Ex Situ Culture of Caribbean and Pacific Coral Larvae Comparing Various Flow-Through Chambers

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ABSTRACT. Coral reefs are some of the oldest and most diverse ecosystems on our planet, yet throughout their range coral reefs are declining precipitously, mainly as the consequence of human activities. In situ conservation practices, such as habitat preservation, are an important way to protect coral reefs. However, reefs now face global threats in addition to local impacts. It is therefore critical that ex situ conservation activities are incorporated into conservation practices for coral reefs. Many coral species reproduce sexually during a limited yearly breeding season. If the resulting larvae are cultured, their husbandry can be very time consuming: time that is often taken away from larval research. Three different types of flow-through larval rearing systems were designed and tested during breeding seasons of the elkhorn coral *Acropora palmata*, the mushroom coral *Fungia scutaria*, and the cauliflower coral *Pocillopora meandrina*. The flow-through systems were tested against static bowl rearing, and no difference was observed in the survival of the larvae in two of the species: $P = 0.12$ for *A. palmata* and $P = 0.99$ for *F. scutaria*. These results suggested that these chambers may result in significant savings of limited research time during a coral spawning event. However, *P. meandrina* larval survival was better in bowls than in the flow-through chamber ($P = 0.03$). Rearing the maximum number of larvae possible with minimal maintenance will enhance opportunities for larval research, settlement, and growth. This is especially important for species that are now threatened, for which time and information are critical during the breeding season.

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INTRODUCTION

Coral reefs are some of the oldest and most diverse ecosystems on our planet. They are essential nurseries and feeding grounds for fish and invertebrates, act as natural storm barriers for coastlines, and are a potential source for novel pharmaceuticals (Colin, 1998). Throughout their range, coral reefs are declining precipitously, mainly because of human activities. These negative influences induce stress and can increase diseases in corals. Even in the most

remote marine bioreserves, such as the northwestern Hawaiian Islands (Maragos et al., 2004), human activities are damaging fragile coral ecosystems (Bellwood et al., 2004). Additionally, other environmental pressures, such as El Niño-Southern Oscillation events, result in bleaching and coral mortality (Glynn and D'Croz, 1990; Glynn, 1996). As greenhouse gases increase, atmospheric and sea-surface temperatures and ocean acidification are also expected to increase (Kleypas et al., 1999; Hoegh-Guldberg et al., 2007). When these effects are coupled with human-induced stresses, reefs will remain in crisis, their existence worldwide increasingly threatened (Hoegh-Guldberg, 1999; Hughes et al., 2003).

Scientists speculate that unless committed efforts are made to remedy this situation functional coral ecosystems may disappear in less than 50 years (World Wildlife Report, 2004; Hoegh-Guldberg et al., 2007). Although all the oceans in the world have corals, reef-building corals in the Caribbean are showing the greatest signs of disease-related mortality, and these corals may have far less than 50 years left to survive (Hoegh-Guldberg et al., 2007). The massive elkhorn coral, *Acropora palmata*, has historically been the most ecologically important reef-building coral in the Caribbean, but its populations have declined 90% to 99% since the mid-1980s, primarily because of disease (Aronson and Precht, 2001). Because of this decline and its critical role for Caribbean reefs, *A. palmata* has been one of the first two corals listed as "threatened" under the Endangered Species Act (*Acropora* Biological Review Team, 2005). As stony corals continue to die, they are being replaced with sponges, gorgonians, and algae (Hughes, 1994; McClanahan and Muthiga, 1998), altering the composition of Caribbean ecosystems.

In situ conservation practices, such as establishment of marine protected areas, are an important way to protect coral reefs. However, reefs now face global rather than just local threats. Therefore it is critical that ex situ conservation techniques are incorporated into conservation actions for coral reefs. Ex situ conservation techniques, defined as protecting organisms outside their native habitat, such as rearing sexually produced larvae in seminatural enclosures for future restoration purposes, hold strong promise for improvements in preserving species and genetic diversity within ecosystems. This stage is particularly needed to help diversify some of the declining endangered populations in Florida where many of the stands of *A. palmata* are genetically identical (Baums et al., 2005).

To address the ex situ conservation needs for coral reefs, SECORE (www.secore.org) was initiated by the Rotterdam Zoo in 2001 with the primary goals of studying sexual coral reproduction, specifically developing ex situ breeding techniques, disseminating techniques among aquarium and research communities through workshops and publications, developing a cooperative international network of public aquariums and research institutions, and establishing breeding programs to help sustain ex situ and field populations. In 2006 and 2007 SECORE members representing several national and international institutions held workshops in Puerto Rico with goals to successfully rear elkhorn coral from spawn produced during the annual mass spawning at Rincón and Bajo Gallardo sites. Gametes were collected and fertilized, producing close to a million larvae, of which hundreds of thousands were raised in the field laboratory and more than 400,000 were brought into captivity, resulting in approximately 2,300 juvenile larval recruits now living in public aquaria around the world (Petersen et al., 2007). These larvae were the first juveniles of this species ever reared in captivity, constituting a major step that will help with the conservation of their genome and restoration of this species in the wild.

Although ex situ conservation practices have yet to be applied to coral populations in conjunction with restoration, extensive work has been conducted in the zoological community on maintaining gene diversity in populations with ex situ techniques (Ballou, 1992; Harnal et al., 2002; Pukazhenthi et al., 2006). In particular, the black-footed ferret was rescued from the brink of extinction, with only 18 individuals remaining in the population, using ex situ conservation practices in parallel with restoration practices (Howard et al., 2003). Enhancing reproductive success of endangered coral through ex situ practices may be key to their future restoration and preservation (Richmond and Hunter, 1990). There are a number of ex situ techniques that have enhanced larval survival and settlement. Heyward et al. (2002) used a seminatural enhancement procedure for maintaining acroporid corals in open floating pools in the ocean. Water was pumped into the pools throughout the larval growth period, and then the contents were pumped into an enclosed area on the sea bottom with conditioned ceramic tiles. Heyward et al. started with $\sim 10.5 \times 10^6$ larvae/pool and after 144 h post-fertilization had $\sim 7.5 \times 10^5$ larvae/pool ($\sim$0.7% survival), resulting in $\sim$1,500 settled recruits in the best treatments versus 0 on the control tiles. Although this settlement rate was relatively low, it was far greater than the natural settlement rate and indicated a robust enhancement of recruits for this area.

Most current coral larval husbandry practices are low-cost efforts, such as bowls or aquaria filled with fil-

tered seawater, and these methods are very successful at rearing larvae (Babcock and Heyward, 1986; Schwartz et al., 1999; Petersen et al., 2007). The problem is that these time-consuming and labor-intensive husbandry practices compete with the limited time available for research during a coral breeding season, especially if the coral species is limited to a single annual breeding, as is *Acropora palmata*. For coral in need of replenishment, rearing the maximum number of larvae possible with the least time invested in husbandry would enhance opportunities for larval growth and settlement (Richmond and Hunter, 1990; Petersen and Tollrian, 2001; Borneman, 2006). The goal of this paper was to design and test simple flow-through systems in the field that would minimize husbandry and yet successfully rear large numbers of coral larvae without compromising survival.

Three species of coral larvae were tested in three different types of rearing chambers. These larvae were selected because they represented a good cross section of coral larval types with different buoyancies, swimming behaviors, and rates of development that might benefit from these chambers. *Acropora palmata* are large lipid-filled floating larvae (Figure 1a) that develop slow swimming ability in the water column after 48 h. *Fungia scutaria* are small negatively buoyant larvae containing modest lipid stores. These larvae develop rapid swimming behavior in the water column within 12 to 24 h (Figure 1b). *Pocillopora meandrina* are negatively buoyant larvae with modest lipid stores (Figure 1c); these larvae develop slow swimming behavior along the bottom after 24 h. In designing and constructing these low-tech chambers, we made an effort to use materials for their components that are affordable and available in most hardware stores throughout the world.

MATERIALS AND METHODS

Larval Collection and Rearing

Acropora palmata eggs and sperm were collected during the annual spawn from Tres Palmas Reserve (Rincon, Puerto Rico) and the offshore submerged bank Bajo Gallardo (Boqueron, Puerto Rico) in August 2007. Egg/sperm bundles were collected in the water over the spawning coral with 1 L plastic Nalgene bottles attached to fine mesh nets. The egg/sperm bundles were brought to shore in the plastic bottles, separated by gentle agitation, and then combined with the eggs and sperm from at least three to four individuals to yield a sperm concentration of approximately 10^6 cells/mL (final concentration in water). The eggs and sperm were gently agitated for 2 h, cleaned with 1 μm-filtered seawater, assessed for fertilization rates, and released into rearing chambers for subsequent development.

Fungia scutaria eggs and sperm were collected from captive animals held in flowing seawater tanks from June through October 2006 at Coconut Island, Hawaii. Animals were prepared for spawning following the methods of Krupp (1983). Briefly, as a female spawned, these eggs were gently moved into a plastic bowl and fertilized with ~150 mL sperm (10^6 cells/mL, final concentration in water) from four or five males. The embryos, resulting from several male and female gametes, were kept in a single plastic bowl (8 L) and left overnight to develop. In the morning the developing larvae were cleaned with four changes of 0.5 μm-filtered seawater and then released into their rearing chambers for subsequent development.

Egg/sperm bundles were collected from *Pocillopora meandrina* fragments in April and May 2008 from Coconut Island, Hawaii. The eggs and sperm were separated

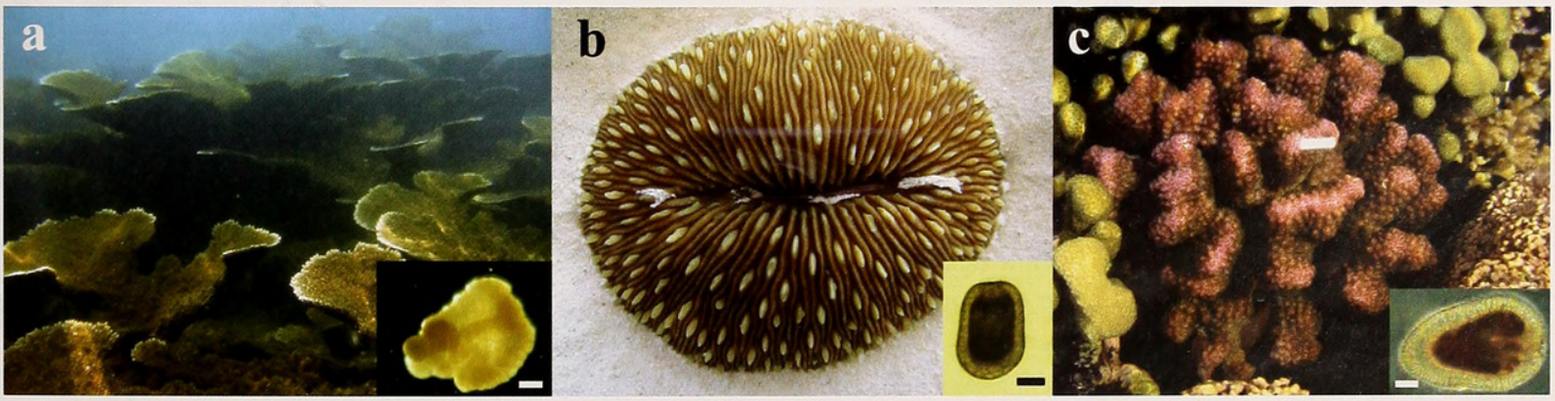

FIGURE 1. Three species of coral were reared in this study. a, *Acropora palmata*, elkhorn coral; inset: larvae at 24 h postfertilization. b, *Fungia scutaria*, mushroom coral; inset: larvae at 96 h postfertilization. c, *Pocillopora meandrina*, cauliflower coral; inset: larvae at 96 h postfertilization. All scales = 50 μm.

by gentle agitation, and then combined with the eggs and sperm from at least three or four individuals to yield a sperm concentration of approximately 10^6 cells/mL. The eggs and sperm were gently agitated for 0.5 h, cleaned with 0.5 μm-filtered seawater, and left overnight to develop. The next morning the developing larvae were cleaned with 0.5 μm-filtered seawater and released into their rearing chambers for subsequent development.

Digital images of the larvae from all three species were captured with an Olympus BX41 microscope with an attached digital camera Sony DFWV300, and the major and minor axes were measured with NIH Image software.

Construction of Rearing Tanks and Measurement of Densities

Larval corals were reared in three different designs of flow-through chambers (Figures 2–4), as well as static bowls that required daily cleaning and water changes. The names of these chambers were chosen to describe the major water movement they provided to the larvae. All developmental times reported throughout the paper are in hours postfertilization.

Up-Flowing Tanks

These tanks were made from 20 L heavy-walled plastic pans (U.S. Plastics Corp., Lima, Ohio) modified by covering the handles in a buoyant foam and removing four panels from the bottom and replacing them with nylon screening (240 μm mesh). A central cross-shaped area was left intact to create an inlet for upward-directed water flow; then additional shear flow was added with four additional adjustable water inlets around the edges approximately 16 cm above the bottom, yielding a final volume in the chambers of ~23 L (see Figure 2). All flow was regulated by valves to optimize the slow tumbling movement of the larvae in the chamber. The floating chambers were immersed in large 2,400 L pools to stabilize their temperature (28°–31°C) and mimic natural temperature cycles throughout a 24 h period. To maintain water quality close to that which the larvae would experience in open water, the chambers were attached to a filtered (1 μm) flow-through system with seawater pumped from the reserve, so that water was completely exchanged in the chambers several times each day. Flow rates through the chambers were maintained at approximately 2 L/min, and the bath of fresh seawater surrounding the chambers was turned over about one to two times per hour. Salinity, temperature, and pH closely mimicked natural conditions without additional effort.

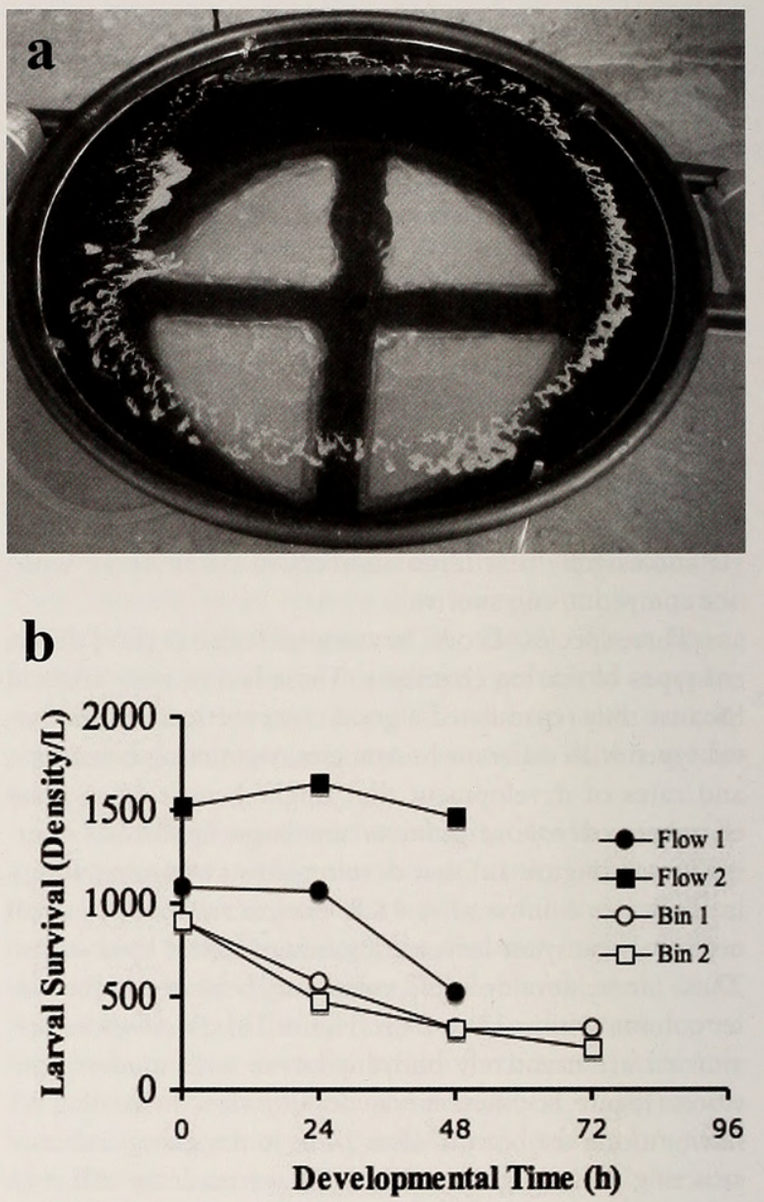

FIGURE 2. Larval rearing of *Acropora palmata* larvae. a, Up-flowing tank used to rear the *A. palmata* larvae. b, There was no significant difference in survival between the up-flowing tanks and static bins (ANCOVA, $P = 0.12$). Points show mean counts for each trial. Larvae in the upward-flow tanks were developing rapidly (~28°C) while those in the static bins were maintained at a slightly lower temperature (~25°C). Larvae in the flow chambers were removed from the experiment one day earlier than those in the bins to ship them to an aquarium for settlement and rearing.

Approximately 1,000 to 1,500 larvae/L were fertilized in 50 mL conical plastic tubes, then placed into either the up-flowing tanks ($n = 2$) or static bins. Counts were taken immediately, and then daily for all groups. Bin density began at ~900 larvae/L, and the two flow chambers contained either 1,100 or 1,500 larvae/L. The fertilization rate for *A. palmata* spawn used for these tests was ~90%.

The static treatments used for comparison with the up-flowing tanks were plastic rectangular bins (length [L] × width [W] = 51 cm × 36 cm) with water depth of 12 cm, yielding a volume of 22 L. These tanks were maintained in an air-conditioned room; the water was maintained at 25° to 26°C and changed twice daily. To keep the floating *A. palmata* larvae from clumping and forming an anoxic layer, the water and floating larvae were stirred every hour with a bubble-wand (2-mm-diameter rigid air line attached to a small air pump) throughout the rearing period. The previous year, a stocking density of approximately 1,000 *A. palmata* larvae/L was used successfully in each bin and we used this same level for these tests. Larvae from the same spawn and bulk fertilization as were used in the chambers were placed in the static bins (n = 2) 2 h after fertilization.

To determine larval survival, the chamber and static containers were stirred to suspend the larvae evenly in the water column, and five 15 mL samples were taken and the number of larvae counted each day. The number of larvae/mL was multiplied by 1,000 to determine the density per liter (density/L). The larvae in both systems were only allowed to develop for two to three days, and then they were packaged for shipment. Approximately 4,000 larvae were placed into a 2 L Nalgene bottle with filtered seawater (FSW); the bottles were filled to the top with FSW and capped leaving no bubbles, taped for security, placed horizontally in a cooler (8–12 bottles were placed in a single cooler), and sent by express mail to aquaria throughout the USA.

Spiral-Flowing Tanks

Small conical fiberglass tanks (~75 L) were fitted with a central standpipe covered with 40 μm nylon mesh to rear *Fungia scutaria* larvae (see Figure 3). To maintain the water quality close to that which the larvae would experience under natural conditions, the conical tanks were attached to a filtered (0.5 μm) flow-through system with seawater

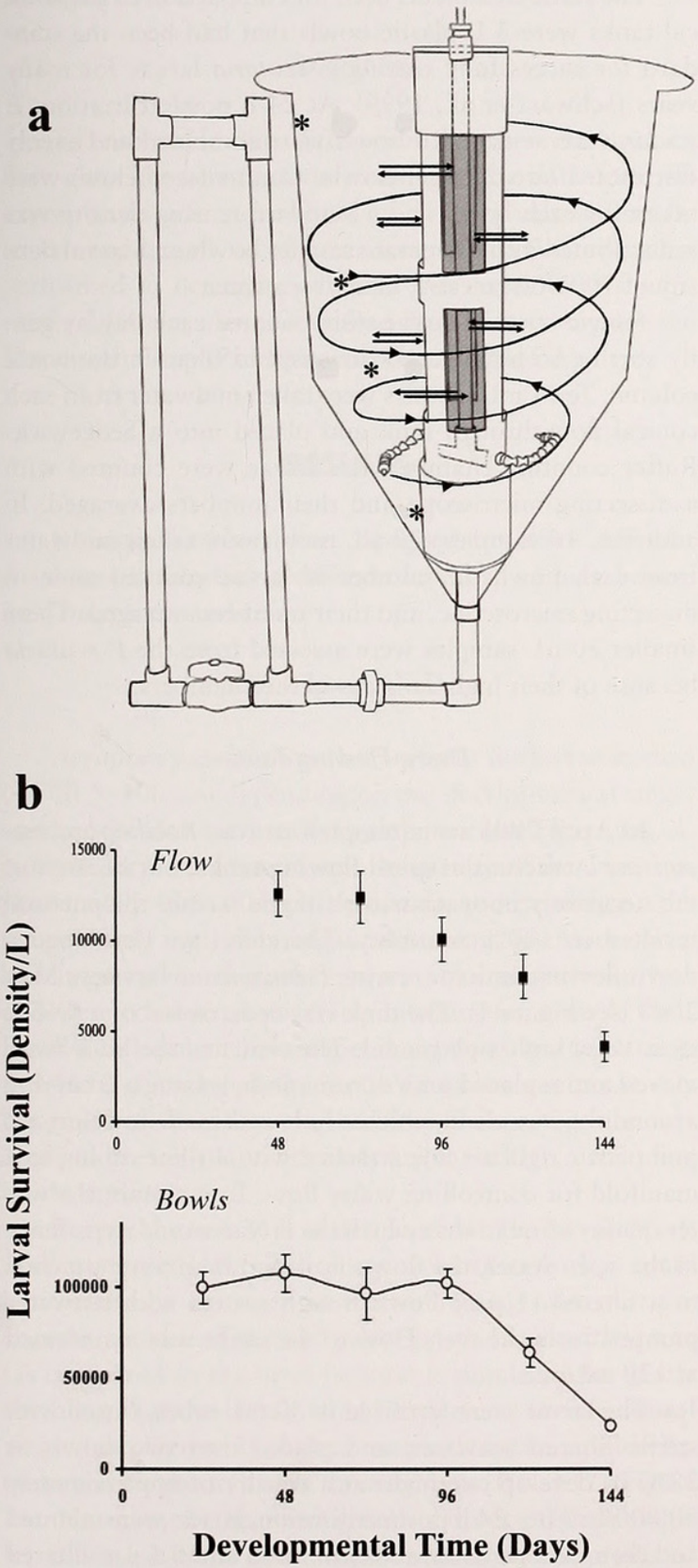

FIGURE 3. Larval rearing of *Fungia scutaria*. a, Drawing of assembled spiral-flowing tank and its flow, mixing, and position of dye experiments. Curved lines with arrowheads indicate direction of water flow spiraling upward from one inlet of a Loc-Line (both inlets had flow, but for simplicity flow from only one is drawn). Double arrows indicate water freely flows into and back out of the mesh areas on the standpipe. Asterisks (*) indicate locations in the water column where dye was injected for dye tests. b, Survival rate of *F. scutaria* larvae maintained in the spiral-flowing tank (upper graph, "Flow") and the static bowls (lower graph, "Bowls") between 24 and 144 h postfertilization. Each point shows mean and standard error. There was no difference in survival of larvae from flow chambers and static bowls (ANCOVA, P = 0.99).

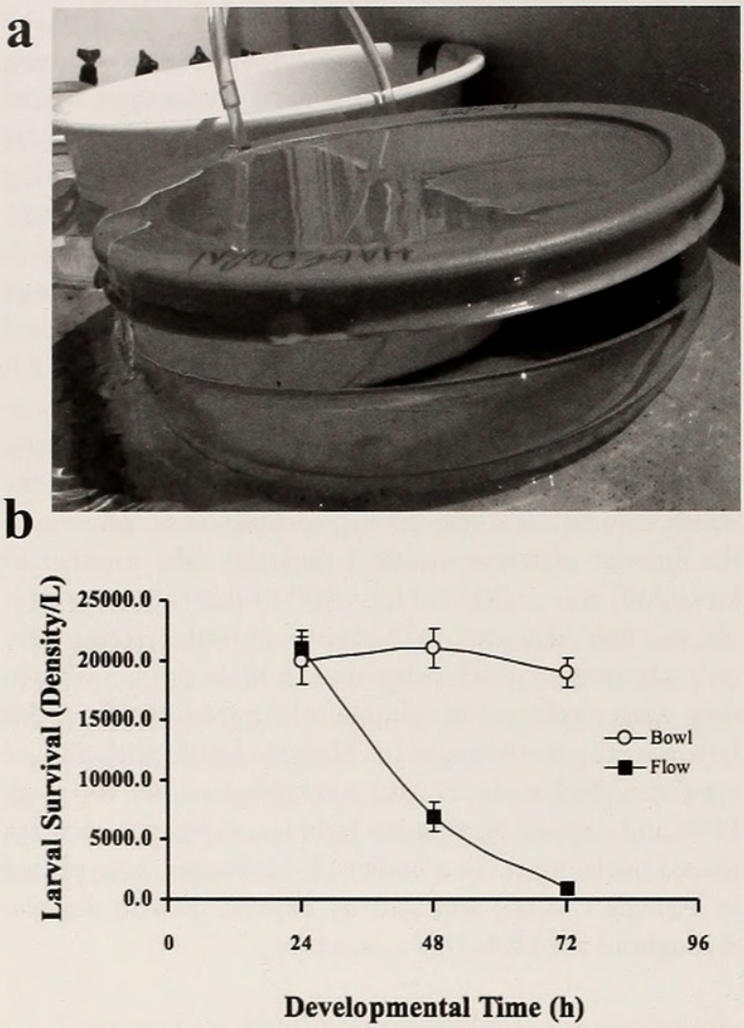

FIGURE 4. Larval rearing of *Pocillopora meandrina*. a, Down-flowing tank used to rear *P. meandrina*. b, Curves for larval survival in the static ("Bowl") and down-flowing ("Flow") tanks did not have the same slope (ANCOVA, $P = 0.03$); bowl rearing for this species produced substantially better survival than the down-flowing tank. Points show mean counts for each trial and standard error of the mean.

pumped from the reef. Flowing filtered seawater entered the top of the central tube and moved through nozzles at the tank base to produce gentle circular movement throughout the water column, and the wastewater exited the tank through the mesh-covered outflow. The flow rate was 150 to 300 mL/min, producing a complete turnover in the tanks approximately every 4 to 8 h. To test whether the conical tank could support the growth and development of *F. scutaria*, approximately 10,000 larvae/L were stocked in the conical tanks ($n = 4$) tanks. To reduce mortality of the early fragile stages (0–24 h postfertilization) from excessive motion in the flow-through chambers, the spiral-flow tanks were tested with 24 h postfertilization larvae. Therefore fertilization rate was not an issue, because all the larvae used for these tests were intact and developing. A comparison of the static bowl method (with daily cleaning) and the flow-through method was then performed to determine survival rate over time (up to 144 h postfertilization).

The static treatments used for comparison to the conical tanks were 3 L plastic bowls that had been the standard for successfully rearing *F. scutaria* larvae for many years (Schwarz et al., 1999). At 24 h postfertilization, *F. scutaria* larvae from 10 bowls were combined and evenly distributed into 2 larger bowls. Counts (see below) were taken for each bowl, and a standard rearing density was redistributed into 8 separate smaller bowls at a larval density of 100,000 larvae/L filtered seawater.

Fungia scutaria larvae were counted each day by gently stirring to homogeneously suspend them in the water column. Ten 1 mL samples were taken midwater from each conical flow-through tank and placed into a Sedgewick-Rafter counting chamber; the larvae were counted with a dissecting microscope and their numbers averaged. In addition, 10 samples (20 μL each) were taken midwater from each bowl, the number of larvae counted under a dissecting microscope, and their numbers averaged. These smaller 20 μL samples were assessed from the *F. scutaria* because of their high densities in the chambers.

Down-Flowing Tank

In April 2008 we attempted to rear *Pocillopora meandrina* larvae in the spiral-flowing tanks, but because of the negatively buoyant nature of the larvae, this method resulted in 100% mortality. Therefore, we developed a down-flowing tank for rearing *P. meandrina* larvae in May 2008 (see Figure 4). The tank was constructed of a 1.65 L glass bowl with a plastic lid. The center of the lid was removed and replaced with 40 μm mesh, leaving a 2 cm ring around the outside in which a hole was made to insert a 2 mm plastic rigid air line attached with air-line tubing to a manifold for controlling water flow. To maintain the water quality close to that which the larvae would experience in the open water, the down-flowing tanks were attached to a filtered (1 μm) flow-through system with seawater pumped from the reef. Flow to the tanks was maintained at 120 mL/min.

The larvae were fertilized in 50 mL tubes, rinsed with sterile filtered seawater, and placed into two bowls at 28°C to develop overnight at a density of approximately 80,000/L. After 24 h postfertilization, larvae were counted and then cleaned using a 40 μm mesh and 0.5 μm-filtered

seawater. One group was placed into the downward-flow chamber at a flow rate of 120 mL/min with a 40 μm mesh top to allow the water to flow out. The other bowl remained static. *P. meandrina* larvae were cleaned (static bowl only) and counted daily from the flow tanks and static bowl (maintained as described for *F. scutaria* above) for comparison.

Statistics

To determine the differences between survival in flow chamber versus static treatment, the data from all experiments was normalized and the *y*-values linearly transformed; analysis of covariance (ANCOVA) was then performed to determine whether the slopes were significantly different, using GraphPad Prism 5 software for the Macintosh GraphPad Software (San Diego, CA).

RESULTS

Rearing chambers were designed for three different coral species exhibiting different larval swimming behaviors, buoyancy, and sizes.

Up-Flowing Tank

Acropora palmata was the largest of the larvae studied (~700 × 500 μm depending on the developmental stage) and had the slowest rate of development (see Figure 1a). *A. palmata* larvae float at or near the surface of the water approximately 48 to 60 h postfertilization (depending on the temperature) until they began swimming. Even once they had begun swimming, they swam at or near the surface and were considered positively buoyant for most of their larval development before metamorphosis and settlement (~144 h). Clearly, all the larvae must become negatively buoyant before settlement; therefore, these categories only apply to the early larval periods (up to ~120 h, depending on the species).

During the first 24 h of development, the larvae developed asymmetrical, small protrusions of cells that could have easily be damaged in the up-flowing tank, but the chamber produced normally developed *A. palmata* larvae (as compared to the bins) because it simulated the gentle tumbling that the larvae would experience in the natural water (see Figure 2). Larval survival in the up-flowing tanks was similar to that in the static bins ($P = 0.12$) (Figure 2a). However, the up-flowing tank did not produce viable larvae for *F. scutaria* and *P. meandrina*. This tank produced 100% mortality in *F. scutaria* larvae within 24 h, even if the larvae were slightly more developed when placed in the chambers.

Spiral-Flowing Tank

Dye injection studies were performed on the spiral-flowing tank, using food coloring to examine the mixing properties of the vessel with an inlet flow of 150 mL/min. Figure 3 illustrates the mixing pattern in the flow-through vessel. Flow into and out of the system was equal, but the open area of the slits in the 10 cm central polyvinyl chloride (PVC) tube dictated the velocity through the screens. The nozzles were angled slightly downward to promote turbulence at the bottom to keep the larvae well mixed. The 180° positions of the nozzles provided rotation within the water column and encouraged mixing. Dye studies with separate injections were made at positions noted by asterisks (*) in Figure 3. At a flow of 150 mL/min, full vertical mixing occurred within minutes.

Developing *F. scutaria* larvae were fairly small (~200 × 100 μm) and fragile during the first 12 h of development and were just negatively buoyant during their early embryonic period (0–12 h postfertilization) (Figure 1b). However, once they began swimming, they were evenly distributed in the water column, and we considered this species to be neutrally buoyant. There was no difference in the survival between the spiral-flowing tank and bowls ($P = 0.99$). Both rearing systems produced similar survival rates in which the densities remained relatively steady through 96 h postfertilization and then dropped off at 120 to 144 h postfertilization (see Figure 3). This decrease in densities may reflect the complete absorption of stored fats (M. Hagedorn, unpublished data), as these larvae did not have zooxanthellae. *P. meandrina* larvae were tested in the spiral-flow tank, but 100% mortality was observed after 48 h postfertilization.

Down-Flowing Tank

Pocillopora meandrina larvae were the smallest of the larvae tested (~120 × 40 μm); they began slow swimming at 24 h but were negatively buoyant for the remainder of their larval development, remaining on or near the bottom (Figure 1c). Similar to *F. scutaria*, *P. meandrina* larvae were relatively susceptible to damage within the first 24 h, so they were reared in 3 L bowls for the first 24 h. The down-flowing tank was used for rearing *P. meandrina*; however, the static bowls appeared superior to the down-flowing tank for this species ($P = 0.03$) (see Figure 4).

DISCUSSION

For many years, large numbers of coral larvae have been reared successfully using simple husbandry methods such as static bowls and tanks. We have demonstrated that species of buoyant and neutrally buoyant coral larvae have similar survival in either static or flow-through chambers (see Figures 2, 3). These devices have proven to be very useful in improving culture conditions to reduce husbandry labor because neither embryos nor fresh water needed to be constantly transferred.

Modified examples of the up-flowing tank have already been used successfully by coral restoration biologists in the field (Margaret Miller, NOAA Southwest Fisheries Center, personal communication). *Montastraea faveolata* and *Diploria strigosa* were reared successfully in the up-flowing tanks and shipped to Columbus Zoo and Aquarium for settlement with 3-month survival as high as 65% and 45% for each species, respectively. Thus, the up-flowing tank has proven to be both practical, in that it can be adapted to the researcher's needs, and valuable, because it reduced husbandry time and facilitated restoration science under field conditions.

In weighing the benefits of each rearing system, one of the biggest factors to consider is time. For species that have only a single breeding season consisting of a few days, time available for conservation and restoration research is precious, and any time savings is a benefit. Moreover, the time remaining for some species that are threatened has become critical, and restoration practices need to be improved. *Acropora palmata* (elkhorn coral) and *Acropora cervicornis* (staghorn coral) were the first corals to be listed as threatened species under the U.S. Endangered Species Act. These major reef-building species once formed dense thickets and stands in the Caribbean. Today, these two species are currently at 1% to 20% of their historical levels throughout their range (Bruckner, 2003). Here we describe only one aspect of an ex situ conservation process, namely improved rearing associated with yielding better time management.

However, both the static and flow-through methods described here have their strengths and weaknesses. The static method was inexpensive to set up in terms of equipment and space. For example, 60 bowls can be maintained in two double-tiered flowing water tables taking up only about 2.5 m^2; however, this method was very expensive in terms of labor needed for cleaning ($\sim$5,000 h $year^{-1}$). The flow-through system was more costly to set up because it required a filtered flow-through water system and specially constructed rearing chambers. The amount of salary needed to pay one person for a season cleaning larvae, however, far exceeds the cost of the filtered seawater system and rearing chambers. The flow-through chambers required more space than the bowls, but each flow-through vessel could maintain almost three times the density (in $\sim$0.25 m^2) than was ordinarily maintained in a static bowl and with little maintenance time required.

One of the major issues facing biologists in rearing coral larvae is how to keep them cool (28°–30°C) under field conditions. During daylight hours, static bins left outside without any cooling mechanism can easily reach 31° to 33°C, which is lethal for most species. The rearing data in Figure 3 reflect some of these issues. These data were not exactly comparable, because they did not have the same developmental temperatures. Had the static bins been maintained at 28° to 30°C (as were the flow chambers), possibly their survival would have been far worse, because their water quality would decay so rapidly. Because *A. palmata* is an endangered species, our goal was to produce the most larvae for captive maintenance in public zoos and aquaria (Petersen et al., 2007), this required having static "backup" bins maintained at a slightly cooler temperature to provide the larvae sufficient development time in transit to reach their respective sites before settlement. However, without an air-conditioned room to cool the bins, this would not have been possible, making this impractical under some field conditions.

Within the first 24 h of development, many coral larvae are susceptible to fragmentation by mechanical disruption. However, the water movement within the up-flowing tank and potential contact with the walls did not cause substantial fragmentation of *A. palmata* during early development, even when the *A. palmata* larvae were placed in the chambers within the first few hours after fertilization. In contrast, *P. meandrina* was far more delicate, did not develop strong swimming behaviors, and could not withstand the water movements in the flow chambers. *F. scutaria* larvae are negatively/neutrally buoyant larvae that develop strong swimming behaviors within the first 12 to 24 h, and the spiral-flow system shown in Figure 3 functioned well for them, because the water flow is upward and any disintegrating unfertilized oocytes and larvae passed through the mesh, allowing for the maintenance of excellent water quality in the rearing chambers. However, no one type of rearing chamber can be applied universally across species. Instead, the type of water flow within the chamber must be matched with the buoyancy and early swimming behavior of the larvae. Regardless, these readily built and easily maintained flow-through chambers may be a substantial aid to coral conservation and restoration.

Acknowledgments

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