity ( $\pm 0.1\text{g L}^{-1}$ ) at weighing depth were recorded. Once set up as described in Herler & Dirnwöber (2011), fragments were hung on the balance's weighing hook using a piece of monofilament line with three loops, two of which were used to hold the fragment. The first stable reading was noted, as well as the first stable tare value that appeared after removing the fragment. Weighing a fragment was repeated until the difference in net weight of two subsequent weightings was  $< 0.1\text{g}$ . This rarely took more than three subsequent weightings. Weights that deviated more than  $0.3\text{g}$  from this series were discarded, remaining weight values were averaged to calculate the buoyant weight (Figure 8).

After the dimensions and buoyant weight were determined, all fragments were attached to white PVC plates. Attaching the corals to a small hard substrate allowed for easy removal from the transplant sites to determine the buoyant weight (described below). The risk of the corals overgrowing and fixing to the actual transplant substrate -thereby rendering them useless for buoyant weight determination- was hereby minimized. The used PVC-plates were  $5 \times 5 \times 0.3\text{cm}$  and had unique numbers punched into them (Fig. 9). The surfaces of the plates were sanded and a  $\varnothing 5\text{mm}$  hole was drilled into one corner to facilitate hanging on a hook. Punched numbers were coloured with an industrial Edding marker to ensure readability of the numbers. Fragments were attached to the plates using marine epoxy (grey Aquascape, D-D The Aquarium Solutions Ltd) by sticking a few ml of well mixed epoxy to the plates, and carefully pressing the middle segment of a fragment into the clot. Care was taken not to crush the polyps which were not embedded in the epoxy. Exposure to the epoxy was minimized to what was necessary to keep the fragments attached. The fragments were then placed on a stable substrate for 1-3 hours to allow the epoxy to harden. They were returned to their respective nursery trees at a depth of 3.8m afterwards.



Figure 9. A fragment is glued to a numbered PVC plate (first number is the code for the genotype origin, second number a fragment identifier) with marine epoxy (left). The fragments are afterwards returned to their tree (right).

Within 24 hours of attaching the PVC plate the fragments were removed from their nursery trees to measure their buoyant weight again. The buoyant weights were determined once more 24 hours before transplantation. The re-weighing within 24 hours allowed for determining the combined buoyant weight of the PVC and epoxy. In 24 hours the epoxy was expected to have fully hardened. The combined weight of the PVC and epoxy can be subtracted from future measurements to find the buoyant weight of each Staghorn fragment. Seawater density (determined via temperature and salinity) at the sites of weighing was never found to deviate enough to necessitate adjusting buoyant weights

(compensation would lead to changes in buoyant weight of much less than 0.05g, while the weighing method had a precision of 0.1g). In some cases the epoxy did not attach to the plates. Therefore, fragments that fell off were reattached to the PVC plate using nylon tie-wraps or PUR Framilon® elastic tape, both of which did not seem to influence growth. The density of these materials is close enough to the density of seawater to not influence the buoyant weighing results (pers. obs.).

#### *Preparation of transplant sites*

*The transplant locations were chosen based on the permits for CRF Bonaire. They are named after the closest recreational dive site. The three locations are 'Jeff Davis' (12° 12.20' N, 68° 18.74' W), 'Tori's Reef' (12° 4.23' N, 68° 16.87' W) and 'Buddy's Reef' (12° 10.21' N, 68° 17.30' W). Three additional nursery trees at the Bonaire nursery were used to serve as a control. This location is described in the previous chapter. All sites are already in use by CRFB and house Staghorn colonies attached to rocky substrate using marine epoxy. The CRFB transplant locations themselves are described in more detail in Experiment 3.*

A steel support structure was used to attach the transplanted corals on. On each site a sandy area of approximately 25m<sup>2</sup> at a depth of 4- 4.5m was selected. The areas were confirmed to contain as little as possible living corals or protruding sponges. At each experimental site nine 90cm Ø12mm steel rebars were hammered vertically into the loose substrate until about half of the rebar was inserted. The rebars were placed in a grid spaced 80 or 120cm apart. A fortified piece of scaffolding pipe (~20kg) was used by a single diver to hammer the rebars into the substrate. Smaller hammers were used to fine tune the depth. Care was taken not to disturb any nearby organism in this process. Three 3m Ø12mm steel rebars were attached horizontally to the top ends of three vertical rebars each, at a depth of 3.8m (Fig. 10). The horizontal bars are placed perpendicular to the shore/drop-off, i.e. generally parallel to incoming waves to minimize their impacts. A finished structure is shown in Figure 11. Building a structure took a 30-45 minute dive for two or three divers, including transport of the materials to the building site.



*Figure 10. Building a rebar structure at Tori's Reef. One researcher inserts the vertical support bars while the second readies the next vertical rods (left). Horizontal rebars are secured with strong tie-wraps (right).*

At Jeff Davis the transplant location for this experiment (Fig. 11) was situated less than 20m northwest of the CRFB site.



*Figure 11. Transplant rebar structure and its surroundings at Jeff Davis, already housing experimental fragments. The CRF Bonaire transplant site is on the left, but out of the frame. De drop-off is approximately 10m behind the rebars.*



At Tori's Reef the rebar structure was placed 50m west of a Cargill Salt Bonaire N.V. effluent outfall. The sandy substrate found here is bordered by a large rubble patch consisting of dead Staghorn remains covered by living *Millepora* spp. (Fig. 12). Living Staghorn colonies can be found in the vicinity indicating that the habitat is still favorable for the growth of new Staghorn colonies.



Figure 12. Transplant structure and its surroundings at Tori's Reef. The surrounding rubble field with *Millepora* spp. can be seen on the sides. The crates next to the structure contain *A. cervicornis* fragments ready to be transplanted.

At Buddy's Reef the transplant site is located next to a larger steel rebar structure housing Staghorn transplanted by CRFB staff. The substrate is coral rubble mixed with sand with very little living coral cover (Fig. 13).



Figure 13. Transplant structure with transplanted corals and its surroundings at Buddy's Reef.

#### *Transplantation of *Acropora* fragments*

At the day of transplantation, fifteen (in one case accidentally fourteen) prepared fragments of each of the three F0 genotypes were haphazardly selected and moved from their nursery tree to plastic crates. Care was taken to minimize contact between fragments. The crates were loaded onto a motorboat where the fragments were quickly transferred to large, black tubs filled with fresh sea water (Fig. 13). Fragments were completely submerged and contact with each other was prevented as much as possible. The tubs were covered with wet cloths to reduce light and heat stress. The 44/45 fragments were then transported by boat to the transplant site, transferred to the crates and quickly put back in the ocean again to a depth of 3.8m. The total time spent outside the ocean was less than 45 minutes in all transfers, with less than 2 minutes of air exposure in total.



One rebar contained all three genotypes and colonies were evenly distributed over the length of the rebar, being attached to the rebar with small elastic bands (Framilon®, a PUR based elastic tape that is resistant to long term UV and seawater exposure). These bands only touched the PVC plates and rebar (Fig. 13). The PVC plate forms a barrier for the coral to prevent it from attaching itself permanently to the rebar structure, which would make it impossible to measure its weight increase.

Table 3. Distribution of *Staghorn* fragments over the three transplant sites for each genotype.

Transplant location	Origin			
	n <sub>Red Slave</sub>	n <sub>North Belnem</sub>	n <sub>Karpata</sub>	n <sub>total</sub>
Jeff Davis	14	15	15	44
Tori's Reef	15	15	15	45
Buddy's Reef	15	15	15	45
Bonaire nursery	16	15	15	46
<b>Totals</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>180</b>



Figure 14. *Acropora* in tubs on the transport boat (left, top) ready for transport. Randomly picked fragments are evenly distributed over the rebar structure (right, top). Corals are evenly spaced and well-secured to the rebar with elastic bands. The clear bands are barely visible (bottom).

Directly after placing, the loose ends of monofilament line, used to hang the fragments in the nurseries, were cut off. The location and orientation of fragments on rebars was recorded and sustained during the entire experiment. Transplantation was repeated for each transplant site, but at Buddy's Reef, as the distance to the transplant frame was only about 50m transplants were not taken out of the water, but the crates brought over by divers. Fragments staying in the nursery trees were moved to horizontal branches at equal depths (by moving them up mostly less than 20cm) and spacing them similarly as on the transplant structures. The final distribution of *Staghorn* fragments over the transplant sites was as shown in Table 3 below.

### Treatment and measurements

The corals used in this experiment were allowed to grow under natural conditions including potential predation. For the first five days after transplantation, the rebar structures and fragments were checked for the presence of predating snails and worms. After the first five days this procedure was repeated once a week for an additional 5 weeks. Detected predators were moved to locations more than 20m away using pliers.

Predator monitoring was done within two hours after sunset as predator abundance was then found to be most noticeable. A SL4 eLED L1 Blue-455 dive light (Underwater Kinetics Europe GmbH) was used in combination with yellow, clear plastic light filters during these checks. The blue light emitted by this dive light triggers fluorescence in certain organic materials. In this case fireworm, *Hermodice carunculata*, tissue was the target of fluorescence, lighting the animal up in distinct green or orange colors. Predating snails did not show fluorescence but are more easily identified. Sudden increase of light intensity did not seem to effect predator behaviour directly (pers. obs.). The limited exposure of zooxanthallae to our dive lights is not expected to have a noticeable influence on their light-dark cycle. See also Figure 14 below for an impression of the fluorescence effect. On each checking of the sites, fragments which had turned or fallen off the structure were returned to their original position. 10-15 fragments fell or turned during the entire experiment. Fouling on the PVC plates was minimal, and only removed when length and weight measurements were performed. Fouling removal was done using a toothbrush and small knife.

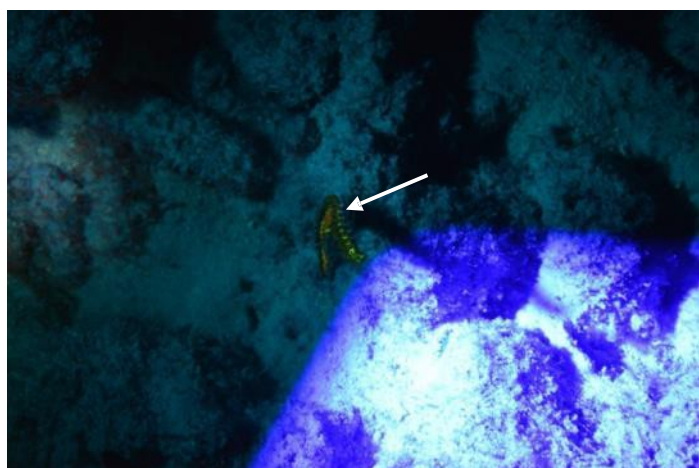


Figure 15. Tissue of the corallivorous *Hermodice carunculata* exhibits fluorescence, making the animal easy to spot against a non- fluorescing background. The camera lens is only partly covered by a yellow filter, hence the bright blue illumination of the lower right corner.

The fragments on all sites and structures were measured and weighed at 2 weeks intervals. For measurement procedures see page 12. Fragments were taken from their structure, cleaned of fouling, measured and weighed, and put back on their structure in the same location and orientation. The total process took less than 3 hours for all transplants at each site, mostly accomplished in two 90 minute dives with two or three persons. All fragments from a transplant site were measured and weighed on the same day. Not all length measurements were performed at all times, due to inconsistent results of the used method. The following table shows the taken measurements at specific times. After the experiment ended, approximately 8 weeks after transplantation, all fragments were returned to Buddy Reef's transplant sites and nursery trees, and most PVC plates were removed. The fragments were allowed to continue growing in these locations. The rebar structures at Jeff Davis and Tori's Reef were removed; the structure at Buddy's Reef was expanded and reorganized to house all 10 available FO's origins.

Table 4. Type of measurements taken at different times during the experiment. "X" indicates the measurement in the column header was taken at the time in the row header. Cnt indicates a count; the order of side branches of counted is also given. L. = length, W. = width.

Time	Buoy. wt.	Length 1 <sup>ary</sup> branch	Length 2 <sup>ndary</sup>	Count 2 <sup>ndary</sup>	L. 3 <sup>rd</sup> and up	Ct. 3 <sup>rd</sup> and up	Max. L. & W., Ø 1 <sup>ary</sup>
T=-1 (preparation)	X	X	X	X	X	X	X
T=0 (-1d to transplant)	X						
T=1 (14-16d)	X	X	X	X		X	X
T=2 (35-36d)	X						X
T=3 (48-49 d)	X	X		X		X	X

### 2.3 Experiment 3: Stimulation of recovery

The main goal of this experiment was to determine whether transplanted Staghorn branch tips devoid of living tissue and overgrown by other organisms (e.g. turf algae) could be recolonized by living tissue when the tissue bordering the "dead" branch tip was stimulated/irritated. The tissue cover over small, un-colonized branch parts was monitored in multiple fragments over a period of weeks. The possible effects of colony origin, depth and colony's location were taken into account. This experiment was performed on three existing transplant locations along the

coast of Bonaire (see page 13). Locations are named after the dive sites they are closest to. At “Buddy’s Reef” clusters of large colonies, organized by FO origin, are spread out over ca. 250m<sup>2</sup> of sandy rubble substrate with little coral cover. All 10 genotypes were transplanted here. This site was the first to be built (June 2013) and suffers from heavy predation by fireworms (*Hermodice carunculata*). “Tori’s Reef” is situated south of a loading pier and an effluent outfall of Cargill Salt Bonaire N.V.. The depth at this site ranges from 6.3 to 6.7m. Low growing (<1m) gorgonians provide some structure on this sand covered terrace. Staghorn colonies descending from seven different genotypes are spread over approximately 400m<sup>2</sup>, clustered by origin. “Jeff Davis” is a smaller site, occupying approximately 50m<sup>2</sup> and housing three clusters from different FO descendants. The area has a rubble substrate and is closely surrounded by large (>1m) gorgonians and smaller *Faviidae*. The depth is 4.0-4.9m. Figure 16 below shows the Jeff Davis transplant site and a close-up of a marked fragment in Buddy Reef’s transplant site.



Figure 16. Transplant site at Jeff Davis (left) and a close-up of a transplanted fragment on Buddy’s Reef (right).

#### Preparation, treatment and measurements

At all three locations attempts were made to find five tissue-less branch tips of at least 2cm of each genotype. Number of colonies used are given in Table 5. Local names are used for easy reading. At Buddy’s Reef this experiment was performed twice with the same branch tips, hence the superscript in the table below. For an exact origin location of a FO’s origin, see Table 1.

Table 5. Number of branch tips per FO’s origin used to determine the effectiveness of stimulation for tissue recovery.

Transplant location	FO’s original location	N <sup>1</sup>	N <sup>2</sup>
Buddy’s Reef	(1) Lac Bay	5	1
	(3) Red Slave	5	4
	(4) Sweet Dreams	5	5
	(5) Vista Blue	5	2
	(6) North Belnem	5	4
	(7) 1000 steps	5	5
	(8) Karpata	5	4
	(9) Nearest point	5	4
	(10) Rock pile	5	4
Jeff Davis	(1) Lac Bay	5	
	(2) Lac Bay	5	
	(3) Red Slave	4	
Tori’s Reef	(4) Sweet Dreams	4	
	(7) 1000 steps	2	
	(8) Karpata	3	
	(9) Nearest point	5	
	(10) Rock pile	5	

After the appropriate branch tips were found, they were marked with earlier made labels. These markers consisted of a small, red or black (to appear unattractive to animals), plastic label made with a Dymo embossed label maker containing a unique code (folded in half, showing the unique number on both sides), a piece of Ø0.5mm monofilament line and two aluminum crimp sleeves (Fig. 17). The label was pierced by the monofilament line and secured with an aluminum crimp sleeve. In the field the monofilament line was looped finger tight around a “dead”



branch tip, approximately 2cm from the living *Acropora* tissue. The line was secured in place with the second aluminum crimp sleeve and was checked not to be moving freely. Loose line ends were cut off and disposed of properly.

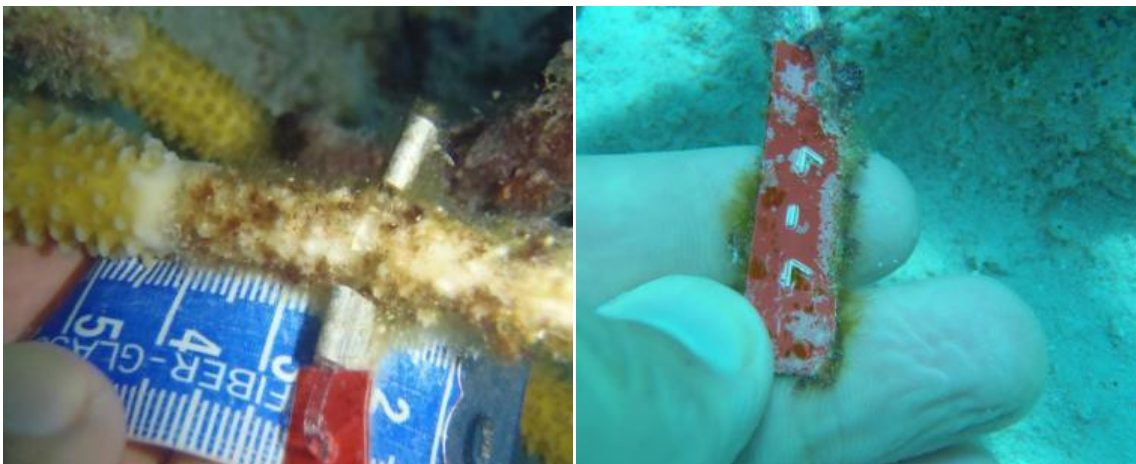


Figure 17. Markers attached to "dead" *A. cervicornis* branch tips. The markers are subject to overgrowth and fouling.

"Dead" tips were cleaned from fouling using a small (tooth)brush before the markers were attached and before each handling and measurement. After the markers were put in place the distance between the monofilament line and the living *A. cervicornis* tissue was measured in mm using fibre glass tape measure. Data was recorded on white PVC underwater slates with pencils. Because the monofilament line and tissue edge were not always parallel to each other, only the distance over the dead branch part directly facing the water surface (upside) was measured in all experimental units. These distances were compared to the same distances measured again 14 days later, to control for unaided recovery of tissue. When no recovery was found, a stainless steel Aquaholland Heavy Duty coral cutter (see Figure 5) was used to slightly damage the living tissue edge on the branch tips. This was done by carefully putting the pliers around the outer 1mm of the living tissue edge and, while slightly pinching, moving the pliers around the central axis of the branch tip. The process caused a shallow (<1mm) cut in the edge of the tissue. The distance between the tissue edge and monofilament line was measured again. In the following weeks the branch tips were allowed to recover on their own. The branch tips were freed of fouling once a week at the same time the distance between the tissue edge and monofilament line was measured.

## 2.4 Experiment 4: Side branch frequency

The main goal of this experiment was to test if there is a difference in the number of side branches between nursery-grown fragments versus "natural" Staghorn colonies and between different genotypes. The comparison was done by measuring the lengths of and counting several categories of side branches in nursery-grown fragments and in colonies currently growing at the original F0 sites. While the colonies currently present in Lac Bay (two of the ten F0's origins present in the CRF nurseries) were not measured, two additional locations currently accommodating small patches of *Acropora cervicornis* were studied. These locations are close to the recreational dive sites Jeff Davis and Tori's Reef. Especially colonies at Tori's Reef are remarkable since they thrive while growing directly in front of the salt company's effluent outfall. This outfall sometimes leads to clearly visible stratification and slow mixing of warm, highly saline and relatively cool, natural ocean water in the direct area of this outfall. For the exact locations of the other F0-origin sites see Table 1. Figure 18 shows two of the original collection sites (March-May 2014).



Figure 18. A large natural patch of Staghorn corals on the dive site Rock Pile (Klein Bonaire) (left) and less dense patches of natural Staghorn corals spread out over the terrace at the dive site Tori's Reef (Bonaire) (right).

## Measurements

At the origin sites 5-10 branches of 20-40cm length and 1.5-3cm diameter, originating from a thick main branch were randomly selected. The lengths of these branches (now called primary branch) were measured with fibre glass tape measure, following the branches curves from the attachment point to the main branch to the tip of the primary branch's apical polyp. The secondary branches were also measured per primary branch, from the node connecting it to the primary branch along the branch's curve to the tip of its apical polyp. The number of tertiary and higher order branches per primary branch was recorded per branch order, but lengths were not measured. A single origin site was so processed in 15-20 minutes with two persons. For the data of nursery-grown fragments, length data collected at the start of experiment 1 (Regeneration of tissue) was re-evaluated. The data from each F2.max and F2.min pair was pooled, the pairs primary branch lengths summed, now representing the side branch lengths and frequencies for all F1 fragments (nursery-grown) from experiment 1. Average "side branch densities" (represented as the number of secondary branches per cm of primary branch of a fragment, as well as the number of tertiary branches per cm of secondary branch of a fragment) are determined using this dataset.

## 2.5 Data analysis

All data was analyzed using R (R Core Team, 2014), with packages agricolae (Mendiburu, 2014), car (Fox & Weisberg, 2011), latticeExtra (Sarkar & Andrews, 2013), nlme v.3.1-111 (Pinheiro et al., 2013) and their dependencies. In all analyses Type I error rate  $\alpha=0.05$ . Microsoft Excel 2010® was used to prepare and organize raw data and determine growth rates (exponential for weight and linear for length of primary branch, via least squares fitting).

### Regeneration data

Data (i.e. number of days) was log (base e) transformed for time until full recovery and time of first visible apical polyp for subsequent analyses, but not for time until color appearance which seemed to follow a normal error distribution without transformation. Three statistical linear models were tested using time to full recovery, time to first zooxanthellae, and time till first apical polyp development (dependent variables). In each model the influence of genotype (collection location), nursery location, their interaction, the facing direction of the cut and the initial fragment length were assessed. Scatterplots were used to identify general trends and likely erroneous outliers, which were only removed when they could be identified as an error and where otherwise left in the model. After model fitting, model assumptions were checked graphically.

### Growth rates and survival data

Survival data was not analyzed, since survival was 100%. Growth rates (exponential for weight and linear for length of primary branch) of individual fragments were determined using least squares fitting in Microsoft Excel 2010. Figure 19 below sketches model fits of three haphazardly chosen fragments. In this figure the rounded exponential growth rates can be found in the exponent.

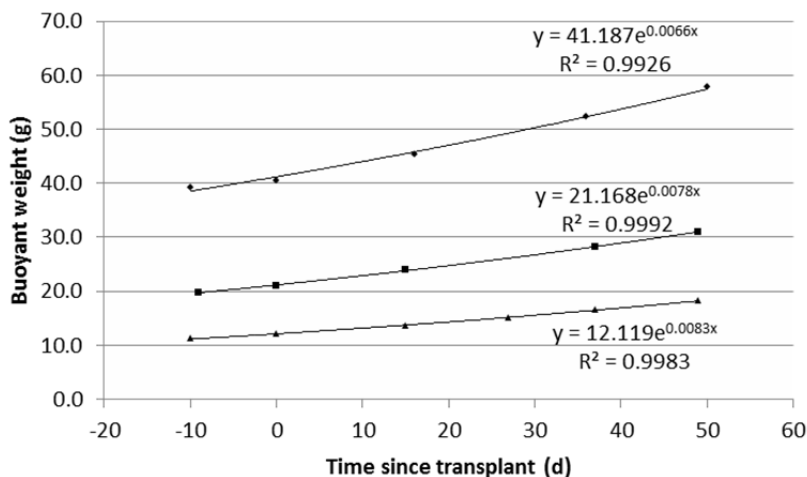


Figure 19. Least squares fitting of *A. cervicornis* exponential growth rates.

Two linear mixed-effect models were built using both exponential (weight) and square roots of linear (primary branch length) growth rates. Both models assessed the influence of FO's origin, transplant location, their interaction (fixed) and individual transplant tree/rebar (random) (independent variables). Model assumptions were checked graphically. The maximum dimensions, diameter and side branch lengths were also measured, but are not used in this report.

### Stimulation of recovery data

The differences in tissue-covered surface (linear) after 17-21 days were categorized into one of two groups: "recovery", if tissue had grown ( $T0 - T17-21d > 0$ ), or "deterioration", if tissue had not grown or had receded. The hypothesis that recovery frequency was not independent from transplant location was tested with Pearson's chi-square test on the categorized data. Additionally, the difference in tissue-covered surface was compared between FO's origin and transplant locations. A linear mixed effect model was built to assess these factors, using transplant

location as a fixed factor and F0's origin as a random factor.

#### *Side branch frequency data*

Primary branch lengths were compared using Welch's anova (which is more robust than standard anova's for homogeneity of variance violations) to verify if similar branches were measured. Secondary side branch densities were compared to identify the influence of the current location (wild versus nursery) and F0's origin. Data was log transformed to ensure a normal error distribution. F0's origin is modelled as a random factor to compensate for observation autocorrelation. Tertiary side branch densities are compared as above, with the exception that no transformation of the data is performed before analysis.

#### *SCUBA diving*

All work involving preparing, handling and measuring the corals in this experiment was done using standard SCUBA equipment plus mentioned tools by two to up to five researchers per dive. Depth measurements were done with a diving computer (Suunto Vyper).



## 3 Results

### 3.1 Experiment 1: Regeneration of damage

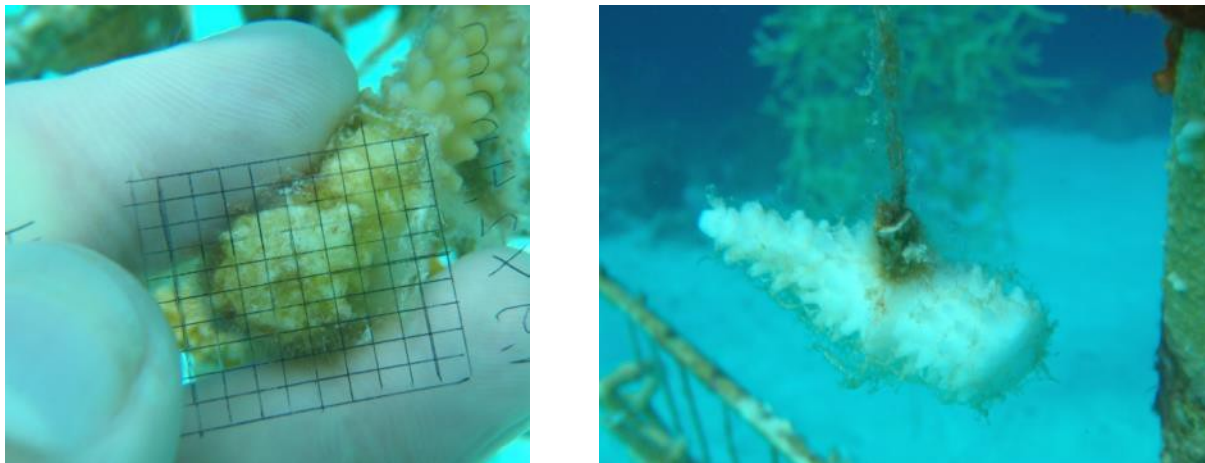


Figure 20. A single fragment failed to recover from the cut. The damaged area was overgrown by algae before it could be covered by new tissue. Picture taken 37 days after cutting, a cell on the grid measures 1.9x2.4mm (left). Another fragment suffered from white band diseases. Picture taken 40 days after cutting (right).

234 Staghorn fragments were used in this experiment. In 233 fragments (99.5%) the experimental cuts showed new tissue cover within the 47 days duration of the experiment. One single fragment did not recover from the cut and was located in the Bonaire nursery originating (F0) from Lac Bay(2). The cut on this fragment suffered from overgrowth by algae, however, the fragment was still growing at the end of the fieldwork period. The fragment was exceptional in that epilithic algae were already present within 2cm from the cut at the moment of cutting (Fig. 20).

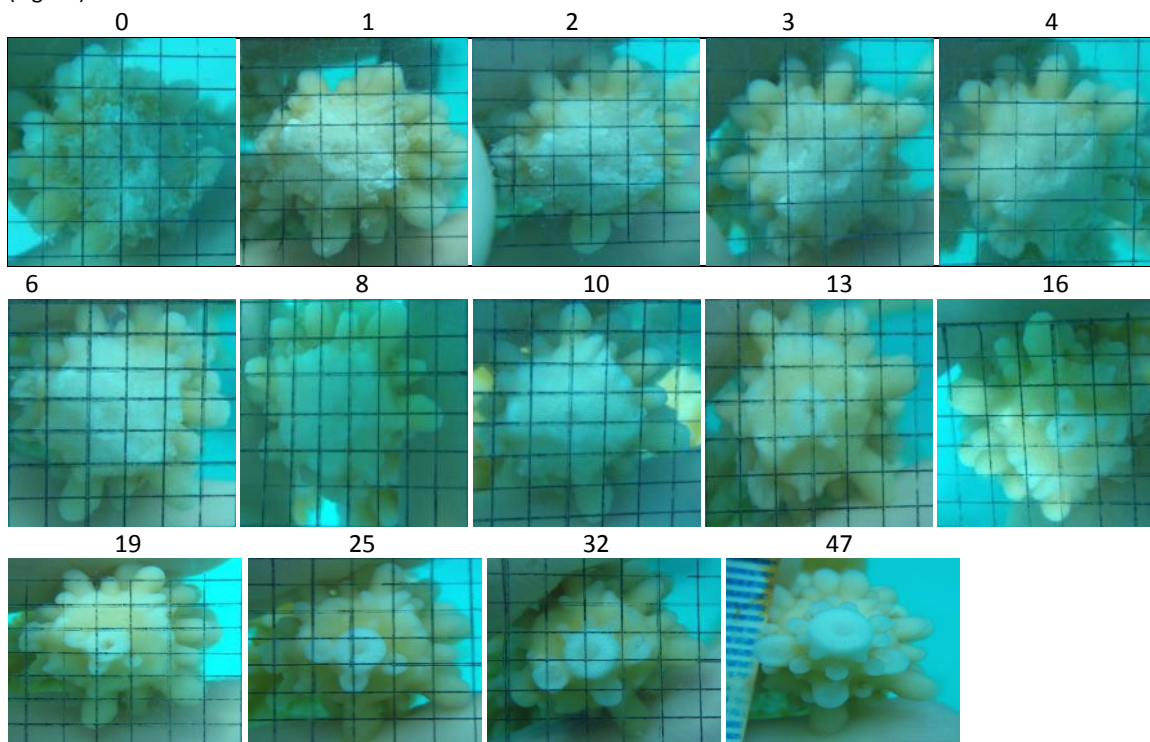


Figure 21. Time series of the recovery of a cut Staghorn fragment from F0's origin Vista Blue (5) in the Klein Bonaire nursery. The numbers above each photograph indicate the number of days passed since the cut was made. Tissue seems fully returned after 4 days (visible under magnification), while zooxanthallae pigments and the first signs of apical polyp development both occur after 13 days. In each photograph a grid cell measures 1.9x2.4mm.

Full mortality of fragments due to cutting was not observed during this experiment. However, a single fragment in the Klein Bonaire nursery (F0's origin Red Slave(3)) suffered from white band disease and died completely approximately 3 weeks after full recovery of the cut, 5 weeks after cutting (Fig. 20). This could mean that fragments are more sensitive to WBD while regenerating damage. This was however the only fragment that died. Cutting fragments resulted in a recovery of 99.6% of the fragments in our experiment. Survival until recovery was 100%. A typical recovery is shown in a time series using photographs taken during the experiment (Fig. 21).

Table 6. Number of days until complete tissue regeneration, visible zooxanthellae and first apical polyp development. Values are days with 95% confidence intervals. BR, Buddy's Reef; KB, Klein Bonaire.

	F0's origin	Lac Bay(1)	Lac Bay(2)	Red Slave(3)	Sweet Dreams(4)	Vista Blue(5)
<b>Tissue recovery</b>	BR	6.8 (6.0-7.7)	6.4 (5.5-7.3)	6.6 (5.8-7.6)	6.0 (5.1-7.1)	4.9 (4.3-5.5)
	KB	6.9 (5.5-8.7)		4.7 (4.1-5.5)		6.0 (5.2-7.0)
<b>Color appearance</b>	BR	17.4 (15.7-19.3)	18.7 (16.0-21.9)	17.7 (15.6-20.2)	12.5 (10.8-14.5)	9.5 (8.6-10.5)
	KB	14.4 (12.2-17.1)		18.2 (15.3-21.7)		14.7 (12.3-17.4)
<b>Apical polyp</b>	BR	14.1 (11.6-16.5)	13.8 (11.1-16.5)	15.3 (12.5-18.1)	15.6 (12.1-19.0)	8.4 (7.1-9.8)
	KB	15.4 (12.1-18.6)		11.0 (9.0-13.0)		12.4 (8.5-16.4)

Table 6 continued

	F0's origin	North Belnem(6)	1000 Steps(7)	Karpata(8)	Nearest Point(9)	Rock Pile(10)
<b>Tissue recovery</b>	BR	5.3 (4.7-5.9)	5.4 (4.8-6.1)	6.3 (5.4-7.3)	5.3 (4.8-5.9)	5.1 (4.3-6.1)
	KB		5.2 (4.0-6.7)		4.3 (3.6-5.2)	
<b>Color appearance</b>	BR	12.6 (10.7-14.8)	12.8 (11.3-14.5)	17.5 (15.6-19.6)	11.6 (10.5-12.7)	14.8 (12.7-17.4)
	KB		15.4 (11.7-20.2)		12.5 (9.9-15.9)	
<b>Apical polyp</b>	BR	12.9 (10.9-14.9)	10.9 (8.5-13.3)	13.4 (11.1-15.6)	10.4 (9.2-11.6)	11.7 (9.9-13.5)
	KB		13.4 (6.9-19.9)		13.4 (8.0-18.8)	

#### Time until recovery of tissue

For time until recovery genotype was found to have a significant effect ( $p < 0.001$ ). Table 6 and Figure 21 show means and confidence intervals for all combinations of F0's origin. Since fragments of all genotypes were not generated on all locations, we did not investigate if regeneration of tissue did differ between nurseries. For the genotypes that were available, data do suggest that there is no difference (Table 6).

#### Time until color appearance

For time until color appearance, F0's origin ( $p < 0.001$ ) and cut orientation ( $p < 0.05$ ) were found to have a significant effect on the time until color appearance. Tukey's HSD distinguished two groups of orientation with different times until color appearance, as described in Table 7 below. The group descriptions can be simplified to "cut facing roughly upwards" and "cut facing roughly side & downwards", where cuts facing roughly upwards take more time to develop color.

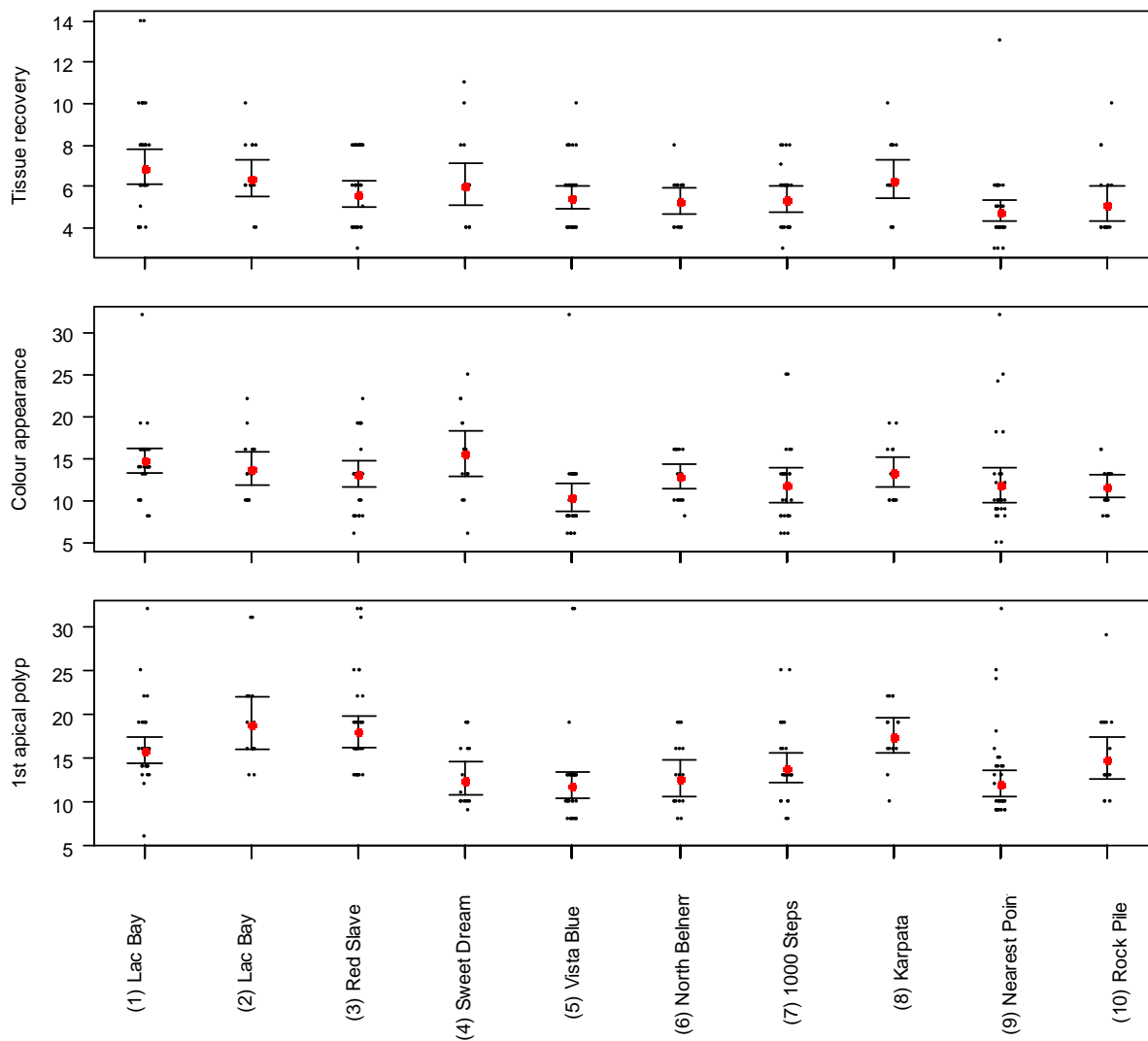


Figure 22. Plots of the number of days until tissue recovery, first color appearance, and first visible apical polyps in cut fragments. Dots indicate individual measurements, larger red dots are means. Whiskers indicate 95% confidence limits.

Table 7. Tukey's HSD comparisons of cut orientation effect on time until colour appearance in cuts. Same group means similar effect of the cut orientations (left column) on time until colour appearance.

Cut orientation	Time until color appearance	Group
UpSide	16.1	a
Up	14.0	ab
Side	12.5	b
Down	11.1	b
DownSide	11.4	b

#### Time until first apical polyp development

The same approach was used to compare times until first visible apical polyp (Table 6 and Figure 21). The nursery location and genotype (original collection location) were found to be significant factors ( $p < 0.05$ ). There was quite a lot of individual variation in the duration of the different stages between fragments, but average values and the statistical analyses indicate that place of origin (assumed genotype) is an important factor. There is not a clear correlation between the rate of tissue recovery, coloration, and apical polyp appearance as genotypes that show fast tissue recovery, may have a slow apical polyp development or vice versa (for example colonies originating from Red Slave and Sweet Dreams).

### 3.2 Experiment 2: Growth and survival in nurseries and field

The growth in terms of both weight and branch lengths was monitored in 180 fragments for this experiment. All fragments survived the experimental period.

#### *Growth rates expressed by buoyant weight*

The growth rates (weight) were determined for 176 fragments. In four fragments buoyant weight determinations failed due to inconsistencies (decreasing weight without visible damage or unexplainably large increases). These data points were removed from further analyses. Interaction between F0's origin and location was significant ( $p < 0.02$ ), indicating that the different genotypes behaved differently on the 4 different sites. For example colonies that originated from Karpata (genotype 8) appeared to have on average the highest growth rate irrespective of the site where they were growing. Among the different sites there is not a clear difference in growth that is consistent over the genotypes. Confidence limits within sites are all substantially overlapping. The individual rebar that colonies were attached to did not matter (no significant random effect). Figure 23 and Table 8 below show the growth rates of the fragments in different combinations of F0's origin and location. The effect of genotype (origin) is generally much higher than that of growth location. On average a fragment increased each day 0.7% in weight, meaning that an average fragment of 25gr would become 12 times its weight within 1 year.

Table 8. Buoyant weight (BW) growth rates (% BW increase per day) including 95% CI.

F0's origin	Buddy's Reef	Buddy's Reef Nursery	Jeff Davis Memorial	Tori's Reef
<b>(3) Red Slave</b>	0.71 (0.63-0.79)	0.63 (0.55-0.71)	0.67 (0.57-0.77)	0.71 (0.66-0.77)
<b>(6) North Belnem</b>	0.58 (0.52-0.64)	0.69 (0.65-0.73)	0.63 (0.57-0.68)	0.65 (0.61-0.70)
<b>(8) Karpata</b>	0.77 (0.71-0.83)	0.82 (0.80-0.84)	0.80 (0.77-0.84)	0.75 (0.70-0.80)

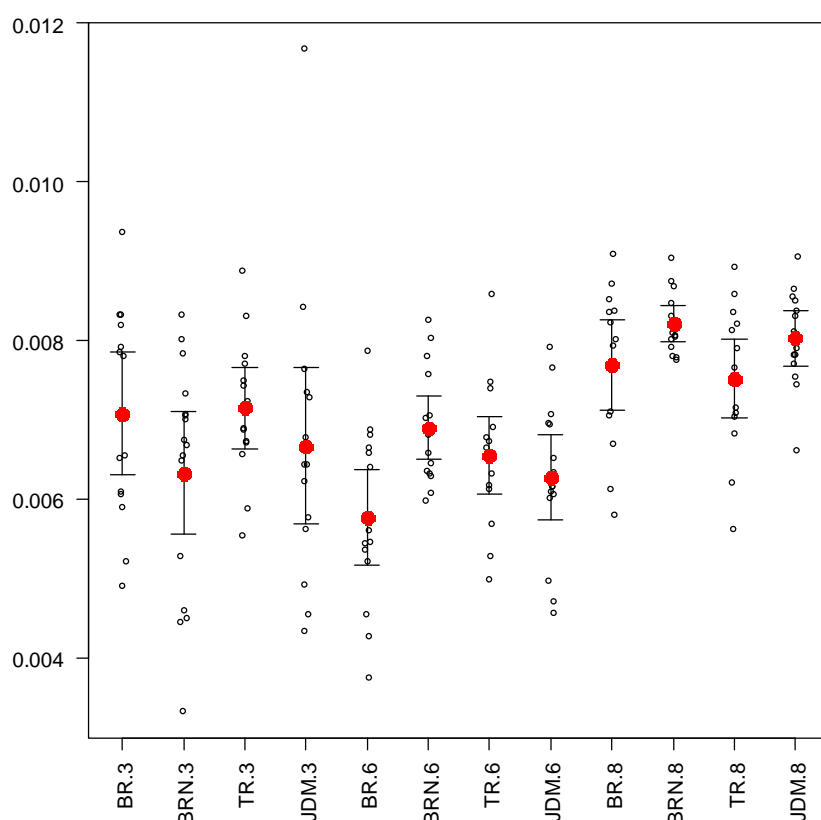


Figure 23. Plot of buoyant weight growth rates. Small dots symbolize individual fragments. Red dots indicate means per F0's origin x location combination. Whiskers show 95% confidence intervals. Growth rates are expressed as slopes of a linear growth model based on ln-transformed data.

### Growth rates expressed by length

The growth of primary branches was monitored in 157 fragments. 23 fragments showed inconsistent measurements (decrease in length without visible damage, or increases of more than 5cm per month) and were therefore removed from further analyses. None of the factors FO's origin, location, their interaction or individual tree/rebar were found to have a significant effect on the linear growth rates of the primary branches, possibly the test had low power as a consequence of increased variation.

Table 9. Mean growth rates (mm d<sup>-1</sup>) of Staghorn coral primary branches including 95% confidence intervals. Means are split per location and FO's origin.

FO's origin	Buddy's Reef	Buddy's Reef Nursery	Jeff Davis Memorial	Tori's Reef
<b>(3) Red Slave</b>	0.092 (0.057-0.126)	0.061 (0.030-0.092)	0.079 (0.060-0.098)	0.060 (0.046-0.074)
<b>(6) North Belnem</b>	0.073 (0.049-0.097)	0.079 (0.027-0.131)	0.087 (0.063-0.111)	0.090 (0.048-0.133)
<b>(8) Karpata</b>	0.076 (0.037-0.115)	0.063 (0.041-0.085)	0.061 (0.041-0.082)	0.093 (0.041-0.146)

Table 9 and Figure 24 below show the average growth rates (mm d<sup>-1</sup>) per FO's origin and location. On average this is 0.076 (0.068-0.085) mm d<sup>-1</sup> (2.3 cm month<sup>-1</sup> or 27.7 cm y<sup>-1</sup>). Growth is in two directions since a branch tip develops a new growth tip at the cut surface.

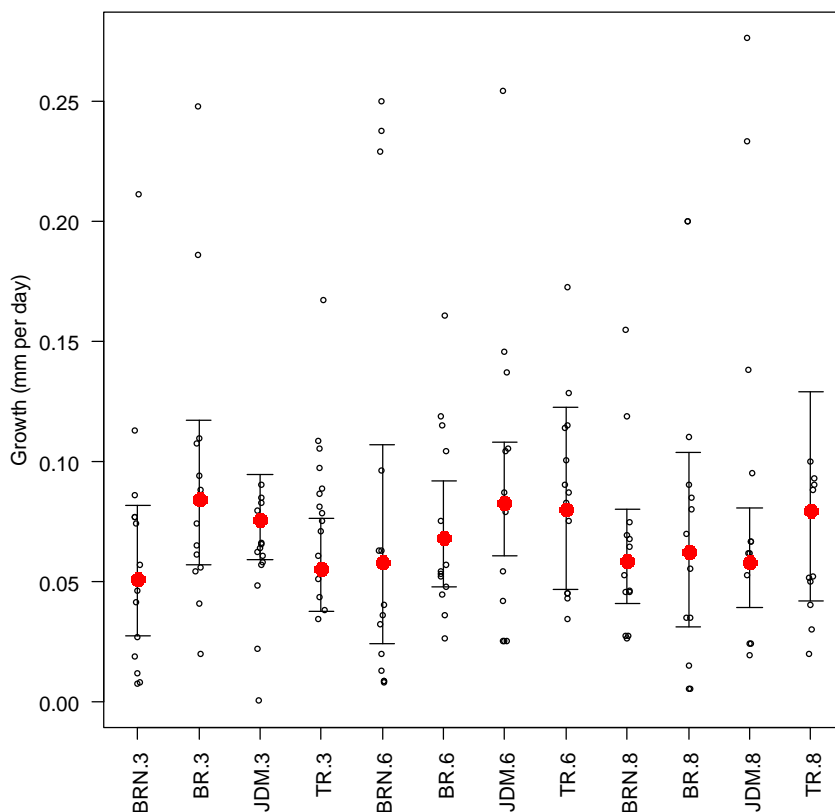


Figure 24. Plot of primary branch growth rates. Small dots denote individual fragments. Red dots indicate means per FO's origin location combination. Whiskers show 95% confidence intervals. Data were square root transformed before means and confidence limits were calculated.

### 3.3 Experiment 3: Stimulation of recovery

In total 83 fragments were available for analysis. 48 Fragments showed an increased tissue cover (tissue had grown >0mm) within 17-20 days after tissue stimulation. 13 Fragments did not show recovery, and in 22 the tissue withdrew further. Since all fragments showed no recovery at the start of the experiment, inflicting damage meant that the regeneration process could be reinitialized in almost 58% of the fragments leading to additional recovery.

#### Recovery frequencies

Transplant location was not found to have a significant influence on the frequency of “recovery” versus “deterioration” ( $0.05 < p < 0.1$ ). It must be noted that sample size was relatively small (power to detect a difference may have been small). Table 10 shows these frequencies.

Table 10. Frequency table for recovery of damaged Staghorn branches. For the analysis columns “No growth” and “Deterioration” were summed.

	Recovery	No growth	Deterioration
Buddy’s Reef <sub>1</sub>	21	6	16
Buddy’s Reef <sub>2</sub>	16	1	2
Tori’s Reef	4	2	2
Jeff Davis	7	4	3

#### Tissue extension

Statistical analysis (GLMM) revealed that neither genotype (origin) nor transplant location had a significant influence on the tissue recovery, although on average tissue had grown 0.092cm (95%CI: 0.026- 0.157cm). Figure 24 below gives a visual impression of the tissue status on the varying locations. Note that a large number of tips showed receding tissue cover, especially in Buddy’s Reef 1.

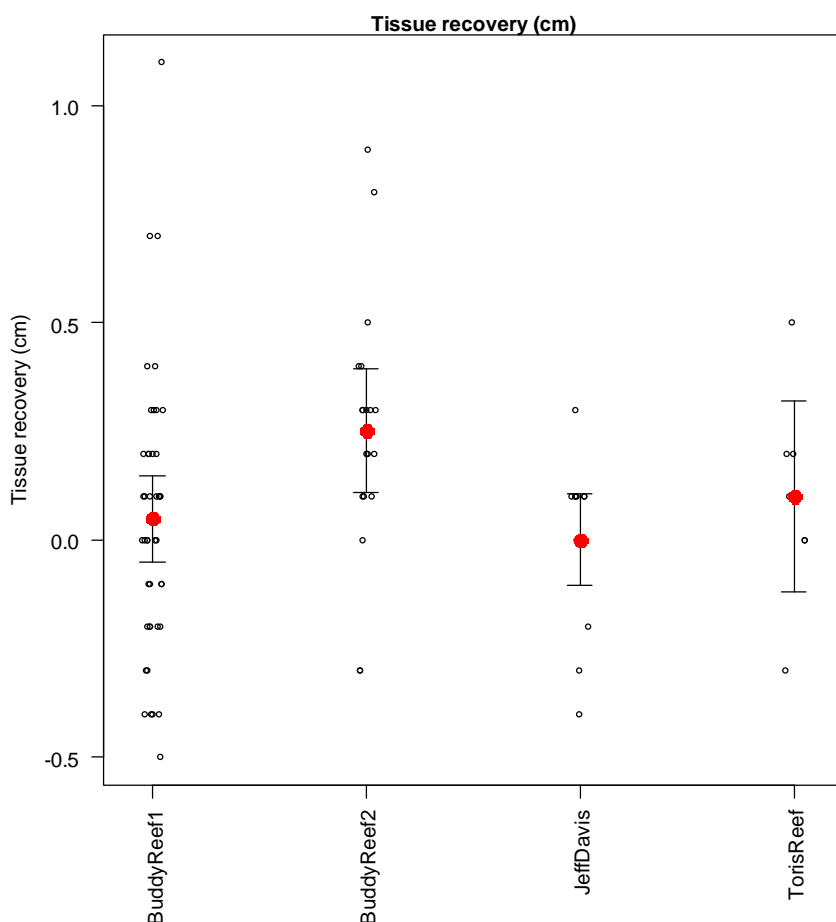


Figure 25. Tissue regeneration re-initialization in transplanted Staghorn fragments. Red dots indicate means, whiskers 95% confidence intervals. Small dots individual fragments. Different FO’s origins are pooled.



### 3.4 Experiment 4: Side branch frequency

201 fragments were measured for this experiment, originating from 12 F0's origins (collected from 12 locations around the island of Bonaire). The number of fragments measured at each location is given in Table 11, for exact locations see also Table 1.

Table 11. Sample sizes of Staghorn colonies originating from different F0's origins for comparing side branch frequencies.

→ Current location ↓ F0's origin	Buddy's Reef Nursery	Klein Bonaire Nursery	Origin site
(1) Lac Bay	8	8	0
(2) Lac Bay	8	0	0
(3) Red Slave	8	8	8
(4) Sweet Dreams	8	0	9
(5) Vista Blue	8	8	10
(6) North Belnem	8	0	10
(7) 1000 steps	8	5	8
(8) Karpata	8	0	10
(9) Nearest point	8	8	5
(10) Rock pile	8	0	8
(15) Tori's Reef	0	0	8
(50) Jeff Davis	0	0	8

First, we compared the primary branch lengths of wild and nursery (Buddy's Reef and Klein Bonaire pooled together) grown fragments (Fig. 26). As can be seen below, the fragments measured in the "wild" are slightly larger than those measured in the nursery ( $p < 0.001$ ,  $F_1 = 47$ , likelihood ratio test data corrected for heteroscedasticity). Model estimated means with 95% confidence limits were 18.5 (17.5, 19.5) and 26.0 (24.2, 27.8) for respectively nursery and wild colonies.

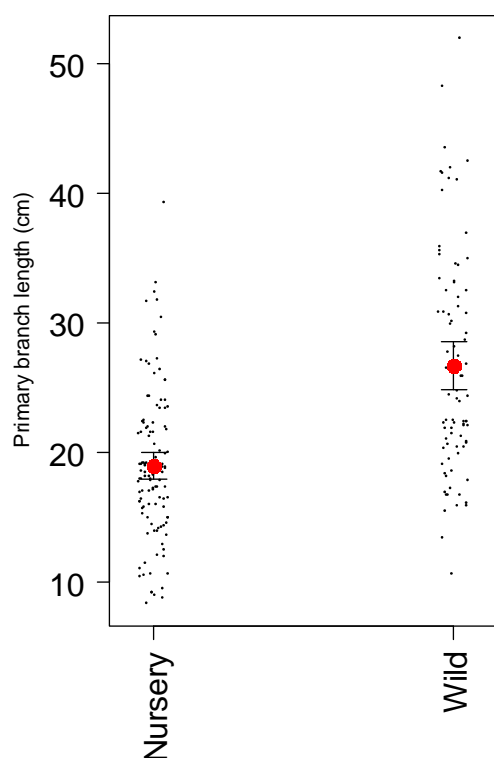


Figure 26. Plot of primary branch lengths of colonies used in this experiment. Small dots represent individual fragments. Red dots indicate means per current location. Whiskers show 95% confidence intervals.

### Secondary side branch density

For further analyses the ratios of secondary branches per cm of primary length were  $\ln$  transformed and a linear mixed-effects model was used to compare the branching frequency of colonies in the wild with branch frequency of colonies in nurseries (Fig. 27). The effect of the current location was highly significant ( $p < 0.001$ ) indicating that branch frequency in nurseries is about twice as high than in the field). Means (plus 95% Confidence limits) are 3.8 (3.3, 4.3), and 1.9 (1.7, 2.2) for nursery versus wild branch frequency per 10cm of branch length. The random effect was also significant, but appeared to be best if applied not only to genotype, but to the combination of genotype and location. This indicates that variation of branch frequency that remains after the location effect is removed is probably also due to differences in the local environment the colonies are in. This can also be seen in the data. For example colonies that appear to have a higher frequency in the nursery (e.g. 2, 6, 10), may have a lower frequency in the wild (6 and 10). The more side branches develop, the harder a colony grows, so colonies in nurseries should increase much faster in weight than colonies in the field.

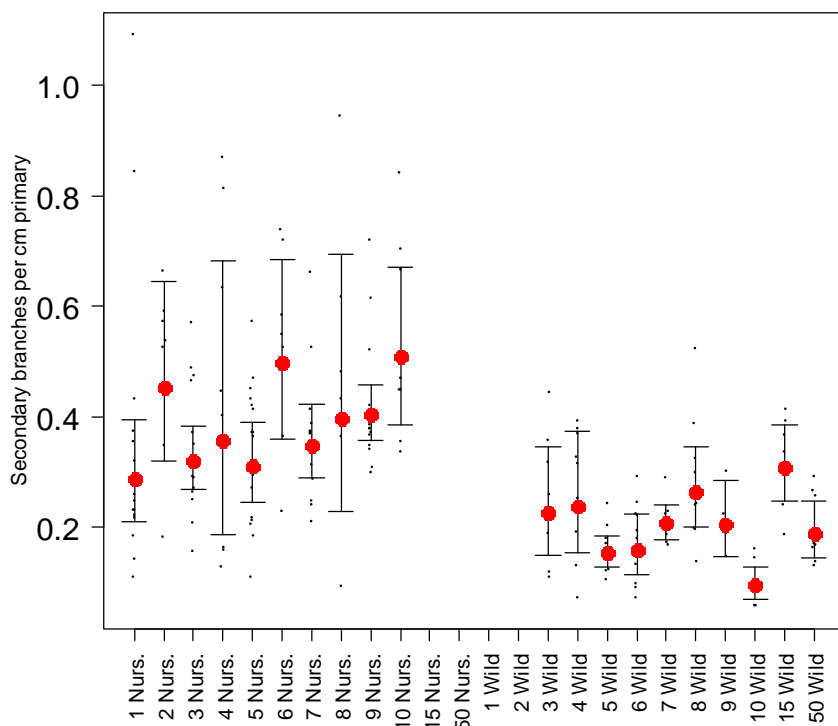


Figure 27. Plot of secondary side branch densities. Small dots denote individual fragments. Red dots indicate means per current location. Whiskers show asymmetrical 95% confidence intervals (symmetrical when  $\ln$  transformed). Larger confidence intervals in even numbered F0's origins are caused by smaller sample size (8 vs 16).

### Tertiary side branch density

The data for numbers of tertiary branches per unit of length of the secondary branches were log transformed before analysis. A single outlier (F0's origin 8, nursery-grown) was removed leaving 200 measured fragments in the analysis. There was some weak evidence for a location effect ( $p = 0.03$ ), but 95% confidence limits on the branching frequency were actually overlapping. Means (95% CL) were 1.1 (0.9, 1.4) versus 0.9 (0.7, 1.1) for nursery versus wild colonies. So there is no conclusive evidence that tertiary branches also develop more frequently in nurseries. This may however be due to the fact that branch lengths in the nurseries are often smaller and tertiary branches may not yet have developed.

## 4 Discussion

### Regeneration of damage

Our study shows that the cuts on new fragments will generally heal completely within two to three weeks. Most studies on regeneration of damage in Caribbean *Acropora* species focus on *A. palmata* (e.g. Bak 1983, Meesters & Bak 1995, Lirman 2000). These papers describe an exponentially decreasing rate of damage regeneration, i.e. regeneration starts fast but slows quickly over time. Our own data, although gathered similarly to Lirman (2000), however, proved insufficient to verify such a regeneration model for *A. cervicornis*. This was mainly due to the fact that the very quick covering of the lesion with a transparent veneer of tissue was impossible to measure. Our results, however, are generally similar to tissue recovery descriptions in other papers and indicate an extremely fast starting regeneration rate (see for example Meesters and Bak 1995 for *Acropora palmata*). Cover by tissue was within approximately 1 week and new polyps would emerge within the second week. Regeneration could also restart in transplanted colonies after it had halted by damaging the edge of the living tissue. This was successful in 58% of the colonies, however, the effect in terms of extra tissue recovery appears marginal. It may be more worthwhile to fragment the colony a little under the dead tip and let the lesion heal. The surface area that then needs to be recovered is only a fraction and the chances of success are probably much higher. In fact this is what CRFB is now practicing. Different zooxanthellae clades can be present in the fragments which may alter harvested light and energy budgets, or intrinsic differences in tolerance limits for water quality or water temperature. These factors were not researched here but may influence the regeneration, growth and survival rates.

### Survival

In terms of survival, fragments on Bonaire seemed to perform better than in most other published studies on nursery-grown *Acropora*. For example, Lirman et al (2010) found in a 4-month experiment that survival of *A. cervicornis* branches was not related to their size if larger than approximately 4cm. However, survival did seem to be related to handling method, where transport of the fragments to a nearby boat and back caused higher mortality (up to 87% for 2.5cm fragments, 13% for 3.5cm fragments versus 8% for 2.5cm and 0% for 3.5cm fragments in case they were not transported but replanted directly). Another transplant of *A. cervicornis*, described in Herlan & Lirman (2008), resulted in a mortality rate of 17.3% in the first two months. Putchim et al. (2008) report mortalities of 4.2% and 5.2% in transplanted (non-Caribbean) *A. grandis* and *A. muricata* respectively after two months. Hollarsmith et al. (2012) found an average tissue mortality of 7% and disappearance of 10.9% of *A. cervicornis* one year after transplant.

In our experiments (experiment 1 and 2) mortality due to handling and transport was basically absent. A single fragment (out of over 400 fragments used) died, but this was a month after recovery from treatment had occurred. In case of the reinitiating regeneration experiment (experiment 3), survival, if defined as maintaining or increasing their tissue edge over the study time, was lower (73% after 2.5-3 weeks).

Most likely survival is not only related to size, handling and transport, but also to the grow-out structure used in a nursery and on the subsequent transplant site. The majority of *Acropora* fragments used in the mentioned studies above were cemented to the reef using epoxy or cement. The fragments used by Putchim et al (2008) were attached to the transplant substrate (steel bars) with tie-wraps. In all of the described transplants the fragments or their structures were in close contact with the substrate. In our experiment, both nursery and transplant fragments were kept a considerable distance from the underlying substrate and a minimum of contact points between structure and substrate was used. The mechanism behind mortality is unlikely to be solely sedimentation, as Enochs et al (2014) describe sedimentation rates of up to 200 mg cm<sup>-2</sup> per 45 days as not having deleterious effects on the growth of Staghorn coral. In experiment 2 we observed that one of the locations frequently experienced periods of high turbidity, which was also not reflected in the growth rates. Thus other factors which may influence survival are likely responsible, such as predation or disease, in which case the substrate may serve as a vector to give the predator easy access. Long-term survival should be investigated more since survival may change over time.

### Location and grow-out structure

Growth in terms of weight increase was exponential. Our results indicate that neither transplant location nor grow-out structure had an influence on the survival and growth rate of Staghorn fragments. This means that environmental conditions are generally good for growth of Staghorn fragments. This view combines well with the observations of e.g. Cairns (1988), Lasker & Coffroth (1999) and Fautin (2002) that Staghorn corals' main method of propagation is fragmentation. New fragments, individuals (ramets), will grow independently of their parents, but the genetic information is the same (genets or clones). Fragmentation brings the risk of smothering downside polyps in substrate (frequently sand) after a breakage, but new tips will develop quickly and the coral will grow into a new direction away from the bottom. Gilmore & Hall (1976) propose that the branching of *A. cervicornis* leads to a certain type of three dimensional structure, which assists in preventing large areas from touching the bottom after falling, thus reducing the risk of smothering. Additionally, this type of structure would also increase susceptibility to fragmentation and overthrowing by waves, leading to increased growth and dispersion. Since fragmentation in *Acropora* species is adaptive Highsmith (1982), this fits with the life history of the species. Personal observations suggest that Staghorn corals can grow a very high rates irrespective of frequently changing environmental conditions on the different sites with regards to temperature, salinity, waves and current action.

Growth measured as linear extension of the primary branch was much more variable. This may have been due to misidentified primary branches because while growing other branches may become the primary branch. But measuring the primary branch only captures a part of the growth process.

Limited data exist water quality around Bonaire, gathered in 2011-2013 and described by Slijkerman et al (2013). Results indicate DIN-concentrations generally below the environmental threshold of 1 µmol l<sup>-1</sup>, but total P generally exceeds the 0.1 µmol l<sup>-1</sup> threshold for organic phosphorus. Spatial variation in nutrients between our study sites seems limited because the study was limited to the dry season and most variation in water quality was found in the month November, in the middle of the rainy season.

### Side branch formation

We found that nursery grown fragments showed higher, almost double rates of side branch formation. Limited published studies are available on this topic. Xin et al (2013) found a difference in side branch formation in *A. formosa* growing on two different islands in Malaysia but did not propose any specific mechanism behind this difference. The most frequently branching colonies in their study were situated in the shallower, warmer water and experienced the highest light intensity. Soong & Chen (2003) conclude that apical polyps can only be newly formed (i.e. not transformed from radial polyps) and may be related to available resources. Location on the parent branch seems less relevant. According to these researchers an upward orientation instead of downward results in apical branch formation more often over a four month period. This points to an effect of orientation. Possibly, the small movements that a fragment experiences while hanging in a tree may stimulate the development of apical polyps. Maybe this is a response to movement and developing more apical polyps will generally lead to quicker fixation and potential risk of damage. Side branch formation may be the result of restricted water movement around the fragments (hanging fragments will move *with* the water in currents or swell, whereas stable fragments experience water flowing through their branches) or unstable light conditions caused by frequent turning of the fragments. It is interesting to note that in some of the nursery grown fragments multiple side branches protrude from a single point (e.g. Figure 13, bottom picture, fragment 8/20). The similar lengths of clustered side branches may hint at simultaneous formation. This was not observed in wild colonies. A temporary change in currents would have affected wild and nursery grown fragments similarly and is an unlikely single candidate, but the constantly changing orientation may play a role.

The available published literature does not appear to have taken into account the effect fragment age may have on side branch frequency. In this study and the two described above relatively small and young fragments were used, and the nursery fragments were somewhat smaller than the wild fragments. Personal observations of older (indicated by larger diameter branches and longer branches), wild *A. cervicornis* hint at a decreased side branch frequency with age, since relatively few young, small side branches were present on these colonies. Perhaps side branch formation is limited by age (thickness) of a branch or regenerative capabilities decrease with distance from the growth tips similar to *A. palmata* (Meesters & Bak, 1995). Slight damage might also trigger side branch formation, as some form of overcompensation for damage recovery. Soong & Chen (2003) found damaging *A. pulchra* tissue often leads to apical polyp formation at that site, but the difference between damaged and undamaged fragments becomes smaller over time (i.e. 3-4 months). Minimal damage is experienced regularly by nursery fragments due to maintenance and collisions with other fragments. While collisions with other fragments generally occur on the sides of branches, we saw that collisions due to maintenance mainly occurred on branch tips (contact with tools, persons etc.).



Figure 28. Atypical apical polyp formation after slightly damaging an *A. cervicornis* branch.

Our experiment 1 often resulted in multiple apical polyps forming at the sites of cuts. However, side branch formation was not observed in experiment 3 where transplanted fragments were purposefully damaged to observe recovery. Of the two experiments, in experiment 3 the damage was most similar, albeit still excessive, to that normally experienced by nursery grown fragments. In this experiment only one fragment out of 91 (not all were analysed) showed apical polyp formation (Fig. 28). This was a unique case and the fragment was not part of the main experiment for different reasons. A third cause for intensive side branch formation might be sudden changes in light intensity. This is not often experienced by wild colonies, except maybe after breakage. In the CRF nurseries, fragments are regularly moved through their tree to facilitate maintenance. A movement to a position higher in the tree lets the concerned fragment experience more light intensity and thus gives the fragment the potential to collect and assimilate more solar energy. The additional energy can be invested in new side branch formation. It appears advantageous to let fragments develop many side branches in the nursery trees before transplanting them to the reef to optimize the number of directions the colony is growing in and thereby maximizing its survival.

## Conclusions

This report shows that growth of Staghorn corals in nurseries and on transplant sites on Bonaire is very high (almost 14cm per year per growth tip and between 0.5-0.8% in weight per day). Survival in nurseries and in the field was 100% after 7 weeks. Cut surfaces on fragments and mother colonies close within 1 week and are fully regenerated including color and polyps within 2-3 weeks. An average fragment will become 12 times its weight within one year assuming growth continues as measured during the 7 weeks of the experiment. It should be realized that in the field when a Staghorn field becomes very dense it is likely that growth will decrease somewhat due to resource limitations. Fragments show twice as much branching in nurseries than in transplant sites without effect on the density of the corals.

There are clear indications that genotypic variation (corresponding here to the original location where samples were collected) is significant in several aspects that are important for restoration. Genotype proved to be a significant factor in the regeneration of damage and growth. Current restoration practices are very successful in the short term. Long-term survival should be studied in more detail as incidental predation on transplanted corals may still lead to high mortality, as was observed in the field on colonies that were attached on the reef bottom with epoxy compared to colonies attached to rebars and kept off the bottom.

The regeneration of damage can be restarted by creating a small amount of damage. This means that Staghorn tips that have died from predation can be broken off so that new tissue may be formed at the fracture. This way colony integrity can be restored and the colony can remain its exponential growth. This technique has already become common practice by CRFB.

Nursery grown fragments of Staghorn coral had significantly more secondary branches per cm primary length than their "wild" counterparts. This may be advantageous for colony survival in the field as the potential number of growth directions is high.

## Recommendations

- Long-term growth and survival of transplanted corals should be studied. It is hypothesized that survival of Staghorn corals is guaranteed if a colony has reached a certain size and number of side branches. Death by natural mortality is always possible, but once a certain number of branches have developed it is highly unlikely that the whole colony will disappear excluding certain disastrous events (e.g. extreme bleaching or hurricane).
- Recovery of tissue on removed dead tips should be further studied. Clipping dead tips from fireworm predation appears to be a reasonable method to restore whole colony integrity, however, this needs to be determined in the field.
- Genetic analyses should be carried out to test if used genotypes are different and whether they are resistant against White Band Disease. Selecting WBD resistant genotypes will limit the possibility of whole colony mortality.
- Observations suggest that high densities in nursery trees may weaken colonies (for example through shading effects or continuous occurrence of small damages). This could be further studied. It could also be an age effect, but at this moment it is unclear whether it happens and if so what is the cause.

A minimum size for pruning and transplanting is suggested to limit potential weakening effects in the tree with  $Size_{pruning} < Size_{transplanting}$ .

- Restoration of Elkhorn corals should be further developed. It is clear that restoration of Elkhorn corals is not as much developed as Staghorn. However, there are promising results from other restoration teams (e.g. Bowden-Kerby 2014).
- Determine minimal "critical forest mass" at transplant sites. We suggest that negative impacts of predation, storm damage or disease will be mitigated or compensated completely by sustained growth of the surviving fragments if the total amount of living branches has reached a certain number and density. At this density the colonies will provide protection to many different animals that will defend the colonies against predators. Also, colonies will intertwine and fuse creating more stable structures that can withstand waves of significant force. Monitoring growth and survival of several restored coral patches of 1, 4, and 9 square meter would provide important insights.
- Selective placement of different FO's origins should be considered. E.g. a fast growing genotypes at the borders of patches to stimulate outgrowth and slower growing colonies on the inside. Better regenerating genotypes where damage or predation is more likely to occur.
- The strong side branching tendency of nursery-grown fragments can be exploited to, e.g., let fragments develop a multitude of branch tips before they are returned to the reef. This can provide immediate shelter for local fish and (in)vertebrates, increase fragment stability on the sediment, and optimize survival because of the large number of growth directions. Densely branching fragments may be more able to withstand considerably stronger wave actions and thus may grow in environments that are too harsh for other fragments.

## 5 References

- Bak RPM (1983) Neoplasia, regeneration and growth in the reef-building coral *Acropora palmata*. *Mar Biol* 77:221-227
- Bak RPM, Brouns JJWM, Heys FML (1977) Regeneration and aspects of spatial competition in the scleractinian corals *Agaricia agaricites* and *Montastrea annularis*. *Proc 3rd Int Coral Reef Symp* 2:143-148
- Bak RPM, Engel MS (1979) Distribution, abundance and survival of juvenile hermatypic corals (Scleractinia) and the importance of life history strategies in the parent coral community. *Mar Biol* 54:341-352
- Bowden-Kerby, A (2014) Best Practices Manual for Caribbean *Acropora* Restoration. Technical report pp. 39
- Cairns SD (1988) Asexual reproduction in solitary Scleractinia. *Proc 6th Int Coral Reef Symp* 2:641-646
- CRF Bonaire (2014) Reef Restoration Program. Available at: <http://www.crfbonaire.org/#phase3>
- Duyf FC van (1985) Atlas of three living reefs of Curacao and Bonaire (Netherlands Antilles). Foundation for Scientific Research in Surinam and the Netherlands Antilles
- Enochs IC, Manzello DP, Carlton R, Schopmeyer S, van Hooidek R, Lirman D (2014) Effects of light and elevated pCO<sub>2</sub> on the growth and photochemical efficiency of *Acropora cervicornis*. *Coral Reefs* 33:477-485
- Epstein N, Rinkevich B (2001) From isolated ramets to coral colonies: the significance of colony pattern formation in reef restoration practices. *Basic Appl Ecol* 2:219-222
- Fautin DG (2002) Beyond Darwin: Coral Reef Research in the Twentieth Century. In: *Oceanographic History: the Pacific and beyond*. University of Washington Press, pp. 446-449.
- Fox J, Weisberg S (2011). *An {R} Companion to Applied Regression*, second edition. Thousand Oaks, California: Sage.
- Gilmore MD, Hall BR (1976) Life-history, growth habits, and constructional Roles of *Acropora cervicornis* in patch reef environment. *J Sediment Petrol* 46:519-522
- Google Maps, 2014. [Online] Available at: <https://www.google.nl/maps/@12.133219,-68.2823656,11z> [Accessed 19 08 2014].
- Herlan J, Lirman D (2008) Development of a coral nursery program for the threatened coral *Acropora cervicornis* in Florida *Proc 11th Int Coral Reef Symp, Florida* 2:1249-1252
- Herler J, Dirnwober M (2011) A simple technique for measuring buoyant weight increment of entire, transplanted coral colonies in the field. *J Exp Mar Biol Ecol* 407:250-255
- Highsmith RC (1982) Reproduction by Fragmentation in Corals. *Mar Ecol Prog Ser* 7:207-226
- Hollarsmith JA, Griffin SP, Moore TD (2012) Success of outplanted *Acropora cervicornis* colonies in reef restoration. *Proc 12th Int Coral Reef Symp, Cairns, Australia* 20A Restoration of coral reefs
- Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265:1547-1551
- Lasker HR, Coffroth MA (1999) Responses of clonal reef taxa to environmental change. *Am Zool* 39:92-103
- Lirman D (2000) Lesion regeneration in the branching coral *Acropora palmata*: Effects of colonization, colony size, lesion size, and lesion shape. *Mar Ecol Prog Ser* 197:209-215
- Lirman D, Thyberg T, Herlan J, Hill C, Young-Lahiff C, Schopmeyer S, Huntington B, Santos R, Drury C (2010) Propagation of the threatened Staghorn coral *Acropora cervicornis*: methods to minimize the impacts of fragment collection and maximize production. *Coral Reefs* 29:729-735
- Meesters EH, Bak RPM (1995) Age-related deterioration of a physiological-function in the branching coral *Acropora palmata*. *Mar Ecol Prog Ser* 121:203-209
- Meesters EH, Bos A, Gast GJ (1992) Effects of sedimentation and lesion position on coral tissue regeneration. *Proc 7th Int Coral Reef Symp* 2:681-688
- Meesters EH, Noordeloos M, Bak RPM (1994) Damage and Regeneration - Links to Growth in the Reef-Building Coral *Montastrea-Annularis*. *Mar Ecol Prog Ser* 112:119-128
- Meesters EH, Pauchli W, Bak RPM (1997) Predicting regeneration of physical damage on a reef-building coral by regeneration capacity and lesion shape. *Marine Ecology-Progress Series* 146:91-99
- Meesters EHWG, Smith SR, Becking LE (2014). A review of coral reef restoration techniques. IMARES report C028/14. Pp. 37.
- Mendiburu, F de (2014). *Agricolae: Statistical Procedures for Agricultural Research*. R package version 1.2-0. <http://CRAN.R-project.org/package=agricolae>
- Miller MW, Marmet C, Cameron CM, Williams DE (2014) Prevalence, consequences, and mitigation of fireworm predation on endangered Staghorn coral. *Mar Ecol Prog Ser* 516:187-194
- Pinheiro JC, Bates DM (2000) *Mixed-Effects Models in S and S-PLUS*. Springer
- Putcham L, Thongtham N, Hewett A, Chansang H (2008) Survival and growth of *Acropora* spp. in mid-water nursery and after transplantation at Phi Phi Islands, Andaman Sea, Thailand *Proc 11th Int Coral Reef Symp, Florida* 2:1263-1266
- R Core Team (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Rinkevich B, Loya Y (1983) Short-Term Fate of Photosynthetic Products in a Hermatypic Coral. *J Exp Mar Biol Ecol* 73:175-184
- RStudio Team (2015). *RStudio: Integrated Development for R*. RStudio, Inc., Boston, MA URL <http://www.rstudio.com/>.



- Sarkar D, Andrews F (2013). latticeExtra: Extra Graphical Utilities Based on Lattice. R package version 0.6-26. <http://CRAN.R-project.org/package=latticeExtra>
- Slijkerman D, de León R, de Vries P, Koelemij E (2013) Waterquality of the coastal zone of Bonaire. IMARES report C158/13.
- Sale PF, Szmant AM (2012) Reef Reminiscences: Ratcheting back the shifted baselines concerning what reefs used to be. United Nations University Institute for Water, Environment and Health, Hamilton, ON, Canada, 35 pp.
- Soong K, Chen T-a (2003) Coral Transplantation: Regeneration and Growth of *Acropora* Fragments in a Nursery. *Restor Ecol* 11:62-71
- Tunncliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. *Proc Natl Acad Sci U S A* 78:2427-2431
- Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth Edition ed. New York: Springer.
- Xin LH, Hyde J, Cob ZC, Adzis KAA (2013) Growth study of branching coral *Acropora formosa* between natural reef habitats and in situ coral nurseries. *AIP Conference Proceedings* 1571:505-511

## Justification

Rapport C152/15

Project Number:

4312810007

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved:

Dr. L. Becking  
Researcher



Signature:

Date:

1-12-2015

Approved:

Drs. J. Asjes  
Head of department Ecosystems



Signature:

Date:

1-12-2015