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Original research article

Restoration of critically endangered elkhorn coral (*Acropora palmata*) populations using larvae reared from wild-caught gametes

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ABSTRACT

Elkhorn coral (*Acropora palmata*) populations provide important ecological functions on shallow Caribbean reefs, many of which were lost when a disease reduced their abundance by more than 95% beginning in the mid-1970s. Since then, a lack of significant recovery has prompted rehabilitation initiatives throughout the Caribbean. Here, we report the first successful outplanting and long-term survival of *A. palmata* settlers reared from gametes collected in the field. *A. palmata* larvae were settled on clay substrates (substrate units) and either outplanted on the reef two weeks after settlement or kept in a land-based nursery. After 2.5 years, the survival rate of *A. palmata* settlers outplanted two weeks after settlement was 6.8 times higher (3.4%) than that of settlers kept in a land-based nursery (0.5%). Furthermore, 32% of the substrate units on the reef still harbored one or more well-developed recruit compared to 3% for substrate units kept in the nursery. In addition to increasing survival, outplanting *A. palmata* settlers shortly after settlement reduced the costs to produce at least one 2.5-year-old *A. palmata* individual from $325 to $13 USD. Thus, this study not only highlights the first successful long-term rearing of this critically endangered coral species, but also shows that early outplanting of sexually reared coral settlers can be more cost-effective than the traditional approach of nursery rearing for restoration efforts aimed at rehabilitating coral populations.

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1. Introduction

Caribbean coral communities were historically dominated by elkhorn corals (*Acropora palmata*) between depths of 0–5 m, where this species was so abundant that shallow Caribbean reef habitats were classically described as the "palmata zone" (Bak, 1975; Goreau, 1959). Well-developed *A. palmata* populations contribute to important ecological processes and services of Caribbean coral reefs such as habitat provisioning for a variety of reef organisms, coastal protection, gross community calcification, and nitrogen fixation (Gladfelter and Gladfelter, 1978; reviewed in Harborne et al., 2006; Nagelkerken, 1974). During the mid to late 1970s, a Caribbean-wide outbreak of white-band disease (WBD) reduced the abundance of *A. palmata* by more than 95% (Acropora Biological Review Team, 2005; Aronson and Precht, 2001; Gladfelter, 1982; Jackson et al., 2014). After this period, populations that survived the disease were subsequently impacted by hurricanes (Goreau, 1992), coral bleaching (Williams and Bunkley-Williams, 1988), algal over-growth (McClanahan and Muthiga, 1998), and predation by corallivorous snails and damselfish (Williams and Miller, 2012). The species' decline without indications of recovery and current lack of recruitment (van Moorsel, 1989; Vermeij et al., 2011) resulted in its listing as "critically endangered" on the Red List of Threatened Species in 2008 (IUCN, 2013).

Lower coral species diversity in the Caribbean compared to the Indo-Pacific (Miloslavich et al., 2010; Roberts et al., 2002; Spalding et al., 2001; Veron, 2000) limits functional redundancy in this region, thus the probability that the local regional species pool harbors species capable of replacing others that have declined in abundance is quite low in the Caribbean (Bellwood et al., 2004; Fonseca and Ganade, 2001). This is particularly true for the members of the Caribbean coral genus *Acropora* which consists of only three species. In contrast, Indo-Pacific acroporids comprise more than a hundred species (Veron, 2000), making it unsurprising that the local decline of one or a few Indo-Pacific *Acropora* species is generally followed by rapid colonization and regrowth of others (Kojis and Quinn, 2001). In contrast, no Caribbean coral species has colonized the habitat in which *A. palmata* was formerly abundant (Nagelkerken and Nagelkerken, 2004). Since no other Caribbean coral species resembles *A. palmata* morphologically or ecologically, the return of *A. palmata* itself appears to be the only pathway by which shallow Caribbean reefs could regain their former composition and function.

To assist the recovery of *A. palmata* populations, restoration efforts were initiated throughout the Caribbean region using asexual propagation or "coral gardening" approaches whereby fragments are cultured from donor colonies in nurseries before they are outplanted on the reef (reviewed in Young et al., 2012). While asexual propagation of Caribbean acroporids has been successful (e.g., Bowden-Kerby et al., 2005; Johnson et al., 2011; Nedimyer et al., 2011; Quinn and Kojis, 2006), it requires that fragments are harvested from otherwise healthy colonies. It also limits the formation of new genotypes through genetic recombination, which may hamper the generation of genotypes better adapted to the altered environmental conditions on modern-day Caribbean reefs (Baums, 2008; Baums et al., 2013; Reusch et al., 2005). Using sexually- instead of asexually-produced offspring avoids these issues. Moreover, the use of eggs rather than fragments could yield a much larger number of individuals that can be reared for restoration efforts. Attempts to raise sexual recruits of *A. palmata* in closed-system aquaria has proven partially successful (Petersen et al., 2008), but has only led to the outplanting of a small number of individuals and lacks evidence of significant long-term survival (Miller, 2014; Szmant and Miller, 2006; A. M. Szmant and M. W. Miller, personal communication). Presently, successful rearing and outplanting of large numbers of sexually produced larvae followed by long-term survival (>6 months) has only been reported for three Indo-Pacific acroporid species (Guest et al., 2014; Nakamura et al., 2011; Omori et al., 2008; Villanueva et al., 2012), whereas studies achieving similar success for any Caribbean coral species do not exist.

Reared coral larvae are generally settled onto artificial substrates and kept in land-based or ocean nurseries for several months to years before they are outplanted (Baria et al., 2012; Guest et al., 2014; Nakamura et al., 2011; Villanueva et al., 2012). Land-based nurseries are generally assumed to offer stable and more protected environments for coral settlers relative to actual reef environments due to reduced fish predation, algal competition, and sedimentation (Nakamura et al., 2011). Because increased size corresponds to lower mortality in recently settled corals (Vermeij and Sandin, 2008), extended grow-out periods are expected to increase the success of restoration efforts by allowing settlers to grow before they are outplanted on a reef. Alternatively, outplanting coral offspring soon after settlement might select for genotypes capable of coping with the conditions at the outplant site. Exposing recently settled corals to moderate stress conditions could also lead to increased tolerance to more severe stress conditions experienced later in life (van Oppen et al., 2015), a process equivalent to "hardening" in plants (Beck et al., 2004). Keeping settlers in nurseries for long periods of time or by outplanting them soon after settlement are consequently two different approaches that each have specific advantages for restoration purposes.

Reduced nursery periods would also aid to make restoration efforts more economically viable. Large scale restoration efforts are currently extremely expensive due to the high costs associated with nursery maintenance and the outplanting of artificial substrates by hand. The costs to rear and outplant one artificial substrate containing at least one coral recruit ("recruit–substrate unit", RSU) currently range from $5.40 USD (Villanueva et al., 2012) to $163 USD (Nakamura et al., 2011). Such estimates only include expenditures until the initial outplant of the RSUs and assume that all outplanted RSU will become one adult colony. Because not all outplanted recruits will survive, a much larger number of RSUs is needed to repopulate an area, which further increases the costs of any restoration effort. Reducing the time that settlers spend in a nursery could lower the costs associated with restoration efforts, but it is currently not known whether early outplanting of settled coral larvae represents a more effective and cost-efficient restoration approach compared to traditional nursery-based methods.

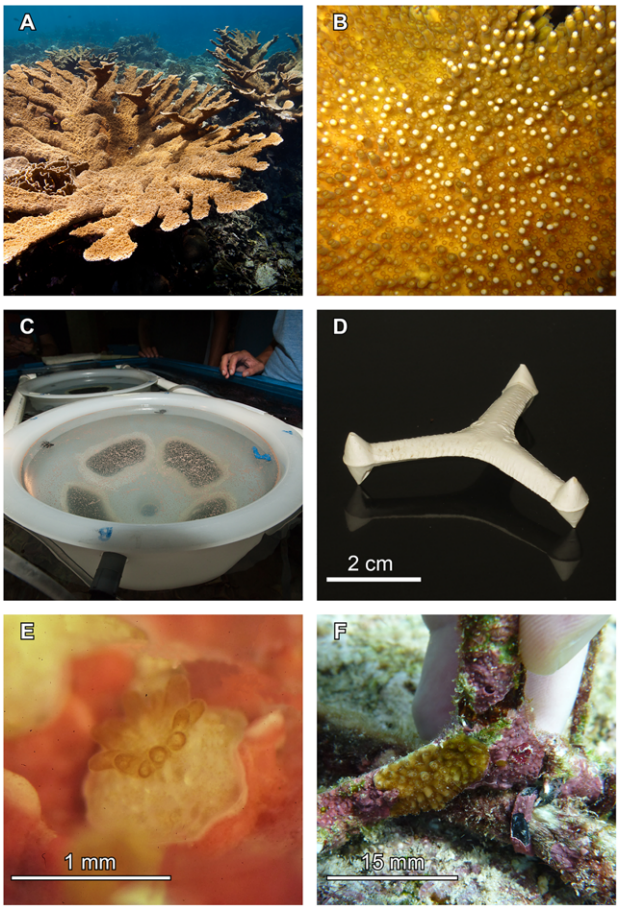

Fig. 1. (A) Healthy *A. palmata* population at the Curaçao Sea Aquarium reef; (B) *A. palmata* colony releasing egg-sperm bundles; (C) the kreisel system in which *A. palmata* embryos developed; (D) tripod made of clay for larval settlement; (E) two-week-old *A. palmata* settler; and (F) one-year-old *A. palmata* recruit outplanted to the reef two weeks post-settlement. Photo credits: (A and C): P. Selvaggio, (D, B and E): V.F.C, (F): D.P.

Here we describe the first successful rearing, outplanting and long-term (2.5 yr) survival of *A. palmata* recruits that were reared from gametes collected in the field. We tested whether the effectiveness of restoration efforts for this critically endangered Caribbean coral species could be improved by shortening *ex situ* grow-out periods to two weeks before outplanting settlers on the reef and determined whether post-settlement survival, colony growth and cost-effectiveness was significantly different from those of recruits reared in land-based nurseries over a 2.5 year period.

2. Material and methods

2.1. Study location and nursery set-up

This study was carried out on the island of Curaçao (12°N, 69°W) in the Southern Caribbean. Reproductively active *A. palmata* populations are abundant at our study site near the Curaçao Sea Aquarium (12°04′59″N, 68°53′44″W) (Fig. 1(a)). In 2010, a land-based facility to rear corals and their larvae was built at this site, consisting of five individual, flow-through aquaria (acrylic, 215 × 69 × 64 cm, L × H × W). Two centrifugal pumps (Hayward Super-pumps, 1.5 HP, NJ, USA) pumped seawater through a 100 m long polypropylene pipe (Ø10 cm) with an off-shore intake at 7 m depth through each aquarium at a rate of ∼2300 L hr^{-1}. The continuous pumping of seawater ensured that the water temperature in the five aquaria followed natural fluctuations in sea surface temperatures (SST). Seawater entering each aquarium was first filtered through a bag filter (200 μm, Pentair Aquatic Eco-Systems Inc.) to reduce the accumulation of sediments and debris in the tanks. Filters were cleaned every two days. Additional water movement in each aquarium was provided by a recirculation pump (∼1823 L hr^{-1}, Sweetwater® High-Efficiency Pump, FL, USA, SHE 2.9). All tanks were placed under a UV-permeable acrylic (70% UV transmission, Solacryl SUVT, Spartech Polycast) roof to expose corals to natural light/dark cycles. Every week, sediments and algae were manually removed from the aquaria throughout the study period. Algal growth was also suppressed by juvenile doctorfish (*Acanthurus chirurgus*, <5 cm total length, ∼10 per aquarium) and blue-legged hermit

crabs (*Clibanarius tricolor*, ∼100 per aquarium). Visual assays at the beginning of the study confirmed that these species did not interact with or fed on coral recruits.

2.2. Gamete collection, larval rearing and settlement

A. palmata is a hermaphroditic broadcasting coral species that releases gametes once or twice a year in the fall (Szmant, 1986; Fig. 1(b)). Three days after the full moon in August 2012, we collected egg–sperm bundles from four colonies between depths of 1–5 m. The colonies spawned 3.5 hrs after sunset and gametes were collected using cone-shaped nylon nets, in which the floating egg-sperm bundles concentrated at the top into a removable 50 mL Falcon tube. Collected bundles were immediately transported to the lab, and after they had broken apart, we mixed sperm and eggs from all colonies in one 2.0 L plastic bowl (Sterilite). We added filtered seawater (GF/F) to obtain a sperm concentration of $\sim 10^6$ cells/mL following Hagedorn et al. (2009). Fertilization was allowed to take place for 1.5 hrs after which we rinsed eggs and embryos twice over a 100 μm plankton mesh with filtered seawater (GF/F) to remove excess sperm. We then transferred the embryos to specially-designed kreisels that were used as larval rearing devices (Hagedorn et al., 2009; Fig. 1(c)). These kreisels consisted of 18 L heavy-walled polyethylene drum funnels (Scienceware, USA) with four 13.0 × 4.5 cm nylon screen mesh (240 μm) covered openings at their undersides. The kreisels were placed at the surface of the aquaria described above and stable temperatures (28–29 °C) and salinities (∼35 ppt) within each kreisel were ensured through continuous water exchange with the aquarium water. An adjustable upward-directed water flow was generated from the bottom center of the kreisels by a submersible water pump (Danner Manufacturing Magnetic Drive Pump, 700 gph, model 7, USA) to ensure that developing embryos were distributed evenly throughout the kreisels. Four adjustable water spigots were mounted around the side of each kreisel to create a rotating water flow that prevented developing embryos from sticking to the kreisel walls. Each kreisel contained 15 L of seawater and water inside each was refreshed at approximately 2 L min^{-1}. During the early stages of embryo development till the end of gastrulation water flow was kept lower (1 L min^{-1}) to maintain embryo integrity (Heyward and Negri, 2012). Embryo density in the kreisels was kept low (600–700 embryos L^{-1}) to prevent the build-up of bacteria that thrive on the substances released (mainly lipids) by dying embryos and larvae. The percentage of successfully fertilized eggs was determined 3h after fertilization by quantifying the proportion of eggs going through cell divisions. Three days post-fertilization, we transferred all larvae to four plastic containers (36 × 31 × 24 cm, L × W × H, Sterilite) to allow larvae to settle. Each container contained ∼23 L of filtered seawater (50 μm) and 80 clay pottery tripods (Fig. 1(d): Ø6 cm, Carl Jaeger Tonindustriebedarf GmbH, Germany). The tripods provide different surface orientations for settlement, and their low height (Ø0.8 cm) ensures that growing recruits can attach to the reef substrate early on. The tripods had been cured for two months in the aquarium system to allow the development of biofilms known to induce larval settlement in corals (Ritson-Williams et al., 2010). Water inside the settlement bins was exchanged daily (75%) to maintain water quality and kept at 28–29 °C by partly submerging the bins inside the culture aquaria. Airlifts placed at opposite corners of the containers ensured water movement inside the settlement containers. Approximately 2500 larvae were added to each settlement bin and allowed to settle for five days after which we sub-sampled 80 tripods and assessed settlement rates under a blue light (Nightsea, MA, USA). All tripods with ≥1 settler (henceforth described as a recruit–substrate unit; RSU) were placed inside one of the large flow-through aquaria to allow further development.

2.3. Outplanting experiment

To assess the effect of shortened grow-out periods, we compared the survival and growth of settlers transferred to the reef at the age of two weeks (Fig. 1(e)) to that of settlers raised in the land-based nursery over a period of 2.5 years. To minimize potential confounding effects of density dependent processes (Edwards et al., 2015; Suzuki et al., 2012), we only used RSUs with roughly similar settler densities (mean settler density 11.1 per substrate unit; SD 4.4). We recorded the exact location of each settler on 60 RSUs of which 30 were randomly assigned to the reef and 30 to the land-based nursery. This allowed us to track the initial settlers during subsequent surveys and identify *A. palmata* recruits that could have recruited naturally to the RSUs after the initial outplant. We transported the RSUs to the reef in Ziploc bags and attached them with zip-ties at 50 cm intervals to three pre-installed nylon ropes (5 m) at approximately 2.5 m water depth. The tripods were attached so that the side facing up during the settlement experiment was also facing upwards on the reef. Tripods were not permanently fixed to the reef so that they could be returned to the laboratory to assess the survival rates of all known settlers after one month. Hereto, we carefully detached the tripods by cutting the zip-ties and quantified the number of surviving *A. palmata* settlers *ex situ* under a dissecting microscope, after which all RSUs were returned to their original locations on the reef. The RSUs kept in the land-based nursery were assessed similarly. We quantified the proportion of live settlers after 6, 11, 17, and 31 months *in situ* because settlers had grown sufficiently large (>3 mm) to be counted by the naked eye. In addition to recruit survivorship, we calculated the proportion of substrate units that still harbored ≥1 settler (i.e., represented a RSU) through time for both treatments. We also calculated the proportion of the total number of settlers that died between surveys. Recruit size was measured after 17 and 31 months by photographing individual colonies in planar view against a ruler for scale so that their size could be measured using the imaging software ImageJ (Abramoff et al., 2004).

Table 1
Overview of the monetary costs to produce 4000 recruit–substrate units (RSUs) separated by the costs for nursery maintenance costs, larval rearing and outplanting/monitoring.

	Specifications	Quantity	Cost per item[a]	Total cost[a]	Percent
Nursery maintenance costs (per year)					
Labor[b]		*(hours)*			
Maintenance	1 aquarist, 2 hrs/day, 365 days	730	10.5	7665	60%
Repairs	1 construction worker, 2 hrs/mo, 12 mo	24	8.75	210	2%
Utilities					
Electricity & (fresh) water				5000	21%
			Total	12 875	100%
			Cost per month	*1073*	
Larval rearing costs					
Labor		*(hours)*			
Gamete collection	4 divers, 3h/day, 4 days	48	10.5	504	6%
Rearing work	2 aquarists, 8h/day, 10 days	160	10.5	1680	19%
Materials		*0*			
Gamete collection	SCUBA gear	4	400	1600	18%
Gamete collection	SCUBA air tanks	16	5	80	1%
Gamete collection	Nylon nets	30	15	450	5%
Larval culture	Kreisels	20	80	1600	18%
Settlement substrates	Tripods	4000	0.6	2400	27%
Consumables	Various (e.g., pipettes, filter bags, plastic containers)	1 (order)	500	500	6%
			Total	8814	100%
Outplanting[c] and monitoring[d]					
Labor		*(hours)*			
Rope installation	2 divers, 3 dives/day, 6h/day, 9 days	108	10.5	1134	18%
RSU outplant	2 divers, 3 dives/day, 6h/day, 14 days	168	10.5	1764	28%
Monitoring	2 divers, 3 dives/day, 6h/day, 6 days	72	10.5	756	12%
Materials		*(number)*			
Rope installation	SCUBA air tanks	50	5	250	4%
Rope installation	1000 m nylon rope	1	500	500	8%
Rope installation	U-shaped stainless steal nails	3000	0.1	300	5%
RSU outplant	SCUBA air tanks	80	5	400	6%
RSU outplant	Cable-ties	4000	0.25	1000	16%
Monitoring	SCUBA air tanks	36	5	180	3%
			Total	6284	100%

[a] Costs are in US dollars.
[b] Wages are based on standard Curaçaoan allowances at the time the project was started in 2010 (Curaçao GDP per capita is $15,000 Central Intelligence Agency, 2015).
[c] Assuming that one diver secures 20 m of rope to the reef substrate, or outplants 50 RSUs per 1h dive.
[d] Assuming that 10% of the outplants are monitored after 6, 12, and 18 months, and that one diver monitors 50 outplants per 1h dive.

2.4. Cost-effectiveness analysis

The cost-effectiveness of the two restoration methodologies was calculated following Edwards et al. (2010). These costs pertained to (1) nursery maintenance, (2) larval rearing and settling and (3) RSU outplanting and monitoring (Table 1). The costs to build our land-based nursery facility amounted to US$52,100.00 (see Appendix A) but were not included in the cost-effectiveness analysis. Even simple flow-through aquarium systems such as the one we used are not essential for mass culture of coral larvae. Equal larval rearing success has been achieved using low-technology equipment such as plastic containers filled with static filtered-seawater (Edwards et al., 2010, Guest et al., 2014; Villanueva et al., 2012). If the bottom of each aquarium would have been maximally covered, the maximum number of RSUs that could be produced in our experimental setup was 4000. We used this number in our cost-effectiveness calculations assuming that the full capacity of our nursery system would normally be used to support large scale restoration efforts. We calculated the fraction of the initial 4000 RSUs that would remain after 1, 6, 11, 17, and 31 months assuming similar survival rates observed for the RSUs that were outplanted to the reef and those kept in the nursery system.

The cost-calculations for rearing and settling larvae were identical for both treatments. For RSUs kept in the nursery for 2.5 yrs, the monthly maintenance costs of the nursery were included in the costs to produce one RSU. For RSUs that were outplanted on the reef two weeks after settlement, only one month of nursery maintenance was included to account for its use during initial larval rearing and settling. All 4000 RSUs were simultaneously outplanted two weeks after settlement resulting in a fixed one-time cost. The cost of outplanting one nursery-raised RSU at 1, 6, 11, 17 and 31 months was set to 1/4000th of the costs to outplant 4000 RSUs to the reef at once. This underestimates the true costs of outplanting the increasingly smaller number of available RSUs through time, but is assumed to suffice as an estimate within the context

of our study. Finally, we performed a cost breakdown analysis to identify which elements contributed most to the total production costs for 2.5 year old RSUs reared on the reef and in the nursery.

2.5. Statistical analysis

We used a maximum likelihood (ML) approach to test if settler survival rates differed between the two treatments. We assumed that the probability of survivorship for individuals that settled on the same tile was a function of experimental conditions, but that survivorship of each individual was independent of the fate of neighbors on the same substrate unit. As such, we assumed that tile-specific survivorship (i.e., proportion of surviving settlers relative to the starting density) was binomially distributed. We assumed that survivorship within each treatment was determined by a single parameter, s_x, such that the proportion of settlers surviving at the end of the experiment was binomially distributed around the expectation, $n_{j.x}s_x$, where n_{jx} is the initial density on tile j exposed to treatment x. The best-fit values of all distinct parameter combinations were estimated and the best combination of parameters was selected using Akaike's Information Criteria (when the number of parameters was different) and based on an assumption of equal Bayesian prior expectations (when the number of parameters was the same). See Hilborn and Mangel (1997) or Vermeij and Sandin (2008) for more details on this statistical approach.

Differences in the probability that one substrate unit still harbors at least one settler through time (i.e., proportion of remaining RSUs) between the reef and the nursery were tested with Fisher's exact test. Because settler mortality rates (i.e., the average proportion of settlers dying per month) and size data did not meet the assumption of a normal distribution (Shapiro–Wilk, $p < 0.05$), we tested for differences in growth and mortality rates between corals reared *in situ* and those kept in the nursery using a one-way non-parametric analysis of variance (PERMANOVA; Anderson, 2001) in PAST 1.97 (Hammer et al., 2001).

3. Results

3.1. Outplanting experiment

80% of the collected eggs were fertilized, resulting in a total of approximately 10 000 *A. palmata* larvae three days after spawning occurred. Approximately 4000 larvae were placed in the settlement containers and settled on 320 tripods at an average density of 12.5 settlers per tripod ($SD = 9.7$; range; 1–41; $n = 80$ tripods). Settlement most commonly occurred on the undersides of the tripods (79.6%; $SD = 18.4$; $n = 80$ tripods). During the first month after outplanting, the settlers returned to the reef at the age of two weeks showed 15% lower survival than those kept in the nursery (ML: $p < 0.05$), but their survival was 3%–9% higher for each subsequent time point (ML: $p < 0.05$) (Fig. 2(a)). As a result, the average survival of *A. palmata* settlers after 31 months was 6.8 times higher for settlers grown on the reef (3.4%; $SE = 1.3$) than for those kept in the nursery (0.5%; $SE = 0.5$). Outplanting recently settled larvae also resulted in 10 times more RSUs that could be used for restoration purposes (Fig. 2(b); Fisher's exact test: $p < 0.05$), as 32% of the tripods on the reef still harbored at least one recruit after 31 months versus only 3% in the land-based nursery.

Mortality rates in our land-based nursery gradually decreased through time, whereas mortality rates of settlers on the reef increased or decreased between subsequent surveys (Fig. 2(c)). The highest mortality rate for settlers returned to the reef at the age of two-weeks occurred during the first month after outplanting, during which 34.2% (SE 4.2) of the settlers died (Fig. 2(c)). The survival rate of larvae that had settled on the undersides of the tripods was twice as high during this month (72.0%; SE 5.1) than that of individuals that had settled on the topsides of the tripods (35.2%; SE 7.9) (ML: $p < 0.05$). Mortality rates of outplanted settlers remained high during the following five months after which 15.3% (SE 2.5) of the settlers were still alive (Fig. 2(a) and (c)). Mortality rates rapidly decreased thereafter and remained low (average monthly mortality rate: 2.8%; SE 1.2; Fig. 2(c)) until settlers were 11 months old. Mortality rates increased between 11 and 17 months during which 8.6% of the remaining settlers died every month on average (SE 1.6; Fig. 2(a) and (c)). After 17 months mortality rates declined again and remained low until the end of the study (Fig. 2(c)).

Settlers in the nursery experienced equal or higher mortality rates compared to outplanted settlers, with the exception of the first month after settlement when mortality rates of settlers kept in the nursery were 1.8 times lower than those of outplanted settlers (PERMANOVA: $p < 0.01$). Mortality rates of 6–11-month-old settlers were 4.4 times higher in the nursery than on the reef (Fig. 2(c)), which resulted in a threefold reduction in both the number of live settlers and number of remaining RSUs in the nursery compared to the reef after 11 months (Fig. 2(a) and (b)). Higher mortality in the nursery during the first year of this study thus caused the resulting lower number of surviving settlers and RSUs in this treatment after 11 months until the end of the study, despite the fact that mortality rates on the reef and in the nursery were similar after 11 months.

The size of settlers outplanted to the reef and those kept in the nursery did not differ after 17 and 31 months (PERMANOVA: 17 mo, $p = 0.81$; 31 mo, $p = 0.61$). After 31 months, settlers on the reef measured 16.7 cm^2 (SD 20.1; $n = 9$) whereas the only individual remaining in the nursery measured 12.9 cm^2. After 31 months, 6 out of the 9 remaining colonies on the reef had started forming upright branches that were 2.85 cm (SD 1.44; $n = 6$) tall on average. After 17 months, two out of 17 settlers growing on tripods had attached to the reef substrate and this number increased to three out of nine after

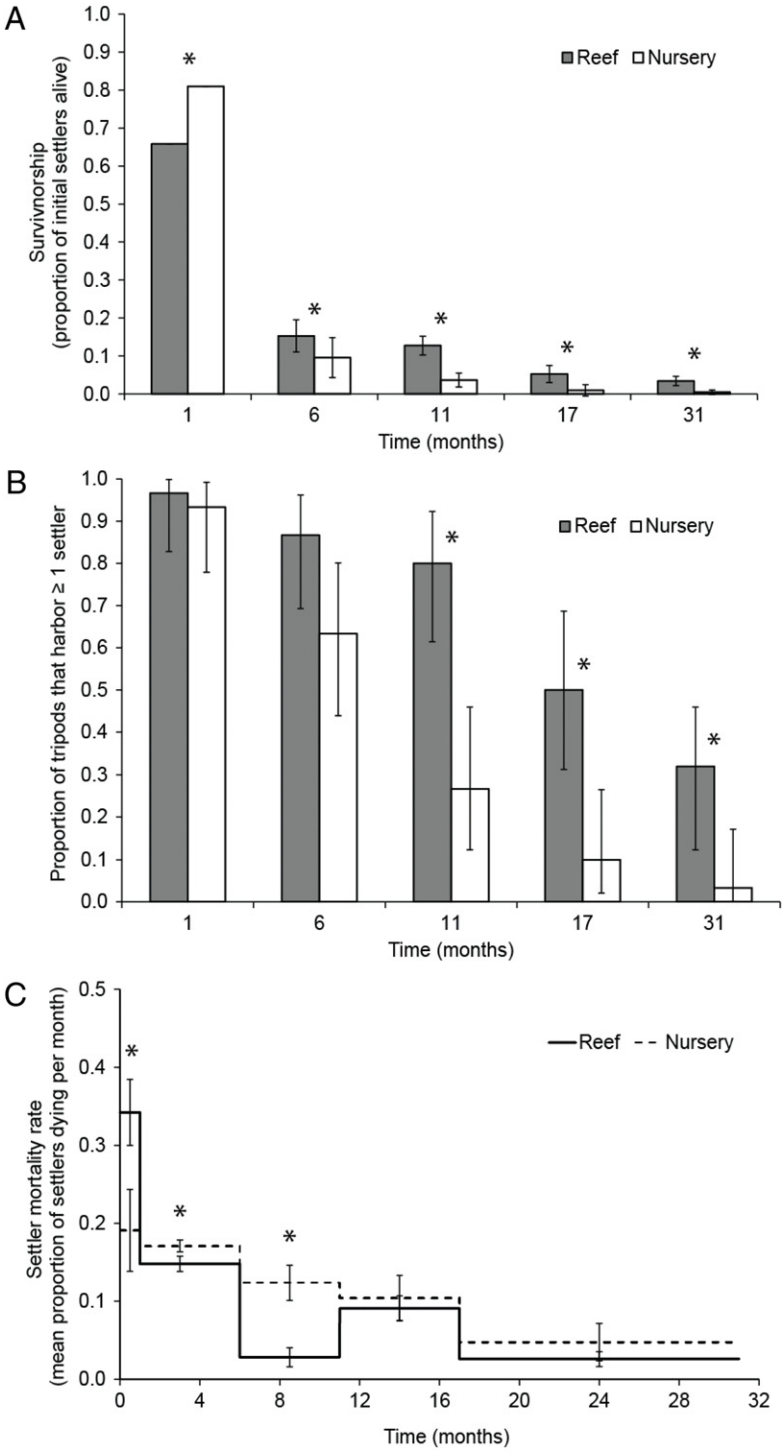

Fig. 2. (A) Proportion of *A. palmata* settlers alive on the reef and in the land-based nursery after 1, 6, 11, 17, and 31 months. Error bars represent standard error and asterisks indicate statistically distinct groups as determined by a maximum likelihood analysis with $p < 0.05$ ($n = 30$ substrate units); (B) proportion of substrate units harboring at least one settler (proportion of remaining RSUs) on the reef and in the land-based nursery after 1, 6, 11, 17, and 31 months. Error bars are 95% confidence intervals (Clopper–Pearson's exact method) and asterisks indicate statistically different groups (Fisher's exact test of independence with $p < 0.05$) ($n = 30$ substrate units); (C) settler mortality rates on the reef and in the nursery throughout the 31 months study period. The proportion of settlers that died between surveys was standardized to a monthly mortality rate. Error bars represent standard error and asterisks indicate statistically different groups (PERMANOVA with $p < 0.05$) ($n = 30$ substrate units).

Table 2
Comparison of the cost-effectiveness and total cost breakdown for RSUs outplanted to the reef two weeks after settlement versus RSUs kept in the nursery for extended periods of time.

	Time (in months)	0	1	6	11	17	31
Nursery							
	Remaining RSUs	*100%*	*93%*	*63%*	*27%*	*10%*	*3%*
	Cost per RSU[a]	3.5	3.9	7.4	21.3	71.3	324.6
Reef							
	Remaining RSUs	*100%*	*97%*	*87%*	*80%*	*50%*	*32%*
	Cost per RSU[a]	4.0	4.2	4.7	5.1	8.1	12.6
	Cost breakdown[a] (for a 31-mo-old RSU)	Nursery maintenance		Larval rearing		Outplanting & monitoring	
Nursery		79.2%		20.3%		1.0%	
Reef		6.6%		54.5%		38.9%	

[a] Costs are in US dollars.

31 months. Because tripods were attached to a rope rather than directly attached to the substrate, most of the tripods were not entirely in contact with the reef substrate and could move, albeit slightly, on the rope, especially during storms. This likely also explained the loss of five tripods that had detached from the ropes between 17 and 31 months. None of the recruits attached to the aquarium surface in the nursery because the tripods had to be regularly moved for aquarium maintenance.

3.2. Cost-effectiveness analysis

At maximum capacity our system holds 4000 RSUs, of which 133 (3.3%) would still have $\geq$1 settler after 31 months based on the data from our experiment (Table 2). The number of remaining RSUs on the reef exceeded that in the land-based nursery almost 10-fold (32.0%) at the end of the 2.5 yr study period (1280; Table 2). Combining these data with the costs overviewed in Table 1, a 2.5-year-old RSU reared on the reef cost $13 USD, whereas rearing one RSU in the nursery for the same period of time cost $325 (Table 2). The nearly 30-fold higher costs to produce one nursery reared RSU mainly resulted from the costs associated with operating the nursery system, which accounted for 79% of the total costs of producing RSUs in the nursery (Table 2). Larval rearing and outplanting contributed 55% and 39% to the total costs per RSU for RSUs that were returned to the reef two weeks after settlement (Table 2). Purchasing tripods accounted for almost one third of the rearing costs (27%), while manually outplanting 4000 RSUs (i.e., air tank rental and labor) accounted for 57% of the total outplanting costs (Table 1).

4. Discussion

This is the first study to report successful outplanting followed by long-term survival and growth of *Acropora palmata* settlers reared from gametes. Our results show that *A. palmata* larvae can be settled and outplanted, and that post-settlement survival rates were sufficiently high that, if produced in greater numbers, RSUs can be used for restoration purposes. One third of the outplanted RSUs harbored more than one juvenile colony after 2.5 years (Fig. 2(b)) despite high settler mortality during the first six months (86%; Fig. 2(a)). While seemingly high, mortality estimates for *A. palmata* in this experiment were similar to mortality rates for presumably more robust Indo-Pacific *Acropora* species (Edwards et al., 2010; Guest et al., 2014) and exceeded natural recruitment of *A. palmata* which currently approaches zero relative to historical baselines (van Moorsel, 1989; Vermeij et al., 2011).

The geometry of the tripods (Fig. 1(d)), which allowed for settlement on undersides, appears to be an important element contributing to the long-term survival of the outplanted *A. palmata* settlers. The undersides of artificial settlement substrates are slowly colonized by cryptic communities that act as better refuges for coral settlers during the initial successional stages of these artificial substrates (Raimondi and Morse, 2000; Vermeij, 2006) compared to the upper surfaces. These more exposed upper surfaces are rapidly colonized by turf algae (Fricke et al., 2011) known to impair survival and growth in young corals (Babcock and Davies, 1991; Babcock and Mundy, 1996; Fabricius, 2005; Miller, 2014; Smith et al., 2001). Cryptic habitats such as the tripods' undersides represent only a relatively small proportion of the total surface provided by settlement substrates in previous studies, and/or settlers preferring this habitat were killed when those substrates were attached to the reef (Guest et al., 2014; Miller, 2014; Nakamura et al., 2011; Petersen et al., 2005; Villanueva et al., 2012). In contrast, in this study the thin legs of the tripod substrates allowed older settlers on the cryptic undersides of the tripods to grow onto the exposed upper surfaces where their survival and growth benefited from higher light levels (Babcock and Mundy, 1996; Maida et al., 1994; Miller, 2014). These observations illustrate the importance of including cryptic microhabitats into the design of artificial settlement substrates used for restoration purposes.

A. palmata recruits on the reef suffered unexpectedly high mortality sometime between 11 and 17 months post-outplanting (Fig. 2(a), (b)), most likely caused by high wave action. Because tripods were attached to a rope instead of permanently secured to the reef, growing recruits could not attach to the reef substrate as most tripods were not in full

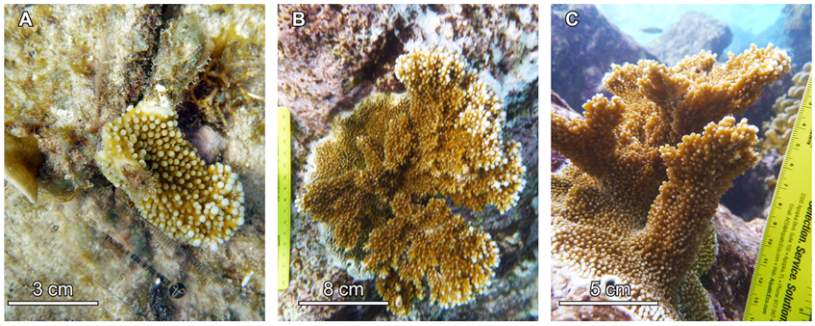

Fig. 3. Juvenile *A. palmata* colonies outplanted at the Curaçao Sea Aquarium reef: (A) example of the 2.5-year-old colonies that were attached to a nylon rope in this study and which were ∼20 times smaller than (B–C) equally old colonies that were secured to an artificial breakwater with epoxy. Photo credits: V.F.C.

contact with the reef framework (Fig. 1(f)) and moved back and forth during high wave action. The importance of firmly stabilizing outplanted recruits is confirmed by our observations from a simultaneously conducted experiment, whereby one-year-old *A. palmata* recruits were permanently stabilized onto an artificial breakwater consisting of large limestone boulders with epoxy (Star Brite marine epoxy putty, USA). Here, all recruits had overgrown the tripods after only six months, and after 1.5 years colonies had higher growth (∼20 times) and survival rates (67% after 2.5 years; $n = 9$) compared to equally old settlers used in this study (V.F.C and D.P., unpublished data; Fig. 3). Combined, these observations indicate that securing RSUs to the reef substrate would have improved the effectiveness of our current study.

Land-based nurseries are generally assumed to offer stable and more protected environments for coral settlers relative to actual reef environments due to reduced fish predation, algal competition, and sedimentation (Nakamura et al., 2011). Nevertheless, *A. palmata* settlers that were transferred to the reef shortly after settlement survived 6.8 times better compared to settlers that were kept in our land-based rearing facility. Petersen et al. (2006) also found that the success of rearing *Acropora* recruits in aquaria is highly unpredictable due to the occurrence of unforeseen and uncontrollable changes in (a)biotic factors. Diseases, predators and parasites can reach unnaturally high abundances in confined aquarium environments when their natural enemies or environmental controls are absent (Borneman, 2008; Petersen et al., 2006). In our nursery tanks, *A. palmata* recruits suffered from unexpected forms of predation and competition. A sudden ciliate infection caused ∼25% of one-year-old colonies to die within 48h and the number of anemones and colonial hydroids occasionally explosively increased resulting in overgrowth and subsequent mortality of settlers in our nursery tanks (V.F.C., personal observation). Additionally, the trade winds that normally cooled our nursery ceased to blow in October 2012 coinciding with the annual maximum in seawater temperature (29.5 °C; NOAA Coral Reef Watch, 2000). This caused water temperatures inside the nurseries to rise to 31 °C. While this temperature is not directly lethal for *A. palmata* (DeSalvo et al., 2010; Polato et al., 2013) settlers did experience suboptimal temperature conditions for one month. Lastly, *A. palmata* typically occurs in areas with strong wave action (Bak, 1975) and the growth rate of *Acropora* increases with increased water flow (Nakamura and Yamasaki, 2005). Rearing species such as *A. palmata* that require highly hydrodynamic environments may therefore be challenging in aquarium systems where high wave action is difficult or impossible to generate. In sum, the combined effects of unforeseen stressors (diseases, competition, and physical factors) likely explain the lower survival rates observed in our land-based nursery compared to that of settlers that were immediately outplanted to the reef. Extended grow-out periods in land-based nursery facilities therefore do not guarantee that more coral recruits can be reared for restoration purposes compared to much cheaper *in situ* approaches.

Our cost-effectiveness analysis demonstrated that rearing coral offspring in land-based facilities over extended periods of time was expensive and consequently economically unviable. Keeping one RSU in our nursery for 2.5 years cost $325, of which 79% covered the operational costs of the nursery (Table 2). These estimates are in the same order of magnitude as those reported by Nakamura et al. (2011) who calculated that producing one 10-mo-old RSU reared in an outdoor nursery cost $163 (*A. tenuis*). Shortening grow-out periods from 2.5 years to two weeks significantly lowered the costs of producing *A. palmata* RSUs. After 2.5 years, a RSU on the reef cost $13 compared to $325 for those kept in the nursery. The cost breakdown analysis showed that the cost-effectiveness of restoration methods could be further improved by using cheaper settlement substrates and more efficient outplanting methodologies. In the end, the cost at which restoration efforts can be considered affordable is extremely subjective. The estimated costs to restore one hectare of degraded reef are extremely variable and range from US$13,000 to transplant fragments in Tanzania to millions of US$ per hectare to restore a ship grounding site in the Florida Keys (Spurgeon, 2001). The resources that one nation or organization is willing to allocate to restoration and the spatial extent of planned restoration efforts will ultimately define the economic viability of restoration practices on a case-by-case basis (Spurgeon, 2001).

Shortening grow-out periods for *A. palmata* settlers shortly after settlement not only reduced costs but also yielded 10 times more RSUs that could be used for restoration, though we stress that such findings will depend on the quality of both the aquarium system and the reef chosen for outplanting. Nevertheless, exposing two-week-old *A. palmata* settlers to natural reef conditions at our study site did not negatively impact their survival and increased the long-term success of

this restoration approach (Miller, 2014; Ritson-Williams et al., 2010). While post-settlement survival was relatively high in our study, the factors determining post-settlement survival differ across space (Vermeij, 2006) so that the success of restoration efforts can be expected to differ among locations. To illustrate, less than 10 out of several hundred *A. palmata* settlers that were outplanted in the Florida Keys shortly after settlement survived beyond one year (M.W. Miller and A. M Szmant, personal communication). Two of these settlers reached the age of seven years before they succumbed to a disease outbreak (M.W. Miller and A. M Szmant, personal communication). While the aforementioned study did not employ a fundamentally different approach from the one used here, local reef conditions are likely an important determinant of local restoration success. Reef communities on Curaçao are less degraded than those in the Florida Keys (Jackson et al., 2014) which likely explains why, despite the use of largely similar techniques and approaches, survival of outplanted *A. palmata* reared from sexually produced larvae on Curaçao is higher than in the Florida Keys.

5. Conclusions

Our findings show that the rearing of sexually produced larvae is possible for Caribbean coral species despite the fact that previous attempts to raise and outplant settlers of the critically endangered *A. palmata* colonies have been effectively unsuccessful. The combination of novel rearing techniques (kreisels and settlement substrates providing a variety of microhabitats), outplanting of recently settled individuals and suitable conditions at our experimental site likely underlie the results obtained in this study. While encouraging, outplanting sexual coral recruits will not "restore a reef" by itself and requires that other causes of degradation are minimized at the restoration site prior to outplanting (Mumby and Steneck, 2008). Nonetheless, our findings, combined with other case studies (Guest et al., 2014; Nakamura et al., 2011; Omori et al., 2008; Villanueva et al., 2012) show that if applied on larger scales and in combination with other management tools such as fishing quotas, coastal protection, and pollution regulations, sexual coral propagation or "assisted recruitment" could contribute to restoring tropical reef communities in the future.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.gecco.2015.10.005.

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