Journal of Experimental Marine Biology and Ecology 549 (2022) 151693

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

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

Alwin Hylkema [a,b,*], Adolphe O. Debrot [b,c], Marit Pistor [d], Eelke Postma [a], Stacey M. Williams [e], Kimani Kitson-Walters [f,g]

[a] University of Applied Sciences Van Hall Larenstein, P.O. Box 1528, 8901 BV Leeuwarden, the Netherlands
[b] Marine Animal Ecology group, Wageningen University and Research, P.O. Box 338, 6700 AH Wageningen, the Netherlands
[c] Wageningen Marine Research, Wageningen UR, P.O. Box 57, 1780 AB Den Helder, the Netherlands
[d] St. Eustatius National Parks Foundation (STENAPA), Oranjebaaiweg 59, St. Eustatius, Caribbean Netherlands, the Netherlands
[e] Institute for Socio-Ecological Research, P.O. Box 3151, Lajas, Puerto Rico
[f] Caribbean Netherlands Science Institute (CNSI), P.O. Box 65, St. Eustatius, Caribbean Netherlands, the Netherlands
[g] NIOZ Royal Netherlands Institute for Sea Research, Utrecht University, P.O. Box 59, 1790 AB, Den Burg, Texel, the Netherlands

ARTICLE INFO

Keywords:
Sea urchin settlement
St. Eustatius
Restoration
Assisted natural recovery
Saba

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*. *D. antillarum* settlement 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.

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 coral and crustose coralline algae (CCA) cover decreased in the months after the die-off (De Ruyter et al., 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 and Edmunds, 2006) and sheltered locations

* Corresponding author at: University of Applied Sciences Van Hall Larenstein, P.O. Box 1528, 8901 BV Leeuwarden, the Netherlands
E-mail address: alwin.hylkema@hvhl.nl (A. Hylkema).

https://doi.org/10.1016/j.jembe.2022.151693
Received 4 January 2021; Received in revised form 10 November 2021; Accepted 4 January 2022

like harbors and breakwaters (Debrot and Nagelkerken, 2006). On these few places where *D. antillarum* naturally recovered, their grazing reversed the phase-shift by significantly reducing algal cover (Carpenter and Edmunds, 2006; Edmunds and Carpenter, 2001). The benthic cover of CCA and bare substrate increased, apparently giving coral larvae a chance to settle and survive (Carpenter and Edmunds, 2006; Edmunds and Carpenter, 2001; 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 and Levitan, 1990; Williams et al., 2011; Mercado-Molina et al., 2015). Low fertilization success (Lessios, 1988; Feehan et al., 2016), lack of an upstream source population (Roberts, 1997), the lack of suitable settlement substrate (Rogers and Lorenzen, 2008) or reduced survival of post-settlers (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 in the water column (Williams et al., 2010, 2011). Large differences in settlement rates (*e.g.* Miller et al., 2009; 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; Williams, 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 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 collectors deployed at mid-water levels (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. To assess the effectiveness and reproducibility of multiple settlement collectors, we included artificial turf, bio ball, frayed rope and doormat 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.

2. Methods

2.1. Experiment 1: settlement on different collectors around St. Eustatius

As *D. antillarum* settlement is known to differ greatly in time and space (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 (Fig. 1). Locations were selected based on the following criteria: 13-15 m depth and a sandy bottom for at least 5 m around the experimental set-up. During the first two months of the monitoring period, collectors were analyzed monthly. When the first

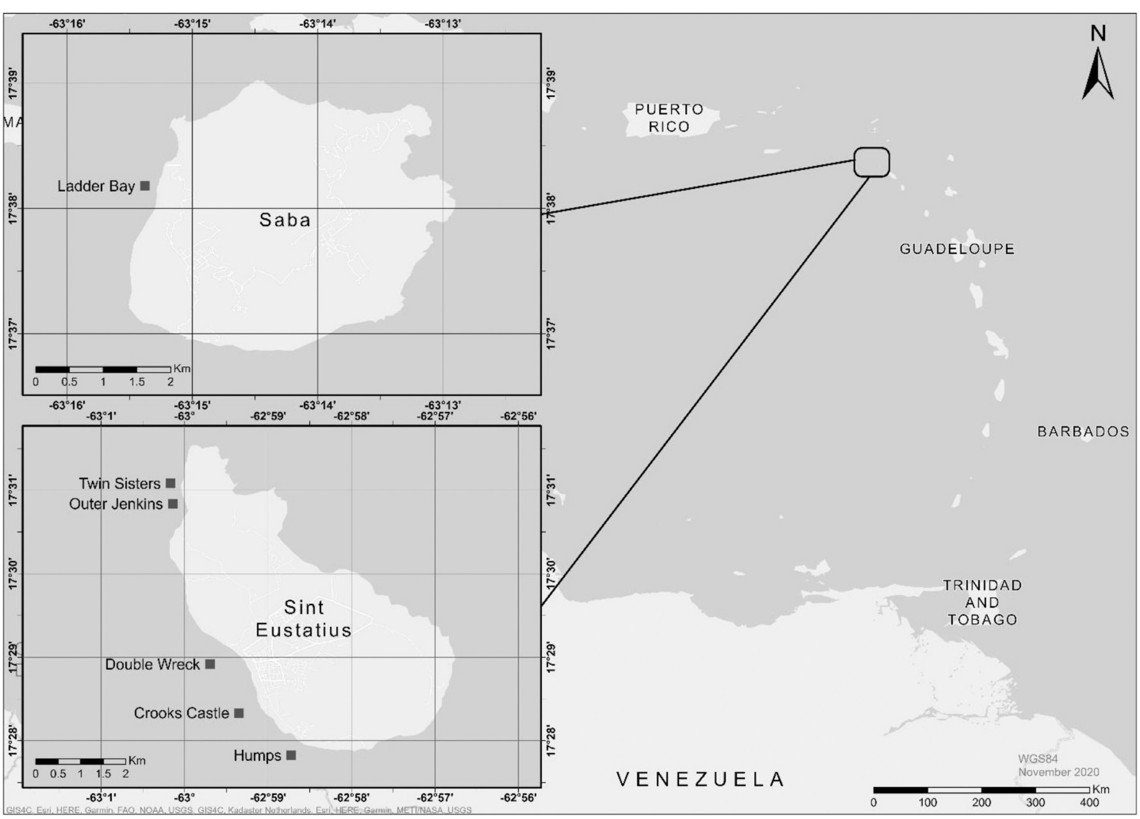

Fig. 1. Experimental locations around St. Eustatius and Saba.

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 (Fig. 2). The lines were connected to two anchors and placed 6.5 m apart on the seabed. A third rope was placed horizontally between the vertical ropes at 9 m depth, which is the optimal depth for *D. antillarum* settlement (Williams et al., 2011). Every 50 cm, 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 50 cm above the seabed on two pieces of rebar that were cast in one of the concrete anchors.

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

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 2 cm of sample were measured and subsequently used to determine the actual surface area of the 20 cm-long collector. To determine actual surface area of the doormat, all threads of a 10 cm × 1 cm sample were separated. The total length and diameter of these threads was measured and used to determine the actual surface area of a 10 cm 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.

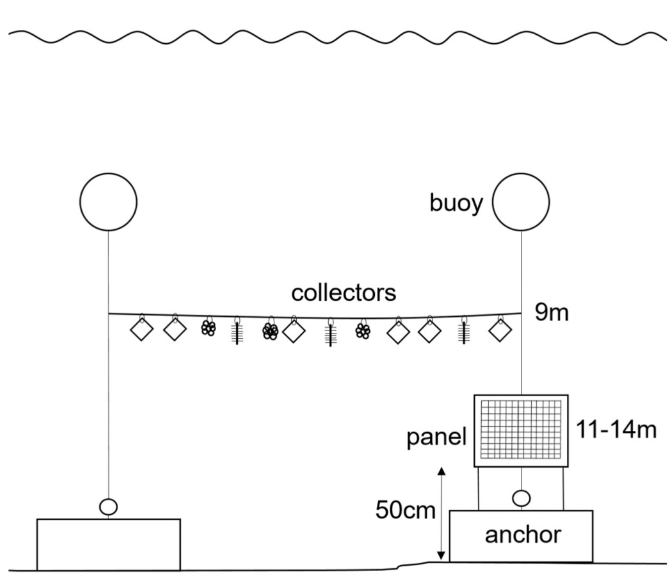

Fig. 2. Experimental set-up used at the locations around St. Eustatius in 2019.

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.

2.2. *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 10 m 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).

2.3. *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 (Fig. 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 12 m depth. Each rope had a loop at 8 m, 9 m and 10 m 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.

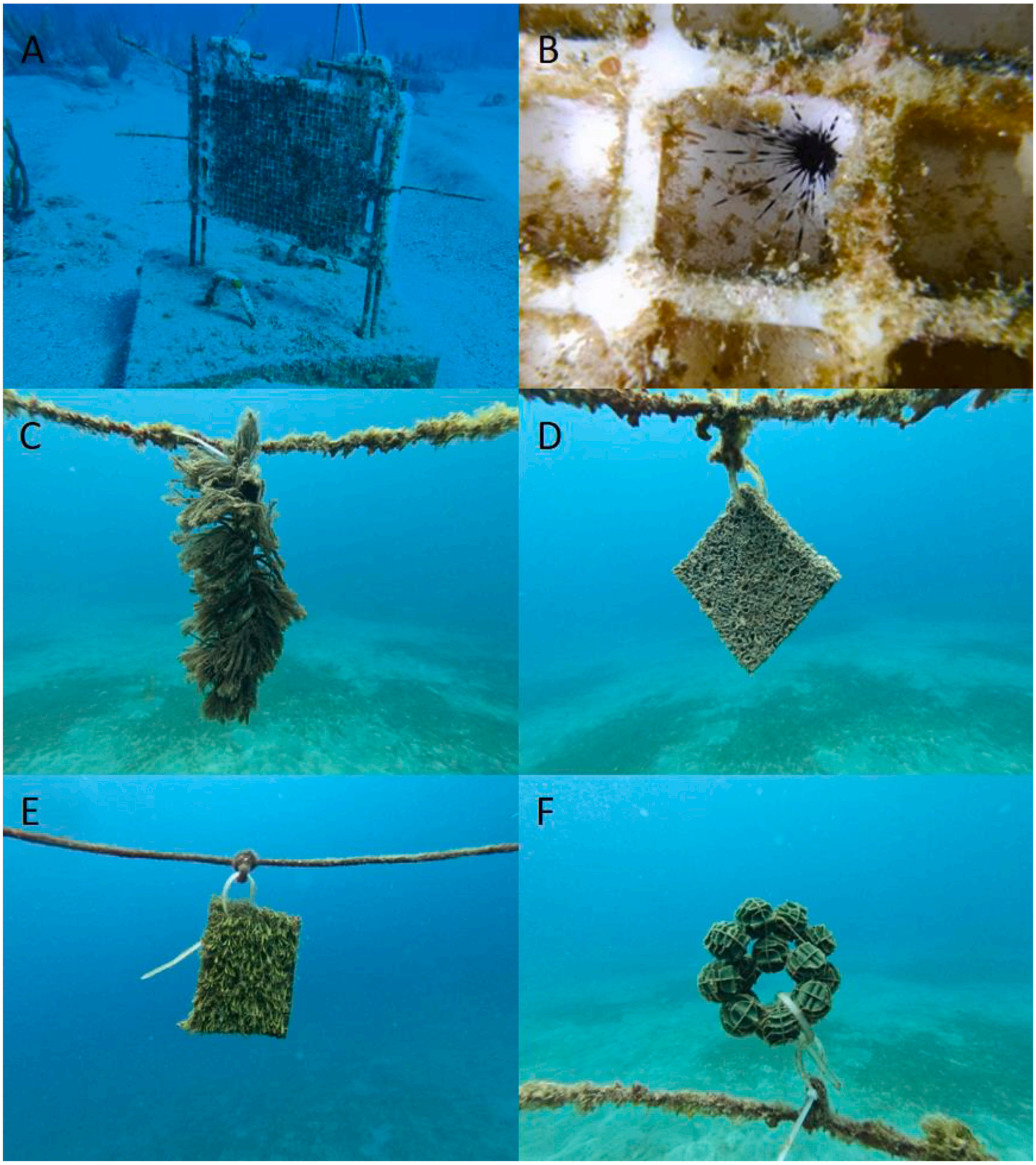

Fig. 3. Settlement collectors: a. panel, b. *D. antillarum* settler on panel, c. frayed rope, d. doormat, e. artificial turf and f. bio balls.

3. 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. Generalized Linear Models (GLMs) with a negative binomial error distribution were used to test the effect of treatment, location and week number on the monthly 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 et al., 2018).

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 (Fig. 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

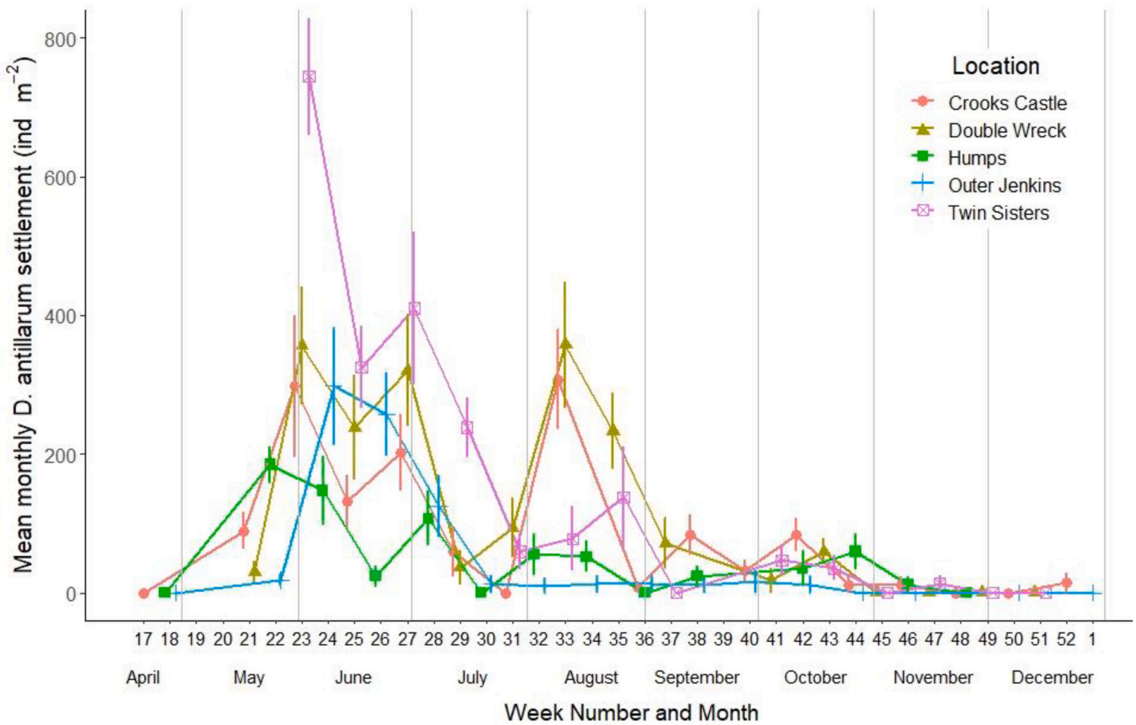

Fig. 4. Monthly *D. antillarum* settlement per m^2 in time per location averaged over doormat, artificial turf and bio balls collectors. Error bars show 95% CI interval.

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 (Fig. 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 (Fig. 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.

4. Results

4.1. 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. Fig. 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.

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, $P < 0.001$). Fig. 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.

The actual surface area, including all chambers, side strings and internal spaces was highest on the panels and lowest on the bio balls (Table 1). 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

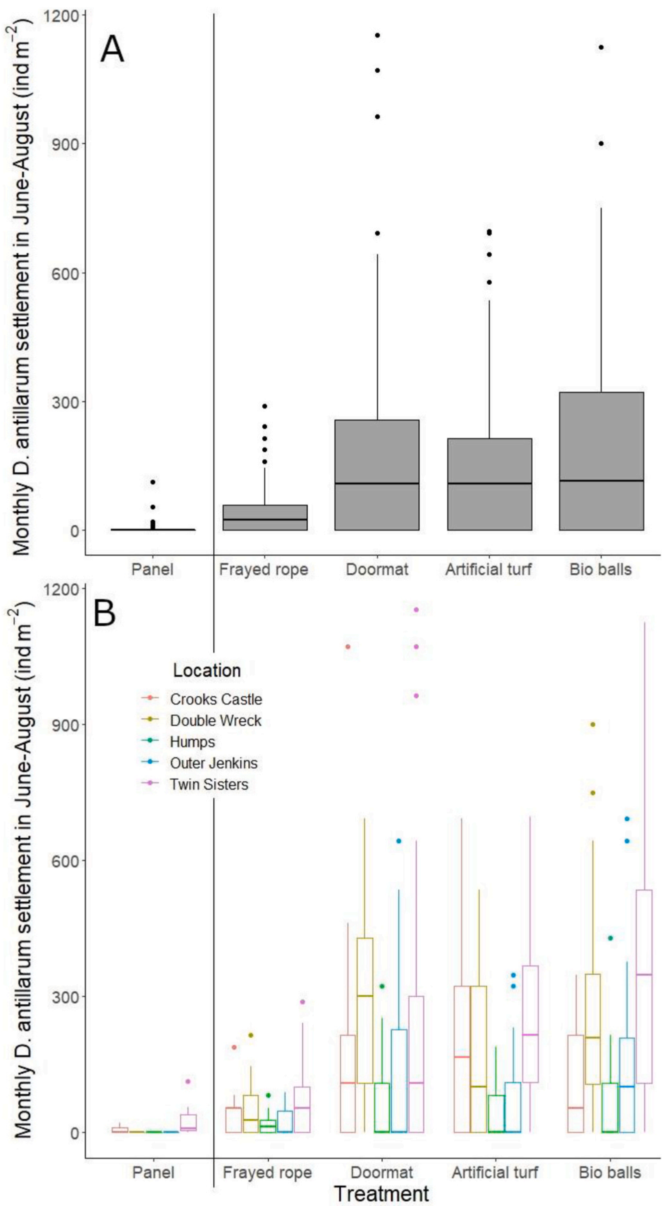

Fig. 5. Monthly *D. antillarum* settlement per m^2 on five types of settlement collectors during months of high settlement (June–August) **A.** overall and **B.** detailed per location. The boxplots show the median (black or colored line), the first and third quartiles (box) and the lower and upper extremes,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

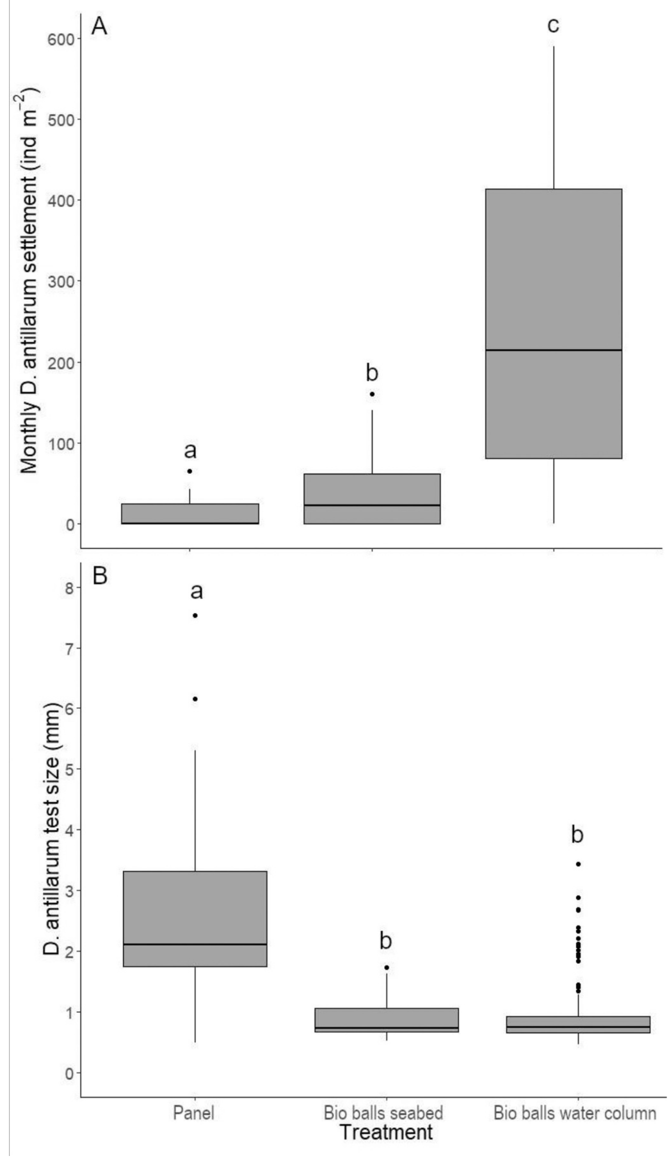

Fig. 6. A. Monthly *D. antillarum* settlement per m^2 and **B.** test size of *D. antillarum* settlers per treatment. The boxplots show the median (black line), the first and third quartiles (grey shaded box) and the lower and upper extremes, black dots represent outlier values (> 1.5 inter-quartile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

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 1). The panels had the highest coefficient of variation, meaning that these observations were least consistent.

4.2. 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,

Table 1
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%

followed by the bio balls close to the seabed and finally, the panels (Fig. 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 (Fig. 6). Location Twin Sisters had slightly larger settlers than Crooks Castle.

4.3. 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 ($P = 0.345$, Fig. 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 ($t(8) = -3.025$, $P = 0.016$, Fig. 7).

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 and Edmunds, 2006; Debrot and 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 and 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

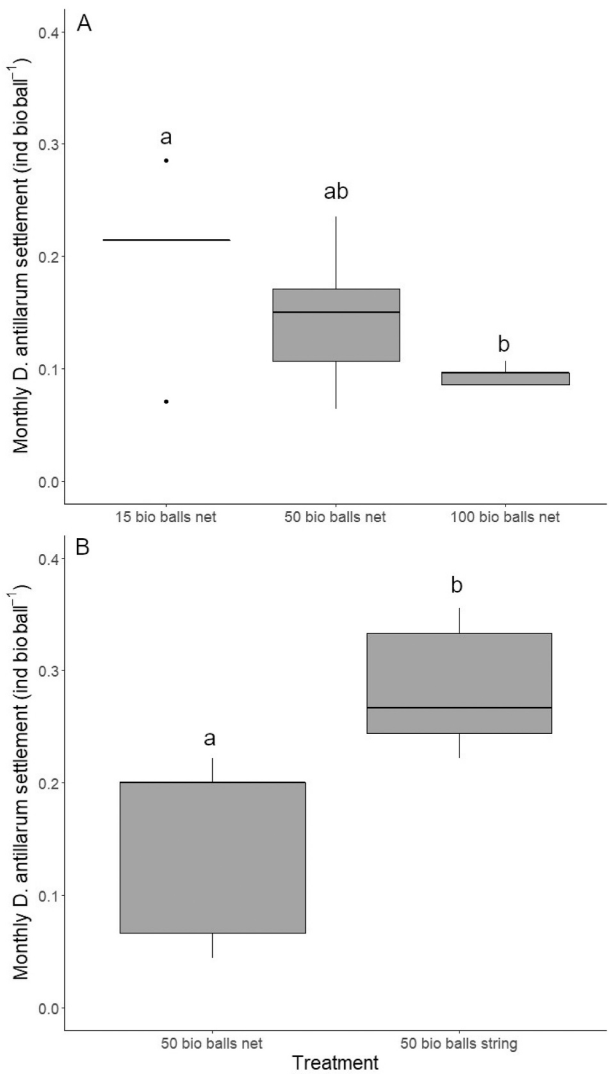

Fig. 7. Mean monthly *D. antillarum* settlement per bio ball for **A.** 15, 50 and 100 bio balls per net and **B.** 50 bio balls in a net and 50 bio balls on a string. The boxplots show the median (black line), the first and third quartiles (grey shaded box) and the lower and upper extremes, black dots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

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 than 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 buoyancy 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 (2-4 cm) (Williams, 2017; Williams, 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research is conducted in the context of the Diadema project (project# RAAK.PRO03.005), which was partly funded by SIA, part of the Dutch Research Council (NWO). We thank David Slieker, Floris Pauw, Oliver Klokman, Martijn Hofman, Anouk Kattenberg and Sander de Hoop for their help processing the collectors and Alex van der Last, Mika de Breuyn, Marnik Lehwald and Koen Crum for their assistance in determining actual surface area of the collectors. We also thank the CNSI, STENAPA, SCF and SBMU staff for providing fieldwork assistance, Callum Reid for making the map and Wortel Product Design for developing the panels. The contributions by A.O. (Dolfi) Debrot were funded by Wageningen Marine Research through project 4311500013 R&D Wetenschapsplan. Wageningen Marine Research is also acknowledged for provided internship funding for several students contributing to this work. The Ministry of Agriculture, Nature and Food Quality provided the funding for contributions of Kimani Kitson-Walters through the Data Monitoring Project on St. Eustatius. This work benefited greatly from comments by Dr. W. White and anonymous reviewers.

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