Pilot study: a novel approach to the rearing of *Diadema antillarum* from larval settlers to sub-adults in situ

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Abstract. The prevalence of herbivores plays a paramount role in cultivating and maintaining a flourishing and synergistic coral reef ecosystem. *Diadema antillarum* is a keystone herbivore that facilitates the critically important role of grazing macroalgae on coral reefs throughout the Caribbean. This species in particular has undergone two region-wide mass mortality events since the 1980s, with losses of up to 99%. In the Florida Keys, population recovery has been slow to absent after nearly 4 decades. In an attempt to increase *D. antillarum* populations, a novel method for producing juvenile *D. antillarum* urchins that could be used for population enhancement within the Florida Keys was explored. This method assessed the feasibility of rearing *D. antillarum* using in situ midwater grow-out systems with urchins sourced from larval settlement collectors. Between October 2022 to April 2023, a total of 113 *D. antillarum* were collected from settlement collectors. These *Diadema* were transferred to the midwater grow-out systems where they exhibited a 38.9% survival rate. At the end of the six-month study, the *D. antillarum* reared in the grow-out systems achieved a mean test size of 22 mm (max = 51 mm). This project demonstrated that *D. antillarum* can be collected in the water column with artificial settlement collectors and reared to sub-adult sizes using in situ midwater grow-out systems.
Key Words: Caribbean, coral reefs, mariculture, restoration, sea urchin.

Introduction. The long-spined sea urchin, *Diadema antillarum*, is a keystone species due to its role as one of the primary reef-grazing herbivores throughout Tropical Western Atlantic coral reef ecosystems (Gardner et al 2003; Furman & Heck 2009; Bodmer et al 2015). Historically, high population densities of *D. antillarum* were found on reefs with a low cover of macroalgae and a high percentage of living hard coral cover (Hughes et al 1987). Unfortunately, in 1983-1984, populations of *D. antillarum* throughout the Tropical Western Atlantic were reduced by 95-99% (Bak et al 1984; Lessios et al 1984) following exposure to an unknown waterborne pathogen exclusively affecting *D. antillarum*.

This mass mortality event altered reef ecosystem dynamics through reduced herbivory and subsequent loss of habitat complexity and hard coral cover. The loss represents a pivotal event that drastically altered the shallow coral reef ecosystem, resulting in a significant decline in coral cover and a disruption of ecological balance, with far-reaching consequences for both the coral and the multitude of species that depend on these vital ecosystems (Hughes et al 1987; Carpenter 1988; Lessios 1988). Soon after the loss of *D. antillarum*, coral reefs in the Caribbean shifted from coral-dominated to alga-dominated communities (Lessios 2016). With this excess alga, the larval settlement and survival of corals were severely hindered (Mumby et al 2006; Speare et al 2019). Over time, as alga outcompeted the corals, living coral cover declined and concurrently the rugosity and complexity of reefs decreased. Natural populations of *D. antillarum* recovery have been patchy (Lessios 2016), and it is widely acknowledged that reduced *D. antillarum* populations have strongly contributed to the ongoing decline of Florida's Coral Reef.

A crucial step in efforts to enhance *D. antillarum* is to develop a rearing system that can take larval/juvenile *D. antillarum* from a variety of sources and rear them to

larger, sub-adult-sized urchins. Larger-sized *D. antillarum* have, in some circumstances, avoided more predation than smaller sized individuals when reintroduced to the local reefs (Williams 2016). Ex situ lab-rearing of *D. antillarum* has been explored in previous studies (Pilnick et al 2021; Wijers et al 2023), as well as in situ larval settlement collection with subsequent transport of settlers to lab-based facilities for rearing (Williams 2016). Additionally, assisted natural recovery (ANR) of *D. antillarum* has been explored through the use of larval settlement collectors to enhance the population (Hylkema et al 2022). However, collecting larval-settled *D. antillarum* and rearing them to sub-adult sizes, completely in situ, has not been explored.

This pilot study investigates the use of larval settlement collectors to collect settlers of *D. antillarum* while utilizing an in situ caged grow-out system to rear these settlers to sub-adult sizes. While previous studies have used cages to study test growth/shrinkage and fecundity rates in *D. antillarum* (Randall 1964; Levitan 1988), rearing newly settled *D. antillarum* in midwater grow-out systems has not been explored. Notably, the urchins in this study were significantly smaller than those typically featured in prior research. With a successful in situ grow-out system for urchins, significant reductions in costs, labor, and spatial efficiency could be achieved. This is an important step toward scaling restoration practices for coral reef restoration practitioners.

Material and Method

Larval settlement collectors. Larval settlement collectors modified from previous designs (Bak 1985; Hernandez et al 2006; Miller et al 2009; Vermeij et al 2010; Williams et al 2010, 2011; Hylkema et al 2022) were deployed mid-water at a depth of 10 m adjacent to a shallow water coral nursery maintained by Reef Renewal USA in Tavernier, Florida. Larval settlement collectors and grow-out systems (as described below) are located within 300' of the following coordinates 24.980275, -80.438931.

Two types of settlement collectors were first deployed in August 2022. These settlement collectors consisted of sheets of polystyrene egg crate and artificial grass door mats. Egg crate collectors (Figure 1) were modified from past experiments (Bak et al 1984; Bak 1985; Miller et al 2009) with HDPE plastic sheeting used as a center substrate instead of plexiglass to allow for easier visual observation of settlers. Holes were drilled in the HDPE sheeting at each corner and cable tied to the egg crating on each side.

The second material, door mats (Figure 1) were modeled after previous experiments (Williams et al 2010, 2011). These door mats were cut, and cable tied back-to-back to increase surface area.

The third settlement collector, primarily made of bio balls (plastic balls commonly used in aquariums as a method of water filtration) (Figure 1), was modified from past experiments (Hernandez et al 2006; Hylkema et al 2022) and deployed in September 2022. Clear, large bio balls were threaded through a monofilament line which was subsequently fixed to a ½" diameter PVC frame. The frames were fixed with 6 monofilament lines threaded with bio balls. Each monofilament line contained 14 bio balls strung through the center hole of the bio ball.

All three styles of settlement collectors, egg crate, door mats, and bio balls were attached to 3/8" triple-strand polypropylene line with cable ties securing the collectors to the untwisted line. Six collectors were placed on each line. The collector lines were attached to anchors in the sea floor and buoyed using subsurface floats. Three collector lines of egg crate and door mat collectors were deployed, while only one bio ball collector line was deployed. All settlement collectors were 60 cm x 30 cm.

Larval settlement collectors were monitored monthly until the first settlers were observed. After initial settlers were observed, monitoring the settlement collectors for settlers was increased to every two weeks. The collectors were inspected using SCUBA for the presence of any visible *D. antillarum* settlers. If any settlers were visible, they were removed from the collectors and placed into the in situ grow-out systems (Figure 2). Every two months, the collector lines were swapped with a set of clean collectors. The fouled settlement collection lines were brought to land and cleaned with a pressure washer. In January of 2023, a Ryobi EZ clean hand-held battery-powered washer was

brought into the field and used to clean the collectors on site. This eliminated the need for a second set of collectors.

Figure 1. Displays the three different types of settlement collectors secured to polypropylene line: 1. egg crating; 2. door mat; 3. bioballs.

Figure 2. Example of a 1 mm settler and small juveniles collected from settlement collectors.

During monitoring events, the presence and size of observed settlers were recorded for each type of collector. The settlers were then carefully removed and placed in different grow-out systems. All settlers were measured to the closest millimeter using brass vernier calipers. Larger urchins (classified as > 4 mm) collected from the settlement collectors were removed by grabbing the urchins by their spines and removing them from the settlement collector. Smaller settlers, as small as 1-3.5 mm in size, were removed with a small 4" cable tie, plastic tweezers, and a turkey baster. The tail end of a cable tie was used to carefully slide underneath the urchin test to remove the urchin from the substrate. The tweezers were utilized to clasp spines and/or carefully grab the urchin test. The turkey baster was used by gently propelling a stream of water at the settler to dislodge it from the collector substrate. These small settlers were transported to a slightly fouled approximately 0.5 liter plastic bottle with 1/8" holes drilled into it. On October 3rd, 2022, the bottle, with no lid attached, was then placed into a bucket grow-out system. This allowed the settlers to move freely from the bottle, although they were not expected to move much during this stage of their life. Small *D. antillarum* settlers do not have a large grazing range in comparison to other species of urchins, primarily feeding on diatoms and biofilms (Dworjanyn & Pirozzi 2008; Mos et al 2011; Rahim et al 2004). On October 15th, 2022, and for the remainder of the experiment, all settlers collected were placed directly into the bucket grow-out systems. During this study, *Lytechinus variegatus* and *Eucidaris tribuloides*, two additional species of urchins, naturally settled within the grow-out systems and were observed.

Grow-out systems. Three different grow-out systems were tested in this study. Each of these grow-out systems consisted of a series of mesh cages or buckets mounted midwater. These caged grow-out systems are used to retain urchins and protect them from most fish predation with the purpose of growing the urchin to a larger size prior to translocation to the reef.

The first grow-out system (Figure 3) consists of a cube made from ½" diameter PVC pipe with 2 crossbars of PVC from corner to corner, that is wrapped with ¼ inch vinyl coated wire mesh. To suspend the cage, two sets of 200 lb. monofilament loops were fixed at two corners on the top and bottom of the cube which were used to attach a tether rope to a set of floats and anchor point. At the top of the cube, a section of the mesh was cut to act as a lid. A 1/8" elastic cord was used to keep the lid closed. The dimensions of the cube were 60 cm x 60 cm x 60 cm.

The second grow-out system consisted of a 1 1/2" diameter square PVC frame, 60 cm x 60 cm (Figure 3). This system was fixed midwater using two sections of 3/8" polypropylene rope, fastened to the PVC frame and connected to both floats and an anchor point. The PVC frame was used to support a ½" vinyl-coated wire mesh section, affixed together in a rectangular box measuring 60 cm x 60 cm x 20 cm. The PVC frame and wire mesh were then affixed together to act as one unit. Two sections of 3/8" rope was tied to the PVC frame to tie to the floats and anchor. Cut sections of garden hose were used to provide a chafing guard, so the vinyl-coated wire would not fray the suspension ropes. This larger-sized mesh was used for larger-sized urchins as the settlers grew.

The third type of grow-out system used was 5-gallon buckets (Figure 3). Four buckets were mounted vertically, and three buckets were mounted horizontally (7 buckets total). Additionally, the buckets were deployed with port holes cut out of the lid and bottom. These port holes were subsequently replaced with ¼" mesh vinyl coated wire. These holes allow for visibility, water movement, and fecal discharge of the urchins. The buckets were mounted mid-water in the water column by affixing a ¼" thick rope to drilled holes within the bucket. Floats were then affixed to that ¼" thick rope. The process was repeated on the bottom of the bucket for a tether rope attached to an anchor point.

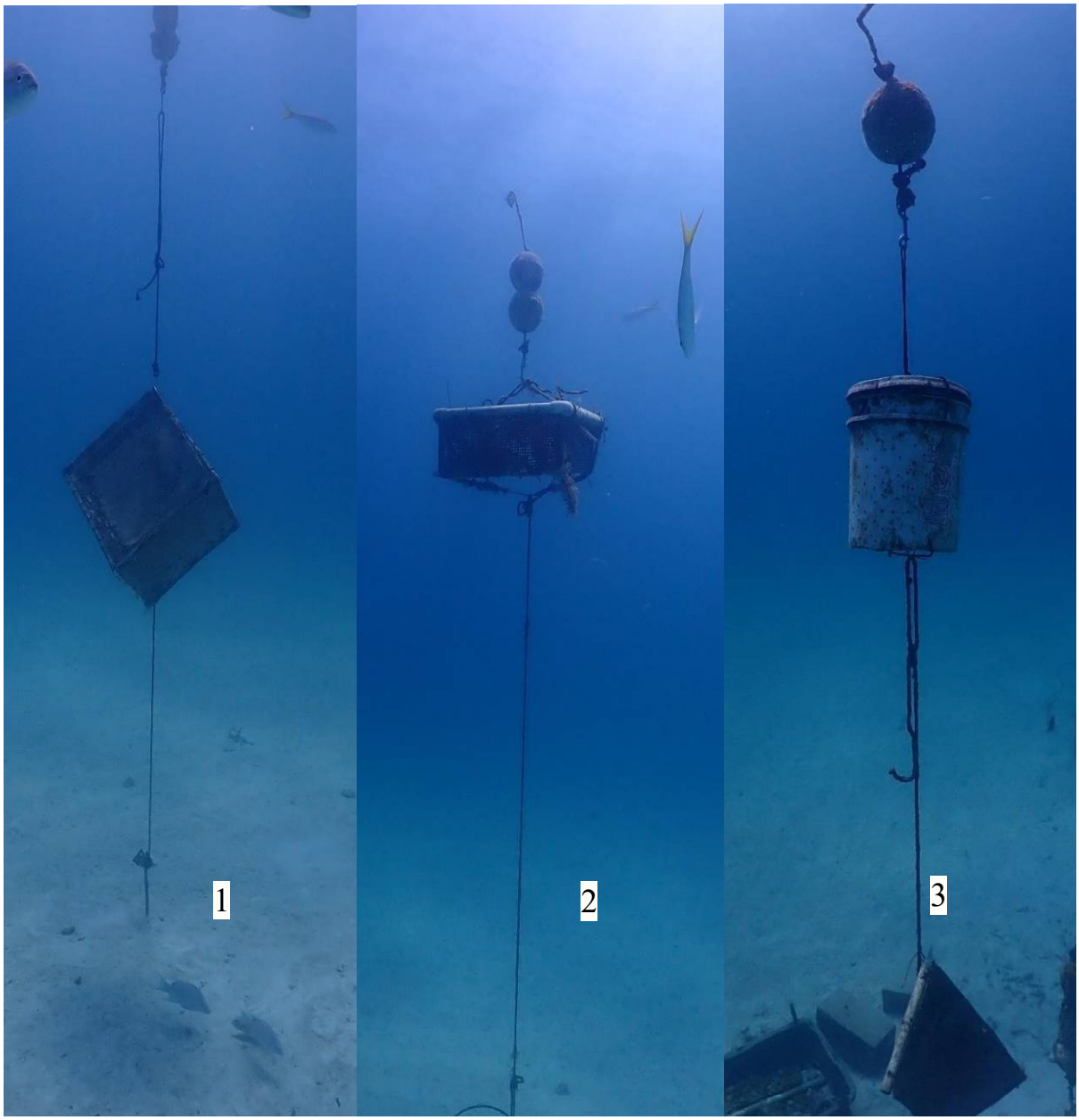

Figure 3. This figure shows examples of the various styles of grow-out systems used: 1. PVC cube with ¼" mesh; 2. PVC frame with ½" mesh section; 3. 5-gallon bucket with ¼" mesh.

To feed the urchins, approximately one liter of fouled coral rubble was placed inside each of the grow-out systems during each monitoring event. The grazed rubble was then swapped with fouled rubble at ~15-day intervals. The coral rubble consisted of dead, storm-generated *Acropora cervicornis* and *Acropora palmata* skeletons collected from the adjacent coral nursery. All rubble was inspected for micro predators, particularly crabs, which were removed and placed in neighboring rock piles prior to placing the rubble inside the grow-out systems.

Throughout this pilot study, *D. antillarum* were moved between the different grow-out systems according to urchin test size and vinyl mesh size. Additionally, urchins were moved between systems to maximize algal availability.

Results. A total of 113 settlers were collected throughout 6 months (Table 1). Over the duration of the study, approximately four times as many *D. antillarum* were observed and collected on the egg crates as compared to door mats (78 vs 15) even though the same number and approximately the same size collectors were used. The egg crate collectors were easier to inspect compared to the door mat collectors, which were very

difficult to examine in an in situ setting. Bio ball collectors were also successful, but fewer were deployed, making comparisons with egg crates or door mats difficult. A total of 44 *D. antillarum* were observed within grow-out cages at the end of the study (38.9%) out of the 113 settlers collected. At the conclusion of this experiment, *D. antillarum* test sizes ranged from 9 to 51 mm (mean = 22 mm).

Table 1

Collector substrates and dates corresponding with the amount of *D. antillarum* collected

Date	*Egg crate collectors*	*Doormat collectors*	*Bio ball collectors*
8/12/2022	Deployed	Deployed	Not deployed
9/6/2022	0	0	Not deployed
9/16/2022	0	0	Deployed
10/3/2022	14	4	0
10/15/2022	13	0	0
11/16/2022	26	2	14
12/6/2022	15	0	6
12/23/2022	0	0	0
1/16/2023	10	0	Not deployed
2/3/2023	0	9	Not deployed
2/19/2023	0	0	Not deployed
3/11/2023	0	0	Not deployed
Total:	78	15	20

Table 1 illustrates the total amount of settlers collected on all substrates at each monitoring event and mean test size of collected urchins. Table 2 illustrates total *D. antillarum* collected with the corresponding date and overall average of test size of all settlers/juveniles.

Table 2

Mean test size of urchins collected and amount at each event

Date	*Mean test size*	*Total D. antillarum collected*
8/12/2022	Deployment	0
9/6/2022	0	0
9/16/2022	0	0
10/3/2022	2.25 mm	18
10/15/2022	3.2 mm	13
10/31/2022	0	0
11/16/2022	2.5 mm	42
12/6/2022	3.14 mm	21
12/23/2022	0	0
1/16/2023	3.1 mm	10
2/3/2023	6.13 mm	9
2/19/2023	0	0
3/11/2023	0	0

Discussion. This study explored the use of settlement collectors and in situ grow-out systems to rear *D. antillarum* to sub-adult sizes. For the duration of the study, a total of 78 individual *D. antillarum* were collected on the egg crate style settlement collectors versus 15 individuals collected from the door mat settlement collectors. Using the white HDPE plastic sheeting instead of plexiglass made in situ survey monitoring more efficient and effective for the egg crate collectors. Compared to prior studies, which involved bringing settlement collectors back to a laboratory for surveying purposes (Williams et al 2010, 2011; Hylkema et al 2022), the door mat collectors exhibited rapid fouling and proved more challenging to inspect for urchins. This difficulty may account for the observed lower recovery rates of *D. antillarum* on this collector type. Our approach

employs in situ surveying methods, emphasizing the importance of substrate visibility in data collection. The door mat collectors became fouled very quickly and were much more difficult to inspect for urchins, which may explain the low recovery rates of urchins on these structures. The urchins that were found on the door mat collectors were ≥ 5 mm which were much more visible than the smaller settlers found on the egg crate. Only one bio ball collector settlement line was put into trial, and therefore, comparisons to egg crate and door mat collectors are difficult. However, based on the captured data, it appears that bio-ball collectors demonstrate high efficiency in collecting *D. antillarum* settlers, aligning with the findings of Hylkema et al (2022). The bio ball collectors took significantly more time to manufacture, which is the reason only one was deployed. However, the collector line did collect 20 *D. antillarum* during its 3-month deployment period.

Larval settlement collectors are prone to fouling and will need to be cleaned periodically to maximize settlement of *D. antillarum*. In the location used for this experiment, offshore Tavernier located in the Florida Keys, the cleaning of nursery hardware should take place approximately every two months. The Ryobi EZ-clean power washer was used to remove fouling from settlement collectors. This device proved to be much more time efficient and did not require the use of a second set of settlement collectors at the 2-month monitoring events because the fouled collectors could be cleaned on-site.

With high densities of urchins, the amount of fouled coral rubble becomes insufficient to adequately feed the growing urchins. To address this deficiency, midwater mesh screens could be deployed outside the cages to collect algal biomass, then introduced into the cages with each new addition of coral rubble.

Once the larval settlers reached ~ 6 mm test sizes the survival rates of the settlers increased dramatically (personal observations). Larger settlers that were collected may have higher survival rates than the very small < 4 mm settlers. The larger settlers may be able to better endure the transfer process from the settlement collector to the grow-out systems.

In these grow-out systems, the potential exists for cultivating a diverse range of urchin sources. The pilot study successfully reared two additional species, *L. variegatus* and *E. tribuloides*, to larger sizes, suggesting the adaptability of these systems. These floating systems effectively nurture urchins sourced from larval settlement collectors, indicating a consistent supply throughout the year, especially during their peak settlement seasons.

Furthermore, these in situ grow-out systems not only offer a controlled and secure space for urchins collected from settlement collectors but also have the potential to serve as a valuable grow-out and staging area for urchins settling in the volatile environment of rubble zones. In the conditions of the Florida Keys, where urchins settling within rubble-filled zones face constant peril, particularly during storm events that can disrupt their habitats, these systems may enhance the survivability of settling urchins. The dual functionality of these systems reinforces their versatility, providing a safe haven for the growth and development of juvenile urchins.

Moreover, there is promising potential for the grow-out of lab-reared urchins in these systems, adding another dimension to their utility. This not only ensures a consistent supply but also contributes to the resilience of struggling urchin populations in degraded marine environments. These in situ grow-out systems play a crucial role in both supporting natural settlement and providing a protective environment for lab-reared urchins, ultimately fostering the overall health and sustainability of urchin populations.

In situ grow-out systems represent an economically sound approach to urchin rearing, with several distinct advantages over land-based facilities. The utilization of the natural oceanic environment minimizes the need for extensive human intervention, simplifying operations and reducing labor and resource costs. The ocean itself acts as a self-sustaining habitat for these marine organisms, requiring only minimal support from humans.

One of the most notable strengths of in situ grow-out systems lies in their scalability and ease of expansion. Unlike land-based facilities that necessitate significant

infrastructure development, oceanic grow-out systems can be incrementally expanded with relative ease. This inherent flexibility allows us to respond to changing demands effectively. As the need for urchin production increases, in situ operations can readily accommodate this growth by scaling up existing systems or establishing new ones in strategically chosen locations. This adaptability not only ensures the ability to meet growing demand for urchins but also reduces the lead time and costs associated with facility expansion.

Conclusions. This pilot study demonstrates that with the use of settlement collectors and in situ grow-out systems, *D. antillarum* can be reared from post larval settlers to sub adult urchins in situ. Mid-water grow-out systems can be implemented as a low-cost scalable mariculture method to bolster the wild populations of *D. antillarum*. The mid-water systems utilized in this experiment experienced tropical storm conditions with high seas and winds up to 40-50 mph, and remained completely intact. Additionally, multiple midwater grow-out systems can be stacked on one tethered line. This decreases the cost of anchors and buoys. In addition to the *D. antillarum* reared during this study, several settlers of other urchin species, *Lytechinus variegatus* and *Eucidaris tribuloides* were collected and reared to 13 mm+ test size by the end of the experiment. Therefore, it is plausible to use this methodology for other urchin species. Additionally, these midwater grow-out systems could be used as a low-cost method for the grow-out of lab-reared D. antillarum.

Acknowledgements. Sustainable Oceans and Reefs would like to thank Mike and Lois Brittsan, Gabrielle Rosenbacher, Joe Tong, Mark Trinoskey, Philipp Rauch, Kevin Macaulay, Matt Finn, and volunteers who helped throughout the years. Thanks to Reef Renewal for the use of their coral nursery for this project. Their holistic approach to coral reef restoration is necessary and greatly appreciated. Thanks to the state of Florida for partially funding this project through State Wildlife Grant, 21346. The funding source was not involved in the analyses or interpretation of the results, preparation of the manuscript, or the decision to submit the article for publication. SOAR would like to thank the Florida Keys National Marine Sanctuary for their continued support of this project. The author James Brittsan is the inventor of this in situ mariculture system.

Conflict of 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.

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Received: 28 November 2023. Accepted: 17 December 2023. Published online: 30 December 2023.
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How to cite this article:
Brittsan J., Nedimyer K., Bruckner A., 2023 Pilot study: a novel approach to the rearing of *Diadema antillarum* from larval settlers to sub-adults in situ. AACL Bioflux 16(6):3397-3406.