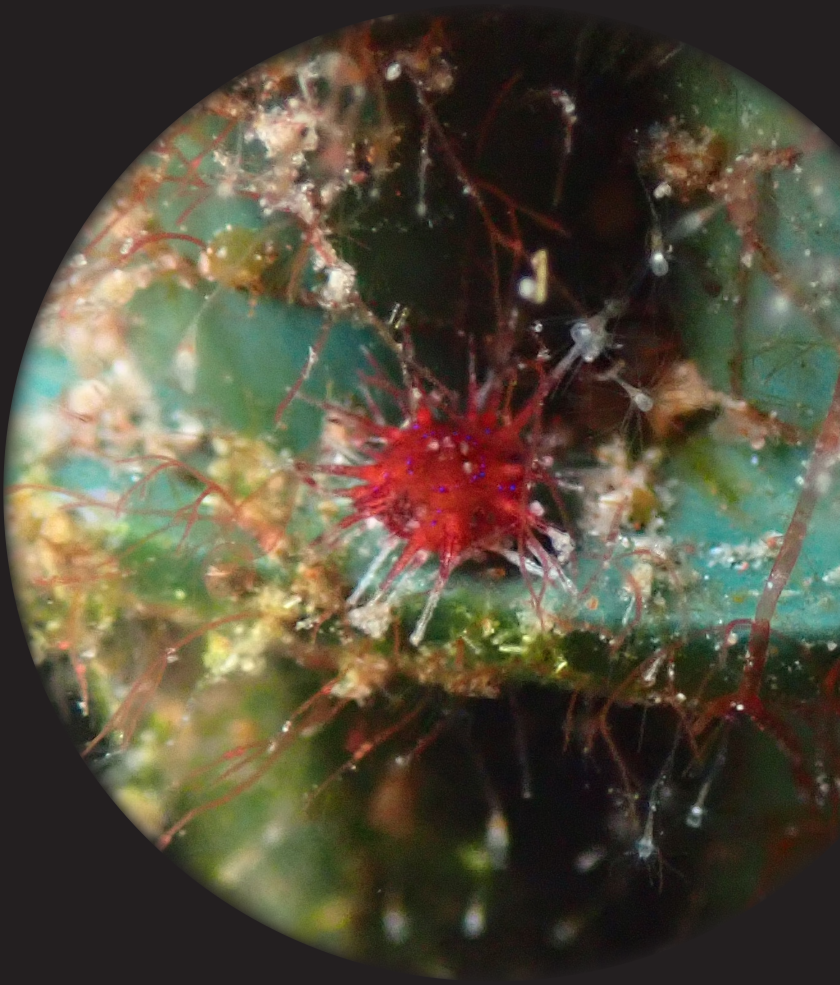
Active interventions to rehabilitate Caribbean coral reefs

The use of artificial substrates to increase the abundance of herbivorous fish and sea urchins



Alwin Hylkema

Propositions

1. The natural recovery of *Diadema antillarum* is mainly constrained by a lack of suitable settlement substrate (this thesis).
2. Artificial reefs that are not protected from fishing pose a risk to the surrounding natural ecosystem (this thesis).
3. Since invasive species are currently the biggest threat to the biodiversity of small islands, stricter regulations and enforcement are necessary to prevent new introductions.
4. Pulse fishing is ecologically less destructive than current bottom trawling methods.
5. Freedom of education based on religion, a right embedded in the Dutch constitution, results in segregation and polarization and should be abandoned.
6. The use of crypto currency based on “Proof of stake”, which is a much more energy efficient process than mining, should be encouraged.

Propositions belonging to the thesis, entitled

Active interventions to rehabilitate Caribbean coral reefs

Alwin Hylkema
Wageningen, 8 July 2022

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Active interventions to rehabilitate Caribbean coral reefs

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Alwin Hylkema

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Chapter 1

General introduction

Degradation of Caribbean coral reefs

Coral reefs have long been recognized as one of the most productive and biodiverse ecosystems on earth (e.g. Odum & Odum 1955). They provide us with ecosystem services such as fisheries, coastal protection, recreation and tourism and, through these ecosystem services, sustain millions of people in their livelihoods (Moberg & Folke 1999, Woodhead et al. 2019). Unfortunately, coral reefs are declining because of overfishing, destructive fishing methods, pollution, disease, coastal development and climate change (Burke et al. 2011, Hughes et al. 2017). Worldwide, most coral reefs are already severely degraded. It is estimated that, even without the effects of climate change, 75% of all reefs are at risk of severe ecosystem degradation in the near future (Burke et al. 2011).

The Caribbean region has been particularly severely affected and coral reef degradation started centuries ago by sequentially overfishing top predators, meso predators and finally herbivores (Jackson et al. 2001). Degradation started to accelerate in the 1970s when white band disease (WBD) decimated the most important reef building corals of the genus *Acropora* (Gladfelter 1982, Aronson & Precht 2001). In the decades that followed, other threats, such as hurricanes, pollution, more coral diseases and mass bleaching events associated with global warming further reduced coral cover (Gardner et al. 2003, Jackson et al. 2014). However, what really sets Caribbean reefs apart from those of the rest of the world, is their low resilience to disturbance (Hughes et al. 2010). In the Indo-Pacific coral recovery after disturbances is relatively common (*eg* Adjeroud et al. 2009, Gilmour et al. 2013). The most striking example of recovery was recently observed on the great barrier reef (GBR). After mass bleaching events in 2016, 2017 and 2020, average coral cover on the GBR was reduced with 50%. In 2021, coral cover remarkably recovered, although the species composition was different compared to 2016 (AIMS 2022). Contrary to other regions, reports of large scale recovery are rare in the Caribbean region (Steneck et al. 2019) and coral cover continues to decline (Jackson et al. 2014).

An important explanation for the severe degradation and low resilience of Caribbean coral reefs is their low species diversity compared to their Indo-Pacific counterparts (Bellwood et al. 2004). The low number of species present leaves few options for functional redundancy, meaning that a healthy ecosystem needs almost all the available species. Under such conditions, even the decline of a single species can have detrimental effects because there are often no other species to replace it (Bellwood et al. 2004). The effect of WBD is a good example; while Indo-Pacific reefs have over a hundred fast-growing branching coral species, Caribbean reefs are dependent on only two: *Acropora palmata* and *A. cervicornis*. When these species were decimated by WBD, there was no other genus or species that could fulfill their niche. After their demise, the boulder coral *Orbicella* became the most important provider of structural complexity, but suffered from more recent declines (Hughes and Tanner 2000, Bruckner and Bruckner 2006). Hard coral species, such as *Acropora* and *Orbicella*, are critical in providing the three-dimensional structure of coral reefs and their disappearance has resulted in a decrease of coral reef rugosity, a process called "reef flattening" (Alvarez-Filip et al. 2009). A flattened coral reef provides less shelter and can sustain fewer niches resulting in decreased biodiversity (Newman et al. 2015), fish abundance (Paddack et al. 2009) and related fisheries productivity (Rogers et al. 2014).

Another example of how the decline of a single species can have detrimental effects on Caribbean coral reefs is the mass die-off of the sea urchin *Diadema antillarum* in 1983 and 1984 (Lessios et al. 1984). *D. antillarum* is a key herbivore and was especially important because other herbivores had been previously severely overfished (Jackson et al. 2001, Pandolfi et al. 2003). With no other grazers that could fill the gap, turf and macroalgae cover started to increase within days after the *D. antillarum* die-off (Carpenter 1985). The results of this die-off were catastrophic for the already-stressed coral reefs: coral and CCA cover decreased, while turf and macroalgae cover increased (De Ruyter van Steveninck & Bak 1986, Hughes et al. 1987). Macroalgae compete with adult corals for space and can overgrow juvenile corals (Jompa & McCook 2002, Box & Mumby 2007). Although their role in large-scale coral mortality is ambiguous (Precht et al. 2020), it is clear that macroalgae can inhibit coral recruitment by occupying available settlement substrate (McCook et al. 2001, Box & Mumby 2007). This way, macroalgae prevent recovery from perturbations (Adam et al. 2015, Precht et al. 2020) and severely reduce the resilience of Caribbean coral reefs (Hughes et al. 2010, Adam et al. 2015).

The combination of large-scale coral mortality and low recovery resulted in up to 80% reductions in Caribbean coral cover: from an average cover of 35% in the 1970s to only 14–16% in the 2000s (Gardner et al. 2003, Jackson et al. 2014). In 2016, a new coral disease, stony coral tissue loss disease (SCTLD), has appeared in Florida (Precht et al. 2016). This new disease affects several species and seems to be both more contagious and more lethal than other coral diseases (Precht et al. 2016). SCTLD is spreading throughout the Caribbean and reduces the remaining coral cover by up to 60% (Heres et al. 2021). Degradation of Caribbean coral reefs is often so severe and the threats so many, that it is uncertain whether these coral reefs will be able sustain their many important ecosystem services in the near future (Woodhead et al. 2019). The coral reefs of the Dutch Caribbean islands of Saba and St. Eustatius, where most of the research described in this dissertation has been conducted, are no exception to the Caribbean-wide trends in coral reef degradation. Average coral cover on both islands decreased to less than 5%, while macroalgae cover increased to more than 25% (Kitson-Walters 2021, Lehwald 2021).

Passive conservation vs active intervention

Coral reef conservation has long focused on the implementation of marine protected areas (MPAs) (Mumby & Steneck 2008), in which human activities are regulated within defined borders (Sale 2008). The implementation of MPAs is an example of passive management (Rinkevich 2008): by reducing stressors, such as fishing, anchoring and diving, direct damage is prevented and the ecosystem is assumed to get the chance to recover. The effectiveness of an MPA is dependent on many factors, including its current ecosystem status, stakeholder participation, geographic extent, enforcement and upstream availability of source populations of fish and invertebrates that can serve to supply larvae (Fox et al. 2012). When implemented well, MPAs have often been found to result in higher abundance of fish and other marine organisms (Roberts 1995, Halpern & Warner 2002, Mumby et al. 2006, Stockwell et al. 2009, McCook et al. 2010). With good reason they are seen as one of the most important management tools in marine conservation (Halpern & Warner 2002). Corals can probably also benefit from MPAs because the increased abundance and biomass of fish herbivores reduce macroalgae cover and indirectly enhance coral recruitment (Selig & Bruno 2010).

However, the implementation of an MPA is not always enough to reverse coral reef degradation trajectories (Coelho & Manfrino 2007, Bruno et al. 2019). This can be explained by the fact that many factors that negatively affect reef-building corals, such as climate change, coastal development and eutrophication are often not resolved by the implementation of an MPA, resulting in continued degradation of the ecosystem. Even if all local threats would be removed, the ecosystem might still not recover. This is the result of a phenomenon called hysteresis, in which the reverse trajectory has a different tipping point than the degradation trajectory (Hughes et al. 2010). This means that simply removing the last stressors, which made the ecosystem change from coral dominance to dominance by macroalgae or other benthic groups, might not be enough to tip back to coral dominance. In order for that to take place, first the negative feedback loops that keep the reefs in an algae-dominated state have to be weakened (Hughes et al. 2010). Active restoration tools can weaken these negative feedback loops and should therefore be considered as a supplement to passive management measures (Rinkevich 2008, Sale 2008).

Already in 2000, convincing arguments were presented that passive protection is not always sufficient to achieve ecosystem recovery and that active restoration interventions have become crucially needed (Yap 2000). Worldwide, coral propagation and outplanting are so far the most practiced and researched forms of active intervention for coral reefs (Boström-Einarsson et al. 2020). Other forms of active intervention often fall under the category of substrate enhancement, which includes substrate stabilization and the deployment of artificial reefs (Boström-Einarsson et al. 2020). In the Caribbean, restoration of populations of the keystone herbivore *Diadema antillarum* is seen as a priority for reef restoration (Lessios 2016), as *D. antillarum* can reduce macroalgae cover and, by doing so, stimulate coral recruitment and survival (Edmunds & Carpenter 2001, Carpenter & Edmunds 2006, Myhre & Acevedo-Gutiérrez 2007, Idjadi et al. 2010). Since active intervention in marine ecosystems is still a relatively new field of study, many potential solutions still remain insufficiently explored (Friedman et al. 2020). This dissertation focusses on optimization of two active intervention methods: the deployment of artificial reefs and the rehabilitation of the sea urchin *D. antillarum.*

Artificial reefs

Artificial reefs are structures deliberately placed on the seabed with the purpose to mimic one or more functions of a natural ecosystem (Baine 2001) and can restore part of the lost three-dimensional structure of coral reefs (Rinkevich 2005). The practice of using artificial reefs is already thousands of years old and for a long time creating new fishing grounds or increasing fishing efficiency were the two most important purposes of artificial reef deployment (Polovina 1991). However, the use of artificial reefs increased exponentially during the last few decades (Lima et al. 2019). Although increasing fishing yields has still been an important objective, artificial reefs have more and more been deployed for other purposes, such as creating new dive sites or for ecosystem restoration (Lima et al. 2019).

To cater to these different purposes, many different types of artificial reefs have been developed and deployed. Conservationists, marine park managers and researchers need to be able to substantiate their choices if they consider the construction of artificial reefs for a specific purpose. Acquired knowledge about results and experiences should therefore be widely shared. However, very few of the deployed reefs have been or are being systematically monitored and their success evaluated. It therefore still remains unknown how most artificial reefs perform and especially those deployed in the Caribbean have been poorly researched (Lima et al. 2019). While reviews exist on artificial reefs in Europe (Jensen 2002, Fabi et al. 2011) and Asia (Chou, 1997), no such reviews exist for artificial reef deployment in the Caribbean Sea.

Ideally, hard corals should recruit, survive and grow on artificial reef structures and expand them, leading to a gradual increase in three-dimensional structure and improvement of the habitat function of the artificial structures for other marine organisms (Cabaitan et al. 2008, Yap 2009). Coral larvae require suitable substrate for settlement and especially turf and macroalgae are known to prevent coral recruitment by reducing available space (Box & Mumby 2007). To prevent this undesired overgrowth from happening, it is essential to facilitate herbivores that can effectively reduce algae cover on the artificial reef structures (reviewed by Seraphim et al. 2019) thus creating opportunities for coral recruitment (Mumby 2009, Adam et al. 2015, 2018). Previous studies showed that artificial reefs with more shelter availability had a higher fish abundance and species richness (Hixon and Beets 1989, Sherman et al. 2002, Gratwicke and Speight 2005). Such artificial reefs would potentially result in a higher abundance of herbivorous fish, higher grazing intensity and concomitantly more chances for coral development but this effect has never been studied.

Diadema antillarum

The mass *D. antillarum* die-off in 1983-1984 reduced population densities of this important herbivore by 98% (Lessios 2016) and changed the appearance of Caribbean coral reefs. Forty years later, *D. antillarum* are still nowhere near their former densities (Lessios 2016). Although some populations seem to have recovered (Carpenter & Edmunds 2006, Debrot & Nagelkerken 2006, Myhre & Acevedo-Gutiérrez 2007), this recovery is mostly limited to shallow and sheltered locations with a low predation pressure. The average density of *D. antillarum* is estimated to be around 12% of pre die-off densities (Lessios 2016). At the few places where *Diadema* recovered their grazing significantly reduced macroalgae cover and increased the cover of CCA and bare substrate (Carpenter & Edmunds 2006, Myhre & Acevedo-Gutiérrez 2007). Locations with high *D. antillarum* densities indeed were associated with higher coral recruitment, survival and growth (Carpenter & Edmunds 2006, Idjadi et al. 2010) and higher coral cover (Myhre & Acevedo-Gutiérrez 2007) compared to locations with fewer *D. antillarum*. Unfortunately, this is not the case for the majority of reefs and it could take decades before *Diadema* populations recover naturally throughout the Caribbean (Chiappone et al. 2013). Finding and implementing ways to speed-up the recovery of *D. antillarum* populations is therefore a key priority as it could help increase the resilience of coral reefs to future disturbances (Lessios 2016).

To restore *D. antillarum* effectively, it is of utmost importance to identify the factors that currently constrain natural recovery in the Caribbean. As both juveniles and adults are absent from most reefs, it is likely that the bottle-neck in *D. antillarum* recovery occurs in the early phases of the life cycle (Figure 1) (Karlson & Levitan 1990, Mercado-Molina et al. 2015, Lessios 2016). Low fertilization success (Lessios 1988, Feehan et al. 2016), lack of an upstream source population (Roberts 1997), the lack of suitable settlement substrate (Rogers & Lorenzen 2008), a reduced survival of post-settlers (Vermeij et al. 2010, Williams et al. 2011) and high predation pressure on recruits (Harborne et al. 2009) are the most likely factors preventing recovery.

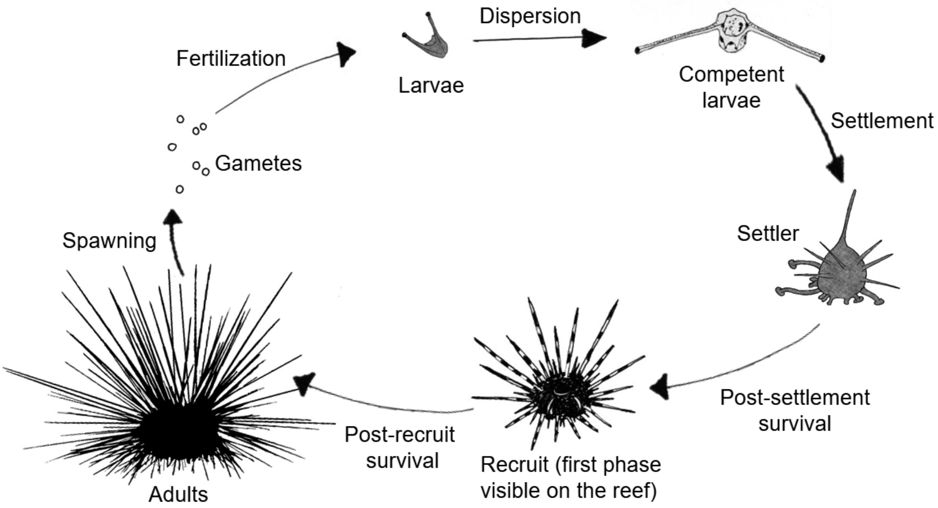

Figure 1: Life cycle of *D. antillarum*.

Although no pre die-off densities have been published for Saba and St. Eustatius, the Caribbean average was ~8 individuals per m^2 (Lessios 2016). Similar to most other locations in the Caribbean, the current density of *Diadema* on the reefs around St. Eustatius and Saba is less than 0.01 individual per m^2 (Kitson-Walters 2021, Lehwald 2021). Interestingly, substantial local *D. antillarum* populations have been reported in isolated areas of shallow waters: in front of Oranjestad at St. Eustatius (2 m water depth) and close to the Fort Bay harbor on Saba (5-8 m water depth). The existence of these shallow-water populations suggest that there is an upstream population supplying larvae to these areas. On nearby reefs without juvenile or adult *D. antillarum* this absence could be due to low settlement rates or low post-settlement survival. Studying settlement rates on artificial collectors makes it possible to distinguish between these processes (Harrold et al. 1991) and can provide insight into what constrains natural recovery. However, settlement rates around St. Eustatius, Saba or elsewhere in the Eastern Caribbean have not yet been measured. In other locations in the Caribbean, settlement rates have been determined both with mid-water collectors (Williams et al. 2010, 2011) and with panels on the seabed (Bak 1985, Vermeij et al. 2010). It remains unclear whether these two methods yield comparable results.

When more is known about the factors constraining natural recovery, new approaches might be able to be developed to restore *D. antillarum* populations. In the past few decades, various approaches have been attempted to restore *D. antillarum* and all have so far relied on restocking. The individuals necessary for restocking can potentially originate from harvest from wild *D. antillarum* populations (Macía et al. 2007, Burdick 2008, Dame 2008), collected settlers that are given a head start in a land-based nursery (Williams 2017, 2021) or laboratory cultured juveniles (Pilnick et al. 2021, Wijers et al. in prep). When using wild *Diadema* for restocking, a local, stable population is needed with thousands of individuals that can be transplanted. This requirement makes it an unsuitable method for most Caribbean islands, including St. Eustatius and Saba. Head starting collected settlers in a land-based facility has been shown successful in Puerto Rico (Williams 2017, 2021). *D. antillarum* cultivation from gametes appears to be the most scalable way to produce juveniles year-round and despite the sensitive nature of the larvae and the relatively long larval phase (Eckert 1998, Bielmyer et al. 2005), several culture runs have recently produced over 100 settlers (Pilnick et al. 2021, Wijers et al. in prep). In contrast to collecting wild individuals, these culture methods seem suitable and scalable, but the costs per juvenile will remain relatively high, limiting their practical restoration utility.

A viable approach to *D. antillarum* restoration should be applicable on a large scale at low costs. For forest restoration, such approaches have been developed under the flag of assisted natural recovery (ANR, Hardwick et al. 2004, Shono et al. 2007). ANR aims to accelerate succession by reducing barriers to natural ecosystem recovery. As the success of ANR depends on natural recovery processes, it only functions if some form of natural succession is already in progress (Hardwick et al. 2004, Shono et al. 2007). For forest regeneration, it often means the introduction of seed banks, the removal of competitors and minimization of stressors and disturbances (Hardwick et al. 2004, Shono et al. 2007). With more insight in the factors that constrain the natural recovery of *D. antillarum*, an analogous ANR approach could be developed with the purpose of removing these barriers to natural recovery. This could help make *D. antillarum* restoration applicable on a far larger scale than is possible with current methods.

General objective and outline PhD thesis

Based on the knowledge gaps outlined above, the general objective of this study was to increase our understanding of the most effective interventions to increase herbivorous grazing intensity, reduce algae cover and increase coral recruitment, survival and growth. These interventions could subsequently be applied to kick-start coral reef recovery by facilitating effective herbivory.

Aside from this general introduction **(Chapter 1)**, this dissertation consists of five research chapters, and concludes with a general discussion including future perspectives.

Chapter 2 reviews artificial reef use in the Caribbean and evaluates the monitoring of their success and their management status. A database was compiled of artificial reefs described in both the gray and scientific literature. This database was used to link reef type, material and intended purpose to the ecological development of the artificial reefs over time. A spatial analysis was conducted to determine if and how fishing on artificial reefs in the Caribbean was regulated. Knowledge gaps to effectively apply artificial reefs in the Caribbean are identified in this chapter.

Chapter 3 describes the fish assemblages of three different types of artificial reefs during early colonization. The comparison of multiple artificial reef types was identified as knowledge gap in Chapter 2. At three locations around St. Eustatius and Saba, the fish assemblages of “reef balls”, “layered cakes” and “rock piles” after a year of deployment were compared to the assemblages found on bare sand controls.

Chapter 4 examines whether differences in fish assemblages of reef ball and layered cake reefs persisted over time. The effect of herbivory and territorial behavior on the benthic community development of the artificial reefs was assessed over 2.5 years and correlated with coral recruitment, survival and growth.

Chapter 5 describes spatial and temporal variability of *D. antillarum* settlement at five locations around St. Eustatius in 2019. At these locations, *D. antillarum* settlement on panels placed at the seabed were compared to settlement on four different settlement collectors deployed mid-water. Subsequently, the best working settlement collector was further optimized in follow-up experiments on Saba in 2020. The optimized collector, a string of bio balls, was used in Chapter 6 to provide settlement substrate for *D. antillarum* larvae on patch reefs.

Chapter 6 proposes a new approach to restore *D. antillarum* populations termed “assisted natural recovery”. To remove one of the barriers to natural recovery, which was identified in Chapter 5 as limited availability of suitable settlement substrate, artificial substrate was attached to the reef prior to the settlement season at a location with known high *D. antillarum* settlement rates. Subsequently, it was determined whether this new intervention indeed resulted in increased numbers of *D. antillarum* recruits on the reef.

Finally, in **Chapter 7**, the results of this dissertation are summarized, the approach discussed and put into perspective using existing literature. This is the basis for recommendations for future research and discussion of the future perspectives of a new artificial reef design, termed “Moreef”, providing optimized facilitation of herbivores.

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Chapter 2

Artificial reefs in the Caribbean: A need for comprehensive monitoring and integration into marine management plans.

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2.1 Abstract

Caribbean coral reefs are in decline and the deployment of artificial reefs, structures on the sea bottom that mimic one or more characteristics of a natural reef, is increasingly often considered to sustain ecosystem services. Independent of their specific purposes, it is essential that artificial reefs do not negatively affect the already stressed surrounding habitat. To evaluate the ecological effects of artificial reefs in the Caribbean, an analysis was performed on 212 artificial reefs that were deployed in the Greater Caribbean between 1960 and 2018, based on cases documented in grey (n=158) and scientific (n=54) literature. Depending on the availability of data, reef type and purpose were linked to ecological effects and fisheries management practices around the artificial reefs. The three most common purposes to deploy artificial reefs were to create new dive sites (41%), to perform research (22%) and to support ecosystem restoration (18%), mainly by stimulating diversity. Ship wrecks (44%), reef balls© (13%) and piles of concrete construction blocks (11%) were the most-often deployed artificial reef structures and metal and concrete were the most-used materials. The ecological development on artificial reefs in the Caribbean appeared to be severely understudied. Research and monitoring has mostly been done on small experimental reefs that had been specifically designed for science, whereas the most commonly deployed artificial reef types have hardly been evaluated. Studies that systematically compare the ecological functioning of different artificial reef types are virtually non-existent in the Caribbean and should be a research priority, including the efficacy of new designs and materials. Comparisons with natural reef ecosystems are scarce. Artificial reefs can harbor high fish densities and species richness, but both fish and benthos assemblages often remain distinct from natural ecosystems. Studies from other parts of the world show that artificial reefs can influence the surrounding ecosystem by introducing non-indigenous species and by leaking iron. As artificial reefs attract part of their marine organisms from surrounding habitats, intensive exploitation by fishers, without clear management, can adversely affect the fish stocks in the surrounding area and thus counteract any potential ecosystem benefits. This study shows that over 80% of artificial reefs in the Caribbean remain accessible to fishers and are a risk to the surrounding habitat. To ensure artificial reefs and their fisheries do not negatively affect the surrounding ecosystem, it is imperative to include artificial reefs, their fisheries and the surrounding ecosystem in monitoring programs and management plans and to create no-take zones around artificial reefs that are not monitored.

Keywords:

Man-made structure, fish aggregation, ecosystem services, fisheries, ecosystem restoration

2.2 Introduction

Since the 1970s, the amount of living reef-building coral cover on Caribbean coral reefs has been greatly reduced due to coral mortality caused by diseases (Gladfelter 1982, Aronson & Precht 2001), water pollution, hurricanes and periods with higher than average seawater temperature (Hughes 1994, Gardner et al. 2003, Jackson et al. 2014). In addition, Caribbean coral reefs are severely overfished (Hughes et al. 1994, Vermeij et al. 2019). The combination of habitat degradation and overfishing has resulted in reduced fish stocks (Paddack et al. 2009), biodiversity and fisheries productivity (Rogers et al. 2014). As Caribbean coral reefs continue to degrade, they are less able to fulfill their important ecosystem functions (Graham et al. 2007, Newton et al. 2007). On a local level, artificial reefs might help to stem the losses as they can restore the lost three-dimensional habitat for fish (Baine 2001, Becker et al. 2018, Lima et al. 2019) and can provide hard substrate for epibenthic communities, including coral recruits and gardened coral fragments (Young et al. 2012).

Artificial reefs can be defined as submerged structures deliberately placed on the seabed to mimic some functions of a natural reef, such as protecting, regenerating, concentrating and/or enhancing populations of living marine resources (Baine 2001). Although the first records of artificial reef use are more than 3000 years old (Riggio et al. 2000), they became common use in 18th century Japan (Lee et al. 2018) and spread to the USA and Europe in the 19th century (McGurrin et al. 1989, Fabi et al. 2011). Although fish attraction was historically the main reason for artificial reef deployment (Polovina 1991), often to create new fishing grounds or to increase fishing efficiency (Lee et al. 2018), they were also used for other purposes such as trawling obstacles for fisheries management and for coastal protection (Lima et al. 2019). In the second half of the 20th century, observations of high fish densities on WWII wrecks resulted in an increase of artificial reef use in Japan and the USA (Lee et al. 2018) and increased fishing yields on these artificial reefs made the practice spread to the rest of the world (Lima et al. 2019). The Caribbean region was relatively late in adopting the use of artificial reefs. The first record we found was an artificial reef made of 800 concrete construction blocks, which was deployed in the US Virgin Islands in 1960 for research purposes (Randall 1963).

Nowadays, increased fisheries yields are still a main reason for artificial reef deployment (Baine 2001, Fabi et al. 2011, Becker et al. 2018), but artificial reefs are also built for ecosystem restoration, habitat protection, as scuba diving object and for many other purposes (Lima et al. 2019). To cater to these different purposes, many different types of artificial reefs have been developed and deployed. Comparing artificial reef performances is complicated, because this depends on a variety of variables, such as size (Tupper & Hunte 1998, Abelson & Shlesinger 2002), material (Fitzhardinge & Bailey-Brock 1989), complexity (Charbonnel et al. 2002), age (Perkol-Finkel & Benayahu 2005), depth (Jaxion-Harm & Szedlmayer 2015), isolation (Shulman 1985), and the geographic region (David et al. 2019) and habitat type in which the reef is deployed (Yeager et al. 2011). Additionally, objectives and success criteria are highly dependent on the intended purpose of the artificial reef (Baine et al. 2001, Fabi et al. 2011). Independent of their purpose, it is crucial that artificial reefs do not harm the surrounding ecosystem. Yet uncolonized artificial structures can, for example, provide a stepping stone for non-indigenous benthic species to colonize the surrounding ecosystem (Glasby et al. 2007, Airoldi et al. 2015). Also, artificial reefs can attract marine life from neighboring habitats, which can subsequently be removed and ultimately even depleted by fishers (Bohnsack 1989). It is yet unknown to what extent fisheries on artificial reefs in the Caribbean are managed and to what extent artificial reefs are located within marine protected areas (MPAs).

Ideally, the knowledge and experiences acquired with artificial reef use should be carefully documented and widely shared to optimize further artificial reef deployment. Unfortunately, however, artificial reefs in the Caribbean have been poorly studied (Baine et al. 2001, Lima et al. 2019) and while reviews do exist on artificial reef deployments in Europe (Jensen 2002, Fabi et al. 2011), the USA (McGurrin et al. 1989) and Asia (Chou, 1997), there are no overviews available yet regarding artificial reefs in the Caribbean. As Caribbean coral reefs continue to decline, the deployment of artificial reefs will be considered more and more often. To support science-based decision making regarding artificial reef deployment, the need for a baseline overview of Caribbean artificial reefs and their effect on the surrounding ecosystem is evident. The objective of this study was to provide such an overview, based both on scientific and grey literature. We included information such as artificial reef type, location, deployment year, intended purpose, material, ecological development and fisheries management status.

2.3 Methods

In this study, we adopted the definition of artificial reefs proposed by Baine (2001): "structures placed on the seabed intentionally with the purpose to mimic one or more characteristics of a natural reef". Marine infrastructure such as oil platforms, piers, marinas, harbours and breakwaters were therefore excluded, as well as floating FADs (fish aggregation devices). Wrecks were included only if they were sunk specifically with the purpose of creating an artificial reef. We focussed on artificial reefs in the Caribbean Sea and included all islands and countries bordering this water body. Because of their similar environmental and geographical characteristics, the Bahamas and the Turks and Caicos islands were included but the Gulf of Mexico and Florida were excluded. Within the greater Caribbean, six distinct regions were distinguished: the Bahamas, Eastern Caribbean, Greater Antilles, Southern Caribbean, Southwestern Caribbean and Western Caribbean (Figure 1).

We conducted an extensive internet search using Google Scholar and Google to find as many artificial reefs as possible, as described in respectively scientific (SL) or grey literature (GL). As search terms we combined the country or island name with “artificial reef”, “man-made reef”, “wreck”, “fish aggregation”, “lobster aggregation”, “lobster casita”, “coral restoration” and “reef ball”. In a pilot study, a longer list of key words was used and combined with one randomly chosen country from each region. All key words that did not yield any extra cases during the pilot study, were not used for the main study. Islands belonging to a larger country were searched individually using the same search terms if they were larger than 300 km^2. The sources compiled were carefully screened to avoid double counting. For countries and islands where French, Spanish or Dutch was the first language, the search was repeated with translated search terms. All artificial reefs included in this study were deployed before 2019. This approach is not exhaustive and a number of artificial reefs have likely been missed. Even so, our results should be sufficient to indicate trends and by clearly specifying our search approach, the results should be replicable and robust.

In a few sources, multiple artificial reefs were described that were deployed at different locations (Haughton & Aiken 1989, Lalana et al. 2007, Wells et al. 2010, Delgadillo-Garzon & Garcia 2009, Tessier et al. 2015, Gittens et al. 2018). Risk (1981), Tupper & Hunte (1998) and Reguero et al. (2018) described more than one type of reef at the same location. For all of these cases, the different reefs were analysed as separate entities. However, if multiple small reefs of the same type were built in close proximity at the same location, the reefs were considered as one entity.

Analysis

The following characteristics were extracted from each separate source: geographic coordinates, material composition, type of reef, year of deployment (age), depth, purpose and number of units forming a single reef. In addition, we determined whether any ecological monitoring was performed and if so, used the results of these studies to link artificial reef type (e.g. ship wrecks or reef balls©) to their fish habitat function and the material used (e.g. concrete or metal) to benthic development. If the characteristics were not described in the first identified source, additional sources were used to identify as many characteristics as possible. As the focus of our study was to assess the effect of artificial reefs on the natural ecosystem, we did not include their socio-economic effects. In order to illustrate source, type of reef and purpose in time, the deployment year of the artificial reefs was used. This was known for 180 of the 212 cases. The other 32 cases were excluded from temporal analysis.

Location descriptions were used to determine the rough GPS position of the artificial reef using Google Earth Pro (V. 7.3.2.5776) in case the exact GPS position was not known. For 9 cases it was not possible to determine a rough GPS position, these reefs were excluded from further analysis. Coordinates were loaded into Arcmap (V.10.6.1.) along with MPA shapefiles downloaded from The Atlas of marine protection (http://www.mpatlas.org/map/mpas/). The ‘Intersect’ tool in Arcmap created output containing only data overlapping each other and was used to determine which of the artificial reefs were located within an MPA. If an artificial reef was located in an MPA, we used publicly available information on the MPA to determine if any fishing regulations were in place. A distinction was made between no take zone (no fishing allowed) and restricted fishing. Our full database is available as an interactive map: https://bit.ly/3hkI1kB.

2.4 Results

Overview

In total, 212 artificial reef cases were identified, 54 from scientific literature (SL) and 158 from grey literature (GL). Most cases were found using English search terms (SL: n=51, GL: n=155), followed by Spanish (SL: n=3, GL: n=2) and French (SL: n=0, GL: n=1). No extra cases were found using Dutch search terms. Most of the artificial reefs we identified (n=76, 36%) were located in the Eastern Caribbean, followed by the Greater Antilles (n=50, 24%), the Bahamas (n=35, 17%), Western (n=26, 12%), Southern (n=18, 8%) and Southwestern (n=7, 3%) Caribbean, respectively (Figure 1). The most commonly described artificial reefs in the Caribbean were ship wrecks, reef balls© and piles of concrete construction blocks, in that order (Table 1). To a lesser extent, also concrete structures (other than reef balls© or construction blocks), plane wrecks and lobster shelters were described. Finally, mineral accretion technique (MAT) structures, other metal structures, piles of natural rocks, motor vehicles and tires were least often described. Metal and concrete were by far the most-used materials for artificial reefs in the Caribbean, due to the common deployment of ship wrecks, reef balls© and concrete construction blocks. Well-defined purposes had been formulated for 205 of the artificial reefs in our database. In declining order, the stated objectives for artificial reefs were found to be: the creation of a new dive site (n=87, 41%), research (n=47, 22%), ecosystem restoration (n=38, 18%), increase fishing yield (n=14, 7%), coastal protection (n=11, 5%), creation of a movie set-up (n=4, 2%), dumping (n=2, 1%), mooring (n=1, 0.5%) and an artificial reef deployment training (n=1, 0.5%). The last four categories are summed under “other purpose” in Table 1. Ecosystem restoration was used as an overarching purpose, for example for restoring three dimensional structure, the fish abundance or biodiversity. Most sources were unclear what they actually wanted to restore.

For 180 of the 212 artificial reef cases, the deployment year could be determined. The first described deployment of an artificial reef in the Caribbean was in 1960 in the US Virgin Islands and consisted of a pile of concrete construction blocks (Randall 1963). Apart from this case, the 1960s had very little artificial reef deployment (Figure 2). Artificial reef deployment in the Caribbean took off in the 1970s, with most cases described in scientific literature. Since the late 1980s, cases described in grey literature increasingly outnumbered cases described in scientific literature. Especially in the last two decades, hardly any new cases were published in scientific literature, while the number of cases described in grey literature continued to increase. The ecological development of only 50 of the 212 reef cases were monitored in some way, of which for 48 cases the results are publicly available, 45 of which in the scientific literature. This means that only limited new information on the ecological development of artificial reefs has recently become available.

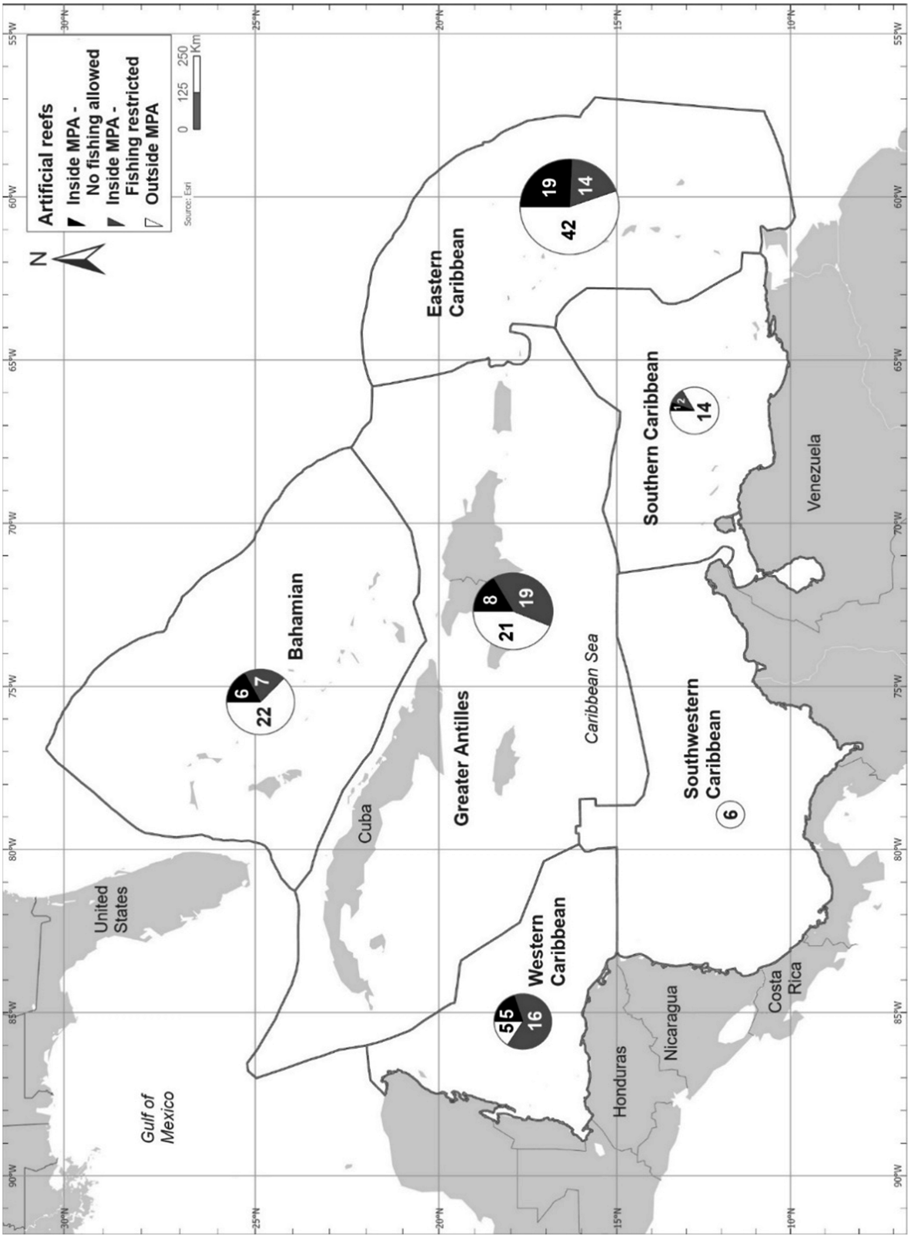

Figure 1: The protection status of artificial reefs in the Caribbean per sub-region. The pie-chart size indicates the total number of reefs in the specific sub-region and the numbers within the pie-chart indicate the number of reefs per protection status.

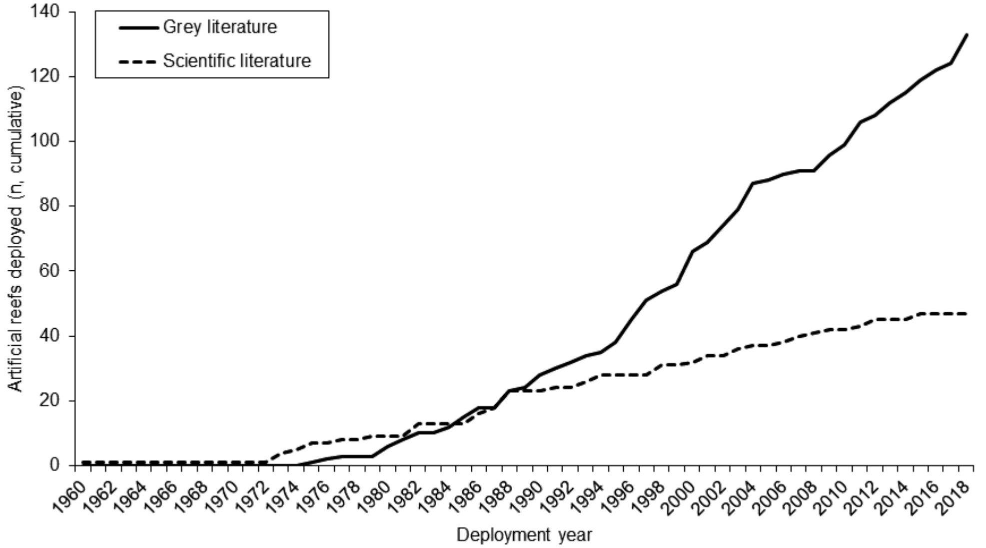

Figure 2: Cumulative number of artificial reefs deployed per year described in grey and scientific literature.

Analysis per artificial reef type

Among the most frequently described artificial reefs in the Caribbean (n=23, 11%) are the "experimental reefs": small sized reefs (around 1m^3), usually made from concrete construction blocks, which were mostly deployed in the Bahamas, Cuba and in the US Virgin Islands. As research was the most frequently reported (87%) purpose to deploy this type of artificial reef, it is not surprising that especially these reefs have been described in the scientific literature (83%). Most construction block reefs were deployed in the 1980s and in the 2010s (Figure 3). The number of building blocks used to make one reef ranged from 3 to 800, but was mostly between 10 and 60, while up to 35 replicate reefs were built in some cases. Some of the studies performed with construction block reefs focused on a pre-selected biological mechanism, for example recruitment patterns of fish (Shulman et al. 1983) and lobster (Lalana et al. 2007) or the effect of habitat complexity and shelter opportunity on the fish assemblage (Beets & Hixon 1994, Gratwicke & Speight 2005). The first study focusing on the fish habitat function of piles of construction blocks simply described the fish density and species richness around this type of artificial reef (Risk 1981), while some follow-up studies also compared the fish assemblage with those of a nearby natural reef. The fish density and species richness at one to two year-old, shallow (2-5m) artificial reefs was lower than the fish density on natural reefs (Carr & Hixon 1997, Zapata 2014), while species composition sometimes was similar (Alevizon et al. 1985, Carr & Hixon 1997) and sometimes different (Beets & Hixon 1994). Systematic comparisons between different types of construction block reefs revealed that reefs with more and smaller holes supported a higher fish abundance, while artificial reefs with a higher rugosity had a higher species richness (Gratwicke & Speight 2005). Studies including the effect of the seascape concluded that artificial reefs placed on dense seagrass beds harbored higher fish abundances than artificial reefs deployed on patchy seagrass beds or bare sand areas (Shulman 1985, Yeager et al. 2011). Piles of concrete construction blocks proved physically not stable over longer periods (Ogden & Ebersole 1981, Beets & Hixon 1994). Therefore, most of the concrete construction-block reefs described in these studies may currently not be functional anymore and this artificial reef type is basically unsuitable for large scale application.

Table 1: Type of described artificial reefs (n) per purpose and in total. The types are sorted based on their used material. *= not elsewhere identified.

Type	Main material(s)	Coastal protection	Create new dive site	Ecosystem restoration	Increase fishing yield	Research	Other purpose	Unknown	Total	% of total
Concrete construction blocks	Concrete	0	0	2	1	20	0	0	23	11%
Reef balls©	Concrete	9	5	8	0	3	2	1	28	13%
Concrete structures*	Concrete	2	2	5	0	1	1	0	11	5%
Ship wrecks	Metal	0	72	10	4	0	1	6	93	44%
Plane wrecks	Metal	0	6	1	0	0	3	0	10	5%
Motor vehicles	Metal	0	2	1	0	1	1	0	5	2%
Mineral accretion technique	Metal	0	0	6	0	3	0	0	9	4%
Metal structures*	Metal	0	0	3	0	2	0	0	5	2%
Tires	Rubber	0	0	0	5	0	0	0	5	2%
Lobster shelters	Wood, metal, concrete	0	0	0	0	10	0	0	10	5%
Piles of natural rock	Natural rocks	0	0	1	3	2	0	0	6	3%
Other	Coral rubble, conch shells	0	0	1	1	5	0	0	7	3%
Total		11	87	38	14	47	8	7	212	100
% of total:		5%	41%	18%	7%	22%	4%	3%	100	

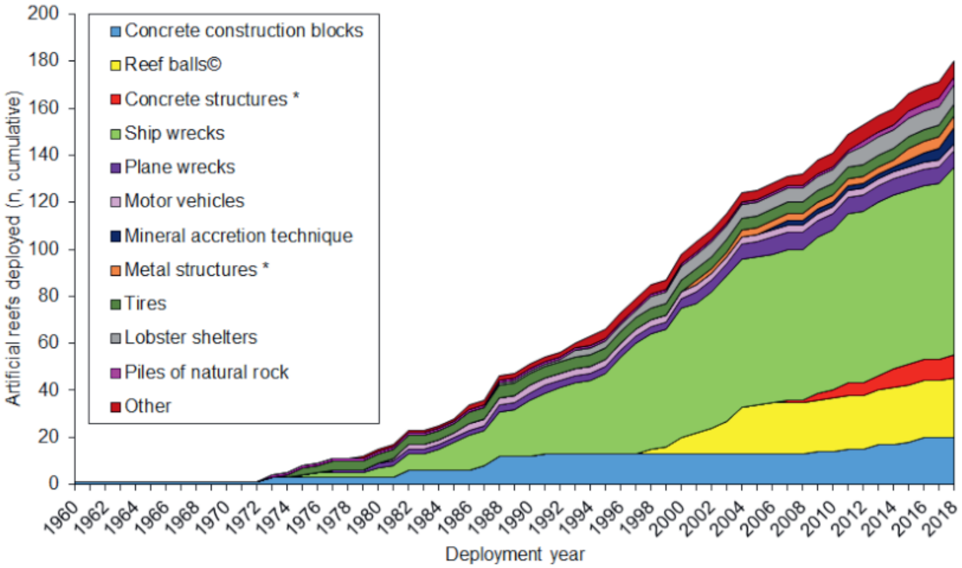

Figure 3: Cumulative number of artificial reefs per type in time, described in grey and scientific literature. * = not elsewhere identified.

Specially made reef balls© are the second common (n=28, 13%) type of artificial reef in the Caribbean and have been deployed in every Caribbean sub-region, especially in the Dominican Republic and Sint Maarten. Reef balls© were almost exclusively deployed in the late 1990s and early 2000s (Figure 3). Coastal protection was the most frequently reported (32%) purpose for deploying reef balls©, although they were also often deployed for ecosystem restoration (29%) and sometimes to create a dive site (18%). Reef balls© were mostly deployed in shallow water, usually between one and four meters deep and sometimes deeper (12 -19 m) for research purposes. Often, many reef balls© were used to form one reef; the largest single reef in our database consisted of 3500 reef balls© and there were 12 other cases reported with more than 150 reef balls©. Reef ball© reefs were mostly (89%) documented in grey literature. Despite their common use, their ecological development has hardly ever been monitored, and if so, it only included survival and growth of coral transplants for a relatively short period of time (Ortiz-Prosper et al. 2001, Cummings et al. 2015). As coastal protection was the main reason to deploy reef balls© in the Caribbean, monitoring of fish abundance and coral cover may often not have been considered relevant. However, also the success of the coastal protection function was never monitored. A few (n=11, 5%) dedicated concrete structures other than reef balls© were described for the Caribbean and all of these were deployed between 2007 and 2018. The most frequently reported (45%) purpose for deployment of these concrete structures was ecosystem restoration, followed by coastal protection and creation of a new dive site (both 18%). Only one of these concrete structures was monitored and this was the only case described in the scientific instead of grey literature. In this study, 10 “Taino” structures, were deployed in a shallow (1m) seagrass bed in Puerto Rico (Sander & Ruiz 2007). Fish density and species richness on the artificial reef was higher compared to natural reefs and seagrass beds in the same depth zone. Especially fish in the smallest size class (1-5cm) were very abundant on the artificial reef, indicating that it was used as a nursery structure (Sander & Ruiz 2007).

Ship wrecks are by far the most commonly (n=98, 44%) deployed type of artificial reef in the Caribbean, in all sub-regions with the exception of the southwestern Caribbean. The first ship was scuttled in 1974 and deploying this type of artificial reef remained popular during the entire period studied (Figure 3). Creating a new dive site was the most frequently (77%) reported purpose for scuttling ships, although it was sometimes done for ecosystem restoration (11%) or even to create a movie set-up. Ships were, in line with their main purpose, deployed in relatively deep water, the average depth of all cases was 24 meter. Despite being a popular choice for creating artificial ecosystems, only one wreck was monitored and described in scientific literature. This wreck, deployed in Guadeloupe at 30 m depth, was monitored for two years after deployment. The wreck had a slightly lower fish biomass than average natural coral reefs (Bouchon et al. 2010), but direct comparisons with nearby natural reefs were lacking.

As with ship wrecks, plane wrecks (n=10, 5%) were mostly (60%) deployed to create a new dive site. In addition, 3 planes (30%) were sunk to create a movie set-up. All plane wrecks were described in grey literature and, to our knowledge, no monitoring was performed on the ecological development on this type of artificial reef. While plane wrecks were always deployed individually, artificial reefs made of motor vehicles (n=5, 2%) usually consisted of 100 or more cars, trucks and sometimes autobuses (Bortone et al. 1988, Friedlander & Beets 1992). Motor vehicle reefs were deployed for a number of purposes (Table 1) and the resulting fish assemblages described for two of them in scientific literature. In Barbados, large (100m^2) artificial reefs made of 10 cars per reef had a comparable fish species composition compared to nearby natural reefs after two years (Tupper & Hunte 1998). In Guatamala, a high fish density but low species richness was reported for a motor vehicle reef deployed on a seagrass bed. However, constant fishing pressure might have affected its fish assemblage (Bortone et al. 1988).

Tailor-made metal structures can be used to make more complex artificial reefs and are lighter and thus easier to deploy than concrete structures (Lima et al. 2019). A few (n=5, 2%) metal reef structures were reported in the Caribbean, all between 2000 and 2015 and all but one were deployed in Colombia. Three of them were meant for ecosystem restoration, and two for research and described in scientific literature (Delgadillo-Garzón & García 2009). For these two reefs, at 16 and 20 m depth, an increase of fish and benthic biodiversity was reported, although no comparisons with nearby natural reefs were performed. A special application of metal structures is the mineral accretion technique (MAT), formerly patented by Biorock^{TM}. MAT exposes metal structures *in situ* to a low voltage electrical current. A MAT system consists of a power supply connected to an anode. The anode is placed in the sea, close to the metal reef structure that serves as a cathode. The current causes electrolysis of the seawater and the accretion of calcium carbonate on the cathode (Hilbertz & Goreau 1996). Applying MAT is claimed to enhance coral recruitment, survival and growth (Goreau & Hilbertz 2005). Nine cases (4%) of MAT treated structures were reported for the Caribbean. The first MAT structures in the Caribbean were deployed around Jamaica in the 1980s, but ecological development of these structures stopped after the power was cut off after two years (Goreau & Hilbertz 2005). Between 2006 and 2018, eight other MAT cases were deployed in Jamaica, Curaçao, Grenada, St. Barths and on the Turks and Caicos islands. Although all MAT structures were intended for ecosystem restoration (67%) or research (33%), with two cases being described in scientific literature, no results on ecological development are publicly available. These two cases were described by Wells et al. (2010) who focused on the hurricane resistance of two MAT structures, deployed at around five meters depth.

Tires were one of the first materials used to create artificial reefs in the Caribbean (Haughton & Aiken 1989, Friedlander & Beets 1992). Being so-called “materials of opportunity”, they were seen as an ideal combination of getting rid of waste items and enhancing fishing yields at the same time (Friedlander & Beets 1992). Most tire reefs in the Caribbean were deployed in the 1970s, although one reef was built in 1986 (Friedlander & Beets 1992). The results of all tire reefs (n=5, 2%) described for the Caribbean were deployed with the purpose of enhancing fishing yields and have been described in scientific literature. Of three of these reefs, ecological results were available. In the US Virgin Island 500 tires were deployed at 35 meters depth. The tires did not form a single structure, but were scattered over the bottom. As a consequence fish densities remained low (Friedlander & Beets 1992). In Jamaica, two tire reefs were constructed with 1000 and 300 tires, which were bound into bundles. The reefs locally increased the fish abundance and species richness, but no comparisons were made with natural reefs (Haughton & Aiken 1989).

Lobster (*Panulirus argus*) shelters or “casitas” were initially introduced as fishing gear, but can also function as an artificial reef to create extra shelter for juvenile lobster (Polovino 1991, Sosa-Cordero et al. 1998, Gittens et al. 2018). Lobster shelters consist of a roof made from concrete or metal that rests on PVC or lumber, creating a low shelter with multiple openings. We found 10 cases (5%) of lobster shelter reefs in the Caribbean, deployed between 1993 and 2012. All but one of them (90%) were deployed for research and described in scientific literature. All cases were from the Bahamas, Cuba or Mexico, the countries in which lobster shelters are commonly used in fisheries (Polovino 1991, Ramos-Aguilar et al. 2003). Lobster shelter reefs were deployed in shallow (2-4m) water and consisted out of 1-18 individual shelters. Monitoring was focused on the habitat function for juvenile lobster, of which lobster shelters were found to harbor high densities, especially if the surrounding habitat was suitable for foraging and limited in natural shelter opportunities (Eggleston et al. 1992, Sosa-Cordero et al. 1998, Briones-Fourzán & Lozano-Álvarez 2001, Lozano-Álvarez et al. 2009). Although lobster shelters can also provide a habitat for fish, this function was never investigated or described.

In only six cases (3%), artificial reefs were made of piles of natural rocks and all of these were deployed in the Eastern Caribbean and Greater Antilles. The most often reported purposes were to increase fishing yields (50%) and research (33%). Although five of the six cases have been described in scientific literature, very little information has become available about rock reefs. A deployment year was described for only three cases (1973, 2012, 2016) and a deployment depth (4m) was only reported for two cases. Risk (1981) is the only study that included ecological observations. Although based on a single replicate, higher fish densities and species richness were found on a natural rock reef compared to a concrete building block reef, while a combination of the two materials yielded the highest fish abundance and species richness (Risk 1981).

Seven cases (3%) were pooled under the category “other” types of artificial reefs. Five of these (77%) were deployed for the purpose of research and described in scientific literature. Forrester (1995) used piles of coral rubble to investigate fish recruitment. Rudolph (2012) described 350 ceramic “Ecoreef” modules deployed in Jamaica at eight meters depth. This artificial reef resulted in a substantial increase in fish density, which was mainly explained by large numbers of French grunts. Berrios & Timber (1994) described three small artificial reefs made from concrete culvert pipes, wood and metal, which were deployed at six meter depth in Puerto Rico. The artificial reefs harbored a higher fish abundance and species richness compared to control plots on bare sand. Finally, Shulman (1985) and Beets (1989) described artificial reefs made from conch shells in the US virgin islands, which were deployed at six meter depth. These conch shell reefs were used to study the effect of distance to the main reef on recruitment of fish to the artificial reef (Shulman 1985) and to study the effect of pelagic streamers on the fish density and species richness of the artificial reef (Beets 1989). Shulman (1985) found that fish densities were higher on artificial reefs close to a natural reefs, but attributed this to the reduced cover of seagrass around these artificial reefs. Beets (1989) showed that pelagic streamers on artificial reefs results in higher fish densities and species richness.

Fisheries management around artificial reefs

The GPS coordinates of 206 artificial reefs were determined and used to assess the fisheries management of the area of deployment. Of these cases, 110 (53%) were located outside MPAs, 58 (28%) were located in a restricted fishing zone of an MPA and only 38 (18%) were located within a no take zone of an MPA (Figure 1). The sub-regions with the highest percentage of artificial reefs within a no take zone of an MPA were the Eastern Caribbean (25%), the Western Caribbean (20%) and the Bahamas and Greater Antilles (both 17%). The Southwestern and Southern Caribbean had zero and one artificial reef, respectively, within a no take zone of an MPA. In the Eastern Caribbean, Greater Antilles and Bahamas, most of the remaining artificial reefs (56, 44 and 63% of the totals for that region, respectively) were located outside an MPA, while in the Western Caribbean most of the remaining artificial reefs (64% of the total for that region) were located in a restricted fishing zone of an MPA. In the Southern and Southwestern Caribbean, 88 and 100% of the artificial reefs were located outside an MPA.

2.5 Discussion

We here provide an overview and assessment of the purpose and deployment of artificial reefs in the Caribbean for the last five decades (1962-2018), whether their ecological development was monitored, and what the fisheries management was of the area they were deployed in. The main materials used for the artificial reefs in the Caribbean (metal: 58%; concrete 29%) contrast sharply with those used in the rest of the world. This can be especially ascribed to the much greater use of ship wrecks in the Caribbean (44%) compared to the rest of the world (8%, Lima et al. 2019). In contrast worldwide, concrete (49%) is the most popular material, while metal (25%) comes in second (Lima et al. 2019). This difference may be explained by the fact that the current study included grey literature, in which many of the metal shipwrecks in the Caribbean were described, while Lima et al. (2019) did not include grey literature in their review of worldwide artificial reef deployment. The exclusion of grey literature might also partly explain the lower percentage of reef balls© worldwide (7% worldwide, 12% Caribbean) (Lima et al. 2019), because the reef ball© cases found in the current study were mostly described in grey literature. Concrete building blocks accounted, worldwide, for 10% of the cases, which is almost similar to our study. This further strengthens the hypothesis that the inclusion of grey literature results in a different relative contribution of types and materials, because concrete building blocks were almost exclusively described in scientific literature. The biggest difference that cannot be related to the inclusion of grey literature is the lower percentage of concrete structures other than reef balls© and building blocks, which was 31% worldwide (Lima et al. 2019) and only 5% in the Caribbean.

Worldwide, the number of scientific studies focusing on artificial reefs increased exponentially since the first publication in 1962 (Lee et al. 2018, Lima et al. 2019). Although artificial reef deployment in the Caribbean is becoming increasingly popular, the number of scientific publications on the subject per year is growing at a much lower rate. Only 11 cases describe reefs currently deployed for purposes other than research and were ecologically monitored as well. Of these, only two included a comparison with natural reefs (Sander & Ruiz 2007, Rudolph 2012), which is essential to put the results in context (Carr & Hixon 1997) and only two compared multiple artificial reef designs (Risk 1981, Hylkema et al. 2020). Although Hylkema et al. (2020) was published outside the time range of our study, we use their conclusions in this discussion.

Fish assemblages on artificial reefs

Results of the few studies that monitored ecological development on artificial reefs in the Caribbean showed that artificial reefs locally increased fish densities and species richness compared to bare sand or seagrass within one to two years after deployment (Haughton & Aiken 1989, Berrios & Timber 1994, Sander & Ruiz 2007, Rudolph 2012, Hylkema et al. 2020). Concrete artificial reefs (Sander & Ruiz 2007) and ceramic artificial reefs (Rudolph 2012) had a higher fish density and species richness than nearby natural reefs. This is in line with results from other parts of the world, which show that artificial reefs can indeed harbor high fish densities, biomass and species richness. Reef balls© in the Caribbean (Hylkema et al. 2020) and in estuarine bays in Australia (Mills et al. 2017, Folpp et al. 2020) had a higher fish abundance and species richness than the surrounding soft sediment habitat. Fish densities and biomass on wrecks were similar (Fowler & Booth 2012) or even higher (Arena et al. 2007) to those on nearby natural reefs. Hylkema et al. (2020) and Abelson & Shlesinger (2002) reported high fish densities and fish species richness for artificial reefs made from natural rock piles in the Caribbean and Red Sea, respectively. The fish density and species richness on rock piles was higher than surrounding bare sediment and comparable to reef balls© (Hylkema et al. 2020) but comparisons with natural reef ecosystems were lacking (Abelson & Shlesinger 2002, Hylkema et al. 2020).

Small and experimental reefs made of concrete building blocks in the Caribbean harbored a species composition comparable to that of nearby natural reefs within two years after deployment (Alevizon et al. 1985, Carr & Hixon 1997). However, in another study, Beets & Hixon (1994) found a difference in fish species composition on the same type of reef compared to nearby natural reefs. In other parts of the world the fish species composition on reef balls© (Folpp et al. 2013, Mills et al. 2017, Komyakova et al. 2019), wrecks (Arena et al. 2007, Fowler & Booth, 2012, Simon et al. 2013) and natural rock reefs (Burt et al. 2013) also were found to be distinct from that of the nearby natural reefs. This can possibly be explained by differences in the structure of the surrounding natural reef or the moment of monitoring after deployment. For wrecks, the similarity to natural reefs increased with wreck age of up to 65 years old (Arena et al. 2007, Fowler & Booth, 2012, Simon et al. 2013). This ageing effect, however, is not the only prerequisite for similarity with a natural reef, as the fish species composition on a very (105 years) old metal ship wreck still was distinctly different from that of a nearby rock-based reef (Simon et al. 2013). It might be that the concrete building block reefs approach the natural reef more closely by providing a more complex and more elaborate shelter availability (Gratwicke & Speight 2005, Hylkema et al. 2020), which is absent in the usually courser built larger reefs. The difference in fish species composition between artificial and natural reefs is not necessarily problematic, but might be undesirable if the purpose of nature restoration is to come as close to the local reef community as possible, as most artificial reef communities differ in various ways from natural reef communities.

Benthic development on artificial reefs

Metal and concrete were the most frequently used materials for artificial reefs in the Caribbean, but none of these artificial reefs were monitored for benthic community development. Corals recruits can settle on metal (Fitzhardinge & Bailey-Brock 1989), so it is no surprise that corals colonize wrecks, both in the Caribbean (Vermeij 2005) and in the Indo-Pacific (Walker & Schlacher 2014). However, just as with fish assemblages, the coral communities on a three year old wreck (Walker & Schlacher 2014) and even on a more than 60 year-old wreck (Vermeij 2005) remained distinct from nearby natural reefs, as was the case with the sponge community (Pawlik et al. 2008). Although wrecks can last for a very long time (>100 year, Arena et al. 2007, Fowler & Booth 2012) the inevitable deterioration of metal surfaces results in rust and flaking, which ultimately inhibits recruitment and survival of sessile organisms

(Fitzhardinge & Bailey-Brock 1989). Interestingly, multiple studies from the Indo-Pacific reported changes in the benthic community of the natural reefs surrounding a metal wreck (Work et al. 2008, Kelly et al. 2012, Carter et al. 2019, van der Schyff et al. 2020). This has been suggested to be the result of iron leakage from the degrading wreck (Work et al. 2008, Kelly et al. 2012). The elevated iron levels in algae surrounding the wrecks (Kelly et al. 2012, van der Schyff et al. 2020) suggest that wrecks can locally alleviate iron limitation occurring on many Indo-Pacific reefs. Higher iron availability can explain observed increases of cyanobacteria (Kelly et al. 2012), turf algae (Kelly et al. 2012), corallimorphs (Work et al. 2008, Kelly et al. 2012) and macroalgae (van der Schyff et al. 2020), all of which can decrease coral cover (Work et al. 2008, Kelly et al. 2012, van der Schyff et al. 2020). Metal wrecks may thus cause iron-induced phase shifts in adjacent natural reefs, turning them into so called “black reefs” (Kelly et al. 2012). Although Caribbean coral reefs are assumed to not be iron-limited in general (Roff & Mumby 2012), it cannot be excluded that increased iron concentrations around degrading iron wrecks may also affect adjacent natural coral reefs in the Caribbean. This has not yet been studied. Furthermore, Indo-Pacific wreck reefs seem to facilitate non-indigenous species. This may happen directly, as organisms attached to the ship hull are placed in a new and disturbed environment after scuttling, or indirectly, if the metal substrate or the leaking iron favors different species than those living in an iron-limited environment. Also, a newly available surface may facilitate settlement of opportunistic species.

Just like metal, concrete is a suitable substrate for coral recruitment (Fitzhardinge & Bailey-Brock 1989, Burt et al. 2009b, Al-Horani & Khalaf 2013). Multiple studies in temperate regions show that concrete marine infrastructure not only exhibits a different community composition than their surrounding natural reefs (Glasby et al. 2007, Bulleri & Chapman 2010, Airoldi et al. 2015), but also harbors a higher number of non-indigenous species (Glasby et al. 2007, Airoldi et al. 2015). This is often attributed to the high initial surface pH of concrete, which would favor alkali-resistant species over others during early succession (Dooley et al. 1999, Guilbeau et al. 2003, Dennis et al. 2018). However, a systematic comparison showed that pH is no main driver of benthic abundance, species richness or species composition (Hsiung et al. 2020), indicating that other factors affect community development on concrete structures. It is not studied to what extent the differences with natural communities could decrease as ecological developement progresses, and opportunistic species disappear again during the course of succession.

High coral cover (Abelson & Shlesinger 2002) and coral recruit densities (Fox et al. 2005) were reported on natural rock piles in the Red Sea and Indonesia. Coral recruit densities on plates made from natural rocks (gabbro and granite) were higher than on most other materials, although experimental location appeared to have a greater effect than substrate (Burt et al. 2009b). Natural-rock breakwaters in the Arabian Gulf developed diverse benthic communities with high coral cover (Burt et al. 2009a, Burt et al. 2011, Grizzle et al. 2016), but the benthic communities were distinct from nearby natural reefs, although breakwaters of more than 25 years old resembled the natural ecosystem more closely than younger breakwaters (Burt et al. 2011).

Artificial reefs as environmental hazard

Although motor vehicle reefs can increase fish abundance (Bortone et al. 1988) and tire reefs can increase fish species richness (Haughton & Aiken 1989), these type of reefs are very unstable and are now considered an environmental hazard (Myatt et al. 1989, Lukens & Selberg 2004, Tessier et al. 2015). While tires can last for centuries, their ballast usually will not and once they become separated, they will start dispersing, potentially damaging coral reefs or littering the seabed habitat. In addition, tires have high heavy-metal concentrations, which can potentially end up in their epifouling organisms and in the food chain (Collins et al. 2002). In other parts of the world, tire reefs were even removed to prevent further damage to the benthic ecosystem (e.g. Morley et al. 2008), but we found no attempts to remove Caribbean tire reefs. Motor vehicles corrode within a couple of years and collapse before they are overgrown by organisms that can keep the structure upright (Lukens & Selberg 2004). Furthermore, they are mostly not cleaned before deployment and therefore can contaminate the local marine environment by leaching petroleum and heavy-metal toxicants (Aleksandrov et al. 2002, Collins et al. 2002, Lukens & Selberg 2004). Because of these drawbacks, no tire or motor vehicle reefs were deployed in the Caribbean anymore after 1986 (Tupper & Hunte 1998), in line with practices adopted in the rest of the world (Tessier et al. 2015).

Fisheries management of artificial reefs

Our study shows that 110 out of the 206 artificial reefs in the Caribbean are currently located outside MPAs and another 58 are located inside an MPA zone with fishing restrictions. Only 38 cases were located within an MPA zone prohibiting fishing. This means that over 80% of the artificial reefs described in this study are somehow fishable, and it is doubtful whether the fishing restrictions in the 38 other cases are truly enforced. In the Southern and Southwestern Caribbean, 100% of the artificial reefs were accessible for fishing, with most reefs being located outside an MPA. Fisheries on artificial reefs can affect both the ecology of the artificial reefs themselves and the ecology of the adjacent natural reefs. Fish presence on an artificial reef may result from local production at the artificial reef (the “Production Hypothesis”) but also from attraction of fish from neighboring areas (the “Attraction Hypothesis”; Bohnsack 1989, Grossman et al. 1997, Pickering & Whitmarsh 1997). The relative contribution of these factors depends on artificial reef size, isolation and age, amongst others, and it is now generally assumed that attraction will co-occur with reef productivity (Grossman et al. 1997, Pickering & Whitmarsh 1997).

As part of the marine life around an artificial reef is attracted from the nearby ecosystem, intensive exploitation of the fish around artificial reefs can potentially lead to depletion of the fish stocks in the surrounding ecosystems (Bohnsack 1989, Brock 1994). Even if artificial reefs are deployed for fishing, possibly also to alleviate pressure from natural reefs, fisheries management and fish monitoring of both the artificial and natural reefs are crucial to prevent indirect overfishing of the surrounding habitat. Unfortunately, we did not find a single artificial reef in the Caribbean of which the surrounding habitat was monitored for this purpose. Also data about fishing pressure on artificial reefs in the Caribbean are, to our knowledge, not available and it seems unknown how many of the artificial reefs that are accessible to fishers are actually fished upon. However, given the reports that Caribbean coral reefs are severely overfished (Hughes et al. 1994, Vermeij et al. 2019), that habitat degradation further reduced fisheries productivity (Rogers et al. 2014, 2018) and that artificial reefs are known and deployed for their high fishing potential (Brock 1994) we hypothesize that most of the artificial reefs in the Caribbean are subject to fishing. The many artificial reefs in the Caribbean that are deployed for diving are well marked, and therefore these also may well suffer from high, unregulated fishing pressure. This would also reduce the recreational value of the diving reefs.

Additionally, artificial reefs deployed with the purpose of increasing fishing yields seem to be underreported in the Caribbean. While increasing fishing yield is the most commonly stated objective of artificial reef programs worldwide (Baine 2001, Fabi et al. 2011, Becker et al. 2018, Lee et al. 2018), only 14 of the reported Caribbean artificial reefs were constructed to increase fishing yields. For example, of the lobster shelters or “casitas” that are commonly used by fishers from the Bahamas, Cuba and Mexico (Polovina 1991, Ramos-Aguilar et al. 2003) with sometimes over 120.000 shelters in a single bay (Polovina 1991), only 10 cases were described in grey and peer-reviewed literature, of which nine that were built for research. Fishers have little incentive to monitor, document or communicate their artificial reef use, unless they are obliged to do this by authorities or paid to aid research. Due to this underreporting of reefs deployed for fishing, the percentage of artificial reefs in the Caribbean accessible for fishing may actually be much higher than the 80% appearing from our study. The likelihood that artificial reefs are currently contributing to the regional general state of overfishing of reef resources is very high. To reduce this potentially very harmful effect of unregulated artificial reefs, more understanding and monitoring of the local reef productivity and the attraction effect are urgently needed. To enable sustainable fisheries, stricter protection and fisheries management around artificial reefs is needed, and monitoring and assessment of the indirect effect of fisheries on natural and artificial ecosystems is crucial. Given the current lack of monitoring and fisheries management, it can be concluded that fisheries on artificial reefs currently represent a serious risk to the ecology of artificial reefs as well as their adjacent natural reefs in the Caribbean.

Conclusions and recommendations for research and management

- Our review revealed that very few artificial reef deployments have been seriously studied and evaluated. Most of the published research of artificial reefs in the Caribbean concerned small experimental reefs, which creates a large discrepancy between the reefs studied and deployed at larger scales in practice. Comparative research is needed to better understand the mechanisms governing reef community development of multiple reef types.
- Due to the typically poor degree of documentation for artificial reefs, including the grey literature is essential to more accurately portray the actual deployment of artificial reef structures.
- Given its potentially adverse effect on the Caribbean coral reefs that already are severely under pressure, large scale deployment of artificial reefs should be considered with caution. Therefore the effects of leaking iron or the

introduction of non-indigenous species, as is reported in the Indo-Pacific, should be an additional research priority for the Caribbean.

- Artificial reefs typically start out having biodiversity and fish densities that equal that of natural reefs within a time frame of a couple of years. The species composition of fish and benthos often differs from those of natural reefs, although this difference may decrease with time.
- Research into new structural designs and materials that may better be able to mimic natural habitat may support development of more natural assemblages within shorter time frames, which may be desired to improve restoration technology. Even small adaptations in structure or materials can result in increased fish densities and species richness (Sherman et al. 2002, Brotto et al. 2006, Hylkema et al. 2020), indicating the potential for improvement of the artificial reef design for dedicated purposes.
- Ship wrecks hardly provide lasting reef structures and may facilitate non-indigenous species and effects on surrounding benthic communities through iron leakage. During hurricanes they can move or collapse on the seafloor causing additional damage to the communities surrounding them. Based on our review, the use of scuttled vessels for habitat restoration purposes should be discouraged.
- Given the paucity of research done on the large number of artificial reefs deployed by fishers without any form of documentation or registry, it is urgent to study positive or negative effects on marine habitats and marine flora and fauna. The use and deployment of fisheries reefs need to be closely monitored and managed at a national level as an integral part of the fisheries management plan. Without adequate scientific studies, and in light of deficient or totally lacking fisheries management, the added value of artificial reefs in terms of habitat and ecosystem restoration remains merely hypothetical and the reefs may presently do more harm than good, even to the region’s fisheries resources.
- Given their tendency to draw in fish from surrounding natural habitat which thus becomes more vulnerable to targeted overfishing, fishing activity on or around artificial reefs needs to be regulated and limited to prevent overfishing of the reef itself as well as the surrounding ecosystem. Such management needs to be supported by fish monitoring studies and no-take zones should be created around artificial reefs that are not monitored.

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Chapter 3

Fish assemblages of three common artificial reef designs during early colonization.

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3.1 Abstract

In this study, we compared the early fish colonization of three types of artificial reefs deployed in the coastal waters of Saba and St Eustatius in the Caribbean: reef balls®, layered cakes and piles of locally obtained basaltic rock. As an indicator of performance, three fish assemblage parameters (abundance, biomass, species richness) were measured using underwater visual censuses at 11 months post-deployment and 4 months after restoration from hurricane damage. All artificial reef plots showed higher values for fish abundance, biomass and species richness than control plots covered by bare sand, which shows that artificial reefs can locally enhance the fish assemblage. However, the effect differed among artificial reef plots. Fish abundance was 3.8 times higher on the layered cake plots compared to the reef ball plots, while fish biomass was 4.6 times higher. Rock pile plots had intermediate values. Species richness did not differ significantly among different artificial reef plots. Three-dimensional modelling revealed that layered cakes had a smaller gross volume, shelter volume and total surface area than reef balls. The availability of multiple small shelters in the layered cake design appeared to be more relevant than other physical parameters, as the layered cake plots had higher fish abundance than the reef balls plots. We concluded that on Saba and St. Eustatius, layered cake plots performed better than reef ball plots after one year of colonization. Rock pile plots, made of local volcanic rock, showed an intermediate performance, and were 4-10 times cheaper to construct. If observed differences are consistent with other locations and persist during further colonization, current efforts to deploy reef balls could better be allocated to deploy artificial reef structures with a higher shelter density.

Keywords:

Reef ball®, layered cake, fish abundance, shelter availability, habitat restoration, artificial reef

3.2 Introduction

Coral reefs are among the most productive ecosystems on earth (e.g. Odum & Odum 1955) and millions of people depend on their ecosystem services (Moberg & Folke 1999). These services are partly the result of the reef's complex framework, which provides a three-dimensional habitat with many niches and refuges from predation. Reef-building corals, the main architects of the reef framework, are adversely affected by local stressors and climate change and their abundance is declining worldwide (Bellwood et al. 2004). In the Caribbean, the degradation of coral reefs began in earnest in the 1980s (Hughes 1994), when diseases decimated the most important herbivore *Diadema antillarum* (Lessios et al. 1984) and dominant reef building corals of the genus *Acropora* (Gladfelter 1982, Aronson & Precht 2001). The ecological extinction of *Diadema* and *Acropora* led to an increase in macroalgae (Hughes 1994, Jackson et al. 2014) and cyanobacterial mats (De Bakker et al. 2016), both groups inhibiting coral recruitment and preventing natural recovery of the reefs (McCook et al. 2001). Other threats, such as hurricanes and periods of higher than average seawater temperatures, further reduced Caribbean coral cover, until by 2014 less than 20% remained (Gardner et al. 2003, Jackson et al. 2014). The large-scale disappearance of corals as the main ecosystem engineers of reefs resulted in a substantial loss of three-dimensional structure (so-called "flattening" of the reef) (Alvarez-Filip et al. 2009). Without its three-dimensional framework, refuge opportunities are reduced and this resulted in a measurable reduction of fish abundance (Paddack et al. 2009), biomass (Rogers et al. 2014), biodiversity (Newman et al. 2015) and fisheries productivity (Rogers et al. 2014, 2018) of Caribbean coral reefs.

The reduced productivity of reef fish and fisheries is not exclusively a Caribbean issue, but a worldwide concern (Graham et al. 2007, Newton et al. 2007). However, the relatively low biodiversity of Caribbean coral reefs makes them even more vulnerable to ecological and economical degradation than their Indo-pacific counterparts, as most functional groups are only represented by one or two species (Bellwood et al. 2004). The degradation of Caribbean coral reefs is severe and threats are prominent, with as a consequence that reefs are practically unable to recover naturally (Goreau & Hilbertz 2005, Mumby & Steneck 2008). Without active intervention and management, coral reefs might not be able to sustain the ecosystem services that millions of people are dependent on (Bellwood et al. 2004). One of the possible intervention methods is the deployment of artificial reefs, structures that are placed on the seabed to mimic certain characteristics of the natural reef ecosystem and help restore the habitat function (Baine 2001). Artificial reefs can instantly increase three dimensional structure and are often used to restore or enhance fish populations or fisheries productivity (Baine 2001, Seaman 2007, Becker et al. 2018). Part of the fish colonizing artificial reefs are the result of enhanced productivity, while others are attracted from neighbouring areas (Grossman et al. 1997, Pickering & Whitmarsh 1997).

Artificial reefs can be constructed from different materials and in multiple designs (Baine 2001, Becker et al. 2018, Lima et al. 2019), which result in a broad variety of artificial reefs currently being deployed. Reef balls ® are one of the most applied artificial reef types (Lima et al. 2019) and over 600,000 reef balls have been deployed worldwide (reefballfoundation.org). The fish assemblages around reef balls are relatively well studied (Sherman et al. 2002, Brotto et al. 2006, Dos Santos et al. 2010, Folpp et al. 2013, Mills et al. 2017). Adding more refuges to the reef ball design, by placing concrete blocks in the central void space (Sherman et al. 2002) or adding extra holes (Brotto et al. 2006) resulted in a higher fish abundance and species richness, indicating that increased shelter availability will support a greater fish diversity on the artificial reefs. Studies in which small experimental reefs were used, confirmed that more shelter availability resulted in a higher fish abundance and species richness (Hixon & Beets 1989, Gratwicke & Speight 2005, Lingo & Szedlmayer 2006). These studies used piles of concrete building blocks or oyster shells, which are easy to deploy and modify, but are unstable over time (Ogden & Ebersole 1981) and therefore not suitable for large scale application. Comparative studies including multiple artificial reef designs that are also used for other purposes than research are scarce (Sherman et al. 2002, Brotto et al. 2006, Hackradth et al. 2011) and totally lacking for the Caribbean. Such comparisons are essential, as they give conservationists, marine park managers, fisheries departments and researchers the opportunity to make science-based choices in the deployment of artificial reefs.

Despite strong indications that the reef ball design may need improvement (Sherman et al. 2002, Brotto et al. 2006), no follow-up studies have been performed and reef balls remain more often-used than alternative designs that provide more shelter opportunities (Lima et al. 2019). One of these alternatives is the layered cake design, which is made with the same outer mold as the reef ball, but has a higher shelter availability. To our knowledge, the layered cake design was never included in any comparative study. The most simple and cheap to construct alternative is to use a pile of rocks. Studies investigating rock pile reefs show high fish (Abelson & Shlesinger 2002) and coral (Abelson & Shlesinger 2002, Fox et al. 2002) densities and conclude that applying rock pile reefs, if available, may be an inexpensive and effective way to restore coral reefs. However, none of these studies compared the fish assemblage of rock pile reefs with alternative artificial reef designs.

The current study aims to compare the fish assemblages of the three different artificial reef designs introduced above: reef balls, layered cakes and rock piles. The reef ball and layered cake designs have a similar gross volume, but are very different in shelter availability. Rock piles have an intermediate shelter availability and are relatively easy and cheap to construct from natural material (rock instead of concrete). As fish colonization of artificial reefs starts immediately after deployment and fish assemblages on small artificial reefs can be stabilized within 150 days (Yeager et al. 2011), fish assemblage descriptors such as abundance, biomass and species richness are useful indicators for the performance of an artificial reef. We hypothesize that based on their higher shelter availability, layered cakes and rock piles will have higher fish abundance, biomass and species richness than reef balls.

3.3 Methods

Construction of artificial reefs

In May 2017, artificial reefs were deployed at 4 locations in the waters surrounding Saba and St. Eustatius, Dutch Caribbean (Figure 1). The locations, Ladder bay (LB) and Big rock market (BRM) on Saba and Twin sisters (TS) and Crooks castle (CC) on St. Eustatius, were selected according to the following criteria: a sandy bottom along the edge of a natural reef, between 12 and 18 m depth and with limited slope. On all locations, 4 plots were set out with a 25 m interval, at 5 m distance from the natural reef. Four different treatments (reef balls, layered cakes, a rock pile and a control plot on bare sand) were randomly assigned to the plots on each location. Two extra rock pile reefs were deployed on the Saba locations.

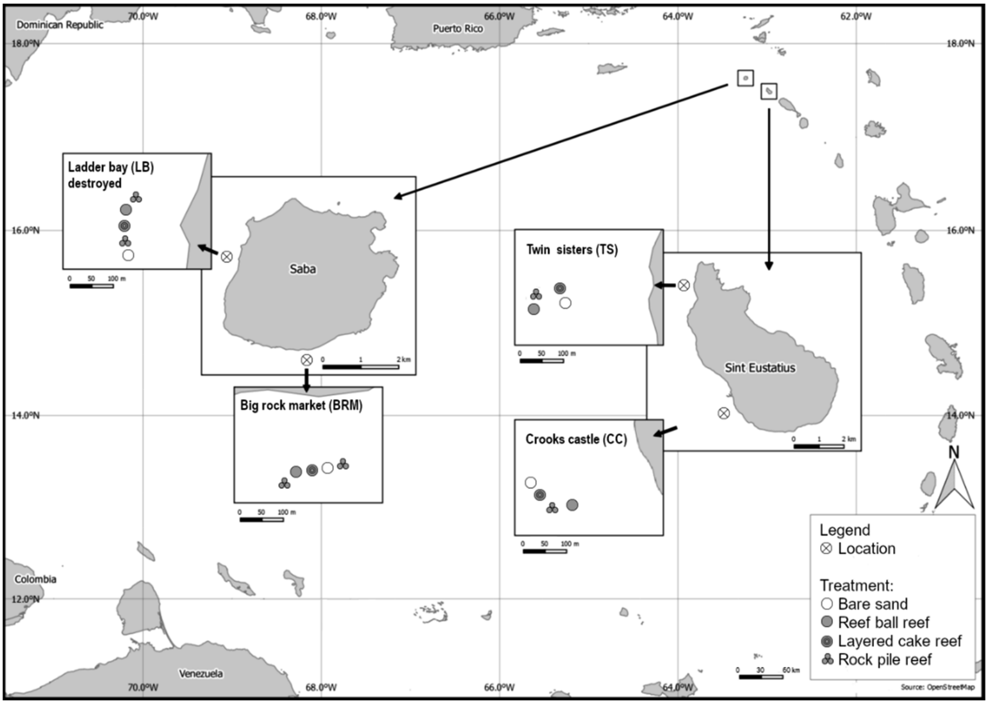

Figure 1: Locations of the experimental plots around Saba and St. Eustatius. All plots at the LB location were covered with sediment during swells and were not included in this study.

Each reef ball or layered cake plot, was composed of respectively 3 reef ball or layered cake units. Reef balls and layered cakes were constructed from concrete using a mold designed for this purpose (Reef ball Foundation, Athens, USA, www.reefball.org). Reef balls have one central void with multiple openings, while layered cakes have different layers of concrete with multiple low, yet contiguous shelters in between (Figure 2). Each reef unit had a bottom diameter of 90 cm, a height of 60 cm high and a weight between 300 and 450 kg. Three units, each covering an area of 0,64 m^2, were placed close together forming one reef plot of approximately 2 m^2. The rock piles were made from natural, previously unweathered basaltic rocks from Saba and St. Eustatius and each rock weighted between 30 and

50 kg. Rock piles were constructed atop an iron concrete wire mesh to evenly distribute the weight of individual rocks (Figure 2C). Rock piles were designed to cover the same seafloor surface area (160x125 cm) and to have the same height (highest point 60 cm) as the other reefs. Habitat architecture differed between rock pile plots and other reef plots, as rock piles formed a single reef while reef ball and layered cake plots consisted of multiple units. Also, the chemical constituency of the used material was different for the rock piles compared to the other two reef plots. These differences were considered part of the specific designs and were therefore not corrected for.

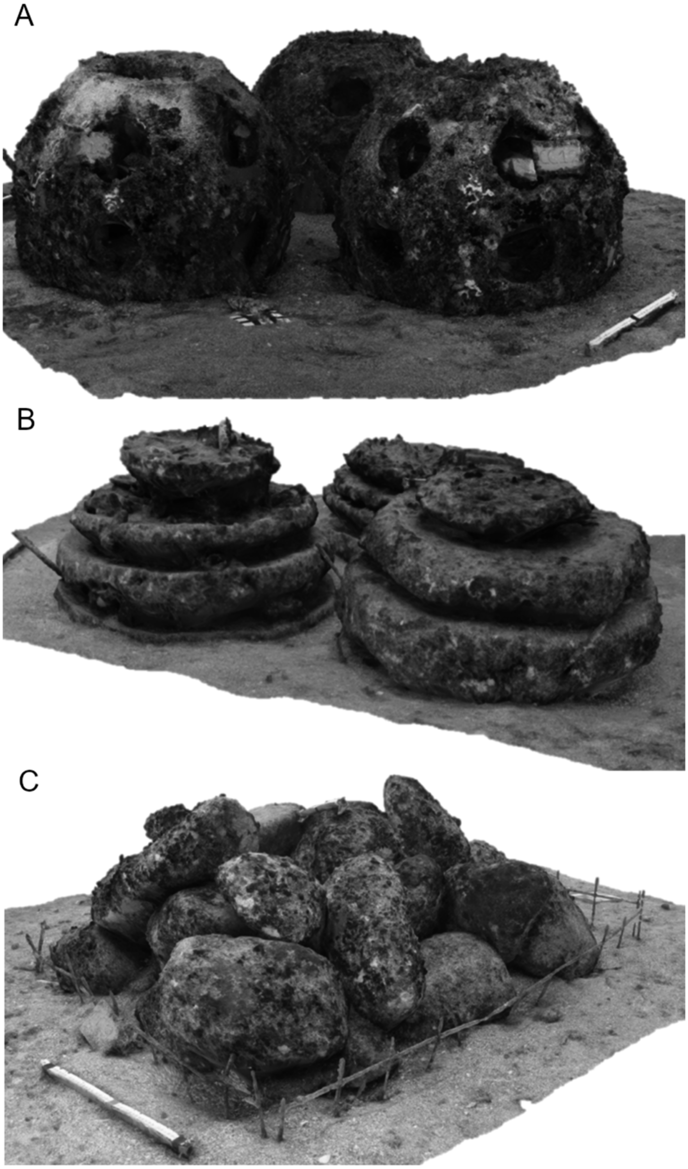

Figure 2: Three different artificial reef design plots, which were compared in this study: reef ball (A), layered cake (B) and rock pile (C) plot. Each plot covers approximately 2 m^2 seafloor area.

In September 2017, hurricanes Irma and Maria hit the islands of Saba and St. Eustatius, resulting in high waves and considerable sediment movement. All artificial reefs became at least partially buried in the sand and became ecologically ineffective. In December 2017, all artificial reefs were cleared of sand and repaired if necessary, after which fish colonization started again. In February 2018, unusual big swells relocated so much sediment that all plots at the LB location were entirely buried under sand. As restoration was not possible, this location was not surveyed and excluded for the remainder of this study. All other locations were unaffected by the swells.

Fish assemblage monitoring

During the months April and May 2018 (11 months post-deployment and 4 months after restoration from hurricane damage), each plot was surveyed 10 times using underwater visual censuses (UVC). Surveys were spread over the two months and the interval between successive surveys was minimally 48 hours. UVC were performed using a modification of the stationary point count (Bohnsack & Bannerot 1986, Lowry et al. 2012), followed by a systematic search of the structures. All surveys were conducted by two researchers using SCUBA. One researcher recorded the fish on underwater paper, while the other filmed the survey for future reference. During each survey, the researchers approached the plot horizontally and started recording fish fleeing from the plot as soon as the structure was within 5 meters. All fish within a virtual cylindrical column, extending 1 meter sideways of the plot and extending 2 meters upward from the bottom were included in the survey. At two meters from the artificial reef, the observers stopped swimming and started the stationary count, first recording all schools and then recording all other fish (Bohnsack & Bannerot 1986). All fish were identified up to species level, counted and categorized using visual estimation of total length (TL) in size classes 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-40, 40-50 and 50+ cm. After three minutes, the stationary point count ended and new fish entering the column were not included in the survey. Subsequently, the plot was thoroughly searched to record all fish residing within the internal spaces of the artificial reef. The survey ended after all fish, with the exception of small (<5 cm TL) cryptic species such as blennies and gobies, had been recorded.

Reef plot modelling to determine physical parameters

For reproducibility, a thorough and quantitative description of the reefs to be compared is essential, but typically absent in most studies. To address this issue, all reef plots on the CC location were three-dimensionally modelled using a diver-held imaging system composed of a DSLR camera (Nikon D850 with a Nikkor 35 mm lens) and four strobes (INON Z-240). Images were acquired at 1 Hz using the camera’s “intervalometer” while circling around the structure to obtain as many angles of view from a constant distance of 1-2 m. For each reef plot, 70-140 images were used to generate a 3D mesh and texture in Agisoft Metashape (Professional Version 1.5). The models were scaled using a measuring stick placed in the scene prior to image acquisition. To measure the gross volume, the total outer volume including shelters, we used a convex hull function in Meshlab (Cignoni et al. 2008).

To measure the total surface area, the combined surfaces of the reef structures (outside and inside) and the sand in between structures within the plot, and net volume, the gross volume excluding shelters, we used the software's "Measure area and volume" function. The shelter volume of each reef plot was obtained by subtracting the net volume from the gross volume.

Cost estimation

The costs to construct a single plot were estimated based on the used materials and the time spend to construct the plots. An hourly wage of $ 20.00 was used to calculate labor costs. As certain materials can be reused and upscaling would reduce the price per unit, we also estimated the costs per 10 plots. We did not include boat use or monitoring costs, as these did not differ between treatments and are highly variable throughout the world, and because monitoring is not always part of an artificial reef program.

Data analysis

Fish abundance (per species, family, trophic group, size class and total) was summed per survey and averages per treatment were calculated using all 30 or 40 surveys (considering 10 surveys per plot on 3 locations and an extra rock pile plot on 1 location). Six major trophic categories (planktivores, herbivores, invertivores, omnivores, carnivores and piscivores) were distinguished, following the classification used by Paddack et al. (2009) and Alvarez-Filip et al. (2011). As only 4 fish greater than 40 cm were observed (1 *Gymnothorax moringa* of 40-50 cm on the layered cakes, 1 *Sphyraena barracuda* of 60-70 cm above the layered cakes and 2 *Gymnothorax moringa* of 60-70 in the rock piles) these recordings were combined into the size class 40+. Fish biomass per treatment was calculated by summing the weight of all species present and averaging total biomass over the 10 surveys. The weight of all species and all size classes was calculated using the length-weight relationship $W = a * TL^b$, where W is the weight in grams, TL is the average total length of the size class in cm, while a and b are species-specific constants obtained from literature (Froese & Pauly 2019). If a and b values were not available, parameters of closely-related species with a similar shape and maximum length were used. If fork length (FL) was needed for the length-weight relationship, a species-specific TL-FL ratio was used (Froese & Pauly 2019). A total of 31 *Heteroconger longissimus* were excluded from the biomass analysis, because they were always observed in their sand burrows and their length could not be estimated. Fish species richness (S) was obtained by summing the total number of species observed during 10 surveys. An average S per treatment was calculated using the 3 or 4 replicates per treatment.

Statistical analyses were performed with R (R Core Team 2019) using R studio version 1.1.463. Generalized Linear Mixed Models (GLMM) with a negative binomial error distribution (lmer.nb function in the R package "lme4") (Bates et al. 2015) were used to test whether fish abundance was affected by treatment or location (fixed factors). To control for the 10 repeated surveys per plot, surveys were included as a random factor. Model selection and validation was performed according to Bolker et al. (2009). The Akaike Information Criterion (AIC) was used to select the best fitting model, which was the model including both treatment and location. Pearson’s residuals were summed to test for over-dispersion. This was the case when a Poisson distribution was used, but was solved by using a negative binomial distribution. Likelihood ratio tests (LRT) were performed for statistical inference of the fixed factors using the drop1 function. Linear mixed models (LMM, lmer function in the R package “lme4”) (Bates et al. 2015) were used to test whether fish biomass was affected by treatment or location (fixed factors). Model selection and validation was performed according to Zuur et al. (2009); the model including both treatment and location had the lowest AIC and was used for further analysis. To control for the 10 repeated surveys, individual reefs were included as a random factor. Residuals of the initial model indicated heteroscedasticity, which was solved after the data were cube-root transformed. For statistical inference, an F-test with Kenward-Roger’s approximation to degrees of freedom was performed using the R packages “lmerTest” (Kuznetsova et al. 2017) and “pbkrtest” (Halekoh & Højsgaard 2014). As species richness only has one value per reef (the total number of species found during 10 surveys), Generalized Linear Models (GLM) with a Poisson distribution were used, including treatment and location as fixed factors. Model selection and validation for GLM was performed according to Zuur et al. (2009); the model including both treatment and location had the lowest AIC. Wald $\chi 2$ tests were performed for statistical inference of the fixed factors (Bolker et al. 2009), using the Anova function of the “car” package (Fox & Weisberg 2019). For all final models Tukey’s post-hoc tests were conducted to examine significance of treatment and location using estimated marginal means (EMM) from the package “emmeans” (Lenth & Herve 2019).

The package “mvabund” (Wang et al. 2020) was used to test whether treatment and location affected the composition of fish species, family, trophic group or size class. The “manyglm” function of this package was used to fit a multivariate GLM, taking the strong mean-variance relationship of abundance data into account (Warton et al. 2012). As it is not possible to include a random factor in this function, data of all surveys per reef were aggregated. Species or families that occurred on fewer than 3 plots were excluded from this analysis, as these contained little information. We first fitted main models and selected the best fitting model based on AIC; this was the model including both treatment and location for all composition descriptors. Residuals were plotted to examine if the model assumptions were met, which was the case when negative binomial distributions were used. Univariate GLMs were then used to asses which taxa or groups drove the main effects. Pairwise comparisons, adjusted for multiple testing (Wang et al. 2012), were conducted to assess which treatment or location had a significant different composition. P values <0.05 were considered statistically significant and reported values are means ± sd.

3.3 Results

In total, 2102 fish representing 48 species were observed during 130 surveys. Treatment (LRT=18.67, df=3, P<0.001) and location (LRT=6.164, df=2, P=0.046) were significant predictors of fish abundance (Figure 3). Layered cake plots had an average abundance of 36.7 ± 14.3 fish, which was significantly higher than the 9.6 ± 7.0 fish on the reef ball plots (P=0.0044). Rock pile plots had an average abundance of 15.5 ± 8.3 fish and neither differed significantly from layered cake (P=0.3018) nor from reef ball (P=0.2672) plots. Fish abundance on all artificial reef plots was significantly higher than the fish abundance on control plots with bare sand (P<0.0001 for layered cake and rock pile plots and P=0.0175 for reef ball plots), which had an average abundance of 3.1 ± 3.7 fish. The only significant difference in fish abundance for combined reef plots at a location was that CC had a significantly higher fish abundance than location BRM (P=0.0353).

Treatment (F=22.05, df=3, P=0.006) and location (F=9.23, df=2, P=0.011) were significant predictors for fish biomass (Figure 3). Layered cake plots had an average fish biomass of 1434 ± 1287 g, which was significantly higher than the average biomass on the reef ball plots (309 ± 273 g fish; P<0.0142) and the control plots with bare sand (35 ± 78 g fish, P=0.0005), but not significantly different from the rock pile plots (459 ± 273 g fish, P=0.1169). The average fish biomass on the rock pile and reef ball plots did not significantly differ from each other (P=0.2980), but differed significantly from the average fish biomass on bare sand (P=0.0381 for reef ball plots and P=0.0033 for rock pile plots). Location BRM had a significantly lower fish biomass for all plots there than location TS (P= 0.0105), but did not differ from location CC. Locations CC and TS did not differ in fish biomass.

Treatment (χ^2=40.15, df=3, P<0.001) and location (χ^2=15.20, df=2, P<0.001) were significant predictors for species richness (S, Figure 3). Average S did not differ among the three artificial reef plots, but all artificial reef plots had a significantly higher S than the control plots with bare sand (P<0.0001, P=0.0001, P=0.0018 for layered cake, rock pile and reef ball plots, respectively). Location BRM had a significantly lower S than locations TS (P= 0.0009) and CC (P= 0.0080). Locations CC and TS did not differ in S.

Average abundance per fish species followed the general trend in fish abundance and most fish species had the highest abundance on the layered cake plots, followed by the rock pile plots, reef ball plots and control plots with bare sand (Table 1). Bluehead wrasses *Thalassoma bifasciatum*, were the most abundant species on all treatments and accounted for 32% of all observations. Fish species composition was affected by location (sum-of-LR=274, df=2, P=0.0001), but not by treatment (sum-of-LR=162, df=3, P=0.063). The key species that drove the location effect were Princess parrotfish, *Scarus taeniopterus,* Redband parrotfish, *Sparisoma aurofrenatum,* Slippery dick, *Halichoeres bivittatus*, Spotted goatfish, *Pseudupeneus maculatus*, and Sergeant major, *Abudefduf saxatilis*. The only species that differed significantly in abundance between treatments was the Coney, *Cephalopholis fulva*. When pairwise comparisons were conducted between locations, none of the comparisons were significant, indicating that the location effects were not very large.

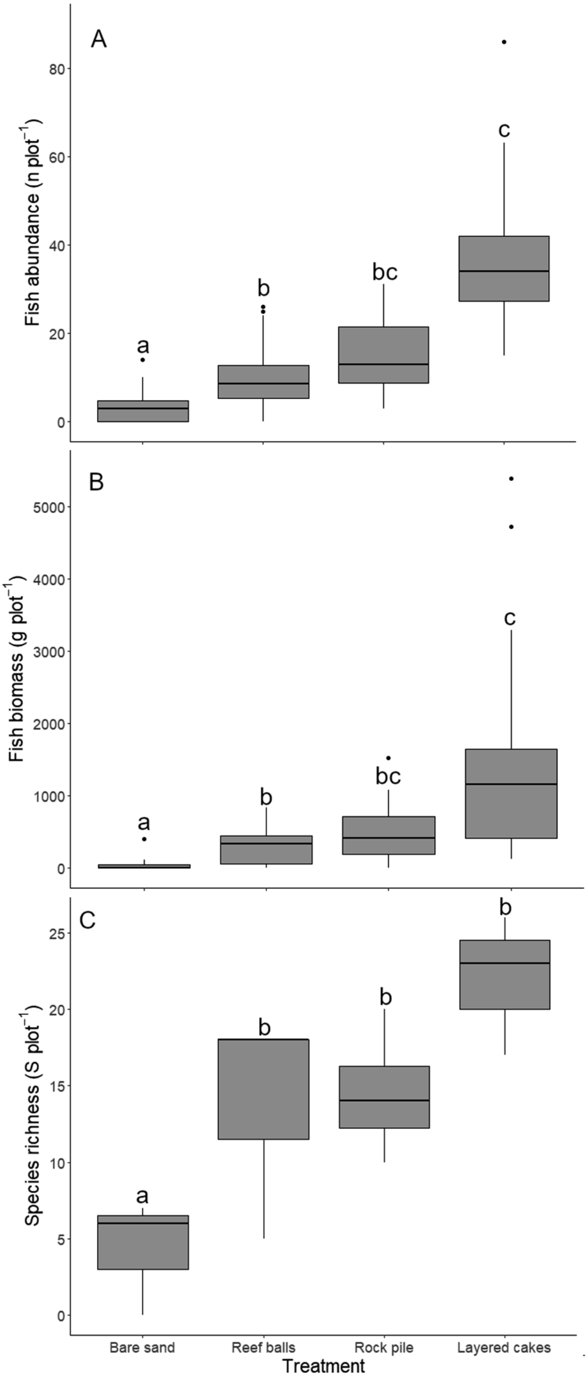

Figure 3: Fish abundance (A), fish biomass (B) and species richness (C) per treatment. The boxplots show the median (black line), the first and third quartiles (grey shaded box), and the lower and upper extremes, black dots represent outlying values (>1.5 inter-quartile range from third quartile). Treatments sharing the same letter are not significantly different (P>0.05).

Table 1: Average fish abundance (n) (±SD) of 20 most common fish species, the sum of all 28 other species and the total average per treatment. Species are sorted based on their overall abundance. *As according to Paddack et al. (2009) and Alvarez-Filip et al. (2011).

				Average fish abundance (n plot^{-1})			
Name	Common name	Family	Trophic group*	Bare sand	Reefball	Rock pile	Layered cake
Thalassoma bifasciatum	Bluehead wrasse	Labridae	Planktivore	0.4±1.1	3.9±4.4	5.4±6.4	10.7±7.8
Holocentrus adscensionis	Squirrelfish	Holocentridae	Invertivore	0.0±0.0	0.3±0.5	0.5±0.5	4.9±3.7
Halichoeres garnoti	Yellowhead wrasse	Labridae	Invertivore	0.0±0.0	0.5±0.8	2.1±4.1	0.8±1.2
Stegastes partitus	Bicolor damselfish	Pomacentridae	Herbivore	0.1±0.3	0.9±1.0	0.9±1.1	1.5±1.0
Myripristis jacobus	Blackbar soldierfish	Holocentridae	Omnivore	0.0±0.0	0.3±0.5	0.1±0.2	3.1±2.4
Halichoeres bivittatus	Slippery dick	Labridae	Invertivore	0.6±1.4	1.2±2.2	0.7±1.9	0.1±0.3
Sparisoma aurofrenatum	Redband parrotfish	Scaridae	Herbivore	0.1±0.3	0.2±0.5	0.5±0.8	1.6±1.7
Acanthurus tractus	Ocean surgeonfish	Acanthuridae	Herbivore	0.0±0.2	0.2±0.5	1.0±1.4	0.7±0.8
Chromis multilineata	Brown chromis	Pomacentridae	Planktivore	0.0±0.0	0.0±0.0	0.0±0.0	2.1±3.5
Cephalopholis fulva	Coney	Serranidae	Carnivore	0.0±0.0	0.2±0.5	0.6±1.3	1.0±1.0
Scarus taeniopterus	Princess Parrotfish	Scaridae	Herbivore	0.0±0.0	0.1±0.5	0.3±0.8	1.4±3.8
Chromis cyanea	Blue chromis	Pomacentridae	Planktivore	0.0±0.0	0.0±0.0	0.0±0.0	1.8±3.5
Haemulon flavolineatum	French Grunt	Haemulidae	Invertivore	0.0±0.0	0.0±0.0	1.3±2.1	0.0±0.0
Haemulon aurolineatum	Tomtate	Haemulidae	Invertivore	0.1±0.3	0.1±0.4	0.5±1.4	0.7±1.9
Apogon maculatus	Flamefish	Apogonidae	Invertivore	0.0±0.0	0.0±0.0	0.1±0.5	1.3±2.8
Acanthurus coeruleus	Blue tang	Acanthuridae	Herbivore	0.0±0.0	0.2±0.4	0.4±0.7	0.6±1.5
Abudefduf saxatilis	Sergeant major	Pomacentridae	Omnivore	0.0±0.0	0.3±0.6	0.1±0.2	0.7±1.4
Heteroconger longissimus	Brown garden eel	Congridae	Planktivore	1.0±1.5	0.0±0.0	0.0±0.0	0.0±0.0
Pseudupeneus maculatus	Spotted goatfish	Mullidae	Invertivore	0.1±0.4	0.2±0.5	0.2±0.4	0.3±0.5
Bodianus rufus	Spanish hogfish	Labridae	Invertivore	0.0±0.0	0.0±0.0	0.2±0.6	0.4±1.3
28 other species				0.7±1.3	1.0±0.9	0.9±1.1	3.2±2.3
Total				3.1±3.7	9.6±7.0	15.5±8.3	36.7±14.3

The species observed in the present study belonged to 27 families (Table 2). Overall, Labridae were the most frequently observed family on all treatments, accounting for 43% of all fish observations. Fish family composition was affected by treatment (sum-of-LR=103, df=2, P=0.020) and location (sum-of-LR=119, df=3, P=0.005). The key family driving the effect of treatment were Serranidae, while Scaridae and Mullidae drove the effect of location. Pairwise comparisons revealed that these effects were not significant when adjusted for multiple testing, indicating that the effect was not very large.

Table 2: Average (n±SD) fish abundance of 10 most common fish families, the sum of all 17 other families and the total average per treatment. Families are sorted based on their overall abundance.

	Average fish abundance (n plot^{-1})			
Family	Bare sand	Reef ball	Rock pile	Layered cake
Labridae	1.6±3.4	5.5±6.2	8.4±9.9	12±8.2
Holocentridae	0.0±0.0	0.7±1.0	0.6±0.6	8.0±4.9
Pomacentridae	0.1±0.3	1.2±1.1	1.0±1.2	6.1±5.9
Scaridae	0.1±0.4	0.3±0.8	0.9±1.4	3.2±5.2
Acanthuridae	0.0±0.2	0.5±0.8	1.5±1.8	1.6±1.9
Haemulidae	0.1±0.3	0.1±0.4	1.7±2.3	0.7±1.9
Serranidae	0.0±0.0	0.3±0.5	0.7±1.4	1.5±1.7
Apogonidae	0.0±0.0	0.0±0.0	0.1±0.5	1.3±2.8
Congridae	1.0±1.5	0.0±0.0	0.0±0.0	0.0±0.0
Mullidae	0.1±0.4	0.2±0.5	0.2±0.4	0.3±0.5
Other	0.0±0.2	0.8±0.6	0.5±0.9	2.1±2.0
Total	3.1±3.7	9.6±7.0	16±8.3	37±14

Average abundance of the six major trophic groups (planktivores, herbivores, omnivores, invertivores, carnivores and piscivores) was highest at the layered cake plots (Table 3), lowest on the bare sand plots and significantly influenced by treatment (sum-of-LR=57 P=0.009), but not by location (sum-of-LR=25 P=0.194). This effect was mainly driven by the abundance of carnivores, although pairwise comparisons revealed no significant differences between treatments.

Table 3: Average fish abundance (n) (±SD) per trophic group and in total per treatment. Trophic groups, according to Paddack et al. (2009) and Alvarez-Filip et al. (2011), were sorted based on their overall abundance.

	Average fish abundance (n plot^{-1})			
Trophic group	Bare sand	Reef ball	Rock pile	Layered cake
Planktivore	1.4±1.6	3.9±4.4	5.4±6.4	15±8.4
Invertrivore	1.4±2.7	3.0±2.4	5.7±3.7	9.5±7.8
Herbivore	0.2±0.6	1.7±1.6	3.3±2.6	6.6±6.1
Omnivore	0.0±0.0	0.7±1.0	0.1±0.4	3.9±3.3
Carnivore	0.0±0.0	0.3±0.5	0.8±1.6	1.5±1.7
Piscivore	0.0±0.2	0.1±0.3	0.1±0.3	0.2±0.5
Total	3.1±3.7	9.6±7.0	16±8.3	37±14

On all artificial reef plots, the 0-5 fish size class was most recorded, while the size class of 5-10 cm was most dominant on the bare sand control plots (Table 4). Fish in the size classes of 25-30 cm and bigger were scarce on all reef plots. Size class composition was significantly affected by treatment (sum-of-LR=80 P=0.010) and location (sum-of-LR=81 P=0.009). These effects were driven by the size classes 0-5 cm and 15-20 cm, respectively. Pairwise comparisons showed no significant differences between treatments or locations.

Table 4: Average fish abundance (n) (±SD) per size class and in total per treatment. *The size class unknown consisted entirely of Brown garden eel Heteroconger longissimus, which were always observed in their burrows.

	Average fish abundance (n $plot^{-1}$)			
Size class	Bare sand	Reef ball	Rock pile	Layered cake
0-5	0.1±0.3	3.8±3.1	8.1±8.5	18±8.9
5-10	1.6±3.3	2.7±4.9	1.3±2.4	2.4±2.4
10-15	0.2±0.4	0.9±1.4	2.5±2.5	5.0±3.7
15-20	0.1±0.4	1.1±1.4	2.8±3.0	8.9±8.6
20-25	0.1±0.4	0.7±1.0	0.7±0.9	2.5±3.9
25-30	0.0±0.0	0.3±0.4	0.1±0.4	0.1±0.3
30-35	0.0±0.0	0.0±0.2	0.0±0.2	0.1±0.3
35-40	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0
40+	0.0±0.0	0.0±0.0	0.1±0.2	0.1±0.3
Unknown*	1.0±1.5	0.0±0.0	0.0±0.0	0.0±0.0
Total	3.1±3.7	9.6±7	16±8.3	37±14

The gross and net volume of the reef ball plots was higher than of the other plots (Table 5). Layered cake plots had the lowest gross volume, while the rock pile plot had the lowest net volume. Layered cakes were made using the same mold (i.e. with the same outer volume) as the reef balls. However, four rocks, functioning as legs, were placed at the bottom of the mold during the construction of every layered cakes. These legs made it easier to deploy the layered cakes, but the legs sunk in the sand after deployment, reducing the gross volume of this design. Shelter volume of the reef ball plots and the rock pile plots was the same, while the shelter volume of the layered cake plots was less than half the size of the other designs. The total surface area covered by the reef ball plots was highest, followed by the layered cake plots and the rock pile plots.

Table 5: Physical parameters of reef ball, layered cake and rock pile plots.

Physical parameter	Reef ball plot	Rock pile plot	Layered cake plot
Gross volume, including shelter (m^3)	1.14	0.98	0.78
Net volume, excluding shelter (m^3)	0.63	0.47	0.55
Shelter volume (m^3)	0.51	0.51	0.23
Total surface area (m^2)	12.23	7.39	8.14
images (n)	131	77	143

The total costs per plot were highest for the layered cake and reef ball plots, while rock pile plots were 10 times cheaper to construct (Table 6). The small difference between layered cake and reef ball plots was due to more material used and more labour needed for construction of layered cake units. Scaling up efforts resulted in a large reduction in costs for a single layered cake or reef ball unit, as molds and lift bags can be reused, but rock piles were still 4 times cheaper.

Table 6: Estimated costs, excluding boat and monitoring costs, in USD for a single and 10 reef ball, layered cake and rock pile plots. *One-time expenses.

Costs	Reef ball plot 3 units	Rock pile plot 27 rocks	Layered cake plot 3 units
Mold*	$ 1,350	-	$1,350
Lift bag for deployment*	$ 700	-	$ 700
Material	$ 78	$ 23	$ 100
Construction	$ 360	-	$ 420
Deployment	$ 360	$ 240	$ 360
Total per plot	$ 2,848	$ 263	$ 2,930
	30 units	270 rocks	30 units
Total per 10 plots	$ 9,927	$ 2,625	$ 10,850

3.5 Discussion

Our analysis shows that all artificial reef plots had a higher fish abundance, biomass and species richness than controls plots of only bare sand. This is no surprise, as any addition of hard substrate on bare sand habitat generally results in an increase in habitat volume and shelter availability, crucial for fish abundance and species richness (Gratwicke & Speight 2005). However, the magnitude of this increase differed greatly depending on the type of artificial reef. Plots with layered cake structures had a higher fish abundance and biomass compared to reef ball plots, while rock pile plots had intermediate fish assemblage parameters. This confirmed our hypothesis that structures that provide more shelter spaces will result in higher fish abundance and biomass. This is in line with previous research, indicating that the fish assemblage is positively affected by shelter availability (Hixon & Beets 1989, Sherman et al. 2002, Gratwicke & Speight 2005, Brotto et al. 2006, Lingo & Szedlmayer 2006 Hackradth et al. 2011). Compared to the layered cake plot, the reef ball and rock pile plots had twice as much total shelter volume than the layered cake plots. Clearly, the central void space of a reef balls provides a single large shelter opportunity, but this is not ideal for attracting big fish assemblages of especially smaller fish. Filling the void space of reef balls with concrete building blocks has been shown to increase the number of fish (Sherman et al. 2002). The rock pile plots had the same total shelter volume as the reef ball plots, but provided many more shelter spaces. Because of the complex nature and connectedness of the rock pile shelters, it was not possible to determine the number of shelters. The high shelter volume combined with a similar total surface area indicates that the shelters of the rock piles were bigger than those in the layered cakes.

Three-dimensional modelling revealed that the reef ball plot had the highest gross volume, followed by the rock pile plot. Despite that artificial reef size is usually positively correlated with fish abundance (Tupper & Hunte 1998, Abelson & Shlesinger 2002) the plots with layered cakes (smaller gross volume) performed better in terms of fish abundance and biomass than the reef ball plots and comparable to the rock pile plot.

Total surface area per bottom area is often used to describe rugosity (Luckhurst & Luckhurst 1978) and is known to positively affect fish abundance on artificial reefs (Gratwicke & Speight 2005). Gratwicke & Speight (2005) kept the shelter availability constant while modifying the rugosity, clearly showing that rugosity alone affects fish abundance. In this study the reef ball plot had 1.5 times more surface area per bottom area than the layered cake or rock pile plot, but harbored respectively lower or comparable fish abundance. This indicates that rugosity, at least in this study, is subordinate to shelter availability in determining fish abundance.

No large differences were detected between treatments in species, family, trophic group or size class composition. Although the fish assemblage composition on layered cake plots appeared to be more homogenous than on reef ball and rock pile plots, this difference was not significant. It could be that we were not able to detect any differences due to low statistical power, which was the result of the low number of replicates per treatment. Other studies showed that artificial reef design can affect fish assemblage composition (Hixon & Beets 1989, Beets & Hixon 1994, Gratwicke & Speight 2005) and that larger shelter sizes can result in a higher abundance of larger fish (Hixon & Beets 1989, Beets & Hixon 1994). Our results did not show this effect. As we observed few fish in this size class on all treatments, possible explanations could be that (1) there was no lack of large shelters on the natural reef, so larger fish were not attracted to the artificial reef plots or (2) the fish assemblage of the surrounding natural reef lacked larger fish, resulting in few fish in this size class on the artificial reefs.

In the current study, one location (BRM) had a significantly lower fish abundance, biomass and species richness than one or both other locations. This confirms the statement of (Baine 2001) that comparing results of different artificial reef studies performed at different locations with distinct environmental parameters cannot be done or are tenuous at best. Also, this underlines the importance of comparative studies in which all studied artificial reef designs are deployed at the same locations. In our study all intra-location comparisons of the plots yielded the same results but distributed over different locations the outcomes would be compromised.

The effects of hurricane Irma on the artificial reefs used in this study show the necessity of making deployed reefs surge and weather-proof. Artificial reefs in dynamic, sandy environments where former reefs have been damaged by natural forces may be quite vulnerable to sinking in, or being smothered by, hurricane or swell-driven sand movement. Careful site selection based on criteria such as sand abundance, depth local knowledge of weather impact helps prevent later failures. Also the need for anchoring should be considered and the opportunities to do so. In areas with expected (increasing) hurricane impact hurricane resistant structures are needed that also could help protect the coast, or rehabilitation should occur only at depths relatively sheltered from impact of hurricanes or surface waves.

Our study shows that artificial reef design can greatly enhance fish abundance and biomass. On Saba and St. Eustatius, layered cake plots performed better than reef ball plots and had higher average fish abundance and biomass, while rock pile plots had intermediate fish assemblage outcomes. Analysis revealed that the availability of multiple small shelters in the layered cake design was responsible for these results and that gross volume (reef size), shelter volume and total surface area (rugosity) were subordinate in determining fish assemblage parameters. Our results also suggest that the cost-benefit ratio of artificial reef implementation for the purpose of reef restoration, could be greatly improved by deploying either layered cake (better performance, similar price) or rock pile plots (similar performance, 4-10 times lower price) instead of the commonly used reef balls. In the coming years we will be monitoring further developments in the reef fish faunas at our experimental plots to study whether the main differences in fish assemblages between the reefs observed during early colonization will persist over time.

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Chapter 4

The effect of artificial reef design on the attraction of herbivorous fish and on coral recruitment, survival and growth

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Submitted

4.1 Abstract

Fish assemblages of artificial reefs can differ greatly in abundance, biomass and composition between reef types, with some reefs harboring over five times more herbivores than others. It is assumed that higher herbivorous fish abundance results in a higher grazing intensity, affecting the benthic community including enhanced reef-building coral recruitment, survival and growth. Territorial fish species might affect this process by chasing away other fish, especially herbivores. In this study we compared the fish assemblage, territorial behavior and grazing intensity by fish on two artificial reef types: reef balls and layered cakes, differing greatly in their fish assemblage during early colonization. In addition, the effect of artificial reef type on the benthic development and coral recruitment, survival and growth, was investigated. Although layered cakes initially harbored higher herbivorous fish biomass, this effect disappeared during consecutive monitoring events. This seems to be the result of the higher territorial fish abundance around the layered cakes and almost four times more chasing behavior was recorded compared to the reef balls. This resulted in a more than five times lower fish grazing intensity compared to the reef ball plots. Although macroalgae were effectively controlled at both reefs, the grazing intensity did not differ enough to cause large structural changes in benthic cover needed for higher coral recruitment, survival or growth. The high turf algae cover, combined with increasing CCA and sponge cover probably reduced coral development. We recommend further research on how to achieve higher grazing rates, for example by facilitating invertebrate herbivores such as the sea urchin *Diadema antillarum* or the Caribbean king crab, *Maguimithrax spinosissimus*.

Key words

Caribbean, herbivores, fish assemblage, reef ball, layered cake, territorial behavior

4.2 Introduction

Caribbean coral reefs are among the most degraded reefs worldwide (Pandolfi et al. 2003, Hughes et al. 2010). Since the 1970s, cover of reef-building coral decreased in many areas with more than 80%. Without the three dimensional structure of the corals, shelter availability (Alvarez-Filip et al. 2009), biodiversity (Newman et al. 2015) and productivity (Rogers et al. 2018) of Caribbean reefs has decreased significantly. Artificial reefs, structures mimicking one or more functions of a natural reef (Baine 2001), are often deployed as alternative fish habitat with the purpose of creating a dive site, to restore ecosystems or to (temporarily) sustain fish catches (Lima et al. 2019, Hylkema et al. 2021). In addition, artificial reefs can provide hard substrate for cultivated coral transplants and for natural coral recruitment, both of which could potentially increase the artificial reefs' habitat value for other marine organisms (Cabaitan et al. 2008, Yap 2009).

After the corals declined, macroalgae and turf algae became the most dominant benthic groups on Caribbean coral reefs (Gardner et al. 2003, Jackson et al. 2014). Algae are important competitors of corals for space, can overgrow them, cause coral mortality (Jompa & McCook 2002, Box & Mumby 2007) and hinder their recruitment (McCook 2001, Box & Mumby 2007, Arnold et al. 2010). This all adds up to reduced coral reef resilience (Hughes et al. 2010). To prevent algae from overgrowing artificial reefs, smothering transplanted corals (van Woesik et al. 2018) and hindering coral recruitment (Box & Mumby 2007), it is essential to facilitate herbivores that can effectively reduce algae cover (reviewed by Seraphim et al. 2019). In the Caribbean, the most important grazers are parrotfish (Mumby 2009, Adam et al. 2018), surgeonfish (Burkepile & Hay 2010, Adam et al. 2015) and the long spined sea urchin *Diadema antillarum* (Carpenter 1986). The importance of the latter has substantially decreased since the mass mortality of *D. antillarum* in the 1980s (Lessios 2016). The density of grazers is positively correlated with coral recruitment on natural reefs (Edmunds & Carpenter 2001, Mumby 2009, Adam et al. 2015, 2018), suggesting their presence can positively influence coral recruitment and growth on artificial reefs.

Most artificial reef studies focused on their habitat function for fish (reviewed by Lima et al. 2019) and clearly higher shelter availability results in a higher fish abundance (Hixon & Beets 1989, Sherman et al. 2002, Gratwicke & Speight 2005, Brotto et al. 2006, Hylkema et al. 2020) including potentially more herbivorous fish. Few artificial reef studies reported on development of coral (Perkol-Finkel & Benayahu 2005, 2007) or the relationship between the benthic community and the presence of corals (Miller et al. 2009). Studies integrating the herbivorous fish assemblage, corals and their interaction are, to our knowledge, not yet available.

This study investigates how the herbivorous fish assemblage on artificial reefs may mediate coral recruitment, survival and growth. Two different artificial reef designs were found to harbor very different fish assemblages after early colonization, both in terms of abundance and biomass (Hylkema et al. 2020). The two concrete artificial reef designs were made with the same mold: reef balls and layered cakes. Reef balls are domes with a single void space with multiple holes, while layered cakes have different layers of concrete with multiple contiguous shelters in between. After one year of colonization, 3.6 times more fish were observed on the layered cakes compared to reef balls (Hylkema et al. 2020). More specifically, the layered cakes harbored over 10 times more parrotfish and over three times more surgeonfish, suggesting that grazing intensity at the layered cakes must be significantly higher than at the reef balls. The higher herbivorous fish abundance at the layered cakes is expected to differentially affect the benthic community and more specific, to enhance coral recruitment, survival and growth when compared to the reef balls. Interestingly, the abundance of territorial damselfish on the layered cakes was also over five times higher than on the reef balls (Hylkema et al. 2020). Damselfish are territorial, farm turf algae (Ceccarelli et al. 2011, Arnold et al. 2010) and chase away other herbivores (Ceccarelli et al. 2001) thereby reducing grazing intensity that could negatively impact coral recruitment (Arnold et al. 2010). Potentially, coral recruits on artificial reefs can also be affected by corallivorous fish (reviewed by Seraphim et al. 2019). However, the reef balls and layered cakes in the study of Hylkema et al. (2020) exhibited very low densities of corallivores.

In this study we determine 1) to what extent differences in herbivorous and territorial fish abundances between artificial reef designs found during early colonization persist over time, 2) how herbivorous fish biomass relates to overall grazing pressure on the artificial reefs, 3) the relationship between grazing pressure and the composition of the benthic community and 4) how these parameters influence coral recruitment, survival and growth. We hypothesize that the higher herbivorous fish abundance on layered cakes will persist and that this higher abundance will positively affect the coral recruitment parameters.

4.3 Methods

Construction of artificial reefs

Artificial reefs deployment for this study was described by Hylkema et al. (2020). In short: on four locations around Saba and St. Eustatius, Dutch Caribbean, four experimental plots were set out on sand, 5m from the natural reef and 25m from other artificial reef plots. Four different treatments (reef balls, layered cakes, a rock pile and a control plot on bare sand) were randomly assigned to the plots on each location, i.e. one replicate per treatment per location. All locations were damaged by hurricanes Irma and Maria in September 2017 but cleared from sediment and repaired where necessary in December 2017. Big swells in February 2018 buried all artificial reefs at Saba's Ladder Bay location, but did not affect the other locations. Sand scouring at one of the St. Eustatius locations (Twin Sisters) resulted in the artificial reefs at this location slowly sinking into the sand over the course of this study. The layered cakes and reef balls could be cleared relatively easily, but this was not possible for the rock piles. As the rock piles on other locations also remained instable, it was decided to exclude the rock piles from this study and to focus on the reef ball and layered cake plots at the tree remaining locations: Twin Sisters (TS) and Crooks Castle (CC) on St. Eustatius and Big Rock Market (BRM) on Saba.

Each reef ball or layered cake plot was composed of respectively three reef ball or layered cake units (pictures in Hylkema et al. 2020). Reef balls and layered cakes were constructed from concrete using a mold designed for this purpose (Reef Ball

Foundation, Athens, USA, www.reefball.org). Each reef unit had a bottom diameter of 90 cm, a height of 60 cm and a weight between 300 and 450 kg. Three units, each covering an area of 0.64 m^2, were placed close together forming one reef plot of approximately 2 m^2. The total hard substrate surface area of a reef ball plot was slightly more than 12 m^2, while the total hard substrate surface of a layered cake plot was slightly more than 8 m^2 (Hylkema et al. 2020). These differences were considered part of the specific designs and were therefore not corrected for.

Fish assemblages

The fish assemblage at each plot was surveyed 10 times during the months April and May 2018,10 times during November and December 2018, 10 times in April and May 2019 and 3 times in December 2019. This resulted in 33 surveys per artificial reef plot, starting 1 year after deployment (4 months after restoration from hurricanes) and continuing till 2.5 years after deployment. For each survey, an underwater visual census (UVC) was conducted consisting of a 3 minute stationary point-count followed by a thorough search of the internal spaces of the artificial reefs to record all hiding fish (Hylkema et al. 2020). All fish within a virtual cylindrical column, extending 1 m sideways of the reef and extending 2 m upward from the bottom were included in the survey. Fish were identified up to species level, counted and categorized using visual estimation of total length (TL) in size classes 0–5, 5–10, 10–15,15–20, 20–25, 25–30, 30–40, 40–50 and 50+ cm. Grazing intensity is considered to depend on the biomass of the herbivores (Korzen et al. 2011). To determine herbivorous fish biomass, fish were categorized in major trophic groups, following Alvarez-Filip et al. (2011). The weight of all herbivorous species and size class combinations was calculated using the middle of the size class and known length-weight relationships (Froese and Pauly, 2019). Herbivorous fish biomass per species and in total were averaged per treatment, using the 33 surveys as replicates. Contrary to grazing intensity, territorial behavior is often determined by abundance instead of biomass. Territorial fish species were identified using remote video surveys (see below) and territorial fish abundance per species and in total was averaged over treatment.

Grazing intensity and territorial behavior

To determine herbivorous grazing impact and territorial behavior, remote video surveys were conducted in November and December 2018. For this purpose, a buoy with camera (GoPro HERO+) was mounted, using ropes and anchors, 2 m above every artificial reef plot. The GoPro was facing downwards, capturing the entire artificial reef plot in the video. After mounting and activating the GoPro, the researchers left the dive site to minimize disturbance, and picked up the camera later that day using scuba. Per artificial reef plot, 6-11 videos with a total length of 6:17-7:32 hours were recorded (Table 1) during daylight (between 10:00 and 14:00). The number of videos per plot and length of each video differed due to logistic reasons and due to the GoPro sometimes erroneous stopping the recording.

Table 1: Number of videos and total time recorded per location and per treatment to determine grazing intensity and territorial behavior.

Location	Treatment	Videos (n)	Time recorded (hh:mm:ss)
Crooks Castle	Reef ball	9	06:17:26
Crooks Castle	Layered cake	8	06:32:44
Twin Sisters	Reef ball	11	07:31:56
Twin Sisters	Layered cake	10	06:45:10
Big Rock Market	Reef ball	6	07:12:00
Big Rock Market	Layered cake	7	06:53:00
Total		51	41:12:16

Videos were checked for visible “bites”, defined as contact between fish mouth and algae, to determine grazing intensity. Rapid bites in quick succession that could not be separated were counted as a single bite (Mantyka & Bellwood 2007, Korzen et al. 2011). For each bite, the conducting fish was identified up to species level and categorized using visual estimation of total length (TL) in size classes of 5 cm (0–5, 5–10, etc). The top of the artificial reef modules was used as a size reference. The weight of all species and size-class combinations was calculated using known species specific length-weight relationships (Froese and Pauly, 2019). A standardized “bite impact” was calculated per species and size class combination by: total bites within that species and size class combination × body mass in grams following Korzen et al. (2011) and was corrected for the duration of the video. Standardized bite impacts were summed and averaged per species and per survey. For some surveys, a change in current during the survey resulted in a part of the reef being out of view. This was taken into account by dividing the bite impact by the part of the reef that was visible, this was always at least 90%.

Territorial behavior was defined as one fish swimming rapidly towards another fish and chasing it away from a certain area of the artificial reef plot (Canterle et al. 2020). For every chase, the fish chasing and being chased were identified up to species level. It was also recorded if the chased fish left the artificial reef plot after the chase. Chases were expressed as number per hour by correcting for the duration of the video while territorial behavior per survey was calculated by summing all chases of that survey. The fishes being chased were grouped per family and the chasing fish per species.

Benthic community composition

To examine succession of main benthic groups on both treatments, photo quadrats were made in April 2018, November 2018, May 2019 and December 2019. Two reef ball modules, one at CC and one at BRM had been broken in two parts during the hurricanes and had been covered by sediment for two months, after which they were repaired and colonization had to start again. These two modules were therefore excluded for all benthic community and coral analysis. A quadrat of 40x40 cm was randomly placed three times on the side of each artificial reef module. Quadrats were aligned with the top of the module and never overlapped with other quadrats taken at the same time. Each quadrat was photographed with an Olympus PEN E-PL2 camera and two external strobes (INON S-2000).

Photo quadrats were analyzed with the software CPCe (Coral Point Count with Excel extentions) version 4.1 (Kohler & Gill 2006). For each picture, 45 points were randomly placed and categorized in 16 main benthic groups: bryozoans, crustose coralline algae, cyanobacteria, hydrozoa, hydrocorals, macroalgae, octocorallia, porifera, scleractinia, tunicates, turf algae, zoanthids, sediment, bare concrete, unknown and shelter. The category shelter was used for points that fell in cavities, in the shade or otherwise on substrate not identifiable and this category was excluded for further analysis. The categories hydrocorals, bryozoans, hydrozoa and scleractinia never had more than 2% cover on an artificial reef module and were summed as “other” for further analysis.

Coral recruitment abundance, survival and growth

In April 2018, one year after deployment, all artificial reef modules were thoroughly searched for hard coral recruits using UV lights (Nightsea BW-1). Since most hard corals exhibit fluorescence, the UV lights made it possible to detect even the smallest recruits. To reduce the amount of ambient light and make detection of corals easier, these dives were conducted around sunset with the use of a filter visor that removed the blue light and transmitted the fluorescence (Nightsea BlueBlock Filter Visor). All coral recruits were mapped and revisited the next day during daylight to make a picture (Olympus PEN E-PL2) with ID number and size reference of each coral. Using the pictures, the recruits were taxonomically identified. As classification of very small colonies is sometimes difficult, *Porites astreoides* and *P. Porites* were pooled as *Porites* spp. and *Agaricia agaricites and A. humilis* were pooled as *Agaricia* spp. (Vermeij 2006). All *Porites* spp. recruits, by far the most abundant recruit on the artificial reef modules, were mapped during the initial monitoring in April 2018 and were searched for again and photographed with a size reference in November 2018 (1.5 year after deployment), May 2019 (2 years after deployment) and December 2019 (2.5 years after deployment) to determine their survival and size. *Porites* spp. recruits that were not found back after 5 minutes of searching with UV lights were considered to have died. *Porites* spp. recruit surface area was determined using ImageJ version 1.52a (Abràmoff et al. 2004) and growth was determined by dividing the increase in surface in between monitoring events by the growth period in years.

Additional coral recruit counts were conducted around sunset using UV lights in November 2018, May 2019 and December 2019 (1.5, 2 and 2.5 years after deployment). These counts included the earlier-mapped *Porites* spp. recruits and were used to determine the total coral recruit abundance per artificial reef module.

Statistical analysis

For all response variables (Table 2), there was some form of dependence in the data collection, by measuring the same reef plot, artificial reef module or coral repeatedly in time. To account for this dependency, mixed modelling approaches (Zuur et al.

2009) were used. All response variables were initially modelled using linear mixed models (LMMs, lmer function in the R package “lme4” (Bates et al., 2014). The strong mean to variance relationship of count data (Warton et al. 2012) often resulted in a cone shaped graph when residuals were plotted against fitted values, which is an indication for heterogeneity of the variance. If this was the case, the model was refitted with a cube root transformed response variable. If this did not resolve the issue, generalized linear mixed models (GLMMs) with a Poisson distribution were fit and checked for overdispersion. This was the case for all the Poisson GLMMs and was solved by using GLMMs with a negative binomial error distribution (glmer.nb function in the R package “lme4”). Interactions for all models were only included if data visualization clearly indicated such an effect (Zuur et al. 2009), which was only the case for the interaction between treatment and time after deployment for the fish biomass and abundance models. For these models, treatment was contrasted within every monitoring event using the package “emmeans” (Lenth and Herve, 2019) to find out when treatments differed significantly. All model selection was done based on Akaike’s Information Criterion (AIC) (Zuur et al. 2009, Bolker et al. 2009).

LMMs were used to test whether herbivorous fish biomass was affected by treatment, location, time after deployment or to test the interaction of treatment and time after deployment (fixed factors). To control for repeated surveys of the same reefs, individual reef ID was included as a random factor. Residuals of the initial model indicated heteroscedasticity, which was resolved after the data were cube-root transformed. For statistical inference, an F-test with Kenward-Roger's approximation to degrees of freedom was performed using the KRmodcomp function of the R package “pbkrtest” (Halekoh and Højsgaard, 2014).

GLMMs with a negative binomial error distribution were used to test whether grazing intensity and territorial behavior were affected by treatment or location (fixed factors). To control for the repeated surveys per plot, reef ID was included in the models as a random factor. The model including both treatment and location, which were the two considered variables, had the lowest AIC. Likelihood ratio tests (LRT) were performed for statistical assessment of the fixed factors using the drop1 function.

Relative cover of main benthic groups showed a strong mean to variance relationship, which is one of the properties of count data (Warton et al. 2012). In order to take this mean to variance relationship into account, the package “mvabund” (Wang et al., 2020) was used to test whether treatment, location and time after deployment affected the composition of the benthic community. The “manyglm” function of this package fitted multivariate GLMs with a negative binomial distribution. The best-fitting models were selected based on AIC; these were the models including treatment, location and monitoring period. Residuals were plotted to examine if the model assumptions were met, which was the case when negative binomial distributions were used. Univariate GLMs, adjusted for multiple testing, were then used to asses which benthic groups drove the main effects (Wang et al., 2012).

GLMMs with a negative binomial error distribution were used to determine if the number of coral recruits per module was affected by treatment, location or time after deployment (fixed factors). The low number of replicates did not allow the consideration of any interaction in the model. To control for the repeated surveys per module, module ID was included as a random factor. Statistical inference was conducted following procedures described above for grazing intensity. The best fitting model, with the lowest AIC, included all three considered variables. When the final model showed a location effect, Tukey's post-hoc tests were conducted using estimated marginal means (EMM) from the package “emmeans” (Lenth and Herve, 2019).

Table 2: Modelling approach, fixed and random factors of best fitting model and replication per response variable.

Response variable:	Modelling approach:	Fixed factors best fitting model:	Random factor best fitting model:	Replication:
Total fish biomass (g plot^{-1})	LMM	Treatment + Location + Time after deployment + Treatment * Time after deployment	Reef ID	10 surveys at 3 locations during 3 monitoring periods and 3 surveys at 3 locations during 1 monitoring period = 99 surveys per treatment.
Herbivorous fish biomass (g plot^{-1})	LMM	Treatment + Location + Time after deployment + Treatment * Time after deployment	Reef ID	
Total fish abundance (n plot^{-1})	GLMM	Treatment + Location + Time after deployment + Treatment * Time after deployment	Reef ID	
Territorial fish abundance (n plot^{-1})	GLMM	Treatment + Location + Time after deployment + Treatment * Time after deployment	Reef ID	
Territorial behavior (chases hour^{-1})	GLMM	Treatment + Location	Reef ID	6-11 videos at 3 locations = 26 reef ball videos and 25 layered cake videos.
Grazing intensity (g hour^{-1})	GLMM	Treatment + Location	Reef ID	
Benthic cover (% per major group)	multivariate GLMs	Treatment + location + Time after deployment	-	2-3 modules at 3 locations during 4 monitoring periods = 28 reef ball surveys and 36 layered cake surveys.
Coral recruit abundance (n module^{-1})	GLMM	Treatment + location + Time after deployment	Artificial reef module ID	
Coral recruit survival (n module^{-1})	GLMM	Treatment + location + Time after deployment	Artificial reef module ID	
Coral recruit size (mm^{2})	LMM	Treatment + location + Time after deployment	Coral ID	16 – 48 corals on layered cakes and 19- 38 corals on reef balls during 4 monitoring events.*
Coral recruit growth (mm^{2} year $^{-1}$)	LMM	Treatment + location + Monitoring interval	Coral ID	16 – 50 corals on layered cakes and 19- 38 corals on reef balls during 3 monitoring intervals.*

* exact n-values per monitoring event or interval are indicated in results section.

LMMs were used to determine if *Porites* survival, size and growth were affected by treatment, location and time after deployment (fixed factors). To control for the repeated surveys per coral, coral ID was included as a random factor. Statistical inference was conducted following procedures described above for herbivorous fish biomass. Part of the *Porites* spp. recruits disappeared during the course of a monitoring interval and growth rates could only be calculated for the recruits that were alive at the beginning and the end of a certain monitoring interval. This results in a slightly different dataset than the *Porites* spp. recruit size dataset, where every recruit alive at a certain monitoring event contributed to the dataset.

All statistical analyses were performed with R (R Core Team, 2021) using R studio version 1.2.5001. P-values <0.05 were considered statistically significant and reported values are means ±sd, with the exception of the fish biomass and fish abundance graphs, where mean ±95%CI was used.

4.4 Results

Total and herbivorous fish biomass

Total fish biomass (Figure 1) at the artificial reef plots was significantly affected by time after deployment ($P < 0.001$, Table S1) and the interaction between treatment and time after deployment ($P = 0.001$). Treatment and location did not affect the total fish biomass. Pairwise comparisons of reef type per monitoring event (time after deployment) revealed that total fish biomass differed significantly between reef balls and layered cakes after one year ($P = 0.006$) and 1.5 year (0.044), but not after two and 2.5 years.

Herbivorous fish biomass (Figure 1) was significantly affected by the interaction between treatment and time after deployment ($P < 0.001$, Table S1), but not dependent on location. Pairwise comparisons of reef type per monitoring event revealed that layered cakes had significant higher herbivorous fish biomass than the reef balls after one year ($P < 0.001$). This difference became smaller in consecutive monitoring periods and was not significant anymore after 1.5 year. The relative contribution of herbivorous fish to the total fish biomass decreased over the course of the study. One year after deployment, average herbivorous biomass was 33% of the total fish biomass at the layered cake plots and 22% at the reef ball plots (Table 3). After 2.5 years, the relative contribution had declined to 12% at the layered cake plots and 10% at the reef ball plots. *Sparisoma aurofrenatum* (redband parrotfish), *Acanthurus coeruleus* (blue tang), *Scarus taeniopterus* (princess parrotfish) and *Acanthurus tractus* (ocean surgeonfish) contributed most to herbivorous fish biomass.

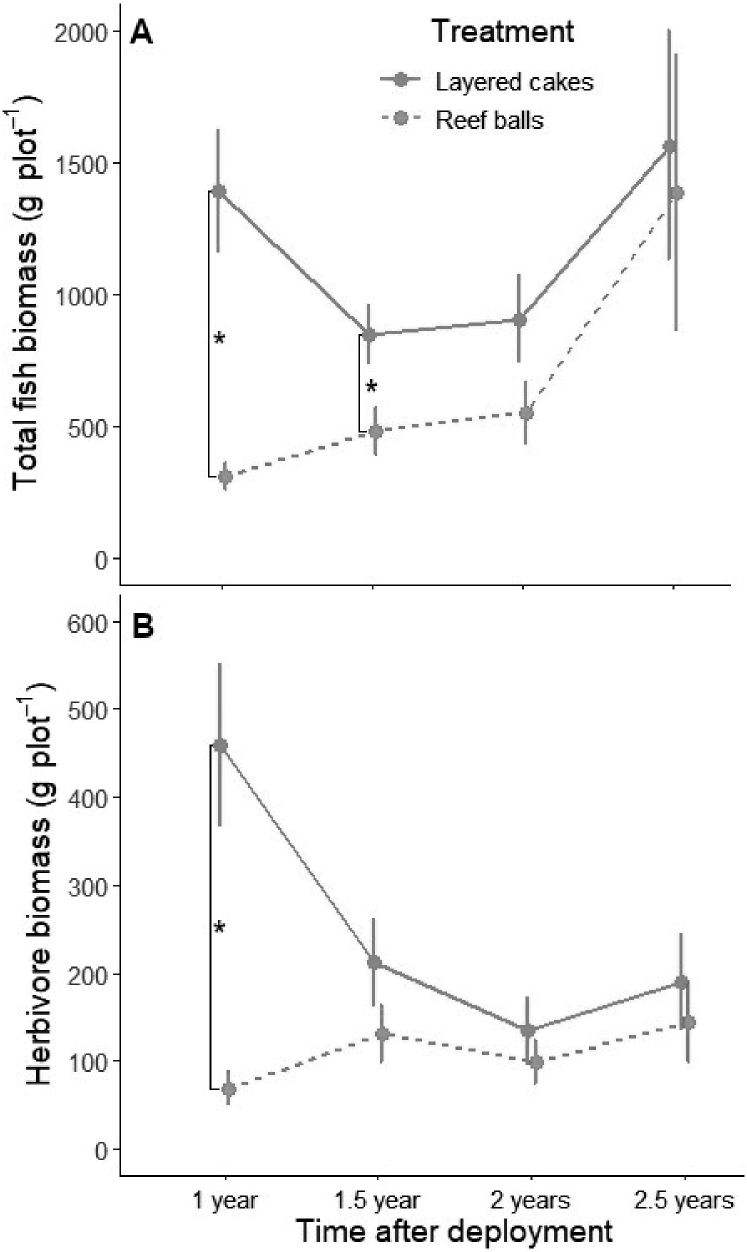

Figure 1: Average total fish biomass (A) and herbivore fish biomass (B) (± 95% CI) per treatment in time after deployment. * indicates a significant difference between treatments for that specific time after deployment.

Table 3: Average herbivorous biomass (g plot^{-1} ± SD) per species, in total and the relative contribution of herbivorous fish to the total fish biomass per treatment in time after deployment. Species are sorted based on their overall biomass. n.o. = not observed

		1 year		1.5 year		2 year		2.5 year	
Name	Common name	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake
Sparisoma aurofrenatum	Redband parrotfish	24.9 ± 61.1	177.9 ± 182.6	57.3 ± 148	73.3 ± 154.2	11.8 ± 43.3	1.6 ± 7.7	44.9 ± 78.1	63.2 ± 52.4
Acanthurus coeruleus	Blue tang	11.4 ± 32.0	70.3 ± 176.2	25.2 ± 52.6	21.7 ± 56.0	18.0 ± 41.5	44.7 ± 133.3	27.0 ± 81.0	51.8 ± 89.4
Scarus taeniopterus	Princess Parrotfish	9.9 ± 54.5	111.7 ± 295.9	3.5 ± 13.9	81.6 ± 190.4	12.1 ± 40.9	18.4 ± 43.0	13.9 ± 33.0	16.8 ± 27.8
Acanthurus tractus	Ocean Surgeonfish	8.7 ± 25.8	34.3 ± 45.3	27.3 ± 46.8	24.6 ± 36.6	6.5 ± 16.1	11.2 ± 22.9	29.8 ± 47.4	52.3 ± 83.2
Sparisoma chrysopterum	Redtail Parrotfish	n.o.	12.8 ± 48.9	16.4 ± 74.1	n.o.	31.6 ± 76.6	41.0 ± 96.8	n.o.	n.o.
Acanthurus chirurgus	Doctorfish	6.4 ± 24.5	24.6 ± 65.0	n.o.	6.8 ± 24.2	17.1 ± 46.7	3.4 ± 16.7	9.1 ± 27.3	4.5 ± 13.6
Stegastes partitus	Bicolor Damselfish	0.3 ± 0.3	3.5 ± 4.8	0.7 ± 1.9	4.0 ± 5.6	2.2 ± 5.3	13.8 ± 12.0	n.o.	1.5 ± 3.4
Sparisoma viride	Stoplight Parrotfish	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	19.3 ± 57.8	n.o.
Sparisoma rubripinne	Yellowtail Parrotfish	7.2 ± 39.3	10.2 ± 40.9	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Scarus vetula	Queen Parrotfish	n.o.	13.6 ± 58.6	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Total herbivorous fish biomass		69 ± 109	459 ± 508	130 ± 178	212 ± 278	99 ± 119	134 ± 191	144 ± 134	190 ± 164
Total fish biomass		309 ± 273	1390 ± 1278	480 ± 485	848 ± 634	551 ± 590	906 ± 826	1385 ± 1570	1563 ± 1299
Percentage herbivores (of total biomass)		22%	33%	27%	25%	18%	15%	10%	12%

Total and territorial fish abundance

Total fish abundance (Figure 2, Table 4) was significantly affected by treatment (P < 0.001, Table S1), location (P = 0.004), time after deployment (P < 0.001) and the interaction between treatment and time after deployment (P < 0.001). One year after deployment, the total fish abundance at the layered cake plots was 5 times higher than on the reef balls, which was a significant difference (P < 0.001). Total fish abundance at the layered cakes decreased between one and two years after deployment, but remained significantly higher compared to the total fish abundance at the reef ball plots (P < 0.001 after 1.5 year, P = 0.001 after two years), which increased in this time period. At the end of the study, the fish abundance at the layered cakes was 1.5 time higher than at the reef ball plots, which was significant (P = 0.011).

Total territorial fish abundance (Figure 2), which is the sum of *H. adscensionis*, *A. saxatilis*, *M. jacobus* and *S. partitus* (species identified using video surveys in section 3.3), was significantly affected by treatment (P = 0.029, Table S1), time after deployment (P < 0.001) and the interaction between treatment and time after deployment (P < 0.001). Location had no significant effect on the territorial fish abundance. Average territorial fish abundance at the layered cake plots was 5.5 times and significantly higher compared to the reef ball plots after one year (P < 0.001). Average territorial fish abundance was relatively stable over the course of the study at the layered cakes plots, while average territorial fish abundance steadily increased at the reef ball plots, reducing the difference between treatments, although it remained significant till the end of the experiment (P = 0.034). Over the course of the experiment, the relative contribution of territorial fish to the total fish abundance increased from 19% at the reef ball plots and 28% at the layered cake plots to around 40% at both treatments.

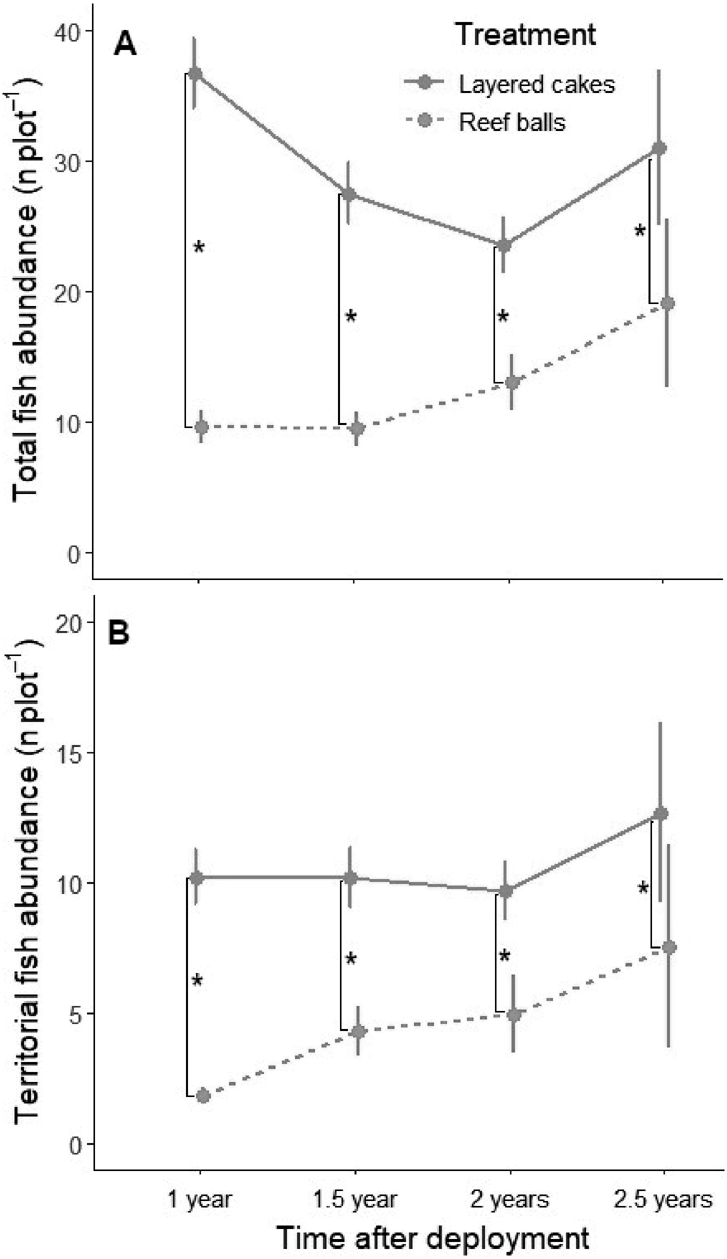

Figure 2: Average total (A) and territorial fish abundance (B) (± 95% CI) per treatment in time after deployment. * indicates a significant difference between treatments for that specific time after deployment.

Table 4: Average territorial fish abundance (n plot^{-1} ± SD) per species, in total and the relative contribution of territorial fish to the total fish abundance per treatment in time after deployment. Species are sorted based on their overall abundance. n.o. = not observed

		1 year		1.5 year		2 years		2.5 years	
Name	Common name	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake
Holocentrus adscensionis	Squirrelfish	0.3 ± 0.5	4.9 ± 3.7	0.3 ± 0.4	4.1 ± 3.7	0.5 ± 0.9	3.1 ± 3.1	0.6 ± 0.9	6.0 ± 5.0
Abudefduf saxatilis	Sergeant major	0.3 ± 0.6	0.7 ± 1.4	0.4 ± 0.9	0.9 ± 2.4	0.8 ± 2.0	1.2 ± 4.9	0.3 ± 0.7	n.o.
Myripristis jacobus	Blackbar soldierfish	0.3 ± 0.5	3.1 ± 2.4	2.7 ± 4.0	3.4 ± 2.9	3.4 ± 5.5	3.1 ± 2.7	6.7 ± 10.3	5,6 ± 7.0
Stegastes partitus	Bicolor damselfish	0.9 ± 1.0	1.5 ± 1.0	1.0 ± 1.0	1.8 ± 1.4	0.3 ± 0.6	2.3 ± 1.4	0.0 ± 0.0	1,1 ± 1,1
Total territorial fish abundance		1,9 ± 1,6	10.2 ± 5.8	4.3 ± 5.1	10.2 ± 6.2	5.0 ± 7.3	9.7 ± 5.5	7.6 ± 11.7	12.7 ± 10.4
Total fish abundance		9,6 ± 7.0	36.7 ± 14.3	9.4 ± 7.1	27.5 ± 12.8	13.0 ± 10.5	23.5 ± 10.7	19.1 ± 19.4	31.0 ± 17.7
Percentage territorial fish (of total abundance		19%	28%	46%	37%	38%	41%	40%	41%

Territorial behavior

Territorial behavior (Table 5) was significantly affected by treatment ($P < 0.001$, Table S1), but not by location. Layered cake plots experienced an average of 31.8 ± 31.9 chases hour^{-1}, which was significantly higher than the 7.9 ± 12.4 chases on the reef ball plots. Exactly 90% of all territorial behavior was conducted by four species: *Holocentrus adscensionis* (Long-spined squirrelfish) (33%); *Abudefduf saxatilis* (sergeant major) (22%); *Myripristis jacobus* (blackbar soldierfish) (23%); and *Stegastes partitus* (bicolor damselfish) (12%) (Table 5). The remainder of territorial behavior was conducted by 16 other species (9%) or unidentified fish (1%). Territorial behavior of all but one species (*M. jacobus*) was much more common on the layered cake plots as compared to the reef ball plots. Territorial behavior at the layered cake plots was 33 times more frequent for *H. adscensionis*, three times more frequent for *A. saxatilis* and 21 times more frequent for *S. partitus* compared to the reef ball plots.

Table 5: Territorial behavior (chases) (±SD) by 4 most common chasing fish species, the sum of 16 other species, chases by unidentified fish and the total average per treatment. Species are sorted based on the overall number of chases.

Name	Common name	Family	Average territorial behavior (chases hour^{-1} plot^{-2})	
			Reef ball	Layered cake
Holocentrus adscensionis	Long-spined squirrelfish	Holocentridae	0.4 ± 0.7	13.2 ± 16.1
Abudefduf saxatilis	Sergeant major	Pomacentridae	2.0 ± 3.5	6.9 ± 11.9
Myripristis jacobus	Blackbar soldierfish	Holocentridae	4.6 ± 8.0	4.4 ± 6.5
Stegastes partitus	Bicolor damselfish	Pomacentridae	0.2 ± 0.3	4.2 ± 4.6
16 other species			0.3 ± 0.2	2.7 ± 1.5
Unidentified fish			0.1 ± 0.1	0.4 ± 0.1
Total			7.9 ± 12.4	31.8 ± 31.9

Almost 80% of all chases were directed to five fish families: Holocentridae (43%), Pomacentridae (24%), Acanthuridae (6%), Labridae (4%) and Scaridae (4%) (Table 6). The remainder of chases were directed towards 13 other fish families (15%) or unidentified fish (4%). All families were chased more often at the layered cake plots as opposed to the reef ball plots. In 17% of all cases, territorial behavior resulted in the chased fish leaving the artificial reef plot, but this percentage differed greatly per family. Holocentridae almost never left the artificial reef plot after being chased (Table 6) in contrast to especially Scaridae and Acanthuridae that left the artificial reef plot much more often after they were chased.

Table 6: Territorial behavior (chased) (±SD) against 5 most commonly chased families, the sum of 13 other families, unidentified chased fish and the total average per treatment. In addition, the percentage of chased fish which were chases off the artificial reef plot (%). Species are sorted based on the overall number of chases received. The assessment was done 1.5 years after deployment.

Family	Experienced territorial behavior (chased $hour^{-1}$ $plot^{-1}$)		% of chased fish leaving the artificial reef plot (%)	
	Reefball	Layered cake	Reef ball	Layered cake
Holocentridae	4.0 ± 7.0	14.2 ± 22.0	1%	3%
Pomacentridae	1.8 ± 3.0	7.6 ± 8.9	10%	22%
Acanthuridae	0.1 ± 0.1	2.1 ± 0.8	48%	32%
Labridae	0.2 ± 0.2	1.4 ± 0.6	0%	21%
Scaridae	0.4 ± 0.4	1.0 ± 0.7	39%	68%
13 other families	1.2 ± 2.1	4.1 ± 2.1	59%	12%
Unidentified	0.1 ± 0.1	1.4 ± 2.1	0%	51%
Total	7.8 ± 12.3	31.8 ± 31.8	14%	16%

Grazing intensity

Treatment was a significant predictor ($P < 0.001$, Table S1) for the standardized bite impact (assessed 1.5 years after deployment), while location was not ($P = 0.051$). The average standardized bite impact at the reef ball plots was 86 ± 24 kg $hour^{-1}$, which was 5.4 times higher than the 16 ± 10 kg $hour^{-1}$ of the layered cake plots (Table 7).

The most important grazers at both the reef ball and the layered cake plots were medium sized Acanthurids: *Acanthurus tractus* (ocean surgeon fish) of 15-20 cm and *Acanthurus coeruleus* (blue tang) of 15-20 cm conducted 60% of all grazing on the reef ball and 50% of all grazing on the layered cake plots. Other important grazers were *Scarus taeniopterus* (princess parrot fish) of 15-20 and 20-25 cm. All species and size classes conducted more grazing at reef ball plots compared with layered cake plots, with the exception of *Sparisoma aurofrenatum* (redband parrotfish) of 15-20 cm.

Grazing sea urchins were never observed on the videos but were observed sporadically during the sunset surveys. Single *Diadema antillarum* were observed at the CC location reef ball plot and the CC layered cake plot in November 2018 and at BRM layered cake plot in December 2019. *Echinometra viridis* was recorded once at the BRM location layered cake plot in November 2018 and once at the BRM reef ball plot in May 2019.

Table 7: Standardized bite impact (g hour^{-1}) (±SD) per species, size class and in total per treatment. The assessment was done 1.5 years after deployment. n.o. = not observed

Name	Common name	Family	Size class	Bite impact (g hour^{-1}) Reef ball	 Layered cake
Acanthurus tractus	Ocean surgeonfish	Acanthuridae	5-10	11 ± 19	23 ± 21
Acanthurus tractus	Ocean surgeonfish	Acanthuridae	10-15	2000 ± 2133	1120 ± 1008
Acanthurus tractus	Ocean surgeonfish	Acanthuridae	15-20	26302 ± 45095	6014 ± 5911
Acanthurus tractus	Ocean surgeonfish	Acanthuridae	20-25	n.o.	849 ± 754
Acanthurus chirurgus	Doctorfish	Acanthuridae	5-10	n.o.	142 ± 245
Acanthurus chirurgus	Doctorfish	Acanthuridae	10-15	n.o.	81 ± 141
Acanthurus chirurgus	Doctorfish	Acanthuridae	15-20	814 ± 1410	n.o.
Acanthurus coeruleus	Blue tang	Acanthuridae	5-10	5.0 ± 8.7	n.o.
Acanthurus coeruleus	Blue tang	Acanthuridae	10-15	281 ± 469	505 ± 72
Acanthurus coeruleus	Blue tang	Acanthuridae	15-20	26239 ± 18614	1933 ± 3295
Acanthurus coeruleus	Blue tang	Acanthuridae	20-25	6356 ± 11009	275 ± 255
Scarus taeniopterus	Princess parrotfish	Scaridae	5-10	n.o.	0.1 ± 0.2
Scarus taeniopterus	Princess parrotfish	Scaridae	10-15	n.o.	195 ± 302
Scarus taeniopterus	Princess parrotfish	Scaridae	15-20	8372 ± 12288	2019 ± 3078
Scarus taeniopterus	Princess parrotfish	Scaridae	20-25	11623 ± 19707	485 ± 530
Sparisoma aurofrenatum	Redband parrotfish	Scaridae	5-10	1.2 ± 2.1	0.3 ± 0.6
Sparisoma aurofrenatum	Redband parrotfish	Scaridae	10-15	618 ± 1062	120 ± 88
Sparisoma aurofrenatum	Redband parrotfish	Scaridae	15-20	1046 ± 1114	1924 ± 2820
Sparisoma aurofrenatum	Redband parrotfish	Scaridae	20-25	930 ± 1543	90 ± 156
Sparisoma chrysopterum	Redtail parrotfish	Scaridae	10-15	8.7 ± 15	n.o.
Sparisoma rubripinne	Yellowtail parrotfish	Scaridae	15-20	998 ± 1728	n.o.
Total				85605 ± 23872	15775 ± 10413

Benthic succession

One year after deployment, turf algae were the dominant benthic group and had a relative abundance of ~80% cover on all artificial reef modules (Figure 3, Table 8). Cyanobacteria, tunicates and CCA were other first colonizers and formed the majority of the remaining cover. Treatment ($P < 0.001$, Table S1) , location ($P < 0.001$) and monitoring period ($P < 0.001$) all had significant effects on benthic community composition. Post-hoc univariate tests revealed that reef ball modules had significant higher tunicate ($P = 0.004$) and sponge ($P = 0.041$) cover and significantly lower sediment cover ($P = 0.008$) compared to layered cake modules during all monitoring events. Location had a significant effect on the cover of cyanobacteria ($P < 0.001$), tunicates ($P < 0.004$), sponges ($P < 0.001$), macroalgae ($P < 0.001$) and CCA ($P < 0.001$). Over the course of study, the cover of turf algae ($P < 0.001$) declined significantly to around 50-60%, although turf algae remained the dominant benthic group. Cyanobacteria ($P < 0.001$) and bare concrete ($P < 0.001$) also declined significantly during the study, while cover of sponges (P <

0.001), CCA (P < 0.001) and other benthic groups (P < 0.001) increased significantly over time.

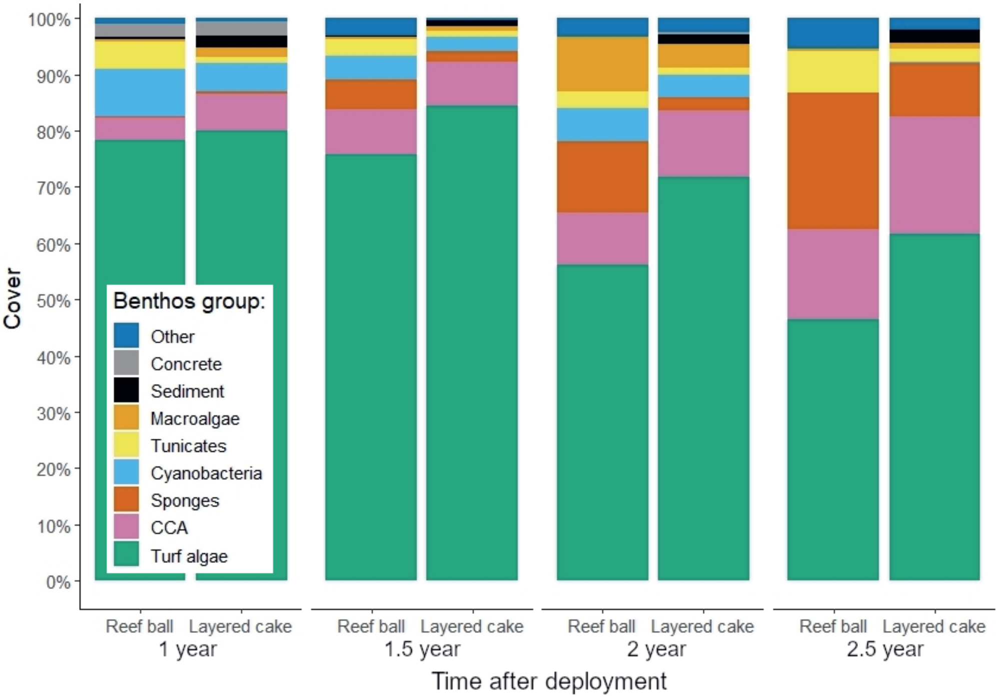

Figure 3: Percentage cover of major benthic groups per treatment per time period after deployment.

Coral recruit abundance

Coral recruit abundance (Table 9) was significantly affected by location (P = 0.023, Table S1) and time after deployment (P < 0.001), while treatment showed no significant effect. The lowest average abundance of coral recruits was 7.4 ± 3.6 coral per reef module, one year after deployment. This was significantly less than at all other monitoring moments (P < 0.001 for all comparisons). After 1,5 years, average coral recruit abundance was significantly higher compared to 2 years after deployment (P = 0.033), but not compared to 2.5 years. Average coral recruit abundance after 2.5 years was not significantly different from 2 years after deployment. The highest average coral recruit abundance was found at location BRM, which was significantly higher than location TS (P = 0.045). neither location differed significantly from location CC, which showed an intermediate number of coral recruits.

Table 8: Relative abundance (%, ± SD) of major benthic groups per artificial reef module over time after deployment.

Time after deployment	Treatment	Turf algae	CCA	Sponges	Cyano-bacteria	Tunicates	Macroalgae	Sediment	Concrete	Other	Unknown
1 year	Reef balls	78.6 ± 13.0	3.9 ± 1.9	0.4 ± 0.6	8.4 ± 9.4	4.9 ± 5.6	0.3 ± 0.4	0.4 ± 0.7	2.5 ± 3.3	0.3 ± 0.6	0.0 ± 0.0
	Layered cake	80.2 ± 9.7	6.5 ± 3.2	0.4 ± 0.6	5.1 ± 7.5	1.0 ± 1.2	1.7 ± 3.2	2.2 ± 3.3	2.5 ± 1.5	0.1 ± 0.3	0.0 ± 0.0
1.5 year	Reef balls	76.9 ± 9.1	8.2 ± 11.2	5.4 ± 8.1	4.3 ± 9.8	2.9 ± 4.6	0.6 ± 0.8	0.1 ± 0.3	0.0 ± 0.0	1.6 ± 0.7	0.2 ± 0.6
	Layered cake	84.6 ± 6.1	7.9 ± 5.6	1.9 ± 2.6	2.5 ± 3.3	1.0 ± 1.0	0.9 ± 1.7	1.0 ± 2.0	0.1 ± 0.3	0.2 ± 0.3	0.0 ± 0.0
2 years	Reef balls	57.1 ± 9.5	9.4 ± 7.3	12.9 ± 14.5	6.0 ± 7.6	3.1 ± 3.9	9.9 ± 12.9	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 0.9	0.0 ± 0.0
	Layered cake	72.6 ± 7.8	12.0 ± 3.6	2.4 ± 2.6	4.1 ± 7.1	1.2 ± 1.8	4.4 ± 5.8	1.6 ± 3.0	0.4 ± 1.0	1.2 ± 1.2	0.1 ± 0.3
2.5 years	Reef balls	47.8 ± 19.0	16.4 ± 11.1	25.0 ± 26.2	0.0 ± 0.0	7.5 ± 4.3	0.6 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 2.0	0.2 ± 0.6
	Layered cake	62.2 ± 12.2	21.3 ± 8.2	9.6 ± 6.9	0.1 ± 0.3	2.3 ± 2.4	1.1 ± 1.6	2.3 ± 3.3	0.0 ± 0.0	1.0 ± 1.1	0.0 ± 0.0

Table 9: Coral recruit abundance (± SD) per artificial reef module, treatment and location in time after deployment

	1 year		1.5 year		2 year		2.5 year	
	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake
Big Rock Market (BRM)	11.5 ± 4.9	7.7 ± 2.3	70.5 ± 3.5	46.7 ± 2.5	22.5 ± 7.8	32.3 ± 9.1	21.5 ± 2.1	34 ± 5.3
Crooks Castle (CC)	10.5 ± 3.5	8.3 ± 3.2	17.0 ± 5.7	27.3 ± 14.6	12.5 ± 2.1	28 ± 5.6	8.3 ± 4.5	16.5 ± 6.4
Twin Sisters (TS)	3.0 ± 0.0	5.7 ± 1.5	8.3 ± 2.9	17.0 ± 5.6	6.7 ± 2.3	19.3 ± 6.0	36.5 ± 3.5	26.8 ± 11.4
Average per treatment	7.6 ± 4.1	7.2 ± 5.0	28.6 ± 19.1	30.3 ± 23.5	12.9 ± 14.9	26.6 ± 8.3	20.1 ± 15.7	26.9 ± 13.2

Porites spp. survival

One year after deployment, during the first monitoring, a total of 103 coral recruits were found on the 16 artificial reef structures. These recruits belonged to three genera: *Porites* spp. (n=88), *Agaricia* spp. (n=13), and *Siderastrea* spp. (n=2). While *Porites* spp. recruits were found on all artificial reef modules at all locations (Table 10), *Agaricia* spp. recruits were found on 6 of the 16 modules and *Siderastrea* spp. recruits were only found on a single layered cake at the BRM location.

Table 10: Average (± SD) coral recruit abundance one year after deployment per artificial reef module, treatment, location and coral genus, one year after deployment. n.o. = not observed

	Big Rock Market (BRM)		Crooks Castle (CC)		Twin Sisters (TS)	
	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake
Porites spp.	8.0 ± 0.0	6.3 ± 3.1	8.0 ± 1.4	5.7 ± 4.0	2.0 ± 1.0	4.7 ± 3.8
Agaricia spp.	3.0 ± 4.2	1.0 ± 1.0	n.o.	0.7 ± 1.2	n.o.	0.7 ± 0.6
Siderastrea spp.	n.o.	0.7 ± 1.2	n.o.	n.o.	n.o.	n.o.

Of the 88 *Porites* spp. recruits found one year after deployment, 35 recruits (40%) were still alive 2.5 years after deployment. The survival of these initial *Porites* recruits (Table 11) significantly decreased in numbers with time after deployment (P < 0.001, Table S1), but did not differ by treatment or location. The average *Porites* recruit abundance, 1 year after deployment, was 5.4 ± 3.3 per reef ball module and 5.6 ± 3.2 per layered cake module. After 1.5 year this abundance was significantly lower (P = 0.014). *Porites* spp. abundance further declined between 1.5 and 2 years (P = 0.014, table 11), but not between 2 and 2.5 years after deployment. At the end of the study 2.7 ± 2.6 recruit per reef ball module and 1.8 ± 1.9 per layered cake module were remaining of the initial *Porites* spp. recruits.

Of the 88 *Porites* recruits, 53 (60%) had disappeared by the end of the study. Going back to the last live picture of each recruit revealed that, at their last picture, 13 recruits were overgrown by CCA, four by turf algae, two by a sponge and two by a tunicate. Of 32 disappeared recruits, there was no clear indication of overgrowth by a competing organism, although all recruits were surrounded by other benthic organisms.

Porites spp. recruit size and growth

Porites spp. recruit size (Table 12) significantly increased over the course of the study (P < 0.001, Table S1), was affected by location (P < 0.001), but not by reef type. As a consequence, growth of *Porites* spp. recruits (Table 12) significantly differed between monitoring intervals (P = 0.003), locations (P < 0.001), but not between reef types. Growth rates at location CC were significantly higher than growth rates at BRM (P < 0.001), but did not differ from TS. Growth rates at TS and BRM did not differ significantly either. Monitoring interval 1.5 – 2 years had significantly higher growth rates compared to 1 – 1.5 year (P = 0.019) and 2 – 2.5 years (P = 0.002), which did not differ among each other. Growth rates at location BRM were negative from 1.5 year till two years after deployment, after which the growth rate became positive again. Growth rates at location TS were negative from 1.5 year after deployment till the end of the experiment (2.5 years).

Table 11: Average *Porites* spp. recruit abundance (± SD) per artificial reef module and percentage of initial recruitment surviving, per location and in total per treatment in time after deployment.

		1 year		1.5 year		2 years		2.5 years	
		Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake
Big Rock Market	recruits	8.0 ± 0.0	6.3 ± 3.1	5.0 ± 1.4	5.3 ± 3.5	3.5 ± 0.7	2.0 ± 1.0	2.5 ± 0.7	1.0 ± 0.0
(BRM)	% surviving	100%	100%	63%	84%	44%	32%	31%	16%
Crooks Castle	recruits	8.0 ± 1.4	5.7 ± 4	7.0 ± 1.4	3.7 ± 2.1	6.5 ± 2.1	2.0 ± 1.7	5.5 ± 3.5	2.0 ± 1.7
(CC)	% surviving	100%	100%	88%	65%	81%	35%	69%	35%
Twin Sisters	recruits	2.0 ± 1.0	4.7 ± 3.8	1.7 ± 0.6	3.3 ± 4.0	1.0 ± 1.0	3.0 ± 3.5	1.0 ± 1.0	2.3 ± 3.2
(TS)	% surviving	100%	100%	83%	71%	50%	64%	50%	50%
Total	recruits	5.4 ± 3.3	5.6 ± 3.2	4.1 ± 2.6	4.1 ± 3.0	3.3 ± 2.7	2.3 ± 2.1	2.7 ± 2.6	1.8 ± 1.9
	% surviving	100%	100%	76%	74%	61%	42%	50%	32%

Table 12: Average size (mm^2 ± SD) and growth (mm^2 year^{-1}, ± SD) of *Porites* spp. recruits per location and in total per treatment in time after deployment. Growth is measured between two monitoring events and is described in the column where this growth period ended. Note that the growth could only be determined for the individuals alive at the end of the growth period (see methods section).

		1 year		1.5 year		2 years		2.5 years	
		Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake	Reef ball	Layered cake
Big Rock Market (BRM)	n	16	19	10	16	7	6	5	3
	Size (mm^2)	17 ± 8.5	11 ± 3.0	52 ± 29	47 ± 48	44 ± 25	30 ± 20	82 ± 43	64 ± 30
	Growth (mm^2 year^{-1})			61 ± 25	62 ± 87	-17 ± 48	-67 ± 95	71 ± 48	30 ± 54
Crooks Castle (CC)	n	16	17	14	11	13	6	11	6
	Size (mm^2)	21 ± 12	21 ± 14	95 ± 68	99 ± 66	101 ± 83	123 ± 64	239 ± 192	152 ± 95
	Growth (mm^2 year^{-1})			128 ± 96	132 ± 94	7.7 ± 67	20 ± 125	221 ± 217	51 ± 156
Twin Sisters (TS)	n	6	14	5	10	3	9	3	7
	Size (mm^2)	34 ± 21	19 ± 11	85 ± 63	86 ± 51	78 ± 54	77 ± 58	62 ± 37	72 ± 55
	Growth (mm^2 year^{-1})			91 ± 80	115 ± 69	-20 ± 82	-21 ± 87	-28 ± 133	-29 ± 96
Total	n	38	50	29	37	23	21	19	16
	Size (mm^2)	22 ± 13	17 ± 11	79 ± 58	73 ± 56	76 ± 66	82 ± 52	155 ± 144	123 ± 66
	Growth (mm^2 year^{-1})			98 ± 80	99 ± 89	-9.2 ± 62	-16 ± 101	126 ± 197	42 ± 117

4.5 Discussion

As described by Hylkema et al. (2020) layered cake plots were more attractive to fish than reef balls, one year after deployment. This resulted in a significantly higher fish abundance and herbivorous and total fish biomass compared to the reef ball plots (Figure 1). This study aimed to investigate the effect of the herbivorous fish biomass on coral recruitment, survival and growth on the artificial reefs. The herbivorous fish biomass at the layered cake plots was half a year later, so 1.5 year after deployment, greatly reduced and no longer significantly different from that at the reef ball plots. Herbivorous biomass at both artificial reef types remained relatively stable during the rest of the study. Initially, total fish biomass followed the same trend and decreased at the layered cake plots, while increasing at the reef ball plots and did not differ between treatments from 2 years after deployment on. From 1.5 year after deployment on, total fish biomass at both treatments increased until the end of the study. Therefore, the relative contribution of herbivorous fish biomass to the total fish biomass decreased for both treatments.

One year after deployment, layered cake plots had five times more fish compared to the reef ball plots (Figure 2). This difference became smaller over the course of the study, but total fish abundance was still significantly higher at the layered cakes compared to the reef ball plots at the end of the study. Since total fish biomass became similar at both treatments, reef balls had on average bigger fish, which might be related to their bigger shelter size (Hixon & Beets 1989, Beets & Hixon 1994). Average territorial fish abundance was significantly higher at the layered cake plots compared to the reef ball plots at every monitoring period and increased on both treatments over the course of the study. The territorial fish abundance increased relatively more than the total fish abundance. The large differences in fish abundance and biomass between one and 2.5 years after deployment indicate that the fish assemblages at the artificial reefs were still developing one year after deployment. The disruption by hurricanes in September 2017 required restoration of the artificial reefs in December 2017, which might have reset fish colonization. Although fish assemblages on artificial reefs have been suggested to stabilize within a year (Yeager et al. 2011), we have the impression that the initial colonization still was in progress, and the fish assemblages appeared more stabilized during consecutive monitoring.

The herbivorous fish abundance might have been reduced by the territorial behavior from other fish. At 1.5 year after deployment we documented significantly more territorial behavior at the layered cake plots compared to the reef ball plots (Table 5). Especially *Holocentrus adscensionis*, *Abudefduf saxatilis* and *Stegastes partitus* made many more chases at the layered cake plots compared to the reef ball plots. *Myripristis jacobus* conducted a similar number of chases on reef ball and layered cake plots. *A. saxatilis* are generally not territorial, except when guarding a nest (Cummings 1968). Although nests could often not be identified on the remote videos, the behavior of territorial *A. saxatilis* indicated they were guarding eggs, which was confirmed during the visual underwater surveys. *S. partitus* maintain permanent territories and defend them against much larger fish, especially during reproductive periods (Myrberg 1972, Luckhurst & Luckhurst 1978). *M. jacobus* are known to chase other fish away from their shelter, likely because of refuge protection (Canterle et al. 2020). No observations in literature were found about territorial behavior of *H. adscensionis*, but given the relatedness and a lifestyle similar to *M. jacobus*, it is likely that this species was also guarding its hiding place.

The higher number of chases at the layered cake plots probably is due to greater hiding opportunities facilitating more territorial fish. In addition, *H. adscensionis*, *A. saxatilis* and *S. partitus* appeared more aggressive at the layered cake plots, as the number of chases per fish (chases per species divided by the fish abundance of that species) were respectively 2.1, 1.4 and 11.0 times higher at the layered cake plots compared to the reef ball plots. This might be the combined result of 1) more suitable habitat in the layered cakes worth defending, 2) more breeding activity at the layered cakes and 3) a higher abundance of other fish that had to be chased away.

The nearly fourfold lower chasing intensity at the reef ball plots coincided with a more than five times higher grazing intensity than at the layered cake plots (Table 7). Grazing intensity was determined 1.5 year after deployment, when layered cake plots had a similar herbivorous fish biomass and a much higher territorial fish abundance compared to reef ball plots (Figure 1 and 2). The similar herbivorous fish biomass and higher grazing intensity at the reef balls indicates that the majority of the grazing at the reef ball plots was conducted by herbivores that only visited the artificial reef plots during foraging trips. The visual underwater surveys might have refrained roving herbivores from entering the artificial reef plots, excluding them from the fish surveys. These fish were probably included in the longer video surveys, where they were not disturbed by divers. Since less territorial fish were residing at the reef ball plots, the visiting herbivores were less prone to be chased away, resulting in higher grazing intensity compared to the layered cake plots. The most influential groups of herbivores, Acanthuridae and Scaridae were indeed the groups most affected by territorial behavior. Chases targeting these groups most often effectively induced the grazers to leave the plots (Table 6), thereby effectively terminating their foraging activity.

On natural Caribbean coral reefs macroalgae (Gardner et al. 2003, Jackson et al. 2014), turf algae and cyanobacterial mats (Bakker et al. 2016) often dominate the benthic community. All three groups are recognized as important competitors of corals and known to hinder coral recruitment (McCook 2001, Kuffner et al. 2006, Box & Mumby 2007, Arnold et al. 2010). Macroalgae cover on both artificial reef types was ver low (< 2%) for the majority of the study, with the exception of two years after deployment, where reef balls had 4.4 % and layered cakes had 10% macroalgae cover (Figure 3 and Table 8). On the natural reefs around St. Eustatius macroalgae cover was around 15% in 2020 (Kitson-Walters 2020). This suggests that grazing intensity at both artificial reef types was high enough to keep macroalgae under control. Although the artificial reefs in this study covered only 2 m^2 of seabed, the hard substrate surface area of the plots was more than 8 m^2 for the layered cakes and more than 12 m^2 for the reef balls (Hylkema et al. 2020). Fish swimming one meter around the artificial reef were considered part of the artificial reef fish assemblage and included in the surveys. This makes it hard to compare the herbivorous biomass per m^2 with that of areas which are an integral part of natural reefs. Depending if the biomass per plot is divided by the ground surface of the reef (2 m^2) or the area included in the survey (9 m^2)' the herbivorous biomass per m^2 is estimated to be in the range of 12-75 g m^{-1} for both reef types during most of the study. An exception are the layered cakes with much higher fish density one year after deployment (Figure 1). Even the lower end of this range is considered high for the Caribbean (Roff & Mumby 2012, Edwards et al. 2014) and explains why macroalgae cover on both reefs was relatively low over the course of the study.

On all artificial reef modules, benthic cover was dominated by turf algae, which had an average cover of ~80% one year after deployment. During the study, turf algae cover declined significantly, ultimately being replaced by mostly sponges and crustose coralline algae (CCA). The higher grazing intensity documented at the reef ball plots did not result in significant lower turf algae or macroalgae cover compared to the layered cakes. Reef balls did have a significant higher tunicate and sponge cover, which could be an indication that grazing created more space for other benthic groups than turf algae. Initial cyanobacteria cover at the artificial reefs was lower (5-8%) than the 10% recorded for the natural reef in 2020 (Kitson-Walters 2020) and significantly declined further over the course of the study.

Average coral recruit density was around 10 individuals on both reef ball and layered cake modules one year after deployment (Table 9). The average coral recruit density increased to 30 recruits after 1.5 year and remained relatively stable for the rest of the study. Higher grazing intensities can facilitate coral recruitment (Arnold et al. 2010), but the higher grazing pressure at the reef ball plots did not coincide with differences in coral recruit abundances although benthic community succession was different. Coral recruit abundance overall increased over time and significantly differed among the locations. This could be related to less competition or more suitable growing conditions at some locations. Also other studies reported that location was more determining for coral recruitment than other factors, such as substrate material (Burt et al. 2009). Despite the low cover of cyanobacteria and macroalgae, coral recruits were still often overgrown by other benthic groups, especially turf algae, sponges and CCA.

The most-often observed coral recruits were *Porites astreoides* or *P. Porites*, two brooding species (Fadlallah 1983) of which the juveniles are very common on Caribbean coral reefs (Bak & Engel 1979, Green et al. 2008, Davies et al. 2013). Of the first cohort of *Porites* spp. recruits on the artificial reefs in this study, consisting of 88 individuals, survival and growth rates were followed for 1.5 years. The higher grazing intensity at the reef ball plots did not result in significant higher coral recruit survival or growth (Table 11 and 13), which had been suggested before (Arnold et al. 2010). *Porites* ssp. survival measured over 1.5 year was around 40% on both reef ball and layered cake modules. Coral recruit survival is highly variable and dependent on numerous factors such as recruit size, benthic competition, the species concerned (Vermeij 2006) and unusual situations such as cover by sand from big storm surges. Although difficult to compare, the six month survival rates in the present study seems to be similar to the 70% survival of *P. astreoides* over four months as reported by Davies et al. (2013). Survival rates in the present study were higher than the 18% survival of mostly *Agaricia* spp. and *Porites* spp. over a year as reported by Arnold et al. (2010).

Growth of *Porites spp.* recruits was hampered at the TS location, where reruit size stopped increasing and growth rates became netative between 1.5 years after deployment till the end of the study (Table 12). This could have been the result of many factors, but we noted that quite some coral recruits were buried by sediment when we monitored them after 2 and 2.5 years and sedimentation has been associated to partial coral mortality (Nugues & Roberts 2003). The growth and survival of *Porites spp.* recruits on the concrete reef ball and artificial reef modules was similar to those for *Porites* spp. recruited on materials that are comparable to the natural reef (Vermeij 2006, Arnold et al. 2010, Davies et al. 2013). Therefore, the results from this study suggest that concrete as substrate yields comparable coral growth rates as natural reef materials. This is in accordance with Hsiung et al. (2020), who found no difference between concrete and natural rock material in the associated benthic communities.

We conclude that the higher attraction potential of layered cakes to territorial fish and the subsequent relative increase in territorial behavior of these fish reduced the grazing intensity of roving herbivores on these structures over time. The herbivorous fish biomass on both artificial reef types appeared high enough to effectively control macroalgae and the higher grazing intensity documented on the reef balls did result in significant differences in benthic community development. This difference, however, did not influence coral recruitment, survival or growth. Especially the high turf algae cover might impair coral recruitment, while other benthic groups reduced coral recruit survival by competition for space. Sedimentation was the most likely factor to explain negative growth rates and coral mortality at one of the locations, underlining the importance of proper site selection when the deployment of artificial reefs is considered. We recommend further research on how to achieve higher turf algae grazing efficiency, including trials with invertebrate herbivores, during early colonization of artificial reefs. Promising candidates are for example the sea urchin *Diadema antillarum* or the Caribbean king crab, *Maguimithrax spinosissimus*, both known to effectively reduce turf algae cover and facilitate coral recruitment on natural reefs (Edmunds & Carpenter 2001, Davies et al. 2013, Sparado & Butler 2021).

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Table S1: Main effects of fixed factors and modelling approach per response variable. Significant effects are in bold.

Response variable:	Modelling approach:	Effects: Treatment	Location	Time after deployment	Treatment* Time after deployment
Total fish biomass	LMM	F = 17.18, df = 1, P = 0.054	F = 18.30, df = 2, P = 0.906	**F = 6.05, df = 3, P < 0.001**	**F = 5.66, df = 3, P = 0.001**
Herbivorous fish biomass	LMM	F = 10.26, df = 1, P = 0.065	F = 16.23, df = 2, P = 0.91	F = 1.12, df = 3, P = 0.343	**F = 6.05, df = 3, P < 0.001**
Total fish Abundance	GLMM	**LRT = 10.84, df = 1, P < 0.001**	**LRT = 10.82, df = 2, P = 0.004**	**LRT = 16.48, df = 3, P < 0.001**	**LRT = 36.63, df = 3, P < 0.001**
Territorial fish Abundance	GLMM	**LRT = 4.77, df = 1, P = 0.029**	LRT = 5.86, df = 2, P = 0.053	**LRT = 33.33, df = 3, P < 0.001**	**LRT = 49.38, df = 3, P < 0.001**
Territorial Behavior	GLMM	**LRT = 11.52, df = 1, P < 0.001**	LRT = 31.41, df = 2, P < 0.054	NA	NA
Grazing Intensity	GLMM	**LRT = 13.02, df = 1, P < 0.001**	LRT = 5.92, df = 2, P = 0.051	NA	NA
Benthic cover	multivariate GLMs	**sum-of-LR = 51, df = 1, P < 0.001**	**sum-of-LR = 129, df = 2, P < 0.001**	**sum-of-LR = 193, df = 1, P < 0.001**	NA
Coral recruit Abundance	GLMM	LRT = 3.12, df = 1, P < 0.077	**LRT = 7.53, df = 2, P = 0.023**	**LRT = 114.59, df = 3, P < 0.001**	NA
Coral recruit survival	GLMM	(F = 0.28, df = 1, P = 0.605)	(F = 1.476, df = 2, P = 0.267)	**(F = 23.56, df = 3, P < 0.001)**	NA
Coral recruit size (mm^2)	LMM	F = 3.53, df = 1, P = 0.061	**F = 20.30, df = 2, P < 0.001**	**F = 67.54, df = 3, P < 0.001**	NA
Coral recruit growth (mm^2 year^{-1})	LMM	F = 0.18, df = 1, P = 0.674	**F = 11.33, df = 2, P < 0.001**	Monitoring interval: **F = 6.28, df = 2, P = 0.003**	NA

Chapter 5

High peak settlement of Diadema antillarum on different artificial collectors in the Eastern Caribbean.

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5.1 Abstract

The massive die-off of the herbivorous sea urchin *Diadema antillarum* in 1983 and 1984 resulted in phase shifts on Caribbean coral reefs, where macroalgae replaced coral as the most dominant benthic group. Since then, *D. antillarum* recovery has been slow to non-existent on most reefs. Studying settlement rates can provide insight into the mechanisms constraining the recovery of *D. antillarum*, while efficient settlement collectors can be used to identify locations with high settlement rates and to collect settlers for restoration practices. The aim of this study was to compare pre and post die-off settlement rates and to determine possible settlement peaks in the Eastern Caribbean island of St. Eustatius. Additionally, we aimed to determine the effectiveness and reproducibility of five different settlement collectors for *D. antillarum*. *Diadema antillarum s*ettlement around St. Eustatius was highest in May, June and August and low during the rest of the study. Before the die-off, settlement recorded for Curaçao was high throughout the year and was characterized by multiple settlement peaks. Even though peak settlement rates in this study were in the same order of magnitude as in Curaçao before the die-off, overall yearly settlement rates around St. Eustatius were still lower. As no juvenile or adult *D. antillarum* were observed on the reefs around the settlement collectors, it is likely that other factors are hindering the recovery of the island's *D. antillarum* populations. Of all five materials tested, bio ball collectors were the most effective and reproducible method to monitor *D. antillarum* settlement. Panels yielded the least numbers of settlers, which can partly be explained by their position close to the seabed. Settler collection was higher in mid-water layers compared to close to the bottom and maximized when strings of bio balls were used instead of clumps. We recommend research into the feasibility of aiding *D. antillarum* recovery by providing suitable settlement substrate during the peak of the settlement season and adequate shelter to increase post-settlement survival of settlers. The bio ball collectors could serve as a suitable settlement substrate for this new approach of assisted natural recovery.

Key words:

sea urchin settlement, St. Eustatius, Saba, restoration, assisted natural recovery

5.1 Introduction

The sea-urchin *Diadema antillarum* was the most abundant herbivore on Caribbean coral reefs until a water-borne pathogen wiped out 95-99% of all populations in 1983 and 1984 (Bak et al. 1984, Lessios et al. 1984), resulting in the biggest die-off of echinoids recorded so far (Lessios et al. 1984). The results of this die-off were catastrophic for the already-stressed coral reefs. Cover of macroalgae increased within days (Carpenter 1985), while reef-building coral and crustose coralline algae (CCA) cover decreased in the months after the die-off (De Ruyter van Steveninck & Bak 1986, Carpenter 1990, Hughes et al. 1987). This seriously affected the resilience of the Caribbean coral reefs, as new coral recruits were unable to settle and survive on the algal-dominated reefs (Mumby et al. 2006). So far, recovery of *D. antillarum* populations has been very slow to non-existent (Lessios 2016), although high densities of *D. antillarum* were observed on shallow reefs (Carpenter & Edmunds 2006) and sheltered locations like harbors and breakwaters (Debrot & Nagelkerken 2006). On these few places where *D. antillarum* naturally recovered, their grazing reversed the phase-shift by significantly reducing algal cover (Edmunds & Carpenter 2006). The benthic cover of CCA and bare substrate increased, apparently giving coral larvae a chance to settle and survive (Carpenter & Edmunds 2006, Idjadi et al. 2010). Recovery of *D. antillarum* populations is therefore a key priority for the Caribbean region, as it could increase the resilience of coral reefs to cope with other threats, such as global warming and ocean acidification (Lessios 2016).

It is therefore of the utmost importance to identify what factors are constraining recovery of *D. antillarum* populations. As both juveniles and adults are absent from most reefs, it is likely that the bottle-neck in *D. antillarum* recovery occurs in the first phase of the life cycle (Karlson & Levitan 1990, Williams et al. 2011, Mercado-Molina et al. 2015). Low fertilization success (Lessios 1988, Feehan et la. 2016), lack of an upstream source population (Roberts 1997), the lack of suitable settlement substrate (Rogers & Lorenzen 2008) or reduced survival of post-settlers (Harborne et al. 2009, Vermeij et al. 2010, Williams et al. 2011) are the most likely potential factors preventing recovery. Studying *D. antillarum* settlement rates and early post-settlement processes can provide insight into why *D. antillarum* populations are not recovering. *D. antillarum* settlement rates have been determined with panels on the seabed (Bak 1985, Miller et al. 2009, Vermeij et al. 2010, Maldonado-Sánchez et al. 2019) and settler collectors deployed mid-water (Williams et al. 2010, 2011). Large differences in settlement rates (e.g. Miller et al. 2009 and Williams et al. 2010) indicate that panels and mid-water collectors yield different results, but the methods have never been compared simultaneously.

Studying settlement rates is also meaningful for other purposes, as the collected settlers can be used for *D. antillarum* restoration (Williams (2017, 2021). While the effectiveness of settlement collectors has been studied for other sea urchins (Balsalobre et al. 2016), it is unknown which substrate is most effective for *D. antillarum* settlement. It is also essential to deploy collectors at the right time of the year, as settlement is characterized by distinct peaks (Bak 1985, Williams et al. 2010). While the occurrence and timing of these peaks have been determined for the Southern Caribbean (Curaçao, Bak 1985, Vermeij et al. 2010), Western Caribbean (Mexico, Maldonado-Sánchez et al. 2019), Greater Antilles (Puerto Rico, Williams et al. 2010, 2011) and the Florida Keys (Miller et al. 2009), no data has yet been collected in the Eastern Caribbean region.

In this study, we compared *D. antillarum* settlement rates on five different collectors for 10 months at five locations around St. Eustatius, Dutch Caribbean. Both panels close to the seabed (Bak 1985) and mid-water collectors (Williams et al. 2010, 2011) were included to be able to compare settlement rates and patterns around St. Eustatius with current and pre die-off settlement rates at other locations. We deployed bio balls, artificial turf, frayed rope and doormat collectors to assess the effectiveness and reproducibility of multiple mid-water settlement collectors. A follow-up experiment was conducted to determine if low settlement rates on the panels were the result of the type of collector or their positioning close to the seabed. Finally, another follow-up experiment was conducted to optimize the configuration of the bio ball collectors for settler collection purposes.

5.3 Methods

Experiment 1: settlement on different collectors around St. Eustatius

As *D. antillarum* settlement is known to differ greatly in time and between locations (Williams et al. 2010), settlement rates around St. Eustatius were studied from March until December 2019 at five locations on the leeward side of the island: Humps, Crooks Castle, Double Wreck, Outer Jenkins and Twin Sisters (Figure 1). Locations were selected based on the following criteria: 13-15m depth and a sandy bottom for at least 5m around the experimental set-up. During the first two months of the monitoring period, collectors were analyzed monthly. When the first *D. antillarum* settlers were observed, the monitoring interval was intensified to 14 days to get a higher resolution of settlement. Due to logistic reasons, two locations were monitored in one week and three in the next. At Twin Sisters the initial deployment took place one month later compared to the other locations. Each set-up consisted of 2 ropes, which were kept vertically with buoys (Figure 2). The lines were connected to two anchors and placed 6.5m apart on the seabed. A third rope was placed horizontally between the vertical ropes at 9m depth, which is the optimal depth for *D. antillarum* settlement (Williams et al. 2011). Every 50cm, loops were made in the horizontal rope, on which the experimental settlement collectors were attached with tie-wraps. At each location, three pieces of frayed rope, doormat, artificial turf and bio balls were randomly distributed over the loops, resulting in 3 replicates of these settlement collectors per location. To be able to compare settlement rates on the collectors in the water column with settlement rates found in previous studies (Bak 1985, Vermeij et al. 2010), a single panel was added to the set-up on all five locations. Panels were placed 50cm above the seabed on two pieces of rebar that were cast in one of the concrete anchors.

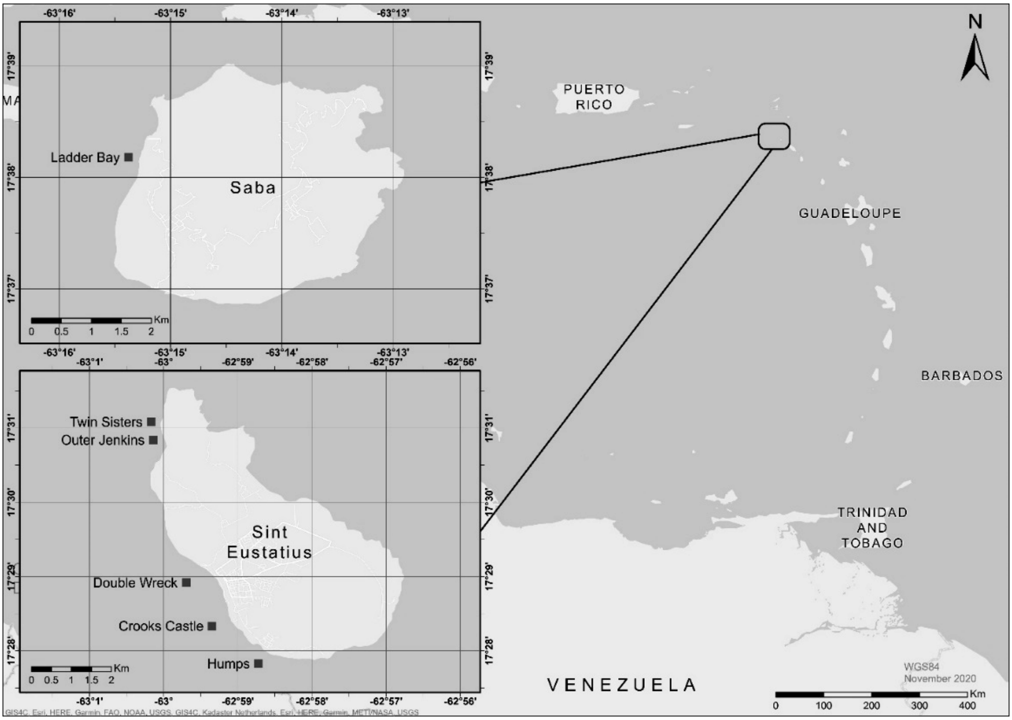

Figure 1: Experimental locations around St. Eustatius and Saba.

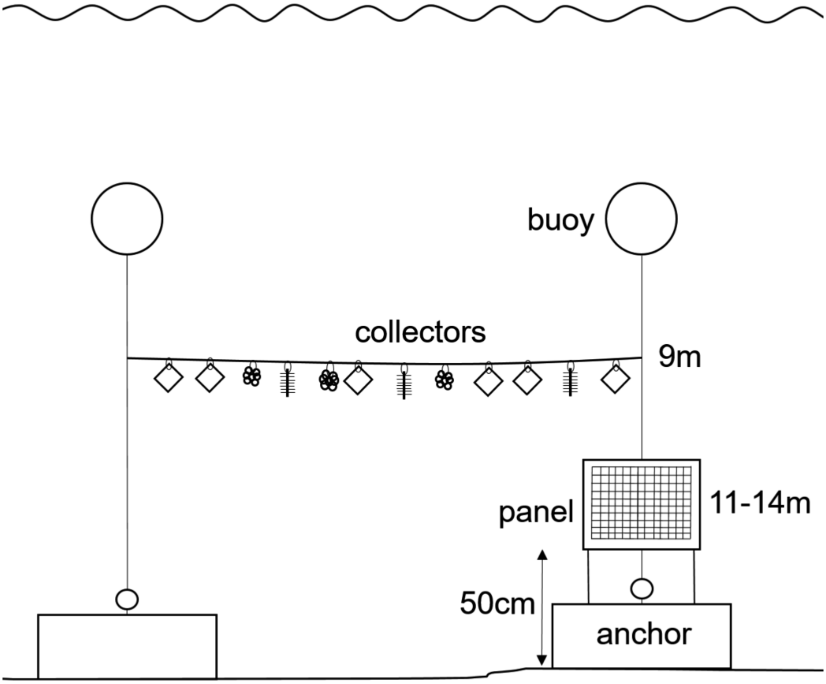

Figure 2: Experimental set-up used at the locations around St. Eustatius in 2019.

The panels were modelled after Bak (1985) and Vermeij et al. (2010) and consisted of a single polyoxymethylene (POM) plate of 49cm x 31cm x 2.5cm with 20 rows and 25 columns of 12mm x 12mm x 10mm small chambers that were milled in the material on both sides (Figure 3). Frayed rope collectors consisted of 20cm long polyester rope with 10cm long side strings, that was designed for mussel seed collection (Molinet et al. 2017). Doormat collectors consisted of 10cm x 10cm x 1cm samples of polyvinyl chloride (PVC) threads with a diameter of 0.5mm that created a spaghetti-like appearance. Artificial turf collectors from polyethylene (PE) and polypropylene (PP) measured 10cm x 10cm with a blade height of 1.5cm. Bio ball collectors consisted of clusters of 15 polypropylene (PP) balls with a diameter of 3cm, kept together by nylon fishing line.

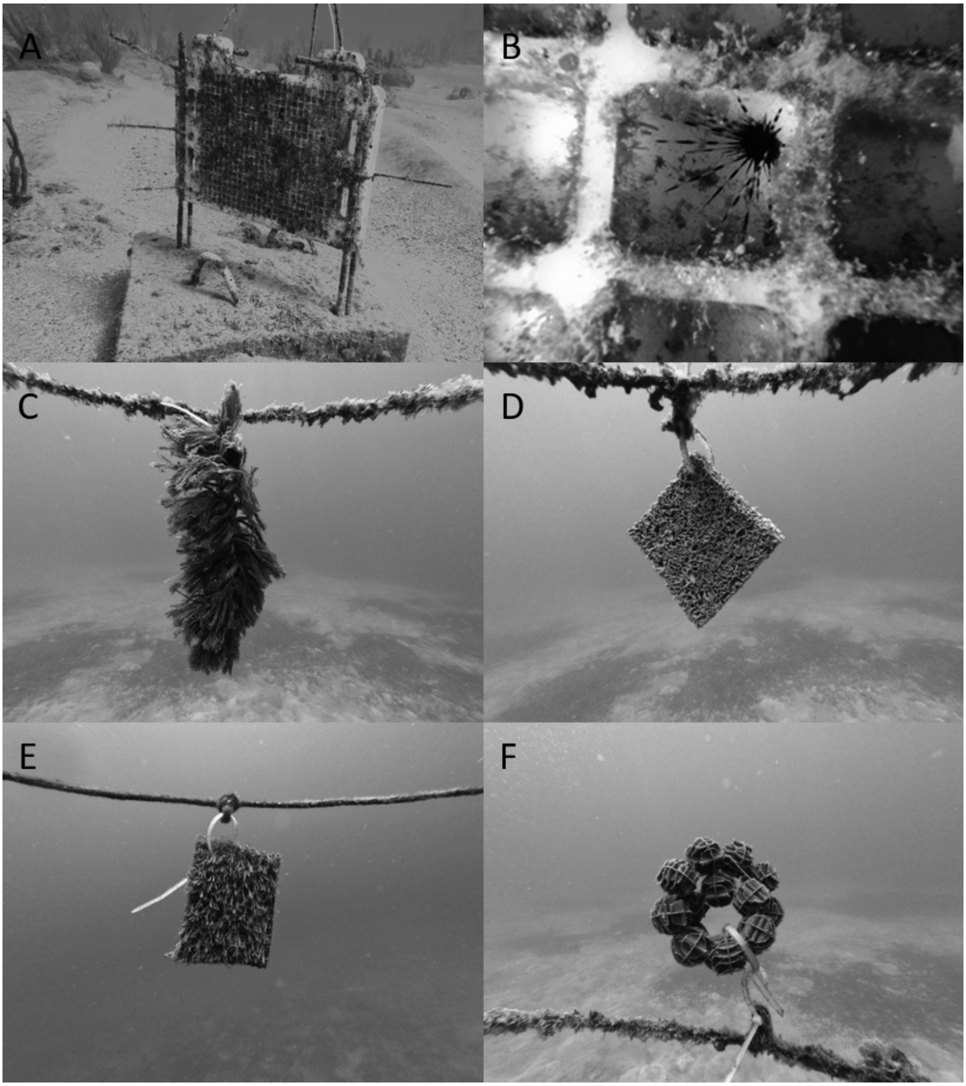

Figure 3: Settlement collectors: a. panel, b. *D. antillarum* settler on panel, c. frayed rope, d. doormat, e. artificial turf and f. bio balls.

The planar surface area of the panels, counting the two-dimensional surface area of both sides, covered 0.31m^2. The planar surface of the frayed rope was 0.08m^2 and the planar surface of all other treatments covered approximately 0.02m^2. The known dimensions of the panel were used to determine its actual surface area (including the surface of all chambers). For the frayed rope, the density, length and diameter of side strings on 2cm of sample were measured and subsequently used to determine the actual surface area of the 20cm-long collector. To determine actual surface area of the doormat, all threads of a 10cm x 1cm sample were separated. The total length and diameter of these threads was measured and used to determine the actual surface area of a 10cm x10cm collector. For the artificial turf collector, the procedure was similar, with the exception that the width of the artificial grass blades was used to determine their combined surface area. A single bio ball was cut into flat and tubular pieces to determine the actual surface area of 1 bio ball and subsequently of the whole collector of 15 bio balls. The “rugosity” of each treatment was determined by dividing the actual surface by the planar surface.

On the panels, *D. antillarum* counts were done by a researcher using SCUBA, an underwater flashlight and tweezers (Bak 1985, Vermeij et al. 2010). All recorded *D. antillarum* settlers were removed from the panel. Counts on both sides of the panel were pooled and the panel was lightly brushed, but not entirely cleaned, to remove excessive benthic growth that could reduce settlement or hamper observations (Bak 1985). All other collectors were enclosed in plastic zip-lock bags and stored in a cooler on the boat. New collectors were immediately attached to the rope. Collectors were analyzed within two hours after collection. Each collector was thoroughly rinsed five times in different white trays. The trays were analyzed for *D. antillarum* settlers, which usually quickly attached to the tray, making it easier to spot them as they would not oscillate with the sediment and other organisms around them. Fouled panels are known to collect more *D. antillarum* settlers compared to clean ones, probably because the biofilm emits important cues for settlement (Bak 1985). The collectors, of which most of the biofilm was removed during rinsing, were therefore stored in sea-water to promote the growth of a new biofilm and were redeployed at the next location that was monitored.

Experiment 2: panels vs. bio balls

To test if low settlement rates on the panels were the result of the collector or its place close to the seabed a follow-up experiment was conducted in May 2020. Four sets of panel and bio ball collectors were deployed at both the Twin Sisters and the Crooks Castle location. Both types of collector were deployed on rebar casted in concrete anchors and were attached 50 cm above the seabed. Another four sets of bio ball collectors were deployed mid-water, so comparisons were possible with settlement rates in 2019. The mid-water bio ball collectors were connected to a rope, which was kept vertically with a buoy, at 7, 8, 9 and 10m depth. Both panels and bio ball collectors were sampled once a month from June-August 2020 following the same procedure as described for experiment 1. As the 2019 sampling indicated that settlers on the panel were larger than settlers on mid-water collectors, the test sizes of all settlers found in this experiment were measured. Each settler was photographed in a Petri dish on millimeter paper and their test size was determined using ImageJ version 1.52a (Abràmoff et al. 2004).

Experiment 3 and 4: bio ball collector configuration

To optimize the bio ball collector for settlement collection, two follow-up experiments were performed in May 2020 at Ladder Bay at Saba (Figure 1). Due to Covid-19 restrictions it was not possible to perform this experiment on St. Eustatius and Ladder Bay was the location with the highest settlement around Saba in 2019 (A. Hylkema, unpublished data). Five sets of anchor and rope kept vertically by buoys were placed five meters apart at 12m depth. Each rope had a loop at 8m, 9m and 10m depth. On each rope, the following treatments were randomly attached to the loops: net with 15 bio balls, net with 50 bio balls and net with 100 bio balls. After one month, the bio balls were collected and analyzed following the procedure described for St. Eustatius. In June 2020, another follow-up experiment was conducted at the same location using the same set-up to test if bio balls deployed along a string of fishing line would collect more settlers than bio balls together in a net. For this purpose, 50 bio balls on a string and 50 bio balls in a net were attached at the same height on all five ropes. After one month, the bio balls were collected and analyzed using the same methods as described earlier.

Data analysis

Statistical analyses were performed with R (R Core Team, 2019) using R studio version 1.2.5001. Settlement collectors around St. Eustatius in 2019 were surveyed every two weeks. To correct for slight differences in soaking time and a different planar surface per collector, settlement rates were expressed as monthly settlement per m^2 (Williams et al. 2010): the number of *D. antillarum* on each collector sample was divided by the number of days the collector was in the water (~14 days), multiplied by 30 (one month) and divided by their planar surface area. To test the effect of treatment and location on the monthly settlement rate Generalized Linear Models (GLM) with a negative binomial error distribution were used to test the effect of treatment, location and week number on the settlement rate in 2019 using the glm.nb function in the R package "MASS" (Ripley et al. 2020). The Akaike Information Criterion (AIC) was used to select the model with the highest goodness of fit (Zuur et al. 2009), which was the model including all three explanatory variables. Model validation revealed that there was no overdispersion, which was earlier the case when a GLM with a Poisson distribution was used. Plotting Pearson residuals against fitted values and explanatory variables revealed no obvious patterns. Likelihood ratio tests (LRT) were performed for statistical inference of the explanatory variables using the drop1 function, while Tukey's post-hoc tests were conducted to examine significance of treatment and location using estimated marginal means (EMM) from the R package "emmeans" (Lenth and Herve, 2019).

As settlement rates on panels and frayed rope were very low compared to the other three much more suitable materials, they were less useful for comparisons of spatial and temporal patterns in settlement. Hence, temporal settlement trends for the different locations (Figure 4) were examined using the combined data from only the doormat, artificial turf and bio balls collectors. Average monthly settlement rates were calculated by averaging settlement estimates over all three collector types, resulting in nine replicates per monitoring event (three settlement collectors with three samples each). As each location was monitored every two weeks, this resulted in two estimates of settlement rates for each month at each location. In comparing differences between settlement collector types and between settlement levels per location, months with very low settlement were not useful. Therefore, these questions were examined using only the data from the months June-August, during which high settlement levels were recorded (Figure 5). These comparisons were based on 90 replicates per settlement collector (three samples per settlement collector monitored twice a month during three months and at five locations) except for the panels which had only one collector per location (and therefore yielding only 30 replicates). To determine the coefficient of variation, a measure of reproducibility (Balsalobre et al. 2016), the standard deviation of each treatment was divided by the mean, using the subset of the data containing the months with high settlement (June-August).

Settlement rates around St. Eustatius in 2020 were expressed as monthly settlement per m^2 following the procedure described above. GLMs with a negative binomial error distribution were used to test the effect of treatment, location and month on settlement rates around St. Eustatius in 2020 and to test the effect of treatment and location on the test size of *D. antillarum* settlers in 2020. Model selection and validation, as well as post-hoc testing was performed as described above: the best fitting models included all considered variables. The full dataset containing 24 replicates per treatment (four samples per settlement collector monitored every month during three months at two locations) was used for illustration purposes (Figure 6).

For the optimization experiments conducted at the Saba location in 2020, the data was expressed as monthly number of *D. antillarum* settlers per bio ball. A one-way ANOVA and Tukey post-hoc test were used to test the effect of number of bio balls in a net collector, while an independent t-test was used to compare monthly settlement rates between strings and net collectors. All graphs were made using the R package “ggplot2” and P values <0.05 were considered statistically significant.

5.4 Results

Experiment 1: settlement on different collectors

From April till December 2019, a total of 893 *D. antillarum* settlers were collected from the settlement collectors around St. Eustatius. Figure 4 shows average monthly *D. antillarum* settlement per location on all substrates, except the frayed rope and panels, because these had significant lower settlement rates per m^2. The first *D. antillarum* settlers were observed in the second half of May, after which settlement rates quickly increased. Settlement rates peaked at the end of May and early June with a mean settlement rate of 200-760 *D. antillarum* per m^2, depending on the location. At some locations a second, smaller peak was observed in the second half of August. In September, settlement rates decreased and almost no settlement was observed from October till December.

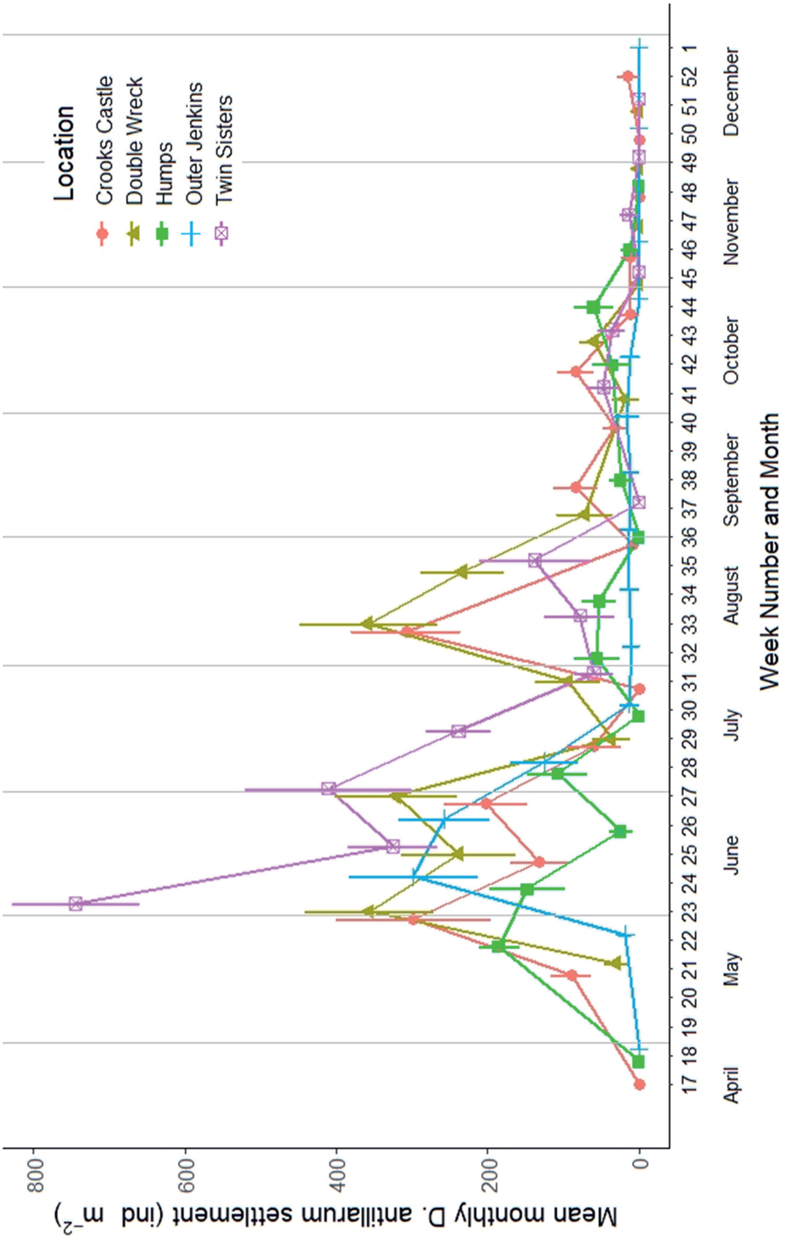

Figure 4: Monthly *D. antillarum* settlement per m^2 in time per location averaged over doormat, artificial turf and bio balls collectors. Error bars show 95% CI interval.

Treatment (LRT=63.18, df=4, P<0.001) and location (LRT=23.84, df=4, P<0.001) were significant predictors of *D. antillarum* settlement. Settlement decreased during the monitoring period, which was reflected by the negative association between settlement and week number (LRT=126.05, df=1, P<0.001). Figure 5 shows *D. antillarum* settlement on different settlement collectors during months of high settlement (June – August). Pairwise comparisons revealed that panels had significantly less settlement per m^2 than all other treatments (P<0.001, except for frayed rope, P=0.043) and this was the case for all locations. Frayed rope collectors had significantly less settlement than doormat, artificial turf and bio ball collectors (P<0.001, for all comparisons), which did not differ significantly among each other. The lowest settlement was found at Outer Jenkins, that had significantly less settlement per m^2 than all other treatments (P<0.001, for all comparisons), which did not differ significantly among each other.

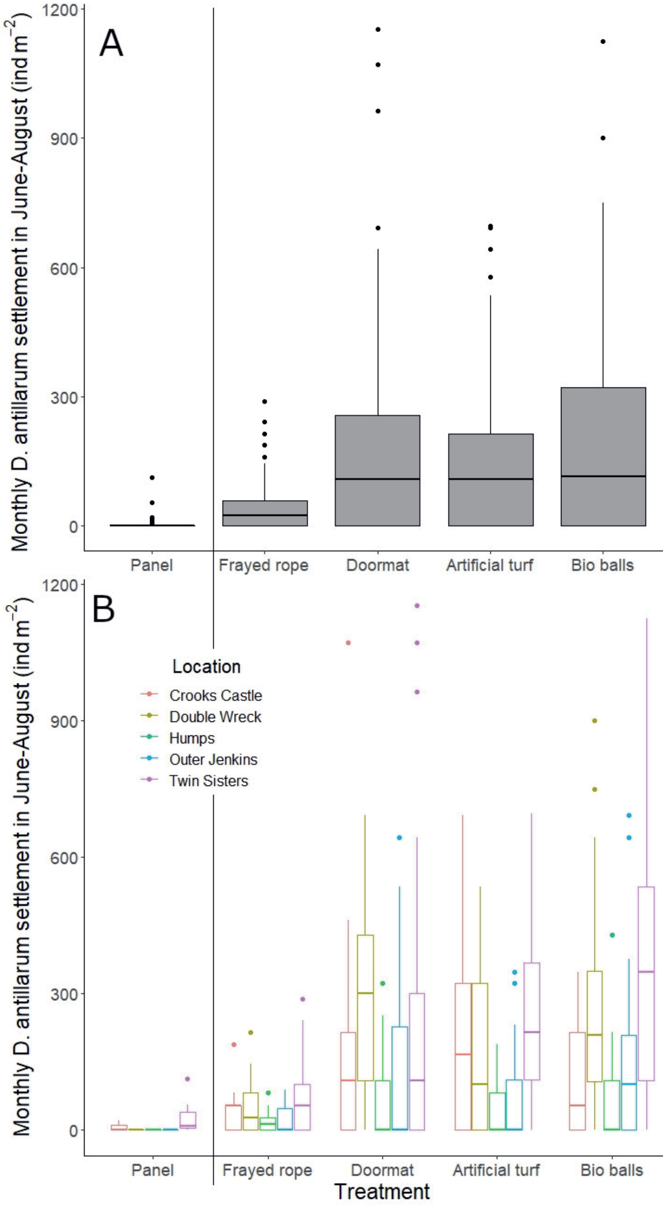

Figure 5: Monthly *D. antillarum* settlement per m^2 on five types of settlement collectors during months of high settlement (June-August) **A.** overall and **B.** detailed per location. The boxplots show the median (black or colored line), the first and third quartiles (box) and the lower and upper extremes, dots represent outlier values (> 1.5 inter-quartile range from third quartile). The panel was positioned closer to the bottom than the other treatments.

The actual surface area, including all chambers, side strings and internal spaces was highest on the panels and lowest on the bio balls (Table 2). The rugosity (actual surface/planar surface) was highest on the artificial turf, followed by the doormat, bio balls, panels and frayed rope. The coefficient of variation, a measure of reproducibility which is calculated by dividing the standard deviation of all settlement rates in the period June-August by the mean of the same period, was lowest on the bio ball collectors, followed by the artificial turf, the doormat and the frayed rope, respectively (Table 2). The panels had the highest coefficient of variation, meaning that these observations were least consistent.

Table 2: Descriptive variables (planar surface, actual surface and rugosity) and the coefficient of variation (standard deviation/mean settlement rate during months of high settlement) for each of the five collector types.

	Planar surface (m^2)	Actual surface (m^2)	Rugosity (Actual surface m^{-2})	Coefficient of variation
Panel	0.31	0.83	2.70	313%
Frayed rope	0.08	0.14	1.80	147%
Doormat	0.02	0.12	5.98	147%
Artificial turf	0.02	0.21	10.29	133%
Bio balls	0.02	0.10	4.50	124%

Experiment 2: panels vs. bio balls

From June until August 2020, 247 *D. antillarum* settlers were collected from the panels and bio ball collectors at the Twin Sisters and Crooks Castle locations at St. Eustatius. Treatment (LRT=62.95, df=2, $P<0.001$), location (LRT=22.99, df=1, $P<0.001$) and month (LRT=15.05, df=2, $P<0.001$) were significant predictors of *D. antillarum* settlement. Settlement was highest on the bio balls in the water column, followed by the bio balls close to the seabed and finally, the panels (Figure 6). Pairwise comparisons revealed that all treatments differed significantly from each other ($P<0.001$ for all comparisons). August and July had significantly higher settlement rates than June ($P<0.001$ for both comparisons), which did not differ significantly among each other.

The test size of the collected *D. antillarum* settlers ranged between 0.46 and 7.54 mm. Treatment (LRT=233.91, df=2, $P<0.001$) and location (LRT=5.49, df=1, $P<0.019$) were significant predictors of *D. antillarum* test size. Pairwise comparisons revealed that *D. antillarum* settlers on the panels were significantly larger than on the bio balls collectors in the water column and close to the seabed ($P<0.001$ for both comparisons), which did not differ significantly among each other (Figure 6). Location Twin Sisters had slightly larger settlers than Crooks Castle.

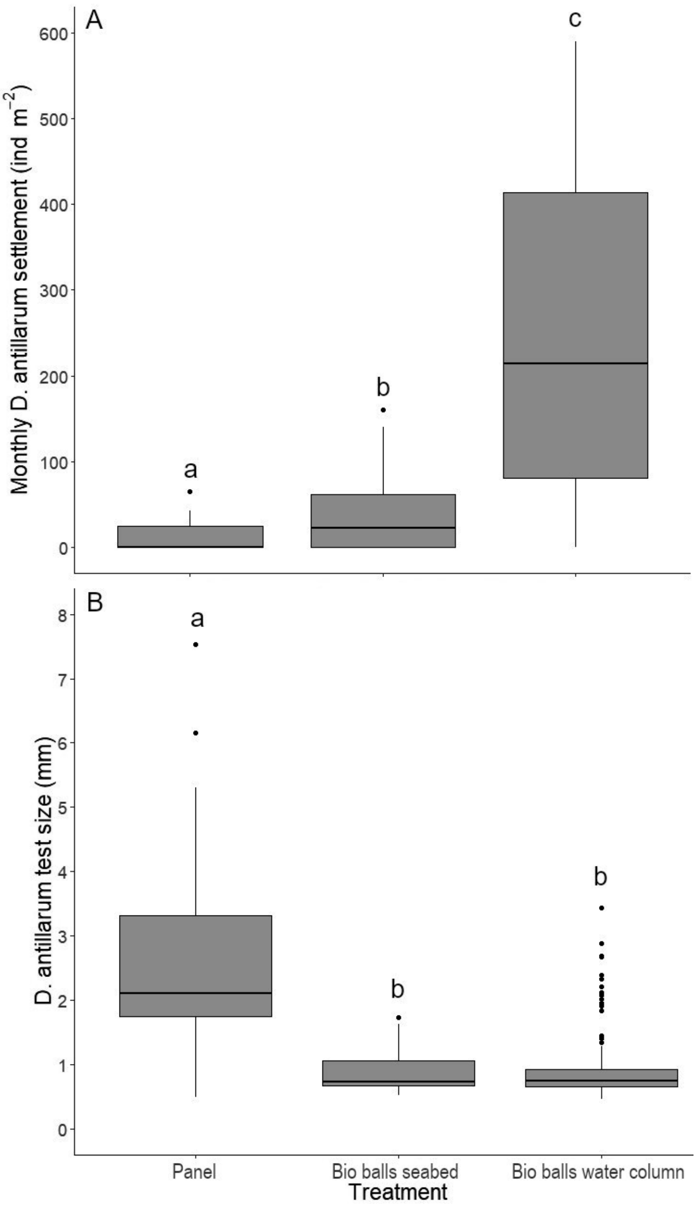

Figure 6: **A.** Monthly *D. antillarum* settlement per m^2 and **B.** test size of *D. antillarum* settlers per treatment. The boxplots show the median (black line), the first and third quartiles (grey shaded box) and the lower and upper extremes, black dots represent outlier values (> 1.5 inter-quartile range from third quartile). Treatments sharing the same letter are not significantly different ($P > 0.05$).

Experiment 3 and 4: bio ball collector configuration

In June 2020, 92 *D. antillarum* settlers were recorded on the bio ball net collectors. Monthly *D. antillarum* settlement rates per bio ball were significantly affected by the number of bio balls in the net collector ($F_{2,12}$=4.03, P=0.0461). A Tukey post-hoc test revealed that the settlement rates per bio ball in collectors with 15 bio balls was significantly higher than those from collectors with 100 bio balls (P=0.037), but not significantly different than those from collectors with 50 bio balls (P=0.345, Figure 7). Settlement rates per bio ball of collectors with 50 and 100 bio balls did not differ significantly (P=0.382). In July 2020, 97 *D. antillarum* settlers were recorded on the net and string bio ball collectors. Monthly settlement rates per bio ball on the string collector were significantly higher than settlement rates on the net collector (t(8)=-3.025, P=0.016, Figure 7).

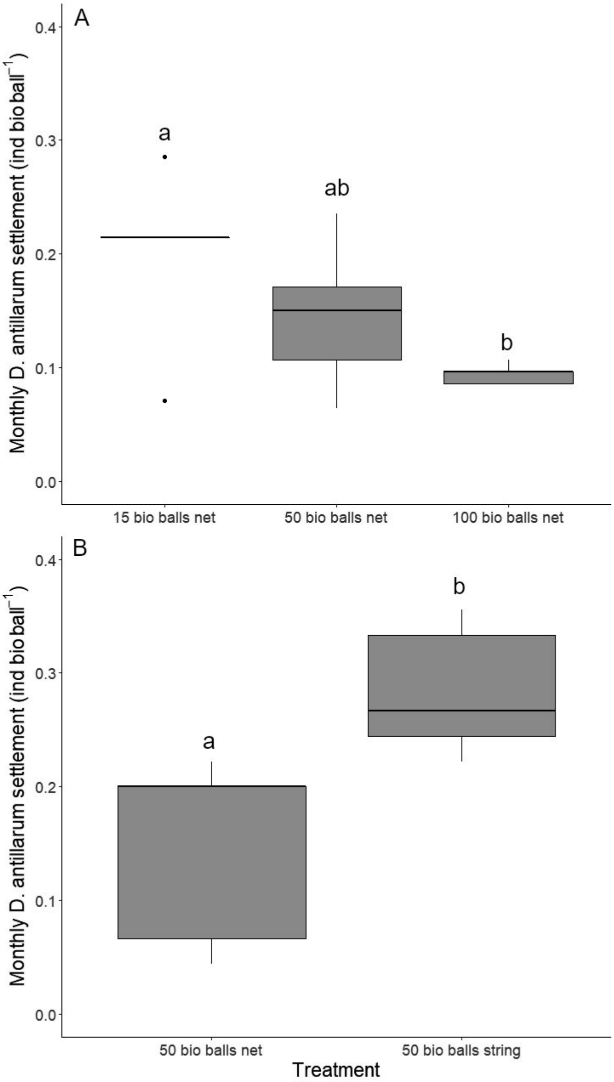

Figure 7: Mean monthly *D. antillarum* settlement per bio ball for **A.** 15, 50 and 100 bio balls per net and **B.** 50 bio balls in a net and 50 bio balls on a string. The boxplots show the median (black line), the first and third quartiles (grey shaded box) and the lower and upper extremes, black dots represent outlying values (> 1.5 inter-quartile range from third quartile). Treatments sharing the same letter are not significantly different (P > 0.05).

5.5 Discussion

D. antillarum settlement around St. Eustatius peaked in early May and June, followed by a smaller peak in August. Settlement was low during the rest of the monitoring period. This pattern is very similar to *D. antillarum* post- die-off settlement in La Parguera, Puerto Rico (Williams et al. 2010) and Curaçao (Vermeij et al. 2010) but different than the pre-die-off settlement pattern observed in Curaçao in 1982 and 1983 (Bak 1985). Before the die-off, settlement around Curaçao was more consistent throughout the year, with peaks in March, June, September and December (Bak 1985). Current settlement patterns may be explained by the fact that most adult populations in the region never recovered. On average, current population densities across the Caribbean region are approximately 12% of those before the die-off (Lessios 2016). While some populations in shallow depths showed at least some recovery (Miller et al. 2003, Carpenter & Edmunds 2006, Debrot & Nagelkerken 2006), most of the populations on deeper reefs have never recovered (Lessios 2016). Therefore, the number of spawning populations is still greatly reduced compared to before the die-off. Larval supply throughout the year might be dependent on the number of adult populations effectively spawning upstream and with few recovered populations this could mean fewer settlement peaks (Hunte & Younglao 1988). This will very likely result in a lower settlement rate throughout the year.

The inclusion of both settlement panels on the seabed and collectors in the water column provided the opportunity to compare settlement rates with studies that used either one of these methods. In Curaçao, settlement was measured with panels before (Bak 1985) and after the die-off (Vermeij et al. 2010). In 1982 and 1983, highest monthly settlement rates were 104 and 243 *D. antillarum* per m^2, respectively. Settlement rates decreased to almost zero after the die-off in 1984 (Bak 1985), but were restored in 2005, with 146 *D. antillarum* per m^2 (Vermeij et al. 2010). Peak settlement rates on panels around St. Eustatius (present study) were 20-104 *D. antillarum* per m^2, which is in the same order of magnitude as reported by Bak (1985) before the die-off. The mean maximum settlement rate of *D. antillarum* on mid-water collectors around St. Eustatius was 200-760 *D. antillarum* per m^2, depending on collector type and location. This is similar to settlement rates observed on mid-water collectors in Puerto Rico in 2006 and 2008, where the mean maximum settlement rates at a single location were 1100 and 220 *D. antillarum* per m^2, respectively (Williams et al. 2010, 2011). Settlement rates around St. Eustatius were higher than around the Florida Keys, where settlement was measured on panels in 2005 and 2006 (Miller et al. 2009) and Mexico, where settlement was measured in 2014 and 2015 (Maldonado-Sánchez et al. 2019). Settlement at both locations was very low throughout the year, with <2 *D. antillarum* per m^2 as the highest monthly settlement rates (Miller et al. 2009, Maldonado-Sánchez et al. 2019).

D. antillarum larvae seem to settle on almost everything in the water column, as we observed settlers on our buoys and other submerged research materials. Bio ball collectors had the highest monthly settlement per m^2, although settlement was not significantly different from doormat and artificial turf collectors. Compared to the other collectors, settlement on bio ball collectors had the lowest coefficient of variation and thus the highest reproducibility. Earlier comparative research on settlement of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* also showed that bio ball collectors had higher numbers of settlers and higher reproducibility than other materials (Balsalobre et al. 2016) and bio ball collectors have also been successfully used for the closely related sea urchin *Diadema africanum* (Hernández et al. 2006). There are practical reasons to use bio balls over other materials in addition to high settlement rates and high reproducibility. Bio ball collectors were the easiest collector to rinse, as this material retained fewer small particles. Rinsing the frayed rope and artificial turf collectors was more time consuming because large amounts of silt were retained in these materials. Panels were the most expensive to make, the most time consuming to process and complicated to place in the field. More time is needed to monitor the panels, as this must be done using SCUBA, while the other collectors can be analyzed on land. We observed that the artificial turf collectors release a small amount of micro-plastics during rinsing, which probably also occurs during incubation in the water. The doormat collectors had none of the above-mentioned disadvantages and would provide a good alternative if the bio ball collectors are not available. Balsalobre et al. (2016) associated the effectiveness of settlement collectors to the rugosity of the material, but that did not seem to be the most decisive factor in the present study. Artificial turf collectors had twice the rugosity of doormat and bio ball collectors, but similar settlement rates, while panels had a higher rugosity than the frayed rope, while their settlement rates were lower.

Settlement rates per m^2 on the bio balls, doormat and artificial turf collectors were 20 times higher than on the panels and four times higher than on the frayed rope during months of high settlement (June-August). The position of the panel above the substrate instead of in the water column might be the main explanation for this difference, as settlement collectors close to the seabed generally yield less settlers (Williams et al. 2011). This was confirmed by our follow-up experiment, in which bio ball collectors close to the seabed yielded seven times less settlers than bio ball collectors in the water column. This might be explained by the fact that larvae in the bottom water layer, close to the reef, will encounter many other potential settlement substrates and receive more settlement cues compared to larvae higher-up in the water column. Alternatively, the lower settlement rates on collectors close to the seabed might also be explained by factors such as hydrodynamics and behavior of larvae, that complicate larval transport to the bottom water layers. If either one, or a combination of both explanations is true, settlement on collectors close to the seabed might be a better indication of the actual settlement on the reef.

Benthic monitoring surveys conducted in 2017-2019 using the recommended guidelines of the Global Coral Reef Monitoring Network (GCRMN), revealed that the mean *D. antillarum* density on the reefs around St. Eustatius was <0.01 *D. antillarum* per m^2 (Kitson-Walters, unpublished data). This density is far below the pre-die-off densities of 0.76 to 14.38 *D. antillarum* per m^2 reported for other Caribbean Islands (Lessios 2016). *D. antillarum* was not observed at any of the locations presented in this study during the 2017-2018 surveys with the exception of Double Wreck (1 juvenile in 2018). During the 2019 surveys, densities of <0.01 individual per m^2 were recorded for all sites except Outer Jenkins Bay which had no individuals. The absence of juveniles indicates that despite high peak settlement rates, post-settlement survival was low, for example possibly due to high predation pressure of micropredators (Harborne et al. 2009, Vermeij et al. 2010, Williams et al. 2011).

D. antillarum settlement rates on panels were significantly lower than on bio ball collectors at the same depth. Settlers on the panels were larger than on bio ball collectors deployed close to the bottom and in mid-water. The panels were visually monitored using SCUBA, while the other collectors were thoroughly rinsed on land. The smallest setters on the panels might have been overlooked and although the panels were brushed off after every monitoring, they were not entirely cleaned, possibly allowing missed settlers to increase in size until the next monitoring period. These settlers spent more time on the panels, increasing the chances of post-settlement predation and possibly resulting in significantly less but larger settlers observed on the panels. This would mean that low settlement rates on the panels might not only be the result of lower larval availability in bottom water layers, but also of post-settlement predation. An alternative explanation for the larger setters on the panels is that *D. antillarum* settlers moved to the panels later, after they settled on the rebar or concrete used to keep the panels upright.

The collection of *D. antillarum* settlers is the key activity of a relatively new reef restoration method in which the settlers are collected in the field, raised in a land-based nursery and then returned to the reefs once they reach a young adult size (2-4cm) (Williams 2017, 2021). To make this method economically feasible, it is important to maximize the number of settlers that can be collected. Bio balls and doormat should be used and deployed in mid-water layers to maximize the collection of D. antillarum settlers. Our follow-up experiments that were conducted around Saba in 2020, showed that bio ball collectors become less effective when the number of bio balls in a net is larger. This is probably because their combined planar surface does not increase as much as their combined volume. The higher number of bio balls reduces the net individual bio ball exposure to currents and thereby also the contact with the late-staged larvae in the water column. This was confirmed by comparing strings and nets with bio balls: bio balls on strings collected two times more settlers than bio balls clumped together in nets and are therefore recommended for the purpose of settler collection.

In conclusion, this study shows that *D. antillarum* settlement around St. Eustatius is generally still lower than settlement rates measured before the die-off around Curaçao, even though, peak settlement rates did attain the same order of magnitude. As almost no juvenile or adult *D. antillarum* were observed on the reefs around the settlement collectors, it is likely that other factors are hindering the recovery of *D. antillarum* populations. Bio balls are the preferred settlement collector because of their effectiveness and efficiency, but doormat collectors can also be used. To optimize settler collection, bio ball collectors are best deployed in mid-water layers and as strings instead of clumps. Panels yielded the lowest numbers of settlers, which can partly be explained by their position close to the seabed. The low yields of settlement collectors in bottom water layers indicates that collection of settlers in mid-water layers followed by transplantation to suitable bottom habitat is an essential step in restoration. The high peak settlement rates around St. Eustatius show potential for recovery of *D. antillarum* populations and we recommend research into the feasibility of aiding recovery by providing suitable settlement substrate and adequate shelter on locations with high settlement rates during the peak of the settlement season.

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Chapter 6

Assisted natural recovery: a novel approach to enhance Diadema antillarum recruitment

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6.1 Abstract

The massive die-off of the sea urchin *Diadema antillarum* in 1983-1984 is one the main reasons for low coral recruitment and little coral recovery in the Caribbean. As natural recovery of *D. antillarum* is slow to non-existent, multiple restoration studies have been attempted. There are currently three different approaches to obtain individuals for restocking: translocation of wild-collected juveniles or adults, lab reared juveniles cultured from wild-collected settlers, or lab reared juveniles cultured from gametes. All three methods are costly and can only be applied on a relatively small scale. We here propose a fourth, new approach, which we term assisted natural recovery (ANR) of *D. antillarum* populations. ANR, a concept already applied in terrestrial restoration to restore forests and grasslands, can accelerate succession by removing barriers to natural recovery. In this study, performed on the Dutch Caribbean island of Saba, suitable settlement substrate was provided in the form of bio ball streamers that were attached to the reef shortly before the settlement season. At the end of the experiment, reefs with streamers had significantly higher *D. antillarum* recruit densities than control reefs without additional settlement substrate, indicating that the lack of settlement substrate is an important factor constraining natural recovery. However, the *D. antillarum* recruit abundance was low compared to measured settlement rates, possibly due to low post-settlement survival. The size distribution of recruits showed that recruits almost never became larger than 20 mm, which is likely due to predation. We conclude that, next to low settlement availability, also low post-settlement survival and high predation on recruits constrain natural recovery of *D. antillarum* populations on Saba. To improve survival of settlers till adults we propose to 1) reduce predation on settlers by using bio balls or other substrates that can provide shelter to larger individuals, 2) optimize the reef habitat by removing macroalgae, either manually or by facilitating other herbivores and 3) choose sites with a known lower predation density. The combination of these measures could improve prospects for ANR and we expect this new approach to contribute to recovery of *D. antillarum* populations in the future.

Key words

accelerated natural recovery, Saba, coral reef, sea urchin, Caribbean

6.2 Introduction

The accelerated degradation of Caribbean coral reefs started in the 1970s, when diseases decimated the most important reef-building corals (Gladfelter 1982, Aronson & Precht 2001). The decennia that followed were characterized by sequential losses in coral cover by hurricanes, global warming and additional disease (Jackson et al. 2014). The degradation of Caribbean coral reefs proceeded quicker than in the Indo-Pacific region, largely because of exceptionally low coral recruitment rates (Precht et al. 2020). This was mainly a consequence of overfishing of herbivorous fish (Jackson et al. 2001, Pandolfi et al. 2003), followed by a massive die-off of the sea urchin *Diadema antillarum* in 1983 and 1984 (Lessios et al. 1984). At the time of the die-off, *D. antillarum* was the most abundant remaining herbivore on Caribbean reefs. After 98% of the population had succumbed (Lessios 2016), the cover of macroalgae increased within days (Carpenter 1988) and macroalgae have remained dominant on many Caribbean reefs since then (Jackson et al. 2014). Macroalgae are principal competitors of adult corals (Jompa & McCook 2002, Box & Mumby 2007) and limit coral recruitment (McCook 2001, Box & Mumby 2007, Arnold et al. 2010), thereby reducing the ability of the coral reef to recover from disturbances.

With a few exceptions (Carpenter & Edmunds 2006, Debrot & Nagelkerken 2006, Myhre & Acevedo-Gutiérrez 2007), mostly limited to shallow and sheltered waters, the recovery of *D. antillarum* populations has been very slow to non-existing (Lessios 2016). The underlying factors constraining the recovery of *D. antillarum* are not exactly known and will very likely differ per location (Lessios 2016). For instance, studies of *D. antillarum* settlement rates show that at some locations, for example around Puerto Rico (Williams et al. 2011), the Florida Keys (Miller et al. 2009) and Mexico (Maldonado-Sánchez et al., 2019), settlement rates can be very low throughout the year. At these locations, low fertilization success (Lessios, 1988, Lessios 2005, Feehan et al., 2016) and lack of an upstream larval source-population (Roberts, 1997) possibly constrain recovery. At other locations, for example around Curaçao (Vermeij et al. 2010), Puerto Rico (Williams et al. 2010, 2011), St. Eustatius (Hylkema et al. 2022) and Saba (Klokman & Hylkema, in prep) peak settlement rates are in the same order of magnitude as measured before the die-off around Curaçao (Bak 1985). Hence, at these locations other factors seem to constrain natural recovery of *D. antillarum*.

At the few places where *D. antillarum* densities on the reef actually recovered, this was correlated with a decrease in macroalgae and an increase in coral recruitment (Edmunds & Carpenter 2001, Carpenter & Edmunds 2006, Myhre & Acevedo-Gutiérrez 2007, Idjadi et al. 2010). These results highlight the importance of the recovery of *D. antillarum* for increased resilience of Caribbean coral reefs (Lessios 2016). However, since it could take decades before *D. antillarum* populations recover naturally throughout the Caribbean (Chiappone et al. 2013) and a new die-off is reducing population densities in parts of the Caribbean in 2022 (AGGRA 2022) active intervention might help accelerate *D. antillarum* recovery (Adam et al. 2015). Three different methods have so far been attempted to help restore *D. antillarum* populations and all rely on the restocking of juvenile or adult individuals. These are either obtained by translocating wild *D. antillarum* from recovered populations

(Macía et al. 2007, Burdick 2008, Dame 2008), by collecting settlers and head-starting them till juveniles in a land-based nursery (Williams 2017, 2021) or by culturing juveniles in captivity through their larval stages starting from gametes (Pilnick et al. 2021; Wijers et al. in prep).

When using wild *D. antillarum* for restocking, a local, stable population is needed with thousands of individuals that can likely be transplanted without too much consequences for the donor location itself. This is seldom the case. Collecting juvenile *D. antillarum* with settlement collectors and raising them in a land-based facility appears more feasible and has already been successfully achieved on Puerto Rico (Williams 2017, 2021). *D. antillarum* cultivation from gametes is an alternative method to produce juveniles year-round. Despite the sensitive nature of the larvae and the relatively long larval phase (Eckert 1998, Bielmyer et al. 2005), several lab-based culture runs have recently produced over 100 settlers (Pilnick et al. 2021, Wijers et al. in prep). In contrast to using wild-caught individuals, head-starting collected settlers and culturing juveniles in the laboratory from gametes appears scalable, but the costs per juvenile remain relatively high, limiting the large scale restoration potential of these methods. To circumvent the limitations of current restoration techniques, we here propose assisted natural recovery (ANR) as a new, scalable approach for restoring *D. antillarum* populations.

ANR is practiced in terrestrial ecosystems to aid the recovery of grasslands (Coiffait-Gombault et al. 2011) and forests (Mackenzie & Naeth 2010, Hardwick et al. 2004, Shono et al. 2007). ANR aims to accelerate succession by reducing barriers to natural ecosystem recovery. It is an alternative to replanting practices and can be applied on a large scale at a relative low cost. As the success of ANR is dependent on natural recovery processes, it only functions if some form of natural succession is already in progress (Hardwick et al. 2004, Shono et al. 2007). For forest regeneration, it often means the introduction of seed banks, the removal of competitors and minimization of disturbances (Hardwick et al. 2004, Shono et al. 2007). There are some analogies between forest regeneration and *D. antillarum* restoration. Just like trees can be replanted, restocking *D. antillarum* is possible. However, it is often resource-consuming and therefore remains relatively limited in scale. At the same time, essential conditions for natural recovery, indicated by high *D. antillarum* settlement rates on artificial substrates (Vermeij et al. 2010, Williams et al. 2010, 2011, Hylkema et al. 2022), appear to be present at certain locations. However, high settlement rates on artificial substrates do not always result in natural recovery of *D. antillarum* populations. This discrepancy can most likely be explained by two barriers to natural recovery, either low natural settlement substrate availability (Rogers and Lorenzen 2008) or low post-settlement survival, for example through high predation pressure or low shelter availability (Bechtel et al. 2006, Vermeij et al. 2010, Williams et al. 2011, Hylkema et al. 2022).

ANR of D. antillarum might be possible if suitable settlement substrate is provided and/or if post-settlement mortality is reduced. Hylkema et al. (2022) compared five different types of D. antillarum settlement collectors. It was concluded that strings of plastic bio balls (Figure 2; hereinafter termed “bio ball streamers”) deployed mid-water were the most effective and reproducible method to monitor D. antillarum settlement. This study will investigate if bio ball streamers attached to the reef shortly before the settlement season in an area with high potential settlement rates, can enhance actual settlement and will result in increased recruitment of D. antillarum. As such, this study will provide insight whether availability of suitable substrate is the major barrier in the recovery of D. antillarum.

6.3 Methods

In April 2021, six patch reefs were selected at the Southwestern corner of Saba, Caribbean Netherlands, at the dive site locally known as Ladder Labyrinth. Coral reefs at this location developed on hard substrate of volcanic origin (Polunin & Roberts 1993). The patch reefs were selected to be similar in terms of high shelter availability, a longest dimension of 6-12 m and at a depth of around 15 m. This resulted in the selection of 2 smaller reefs and 4 larger reefs at this site at a depth of 12-18 m (Table 1). To assign experimental treatments, each reef was paired to the reef with the most similar largest dimension. For each pair, treatment and control were randomly assigned, resulting in three treatment and three control reefs (Figure 1). Permanent markers were attached to both sides of the widest cross-section of each reef to mark the monitoring area.

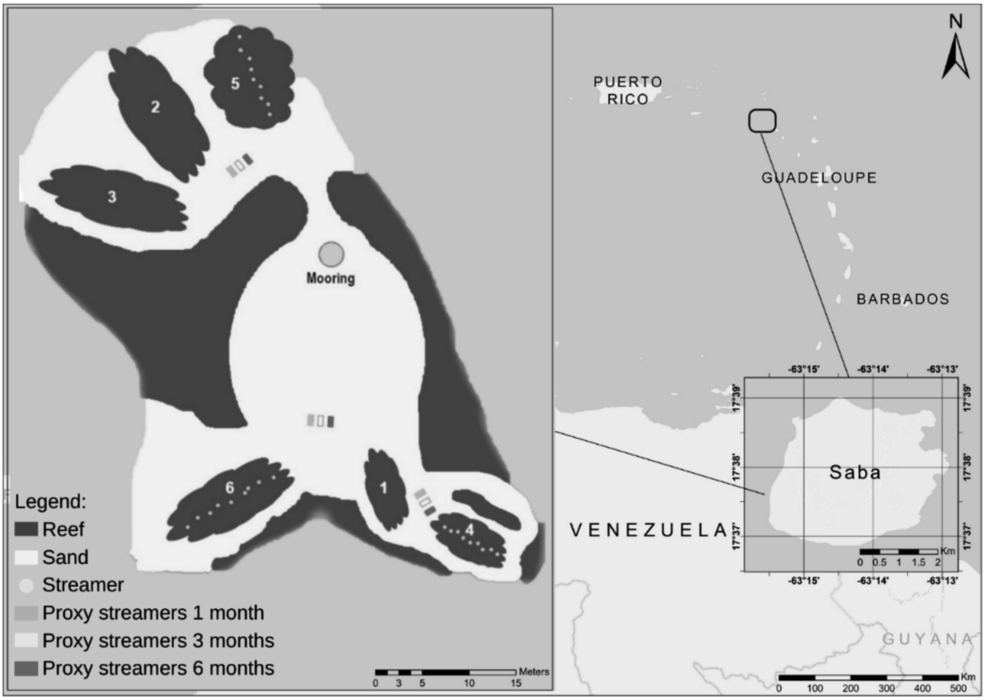

Figure 1: Location of Saba, Caribbean Netherlands in the Caribbean region and location Ladder labyrinth, showing the positions of the experimental reefs and proxy streamers.

Table 1: Perpendicular dimensions, depth and experimental surface area of the patch reefs.

Reef number	Longest dimension (m)	Shortest dimension (m)	Highest point above sediment (m)	Depth deepest point (m)	Experimental area (m^2)	Treatment/ Control
1	6.6	3.0	1.3	13	13.2	Control
2	12.0	5.2	3.0	17	24	Control
3	12.0	4.4	2.8	17	24	Control
4	6.5	1.8	1.5	12	13	Treatment
5	8.5	8.0	3.0	17	17	Treatment
6	11.0	4.3	2.0	15	22	Treatment

To enhance *D. antillarum* settlement, nine streamers, consisting of 30 bio balls stringed on fishing line (Figure 2), were attached to each treatment reef right before the start of the settlement season (Hylkema et al. 2022) in May 2021. Bio balls were 3 cm in diameter and made from polypropylene (PP). The streamers were attached to stainless steel rings, which were epoxied into the reef matrix and kept upright using a small buoy on top. Streamers were approximately 1 m long and had a planar surface of 0.04 m^2. The streamers were placed close to the transect line that was deployed between the two permanent markers. The experimental area was a band of reef one meter to each side of the transect line. Control reefs were left undisturbed.

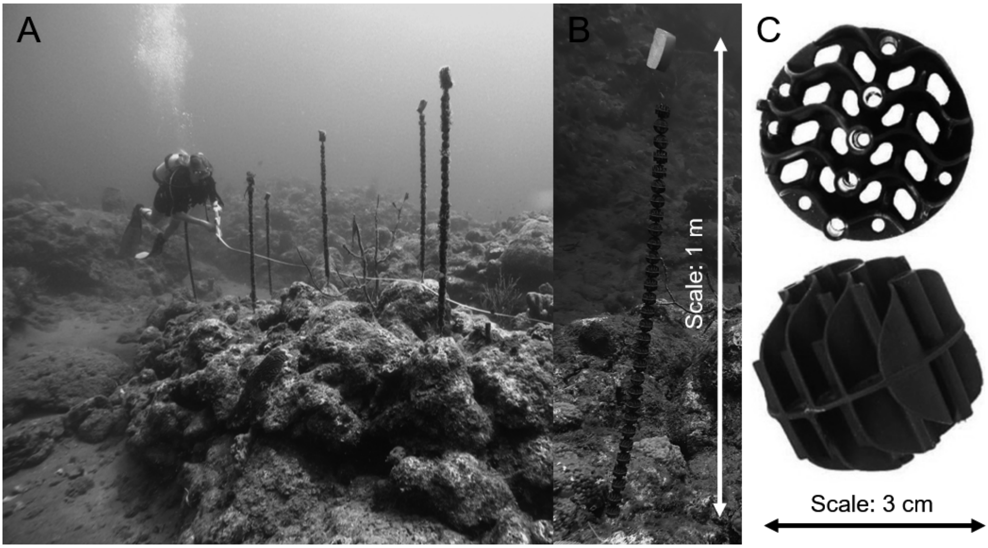

Figure 2: A. Patch reef (no. 4) with streamers on which the transect tape is removed after monitoring, B. Streamer with buoy and C. Close-up of bio balls.

To get an indication of settlement on the streamers attached to the reefs, three extra sets of three streamers were attached to separate cinder blocks. These streamers functioned as proxy for the streamers on the reef and were replaced monthly to get an indication of settlement on the streamers attached to the reef. Each cinder block was placed at 2 m distance from a treatment reef on sand. Extra fishing line was used to position each set of proxy streamers at the same depth as the streamers attached to the reef. To determine settlement on the proxy streamers divers removed the streamers from the cinder block, stored them in a ziplock bag and immediately attached new streamers. Once on the boat, the ziplock bags were stored in a cooler and transported to land. Each proxy streamer was shaken vigorously in five different white trays and the number of *D. antillarum* settlers in each tray were precisely determined (Hylkema et al. 2022). Used streamers were rinsed in fresh water for 24 hours and sun dried before they were redeployed at the next monitoring. To provide insight in the period that *D. antillarum* settlers remained on the streamers and the suitability of streamers with a longer soaking time as settlement substrate, six additional sets of three proxy streamers were deployed at the start of the experiment using the above-described methods. Three of these sets were retrieved and analyzed after three months and three after six months, at the end of the experiment.

The abundance of *D. antillarum* recruits on all six experimental reefs was determined every month, starting at the beginning of the experiment (May 2021) and ending six months later (November 2021) for a total of seven monitoring events. Monitoring was conducted during the day. A transect tape was positioned between the two permanent markers and the area from 1 m left to 1 m right of the transect was thoroughly searched by trained observers for *D. antillarum* recruits using underwater flash lights. The test size of all recruits was estimated to the nearest mm using long jawed calipers. The search time for recruits was standardized to 10 minutes per reef, excluding the time needed to record and measure the observed *D. antillarum*. After the monitoring, all *D. antillarum* were left undisturbed on the reefs.

To determine in which month *D. antillarum* recruits on the reefs could have settled, we used published settlement sizes and growth rates. Cultivation experiments in the laboratory shows that the size of *D. antillarum* settlers is 0.5 mm (Eckert 1998) and growth rates are linear for the first year (Idrisi et al. 2003). Initial growth in the laboratory was on average 3 mm per month (Idrisi et al. 2003), while observations in the field show that small juveniles in the size range of 4-11 mm can grow up to 6.7 mm per month (Randall et al. 1964). The predicted size ranges per monitoring and settlement month based on settlement and growth parameters from the literature are given in Table 2.

Table 2: Estimated size range, based on growth rates from Idrisi et al. (2003) and Randall et al. (1964), per settlement and monitoring month.

		Settlement month					
		April	May	June	July	August	September
Monitoring month	May	0.5 – 3.5	-	-	-	-	-
	June	3.5 – 10.2	0.5 – 3.5	-	-	-	-
	July	6.5 – 16.9	3.5 – 10.2	0.5 – 3.5	-	-	-
	August	9.5 – 23.6	6.5 – 16.9	3.5 – 10.2	0.5 – 3.5	-	-
	Sept	12.5 – 30.3	9.5 – 23.6	6.5 – 16.9	3.5 – 10.2	0.5 – 3.5	-
	October	15.5 – 36.9	12.5 – 30.3	9.5 – 23.6	6.5 – 16.9	3.5 – 10.2	0.5 – 3.5

Statistical analysis

A one-way ANOVA was used to determine if settlement on the proxy streamers differed by month. Initial model validation, using untransformed data, indicated non-normality of the residuals and heterogeneity of the variance, which was corrected for using a square root transformation of the *D. antillarum* counts per streamer. Tukey post-hoc tests were performed to identify months with significantly different settlement rates.

A Kruskal-Wallis test was conducted to determine if soaking time of the streamers had an effect on the number of *D. antillarum* per streamer. Streamers with a soaking time of three (and six months (respectively collected in August and November, 2021),were compared with streamers with a soaking time of only one month (collected in June, 2021). A post-hoc Dunn's test was performed to detect differences in settlement densities for different soaking times. To assess the suitability of streamers with a longer soaking time on new settlement, Mann-Whitney U tests were conducted to compare settlement on streamers with a soaking time of three and six months with streamers that had a soaking time of only one month, all of which were collected on the same day.

Linear mixed models (LMMs) were used for statistical inference of the monthly *D. antillarum* abundance and recruit size on the patch reefs. Treatment, monitoring month, monitoring area and the interaction between treatment and monitoring month were considered as fixed factors. To correct for the monthly repeated counts at the same reefs, reef ID was included as a random factor. Models were run using the lmer function in the R package "lme4" (Bates et al., 2014) and model selection and validation was performed according to Zuur et al. (2009). The Akaike Information Criterion (AIC) was used to select the best-fitting model. For the recruit density, this was the model including treatment, monitoring month and their interaction. For the recruits size, this was the model including only the monitoring month. Plotting the residuals versus the fitted values revealed heterogeneity of variance, while a qqplot showed that the residuals were not normally distributed. Both issues were resolved when density and size were square root transformed. To test in which months treatment significantly affected the *D. antillarum* density, treatment was contrasted within month using the package "emmeans" (Lenth and Herve, 2019).

All statistical analyses were performed with R (R Core Team, 2021) using R studio version 1.2.5001. All graphs were made using the R package "ggplot2". P values <0.05 were considered statistically significant and reported values are means ± standard deviation, except for the graphs, in which the standard error was used.

6.4 Results

Settlement on the proxy streamers

After one month (in June 2021), the first *D. antillarum* settlers were observed on the streamers (Figure 3). Average number of *D. antillarum* settlers on the proxy streamers (Figure 4) differed significantly per month ($P<0.001$, Table 3). Tukey post-hoc tests revealed that May ($P < 0.004$ for all comparisons) and June ($P < 0.001$ for all comparisons) differed significantly from all other months, but not from each other ($P = 0.239$). Average number of *D. antillarum* settlers was 8.6 ± 3.2 streamer^{-1} in May, 14.9 ± 10.8 streamer^{-1} in June and decreased to around 2-3 settlers per streamer for the remainder of the study. No significant differences were found between other months.

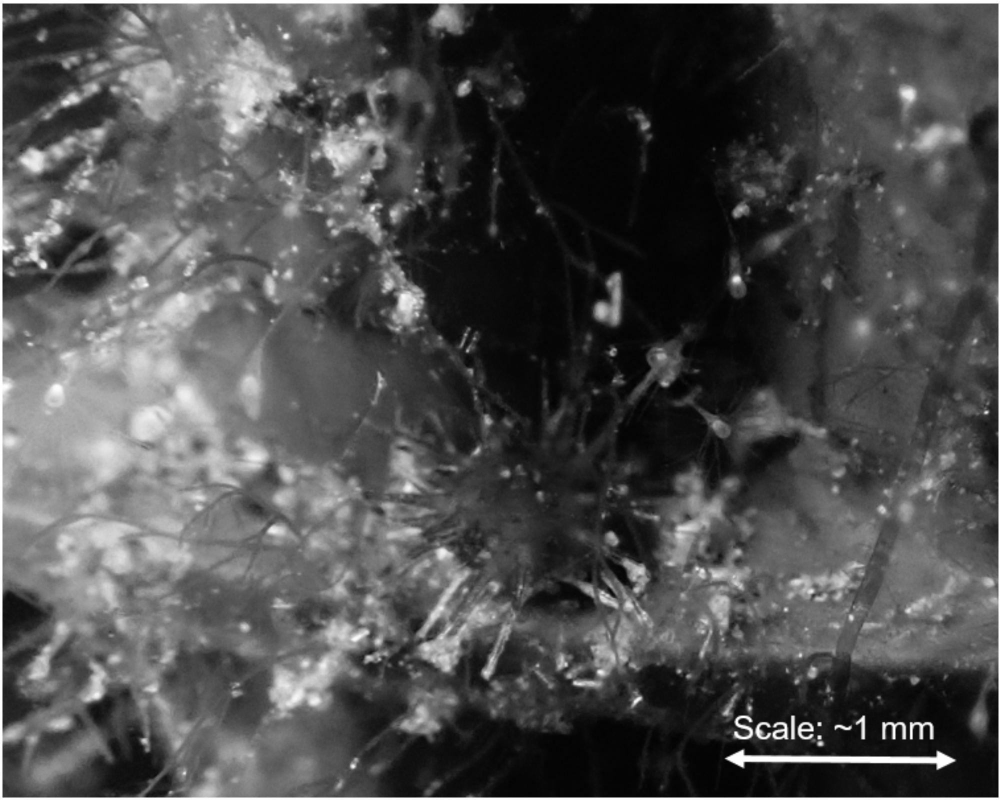

Figure 3: *D. antillarum* settler on a bio ball.

Table 3: Statistical inference, explanatory variables, test statistics, degrees of freedom and P values per response variable. Significant effects are in bold.

Response variable	Statistical inference	Explanatory variable(s)	Test statistic	Degrees of freedom	P value
Settlers per proxy streamer	ANOVA	Month (1-6)	F = 20.94	5, 48	**<0.001**
Settlers per proxy streamer	Kruskal-Wallis	Soaking time (1, 3 and 6 months, same day of deployment)	H = 19.75	2	**<0.001**
Settlers per proxy streamer	Mann-Whitney U test	Soaking time (1 and 3 months, same day of retrieval)	W = 71.5	na	**0.004**
Settlers per proxy streamer	Mann-Whitney U test	Soaking time (1 and 6 months, same day of retrieval)	W = 50.0	na	0.376
Recruits per reef	LMM	Treatment	F = 10.34	1	**0.032**
		Month	F = 5.50	6	**0.001**
		Treatment * month	F = 2.64	6	**0.042**
		Monitoring area	not included in best fitting model		
Recruit size	LMM	Month	F = 2.09	6	0.068
		Treatment	not included in best fitting model		

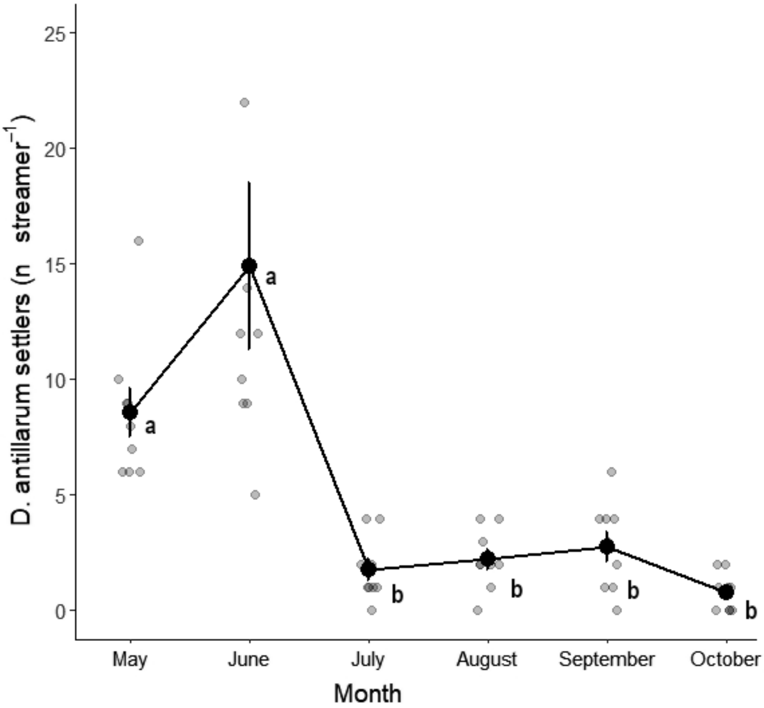

Figure 4: *D. antillarum* settlers per streamer per month. The line connects averages (± 95%CI) and grey points represent the replicate streamers. Averages sharing the same letter were not significantly different. A single result of 42 *D. antillarum* documented from a streamer in June falls outside the plotted y-axis limits, but was used to calculate average (± 95%CI).

Apart from the proxy streamers that were replaced monthly, 18 additional streamers were deployed at the start of the experiment, of which nine were retrieved after three months and nine were retrieved after six months. Soaking time had a significant effect on the number of settlers (P < 0.001, Table 3). Streamers with a soaking time of one month, retrieved in June 2021, had 8. 6 ± 3.2 *D. antillarum*. This decreased significantly to 0.2 ± 0.4 after 3 months, collected in August 2021, and to 0.4 ± 0.7 after 6 months, collected end of October 2021 (P < 0.001 for both comparisons). Streamers retrieved after 3 and 6 months did not differ in their number of *D. antillarum* settlers.

Settlement on streamers with a soaking time of three and six months was also compared with streamers that were collected at the same day, but had a soaking time of only one month. In July 2021, streamers with a soaking time of one month had 1.8 ± 1.4 *D. antillarum* settlers per streamer, which was significantly higher than the 0.2 ± 0.4 per streamer with a soaking time of three months (P = 0.004, Table 3). In October 2021, streamers with a soaking time of one month had 0.8 ± 0.8 *D. antillarum* settlers per streamer, which was not significantly different to streamers with a soaking time of six months, which had 0.4 ± 0.7 *D. antillarum* settlers per streamer.

Recruitment on the patch reefs

D. antillarum recruit abundance on the patch reefs (Figure 5) was significantly affected by treatment (Streamers vs. control, P = 0.032, Table 3), month (P = 0.001, Table 3) and their interactive effect (P = 0.042, Table 3). Monitoring area had no significant effect on the *D. antillarum* recruit abundance, as it was not included in the best fitting model. At the start of the experiment, reefs with and without streamers both had zero *D. antillarum*. The average *D. antillarum* recruit abundance increased on all six patch reefs, independent of treatment, and remained similar till month four, when reefs with streamers had an average of 4.3 ± 3.2 *D. antillarum* per reef and control reefs had an average of 2.7 ± 2.1 *D. antillarum* per reef. In month five, average *D. antillarum* density at the treatment reefs was, for the first time, significantly higher than at the control reefs (P = 0.007). One month later, at the end of the experiment, this effect had strengthened: reefs with streamers had on average 6.3 ± 5.9 *D. antillarum* recruits, which was more than 20 times higher compared to control reefs without streamers, which had an average of 0.3 ± 0.6 *D. antillarum* recruits per reef. This difference was highly significant (P = 0.001).

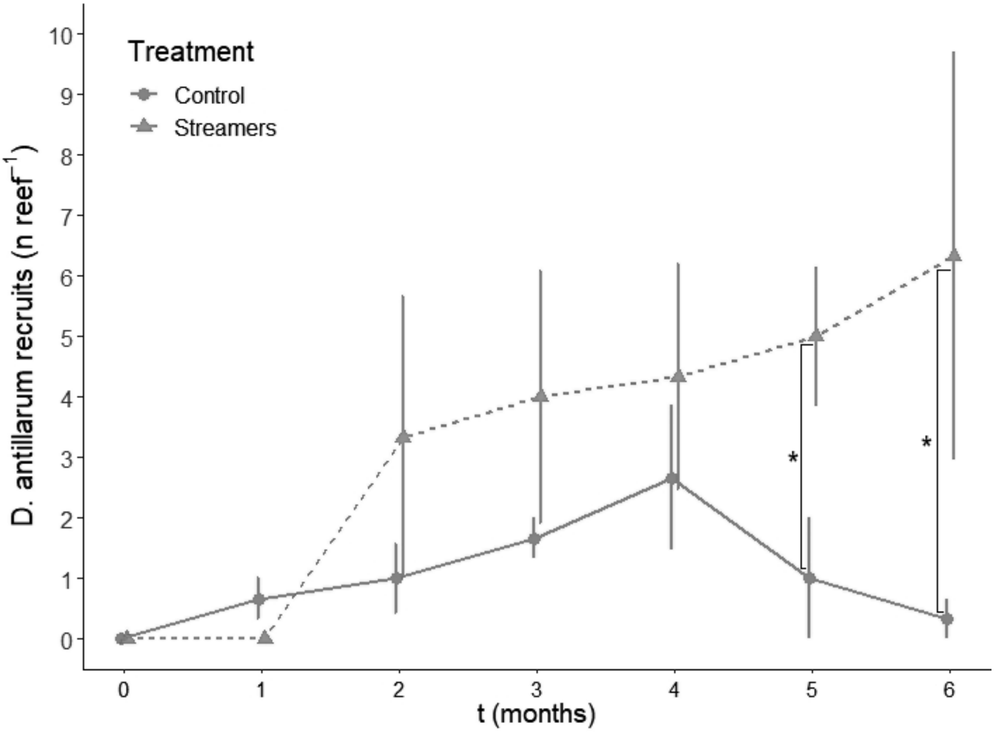

Figure 5: *D. antillarum* density on treatment (with streamers) and control (without streamers) reefs as a function of time. Lines connect averages ± 95%CI and * indicate a significant difference in treatments for that month.

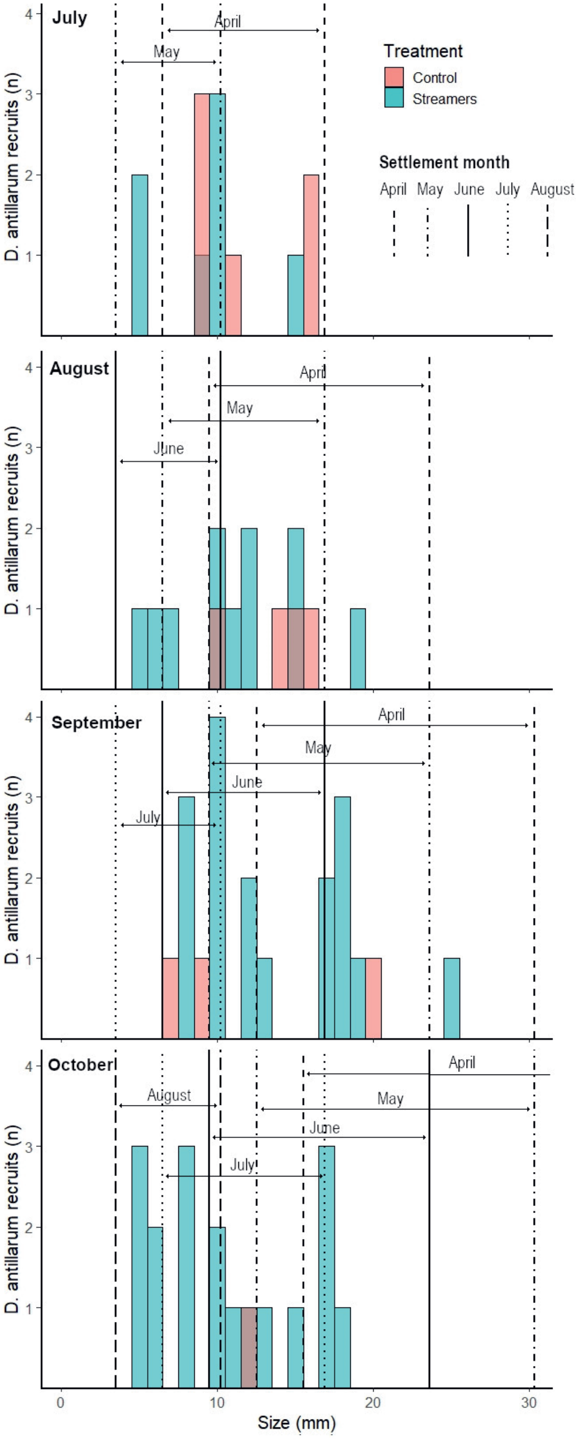

Figure 6: *D. antillarum* recruit size distribution on control and treatment reefs per monitoring month. The area between vertical lines of the same type (dashed, solid, dotted, etc) indicate estimated size range per settlement cohort, based on growth rates from Idrisi et al. (2003) and Randall et al. (1964).

Size distribution on the patch reefs

Figure 6 shows the *D. antillarum* recruit size-distribution per monitoring month (July-October). The average *D. antillarum* recruit size was not affected by monitoring month (P = 0.068, Table 3) or treatment (not included in the best fitting model). In July, average *D. antillarum* recruit size was 10.3 ± 3.5 mm, which corresponded with settlement in April. In August, recruits had a similar average size (11.8 ± 4.0 mm) and their size distribution corresponded most closely with settlement in May. In September, average recruit size was 13.4 ± 5.1 mm. Half of the recruits were around 20 mm, these where likely the remaining recruits from the cohort settling in May, or possible April. The other half of the recruits were smaller and had probably settled in June or July. In October, the average recruit size was 10.7 ± 4.5 mm and most of the recruits corresponded in size most closely to the predicted size range for settlement in July or even August.

6.5 Discussion

Settlement on the proxy streamers revealed that the experiment was conducted at the right time of the year and very likely had been started right before the yearly settlement peak. Settlement on the proxy streamers was highest in May and June and was significantly lower during subsequent months. Settlement on the neighboring island of St. Eustatius in 2019 was also highest in May and June (Hylkema et al. 2022). In La Parguera, Puerto Rico, *D. antillarum* settlement was highest in July in 2006 (Williams et al. 2010) and in September in 2008 (Williams et al. 2011). Highest average *D. antillarum* settlement in this study was 14.9 ± 10.8 *D. antillarum* streamer $^{-1}$. The planar surface area of a streamer with 30 bio balls was 0.04 m^2, resulting in a settlement density of 372 ± 270 *D. antillarum* m^{-2}. This density is in the same order of magnitude as the settlement on collectors documented for most of the locations studied around St. Eustatius in 2019 (Hylkema et al. 2022), as well as compared to the location with highest settlement rates near Puerto Rico (Williams et al. 2010, 2011). These results indicate that the bio ball streamers were able to provide a suitable settlement substrate for *D. antillarum* larvae and thereby potentially able to help circumvent one of the key potential barriers to natural recovery (Rogers and Lorenzen 2008).

The proxy streamers with a soaking time of three and six months (collected in August and November) contained significantly less settlers compared to proxy streamers with a soaking time of 1 month (collected in June), indicating that *D. antillarum* settlers remained on the streamers less than three months. *D. antillarum* settlers have a test diameter of ~0.5 mm (Eckert 1998) and grow 3-7 mm month^{-1} (Idrisi et al. 2003, Randall et al. 1964). The space in between bio ball layers is 5 mm, meaning that settlers outgrew their shelter and probably moved to the outside of the bio balls 1-2 months after settlement. They likely migrated to the reef on their own accord or may have been flushed off the bio balls by currents or were eaten by predators. Proxy streamers with a soaking time of three months had significantly less settlers compared to streamers with a soaking time of one month that were collected on the same day. Bak (1985) observed that *D. antillarum* settlers appear to have a preference for relatively clean surfaces with a fresh biofilm. For other sea urchins, biofilm age also has been found to be important in determining settlement (Rahim et al. 2004, Pearce & Scheibling 1991). We observed abundant turf and macroalgae growth on the three and six month old bio balls, which probably made the bio balls less suitable for settlement. In practice this means that streamers older than 2 months attract less new *D. antillarum* settlers.

Monthly monitoring revealed an increase of *D. antillarum* recruits on both control and streamer reefs during the course of the experiment. However, this increase was largest at the treatment reefs, where recruit densities continued to increase till the end of the experiment. In contrast, recruit densities on the control reefs started to decrease half way the experiment, after three months. This resulted in a significantly higher *D. antillarum* recruit abundance on reefs with streamers as opposed to reefs without streamers. The highest number of *D. antillarum* recruits counted on the monitored surface of a single treatment reef was 13 individuals. This is an order of magnitude lower than what would have been expected based on the total settlement on the nine streamers with each ~15 *D. antillarum* settlers in the month with the highest settlement. The latter would have resulted in 135 settlers in a single month if all settlers had remained. During monitoring we sometimes observed substantial currents at the research location, which might have washed juveniles off the streamers, off the monitored area and possibly even away from the experimental reefs. As control and treatment reefs were located close to each other, strong currents could even be responsible for depositing some of *D. antillarum* juveniles on the control reefs. However, the reefs just outside the transects were inspected at the end of the experiment and only few *D. antillarum* recruits were observed there. It is therefore likely that, next to dislocation by currents, other processes reduced the survival of settlers that outgrew the bio balls and colonized the reefs. Macroalgae, that covered large areas of the reefs during the experiment, are known to harbor large numbers of micropredators such as crabs, shrimps and worms (Bechtel et al. 2006), which could be responsible for high post-settlement mortality. In addition, the dense turf and macroalgae cover of the patch reefs might be suboptimal habitat for settlers in terms of food (Vermeij et al. 2010), while it might also reduce shelter availability by filling small crevices (Spadaro & Butler 2021).

Although new recruits were observed on the treatment reefs every month, the total number of recruits barely increased, indicating a low long term retention of the recruits. This is confirmed by the average *D. antillarum* test size, which did not increase during the course of the experiment. Size distributions showed that very few recruits made it past a test size of 20 mm and every month from the period July - October, most recruits were derived from a new cohort of settlers. The low retention of *D. antillarum* recruits could be attributed to migration to other parts of the reef or to predation. Monitored recruits mostly showed a high shelter fidelity and were often seen in the same crevice during multiple monitoring events. They were increasing in size, appeared healthy, were not yet outgrowing their shelter space and then suddenly had disappeared, suggesting that predation rather than migration is the most likely factor explaining the limited long term increase in *D. antillarum* recruit density. Among many potential predators, especially Queen Triggerfish (*Balistes vetula*), Spanish Hogfish (*Bodianus rufus*), Pudding Wife (*Halichoeres radiatus*), Black Margate (*Anisotremus surinamensis*), Spanish Grunt (*Haemulon macrostomum*) and Jolthead Porgy (*Calamus bajonado*) are known to prey upon *D. antillarum* (Randall et al. 1964). All these species, with the exception of *H.*

macrostomum, were frequently observed on the experimental site and Harborne et al. (2009) suggested that even a relatively low predation pressure can be sufficient to prevent *D. antillarum* recovery. It could therefore well be that the predation pressure at the experimental site was too high or the shelter availability too low (Bodmer et al. 2021) to support *D. antillarum* population recovery, notwithstanding increased settlement due to the streamers.

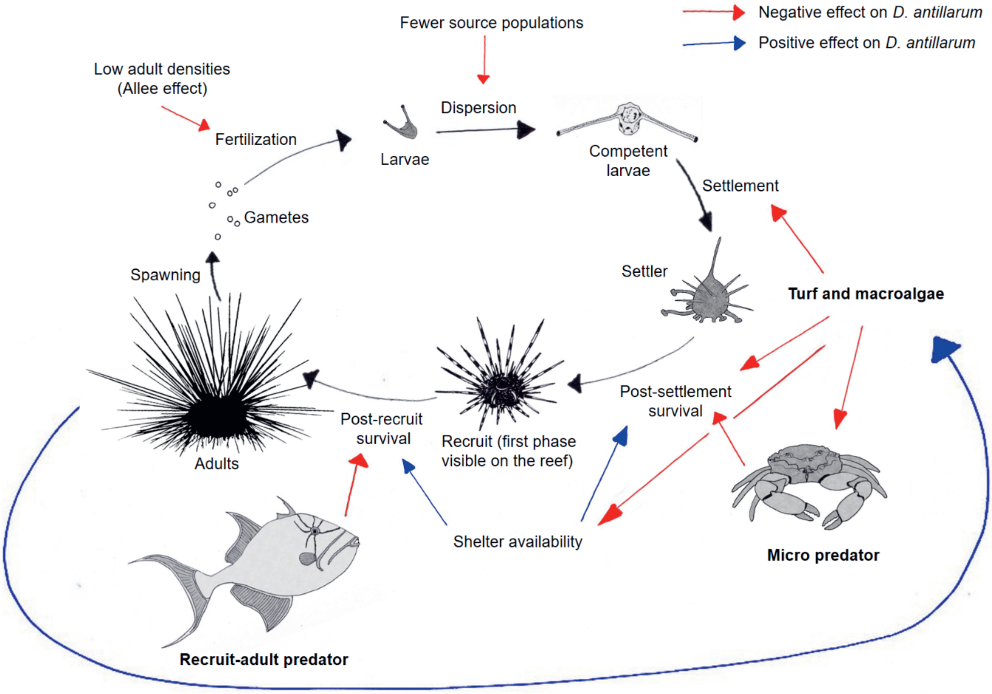

Figure 7: Life cycle of *D. antillarum* (black arrows) and factors constraining (red arrows) or enhancing (blue arrows) *D. antillarum* population development. Factors in bold were confirmed by the present study.

Figure 7 summarizes the life cycle of D. antillarum and the possible factors constraining its natural recovery. Hylkema et al. (2022) showed high peak settlement rates on Saba at certain times of the year, indicating that limited fertilization of gametes (Feehan et al. 2016) and lack of upstream adult populations were not the main factors constraining natural recovery. The addition of bio ball streamers in the current study resulted in increased D. antillarum recruitment, confirming that the availability of suitable settlement substrate was limited on the natural reef (Rogers and Lorenzen 2008). This can be explained by the high turf and macroalgae cover, limiting the amount of fresh biofilm on the patch reefs. The algae dominated habitat might also have reduced post-settlement survival, as the algae fill potential shelter spaces (Spadaro & Butler 2021), do not provide the right food for D. antillarum settlers (Vermeij et al. 2010) and harbor high densities of micro-predators (Bechtel et al. 2006). Finally, high predation of larger juveniles might have reduced post-recruit survival and prevented D. antillarum population recovery.

We conclude that our new approach of assisted natural recovery (ANR) successfully provided suitable settlement substrate and significantly increased D. antillarum recruit densities on patch reefs. However, before ANR can be implemented on a large scale, post-settlement and post-recruit survival should be improved. To optimize prospects for ANR, we propose to: 1) employ bio balls or other substrates that can provide shelter suitable to larger juveniles, 2) optimize the habitat by removing macroalgae, 3) choose sites with a lower predation pressure and 4) place corrals around the streamers. Larger bio balls that, in addition to the current 5 mm shelters, also provide larger shelters, might be able to maintain D. antillarum juveniles safe in mid water during the intermediate phase between settlement and their ultimate descent to the larger shelter spaces of the reef, thereby improving post-settlement survival. By removing macroalgae from the reefs at the start of the settlement season, the habitat might also become more suitable for settlers and less suitable for micro predators, also increasing post-settlement survival. Macroalgae could be removed manually or by restocking adult D. antillarum or other herbivores such as reef urchins (Echinometra viridis) (Bechtel et al. 2006) or Caribbean king crab (Maguimithrax spinosissimus) (Spadaro & Butler 2021). To select restoration sites with a low predation pressure, it might, paradoxically, be recommended to start ANR outside marine reserves, where fishing pressure can reduce predator densities (Harborne et al. 2009, Edmunds & Carpenter 2001). Alternatively, shallower reefs (Carpenter & Edmunds 2006; Debrot & Nagelkerken 2006, Myhre & Acevedo-Gutiérrez 2007) or reefs with a high shelter availability (Bodmer et al. 2021) might increase post-recruit survival and increase the chance of successful D. antillarum recovery. Finally, post-recruit survival of D. antillarum on locations with a high predation pressure might be enhanced using corrals placed around the streamers, which can protect the juveniles during their first months on the reef (Williams 2017, 2021). The combination of these measures could result in a comprehensive ANR approach. If settlement densities are not significantly reduced by the new D. antillarum die-off taking place in parts of the Caribbean in 2022 (AGGRA 2022) we expect ANR to contribute substantially to D. antillarum population recovery in the near future.

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Chapter 7

General discussion

The continuing degradation of Caribbean coral reefs is so extensive and severe that it is unlikely that they will be able to sustain their important ecosystem services in the near future (Woodhead et al. 2019). The implementation of MPAs, termed passive conservation (Rinkevich 2008), is currently the most implemented tool used in the management and conservation of these important ecosystems. However, passive conservation alone is sometimes not enough to reverse current trajectories and often passive measures are only able to temporarily reduce the pace of degradation (Coelho & Manfrino 2007, Bruno et al. 2019). Therefore, more active intervention measures are urgently needed to supplement passive conservation in order to ultimately achieve ecosystem rehabilitation (Rinkevich 2008). However, the science of active intervention in coral reef ecosystems is a new field of inquiry and many potential solutions needed to restore healthy marine ecosystems still remain insufficiently explored (Friedman et al. 2020). Two of the most widespread active intervention measures in the Caribbean are the deployment of artificial reefs and efforts to restore populations of the herbivorous sea urchin *D. antillarum*. Based on the knowledge gaps outlined in the introduction, the general objective of this dissertation was to increase our understanding of the most effective use of these interventions to increase herbivorous grazing intensity, reduce algae cover and increase the recruitment, survival and growth of reef-building corals.

The first knowledge gap addressed in **Chapter 2**, was the type, purpose, protection status and ecological functioning of artificial reefs in the Caribbean. The literature review showed that the ecological development of artificial reefs in the Caribbean was seldomly assessed. Studies comparing multiple artificial reef types are essential to enable substantiated choices in artificial reef design, but these type of studies were non-existant. Therefore, the fish assemblage of three artificial reef designs, reef balls, layered cakes and rock piles, were compared in terms of early succession in **Chapter 3**. To test if observed differences in the fish assemblages persist over time, the continued colonization of reef balls and layered cakes was studied in **Chapter 4**. In this chapter, artificial reef design was also related to grazing intensity and territorial behavior as well as to to benthic community development, coral recruitment, survival and growth. This resulted in the recommendation that, in addition to fish herbivores, facilitating invertebrate herbivores might help enhance coral recruitment. In **Chapter 5** the timing and magnitude of *D. antillarum* settlement was investigated on five different materials at five locations around St. Eustatius. The best-performing substrate was subsequently applied for the research described in **Chapter 6** providing a suitable settlement substrate for *D. antillarum* larvae on the reef. This new approach to *D. antillarum* rehabilitation was termed assisted natural recovery (ANR).

In this chapter I discuss and integrate the obtained results in the context of existing literature and provide recommendations for future research.

Artificial reefs in the Caribbean

The deployment of artificial reefs is increasingly considered as a measure to help sustain the ecosystem services provided by the degrading natural reefs. Regardless of their intended purpose, it is of the utmost importance that artificial reefs do not harm the already-stressed natural ecosystem. In **Chapter 2,** the design, purpose and protection status of 212 artificial reefs deployed in the greater Caribbean was reviewed. This study showed that less than 20% of these artificial reefs were located within management zones in which fishing was restricted or prohibited. As management is not always properly enforced, this means that more than 80% of the artificial reefs in the Caribbean are fishable in some way. The fish assemblage of an artificial reef is, necessarily, the combined result of attraction and local production (Grossman et al. 1997, Pickering & Whitmarsh 1997). The relative contributions of these two sources of fish assemblages differ per artificial reef and are dependent on many factors such as age and size of the artificial reef, as well as distance to natural reefs. As part of the fish assemblage is derived from the surrounding ecosystem, artificial reefs can function to concentrate marine organisms or in essence as fish aggregating devices on the seabed. By exploiting artificial reefs, the surrounding ecosystem might then indirectly be depleted, even if not fished directly (Bohnsack 1989, Brock 1994). To prevent further overfishing of Caribbean coral reefs, it is recommended to establish no-take zones around artificial reefs. If fishing is the purpose of the artificial reef deployment, the fish populations and fisheries landings on and around artificial reefs should be carefully monitored and managed to avoid indirect overexploitation of surrounding habitats.

Very few of the artificial reefs deployed in the Caribbean have been ecologically assessed and the most commonly-used designs (ship wrecks and reef balls) have not been assessed for this purpose at all. The only type of artificial reef type that have been more-extensively investigated are small piles of concrete construction blocks (e.g. Beets & Hixon 1994, Gratwicke & Speight 2005). These reefs were usually constructed for the sole purpose of research and, because they have proven to be unstable in storms (Ogden & Ebersole 1981, Beets & Hixon 1994), are unsuitable for most other purposes. It was therefore concluded that there has been a major discrepancy between the reef types used for research and those used for other purposes. As a consequence, managers and conservationists in the Caribbean still have very scant scientific evidence to substantiate their choices regarding artificial reef deployment, use and management. Hence, comparing the results of multiple artificial reef designs is a research priority. In **Chapters 3 and 4,** headway along these lines was made by comparing several aspects of a number of commonly used artificial reef designs.

Effect of artificial reef design on fish and benthic communities

After one year of colonization, layered cake reefs had a significantly higher fish abundance and biomass compared to reef balls (**Chapter 3**). Rock piles had intermediate levels of fish abundance and biomass and did not differ significantly from the other designs. The difference in fish assemblages between layered cakes and reef balls was attributed to the higher shelteravailability and diversity of the layered cakes. The latter results are consistent with previous studies that also demonstrated that a higher availability of shelter can result in a higher fish abundance and biomass on artificial reefs (Hixon and Beets 1989, Sherman et al. 2002, Gratwicke and Speight 2005). Gross volume (reef size), shelter volume and total surface area per area of seabed covered (rugosity) are also known to increase the fish abundance (Abelson and Shlesinger 2002, Gratwicke and Speight 2005). However, all these parameters were larger at the reef balls and it was therefore concluded that these parameters were, in the current study, less important drivers for early colonization than shelter availability and diversity.

Continued monitoring revealed that differences in fish abundance and biomass between reef balls and layered cakes became smaller over time (**Chapter 4**). At the end of the experiment, 2.5 year after deployment, layered cakes hosted a higher fish abundance but similar fish biomass compared to reef balls. This can partly be explained by the larger volume of the central shelter space of the reef balls that attracted fewer but bigger fish with a higher biomass, similar to the results of other studies (Hixon & Beets 1989, Beets & Hixon 1994). However, the fish abundance at the reef balls steadily increased, while the fish abundance at the layered cakes did not increase throughout the course of the study. This appears to have been caused by more territorial behavior at the layered cakes as remote video surveys showed that fish at layered cakes experienced four times more territorial fish chases than fish at the reef balls. No less than 90% of the territorial behavior was due to *Holocentrus adscensionis* (Long-spined squirrelfish), *Abudefduf saxatilis* (sergeant major), *Myripristis jacobus* (blackbar soldierfish) and *Stegastes partitus* (bicolor damselfish). The abundance of these territorial species was higher on the layered cakes compared to the reef balls and increased during the course of the study, which may account for the reduced abundances of other fish species. Roving herbivores in particular were chased off the artificial reef plot to resume their grazing activities elsewhere. As a consequence, grazing intensity was five times higher at the reef balls. It can therefore be concluded that territorial behavior of fish residing on the artificial reefs damped the positive effects of shelter availability on the fish assemblage.

Contrary to expectations, the higher fish grazing intensity recorded at the reef balls did not result in significantly lower macroalgae cover compared to the layered cakes. Both reef types showed high turf algae and low macroalgae cover. Reef balls had a slightly but significantly higher sponge and tunicate cover, which could be an indication of higher availability of space due to grazing on algae. Nevertheless, recruitment, survival and growth of corals were similar on both reef types. Arnold et al. (2010) compared coral settlement, survival and growth inside and outside damselfish territories and concluded that the reduced grazing inside the territories resulted in higher turf algae biomass and lower coral recruitment and survival, but this effect was not measurable on our artificial reefs, despite the higher occurrence of damselfish and other territorial fish on layered cakes. **Chapter 4** shows that, despite relatively high herbivorous fish biomass on both reef types, coral recruit survival was low. Other factors, such as sedimentation, eutrophication and competition from other benthic organisms might prevent healthy coral development. Especially the high cover of turf algae on the artificial reefs might be problematic. As suggested by Roff & Mumby (2012), the grazing intensity of herbivorous fish, without additional grazing by other species groups, might not be high enough to support substantial coral recruitment in the Caribbean. Facilitating invertebrate grazers such as *D. antillarum*, is therefore a priority in artificial reef research.

During the course of the artificial reef experiments, from construction and deployment to monitoring, it became clear that the chosen artificial reef designs had serious disadvantages. Construction of the reef balls and the layered cakes was quite time consuming. Multiple actions had to be performed to build a single reef module, such as placing buoys in the mold for the reef balls and casting sand and concrete on top of each other for the layered cakes. The modules weighed around 300-400 kg, making transportation challenging, especially on the water. In September 2017, when hurricanes Irma and Maria passed very close to Saba and St. Eustatius, some modules broke and others were moved over as much as 20 meters. The rock piles were even less storm-resistant and were also damaged by large swells during the following year. Hence, despite their weight, these reefs proved to be quite vulnerable to storm damage. **Chapter 4** showed that layered cakes had a good habitat for small fish, while reef balls had less fish that were larger. The layers of the layered cake design were higher at the rim and had a depression towards the center. This allowed sediment build up and prohibited benthic development. To summarize the disadvantages, an artificial reef module should be easier to build, lighter, more storm resistant and combine ecological advantages of the reef balls and the layered cakes. To optimize artificial reef use as an active intervention measure for ecosystem rehabilitation, the development and testing of new artificial reef designs should be a research priority.

Factors constraining natural recovery of D. antillarum

At the moment of writing this dissertation, the *D. antillarum* die-off had happened almost 40 years earlier. Despite four decades of natural recovery processes and over 500 papers on the subject (Google scholar), recovery remains mostly restricted to a few sheltered and shallow locations and population densities are nowhere near their former densities (Lessios 2016). To develop active interventions that can truly assist in the recovery of *D. antillarum,* it is essential to understand what is actually constraining natural recovery. By studying settlement rates on artificial substrates, a distinction can be made between settlement and post-settlement survival (Harrold et al. 1991) to allow insight into the factors constraining natural recovery. The settlement rates at five locations on the west side of St. Eustatius in 2019 differed greatly over time and were highest in June and August (**Chapter 5**). Settlement rates were in the same order of magnitude as in Puerto Rico in 2006 and 2008 (Williams 2010, 2011) and in Curaçao in 2005 (Vermeij et al. 2010). Peak settlement rates were even comparable to pre die-off rates measured in Curaçao (Bak 1985), although overall settlement throughout the year still appeared to be lower. The relatively high settlement rates measured around St. Eustatius in certain months suggest that it is not low fertilization rates or lack of upstream source populations (Lessios, 1988, Feehan et al., 2016) that are the main factors constraining the recovery of *D. antillarum* around St. Eustatius (Chapter 6, Figure 7). The results of this dissertation in combination with other studies suggest a lack of suitable settlement substrate on the reef (Rogers & Lorenzen 2008) and/or low survival of settlers due to micro predation (Vermeij et al. 2010, Williams et al. 2010).

A relatively fresh biofilm seems to be an important settlement cue for *D. antillarum* larvae, as settlement rates on older collectors was lower than on monthly refreshed collectors (Bak 1985, **Chapter 6**). Before the 1980s die-off, millions of adult *D. antillarum* scraped large areas of Caribbean reefs clean of any benthic growth, inducing a new biofilm to grow and, by doing so, providing a settlement cue for *D. antillarum* larvae (Bak 1985). This might also explain the high density of juveniles in areas with adults, something that was attributed before to settlement cues from the adult individuals (Lessios 1988) or increased settler protection by adult *D. antillarum* (Miller et al. 2007). After the die-off, bare substrates on the coral reefs of the region were quickly overgrown by macroalgae, turf algae and other benthic organisms, greatly reducing the opportunities for fresh biofilm formation as potential settlement cues for *D. antillarum* larvae. A limited food supply in absence of a suitable biofilm might reduce survival of settlers. *D. antillarum* settlers first feed best on fresh biofilm (Pilnick et al. 2021) and later on shortly-cropped turf algae, both of which have become less available on reefs compared to before the die-off. Also, post-settlement survival might have become reduced by a higher abundance of micropredators such as crabs, shrimps and fire worms, which can attain high densities in macroalgae covered reefs (Bechtel et al. 2006). In addition, dense turf- and macroalgae coverage potentially reduces the shelter availability for *D. antillarum* settlers by filling up small crevices (Spadaro & Butler 2021), making them vulnerable to predation.

Further insight into settlement cues and survival was provided in **Chapter 6** where suitable settlement substrate was provided directly on the reef. This new approach, coined “assisted natural recovery” (ANR) is a new method to restore *D. antillarum* populations and is based on concepts derived from terrestrial ecosystem restoration (Hardwick et al. 2004, Shono et al. 2007). The method aims to accelerate ecological succession by reducing barriers to natural ecosystem recovery. This was achieved in this study by supplementing patch reefs on Saba with additional settlement substrate to overcome the usual lack of suitable settlement substrate. This approach resulted in substantial *D. antillarum* settlement on bio ball streamers and increased recruitment on the reefs to which the streamers were attached. These results confirmed that the availability of suitable settlement substrate is indeed constraining natural recovery of *D. antillarum.* However, recruitment on the reefs with bio ball streamers was relatively low compared to the enhanced settlement, confirming that low post-settlement survival also constrains natural recovery (Chapter 6, Figure 7). Finally, post recruit survival was low, possibly due to high predation pressure. Most of the known predators of *D. antillarum* (Randall et al. 1964) were regularly observed during the surveys. Harborne et al. (2009) found that even low predation pressure can prevent the establishment of new *D. antillarum* populations. In addition to high predation pressure, limited shelter availability which might serve to further exacerbate vulnerability to predation, is another factor that possibly contributes to the constrained recovery of *D. antillarum* on Saba.

Perspectives for Caribbean coral reef reefs

The huge effect of climate change on the rate of coral reef degradation is clear and curbing greenhouse gas emission should be our number one priority to prevent total collapse of coral reefs and other ecosystems worldwide (Hughes et al. 2017, Bruno et al. 2019). Coral reefs of the future will be different than reefs of the past, as climate change will certainly affect certain species more than others (Graham et al. 2014, Toth et al. 2019). It is unlikely that artificial reefs can ever be a full substitute for a healthy and thriving natural reef. However, active intervention measures should be developed, optimized and implemented to steer towards the most biodiverse and productive future reef as possible. In addition, it is necessary to think beyond the current climate change crisis. Even if we manage to keep global warming within 1.5 °C, many Caribbean coral reefs will not be able to recover by themselves due to their impaired resilience. To reverse the trajectory of degradation requires the undoing of the majority of accumulated local and global threats, not just removal of the sequentially last threat that triggered the most visible shift in the ecosystem (Hughes et al. 2010). This is considered the main reason why restoring reefs to a coral dominated system is typically so difficult. Active intervention measures can be essential tools to reverse the current trajectory of decline and reset positive self-reinforcing feedback loops towards ecosystem rehabilitation (Rinkevich 2008).

Optimizing artificial reefs: the Moreef design

One of these active intervention measures of the future could be the Moreef artificial reef, which is an abbreviation for Modular Restoration Reef (Figure 1). To address the shortcomings in artificial reef design issues outlined above, I developed a new artificial reef design, together with product designer Frank Westland (Wortel Product Design). Moreef modules can be casted in a single mold, without any alterations. Their mass is only 150 kg, making them easy to transport over land and sea. Moreefs are designed to have a high weight to volume ratio, making them more storm resistant. Moreef modules can be stacked and connected using under water epoxy, creating a bigger, heavier and more robust reef. Specific holes are included in the design to anchor the bottom modules to the seabed. The design has sloping layers, to prevent accumulation of sediment. Micro shelters, blind shelters and interconnecting shelters cater to the shelter needs of wide selection of marine organisms. To facilitate increased grazing intensity by sea urchins, micro shelters are specifically designed to provide shelter for *D. antillarum* settlers, while blind shelters are suitable shelter for *D. antillarum* juveniles and adults. When stacked, the Moreef modules also provide extra shelter space for larger fish and other marine organisms. The Moreef design proved technically feasible in a field trial (Figure 1B) but has not been ecologically investigated yet. Field trials of the ecological performance of the Moreef compared to other artificial reef designs could help to further optimize artificial reef design for reef restoration purposes.

Just as is the case with any other artificial reef design, there will also be limitations to the use of the Moreef design. Although the Moreef modules are relatively easy to build, they still require substantial time and other resources. Transportation and deployment are easy and fast, but stacking needs to be done under water using SCUBA, which takes time. These factors will reduce the scale at which Moreef modules or other comparable artificial reefs can be implemented. An alternative reef type that does not have this limitation, is the use of piles of natural rock. The rock piles used in **Chapter 3** harbored a fish assemblage that was similar to the layered cakes, but the piles were unstable and got easily destroyed by storms and big swells.

However, with the use of a barge, larger rocks could be deployed than used in the current study.

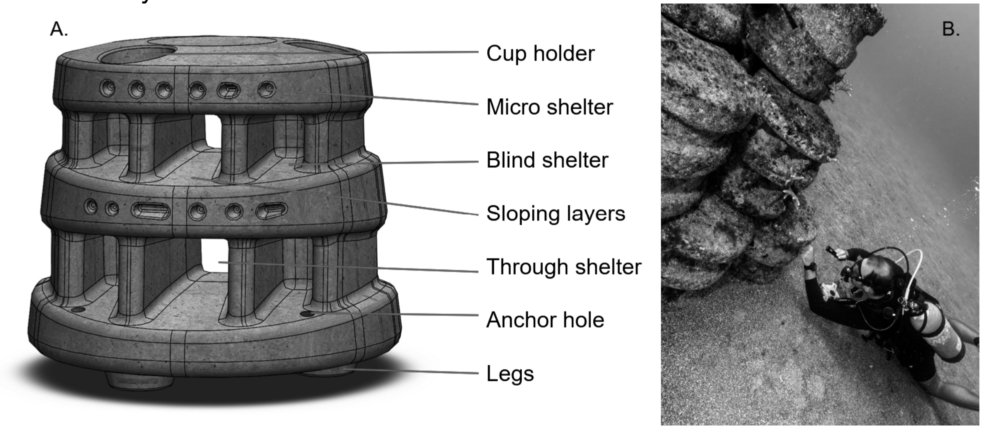

Figure 1: A. Moreef design and characteristics and B. A diver inspecting a large pile of Moreef modules (photo by Joe Snyder).

The combination of smaller and larger rocks will improve the stability of the rock piles and at the same time create diverse shelter availability. Micro shelters could be provided by the use of porous rock. Research performed in the Red Sea shows that breakwaters and specifically-built reefs of natural rock sustain healthy fish and coral assemblages (Abelson & Shlesinger 2002, Burt et al. 2009, Burt et al. 2011), but this has not yet been investigated in the Caribbean region. Because of its potential for large-scale implementation, research into the habitat function for coral and fish for large rock-pile reefs in the Caribbean should also be a research priority.

Optimizing the assisted natural recovery approach

Another active intervention measure of the future could be an optimized form of the ANR approach tested in Chapter 6. This dissertation shows that the following factors constrain natural recovery of *D. antillarum* populations on Saba (see also Chapter 6, Figure 7):

1. Limited availability of suitable settlement substrate.
2. Low post-settlement survival by high micropredator abundance combined with low shelter availability and possibly low food availability.
3. Low post-recruit survival by high fish predator abundance combinted with low shelter availability

The current form of ANR, tested in this dissertation, only addressed the settlement substrate availability barrier. The optimized form of ANR should, preferably, target all three barriers to natural recovery. Firstly, to improve post-settlement survival, larger bio balls that also provide larger shelters in addition to the current 5 mm shelter cavities could be used (Figure 2). These bio balls might be better able to maintain *D. antillarum* juveniles safe from predation after settlement. Also, they could improve post-recruit survival as larger sea their ultimate descent to the larger shelter spaces of the reef as less vulnerable, larger urchins. Secondly, the reef habitat can be optimized to further improve post-settlement survival by facilitating animals that reduce turf and macroalgae cover. This probably will reduce the density of micropredators hiding in the macroalgae while improving shelter availability, as thick algae growth can fill up small crevices. To test this hypothesis, small areas of the reef could be cleaned manually throughout the settlement season, using wire brushes and monitoring post-settlement survival of *D. antillarum*. If proven effective, the stocking of other invertebrate grazers, such as rock boring urchins (*Echinometra viridis*) (Bechtel et al. 2006) or Caribbean king crab (*Maguimithrax spinosissimus*) (Spadaro & Butler 2021) to replace the need for manual removal of algae. The expected reduction of macroalgae might also contribute to *D. antillarum* settlement rates, as bare substrate with a fresh biofilm is expected to increase. Thirdly, ANR might be further improved by proper site selection in order to reduce predation pressure on recruits. Results so far indicate that shallower sites and sites outside MPAs usually have a lower predator density compared to deeper and better-protected sites (Harborne et al. 2009). Low predation pressure on recruits has also been indicated as the most important factor resulting in natural recovery of *D. antillarum* in Jamaica (Edmunds & Carpenter 2001, Carpenter & Edmunds 2006). In addition to this, selecting reefs with a high shelter availability might also improve recruit survival (Bodmer et al. 2021).

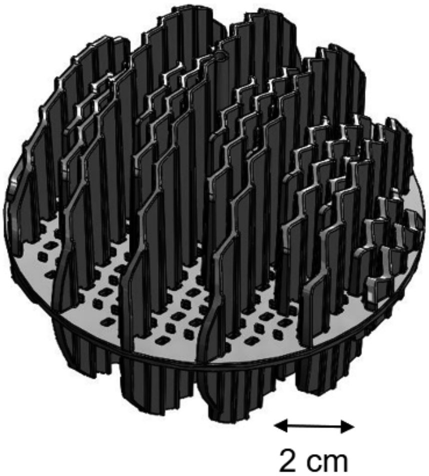

Figure 2: Larger bio ball (diameter: 12 cm) including shelter spaces of 0.5, 1 and 2 cm.

Recommendations for future research

This dissertation aimed to increase our understanding of how artificial reefs and *D. antillarum* restoration can be effectively implemented and combined in synergy. The acquired insights have been used to develop the Moreef artificial reef design and the new approach of ANR to restore *D. antillarum* populations. Future research is necessary to optimize these active interventions and to make it possible to implement them on a larger scale. This dissertation confirms the findings of many other studies that healthy coral development on both natural and artificial reefs in the Caribbean is very difficult without the presence of invertebrate grazers such as *D. antillarum*. Restoring this key herbivore on a large scale should therefore be the number one research priority and the optimized ANR approach, following from this dissertation, should be developed and tested as soon as possible. The artificial reef with most potential for large scale implementation is the large rock pile consisting of large and smaller rocks to increase stability and shelter availability. The ecological comparison of such large rock piles with other natural and artificial reef designs is another research priority. The Moreef design, which still need to be ecologically tested, is one of the possible candidates for this comparison. Stacking interventions together, aiming for their synergistic interaction, for instance by deploying artificial reefs in combination with the stocking of *D. antillarum* or other invertebrate herbivores, might also provide improved habitat for corals, but this remains to be investigated. Both artificial rock piles and the Moreef design might be suitable for this tandem restoration.

This dissertation helps take new innovative steps towards a more effective and combined use of artificial reefs and *D. antillarum* restoration. In doing so it opens the way for additional lines of research and, more importantly, for the active rehabilitation of Caribbean coral reefs.

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Summary

Coral reefs are one of the most productive and biodiverse ecosystems providing ecosystem services such as fisheries, coastal protection, tourism and recreation. However, these services are threatened, as coral reefs worldwide are degrading because of multiple factors including overfishing, pollution, disease and climate change. The Caribbean region has been particularly affected and average cover of reef-building corals has been reduced by more than 80% since the 1970s. As the principal reefbuilders, corals are vital to the three dimensional structure of the reef and provide shelter space for a multitude of marine organisms. However, without living coral, the reef becomes flatter, provides less shelter and cannot sustain as many ecological niches, resulting in a decrease in biodiversity and productivity. Contrary to Indo-Pacific reefs that often recover at least partly after disturbances, average coral cover on Caribbean reefs typically does not recover. This might be the result of the relatively low coral recruitment of Caribbean coral reefs compared to most Indo-Pacific reefs.

Turf and macroalgae are the main competitors of corals for space, light and nutrients. Coral larvae need bare substrate or crustose coralline algae allow successful settlement. Turf and macroalgae can hamper coral recruitment by reducing available space and by outcompeting settlers. Herbivorous fish and invertebrates, in particular sea urchins, play a key role on coral reefs and contribute to coral settlement, survival and growth by grazing away encroaching algae and creating bare substrate. Compared to the Indo-Pacific, Caribbean coral reefs harbor substantially fewer species in general and also fewer herbivores in specific. As a result, a reduction in abundance of only a single species can have detrimental effects on the balance between coral and algae, as with lower species redundancy there are often no other species to serve as a replacement when a key species is lost. This happened in the 1980s, when a unknown disease decimated up to 98% of the *Diadema antillarum* sea urchins that up to then had been a prominent grazer on the reefs. As herbivorous fish were already low in abundance due to centuries of overfishing, Caribbean coral reefs rapidly became covered with algae, reducing coral cover and recruitment. As recovery of *D. antillarum* has been slow to non-existent, Caribbean coral reefs are still mostly dominated by algae or other benthic groups instead of corals.

The establishment of marine protected areas (MPAs) with no take zones, a form of passive conservation, is seen as the most important tool in coral reef restoration. Particularly on Caribbean coral reefs, this has proven to be not enough to reverse the trajectory of degradation in coral cover and generally only serves to slow the pace of deterioration. Therefore, active intervention methods should be considered to supplement passive conservation, but many potential solutions still remain insufficiently explored. This dissertation focusses on optimizing two active intervention methods: the deployment of artificial reefs to increase three-dimensional structure at eroded reef sites and the restoration of the sea urchin *D. antillarum* to increase the grazing pressure on taxa that compete with corals.

Artificial reefs are structures deployed on the sea bottom to mimic one or more ecosystem functions of a natural reef. Worldwide, they are becoming increasingly popular and thousands of structures are being deployed every year. To provide

conservationists, marine park managers and researchers the opportunity to substantiate their choices of artificial reefs interventions, the available knowledge and experiences should be widely shared. In **Chapter 2** we reviewed the purpose and current management of 212 artificial reefs that were deployed in the Greater Caribbean between 1960 and 2018 based on cases documented in gray (n=158) and scientific (n=54) literature. The three most common purposes to deploy artificial reefs were 1) to create new dive sites, 2) to perform research and 3) to restore ecosystems. Ship wrecks, reef balls© and piles of concrete construction blocks were the most-often deployed artificial reef structures. As artificial reefs attract part of their marine organisms from surrounding habitats, their exploitation by fishers, without clear management, can adversely affect the fish stocks in the surrounding area and thus counteract any potential ecosystem benefits. To ensure artificial reefs and their fisheries do not negatively affect the surrounding ecosystem, we recommend to include artificial reefs, their fisheries and the surrounding ecosystem into future monitoring studies and to create no-take zones around artificial reefs that are not monitored. Another finding of the review was that artificial reefs in the Caribbean are seldom monitored and that comparative studies are non-existent.

To test the effect of artificial reef design on its habitat function for fish and coral, we conducted several studies on Saba and St. Eustatius in the Eastern Caribbean. In **Chapter 3** we compared the early fish colonization, one year after deployment, of three types of artificial reefs: reef balls®, layered cakes and piles of locally obtained basaltic rock. All artificial reef plots showed higher values for fish abundance, biomass and species richness than control plots covered by bare sand, which shows that artificial reefs can locally enhance the fish abundance and diversity. However, the effect differed among artificial reef plots. Fish abundance was 3.8 times higher on the layered cakes compared to the reef balls, while fish biomass was 4.6 times higher. Rock piles had intermediate values. Species richness did not differ significantly among different artificial reef types. Three-dimensional modelling revealed that layered cakes had a smaller gross volume, shelter volume and total surface area than reef balls. The availability of multiple small shelters in the layered cake design appeared to be more relevant than other physical parameters. We concluded that layered cakes performed better than reef balls after one year of colonization. Rock piles, made of local volcanic rock, showed an intermediate performance, and were 4-10 times cheaper to construct.

Chapter 3 also showed that layered cakes harbored over five times more herbivorous fish compared to reef balls. We hypothesized that higher herbivorous fish abundance would result in a higher grazing intensity, and favorably impact the benthic community including coral recruitment, survival and growth. To test this hypothesis, we compared the fish assemblage, territorial behavior and grazing intensity at reef balls and layered cakes 2.5 years after deployment in **Chapeter 4**. In addition, the effect of artificial reef type on the benthic development and coral recruitment, survival and growth, was investigated to relate these parameters to possible differences in fish populations and fish behavior. The higher fish biomass on layered cakes during early colonization disappeared during consecutive monitoring events. This might have been due to a higher abundance of territorial fish around the layered cakes: almost four times more chasing behavior was recorded compared to the reef balls. The difference in territorial behavior correlated with a more than five times lower fish grazing intensity on layered cakes. Despite the difference in grazing intensity, macroalgae were effectively controlled at both reef types. Nevertheless, coral recruitment, survival and growth was low at both reef types. Apparently, even the higher grazing intensity at the reef balls was not sufficient to prevent the development of a high cover of turf algae. The high turf algae cover, combined with increasing cover of other benthic groups, probably reduced coral development. Invertebrate grazing, for example by *D. antillarum*, should be facilitated to further enhance grazing pressure on turf algae. However, densities of *D. antillarum* are currently very low around Saba and St. Eustatius.

Studying settlement rates of *D. antillarum* larvae can provide insight into the mechanisms constraining the natural recovery of this urchin species. For this, efficient settlement collectors can be used to identify locations with high settlement rates and to collect settlers for restoration practices. Hence, the aim of **Chapter 5** was to compare pre and post die-off settlement rates and to determine possible settlement peaks for St. Eustatius. Additionally, we aimed to determine the effectiveness and reproducibility of five different settlement collector types for *D. antillarum*. Before the die-off, settlement recorded for Curaçao was high throughout the year and was characterized by multiple settlement peaks. Even though peak settlement rates for St. Eustatius were in the same order of magnitude as in Curaçao before the die-off, overall yearly settlement rates around St. Eustatius were still lower. Of all five collector types tested, bio ball collectors, consisting of plastic balls normally used in aquaculture filters, were the most effective and reproducible method to monitor *D. antillarum* settlement.

High peak settlement rates on artificial collectors in **Chapter 5** indicated that there should be sufficient settlement potential for natural recovery of *D. antillarum*. The fact that very few juvenile and adult *D. antillarum* were observed on Saba and St. Eustatius, suggests that the larvae do not settle on the natural reef and that recovery of *D. antillarum* might be limited by the availability of settlement substrate. In **Chapter 6** we proposed a new approach to restore *D. antillarum* populations which we term assisted natural recovery (ANR). ANR, a concept already applied in terrestrial restoration, can accelerate succession by removing barriers to natural recovery. In this case, the settlement substrate availability barrier was removed by providing bio ball streamers that were attached to patch reefs shortly before the settlement season. Reefs with streamers had significantly higher *D. antillarum* recruit densities than control reefs. However, the recruit density remained low, possibly due to low post-settlement survival of settlers and high predation pressure on recruits, which likely are, next to settlement substrate availability, important constraints to natural recovery.

This dissertation helps take new innovative steps towards a more effective and combined use of artificial reefs and *D. antillarum* restoration. In doing so it opens the way for additional lines of research. As healthy coral development on both natural and artificial reefs in the Caribbean is very difficult without the presence of invertebrate grazers such as *D. antillarum*, interventions for the large scale rehabilitation of this key herbivore should be the number one research priority. The novel approach of ANR could be further optimized by adding extra shelter space to the bio ball design, thereby offering suitable settlement substrate for larger juveniles and improving post-settlement survival. In addition, settlement habitat could be improved by either removing macroalgae manually or by restocking other herbivores. Finally, careful site selection should focus on sites with a low predator density and a high shelter availability. The restoration of *D. antillarum* could possibly be combined with the deployment of artificial reefs. This combined approach should preferably be tested on new artificial reef designs, as the designs used in this dissertation can easily be further improved. With Modular Restoration Reef (Moreef) a new artificial reef design was developed that is easier to build and transport. Under water, modules can be connected, creating a more complex and storm-resistant reef. For large scale and more cost-effective artificial reef deployment, we recommend research into the use of large rock piles constructed from bigger rocks, as rock piles in the current studies harbored a high fish abundance and biomass, but proved unstable over time. Active intervention measures such as outlined above are expected to, next to reducing local and global threats, contribute substantially to the active rehabilitation of Caribbean coral reefs.

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Training and education

With the training and education activities listed below, Alwin Hylkema has complied with the requirements set by the Graduate School of the Wageningen Institute of Animal Sciences (WIAS) which comprises of a total of 32 ECTs (1 ECT equals a study load of 28 hours).

Training and Supervision Plan (TSP)

EDUCATION AND TRAINING	Year	Credits *
A. The Basic Package		1
Course Scientific integrity	2018	
Course Fish welfare ("Welzijn van vissen")	2013	
Course Environmental ethics	2019	
B. Disciplinary Competences		9
Course Design of Experiments	2017	
Course Statistics of the life sciences	2018	
Writing research proposal	2017	
C. Professional Competences		13
Teaching qualification for lecturers in universities of applied sciences (“Basiskwalificatie Didactische Bekwaamheid”), VU Amsterdam	2016	
Course Ecopath with Ecosim, SAMS, Scotland	2014	
Organizing AcroporaNet symposium, Leeuwarden, 7-12-2018	2018	
Course Societal impact of your research	2018	
D. Presentation Skills		3
EcoShape conference, oral presentation, Utrecht 27-6-18	2018	
Acropora.net symposium, oral presentation, Leeuwarden 7-12-2018	2018	
AGGRA Webinar Diadema restoration in the Caribbean, 1-7-2020	2020	
E. Teaching competences		
Teaching at University of Applied Sciences VHL	17-21	6
Education and Training Total (minimum 30 credits)*		**32**

**One ECTS credit equals a studyload of approximately 28 hours*

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