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THE CULTURE, TRANSPLANTATION AND STORAGE OF *MONTASTRAEA FAVEOLATA, ACROPORA CERVICORNIS* AND *ACROPORA PALMATA*: WHAT WE HAVE LEARNED SO FAR

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ABSTRACT

Coral explantation provides new colonies for ship-grounding sites while maintaining the complexity and diversity of the donor site. In our studies, explants included branches (*Acropora palmata* and *A. cervicornis*) and cores (*Montastraea faveolata*). Maintaining explants in closed aquaria, an open seawater system, or on arrays out on the reef was compared. Closed aquaria allow controlled and potentially optimized conditions, however, diseases can quickly eliminate corals. Open seawater systems were found to be viable in the Bahamas (Lee Stocking Island), but less successful in the Florida Keys where nearshore waters are subject to wide temperature variations and turbidity. Corals placed on arrays or directly on the reef substrate had similar survival rates that were not significantly different (*M. faveolata* 2.5 cm, 9 mo: array 100%, substrate 75%; *A. cervicornis*, 7 mo: array 91.7%, substrate 75%). We have begun to examine minimum size requirements for *M. faveolata* explants. Although a single apical polyp of *A. cervicornis* or several polyps of *M. faveolata* survive in aquaria, they are unlikely to do so on a reef. In different experiments, survival rates of 2.5 and 5.1 cm diameter cores of *M. faveolata* on substrate were 75% (11 mo) and 86.1% (12 mo), respectively, and on arrays for 9 mo were 100% and 91.6% (inshore array) — 95.6% (offshore), respectively. With no clear differences apparent, the minimum viable size may be smaller than 2.5 cm in diameter. An ongoing experiment is reexamining this issue with explants of differing size from the same parent colonies.

Over the past 15 yrs, there have been a number of large vessel groundings on coral reefs in the Florida Keys including the MV WELLWOOD (1984), MV ELPIS (1989), the MV MAITLAND (1989), RV COLUMBUS ISELIN (1994), and the Contship HOUSTON (1997). The sizes of the damaged areas range from hundreds to thousands of square meters. Natural recovery from such extensive damage may take decades (Stoddart, 1969, 1974; Cook et al 1993; Precht, 1998). Natural re-establishment of scleractinian corals has been slow in these areas, apparently because of poor sexual recruitment and/or post-settlement survival. Aronson and Swanson (1997) showed that after 10 yrs grounding sites that were not structurally restored were statistically more similar to a hardground than to their initial spur and groove reef configurations (Aronson and Swanson, 1997; Precht, 1998). Core data from spurs on reefs of the Florida Keys indicate that these structures typically represent as much as 4000–6500 yrs of construction (Shinn et al., 1981).

Because of the extremely long natural recovery times and the potential for further damage to surrounding areas due to destabilization, several large grounding site repair projects have been undertaken (all of the above-named vessel sites). Priority has generally been placed on site stabilization by re-securing large coral colonies, removing loose rubble or covering it with concrete matting and structural restoration to prevent further erosion (Curtis, 1985; Hudson and Diaz, 1988). Hard substrates in the Keys are typically a veneer of cemented limestone overlying unconsolidated reefal material. Thus, removal of the limestone veneer allows erosion of adjacent areas with consequent enlargement of the original damage area. Recently, Hurricane Georges was estimated to have doubled the

size of the RV COLUMBUS ISELIN grounding site at Looe Key prior to restoration (R. Spadoni and C. Kruempel, pers. comm.).

Site restoration goals have not been defined on a uniform basis. At locations where tourist visitation is low, stabilization of large coral colonies and the substrate has been considered sufficient. In the case of the RV COLUMBUS ISELIN restoration, the site is a spur-and-groove formation with high visitor use. Here, reconstruction/stabilization and aesthetic appeal are of primary concern. Some attempts have been made to address restoration of reef function, largely through transplantation of corals and other major benthic fauna. The surrounding substrate or similar habitat must be used as a guide for restoration because baseline biological cover data for grounding sites are seldom available (Pearson, 1981).

Transplantation of corals has been suggested as a viable, and possibly essential, methodology of expediting the recovery of a damaged or degraded coral reef (Rinkevich, 1995; Miller et al., 1993). Transplantation of coral colonies (Maragos, 1974; Bouchon et al., 1981; Hudson et al., 1989; Clark and Edwards, 1994; Clark, 1996; Goreau and Hilbertz, 1996; Hudson and Goodwin, 1996; Jaap et al., 1996; Muñoz-Chagin, 1996; van Treeck and Schuhmacher, 1997) and fragments (Yap and Gomez, 1984, 1985; Plucer-Rosario and Randall, 1987; Guzman, 1991; Yap et al., 1992; Bowden-Kerby, 1996; Garcia et al., 1996; Oren and Benayahu, 1997; Lindahl, 1998) has been widely employed for restoration projects and research. The techniques for removal, transportation and re-attachment are fairly straightforward although varying degrees of success have been reported. Reasons for failure may include transport stress, inappropriate species for the restoration site, obtaining donor colonies from an incompatible habitat, poor attachment or subsequent loss in high-energy settings.

However, transplantation of entire colonies from an undamaged reef area (donor site) to a damaged site is essentially redistributing the damage. The focus of our research was to develop and refine methods of coral restoration while minimizing damage to the donor site. We have chosen an asexual approach using coral 'explants', a general term for fragments, cores, nubbins or branches obtained from donor (or parent) colonies. By emplacing coral explants, rather than entire colonies, on ship-grounding sites, the complexity of the donor site can be preserved while accelerating the recovery of the damaged site through overcoming coral recruitment limitations. The major limitation of this approach is that topographic complexity of the restoration site is not significantly increased in the short term.

To minimize damage to donor colonies, we examined the minimum viable size of *Montastraea faveolata* (*annularis* complex; Knowlton et al., 1992) explants, one of the major reef building corals in the Caribbean. In addition, the advantages of using closed aquaria, open system, or field arrays (stable structures of inert materials that maintain the corals off of the substrate) for holding and culturing coral explants have been explored. In cases where reef damage requires time-consuming assessment, engineering, and structural restoration, such approaches could be useful for maintaining the viability of coral fragments generated during the damage. Culture of fast-growing species, such as the acroporids, under optimal conditions may also be useful in producing coral stocks for restoration.

Acropora palmata and *A. cervicornis* are being considered for the endangered species list. If standing stocks of acroporids can be established, this might be useful in preserving these Caribbean corals that have suffered high mortality over the past two decades due to

white band disease, bleaching and predators. Colonies that are still surviving may be assumed to be more resistant to the conditions causing the mortality since they exist in the same conditions that killed their conspecific neighbors. The colonies may eventually be used to reintroduce the species to reefs from which they have disappeared.

Material and Methods

All corals, unless stated otherwise, were transported by the 'dry' method (D. Allemand, pers. comm.). Collected explants are placed in plastic bags, which were emptied upon being brought to the surface. Residual seawater remains in the bag but there is no bulk water. The explants were layered in coolers with padding and several ice blocks placed on top. Closed aquaria (150 gal each) were based on the Jaubert (1989, 1991) Microcean™ system. Lighting by 1000 watt metal halide lamps (5500K) provided ~350 µmol photons m^{-2} s^{-1} near the bottom on a 12 h day/12 h night schedule. Temperature was maintained at 27°C although power problems caused periodic loss of cooling capability and temperatures as high as 30°C occurred.

Experiment 1.—On 29 May 1996, three donor colonies of *M. faveolata* were haphazardly selected at an offshore patch reef within the Looe Key National Marine Sanctuary near Restoration Test Site 2 (RTS2; Fig. 1; This area is now in the Looe Key Special Use Zone of the Florida Keys National Marine Sanctuary). Twelve 5.1 cm (2 in) in diameter cores were extracted from each of the donor colonies using a pneumatic drill fitted with a diamond core bit. The cores were transported back to the Pigeon Key Marine Research Center (PKMRC) in seawater-filled coolers. The holes in the donor colonies were later filled with a mixture of epoxy and fine carbonate sand to prevent disease and bioeroders from affecting the parent colonies and to facilitate aesthetic recovery of the colonies.

The cores were attached to ReefMounts (mushroom-shaped, fired ceramic pedestals) with epoxy (Devcon). The mounted explants were then placed in an aquarium. The buoyant weight of each core was measured and periodically thereafter. The density of aragonite was assumed to be 2.93 (Jokiel et al., 1978). Before each weighing, all calcareous material and organisms were carefully removed from the explants and their ReefMounts.

In May 1997, the explants were cleaned and weighed prior to placing them in the field. Epoxy was applied to any exposed skeleton and then each was reweighed. Two mostly dead *Montastraea* mounds, designated a and b, on the Looe Key forereef were selected as Restoration Test Sites (RTS1; Fig. 1). The explants were randomly assigned to each mound and to one of three orientations: horizontal, vertical, and intermediate. To emplace the explants, holes were drilled (2.54 cm diameter × 7.62 cm deep = 1 in diameter × 3 in deep) in areas with no live coral to accommodate the stems of the ReefMounts. Epoxy was applied sparingly so that the explants could be removed for re-weighing.

The corals were retrieved in May of 1998 and 1999, transported back to PKMRC, cleaned of encrusting organisms and reweighed. A nested MANOVA was performed, testing for treatment, mound, and orientation effects using Statistica. A separate ANOVA was performed to compare colony and treatment effects.

Experiment 2.—This experiment examined the growth of acroporids in an open seawater system and in the field. In July 1997, two donor colonies of *A. cervicornis* and two colonies of *A. palmata* were selected near Lee Stocking Island, Exumas, Bahamas. Twelve branches, each approximately 7 cm long, were collected from each colony. The branches were mounted with epoxy in an upright position onto PVC plates. The buoyant weights of the branches were measured before and after attachment. Photographs were made of each explant using a Nikonos camera with an extension tube. The branches were randomly assigned to one of two treatments. Half of the branches were placed on a PVC frame in a ~1m deep tank continuously supplied with seawater. A mesh screen over part of the tank blocked most afternoon sun. The remaining branches were placed on a stainless steel array on a patch reef near Bock Cay at a depth of ~3 m. This array was a single pole

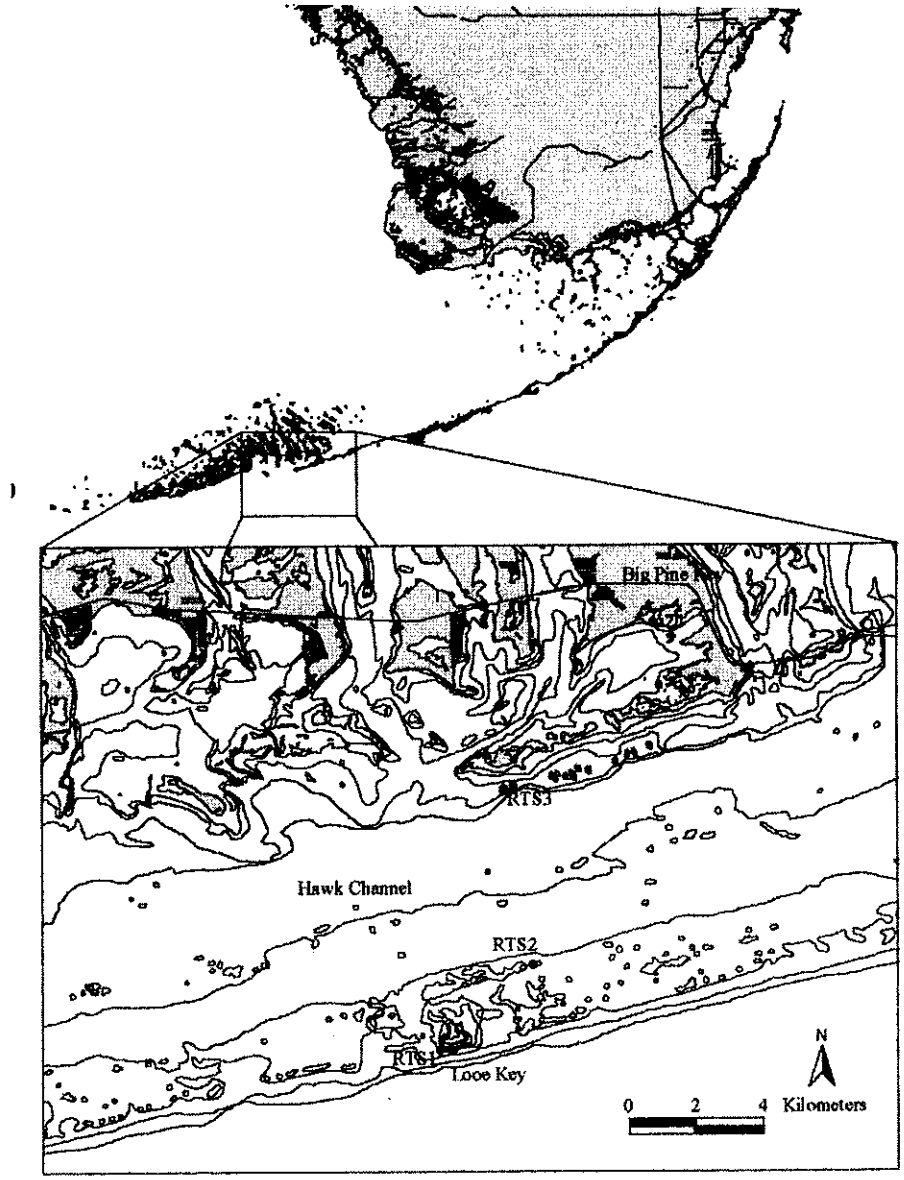

Figure 1. Map showing locations of Florida Keys restoration test sites (Bahamas site not shown). Experiment 1 was conducted at Restoration Test Site 1 (RTS1), at Looe Key Reef. Experiment 3 employed both RTS2, in the Looe Key Special Use Zone, and RTS3, in the Newfound harbor Sanctuary Preservation Area. All were located in the Florida Keys National Marine Sanctuary.

inserted into a pipe hammered into the substrate with two arms formed by a cross bar attached to the upper end of the pole. Each arm had three additional cross pieces creating six smaller branches that held the PVC plates. After 10 mo all corals were collected, photographed, cleaned of encrusting organisms, and reweighed. The corals were then removed from their PVC mounts, returned to near the location of their donor colony and attached to the substrate using epoxy.

The vertical linear extension (measured from the base to the tallest point of the coral) and maximum basal width (maximum diameter of the base) were determined from photographs. Data were examined using ANOVA (Statistica). One dead and two broken *A. cervicornis* branches were excluded from the growth analysis where appropriate.

EXPERIMENT 3.—Growth and survival of *A. cervicomis* and *M. faveolata* in the Florida Keys were compared using three treatments. In August 1997, twelve 2.5-cm (1") cores of *M. faveolata* were extracted from three colonies that were selected haphazardly from an offshore patch reef near the Looe Key Special Use Area. The corals were attached to ReefMounts and the volumes of the cores measured. Four explants from each donor colony were left in aquaria and the others taken back into the field. Four explants from each colony were placed onto a 5.5-m deep array within the Looe Key Special Use Zone (RTS2). The remaining four explants from each colony were placed on a grounding site at an inshore patch reef (RTS3; Newfound Harbor Sanctuary Preservation Area) where holes for the ReefMounts had been drilled. ReefMounts were placed in holes (all vertical) without any epoxy for easy retrieval. The condition and volumes were periodically re-measured through May 1999.

In November 1997, a similar protocol was employed with *A. cervicornis*. Bone cutters were used to collect 12 branches (approximately 7 cm long) from each of three colonies from the same reef area as the *M. faveolata* cores. The branches were attached in an upright position to Key Largo limestone blocks (approximately $4 \times 12 \times 2$ cm) with epoxy. The branches were randomly assigned to one of three treatments: storage on the array at RTS2, in an aquarium, or placed directly onto reef substrate without epoxy near RTS2. The volumes and lengths of the aquarium-stored explants were measured in the laboratory. Measurements were made in situ for field-based explants.

In the field, the volumes were measured by placing the explant on a raised level platform with an inverted funnel coming through the platform. A graduated cup was placed over the coral, which was then filled through the funnel with air from a regulator. Once filled, the cup was carefully lifted off of the coral, the coral removed, and the cup replaced onto the platform. The amount of water in the cup was equal to the combined volumes of the coral and the epoxy. The corals were then reattached to the array or placed back on the reef substrate. Survival was analyzed using the Kruskall-Wallis test (Statistica).

Table 1. The means and standard deviations of $CaCO_3$ accretion (normalized to mg d^{-1}) for the 5.1 cm (2") cores of *Montastraea faveolata* cores after approximately 1 yr in an aquarium and then 1 yr in the field. In the aquarium, all of the colonies were significantly different from each other ($P < 0.01$) and in the field, colony 1 was significantly different from colony 2 ($P < 0.02$). There were no treatment effects or effects of placement in the field.

Colony	Calcium carbonate accretion					
	Aquarium			Field		
	mean (mg d^{-1})	SD	n	mean (mg d^{-1})	SD	n
1	45.85	8.62	12**	19.93	24.58	9 *
2	36.92	5.12	12**	46.60	15.77	12 *
3	26.27	6.56	12**	33.45	21.72	7
Combined	36.35	10.54	36	34.79	22.87	28
	3	2	1	1	3	2

Results

Experiment 1.—All of the explants appeared healthy and survived during their year in the aquaria. The growth rates of all three of the colonies were significantly different from each other ($P < 0.01$, Table 1). After placement at Looe Key, 28 (77.8%) corals were present after 1 yr with no mortality although eight colonies had been physically lost. Calcium carbonate accretion of colony 1 was significantly different from colony 2 ($P < 0.02$) during the first year in the field. After 2 yrs in the field, 23 (63.8%) were present; again several cores were no longer at the site for unknown reasons but none died in situ. The mean growth rate in the aquaria was 36.4 ± 10.5 mg d^{-1}, 32.23 ± 23.26 mg d^{-1} for the first year in the field and 65.17 ± 43.36 mg d^{-1} for the second year in the field. Year 2 in the field was significantly different from the other 2 yrs ($P < 0.001$ for the first year in the field; $P < 0.01$ for the aquarium). The accretion of $CaCO_3$ in cores from colony 2 was significantly greater than colony 3 ($P < 0.02$) during the second year in the field. There were no mound or orientation effects on growth rates during the first year in the field. However, there was a combined year and mound effect in the second year. The growth rates of RTS1b (the mound closest to open water) were significantly greater ($P < 0.05$; Table 1) than those of the first year or RTS1a.

A number of qualitative observations were made regarding growth morphology. Cores with epoxy at the edge of the tissue grew smoothly over the epoxy and onto the ReefMount. Cores that were epoxied at the core base with exposed skeleton developed a mushroom shape leaving the skeleton exposed and colonized by algae and small encrusting invertebrates. In the field, cores that were placed vertically or intermediately had most of their growth on the lower side of the core with almost no growth on the upper side. On the topside there was a layer of fine sediment and filamentous red algae. One core appeared to have 'white plague' disease that affected ~1/4 of the explant area; it recovered. Several explants bleached to varying degrees, none severely, over the summer months of 1998 and subsequently recovered.

Experiment 2.—The accretion of calcium carbonate by *A. cervicornis* in the field (38.59 ± 12.16 mg d^{-1}; Table 2) was not significantly greater than in the tank (24.29 ± 8.85 mg d^{-1}) and there was no significant difference between the two colonies. There was also no significant difference in linear extension between the field (40.15 ± 9.98 mm) and the tank (31.38 ± 12.57 mm), however; maximum basal width was significantly greater ($P < 0.00001$) in the tank (56.00 ± 4.42 mm) than in the field (27.52 ± 2.30 mm). There was no intercolony difference with respect to linear extension ($P = 0.089$).

The accretion of calcium carbonate of the *A. palmata* in the field (111.86 ± 30.70 mg d^{-1}) was greater ($P < 0.01$) than that in the tank (64.62 ± 14.74 mg d^{-1}). There was also a significant difference ($P < 0.001$) in linear extension between the field (40.68 ± 9.85 mm) and the tank (16.18 ± 5.48 mm). As seen with *A. cervicornis*, the basal growth was much greater ($P < 0.0001$) in the tank (84.40 ± 12.46 mm) than in the field (44.53 ± 8.54 mm). There were no significant differences between the two colonies in any of the measured parameters.

One *A. cervicornis* branch died apparently a short time after placement into the open water system since it exhibited no growth. Three of the *A. cervicornis* branches were broken on the array: one completely lost, one down to a short stub, and the third was found in the sand and matched to its base.

Table 2. Means and standard deviations of measurements taken of *Acropora cervicornis* and *A. palmata* branches that were stored in an open seawater system or on an array in the field.

	Acropora cervicornis							*Acropora palmata*						
	Tank			Field				Tank			Field			
	mean	SD	n	mean	SD	n		mean	SD	n	mean	SD	n	
Measurement														
$CaCO_3$ accretion (mg d^{-1})	24.29	8.85	5	38.59	12.16	4	ns	64.62	14.74	6	111.86	30.70	6	**
Vertical extension (mm)	31.38	12.57	5	40.15	9.98	4	ns	16.18	5.48	6	40.68	9.85	6	***
Maximum basal growth (mm)	56.00	4.42	5	27.52	2.30	5	*****	84.40	12.46	6	44.53	8.54	6	****
	Colony A			Colony B				Colony A			Colony B			
	mean	SD	n	mean	SD	n		mean	SD	n	mean	SD	n	
Measurement														
$CaCO_3$ accretion (mg d^{-1})	33.62	15.63	5	26.92	6.66	4	ns	73.45	21.84	6	103.03	38.69	6	ns
Vertical extension (mm)	41.20	11.43	5	27.88	7.85	4	ns	30.83	20.51	6	26.03	7.28	6	ns
Maximum basal growth (mm)	43.52	14.61	5	40.00	17.63	5	ns	62.40	18.52	6	66.53	28.78	6	ns

** $P < 0.01$
*** $P < 0.001$
**** $P < 0.0001$
***** $P < 0.00001$

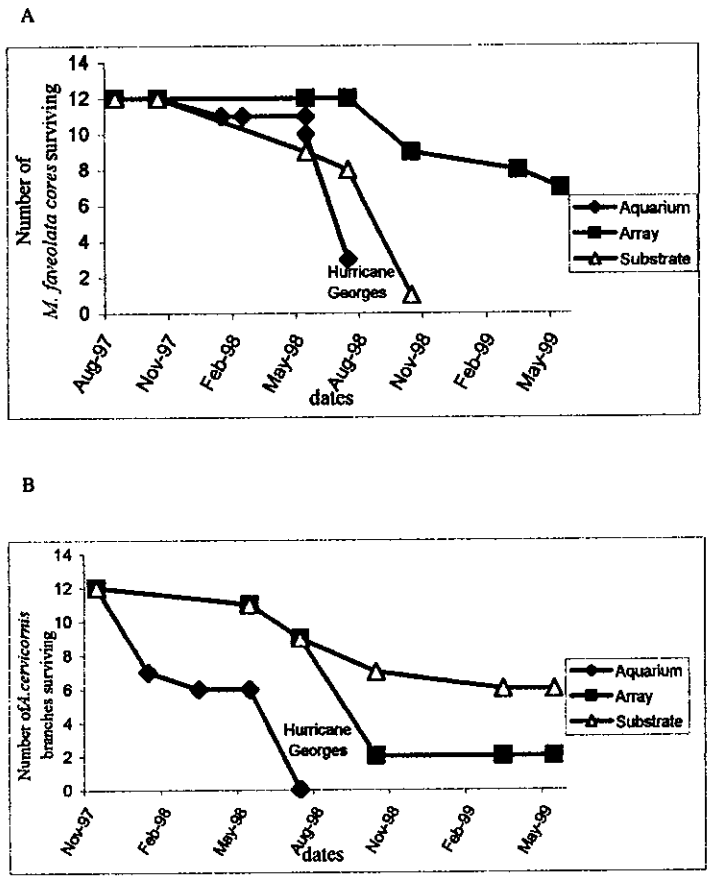

Figure 2. Graphs showing the number of live explants over time in each of the three storage treatments of (A) *Montastraea flaveolata* and (B) *Acropora cervicornis*.

The *A. cervicornis* explants placed in the tank had little or no branching while those on the array had formed 2–6 branches. The basal growth of the explants in the tank was very extensive while the basal growth in the field was minimal. This trend was also observed with *A. palmata*.

EXPERIMENT 3.—The *A. cervicornis* explants in the aquarium began to grow over their epoxy bases within weeks. After a month, they began to lighten in color; some had dark spots inside the calyxes. Necrosis of the tissue began within the next 2 wks, beginning either at the base or the growing tip and progressing toward the opposite end. Some explants no longer had white tips, indicating that they had ceased growing. The four explants from colony A were dead within 2 mo. Those from colony B died by 6 mo while some of the colony C explants survived as long as 8 mo (Fig. 2).

The *A. cervicornis* on the array showed no signs of disease. After 6 mo, one explant was lost and all but two had broken although what remained was otherwise healthy. This rendered the volume method for growth measurement invalid. After 9 mo, Hurricane Georges (25 September 1998) caused the line from a lobster trap to be wrapped around the array,

bending two branches and dislodging the corals. Two branches were retrieved and replaced onto the array; they were healthy as of May 1999 (18 mo into the experiment).

The *A. cervicornis* explants on the substrate showed no signs of disease. After 6 mo, one branch was dead from an unknown cause, one broken and another was missing. The remaining nine corals were dark in color but appeared healthy with several producing new branches. After Hurricane Georges, seven were located with live tissue, including several buried in the sediment. Some had started new branches and most were dark in color. As of May 1999, seven were still alive, dark in color and with new branches. The Kruskall-Wallis ANOVA shows a significant difference in that the aquarium had a lower survival rate than the other two treatments ($P < 0.0001$).

A possible disease was also a factor with the *M. faveolata* cores in the aquarium. After 5 mo, only one core had begun growing over the epoxy while the rest of the cores lost tissue, some down to only four polyps. In several cases, the only visible tissue left was down in the calices of a few polyps with none of the coenosteum apparent. Three of the cores, two from colony F and one from E, died. After 7 mo and the installation of an UV water sterilizer, the cores began to recover. The coenosteum reappeared and polyps reconnected. Tentacles became visible and active. After 2 mo, the cores began to again decline and die. Three more cores died at 9 mo. All of colony F was then dead as were one sample from each of the other colonies. There were other *M. faveolata* pieces in the aquarium with the cores. These had black-green lines around the edges of the live tissue. These were identified as folliculinids, ciliates that usually eat bacteria. In July 1998, there were only three cores left, all from colony E. These were taken from the aquarium and placed at the Newfound Harbor heads (RTS3).

The twelve *M. faveolata* cores on the array were all healthy with tissue growing over the epoxy until May 1999 (21 mo into the experiment). After Hurricane Georges, nine remained. As of May 1999, eight were present. Most of these had grown over the epoxy and most of the ReefMounts.

The *M. faveolata* cores on the substrate at RTS3 were all present and healthy until May 1998. At that point, three were missing and the others healthy. Three of the ReefMounts were broken and needed replacing. The cores were taken back to the PKMRC, repaired, and returned the following day. After Hurricane Georges, only one core was left. Its ReefMount had a broken stem that had been repaired with duct tape and was wedged into the hole. This core had a dead spot, possibly from injury during the storm, but was growing over the epoxy on all but a small section. The core remained there until May 1999 when it was transferred to the array at RTS2. There was notably more tissue covering the ReefMounts of the cores kept on the array than the core that had been at RTS3. A lost core was found a few days later in the surrounding rubble with live tissue and also moved to the array.

The Kruskall-Wallis ANOVA shows a significant difference in survival of the corals on the array over the other two treatments ($P < 0.0001$).

Table 3. The percentage of corals surviving in three different treatments and in two sizes.

Treatment	Location	Period (mo)	Size 2.5 cm	Size 5.1 cm
Transplant				
	† Looe Key	22		*63.8
	‡ Newfound Harbor	8	*75.0	
		14	*8.3	
Array				
	§ Tennessee Reef	12		91.7
	§ Channel #5	12		83.3
	† Looe Key (RA)	8	100.0	
		14	*75.5	
Aquarium				
	† .	10		100.0
	‡	8	25.0	

† Corals from experiment 1.

‡ Corals from experiment 3.

§ Corals from C. Cook et al., 2001

Discussion

Storage Systems.—Each storage method investigated here has potential advantages as well as disadvantages. Closed aquaria allow corals to be maintained under controlled, and potentially optimized, conditions. The potential usefulness of the closed aquaria was demonstrated with the successful storage of the 5.1 cm cores of *M. faveolata* in experiment 1. Although conditions such as temperature and light may not have been optimized, growth rates were comparable to those measured at offshore reef locations. Aquaria can also be used for culture, growing small corals to a size that is viable for transplantation. Compared to field storage, corals can be more easily monitored at virtually any desired frequency. Another advantage is that fouling is much lower and the explants require much less cleaning to weigh than cores that have been out in the field, either on an array or on the substrate. This reduces spatial competition and appears to allow the corals to increase surface area faster.

Disadvantages of closed aquaria include the highest amount of maintenance and cost, both initially and operationally, of the three options examined here. They require daily monitoring and frequent cleaning. If pathogenic organisms are introduced, as may have happened when new *M. faveolata* cores were added for experiment 3, the enclosed system may become an incubation chamber for disease. It is also possible that the pathogenic organisms were present all along but that conditions became stressful for corals and symptoms then manifested themselves. The closed system may also lead to concentrated levels of pathogens, thus helping to overcome coral defenses. The problems occurred after corals had been successfully housed in the aquaria in previous experiments so this is not a persistent problem but an incidental one. The infected aquarium has since been broken down, sterilized, and successfully restarted; corals have been introduced with no sign of the disease recurring. A remedy for this potential problem may be a quarantine tank for observation of disease signs and, possibly, preventive treatment.

It should be noted that the corals that lost tissue did not display signs of previously described diseases. The malady affecting *A. cervicornis* was somewhat like 'white-band' disease (WBD; Gladfelter et al., 1977) except that tissue loss sometimes started at the apical tip, not always at the base as classically described for white-band. However, Precht and Aronson (1997) describe WBD as progressing "usually from base to tip" but give no further description of other patterns. Tissue loss was also much slower than usually reported for white-band. Tissue loss by *M. faveolata* was gradual, leaving isolated patches of tissue. The signs were unlike any diseases described thus far. The presence of folliculinids on the *M. faveolata* may be an indication of increased bacterial levels in the aquarium since they do consume bacteria. Increased bacteria levels may have been either the source or result of disease. Folliculinids have not been implicated in any disease state previously described although they have been found associated with corals in the field (D. Santavy, per. comm.).

The open-water system at Lee Stocking Island worked very well for storage of acroporids although growth rates were generally lower than in the field. With only routine system maintenance, all but one coral survived and appeared to be healthy. However, there are considerations that must be made before using an open seawater system. The seawater source must be of high quality. This was the case at Lee Stocking Island; however, experience with open seawater systems in the Florida Keys has shown that they are not suitable for year-round maintenance of reef corals. Near-shore waters of the Keys vary beyond the tolerances of reef corals with respect to temperature. Salinity variation and periods of high turbidity are also problematic. Unless specifically treated, the incoming seawater provides opportunities for pathogens to be introduced into the system.

The arrays raised the corals off the substrate, thereby reducing the potential for sand scour, predation and competition. One of the 5.1 cm cores and a few of the *A. cervicornis* explants on the reef substrate showed signs of competition with adjacent gorgonians and other benthic organisms. The *A. cervicornis* colonies were moved but those of *M. faveolata* could not be. However, arrays are vulnerable to fishing lines and lobster trap lines moved about during storms; the array on Looe Key had to be repaired twice. The survival rate of the 2.5 cm cores on the array was 100% until the hurricane. In another experiment using the same type of array, 5.1 cm cores had survival rates of 83.3% in nearshore waters and 91.7% in offshore waters over a year (C. Cook et al. 2001; Table 3). These survival rates demonstrate that arrays may be useful for the short to long-term storage of *Montastraea* and, probably, other massive coral species. We did find that the arrays at RTS2 and in the Bahamas had a slight movement at the attachment points that may have contributed to the breakage of acroporids.

The *M. faveolata* cores were not permanently attached to the substrate in experiments 1 or 3 so fully testing the direct transplant approach was not accomplished. The cores were not securely attached because of the measurement techniques used (i.e., buoyant weight and volume). We have no doubt that using adequate amounts of epoxy would have increased the retention rates of the explants placed directly on the substrate. The same applies for the *A. cervicornis* in experiment 3. Placing *A. cervicornis* on the substrate (at 6 m depth) with the Key Largo limestone blocks was successful to a point. This method is best for very short-term storage (days to weeks) during calm conditions. The variance in calcium carbonate accretion by all three species examined was greater in the field than in the aquarium or open seawater system in all three species. This was most evident when in *M. faveolata* as it increased over time. Even though the cores were within meters of each

other, there may have been slight differences in environments. Some bleached more than others did, some were initially affected by fish grazing whiles others nearby were not, and a few were affected by proximity to gorgonians. In the first year in the field, three corals lost mass and two did so in the second year. However, the overall growth rate in the second year in the field accelerated over the two previous years. This probably has little to do with the storage strategy but is probably a consequence of the increasing perimeter and surface area. Variance is also expected to increase as faster growing explants accelerate in their growth rate. This highlights the importance of using corals of equal size (perimeter and area) over relatively short periods of time for testing various conditions.

Finally, a difference was found between the two RTS1 subsites. Even though the mounds were only 7.1 m apart, corals on the mound with closest proximity to the open water grew faster perhaps due to hydrodynamic conditions or plankton availability. There is also the possibility that the difference may simply be an artifact of the low sample size.

Morphology.—Growth morphology was affected by the storage strategy. In the field, *M. faveolata* cores that were placed in vertical or intermediate orientations on the substrate had more growth on the lower side. The tops of the ReefMounts were covered with filamentous red algae and a substantial layer of fine sediment, either of which, or both, may have inhibited the tissue from advancing. All explants in aquaria and on the arrays were maintained horizontally so the effect of the environment versus the angle itself has not been tested.

In experiment 2, the colony morphologies of both *A. cervicornis* and *A. palmata* were definitely affected by how they were stored. Basal growth was significantly higher in the seawater system than on the array for both species. This fact suggests that by placing explants in such a facility the bases would become more secure in a few months, thus adding to the chances of successful explantation.

There are several possible explanations for the morphological responses. The first difference is in water flow, unidirectional vs oscillatory. Branching in an oscillatory environment may be advantageous in that there is increased breakage. Branching fragments survive and produce a new colony, by keeping the fragment slightly off the bottom and reducing its tendency to roll. Another possible explanation is related to the light regimes. In the tank, the corals were exposed to direct sunlight only around noon; the tank walls and the heavy screening (which was fouled with algae), blocked direct light during the morning and afternoon. Also, the tank was black and did not reflect light. Growing a wide horizontal base may be advantageous for optimizing light capture in this situation. On the reef, the corals were exposed to irradiance from all directions. Branching may allow a more efficient capture of scattered light. Finally, the presence of large numbers of snails (unidentified ceriths) may have encouraged the basal growth in the tanks by removing all potentially competing algae. This is consistent with aquarium observations where the reduction of competition for space appears to encourage lateral growth. On the other hand, both species of corals calcified more in the field than in the tank, although only significantly in the *A. palmata* (it may have been significant in the *A. cervicornis* if there had not been the breakage and death that excluded three samples).

Minimum Size.—All three species appeared to survive fragmentation and handling very well; there were no obvious cases of transport or other handling morbidity or mortality. This is not surprising for the acroporids since this is a primary reproductive method for these species. These results are important for restoration efforts with *Montastraea* (Knowlton 1997). This coral is an important reef-building coral that provides much of the

frame-building on reef systems of the Caribbean. There appeared to be no difference in survival of the two different sized cores although these experiments did not provide rigorous testing of the minimum viable size (Fig. 2). The smaller (2.5 cm) cores survived well except where mechanical loss occurred and in the aquaria. This was probably a result of circumstance rather than a size effect. Thus, we conclude that the smallest viable size is probably smaller than the ~2.5 cm tested here. However, smaller sizes would be expected to make the explants more vulnerable to predation by corallivorous fishes and spatial competition. Also, there is probably little value in deploying explants of smaller size.

REEFMOUNTS.—The design of the ReefMounts made it easy to handle cores without touching live tissue. ReefMounts fit readily into inexpensive and easily made PVC racks when maintained in open or closed aquarium systems. This design does make it necessary to drill holes into the substrate for field deployment but this allows for a secure emplacement on virtually any angle (assuming that sufficient amounts of epoxy are used). The design also allows corals to grow over the edge onto surrounding substrate with a natural look.

We acquired the ReefMounts from two different sources. The ReefMounts were made of the same standard ceramic material, however, due to differences in the casting processes, we did experience structural problems with those from one of the sources that were hollow and broke easily where the stem joined the top; this accounted for virtually all of the losses in experiment 3, while this problem was nonexistent in experiment 1 where the better quality mounts were used.

GROWTH MEASUREMENTS.—In experiments 1 and 2 the buoyant weight method was used. This provides a sensitive and well-accepted measure of calcification (Jokiel, 1978; Dodge et al., 1984; Davies, 1989), however, this method also requires retrieving the cores and making measurements with a very stable balance (i.e., on land or on a large ship). Thorough removal of encrusting algae and other biota is also necessary and can be a time-consuming task. Buoyant weight is inappropriate for corals placed permanently on the reef for restoration for obvious reasons but may be appropriate to track the growth of corals while in storage. Measurement of linear extension is only an aspect of growth as is basal growth, thickening, or branching. These individual measurements are important for interpreting the types of growth but provide only a partial picture whereas changes in buoyant weight integrates all types of skeletal accretion.

A volumetric measurement was attempted in experiment 3 as a means of obtaining an integrated measure of growth in the field but was found to be problematic. The *M. faveolata* cores grew too slowly for an accurate measurement of growth. The signal-to-noise ratio was very low and no discernible growth was detected. This method may have utility but it is essential that minimum-sized vessels be used for a given coral. In the case of *A. cervicornis*, branches broke and were lost onto the reef during the experiment making it impossible to repeat measurements. Even if they did not break, branching would make it necessary to use larger vessels with the consequent reduction in the signal-to-noise ratio.

Conclusions

This research examined the minimum size of *M. faveolata* explants and different storage methods for corals. Although we did not determine the minimum viable size for *M. faveolata*, use of explants less than 2.5 cm^2 (1 in core) is probably impractical. Direct transplantation subjects the explant to only one move thus minimizes opportunity for injury. This method is of relatively low cost and maintenance. The explants will be subject to loss if the corals are not securely attached. Arrays are useful for storage and culture of explants. Arrays can reduce competitive and predatory pressures and protect corals from sand scour. They are lower maintenance and cost than aquaria but higher than direct transplantation. Arrays are susceptible to damage from lines because they are raised about the rest of the substrate. Storage in aquariums is an option for holding and culturing corals. Aquarium storage reduces encrustation on epoxy and ReefMounts and potentially provides conditions for optimum growth. Disease is a potential factor in aquariums. Aquaria require higher maintenance and cost than arrays and direct transplantation.

Other conclusions: (1) all three species examined here recovered, (2) the type of storage facility and orientation of cores effects morphology of all three species, (3) the 'dry method' is best for transportation, (4) epoxies do not appear to be toxic, (5) UV sterilizers may reduce disease incidence, and (6) disease-resistant strains may eventually be identified and used to increase the success of transplantation.

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