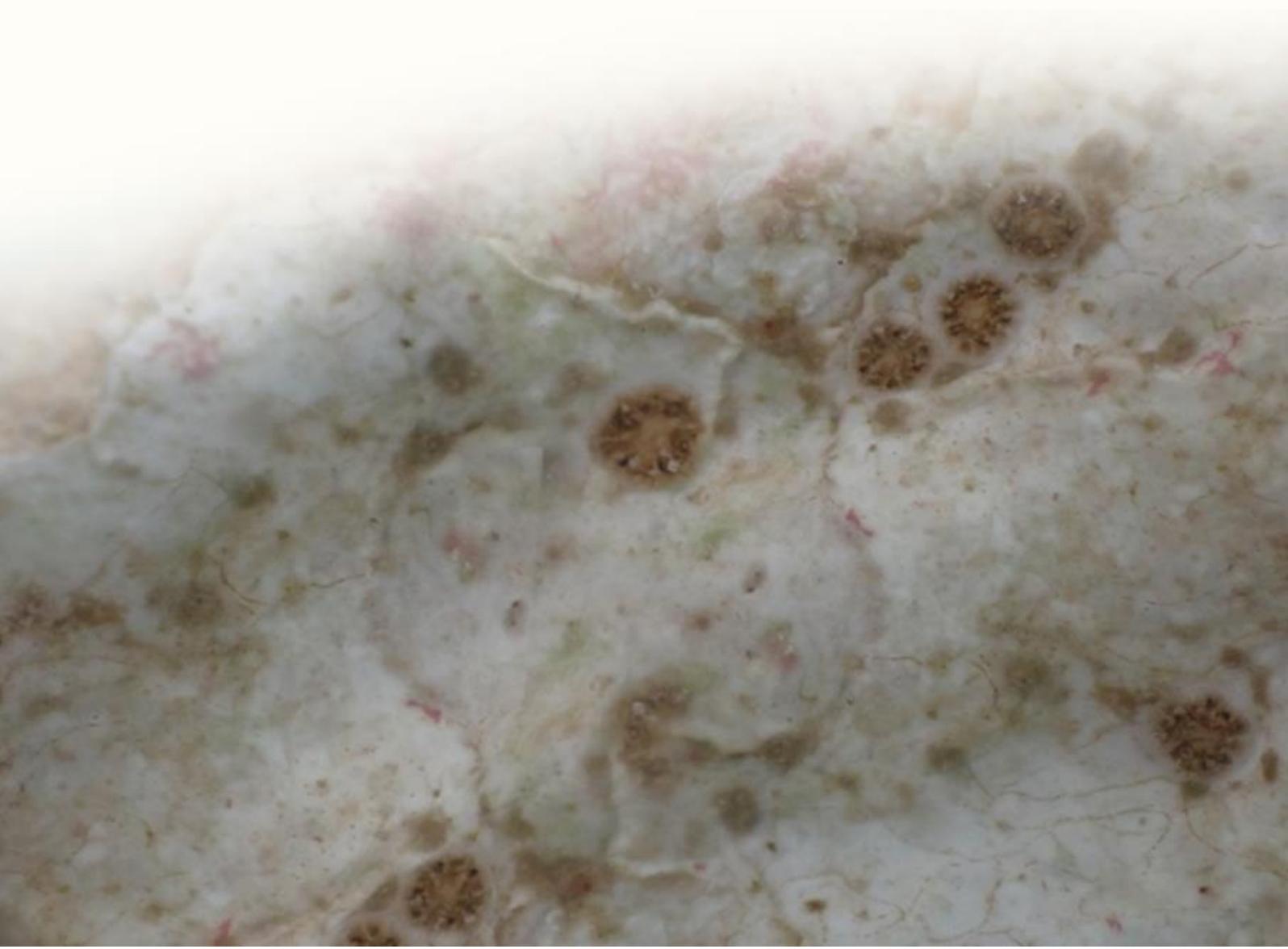


Upscaling sexual coral restoration

*Optimizing survival and growth of *Favia fragum* settlers mass-reared in situ*

Jeroen Schneider



Upscaling sexual coral restoration: Optimizing survival and growth of *Favia fragum* settlers mass-reared *in situ*



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Photo: three-week-old Favia fragum settlers, by Val rie Chamberland

Foreword

This thesis was written as bachelor project for my study; Applied Biology, at the Aeres Hogeschool Almere. The project was done in collaboration with SECORE International on Curacao, a research institute that focuses on restoring coral reefs.

I would not have been able to do this amazing experience without my internship supervisors Valérie Chamberland and Kelly Latijnhouwers. I want to thank them very much for this, not only for being my supervisors but also helping and teaching me with every step I took. I also want to thank Julia van Duijnhoven and Lars ter Horst for collaborating with me on this project and help me learn new things. Working on this project with this team has been an absolute pleasure, providing me with joy, laughter and new friends.

I also would like to thank Kars Klein Wolterink for helping me design this thesis. Finally I would like to thank my internship coach, Danny Meri n, for the guidance during this time.

All pictures in this report are our own pictures , unless suggested otherwise.

Jeroen Schneider
Carmabi, Curacao
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Abstract

Coral reefs are of great importance for social, biological and economical purposes. However, due to global and local threats they are declining rapidly. One of the ways to sustain coral reefs is sexual coral propagation. While the sexual propagation of corals for reef rehabilitation has been successful in a few occasions, the survival of coral settlers after they are outplanted to the reef remains low. New methodologies need to be developed to enhance the survival of settlers. This could for example be achieved by increasing available nutrition sources for young settlers and reducing their competition with detrimental algae. To address this problem, survivorship of settlers of the brooding coral, *Favia Fragum*, was studied in an ocean-based larval rearing pool, in which feeding of *Artemia salina* nauplii (~ 300 nauplii L^{-1}) and grazing by small-sized *Diadema antillarum* (test size: ~ 1.5 to 3.0 cm) was tested. Results showed a six-fold decrease of survival between the grazing treatments (15 - 16%) and the non-grazing treatments (90 - 95%). Feeding did not affect survival, but resulted in an increase in settler size between the feeding treatments (2.1 mm^2) and the non-feeding treatments (1.7 mm^2). These results indicate that grazing activity of *Diadema antillarum* resulted in a high mortality, leading this to be a non-effective method. However, feeding did increase settler size. Settler size is of importance for post-settlement survivorship as bigger settlers have higher chances of survival, thus possibly increasing post-settlement survivorship after outplanting. An alternative method needs to be established in order to reduce competition with detrimental algae. However, the feeding of *Artemia salina* nauplii is an effective method to increase settler size.

Samenvatting

Koraalriffen zijn van groot belang voor sociale, biologische en economische doeleinden, maar door wereldwijde en lokale invloeden worden ze ernstig bedreigd. Eén van de manieren om koraalriffen te herstellen is door middel van seksuele koraalvoortplanting. Terwijl het gebruik van seksuele voortplanting van koralen voor rif-herstel in een paar gevallen succesvol is geweest, blijft de overlevingskans van jonge koraalpoliepen die zijn uitgeplant op het rif, laag. Nieuwe methoden moeten worden ontwikkeld om de overleving van de koraalpoliepen te verbeteren. Dit kan bijvoorbeeld worden bereikt door de toevoeging van extra voedingsbronnen en het minderen van concurrentie met schadelijke algen. Om dit probleem te benaderen, werd het overlevingsvermogen van koraalpoliepen van het broedkoraal, *Favia Fragum*, bestudeerd in een oceaan gelokaliseerde kweekstelsel. Hierin werd het voeden van *Artemia salina* naupliën (~ 300 nauplii L^{-1}) en de begrazing activiteit door kleinschalige *Diadema antillarum* (testgrootte: ~ 1.5 tot 3.0 cm) getest. De resultaten liet een zesvoudige daling zien, tussen de begraasde behandelingen (15 - 16%) en de niet-begraasde behandelingen (90 - 95%). Het bijvoeren had geen invloed op de overleving maar resulteerde in een toename van poliep grootte tussen de bijvoer behandelingen (2.1 mm^2) en de niet-bijvoerbehandelingen (1.7 mm^2). Deze resultaten geven aan dat de begrazing van *Diadema antillarum* een hoge mortaliteit tot gevolg heeft. Echter, door het bijvoeren nam de omvang van de poliep toe, de omvang van koraalpoliepen is van belang voor de overleving na de uitplanting, grotere koraalpoliepen hebben een hogere overlevingskans. Er moet een alternatieve methode worden vastgesteld om de concurrentie met schadelijke algen te verminderen, om deze methode te verbeteren. Echter, het voeden van *Artemia salina* nauplii is een effectieve methode om koraalpoliepen te vergroten.

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1. Introduction

Coral reefs are indispensable for marine and terrestrial life. They participate in the global carbonate cycle (Ridgwell & Zeebe, 2005) and provide habitat and shelter for a wide range of marine species (Graham & Nash, 2013). It is further estimated that coral reefs provide livelihood, food and coastal protection for at least 500 million people (Wilkinson, 2008). Coral reefs are not only of importance biologically and socially-wise, but also economically. It is suggested that on average coral reefs provide \$352,250 USD per hectare annually through tourism, fisheries and as a source of new medicines (de Groot, et al., 2012). However, coral reefs have been degrading in recent times due to global and local threats (Hoegh-Guldberg, et al., 2007; Gordeau, 1992). For instance, climate change causes accelerated changes in the oceans' abiotic and biotic environment such as rising temperatures and acidification which in return has drastic effects on marine species. Moreover, human activity such as pollution and coastal development cause local water eutrophication, allowing harmful algae that thrive on nutrients to dominate coral reefs. This can further be aggravated through overfishing of key herbivorous fish species. (Burkepile & Hay, 2006; Belle, 1992)

Coral reefs cannot adapt fast enough to overcome this rapid change (Pandolfi, et al., 2011), and it is therefore imperative to implement measures to reverse, or to the very least slow the decline of coral reefs. Coral reefs can be protected through indirect interventions in the form of policy and management actions, such as the implementation of marine parks, no fishing zones and regulations of coastal development. If these policies are properly enforced, then direct interventions in the ecosystem may accelerate the process of population recovery. Examples of direct interventions may include the removal of harmful algae, or outplanting coral species through coral propagation. In recent years, coral propagation has gained in popularity as a measure to help the recovery of coral population. (Randall, et al., 2020)

Coral propagation can either be performed via fragmentation or via coral larval propagation. Fragmentation techniques make use of the asexual reproductive system of corals, whereby parts of a colony are fragmented into multiple genetic clones that are replanted to the reef (Highsmith, 1982). While this method is effective for increasing coral cover relatively rapidly, it does not increase genetic diversity of restored coral populations. In contrast, coral larval propagation approaches make use of the sexual reproductive system of corals, thus allowing to increase the genetic diversity of restored populations. A higher genetic diversity makes populations more likely to adapt to global and local threats (Baums, et al., 2019; Baums, 2008). The different steps of coral larval propagation techniques are directly linked to the life cycle of corals. Corals can reproduce sexually either via a brooding or a broadcast spawning strategy (SECORE, 2020). Broadcast spawning species release their eggs and sperm in the water column where fertilization occurs during mass spawning events, once or twice a year. In brooding coral species, fertilization in contrast occurs internally before the release of larvae that are fully developed and competent to settle. Brooders typically have an extended reproductive season throughout the year (Ritson-Williams, et al., 2009). Coral larval propagation is thus achieved by collecting either brooded larvae or broadcasted gametes. The

latter are then reared until they reach competency and are then allowed to settle (i.e., attach) on artificial settlement substrates. During settlement, coral larvae undergo metamorphosis, whereby they transition from motile larvae to sessile polyp (henceforth referred to as settlers). Once settled, the settler forms a mouth and tentacles and is therefore capable of heterotrophic feeding. After a few days of settlement, the concerned substrates can either be kept in a land-based or ocean-based nursery until coral settlers reach larger sizes, or can be directly outplanted to the reef. (Chamberland, et al., 2015; Chamberland, et al., 2017)

While larval propagation techniques have proven to be successful in multiple occasions (Chamberland, et al., 2017; Chamberland, et al., 2015; Guest, et al., 2013; Nakamura, et al., 2011; Villanueva, et al., 2012; Omori, et al., 2008), they are costly and labour intensive to implement, preventing their application on large scales. In addition, current methods yield very low survival rates of settlers once they are outplanted to the reef (Chamberland, et al., 2017; Guest, et al., 2013). Thus, increasing settler survivorship as well as developing tools to upscale operations would make larval propagation efforts more effective.

Recently, ocean-based larval rearing pools (LRPs) were developed by SCORE International to achieve mass rearing of coral embryos and larvae (Chamberland, 2018). An ocean-based LRP is a floating nursery allowing hundreds of thousands of gametes and larvae to be reared and settled at once. Because the LRPs are ocean-based, constant flow of large water volumes allows successful rearing to occur with relatively little maintenance. To date, this method showed to be successful with multiple Caribbean coral species (e.g., *Acropora palmata*, *Colpophyllia natans*, *Pseudodiploria strigosa*, *Orbicella faveolata*, *Diploria labyrinthiformis*), and at multiple locations (e.g., Curaçao, Mexico, Dominican Republic, The Bahamas) (personal communication, Kelly Latijnhouwers), whereby hundreds of thousands of coral larvae could be reared and settled onto up to two thousand substrates at a time. However, the survival of coral recruits after outplanting remains low (<5% after one year; personal communication, Kelly Latijnhouwers). One approach to enhance settler survivorship after the outplanting phase could be to optimize nursery conditions prior to outplanting. For example, settlers can be co-cultured with grazers to suppress the growth of detrimental algae, such as turf and macroalgae (Craggs, et al., 2019). These algae are known to overgrow coral settlers limiting their available space. In Singapore, *Pocillopora damicornis* settlers co-cultured with the sea urchin *Salmacis sphaeroides* (73 ± 2 mm test diameter) in a land-based nursery, had a 13-fold increase in survival compared to settlers without any grazing activity (Toh, et al., 2013). In some cases, however, grazing can negatively affect coral settlers. For instance, *Favia fragum* are highly sensitive to grazing activity, with settlers densities declining to negligible numbers in the presence of full grown *Diadema antillarum* (four urchins m⁻²) (Sammarco, 1980). In contrast, smaller-sized grazers, such as juvenile sea urchins or snails, have shown to be beneficial to coral settlers (Craggs, et al., 2019). Smaller-sized grazers are able to graze more precisely without incidentally grazing or damaging coral settlers (Craggs, et al., 2019). Another approach to improve settler survival may include providing them with extra sources of nutrition in order to enhance their health and growth. Size for early-phased settlers is of great

importance as their chance of survival significantly increases as they grow, i.e. corals follow a type III survival curve (Vermeij & Sandin, 2008). Moreover growth is an indication of health: unhealthy corals often survive, but remain small-sized and with fewer polyps (Vermeij & Sandin, 2008). It was shown that feeding early-staged *F. fragum* settlers with lipid-rich *Artemia* spp. nauplii enhances their growth by tenfold over a time period of five months in a controlled land-based nursery (Petersen, et al., 2008). Outplanting settlers that have been fed during their nursery phase may therefore help to overcome the early post-settlement stage. Yet, to date, both co-culturing of grazers and feeding of settlers with *Artemia* spp. were never tested within ocean-based *in situ* nurseries for coral settlers, with large scale restoration as the main objective.

In this study, we attempted to increase the success of *in situ* nursery phases of coral settlers by applying grazing and feeding treatments within the above mentioned SECORE ocean-based LRP. This was achieved using larvae of the Caribbean coral *Favia fragum* (Esper 1797), a brooding coral species that releases larvae throughout the year, thereby increasing opportunities for research within these ocean-based LRP's. We tested if co-culturing coral settlers with small-sized herbivorous urchins, as well as providing them with a protein and lipid rich diet, within an ocean-based nursery setting, optimize the efficiency of larval propagation techniques. We more specifically aimed at answering the following questions: 'What is the effect of the addition of lab-cultured *Artemia* spp. nauplii on the survivorship and growth of *F. fragum* settlers?', and 'What is the effect of grazing by small-sized, juvenile *Diadema antillarum* urchins on the survivorship and growth of *F. fragum* settlers?'. To answer these research questions, *F. fragum* larvae were settled on artificial settlement substrates and distributed over different feeding and grazing treatments within an ocean-based LRP, after which survival and growth were monitored just before the outplanting phase.

We expected that, by increasing available nutrition sources and by reducing competition with detrimental algae, *Artemia* spp. nauplii feeding and grazing by small-sized *D. antillarum*, respectively, would both enhance the survival and growth of settlers. If these expectations are validated, then these protocols could be adapted and applied to other coral species, possibly increasing the effectiveness of sexual coral restoration techniques for a range of coral species

2. Materials & Methods

2.1 Study species and Larval collection

Experiments were conducted between January and April 2020 on Curaçao (12°N, 69°W), a Southern Caribbean island located 60 km north of Venezuela. *Favia fragum* is a relatively small coral with mature colonies typically less than 100 cm² surface area, and is usually hemispherical in shape with large corallites densely packed together (Figure 1). *F. fragum* occurs in the Caribbean, Florida, Bahamas, southern Gulf of Mexico and Bermuda to the south of Brazil, and on the tropical west coast of Africa (IUCN, 2020). *F. fragum* is commonly found in shallow reefs (0.5-5 m) and has a monthly larval release that follows 6 to 16 days after new moon (Szmant-Froelich, et al., 1985). Unlike other coral species, *F. Fragum* larvae bear zooxanthellae derived from the parent colony (Nunes, et al., 2011). Zooxanthella are unicellular marine algae endosymbiotic with coral species, providing nutrition through photosynthesis (Muscatine, 1980).



Figure 1. *Favia Fragum* colony, approximately >1 years old, used to collect larvae inside the larval collection system.

A larval collection system (Chamberland, et al., 2017) (figure 2A) was used to collect *F. fragum* larvae. This is an enclosed system in which 16 adult colonies were kept overnight during the monthly peaks of larval release. One hour before sunset, daily, colonies were placed in 16 individual beakers supplied with a constant inflow of 100 μ m filtered seawater (FSW), causing the beakers to overflow into a larval collection cup (150 μ m mesh). *F. Fragum* larvae are positively buoyant and therefore forced to overflow into the collection cups upon release. Both the beakers and collection cups were placed in a flow-through aquarium system at a constant temperature of 28°C. Each morning, the larvae were collected between 8:00-9:00, by gently pipetting the larvae out of the collection cups into a 500 mL plastic container filled with FSW.

For this study specifically, larvae were collected during seven consecutive days between 9 and 16 days after the March new moon.

2.2 Larval settlement

In order to allow settlement, larvae that were collected each day were equally divided between two plastic settlement containers (50 × 25 × 15 cm) (figure 2B) filled with 0.5 µm FSW. The settlement containers contained 15 tetrapod-shaped artificial settlement substrates made of cement. These tetrapods (figure 2C) were specially designed for coral settlement and allow for large scale and cost-effective outplanting, because they can be outplanted by simply wedging them in reef crevices. Using tetrapod tiles (henceforth referred to as tile) takes ≤ 7% of the time used with the traditional labour-intensive and costly outplanting techniques using binding materials to secure them to the substrate to the reef (Chamberland et al. 2017). To ensure similar settler densities on each tile, the substrates were inspected daily. *F. fragum* larvae fluoresce bright green or red, which can be seen using a blue light and yellow filter, therefore making the settlers easily detectable. Each tile with ≥ 10 settlers was placed in a container with no larvae and replaced with a new tile in the settlement container. This process was repeated until a total of 96 tetrapods each containing at least 10 settlers was reached. Until the settlement phase was completed, water changes in the containers were done daily to preserve water quality. In order to track survival through time, an initial settler count was performed before the tiles were transferred to the ocean-based LRP (figure 2D). To do so, all settlers on each tetrapod were thoroughly counted using a blue light and a yellow filter.

2.3 *In situ* experiments

The tetrapods harbouring settlers were placed in the ocean-based LRP and divided among four treatments; Control, Feeding, Grazing, and Feeding + Grazing (n = 4 replicate containers per treatment). Each replicate was established in a ~60 L cylinder-shaped replicate container (figure 2E). In order to prevent nutrition exchange and grazer movement between treatments while maintaining water flow within the replicate containers, three removable windows covered with 100 µm mesh were added on each replicate containers. To include approximately the same number of settlers in each replicate container, the tiles were assigned a replicate container based on the amount of settlers they harboured.

For the Grazing treatments, a total of 24 small-sized *Diadema antillarum* (test size: ~1.5 to 3.0 cm) were collected at three reef sites between 0.5 - 6.0 m depth (Piscaderabaai 12.109°N, -68.953°W; Water Factory 12.090°N, -68.904511°W; Marie Pampoien 12.116, °N - 68.964°W) and divided over the eight appropriate replicate containers, so that each replicate container received three urchins of similar size ranges (Figure 2F).

For the Feeding treatments, settlers were fed *Artemia salina* nauplii every other day. To do so, 0.5 g of *Artemia* cysts (*A. salina*; size: 600 µm; Select Brine Shrimp Eggs; Salt Creek, Inc., USA) were hatched in 500 mL of 0.5 µm FSW. A 0.5 L hatchery set-up was used (ArtemioSet, JBL GmbH & Co. KG, Neuhofen, Germany) (figure 2H). The first cysts hatched after 16 hours, and a 90% hatch rate was reached at 26 hours, resulting in ~150,000 *A. salina* nauplii (henceforth referred to as nauplii) in 500 mL. To ensure that each replicate container received equal amounts of nauplii, the water volume was reduced to 400 mL and homogenized, the

culture was then divided over eight 50 mL falcon tubes (Becton, Dickinson and Company, NJ, USA), resulting in ~18,750 nauplii per tube. The nauplii were poured into the 8 replicate containers, resulting in ~300 nauplii L⁻¹ (Petersen, et al., 2008). Settlers were fed for a duration of 1.5 hours every other day, leading to seven feeding time points over the two-week nursery period. 1.5 hours is an estimated optimal feeding period based on studies where nauplii were fed (0.3 to 3.0 hr), in which the nutrition value of nauplii decreased overtime (Léger, et al., 1986).

To prevent uncaptured nauplii from decaying within the replicate containers, the windows of the replicate containers were opened to discard the remaining nauplii after the feeding period of 1.5 hours. During the feeding period, maintenance of the ocean-based LRP was performed, whereby algae were removed from the inside and outside walls using a scrub brush and a sponge.

2.4 Settler survival and size

After two weeks, all settlers were re-scored using a blue light and yellow filter in order to determine their survival. In addition, photographs of settlers next to a 0.1 mm graduated ruler (Figure 2G) were taken using a camera set on microscope mode (TG-6, Olympus) to allow for subsequent size measurements (area, mm²) using Image J (Schneider, 2012).

2.7 Data analysis

Because all data was non-normal, non-parametric Welch's t-test followed by Games-Howell's *post-hoc* test were applied in order to test the individual and combined effects of grazing and feeding on settler survival and growth (Tomarken & Serlin, 1986). A p-value of 0.05 was used as significance threshold for all tests.

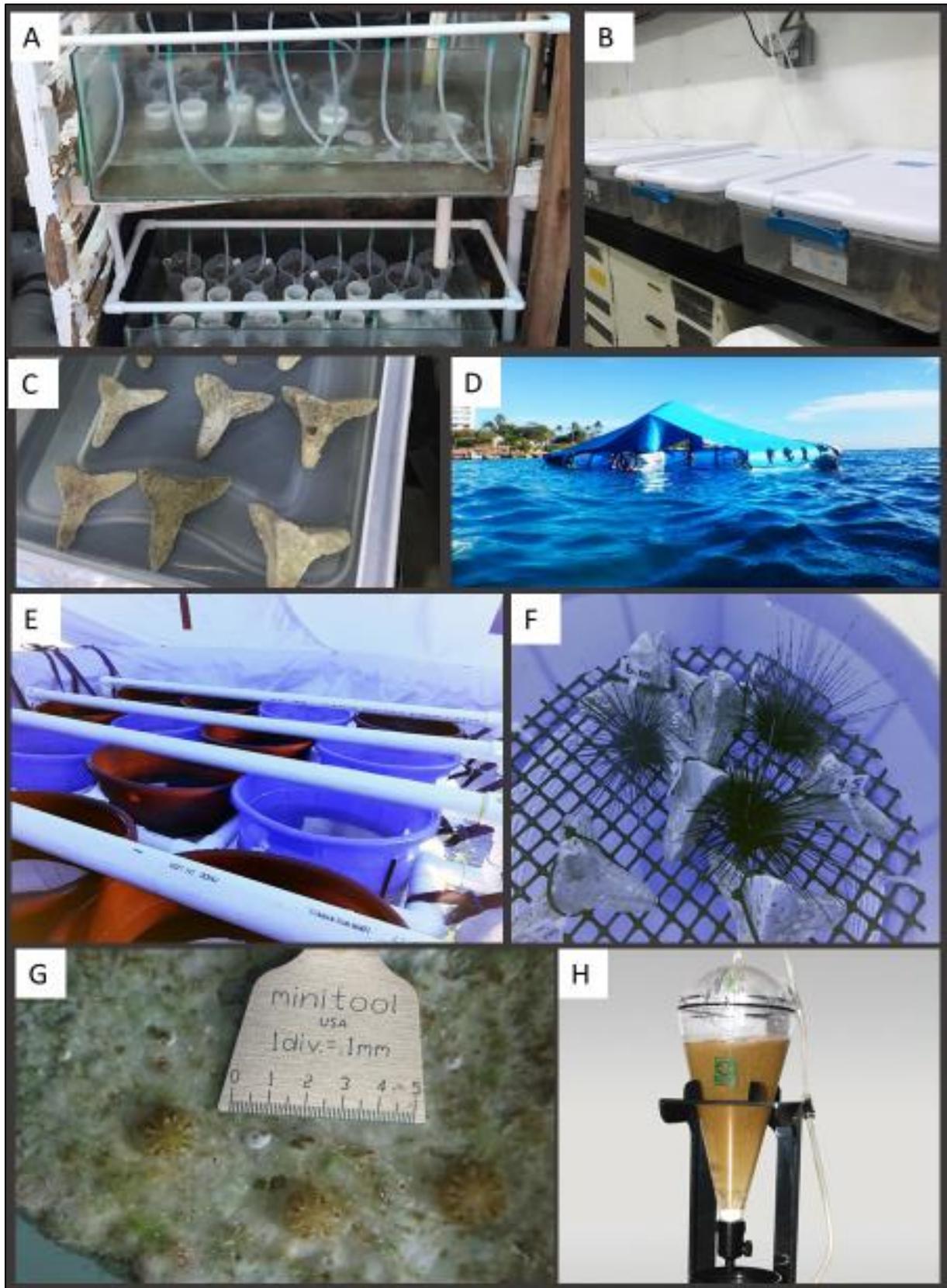


Figure 2. Pictures of methodology. **(A)** Larval collection system. **(B)** Larval settlement container. **(C)** Tetrapod tile, used for larval settlement and outplanting. **(D)** Ocean-based larval rearing pool (LRP). **(E)** Replicate containers placed within the LRP. **(F)** *Diadema antillarum* placed inside a replicate container to obtain grazing activity. **(G)** Photographs of settlers next to a 0.1 mm graduated ruler **(H)** Artemia hatchery set-up.

3. Results

A total of ~6,600 larvae were released by the 16 *Favia Fragum* colonies kept in the larval collection system. Approximately 60% (~3,800) of these larvae settled on 96 tiles, resulting in ~1,000 settlers for each of the four treatments. The amount of settlers per tile averaged 40 ± 32 SD ($n = 96$ tiles).

Settlers grown in the presence of the herbivorous grazers *Diadema antillarum* were six times less likely to survive compared to those reared without urchins (Welch's t-test: $t = 279.6$, $p < 0.05$) (Appendix I-a) (figure 3A). When settlers were reared in the absence of *D. antillarum*, survival was high (90 to 97%) regardless if they were fed or not. While feeding *F. fragum* settlers with nauplii did not influence their survival during the 20-day duration of the nursery phase, feeding did result in a 15% increase in settler size (Welch's t-test: $t = 6.028$, $p < 0.05$) Appendix I-b) (Figure 3B). Moreover the presence of *D. antillarum* did not affect settler size. At the end of the 20-day study period, all *F. fragum* settlers in all treatments still consisted of one polyp, and had therefore not initiated polyp divisions.

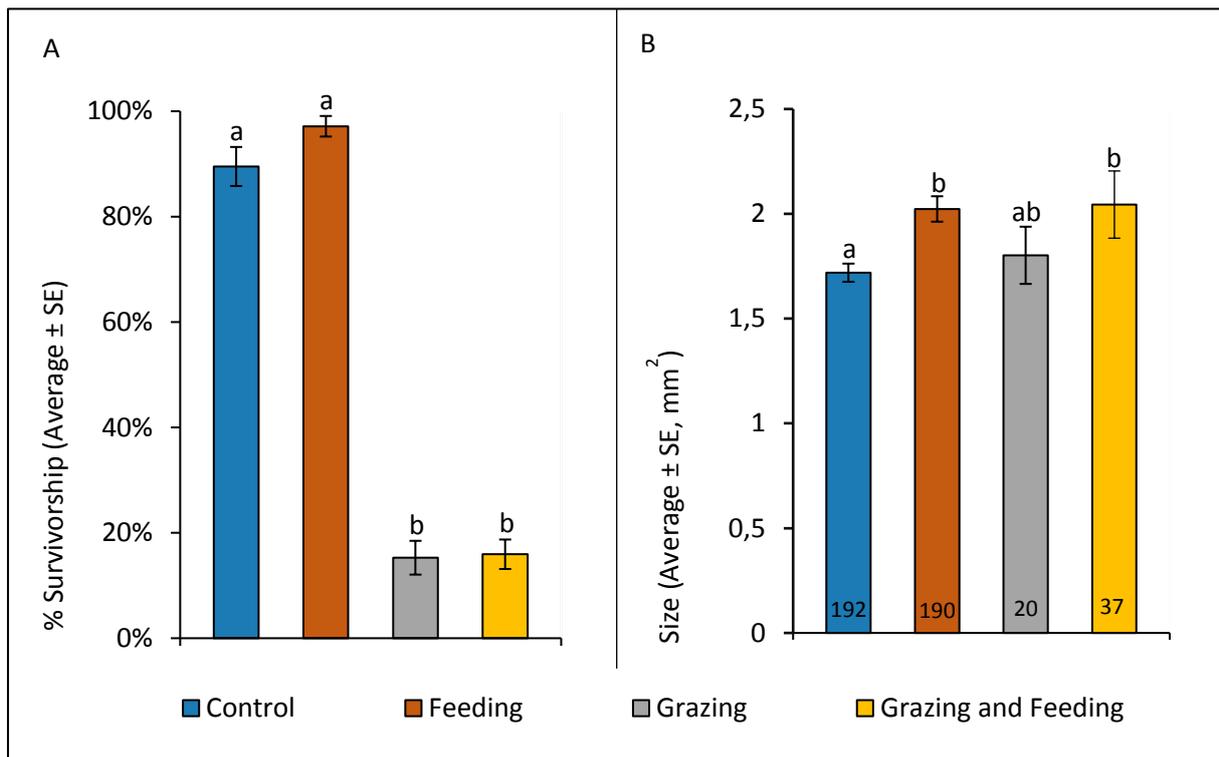


Figure 3. Individual and combined effects of Feeding and Grazing on 3 to 10-day-old *F. Fragum* settlers after a grow-out period of 20 days in an ocean-based larval rearing pool (LRP). **(A)** Survivorship percentage showing average survival of the 4 treatments ($n=24$ tiles per treatment). **(B)** Average settler size (mm²) in each of the 4 treatments. Numbers inside bars are sample sizes.

4. Discussion

Restoring imperilled populations of corals to help preserved coral reef ecosystems is required for social, biological and economical purposes, and this can be achieved via coral propagation. Large scale sexual propagation of corals for reef rehabilitation has been successful in a few occasions (Chamberland, et al., 2017; Nakamura, et al., 2011; Omori, et al., 2008), however post-settlement survivorship remained low. To increase the survivorship of sexually propagated settlers after outplanting, this study investigated different methodologies aimed at increasing available nutrition sources available to young coral settlers and reducing their competitive interactions with detrimental algae.

It was expected that these two approaches would enhance the survival and growth of settlers during their nursery phase. This was tested with three to ten-day-old *Fava fagum* settlers, using an ocean-based larval rearing pool (LRP), a SCORE International developed nursery allowing mass-rearing and settlement of gametes and larvae. In this set up part of Craggs and Petersen's protocols were tested (Craggs, et al., 2019; Petersen, et al., 2008). In contrast to those protocols, this study makes use of Caribbean urchins and are tested in large scale ex situ enclosures (LRP).

4.1 Optimization of post-settlement survivorship

Tiles kept in the presence of *Diadema antillarum* appeared, as expected, to have less harmful algae growing on them then the tiles kept without urchins (Figure 4). This indicates that the grazers functioned as expected. This reduction in algal cover however surprisingly did not result in higher survival of *F. Fragum* settlers, as it appeared that the latter were also grazed by *D. antillarum*. This led to a 6-fold decrease in settler survival in the grazing treatments, indicating that co-culturing *F. fragum* settlers with herbivorous *D. antillarum* resulted in an increased mortality of young settlers. These findings are contradictory to the expectations, despite the fact that small sized urchins were collected for this experiment.



Figure 4. Algae growth on tetrapod tiles after outplanting **(A)** Tetrapod tile derived from grazing treatments **(B)** Tetrapod tile derived from non-grazing treatments.

The results correspond with the fact that *F. Fragum* settlers are sensitive to incidental grazing activity of mature *D. antillarum* (Sammarco, 1980). However, to avoid incidental grazing activity in the present study, only small-sized *D. antillarum* were selected (test size: ~1.5 to 3.0 cm). Nevertheless, it may be possible that even smaller-sized *D. antillarum* should have been used for co-culturing, possibly by using urchins right after they metamorphose. Craggs, et al., (2019) for example used urchins (*Mespilia globulus*) that were reared from spawned gametes, through their planktonic phase. This way the urchins could be used right after metamorphosis. Making this method labour intensive but effective. However, because it was too labour intensive to apply in this study, we instead collected wild *D. antillarum*, possibly still leading to oversized and therefore more detrimental *D. antillarum*. It is further possible that, because the tiles with settlers initially did not contain fleshy algae that normally compose the diet of sea urchins until a certain time after the start of the experiment, urchins in the nursery might have been starving, leading them to predate on *F fragum* settlers. *D. antillarum* is indeed known to predate on corals when experiencing a food shortage (Bak & van Eys, 1975).

When cultured without urchins, *F. fragum* settlers had a very high survival (90-97%), which is comparable to a survivorship of 90% - 95% of *F. Fragum* settlers after 20 days of treatment for *ex situ* small scale settings (Petersen, et al., 2008). This had however never been achieved in a large scale *in situ* setting where conditions are not as controlled as in *ex situ* aquarium facilities.

The settlers where kept ocean-based and contained zooxanthellae providing them with enough nutrients to survive, which means that providing them with *Artemia salina* nauplii is an addition to their diet. This could explain that this treatment did not show an effect in settler survivorship, but it did enhance their growth.

4.2. Optimization of settler size

The size of *F. fragum* settlers in all treatments varied between ~1.7 to 2.1 mm² after 20 days. Sizes ranging between ~0.8 to 1.3 mm² are common for this species when reared regarding the same treatments (feeding and non-feeding) and time period, in land-based small-scale settings (Petersen, et al., 2008). It is plausible that *in situ* rearing, in comparison to *ex situ* facilities, promotes the growth of coral settlers. Conditions in the LRP may be more similar to natural reef conditions, contain more natural conditions, such as additional nutrients (minerals) in the water column, water movement and light cycles, which could be more beneficial than a controlled setting. To confirm if coral settlers actually thrive in ocean-based conditions, a follow-up research could be executed regarding this. Overall, these finding shows that the LRP is not jeopardizing the health and growth for settlers.

F. Fragum bear zooxanthellae as larvae, providing them with photosynthetic products in early settlement phases. However, there are non-zooxanthellae-bearing corals that lack this advantage, and are obliged to provide nutrition themselves until they acquire their symbionts (Nunes, et al., 2011). Which means that feeding these corals in their early stages will have an even larger benefit than for e.g. *F. Fragum*, suggesting that this method may be even more effective on non-zooxanthellae-bearing corals.

Grazing activity of *D. antillarum* has no effect on settler size. A slightly higher average size was measured in settler size of the grazing treatments. However, no significant difference was shown regarding this, possibly because the sample size was not large enough due to high mortality among grazed settlers (n = 20). The decrease of detrimental algae would give the settlers spend less energy into competing and more energy into metabolic processes resulting in growth, supporting this assumption. Perhaps the algae did not grow large enough to compete with the settlers in the 20 days of treatment.

4.3. Post outplanting success

The *F. fragum* settlers were outplanted after the nursery phase. Their survival and growth is currently still being monitored for further examination. Effects of grazing and feeding on the post-outplanting survivorship, in which an in depth analysis of the benthic community on the tiles and the survivorship of the outplanted settlers are tracked.

It is expected that the tiles with settlers that were being fed show an increase in survival. Additionally, we expect that algal succession on tiles that were exposed to grazers will be slower, increasing the chances of survivorship of those settlers as well. These expectations are based on the results of this study and the fact that size and the reduction of algal succession increases survivorship (Vermeij & Sandin, 2008; Craggs, et al., 2019). If the results show to be successful, other species of corals need to be studied to make an increase in the amount of species with which we can do large scale restoration in an LRP.

5. Conclusion

In order to optimize settler survival and growth through grazing, a different approach of the grazers methodology is required. The reason for the use of *D. antillarum* in studies is because this species is a herbivorous urchin common in the Caribbean region which are relatively easy to maintain. However different species could serve for the same purpose, *Trochus maculatus*, an Indo-Pacific snail species, has shown a tenfold increase of the survivorship when co-cultured with the coral species *Pocillopora damicornis* (Toh, 2013). In addition, rearing *Acropora valida* settlers *in situ* in the presence of *Trochus niloticus* resulted in a 15% higher survival (Villanueva, et al., 2013). Furthermore, *Paguristes sp.*, a hermit crab species has been used in aquaculture to control the growth of algae, and has shown no primary or secondary damage to coral settlers (Petersen, et al., 2008). In contrast to *D. antillarum*, these species are less harmful to coral settlers and may provide grazing activity benefitting the settlers. Similar research could be conducted with Caribbean pervious snail species of the same genus *Trochus muricatus*.

Settler size and number of polyps is of great importance for early-stage settlers as corals follow a type III survival curve, which means bigger settlers have higher chances of survival (Vermeij & Sandin, 2008). Growth rates in relation with different types of diets is therefore relevant for post-settlement survivorship. For example, feeding nauplii enriched with omega-3 polyunsaturated fatty acids (PUFAs) increases the ability of corals coping with terminal stress (e.g. coral bleaching). It has further increased growth rates in the Pacific coral species *Duncanopsammia axifuga* and subsequently their survival. (Tagliafico, 2017). It is worth investigating the effect of different lipid-high diets on the rate of polyp divisions and growth. Since growth increases survival an optimum diet may help increase post-settlement survivorship and thus the effectiveness of sexual coral propagation.

This study focused on addition of nutrients and reducing algal growth to achieve a higher post-settlement survivorship, however other factors impacting the health and survival of coral settlers could further be tested. Biotic factors such as mutualistic relationships with bacteria or algae, or abiotic factors such as waterflow, temperature, light or even placement on the reef (vertical or horizontal). These are all factors that could be tested within LRP to further increase the success of *in situ* larval rearing. For example survivorship of *F. Fragum* settlers decreases at temperatures exceeding 28 degrees C° (Randall & Szmant, 2009), which may occur during the warmest periods of the year between June and November (Climate-Curacao, 2020). Thus, rearing *F. Fragum* in during cooler months could be of importance to support settlers trough their early live stages.

The addition of *Artemia salina* nauplii as a source of nutrients, pre-outplanting phase, had no effect on the survivorship of *F. Fragum* settlers. However, it increases settler size, possibly increasing post-outplanting survivorship. The co-culturing with small-sized *Diadema antillarum* had a decreasing effect on the survivorship of *Favia fragum* settlers, regardless of the fact that algal growth appeared to be significantly absent on the grazed tiles. Moreover, grazing did not affect settler size. We conclude that providing coral settlers with a protein and lipid rich diet within an ocean-based nursery setting, helps optimizing the efficiency of larval propagation techniques as it is beneficial for coral settler size. In contrast to co-culturing coral settlers with small-sized *D. antillarum*, which are harmful and therefore not an effective approach to optimize efficiency of larval propagation techniques in this specific setting.

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Appendix

Appendix I-a Post hoc survivorship

Multiple Comparisons

Dependent Variable: Survival

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Games-Howell	Control	Feeding	7,63610	4,18713	,280	-3,6582	18,9304
		Grazing	-74,23942 [*]	4,89879	,000	-87,3071	-61,1717
		Grazing and Feeding	-73,57971 [*]	4,64158	,000	-85,9863	-61,1731
	Feeding	Control	-7,63610	4,18713	,280	-18,9304	3,6582
		Grazing	-81,87552 [*]	3,75435	,000	-91,9614	-71,7896
		Grazing and Feeding	-81,21581 [*]	3,41193	,000	-90,3506	-72,0810
	Grazing	Control	74,23942 [*]	4,89879	,000	61,1717	87,3071
		Feeding	81,87552 [*]	3,75435	,000	71,7896	91,9614
		Grazing and Feeding	,65971	4,25528	,999	-10,6905	12,0099
	Grazing and Feeding	Control	73,57971 [*]	4,64158	,000	61,1731	85,9863
		Feeding	81,21581 [*]	3,41193	,000	72,0810	90,3506
		Grazing	-,65971	4,25528	,999	-12,0099	10,6905

Appendix I-b Post hoc settler size

Multiple Comparisons

Dependent Variable: Size Settler (Area mm2)

Games-Howell

(I) Treatment	(J) Treatment	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
		(I-J)			Lower Bound	Upper Bound
Control	Feeding	-,304258*	,074730	,000	-,49719	-,11133
	Grazing	-,082508	,143238	,938	-,47884	,31383
	Grazing and Feeding	-,324742	,166101	,222	-,76929	,11981
Feeding	Control	,304258*	,074730	,000	,11133	,49719
	Grazing	,221750	,149383	,460	-,18688	,63038
	Grazing and Feeding	-,020484	,171428	,999	-,47710	,43613
Grazing	Control	,082508	,143238	,938	-,31383	,47884
	Feeding	-,221750	,149383	,460	-,63038	,18688
	Grazing and Feeding	-,242234	,210523	,660	-,80041	,31594
Grazing and Feeding	Control	,324742	,166101	,222	-,11981	,76929
	Feeding	,020484	,171428	,999	-,43613	,47710
	Grazing	,242234	,210523	,660	-,31594	,80041

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