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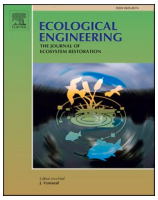

Enhancing survival of ex-situ reared sexual recruits of *Acropora palmata* for reef rehabilitation

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ABSTRACT

Acropora palmata is one of the major reef-building coral species in the Caribbean. The species has suffered drastic declines in abundance and sexual recruitment over the past decades. One method for active rehabilitation of *A. palmata* reefs is by assisting the production of sexual recruits under controlled lab conditions. Within this study, the effect of different aquaculture regimes and culturing periods on the survival rates of these recruits was investigated. In August 2016, coral spawn was collected on a reef nearshore New Providence, Bahamas, cross-fertilized, and reared in mobile laboratory facilities from Van Oord Dredging and Marine Contractors. Larvae were settled on pre-conditioned aragonite plugs.

Sexual recruits were cultured under four different aquaculture conditions: ambient vs. high Total Alkalinity (TA) (~2.8 mEq L^{-1} vs. 4.8 mEq L^{-1}) and with vs. without feeding *Artemia* nauplii. Recruit size was monitored by tracking living tissue area and the number of polyps of a subset of recruits. Plates with recruits were outplanted to a nursery on the reef after 4, 9 and 14 weeks of aquaculture. Survival was determined during the aquaculture phase (at 4, 9 and 14 weeks after settlement), and after outplanting (at 27 and 44 weeks after settlement).

During the aquaculture phase, survival was significantly lower in seawater with increased TA compared to ambient seawater conditions. The average number of polyps per recruit was significantly higher in the treatments with feeding. After outplanting to the reef, both survival and recruit size were highest in the feeding treatments. The most successful aquaculture treatment in this study was a combination of increased TA and feeding during 9 weeks of aquaculture, which resulted in a doubling of survival and recruit size at 10 months after settlement compared to ambient conditions. Ambient conditions did not enhance survivorship nor recruit size at 10 months after settlement, as compared to the other aquaculture treatments. Nevertheless, the success of ambient aquaculture conditions exceeded natural conditions, as no natural recruitment of *A. palmata* was observed in this study. We conclude that feeding during and ex-situ culture period enhances ex-situ growth rates and in situ recruit survival of *A. palmata* juveniles. No positive effects of the aquaculture treatment with only increased TA were found. Building on these results, recommendations are provided for future reef rehabilitation efforts using ex-situ rearing of sexually reproduced *A. palmata* recruits.

1. Introduction

Worldwide, coral reefs are threatened by increasing anthropogenic stress (Hoegh-Guldberg et al., 2007; Hughes et al., 2018) resulting in the decline of reef-building coral populations and live coral cover. Attempts to restore coral populations using asexually produced coral fragments (Shafir et al., 2006; Rinkevich, 2014) have been made to increase live coral cover, biodiversity and/or topographic complexity (Edwards and Clark, 1999). These efforts have limitations that they do not enhance the genetic diversity of a population until the fragments reach sexual

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maturity. Although nursery-grown asexual coral fragments have recently been reported to spawn (Carne and Baums, 2016), recruitment success is often impaired, in particular in the degraded reefs that are targeted for restoration. In addition, the potential to successfully produce offspring depends on the genetic diversity and size of the source population (i.e. overcoming fertilization constraints) (Isomura et al., 2013; Miller et al., 2016, 2018). Successful sexual reproduction (i.e. larval supply) and recruitment (i.e. post-settlement survivorship) are essential to increase the prospects of coral reefs, as genetic diversity is important for evolutionary adaptation to environmental stress. Therefore, restoration efforts should promote genetic diversity among the restored coral populations. The production of sexual recruits under controlled lab conditions could address this. By alleviating some of the early life stage bottlenecks that are likely playing a role in recruitment limitation (e.g. Ritson-Williams et al., 2009; Van Koningsveld et al., 2017), fertilization success, settlement success and post-settlement growth and survivorship can be significantly enhanced.

Significant advances have been made in the techniques for sexual coral restoration, ranging from the knowledge of spawning timing and collection of gametes to the settlement of larvae on suitable substrates (Edwards, 2010; Chamberland et al., 2015). However, not much is known about the optimal conditions for improving growth and survival of recruits in aquaria and their increased survival once outplanted at the reef. As mortality rates of recruits seem to be inversely related to their size (Vermeij and Sandin, 2008; Doropoulos et al., 2012), efforts to enhance recruit size before outplanting to the reef could enhance restoration success. Indeed, longer grow-out periods of sexually propagated corals in protected in-situ nurseries significantly increased survival when outplanted to the reef and this increased survival was related with age/size (Guest et al., 2014). As maintenance costs for extended grow-out periods in land-based or ocean nurseries can be costly (e.g. Edwards, 2010; Chamberland et al., 2015), optimizing growth in a shorter time span or at a larger scale could greatly reduce costs and increase the success of restoration efforts.

The current study focuses on enhancing recruitment success of ex-situ reared sexual recruits of the endangered Caribbean coral species *Acropora palmata*. The populations of this once abundant reef-building coral declined tremendously since the 1980's (Rodriguez-Martinez et al., 2014), which has been mostly attributed to white-band disease (Gladfelter, 1982; Aronson and Precht, 2001) in combination with global warming (Muller et al., 2008; Rogers and Muller, 2012). As a result, *A. palmata* has been classified as a threatened species on the US endangered species act, and as a critically endangered species in the IUCN Red List (Aronson et al., 2008). Natural recruitment rates of *A. palmata* are reported to be very low (Quinn and Kojis, 2005; Williams et al., 2008). Hence, efforts to rehabilitate populations of *A. palmata* (Chamberland et al., 2015) have primarily adopted the sexual approach.

In this study, we tested whether the growth and survival of ex-situ reared sexual recruits of *A. palmata* could be enhanced by offering different aquaculture periods (4, 9 and 14 weeks) and aquaculture conditions (feeding *Artemia* nauplii and addition of bicarbonate). Heterotrophic feeding is known to enhance coral growth (Houlbrèque and Ferrier-Pagès, 2009; Toh et al., 2014; Conlan et al., 2017), while the addition of bicarbonate increases calcification and thereby enhances skeletal growth rates (Marubini and Thake, 1999; Marubini et al., 2001; Jury et al., 2010). We determined whether recruit size and post-settlement survival were significantly different between rearing strategies during the aquaculture phase and after outplanting to a field-based nursery.

2. Material and methods

2.1. Study location and aquaculture facilities

All work involving fertilization, larval rearing, settlement and aquaculture of recruits was carried out at the ReefGuard facilities of Van Oord Dredging and Marine Contractors (Van Koningsveld et al., 2017), set up at the military base of the Bahamian Royal Defence Force at Coral Harbour, New Providence Island, Bahamas (24°58′55.3"N, 77°28′13.5"W). The ReefGuard is a mobile coral-breeding laboratory that can be operated anywhere in the world.

The outdoor aquarium system contains four culture basins of 1500 L and four of 750 L, located in a white tent transmitting 5% of ambient light. Seawater was filtered to 1 μm, UV-sterilized and circulated in a thermostat-controlled 6000 L basin. From there the rearing basins were supplied with a constant flow of filtered seawater (FSW). Water temperature was kept at 28 °C. Daily partial water changes were approximately 10%.

The ReefGuard also includes an indoor aquarium system that contains 3 independent basins of 380 L, each with separate protein skimmer and UV sterilizer. Daily partial water changes with FSW from the main filter container corresponded to approximately 11% of total volume.

2.2. Gamete collection, fertilization and larval culture

Gamete bundles were collected in situ on a healthy *A. palmata* reef near Salt Cay Island, Bahamas (25°6′0.684"N, 77°15′52.092"W) under Permit No. MAMR/FIS/17 for Coral Aquaculture and to Conduct Marine Scientific Research, issued on 22 October 2015 by the Department of Marine Resources for the Minister of Agriculture, Marine Resources and Local Government of the Bahamas. Three teams, each consisting of two divers and one snorkeler, were deployed to monitor 3–4 spatially separated stands of *A. palmata*. Spawn was collected using nets (mesh size 100 μm) with removable collector cups from at least 7 different colonies. Gamete bundles were carefully pipetted off the water surface inside the cups, while avoiding small predators and dirt. They were gently mixed inside a 60 L bin while adding FSW and keeping a visually optimal sperm density (10^5–10^6 sperm mL^{-1}, Oliver and Babcock, 1992). As more gamete bundles and FSW were added, spawn was distributed over two bins. After 30–60 min of spawn collection, the bins were transported to the ReefGuard facilities (1 h drive) while being gently mixed. The fertilization procedure ended 3 h after start of fertilization. Six replicate samples were counted under a stereo microscope to determine the number of fertilized eggs in each bin. Fertilization success was 90.3% ± 0.1SD ($n = 6$). The fertilized eggs were placed in two large culture basins of 1500 L and 750 L at a stocking density of approximately 182 and 287 embryos per liter (rep. 273,240 and 215.400 embryos per culture basin). Larval development was monitored every 4 h for 2 days and fatty residues were skimmed off the water surface when required using plastic foil or paper tissues. Gentle aeration was started at 4 days after fertilization to keep the seawater oxygenated while producing minimal agitation. Average temperature throughout the larval culture period was respectively 28.2 ± 0.6 °C and 27.7 ± 0.7 °C.

2.3. Larval settlement

Approximately 40 thousand aragonite plugs (22.2 mm crown with a 9.5–12.7 mm base) were pre-conditioned on a reef near Coral Harbour (24°58′48.9"N, 77°30′2.592"W) 2.5 months prior to the spawning event, allowing the build-up of a biofilm containing suitable cues for coral settlement (Heyward and Negri, 1999). About a week before spawning, they were recovered and cleaned from sediment and macrobenthic organisms with a high-pressure cleaner using seawater. Until their use for larval settlement, they were kept in aerated 60 L bins filled with FSW to preserve their crustose coralline algae and biofilm. Seawater was partially refreshed daily (ca. 75–85% of water volume).

2.3.1. Ex-situ settlement

Larvae were considered competent for settlement at 4 days after fertilization, when the majority of larvae started moving mid-water column. A total of 108 grey PVC plates (40 × 30 × 2 cm) with 99 holes (9 mm diameter) were filled with the pre-conditioned aragonite

plugs. The plates were stacked into 27 bins (4 plates each) separated by ~8 cm spacers. The upper plate accommodated 99 plugs while the three lower plates accommodated 95 plugs. Each bin was filled with 50 L fresh FSW and provided with aeration. The bins were placed in a lab container where irradiance was between 0 and 1 μmol/m^2/s as measured using a Li-Cor 192 quantum sensor. The 4-day old larvae from the large culture basins were drained through a 100 μm filter and batches were mixed. The total number of settlement-ready larvae was estimated to be 117,500 by means up replicate counts of subsamples under the binocular. Settlement started after adding approximately 4000 larvae to each of the 27 bins (~80 larvae L^{-1}). Temperature, pH and salinity were monitored twice a day. Temperature was maintained at 27.7 ± 0.3 °C, pH at 8.0 ± 0.2 and salinity at 34.4 ± 0.2 ppt. On average, temperature was 28.4 ± 0.3°, pH was 8.0 ± 0.07 pH and salinity was 34.0 ± 0.3 ppt during the 3.5 day settlement period.

After 2.5 days, the seawater was changed in all bins to stop the settlement procedure. Larvae that settled on the top and side of each plug ("exposed settlement") were counted using a BlueStar blue flashlight with yellow filter glasses (NIGHTSEA). Settlement success was calculated per bin (average ± SD per bin, $n = 27$ bins with 4 plates each). On average 59.8% ± 1.8SD of coral plugs within a bin was observed to have at least 1 recruit settled on the exposed part of the plug ("top and side"). The percentage of larvae that settled on plugs per bin was 7.9% ± 0.3SD (n = 27). In total we observed 8577 settled larvae settled on 4669 different aragonite plugs. To provide an estimate for settlement preference ("exposed" vs. "cryptic") also the settlement on the stalk of each plug that was hidden inside the plates ("cryptic settlement") was counted of 6 stacks (24 plates). Within this subsample, "exposed settlement" was 5.7 ± 1.2% and "cryptic settlement" was 9.5% ± 0.3SD (average ± SD per bin, $n = 6$).

2.3.2. In-situ settlement

About a week before spawning, 32 black polycarbonate plates were filled with pre-conditioned aragonite plugs and made into packages consisting of two pairs of sandwiched plates (both facing inwards) that were separated by 6 cm spacers. These packages were placed horizontally on the seafloor at two different reef sites (Coral Harbour and Blue Lagoon, 1308 plugs each). The packages were retrieved about 3 weeks after spawning and checked for recently settled coral larvae using a BlueStar blue flashlight with yellow filter glasses (NIGHTSEA). No natural settlement was detected on the pre-conditioned plugs attached to the polycarbonate plates that were placed at Coral Harbour and at Blue Lagoon.

2.4. Culture of sexual recruits

Coral plugs with settled recruits were redistributed over 54 identical grey PVC plates, making sure that each plate had an equal number of plugs with the same number of recruits. At total of 84 coral plugs were added to each plate, of which 49 had 1 recruit, 20 had 2 recruits, 8 had 3 recruits, 4 had 4 recruits, 2 had 5 recruits and 1 had 6 recruits. Coral plugs were placed on each plate following a pre-calculated randomized layout to enable individual tracking of each coral plug. In addition, one plug with a minimum of 3 recruits specifically designated for growth measurements was added to each plate (Fig. 2). These plugs were omitted from survival analysis as they received more handling.

These 54 plates with recruits were assigned to four different aquaculture conditions (ambient (A), ambient + feeding (AxF), high alkalinity (TA), high alkalinity + feeding (TAxF); see section 2.4.1) in duplicate tanks and for different aquaculture periods (4, 9 and 14 weeks), i.e. six plates per experimental treatment (Figs. 3 and 4).

During the first 4 weeks of aquaculture, all coral recruits were allowed to establish symbiosis in the presence of adult coral fragments (section 2.4.2). After these 4 weeks, the first 6 ambient plates were outplanted to a nursery at the reef (Section 2.5). After 9 weeks of aquaculture, 6 plates from each treatment (3 from each duplicate tank) were outplanted to the reef. After 14 weeks of aquaculture, the remaining 6 plates from each treatment (3 from each tank) were outplanted.

2.4.1. Aquaculture conditions

Temperature, pH and salinity were measured twice a day, 6 days a week using a YSI Pro plus with calibrated sensors: a Pro Series 1001 pH sensor and a 6560 Temperature/conductivity sensor. In addition, temperature in each basin was automatically recorded every 30 min using a TL-G thermologger (Thermodata Pty Ltd). No fouling control was done during the experiment. Light conditions inside the ReefGuard tent were approximately 5% of the natural irradiance levels outside the tent.

2.4.1.1. High total alkalinity (TA). TA was manipulated using Arms & Hammer baking soda ($NaHCO_3$, sodium bicarbonate) and "baked" baking soda (i.e. baking soda that was baked at 200 °C for 1 h, which turns it into Na_2CO_3 or sodium carbonate) in a proportion 4 to 1. TA was measured daily using an Alkalinity Test Kit (Model AL-AP, Hach, resolution ±0.2 mEq L^{-1} using doubled sample size). Ambient FSW had a TA of 2.8 mEq L^{-1}. During the first four weeks of aquaculture, total alkalinity in the TA treatment was kept at 5.6 mEq L^{-1}. Thereafter, TA was kept at 4.8 mEq L^{-1} due to heavy abiotic precipitation. Calcium and magnesium concentrations were monitored using Salifert aquarium test kits (resolution ±20 ppm).

2.4.1.2. Feeding. Coral recruits were fed 3 times a week with micro *Artemia* nauplii (diameter ± 430 μm, Ocean Nutrition) at a concentration of 900 individuals per liter. *Artemia* were hatched for 18 h overnight at 28 °C in 25 ppt, 8.1 pH FSW under dim light. During feeding time, all plates with coral plugs (feeding and non-feeding treatments) were placed in random order for at least 2 h into a bin containing 37 L seawater from the corresponding treatment. Each bin fitted three plates inside that were stacked using 10 cm spacers. Bins were continuously aerated.

2.4.2. Establishment of symbiosis

One hundred coral fragments of ca. 5 cm^2 were collected using a steel bone cutter from 10 spatially separated healthy colonies of *A. palmata* at Elkhorn Garden (25°1′27.12"N, 77°34′22.08"W) under Permit No. MAMR/FIS/17 for Coral Aquaculture and to Conduct Marine Scientific Research, issued on 22 October 2015 by the Department of Marine Resources for the Minister of Agriculture, Marine Resources and Local Government of the Bahamas. They were transported to the ReefGuard facilities in zip lock bags filled with seawater. Fragments were labelled and kept in quarantine in a closed aquarium system under artificial lighting (Aqua Illumination Hydra 26 HD) for 3 weeks until their use as symbiont donors in the experiment.

At the start of aquaculture of the recruits, the fragments were distributed among the 8 experimental tanks containing coral recruits. A period of 3 weeks was given for symbiont uptake, during which the fragments were fed with *Artemia* nauplii to enhance the production and release of symbionts. The establishment of symbiosis was monitored under a binocular stereomicroscope (20×, VisiScope STB250, VWR) 1 and 2 weeks after introduction of the fragments to identify a potential effect of aquaculture condition on the establishment of symbiosis.

After the period for symbiont uptake by the recruits, the fragments were stored until outplanting in the coral nursery (section 2.5). All fragments survived the aquaculture period.

2.5. Outplanting to the reef

A suitable outplanting site was found near Elkhorn Garden (25°1′18.012"N, 77°34′22.764"W) where 6 nursery structures were placed in a sand patch protected by surrounding reef structure. Each nursery structure consisted of 4 vertically placed star pickets (1.5 m

long) connected by 2 horizontally placed stainless-steel rods (2 m long, diameter 1 cm) (Suppl. Fig. 1). The structures were placed within 1 m from the reef base to allow herbivore fish and other reef-associated organisms to visit the structures and consume any growing (macro) algae.

Outplanting of plates with coral plugs took place after 4, 9 and 14 weeks of aquaculture. Each batch of plates were outplanted in a package consisting of one of four plates with coral plugs placed in a random sequence facing a dummy plate, separated by four 10 cm long spacers (Fig. 5). The plug-side of each plate in the package faced the same orientation. Each package was attached to one of 6 outplanting racks and reinforced after tying it to the nursery structure.

2.5.1. Survival during aquaculture and after outplanting

The survival of recruits was determined during the aquaculture phase (at 4, 9 and 14 weeks after settlement), and after outplanting (at 27 and 44 weeks after settlement) (Table 1). Survival was calculated per plate as the percentage of plugs with live recruits (minimal 1) compared to the number of plugs with live recruits at the start of the aquaculture period. Each time, the number of recruits per plug was counted using a binocular stereomicroscope (20×, VisiScope STB250, VWR).

Survival at 4, 9, 14 and 27 weeks was calculated based on 6 replicate plates per treatment. After the counting campaign week 27, all coral plugs with living recruits were randomly redistributed over 4 plates per treatment (instead of 6) and reinstated to 4 outplanting racks at the nursery site. Each of the 4 plates received the same number of plugs. Therefore, at 44 weeks survival was calculated based on 4 replicate plates. To correct for the different number of experimental replicates, the survival over the total experimental period (44 weeks after settlement) was calculated by multiplying the average survival of 0–27 weeks with the average of 27–44 weeks. Data for the different periods were observed to have consistent patterns.

After the last counting campaign at 44 weeks after settlement, the plugs with live recruits were used to stock a permanent Elkhorn coral nursery at Goulding Cay, called the "Coral Engine" (ter Hofstede et al., 2019).

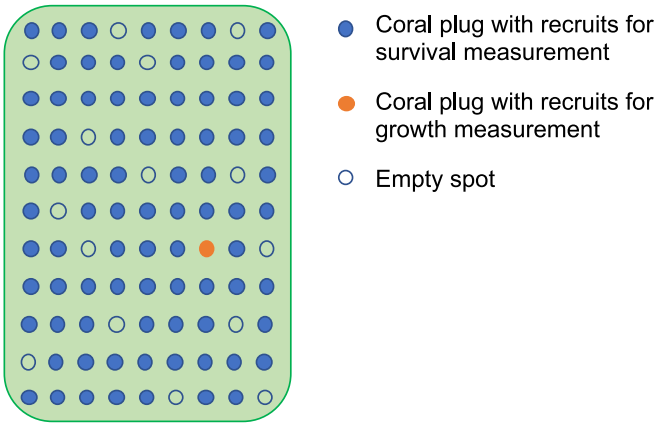

Fig. 2. Schematic overview of a PVC plate with coral plugs for survival measurement (84 plugs), one coral plug for growth measurement and some empty spots.

2.5.2. Recruit size and after outplanting

Recruit size of the recruits present on the designated growth plugs (n = 48 plugs, assigned to a different plate with ~3–4 recruits per plug) was determined at six intervals during aquaculture. Recruit size was determined as the number of polyps per recruit and as living tissue area. The initial number of recruits differed between treatments: n = 42 for ambient, n = 49 for AxF, n = 35 for TA and n = 35 for TAxF. Measurements were based upon pictures that were taken through a

Fig. 1. Overview of the study locations around New Providence Island, The Bahamas: ReefGuard facilities (in-situ experiment), spawn collection site and coral nursery site (ex-situ experiment) (source: Google Earth).

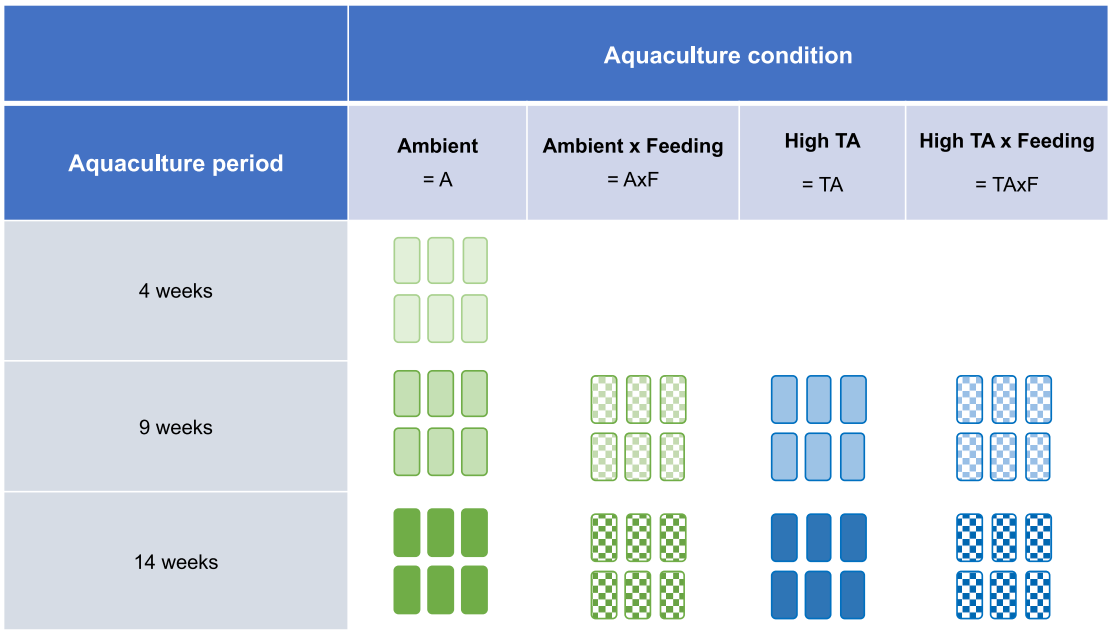

Fig. 3. Overview of the number of plates assigned to each experimental treatment (aquaculture period and aquaculture condition). Plates are color-coded to represent their experiment treatment: ambient (plain green), TA (plain blue), ambient x feeding (blocked green), TA x feeding (blocked blue). The intensity of the color indicates aquaculture period: 4 weeks (light colored), 9 weeks (normal colored), 14 weeks (dark colored). The same color-codes are used throughout this manuscript. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

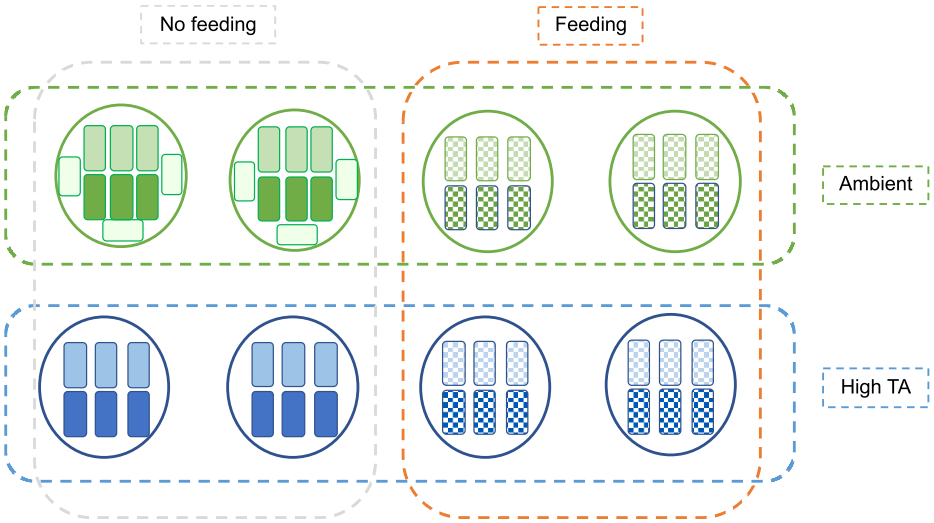

Fig. 4. Schematic representation of the experimental setup during the aquaculture period of the experiment. Color-codes are explained in Fig. 3.

binocular stereomicroscope using a digital camera (Canon Powershot G10) attached to a camera adapter for microscopes (Vixen Universal Digital Camera Adapter). A small ruler was included in every picture to allow for size calibration using image analysis software (ImageJ 1.46r).

Only few of the designated recruits for the calculation of growth rates survived after outplanting, therefore a different strategy was adopted for recruit size observations at 27 and 44 weeks after settlement. Recruit size in terms of the number of polyps per recruit was counted for all recruits observed during the survival counts (n = 73–250 recruits per treatment at week 27, n = 13–119 recruits per treatment at week 44), while recruit size as living tissue area was measured of a subset of recruits randomly selected from each treatment (n = 6 plugs, n = 3–11 recruits at week 27, n = 3–7 recruits at week 44).

2.6. Data analysis

All statistical analyses were performed in R using the R-studio 3.0.1 interface (R Core Team, 2013).

2.6.1. Survival data

Factorial logistic regression analysis of binomial data (success, failure) was performed using the Generalized Linear Model (GLM) with the logit link function (binomial family) in R-studio 3.0.1 (R Development Core Team, 2013), following Schutter et al. (2015). For each model the dispersion parameter was estimated. The quasibinomial family was used if data were significantly over-dispersed. Each model was analyzed using a Type III ANOVA. In the presence of a significant interaction, multiple comparisons were made using user-defined contrasts and Bonferroni correction using the glht() function from the multcomp

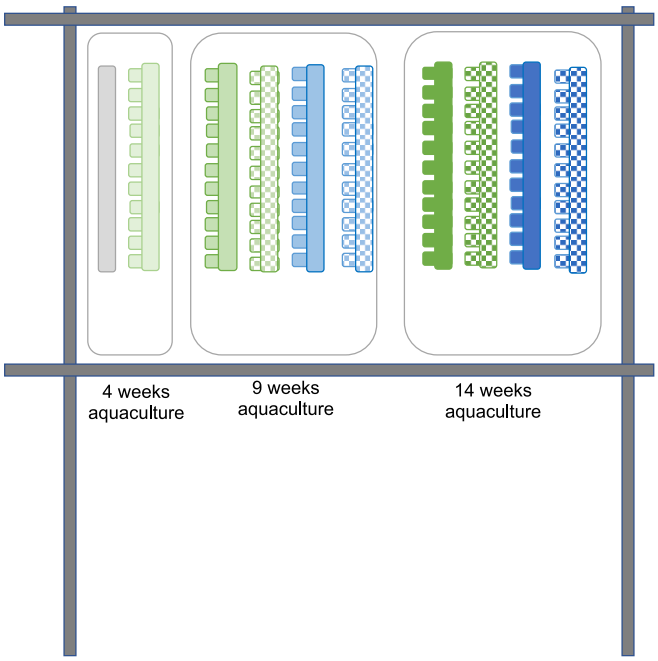

Fig. 5. Experimental layout of plates with coral plugs that were outplanted to each of the six nursery structures at Goulding Cay. See Fig. 3 for legend of color coding used in this graph to indicate aquaculture conditions.

package (Hothorn et al., 2008). Contrasts focused on 1) the effect of treatment within each aquaculture period and 2) the effect of aquaculture period for each treatment. In the absence of interactions but the presence of significant main effect of treatments, Tukey comparisons were performed to test for significance between treatment levels.

2.6.2. Recruit size data

To give a reliable impression of recruit size at each measurement time, all observations were used in the analyses, regardless of whether recruits died at a later stage. All parameters were checked for satisfying the assumptions for ANOVA. An ANOVA was followed by multiple comparison testing using user-defined contrasts. When assumptions were not satisfied, the non-parametric Kruskall-Wallis rank sum test was used and Dunn test in combination with the Benjamini-Hochberg method for multiple comparisons.

3. Results

3.1. Culture of sexual recruits

3.1.1. Water conditions during aquaculture

Concentrations of calcium and magnesium were consistently lower in the TA treatments compared to the ambient treatments (380 ppm ± 33SD ($n = 18$) vs 451 ppm ± 12SD ($n = 17$) Ca^{2+} and 1384 ppm ± 49SD (n = 18) vs 1430 ppm ± 60SD (n = 17) Mg^{2+}), likely as a result of abiotic precipitation of calcium carbonate. pH was also consistently 0.1 pH unit lower in the TA treatments compared to the ambient treatments (Suppl. Table 1).

3.1.2. Establishment of symbiosis

The establishment of symbiosis was visually checked after 1 week and 2 weeks of exposure to coral fragments. After one week, the initiation of symbiosis (i.e. appearance of small brown dots) was observed in all treatments. The percentage of recruits with symbiosis per treatment after one week was on average 41% ± 32SD (range: 30–55%, $n = 40$ recruits per treatment) and after two weeks on average 74% ± 19SD (range: 61–85%, $n = 80$ recruits per treatment). No consistent effect of aquaculture condition on the establishment of symbiosis was observed.

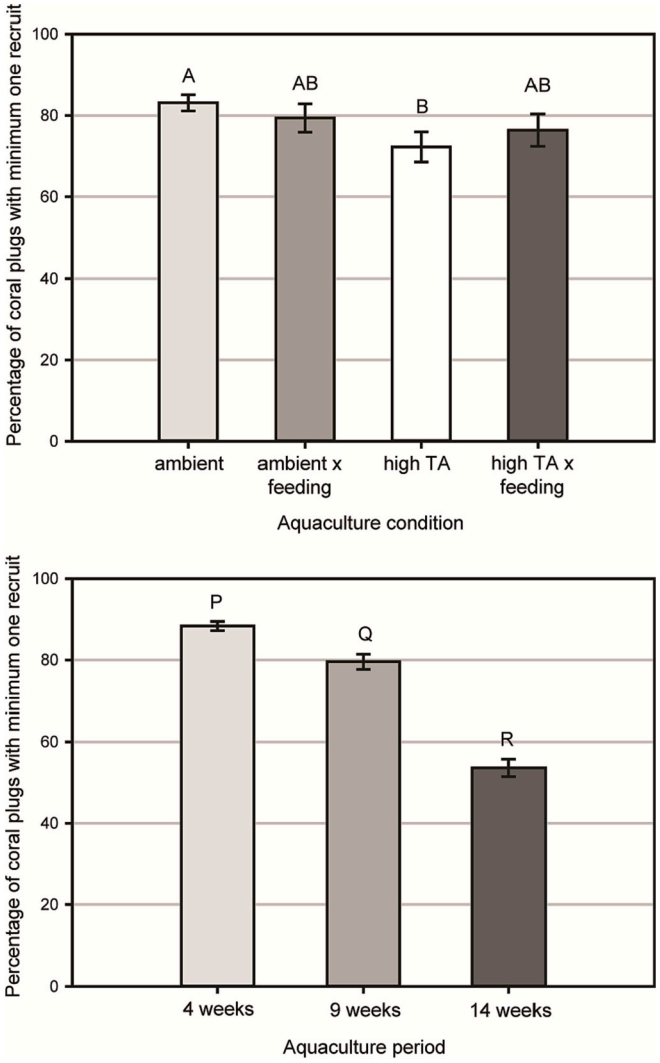

Fig. 6. Bar graphs showing the percentage of coral plugs with at least 1 living recruit after 4, 9 and 14 weeks of aquaculture (A) and under different aquaculture conditions (B) (average ± SE). Treatments sharing the same letter are not significantly different. Capital letters indicate main effects of or aquaculture treatment (ABC) or aquaculture period (PQR). n = 18 for ambient at 4 weeks of aquaculture, n = 12 for the remaining treatments at 4 weeks of aquaculture, n = 6 for the treatments at 9 and 14 weeks of aquaculture.

3.1.3. Survival and recruit size during aquaculture

3.1.3.1. Survival during aquaculture. Both aquaculture period and aquaculture condition had a significant main effect on the survival of recruits (minimum 1 recruit per plug alive) during the aquaculture period (GLM: $X^2(3) = 9.41$, $p < 0.05$ and GLM: $X^2(2) = 16.6$, $p < 0.001$ respectively). Tukey comparisons for aquaculture period indicated that survival decreased significantly over time (Tukey contrasts: $p < 0.05$ for all; See Fig. 6A and Suppl. Table 2): compared to survival after an aquaculture period of 4 weeks (53.6% ± 10.5 SE), survival after 9 and 14 weeks of aquaculture (79.6% ± 1.8 SE and 53.6% ± 2.1 SE) was resp. 1.1 and 1.6 times lower. Tukey comparisons for aquaculture condition indicated that survival was significantly lower in the TA treatment compared to the ambient treatment: 72.3% ± 3.7 SE vs. 83.1% ± 2.0 SE ($p < 0.05$). (Fig. 6B).

3.1.3.2. Recruit size during aquaculture. Recruit size was significantly

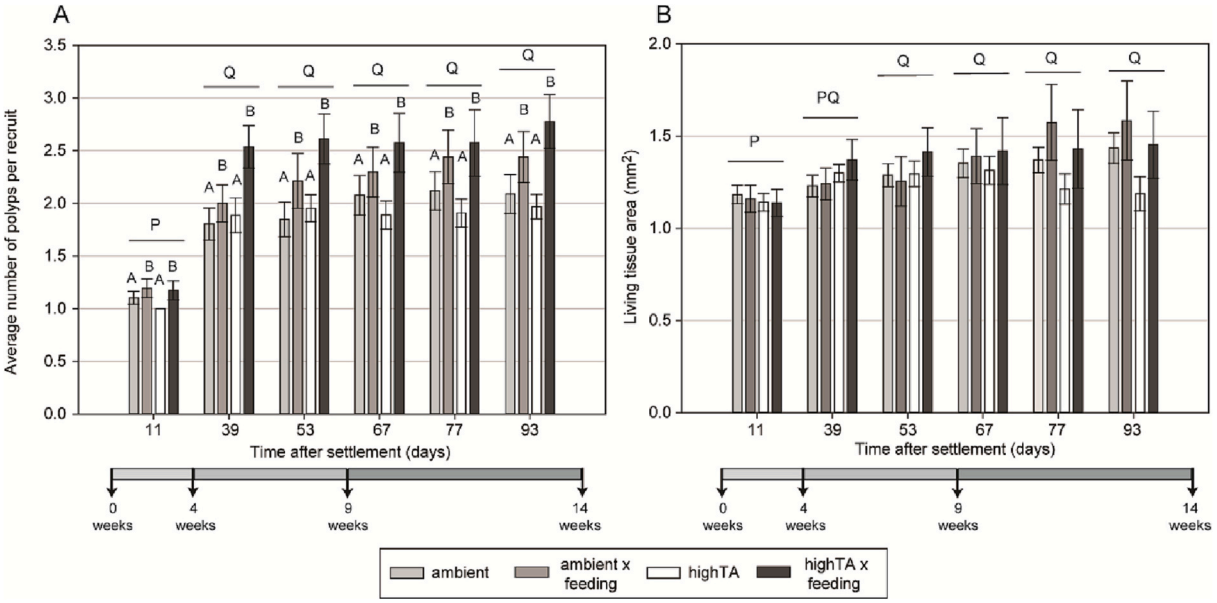

Fig. 7. Average number of polyps per recruit (A) and average living tissue area (B) of a subset of recruits measured at regular intervals after settlement for each aquaculture treatment during the aquaculture period (average ± SE). Treatments sharing the same letter are not significantly different. Capital letters indicate main effects of aquaculture condition (AB) and aquaculture period (PQ). The bar underneath each graph indicates the aquaculture period during which each measurement was done.

affected by time (polyps: $X^2(5) = 97.5$, $p < 0.0001$, tissue area: $X^2(5) = 16.1$, $p < 0.01$). The number of polyps increased significantly between the first and second measurement time (1.8 times increase, from 1.1 ± 0.04 SE to 2.0 ± 0.1 SE) but did not increase significantly between the subsequent observations (Fig. 7A) (Suppl. Table 3). In contrast, living tissue area did not increase significantly between the first and second observation, but between the first and the third observation (1.1 times increase, from 1.15 mm^2 ± 0.03 SE to 1.31 mm^2 ± 0.05 SE). Living tissue area did not increase significantly between the subsequent observations (Fig. 7B) (Suppl. Table 4).

Recruit size in terms of the average number of polyps per recruit was significantly higher in the treatments with feeding (1.2 times increase; no feeding: 1.8 ± 0.1 SE, with feeding: 2.2 ± 0.1 SE, $X^2(1) = 14.15$, $p < 0.001$) (see Fig. 7A). The feeding regime did not affect living tissue area during aquaculture ($X^2(1) = 0.10$, $p = 0.75$). No significant effects of the TA treatment were detected on the number of polyps per recruit or living tissue area ($X^2(1) = 0.53$, $p = 0.46$ and $X^2(1) = 2.07$, $p = 0.15$) (Suppl. Table 3 and 4).

3.1.4. Survival and recruit size after outplanting

3.1.4.1. Survival after outplanting. The combination of aquaculture condition and aquaculture period affected the survival in the field. At 44 weeks after settlement (Fig. 8A) survival was significantly higher for recruits subjected to an *aquaculture period* of 9 weeks compared to a period of 14 weeks for the ambient (3 times higher, 14.5% ± 1.1 SE vs. 4.9% ± 0.7 SE, $p < 0.001$), TA (4.1 times higher, 11.5% ± 1.8 SE vs. 2.8% ± 0.5 SE, $p < 0.0001$) and TAxF treatments (1.7 times higher, 20.4% ± 1.7 vs. 11.7% ± 2.1 SE, $p < 0.05$) (Suppl. Table 5). Similar significant differences were already visible after 27 weeks (ambient: 35.6% ± 2.9 SE vs. 17.0% ± 2.2 SE, $p < 0.05$; TA: 30.4% ± 5.0 SE vs. 12.9% ± 2.0 SE, $p < 0.01$; TAxF: 41.5% ± 3.3 vs. 22.8% ± 4.1 SE, $p < 0.05$) (Suppl. Table 6). Aquaculture period also affected survival under ambient conditions in the laboratory: plates with recruits that remained under ambient conditions for 14 weeks had a significantly lower survival compared to a culture period of 4 weeks (2.7 times lower, 3.8 ± 0.6 SE vs 10.1 ± 1.5 SE, $p < 0.05$). This difference was not reflected in survival rates after outplanting as observed after 27 weeks (22.5% ± 2.9 SE vs. 30.4% ± 4.6 SE, NS).

In addition, survival at 44 weeks after settlement was affected by aquaculture condition. After 9 weeks of aquaculture, survival in the TAxF treatment (20.4% ± 1.7 SE) was significantly higher compared to the ambient and TA treatment (resp. 3.5 and 1.8 times higher, 5.9% ± 1.4 SE and 11.5 ± 1.8 SE, $p < 0.0001$ and $p < 0.01$ resp.). After 14 weeks of aquaculture, survival in the TAxF treatment (11.7% ± 2.1 SE) was significantly higher compared to the other three treatments (2.4–4.2 times higher; ambient: 3.8% ± 0.6 SE, AxF: 4.9% ± 0.7 SE, TA 2.8% ± 0.5 SE, $p < 0.01$) (Suppl. Table 5). These differences in survival from different aquaculture conditions were not reflected in survival rates after outplanting as observed after 27 weeks (Suppl. Table 6).

3.1.4.2. Recruit size after outplanting. At 44 weeks after settlement, multiple comparisons indicated that aquaculture period did not significantly affect recruit size in terms of the number of polyps per recruit in neither the ambient treatment nor the feeding treatments (AxF and TAxF) ($p = 1$). However, recruit size in week 44 of recruits grown in the TA treatment was significantly lower for recruits that had been in aquaculture for 14 weeks (2.5 ± 0.5 SE) than for recruits that had been in aquaculture for 9 weeks (4.9 ± 0.5 SE) (2 times lower; $p < 0.05$) (Fig. 8B) (Suppl. Table 7). This difference was not significant after 27 weeks (2.2 ± 0.2 SE vs 3.0 ± 0.2 SE, $p = 0.09$) (Suppl. Table 8). Recruit size in terms of living tissue area was not significantly affected by aquaculture period at 27 and 44 weeks after settlement (Fig. 8C) (Suppl. Table 9, 10).

In contrast, *aquaculture condition* did significantly affect recruit size in terms of the number of polyps per recruit. After 9 weeks of aquaculture, recruits in the TAxF treatment had a significantly higher number of polyps compared to the ambient treatment (1.9 times higher, 7.8 ± 0.6 SE vs 4.1 ± 0.3 SE, $p < 0.01$). Similarly, after 14 weeks of aquaculture, recruits in the TAxF treatment had a significantly higher number of polyps (9.0 ± 1.2 SE) compared to all other treatments (1.7–3.8 times higher; ambient: 3.0 ± 0.2 SE, AxF: 5.2 ± 0.6 SE and TA: 2.4 ± 0.5 SE, $p < 0.05$). In addition, the recruits in the AxF treatment had a significantly higher number of polyps compared to the TA treatment (2.2 times higher, $p < 0.05$) (Fig. 8B) (Suppl. Table 7). At 27 weeks, similar significant differences were observed for recruits that had been

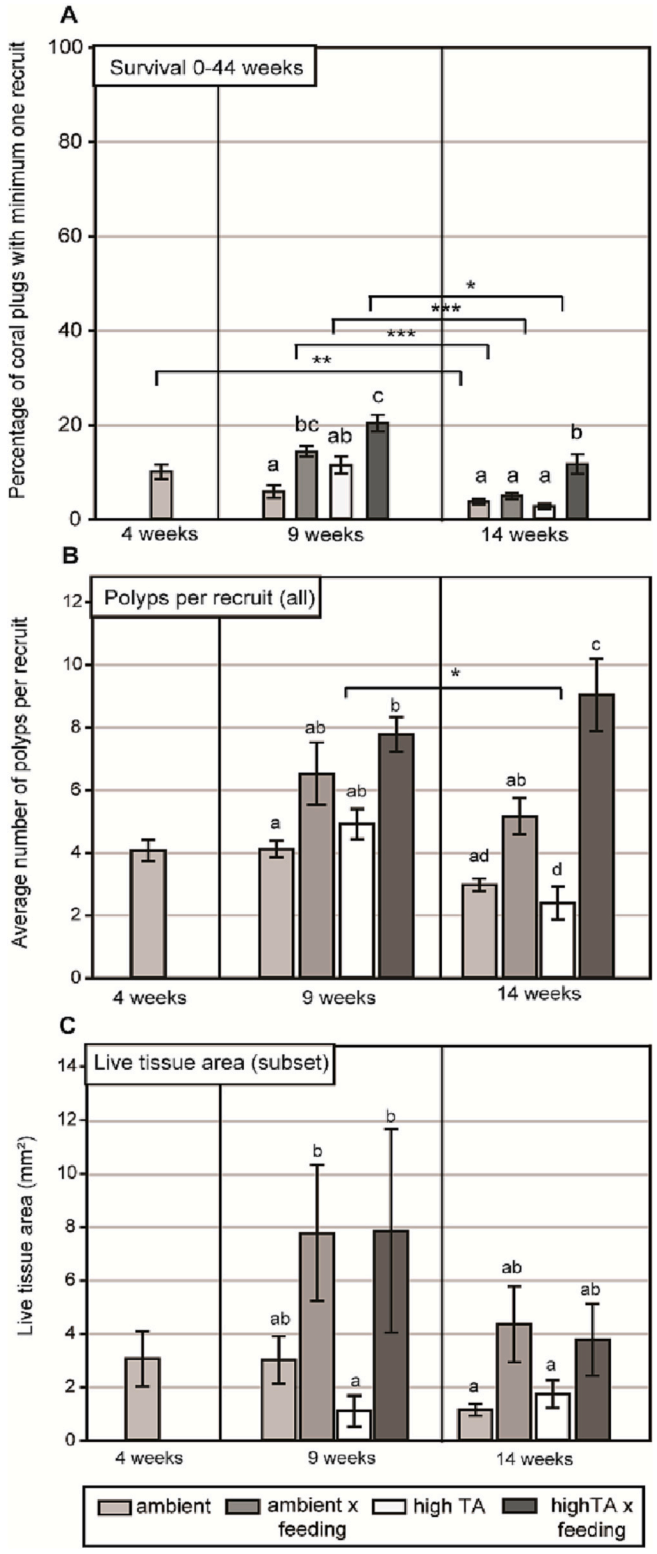

Fig. 8. Bar graphs showing the percentage of coral plugs with at least 1 living recruit (A), average number of polyps per recruit (B) and average living tissue area per recruit (C) at 44 weeks after settlement, after being outplanted after 4, 9 and 14 weeks of aquaculture and having received different aquaculture treatments (ambient, ambient x feeding, high TA, high TA x feeding) (average ± SE). Lower case letters indicate single effects of aquaculture treatment within an aquaculture period (abcd). Treatments sharing the same letter are not significantly different. Significant differences between aquaculture periods are indicated using horizontal bars, with asterisks indicating the level of significance (*** $p < 0.0001$, ** $p < 0.001$, * $p < 0.05$).

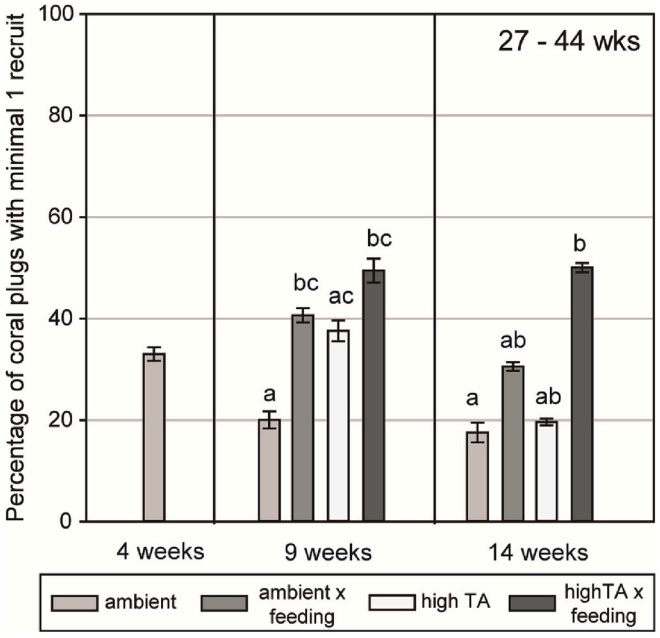

Fig. 9. Bar graph showing the percentage of coral plugs with minimal 1 recruit that have survived in the field between 27 and 44 weeks after settlement after being outplanted after 4, 9 and 14 weeks of aquaculture and having received different aquaculture treatments (ambient, ambient x feeding, high TA, high TA x feeding) (average ± SE). Lower case letters indicate single effects of aquaculture treatment within an aquaculture period (abcd). Treatments sharing the same letter are not significantly different. None of the pre-planned multiple comparisons of aquaculture treatment between aquaculture periods were significant and therefore not indicated. n = 4 plates per treatment.

in aquaculture for 9 weeks (5.1 ± 0.4 SE (TAxF) vs. 2.8 ± 1.1 SE (ambient), $p < 0.0001$) and for recruits that had been in aquaculture for 14 weeks (5.8 ± 0.3 SE (TAxF) vs. 2.1 ± 0.06 SE (ambient), 3.7 ± 0.2 SE (AxF) and 2.2 ± 0.2 SE (TA), $p < 0.001$), Suppl. Table 8). Recruit size in terms of *living tissue area* followed the same pattern as for the number of polyps per recruit (Fig. 8 BCE), though sample sizes were small (n = 3–10 for the different aquaculture periods and conditions). Recruits from the feeding treatments were larger than from the non-feeding treatments, although significance was not always reached, likely as a result of the low sample size and large variation between recruits. One outlier with extreme growth rates was removed from the analysis (a recruit measuring 63 mm^2 after 27 weeks and 102 mm^2 after 44 weeks in the TAxF treatment) (Suppl. Table 9 and 10).

3.1.4.3. Relation between survival and recruit size in the field-based nursery (27–44 weeks). The aquaculture period did not significantly affect survival in the field-based nursery between 27 and 44 weeks (multiple comparison: $p > 0.53$) (Fig. 9). In contrast, aquaculture conditions did significantly affect survival in the field-based nursery between 27 and 44 weeks: in the group that had received 9 weeks of aquaculture, coral plugs with recruits from the TAxF treatment and AxF treatment had a significantly higher survival than the coral plugs from the ambient treatment (2.5 times higher: 49.5% ± 2.6 SE vs. 20.0% ± 1.7 SE, $p < 0.001$ and 1.9 times higher: 40.6 ± 1.4 SE vs. 20.0 ± 1.7 SE, $p < 0.05$). In the group that had received 14 weeks of aquaculture, survival in the TAxF treatment was significantly higher than in the ambient treatment (2.9 times higher: 50.1% ± 0.9 SE and 17.5% ± 2.0 SE ($p < 0.01$) (Fig. 9) (Suppl. Table 11). This corresponds to the patterns of recruit sizes measured as the number of polyps per recruit and living tissue area as presented in Fig. 9A and B and Fig. 10A and B. The average size of the group of recruits that remained alive between 27 and 44 weeks after settlement was significantly higher than the average size of the recruits that had died (ANOVA: $F_{(1,\ 53)} = 9.5$, $p < 0.01$) (Fig. 10, Suppl.

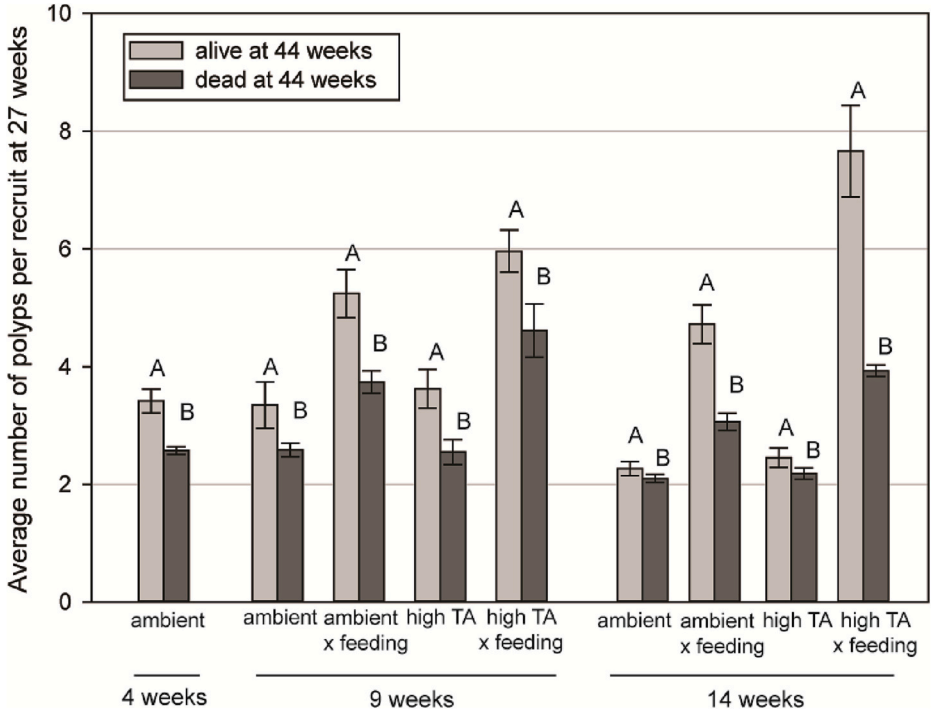

Fig. 10. Average recruit size in terms of number of polyps at 27 weeks after settlement of the group of recruits that remained alive till week 44 and the group of recruits that died before week 44. Capital letters indicate main effects.

Table 1
Overview of time, location and number of plates with coral plugs that were counted for survival.

Time of survival count	Location of counted plates	Number of plates counted (N)
4 weeks	In aquaculture, before outplanting	N = 6 plates *(6 plates per treatment)*
9 weeks	In aquaculture, before outplanting	*N* = 48 plates *(6 plates per treatment)*
14 weeks	In aquaculture, before outplanting	*N* = 24 *(6 plates per treatment)*
27 weeks	At coral nursery in field	*N* = 54 plates *(6 plates per treatment)*
44 weeks	At coral nursery in field	*N* = 36 *(4 plates per treatment)*

Table 12).

4. Discussion

The results from this study indicate that post-outplanting survival and size of *A. palmata* recruits was significantly enhanced after applying specific pre-outplanting aquaculture regimes with feeding and the addition of bicarbonate, as compared to ambient aquaculture conditions. For the most successful aquaculture treatment in this study (TAxF, 9 weeks aquaculture), both survivorship and recruit size after 44 weeks were a factor 2 higher compared to ambient aquaculture conditions (20% vs. 10%, 8 polyps per recruit vs. 4 polyps per recruit). Two mechanisms are likely to have played a role: first, (natural) selection of those recruits that were coping well with the experiment conditions offered, and second, the reinforcing nature of having a larger recruit size.

Ambient aquaculture conditions did not enhance survival nor recruit size at 44 weeks after settlement, as compared to the other treatments. Nevertheless, the success of ambient aquaculture conditions exceeded natural conditions, as no natural recruitment of *A. palmata* was observed in this study, comparable to previous studies (Quinn and Kojis, 2005; Williams et al., 2008; Vermeij et al., 2011). Fertilization and settlement rates in this study were comparable to the rates measured during a previous coral breeding study in the ReefGuard in The Bahamas. Based on our findings, recommendations are discussed for further optimizing growth and survival and subsequent up-scaling for restoration efforts.

4.1. Aquaculture conditions

The addition of *Artemia* nauplii had contrasting effects on survival and recruit size during the aquaculture phase: survival was highest under ambient conditions, while the feeding treatments (AxF and TAxF) resulted in larger recruit sizes. Lower survival in feeding treatments could be explained by increasing available dissolved and/or particulate nutrients in the aquaculture basins as a result of decomposing leftover *Artemia* nauplii, facilitating the growth of nuisance algae and other fouling organisms and consequently increasing competition (McCook et al., 2001; Vermeij, 2006; Box and Mumby, 2007). No fouling control was done during the experiment, as it was not possible to manually remove fouling organisms from the plugs without damaging the recruits. As a result, interactions with fouling organisms likely caused some of the mortality during this phase and therefore reduced survival rates, especially in the feeding treatments. On the other hand, feeding also resulted in larger recruit sizes by increasing coral growth rates (e.g. Houlbrèque et al., 2004). This likely occurred indirectly, as *A. palmata* recruits were not observed to feed on *Artemia* nauplii, while feeding was clearly observed for *Porites asteriodes* recruits that were found on some plugs. Although *A. palmata* is known as a predominantly photo-autotrophic species, relying mostly on the translocated photosynthates from their symbionts (Porter, 1976, 1989; Bythell, 1988), a significant portion of their nitrogen budget is derived from particulate sources (70%, Bythell, 1988) such as bacteria in the water column (Bak, 1998). Future feeding strategies should incorporate these findings.

While survival in the feeding treatments (AxF and TAxF) was lower compared to ambient conditions during the aquaculture phase, after outplanting to the field-based nursery, both survival and recruit size were significantly higher for recruits obtained from the feeding

treatments. As expected, the larger recruit sizes resulted in higher survival (Doropoulos et al., 2012), likely because they were more resistant to the natural biofouling pressures in the nursery. In this study, this was evidenced by the fact that recruits that remained alive at week 44, retrospectively had a significant higher average recruit size at week 27 compared to the ones that had died before week 44 (Fig. 10).

The addition of bicarbonate appeared to be stressful for the recruits, evidenced by a lower survival and recruit size in the TA treatment during aquaculture and after outplanting. As the recruits in the TA treatment had the lowest percentage of symbiosis at two weeks after initiation of symbiosis (61%), mortality resulting from a lack of symbiosis could potentially explain the lower survival in this treatment after 4 weeks. This can neither be confirmed nor excluded in this study. The stressful effect of bicarbonate addition was possibly mediated by the lower pH (Δ0.1) compared to ambient conditions. Combining bicarbonate addition with feeding, however, survival was still low, but under these conditions, the recruits were apparently able to use the additional bicarbonate for growth. After outplanting, survival and recruit size tended to be higher in the TAxF treatment compared to AxF. This trend reached significance at 44 weeks after settlement for recruits maintained in aquaculture for 14 weeks.

Although based on the study by Marubini and Thake (1999) it was expected that bicarbonate addition by itself would increase coral growth rates, the absence of a positive effect of bicarbonate addition on coral growth in this study was possibly mediated by the decreased pH level and/or a limited nutritional status of the recruits. Nevertheless, in agreement with the TA treatment in this study, De Putron et al., 2011 also observed decreased calcification rates with increasing bicarbonate concentrations under nutrient limiting conditions (i.e. no feeding) and reduced seawater pH. Interestingly, Jury et al. (2010) observed the opposite effect under nutrient-replete conditions (i.e. with feeding) and despite low seawater pH (7.6–7.8), which corresponds to our finding in the TAxF treatment. As suggested by De Putron et al. (2011), possibly, increased nutrient availability and/or coral nutritional status can enhance the use of bicarbonate for calcification.

4.2. Aquaculture period

In contrast to our expectations, the longest aquaculture period of 14 weeks did not result in higher recruit sizes or survivorship as measured at 27 and 44 weeks after settlement, when compared to the shorter aquaculture periods. The most likely explanation is the occurrence of suboptimal conditions during the last aquaculture period compared to the natural conditions on the reef in that same period. Factors that might have contributed to suboptimal conditions during the last aquaculture period could have been: 1) larger daily temperature fluctuations due to colder nights, 2) slightly lower ambient light levels due to the time the year, 3) indirect cumulative effects of bicarbonate addition, such as the long-term decreased pH and precipitation of essential macro elements such as calcium and magnesium and/or 4) lasting post-hurricane conditions affecting seawater quality (Smith et al., 2009). Maintaining optimal conditions for rearing sexual recruits in aquaria requires the attention of experienced and dedicated personnel and reliable technical facilities. However, the risk of (partial) system failure or the occurrence of uncontrollable or unforeseen changes in (a)biotic factors can never be completely excluded (Petersen et al., 2006; Chamberland et al., 2015). Limiting the aquaculture phase to a relatively short period of several weeks reduces these risks and costs and has shown to enhance coral growth and survival to enable increased restoration success (this study). In addition, limiting the extent of the aquaculture phase also reduces the potential risk of rearing and selecting for 'laboratory-adapted' corals that may reduce their performance on reefs (Randall et al., 2020).

4.3. Recommendations for future research

Other coral species are expected to respond similarly to the aquaculture conditions applied in this experiment, following the mechanisms suggested (De Putron et al. (2011). Coral species for which sexual recruits are known to feed on *artemia* nauplii are expected to have the largest benefit (Conlan et al., 2017).

The role of recruit size in enhancing survival of early life stages should also be evaluated for coral larvae. Although larval size can reflect genet-specific differences of a gamete source population (Miller et al., 2018) and/or the health of the source population (Michalek-Wagner and Willis, 2001a, 2001b; Marshall and Uller, 2007; Howells et al., 2016), the amount of handling during cultivation and research may also affect larval size through (unintended) fragmentation of coral embryos during embryonic development (Heyward and Negri, 2012).

In addition, as interactions with fouling organisms likely caused some mortality during the aquaculture phase with feeding (Kuffner et al., 2006; Birrell et al., 2008), future research could contribute knowledge about factors influencing the succession of the benthic community. This knowledge could be applied for maintaining, enhancing and/or promoting a healthy benthic community on the substrates during the aquaculture and outplanting phase of a coral restoration project.

In the light of global climate change, it remains to be tested how higher temperatures and lower pH in the coral nursery will affect the growth and survival of recruits cultured under the conditions in this study. A larger recruit size offers a good head-start. However, in addition, the aquaculture phase might have led to unintended pre-selection, resulting either in recruits that are selectively optimized to cope with the conditions offered during aquaculture, but that may be maladapted to other conditions (similar to strong local adaptation, Schiffers et al., 2013, Baums et al., 2022), or resulting in recruits that are resilient and able to adapt to a wide range of environmental conditions including future climate conditions (e.g., Van Oppen et al., 2015). It remains to be studied to what extent cultured recruits might show resilience to future climate conditions.

5. In conclusion

This study shows that an ex-situ aquaculture period in which feeding and the addition of bicarbonate (TAxF) are applied, can give early sexual recruits of *A. palmata* an important head-start for their life on the reef by significantly enhancing recruit size and survival compared to ambient culture conditions. Nevertheless, the addition of bicarbonate without feeding appeared to be stressful to the recruits, evidenced by a lower survival and recruit size in the TA treatment during aquaculture and after outplanting. Despite a larger recruit size, lower survival was also observed in the feeding treatments (AxF and TAxF) compared to ambient conditions during aquaculture. However, after outplanting to the field-based nursery, both survival and recruit size were significantly higher for recruits obtained from the feeding treatments. Strengthening impaired natural populations of *A. palmata* with new genetic individuals through ex situ rearing is an important step towards the recovery of this threatened species. It has the potential applicability to other coral species as well to enhance global reef rehabilitation at relevant scale. Future studies should consider to what extent cultured recruits are able to adapt to future climate conditions.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2023.106962.

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