

ADVANCES IN CAPTIVE HUSBANDRY AND PROPAGATION: AN EASILY UTILIZED REEF REPLENISHMENT MEANS FROM THE PRIVATE SECTOR?

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ABSTRACT

Over the past decade, advances in the husbandry of captive marine habitats in private aquaria has allowed for the breeding and propagation of marine invertebrates to become commonplace. The vegetative propagation of Scleractinia, Octocorallia, Zoantharia, Corallimorpharia and Porifera are accomplished by simple and effective means, and provide for a significant amount of organisms within the private sector. These techniques can be easily utilized for large-scale grow-out facilities, and many such facilities currently exist to provide for the reef aquarium trade. Local efforts commonly include ‘coral fragment trading’ from within the populace. More recently, the cultivation of small benthic and pelagic fauna and macroalgae has also begun. To date, over 150 species of Scleractinia, 100 species of symbiotic Octocorallia, and virtually all available Zoantharia are being propagated regularly by asexual means, with few limitations as to the potential number of species which could be grown. Sexual reproduction of corals in captivity, while once extremely rare and although still not completely predictable, is being reported regularly by aquarists of all levels of expertise. Growth rates easily match or exceed those found in the wild, and organisms can be raised in areas free of storm damage, predation, encroachment, and the resultant economic and time losses inherent to in situ propagation efforts. The use of coral transplantation efforts, by which some amount of both habitat and organism loss results from the transplantation, could be largely overcome by utilizing the simple and inexpensive means already available. A lack of communication between the scientific community, public aquaria, and the private sector may be responsible for under-exploitation of a largely unrecognized, accessible, and non-destructive partial solution to repopulating damaged natural reef communities.

For well over 10 yrs, private ‘reef’ aquarium husbandry has progressed at a rate that has, in many ways, far surpassed the best efforts of public aquaria. Beginning with natural ecosystem modeling using the patterns of the “Berlin” method (Delbeek and Sprung, 1994), Algae Turf Scrubbing (Adey, 1983), and the Jaubert Microcean system (Jaubert, 1989), successful contemporary aquarium methodology utilizes biodiversity to maintain high water quality in closed systems. With these methods, the growth and reproduction of both vertebrate and invertebrate species has become commonplace for many species. Partial and mass sexual spawnings have occurred in established aquaria around the world, and are becoming more frequent (Nilsen, 1998; Tyree, 1994). Successful recruitment during spawning events in captivity is currently low because of the unpredictable nature of the occurrences and the loss of gametes through filtration devices employed in many systems. Highly successful unfiltered natural systems and control over tank parameters and spawning events are increasing, and will likely result in rapid advances in the recruitment rates of such spawning events. More significantly, asexual propagation of corals is regularly utilized on both commercial and local levels. Growth rates of corals in captivity frequently meet or exceed those in the wild (Bates, 1997; Bingman, 1998).

As reefs come under increasing pressures, many papers have focused on the methods and results of reef replenishment (Auberson, 1982; Bak and Criens, 1981; Clark and

Edwards, 1995; Harriott and Fisk, 1988; Plucer-Rosario and Randall, 1987; Rinkevich, 1995; Sakai et. al., 1989; van Treeck and Schuhmacher, 1997; Yap and Gomez, 1985; Yap et. al., 1998). Frequently, transplantation and re-introduction of various species have been utilized, sometimes including entire reef transects (Munoz, 1997). While some efforts have been remarkable, others have not fared as well. Predation, sedimentation, and mortality frequently occur in transplanted colonies (Bak and Criens, 1981; Sakai et al., 1989; Yap and Gomez, 1985; Yap et al., 1998). The efforts of removing colonies to establish viable colonies elsewhere is labor and time intensive, costly, and has the distinct disadvantage of removing species from one area to populate another (Carlson, 1999). Even if fragmentation methods are employed, rather than removing whole colonies, the resultant injury caused by breakage can lead to the partial or total loss of the parent colony from encroachment by invasive and fouling organisms or infection (Bak and Criens, 1981). Utilization of some of the methods outlined in this paper could help to avoid many of these pitfalls; infections, disease, predation, and encroaching organisms can be managed by quarantine, organism removal, direct treatment protocols (Borneman, 2001), and by the lack of such organisms in closed systems.

One of the primary concerns in attempting to re-establish damaged or threatened reefs is the ability of an area to be suitable for re-establishment of local species. In areas damaged by storms or mechanical accidents, replenishment efforts can usually take place successfully and have been documented in studies too numerous to mention. However, in those areas in which chemical insult has occurred, or those areas subjected to high nutrients, sedimentation, or other long-term stresses, utilization of the methods outlined in this paper could act as a test of the ability of a reef to accept re-population or replenishment efforts. This could occur without collateral loss of reef habitat from other areas and without the efforts and costs entailed in the removal and transfer of colonies from other areas. Corals grown by asexual means are of the same genotype and their use in replenishment of the same region where collected could serve to decrease the diversity of the gene pool of that region. However, coral species from various regions and with various genotypes, grown by culture, could be reintroduced to actually increase the diversity or number of damaged, declining, or impoverished reef areas. Additionally, those grown from sexual spawns can be similarly utilized (Petersen and Tollrian, 1999; Richmond, 1999). Finally, species grown in captivity have been overwhelmingly reported to be exceptionally durable, and may, in fact, be more likely to establish themselves and survive adverse conditions as they mature (Becker and Mueller, 1999; Carlson, 1999; Gates and Edmunds, 1999; Tullock, 1999; Wilkerson, 1996b).

This paper will attempt to summarize some of the various methods being used in the private sector to propagate and breed various marine fishes and invertebrates. It is not possible to present either an exhaustive list of species that have been propagated, nor a list of the many variations of the methods used, but an overview will allow anyone involved in reef replenishment to take advantage of these simple and inexpensive techniques. Currently, corals are propagated by asexual means, such as the removal of buds, collection of dropped branchlets, or by fragmentation. Sexual reproduction through the collection, settlement, and rearing of larvae or through captive breeding in fish and some invertebrates is also possible and increasing in importance and occurrence (Petersen and Tollrian, 1999; Richmond, 1999; Rinkevich, 1995). Finally, we investigate the potential advances in technique, which could be made in the very near future, including more frequent use of sexual

spawnings and raising of the various taxa for reef replenishment and sustainable supply of coral reef species.

METHODS AND MATERIALS USED TO AFFIX CORAL FRAGMENTS AND PROPAGULES

Affixing fragments or coral tissue is accomplished by using several types of adhesives and base materials. To avoid using living calcareous substrate, pieces of preformed 'aragocrete' can be made utilizing a mix of Portland cement and aragonite sand (Headlee, 1998; Knop, 1998). This material tends to allow crustose algae to proliferate, whereas other materials tend to enhance the settlement of encroaching and undesirable filamentous algae (Delbeek and Sprung, 1994). Acrylic or other plastics may also be used, and ceramic plates have been used extensively in fieldwork after the description by Harriott and Fisk (1987). Black acrylic and eggcrate are used extensively for an ability to grow coralline algae and for better water flow around the colony, respectively. The adhesives commonly employed are various epoxy putties and cyanoacrylate glues. Two part gel or liquid epoxies have not proven advantageous. Epoxy putties are desirable for their strength, but lack good adhesive properties in salt water and on smooth surfaces, especially where mucus and biofilms may be common. Furthermore, local loss of tissue at the adhesion site can cause a fragmented coral to become loose in the 'mold' resulting from cured putty. It is generally preferable to use high-density cyanoacrylate gels, with certain types displaying much better adhesion and curing properties in salt water than others (Headlee, 1998). A number of high-density cyanoacrylates are provided by the aquarium industry, under such names as 'Super Reef Gel'. Another product occasionally employed bears the trade name 'Ascetator', and is a formula to be mixed with Portland cement that accelerates curing time to well under 5 min. Alternately, calcium chloride can be added in differing amounts to a cement mixture to variably accelerate the curing time. A potentially ideal adhesive is unfortunately not commonly available (except in expensive sterile form), although its properties for use with coral skeletal material are excellent. Surgical Simplex (Howmedica Products, Pfizer) is a surgical adhesive used in orthopedic surgery to affix artificial joints to bone tissue. The adhesive is completely non-toxic, permanent, calcium based, and sets rapidly even when wet or submerged. It has proven to be the strongest and most durable coral fixative to date (pers. observ.). A powder and a solvent are mixed together to form a soft putty that remains flexible for approximately 5 min before undergoing an extremely rapid and complete cure with incredible adhesion. The material itself is very close to the hardness and characteristic of bone or coral skeleton. Similar two-part adhesives are used in dentistry.

PROPAGATION OF SCLERACTINIA.—The Scleractinia were initially among the most challenging to keep successfully in closed system aquaria (Carlson, 1987). However, they are now easily maintained and grow quickly, often requiring pruning to avoid outgrowing the confines of smaller aquaria (Siegel, 1998a,b). The need for pruning gave rise to novel methods of asexual propagation. Species adopting branching growth forms are typically the easiest to propagate and grow, showing similarly high natural survival rates even without care (Tunncliffe, 1981; Bowden-Kerby, 1999). Animal nail shears, metal cutters, or snips are used to break off branch tips or sections, sometimes referred to as 'nubbins' (Davies, 1995). The length of branches that can be grown out for some species can be less than 0.5 in, although almost any length can be used. We have been repeatedly able to produce entire colonies from a single, accidentally broken axial corallite of *Acropora* spp.

by carefully affixing them with superglue. Plating corals can be cut, or snapped like a potato chip. Dremel tools fitted with a cutting blade work very well to shear and divide parent colonies. The Dremel tool also works with other growth forms, and can even be used to divide sub-massive and massive colonies into fragmented daughters. Alternately, coring bits can be used with corals possessing heavier skeletons. While working with massive corals is not as easy, neither are they likely to be species most often used in reef restoration because of their slow linear growth and net effect as reef builders. Broodstock corals heal quickly and can provide a potentially limitless number of clones over time (Petersen and Tollrian, 1999). Survival of fragments by aquarists is nearly 100% even by those attempting such procedures for the first time. A list of captive-grown Scleractinia is shown in Table 1.

Another benefit of stony corals in captivity that can be utilized is their propensity to form satellite colonies by budding. Noted aquarist, Julian Sprung, has suggested that possibly all corals can form buds (Sprung, pers. comm.). While officially undocumented, the observation seems plausible based on personal observations and the collective reports from the aquarist community. Buds are formed regularly on many dozens of corals unre-

Table 1. Commercially available captive-grown Scleractinia.

<i>Acrhelia horrescens</i>	<i>A. valida</i>	<i>M. foliosa</i>
<i>Acropora abrothosensis</i>	<i>A. yongei</i>	<i>M. spongodes</i>
<i>A. austera</i>	<i>Alveopora</i> spp.	<i>M. stellata</i>
<i>A. brueggemanni</i>	<i>Anacropora forbesi</i>	<i>M. spumosa</i>
<i>A. bushyensis</i>	<i>Astreopora</i> spp.	<i>M. tuberculosa</i>
<i>A. carduus</i>	<i>Blastomussa</i> sp.	<i>Oxypora</i> sp.
<i>A. cerealis</i>	<i>Caulastrea curvata</i>	<i>Pavona cactus</i>
<i>A. chesterfieldensis</i>	<i>C. furcata</i>	<i>P. clavus</i>
<i>A. digitifera</i>	<i>Echinopora lamellosa</i>	<i>P. decussata</i>
<i>A. divaricata</i>	<i>Euphyllia cristata</i>	<i>P. frondifera</i>
<i>A. echinata</i>	<i>E. divisa</i>	<i>P. varians</i>
<i>A. elseyi</i>	<i>E. glabrescens</i>	<i>Pocillopora damicornis</i>
<i>A. formosa</i>	<i>E. parancora</i>	<i>P. eydouxi</i>
<i>A. gemmifera</i>	<i>E. paradivisa</i>	<i>P. verrucosa</i>
<i>A. horrida</i>	<i>Favia</i>	<i>P. meandrina</i>
<i>A. hyacinthus</i>	<i>Fungia</i> spp.	<i>P. woodjoneesi</i>
<i>A. kirstyae</i>	<i>Galaxea</i> sp.	<i>Porites antennuata</i>
<i>A. loripes</i>	<i>Goniopora</i> spp.	<i>P. cylindrica</i>
<i>A. lovelli</i>	<i>Hydnophora rigida</i>	<i>P. rus</i>
<i>A. microphthalmalma</i>	<i>H. exesa</i>	<i>Psammocora contigua</i>
<i>A. millepora</i>	<i>Leptosera</i> sp.	<i>Seriatopora hystrix</i>
<i>A. nana</i>	<i>Merulina</i> spp.	<i>S. caliendrum</i>
<i>A. nasuta</i>	<i>Montipora aequituberculata</i>	<i>Stylophora pistillata</i>
<i>A. nobilis</i>	<i>M. angulata</i>	<i>S. mordax</i>
<i>A. pulchra</i>	<i>M. capricornis</i>	<i>Turbinaria reniformis</i>
<i>A. samoensis</i>	<i>M. crassituberculata</i>	<i>T. mesenterina</i>
<i>A. secale</i>	<i>M. danae</i>	<i>T. peltata</i>
<i>A. tenuis</i>	<i>M. digitata</i>	
<i>A. tortuosa</i>	<i>M. efflorescens</i>	

Table 2. Some genera observed forming buds in aquaria.

<i>Alveopora</i> spp.	<i>Herpolitha</i> sp.	<i>Siderastrea</i> sp.
<i>Blastomussa</i> spp.	<i>Hydnophora</i> spp.	<i>Symphyllia</i> spp.
<i>Cynarina</i> sp.	<i>Lobophyllia</i> spp.	<i>Scolymia</i> spp.
<i>Euphyllia</i> spp.	<i>Merulina</i> spp.	<i>Trachyphyllia</i> spp.
<i>Fungia</i> spp.	<i>Nemzophyllia</i> sp.	<i>Tubastraea</i> spp.*
<i>Galaxea</i> spp.	<i>Pavona</i> spp.	<i>Turbinaria</i> spp.
<i>Goniopora</i> spp.	<i>Physogyra</i> sp.	Family <i>Faviidae</i> **
<i>Heliofungia</i> sp.	<i>Plerogyra</i> spp.	

*unique budding; not attached satellite colonies but with settlement away from colony

**positive identification not assured, but no known exceptions

ported in the literature to possess this trait. Buds are easily removed and can be attached to various media in grow-out areas. Some corals produce many buds at a time, and can do so almost continuously (Borneman, 2001; Delbeek and Sprung, 1994). Members of the Caryophyllidae, Faviidae, and Mussidae are especially prolific. Corals such as *Fungia*, which produce many juveniles from their attachment stalk, can also be moved to grow-out areas without concomitant loss of the daughters by burial, etc. (ibid). A number of novel asexual reproductive methods undocumented in the literature have also been observed in many species of both scleractinians and octocorals (Borneman, submitted). A list of Scleractinia that form buds in aquaria is shown in Table 2.

PROPAGATION OF OCTOCORALLIA.—While not typically considered hermatypic, some Alcyonaceans are hermatypic and their sclerites contribute to reef diagenesis (Schuhmacher, 1997). Furthermore, their diversity and presence in many reef areas as important constituents or even dominant taxa, along with their roles in the ecology of the reef and to their associated and commensal organisms cannot be overlooked (Fabricius, 1995). Furthermore, the octocorals produce many known and currently undescribed secondary metabolites of commercial and pharmaceutical importance (Sammarco and Coll, 1987).

Some of the first corals to be propagated were various members of the Alyconaria (Wilkens, 1992; Wilkens and Birkholtz, 1992). Simply slicing a parent colony into parts using a razor blade or similar sharp cutting instrument can asexually propagate these corals. For example, branches can be snipped from *Simularia* spp., or either vertical or horizontal sections cut from *Sarcophyton* spp. Cut areas heal rapidly, often within days. Once healed, the tissue can be placed lightly against suitable substrate and attachment commences rapidly. Attaching freshly cut tissue often results in necrosis, and allowing the cut regions to become re-sealed results in a higher success rate (Knop, 1998). Care must be taken to remove colonies from established systems prior to excisions, as the interstitial fluids and secondary metabolites released from the procedure can be detrimental to the inhabitants. Gorgonians are equally simple to propagate using the same methods. Encrusting gorgonians and stoloniferans can have their coenenchyme cut with a scalpel and lifted from substrate. At this point, the base can be either cemented or tied onto substrate until attachment. Encrusting species can also be 'trained' onto substrate placed adjacent to their spread, simply cutting the tissue once the colony has attached to the desired material. Flaccid or slimy soft corals, such as *Xenia* spp., are somewhat more difficult to manage because they lose much water when cut and cannot be glued with success. Propagated colonies can be made by slicing colonies in the process of longitudinal fission, having branches sliced off, or even by removing individual polyps. Another

method used to separate daughter colonies of many soft corals is the use of rubber bands to exert continuous pressure across a parent colony to be 'cut'. The coral heals as the pressure exerted by the rubber band slowly cuts through the parent colony. This decreases the likelihood of necrosis by invading microorganisms following direct cuts. It is, however, a much slower procedure. Because of their rapid rate of growth and healing capacity, a single Alcyonacean such as a *Simularia* sp. can produce dozens or hundreds of cuttings a year with nearly 100% survival even by beginning aquarists. Furthermore, natural buds (common with Alcyonians around the margins of the capitulum) and branchlet droppings (common with Nephtheids) can be harvested and grown out. As with the Scleractinia, very small fragments of tissue can be grown without difficulty. Many of the asymbiotic octocorals are very difficult to maintain in captivity because of their high plankton requirements, but given a plentiful source of food coupled with strong foam fractionation, similar success may be possible with asexual propagation methods.

If tissue is blotted dry prior to placement on substrate, the cyanoacrylate adhesives work well for attachment. This procedure can be used for virtually any soft coral with substantial spicule support, including the Nephtheids. However, calluses formed, heavy mucus, and the ability of many species to substantially expand their tissue volume renders glue bonds tenuous in some cases. Fishing line has been used to sew glue-resistant species onto substrate until attachment. Toothpicks can also be used to impale the tissue firmly against substrate material, or alternately, to affix the impaled toothpick to substrate with ties which would normally cut through the coral (Knop, 1997). This method also works to affix juveniles to more permanent substrate using the natural crevices and pores in the live rock (loose or broken pieces of reef growth consisting of old coral growth, coralline algae and all the associated organisms that are used as a framework in reef aquaria). Alternately, pea gravel can be used to fill a tray that is removed from strong water movement. The tissue attaches to the pea gravel, which can then be affixed to substrate using conventional adhesive techniques (Headlee, 1998). The octocorals have proven to be extremely durable in captivity and very tolerant of propagation techniques. A list of captive-grown Octocorallia is shown in Table 3.

PROPAGATION OF ZOANTHARIA.—The Zoantharia, although competitors of hermatypic corals, are still important ecological constituents of many reef areas and contribute to biodiversity. Mycosporine-like amino acids (MAA's) from zoanthids (Scelfo, 1985), as well as novel secondary metabolites show potential benefit for commercial applications. They grow so well in captivity that they frequently become 'weed' species. The means to propagate them is by separating individual polyps, stolon-formed daughter buds, or by cutting the encrusting mat in a manner similar to the methods with some of the Octocorallia

Table 3. Commercially available captive-grown Octocorallia.

<i>Alcyonium</i> spp.	<i>Eunicea</i> spp.	<i>Plexaura</i> spp.
<i>Anthelia</i> spp.	<i>Heliopora coerulea</i>	<i>Psuedoplexaura</i> spp.
<i>Briareum</i> spp.	<i>Gorgonia</i> spp.	<i>Psuedopterogorgia</i> spp.
<i>Capnella</i> spp.	<i>Lemnalia</i> spp.	<i>Pterogorgia</i> spp.
<i>Cespitularia</i> spp.	<i>Litophyton</i> spp.	<i>Sarcophyton</i> spp.
<i>Cladiella</i> spp.	<i>Lobophytum</i> spp.	<i>Simularia</i> spp.
<i>Clavularia</i> spp.	<i>Muricea</i> spp.	<i>Tubipora musica</i>
<i>Diodogorgia</i> spp.	<i>Nephtea</i> spp.	<i>Xenia</i> spp.
<i>Erythropodium</i> spp.	<i>Pachyclavularia</i> spp.	

Table 4. Commercially available captive-grown Zoantharia.

<i>Isaurus</i> spp.	<i>Parazoanthus</i> spp.	<i>Zoanthus</i> spp.
<i>Palythoa</i> spp.	<i>Protospalythoa</i> spp.	

(Wilkins, 1992). As with the octocorals, all propagation procedures should take place out of the main aquaria because of the secretions that result. They may also be trained onto substrate material placed adjacent to the colony margins. The attachments of many zoanthids is quite strong, and occasionally the surface layer of substrate must be taken by using a sharp scraping instrument like a paring knife. Because they are heavy mucus-producers, adhesives do not work well unless the tissue is blotted quite dry. Most zoanthid propagation takes place by simply allowing them to grow onto loose rubble or gravel, and then affixing the small base material to more permanent substrate. Toothpicks can be used to assist in stabilizing the polyps. Palythoids that incorporate sediments into their coenenchyme are more easily affixed with adhesives and may not require the additional loose gravel material prior to attachment. Although sexual reproduction in zoanthids is reported in captivity, fast asexual spread by budding and stolon growth easily provides large number of new colonies within months with nearly 100% survival. A list of captive-grown Zoantharia is shown in Table 4.

PROPAGATION OF CORALLIMORPHARIA.—Corallimorpharians are ahermatypic and generally uncommon in reef areas, with large tracts of colonial associations being present on some reefs. Their presence is important in terms of ecological biodiversity. Largely unstudied, the taxonomy of the Pacific corallimorpharians is just beginning (Fautin, pers. comm.). Corallimorpharians are also economically important species to the aquarium trade and have long been mainstays of the industry. They are easily propagated in a manner similar to the zoanthids. Daughter colonies naturally form from the parent base in great numbers, and they may become 'weed' species in aquaria. Daughter colonies can be severed from their parent prior to them moving away from the parent base. Alternately, parent polyps can be removed from the substrate using a scraper or sharp knife. Once separated, the corallimorphs can be propagated by slicing across their surface in sections, like a pie. Incorporating some of the mesenteries is helpful in ensuring the viability of the cut sections. The tissue pieces can then be left in a tray of pea gravel or coarse sand until they heal and form new attachments. Toothpicks can be used to assist in stabilizing the polyps or tissue until attachment (Wilkins, 1992; Headlee, 1998). It is not yet known whether this method would work for the true anemones, but the possibility certainly exists. Sexual reproduction in corallimorphs in private aquaria is reported, although settlement has not yet occurred. Asexual reproduction is done with nearly 100% survival, each individual polyps producing 2–8 daughters per propagation event with complete regeneration within months. A list of captive grown Corallimorpharia is shown in Table 5.

Table 5. Commercially available captive-grown Corallimorpharia.

<i>Actinodiscus</i> spp. (= <i>Discosoma</i> spp.)	<i>Discosoma</i> spp.	<i>Ricordea yuma</i>
<i>Amplexidiscus fenestrafer</i> (= <i>Discosoma</i> spp.)	<i>Ricordea florida</i>	<i>Rhodactis</i> spp.

Table 6. Commercially available captive-grown Porifera.

<i>Adocia</i> sp.	<i>Monanchora</i> sp.	<i>Sycon</i> sp.
<i>Aplysina</i> sp.	<i>Speciospongia</i> sp.	
<i>Haliclona</i> sp.	<i>Spongia</i> sp.	

PROPAGATION OF PORIFERA.—While not hermatypic, sponges are important and conspicuous reef organisms of ecological importance and significantly contribute to reef biodiversity. Sponges bind corals and reef substrate, significantly enhancing coral survival and coral reef regeneration and integrity (Wulff, 1999). Furthermore, their production of secondary metabolites is well documented and may have important pharmaceutical applications. The poriferans are propagated with less success than the cnidarians, simply because they are currently more difficult to maintain in captivity. This is due primarily to collection problems (air exposure, fouling) and inadequate food material in closed systems. Few sponges have yet to be found that grow well or are long-lived in captivity once collected. However, sponge growth within aquaria can be prolific. The advances in aquariology that allow for high quality water without the use of filtration, sterilization, ozone, or foam fractionation have allowed for many species of sponge to thrive (Tyree, 1998). Sponge propagation is commonly done by slicing sections of sponge tissue and tying, banding, or impaling the tissue to substrate. The regenerative and totipotent nature of sponge tissue easily allows for attachment and growth. One method used occasionally (and somewhat experimentally) is to mix sponge tissue with sea water in a blender. The resultant slurry can then be added to an aquarium with sand and rock of high spatial heterogeneity. New sponge colonies are then frequently found throughout the system. No sexual spawns of sponges have been reported in private aquaria. A list of captive-grown Porifera is shown in Table 6.

PROPAGATION AND BREEDING OF OTHER INVERTEBRATES.—The myriad of other organisms that inhabit coral reefs contribute important ecological roles in the reef environment. Estimates of diversity from aquaria equal those from reef communities (Small et al., 1998). Mass mortalities of reef organisms, including organisms playing vital ecological roles such as *Diadema antillarum*, are being reported with increasing frequency (Peters, 1997). Benthic fauna such as errant polychaetes play similarly important roles (Sorokin, 1995). The sexual and asexual culture of these organisms may therefore be of value in some reef replenishment efforts. There are no special techniques for raising many of the small invertebrates that occur as natural fauna in today's reef aquaria. They reproduce prolifically without any special care. *Berghia verrucicornis* are cultured to eradicate *Aiptasia* spp. 'weed' anemones (Borneman, 1998a; Carroll and Kempf, 1990). Spawnings of anemones are reported, although settlement and rearing of most species are not yet reported (Shimek, pers. comm.). *Entacmaea quadricolor* regularly reproduces by fission in captivity (Delbeek and Sprung, 1997). Members of Trochidae, Fissurellidae, Stomatellidae, and other gastropods and mollusks reproduce regularly by sexual spawns (Shimek, pers. comm.). Many polychaetes and small crustaceans are similarly prolific. The hydrocorals, *Millepora* spp., are as easily fragmented as the branching scleractinia (Borneman, 2001). Asymbiotic hydrocorals, such as *Distichopora* spp. and *Stylaster* spp., are not yet being raised in captivity with a high rate of success because of their planktonic requirements (Curry, pers. comm.). As captive aquaria become mature with long-term stability, it is likely that there will be many more invertebrates reproducing with regularity. The raising of various ornamental and commercially important shrimp will not be covered here, but has been accomplished. While many of the smaller benthic fauna are not

Table 7. Commercially available captive-bred and captive-grown invertebrates.

<i>Astraea</i> sp.	<i>Gammarus</i> spp.	<i>Stenoplax purpurascens</i>
<i>Aurelia aurita</i>	<i>Haliotis</i> sp.	<i>Stomatella</i> sp. (<i>S. varia</i> ?)
<i>Berghia verrucicornis</i>	<i>Hippopus hippopus</i>	<i>Strombus gigas</i>
<i>Cerithium litteratum</i>	<i>Hyalina albolineata</i>	<i>Terbellid</i> spp.
<i>Clavelina</i> sp.	<i>Millepora tenella</i>	<i>Tridachia crispata</i>
<i>Entacmaea quadricolor</i>	<i>M. dichotoma</i>	<i>Tridacna</i> spp.
<i>Eurythroë</i> spp.	<i>Mysis</i> spp.	<i>Trochus</i> sp.
<i>Fissurellidae</i> (u.k. species)	<i>Ophiostigma isocanthum</i>	<i>Turbo</i> spp.

typical constituents of reef replenishment projects, their addition to total biodiversity and their role in reef ecology may be beneficial for cases in which extreme environmental insult has occurred. A list of captive-grown and captive-bred invertebrates is shown in Table 7.

BREEDING OF MARINE FISHES.—It is unfortunate that more progress has not been made in the breeding of fishes. The low-cost availability of wild-collected specimens has somewhat thwarted widespread advances in captive breeding programs because of their limited commercial viability. Nonetheless, many species are being bred in closed systems on a local and commercial scale. Most notable are the various *Pseudochromis*, *Amphiprion*, *Premnas*, *Gobiodon*, *Gobiosoma* and *Pterapogon* spp. (Wilkerson, 1996a,b, 1998) The methods and references used in the breeding of these fishes are readily available (Tellock, 1996), and they are not discussed here. While technically not part of present reef replenishment projects, overfishing of key species, especially of herbivorous fishes, could make this an important area of future work. A list of captive-bred fishes is shown in Table 8.

Table 8. Commercially available captive-bred fishes.

<i>Amblygobius rainfordi</i>	<i>Gobiosoma oceanops</i>
<i>Amphiprion akallopsis</i>	<i>G. prochilus</i>
<i>A. akindynos</i>	<i>G. genie</i>
<i>A. clarkii</i>	<i>Meiacanthus atrodorsalis</i>
<i>A. ephippion</i>	<i>Ogilbyina novaehollandae</i>
<i>A. frenatus</i>	<i>Opistognathus aurifons</i>
<i>A. melanopus</i>	<i>Pomacanthus maculosus</i>
<i>A. ocellaris</i>	<i>Premnas biaculeatus</i>
<i>A. perideraion</i>	<i>Pseudochromis aldabrensis</i>
<i>A. percula</i>	<i>P. flavivertex</i>
<i>A. polymnus</i>	<i>P. fridmani</i>
<i>A. sandaricinas</i>	<i>P. fuscus</i>
<i>Callioplesiops altivelis</i>	<i>P. olivaceus</i>
<i>Chiloscyllium punctatum</i>	<i>P. paccagnellae</i>
<i>Coryphopterus personatus</i>	<i>P. porphyreus</i>
<i>Gobiodon citrinus</i>	<i>P. sankeyi</i>
<i>G. okinawae</i>	<i>P. splendidens</i>
<i>Gobiodon oceanops</i>	<i>P. springeri</i>
<i>G. xanthipora</i>	<i>Pterapogon kaudneri</i>
<i>Gramma loreto</i>	<i>Sepia officianalis</i>
<i>G. melacara</i>	<i>Sphaeramia nematoptera</i>
<i>Hippocampus (erectus, reidi, zosteræ)</i>	

PROPAGATION AND BREEDING OF MARINE ALGAE AND MARINE PLANTS.—Coralline algae, impacted by the CLOD pathogen (Littler and Littler, 1995), are important framework builders and provide the chemical cues for settlement of many invertebrate and coral species (Morse et al., 1999). Seagrass meadows provide important nutrient uptake for reef communities, act as buffer zones, and as breeding areas for marine species. These areas have been substantially impacted over the past decades (Durako et al., 1987). While perhaps not a typical constituent of reef replenishment projects, the overfishing of various predator populations could warrant their inclusion in future efforts. They could also be used to re-seed damaged indigenous populations, provide food for attracting the immigration of herbivores, or simply increasing natural biodiversity. Marine algae are easily grown in captive systems and do not require any special protocol other than the conditions provided by contemporary natural aquaria methods. Iron supplements are frequently employed to increase growth rates, and iodine additives are available for intensive culture of certain brown algae. Sexual reproduction and recruitment in the algae, especially *Caulerpa*, *Halimeda*, *Penicillus* and *Ulva* spp., are extremely common (Lidster, pers. comm.). The seagrasses, including *Thalassia* sp., are also easily grown under strong lighting combined with a deep substrate of organically enriched and fine particulate substrate. Settling of particulate matter from borings, coral mucus, and other metabolic processes of reef aquaria has proved adequate to sustain the nutrient requirements of most available seagrasses for their growth and spread. Flowering also occurs, although the sexual reproduction of seagrasses in captivity has not yet been documented to our knowledge (Delbeek, pers. comm.). The propagation of seagrasses may be an important means to replenish the many threatened seagrass meadows in the wild (Durako et al., 1987). A list of captive-grown and captive-bred algae and plants is shown in Table 9.

SEXUAL REPRODUCTION OF THE ANTHOZOA AND FUTURE GOALS.—Today, the partial or mass spawnings of corals in closed system aquaria is still somewhat uncommon. Most reports of mass spawnings occur in systems established for many years, but it is hoped that advances being made in captive husbandry will increase the frequency of sexual spawnings in captivity (Tarrant, 1997; Tyree, 1994; Nilsen, 1998). This is important for increasing the genetic diversity of propagated animals for both aquaria and reef replenishment uses (Petersen and Tollrian, 1999; Richmond, 1999). More frequently, individual spawnings of species occur, although the degree to which these occurrences are a normal behavior vs a stress-induced response has not yet been fully established. As plankton availability is typically magnitudes of order less than in the wild, it appears that most energy allocation is put toward growth rather than the development of gonads. *Pocillopora damicornis* does, however, planulate regularly with successful settlement. The use of plankton-culturing refugia is one solution being commonly employed to limit this drawback. One potential drawback that has been mentioned, in light of the success of captive asexual breeding, is the lack of genetic variety influencing fragmented corals through Mueller's ratchet (Toonen, 1998). While new broodstock could be a remedy to existing lines, increasing occasional sexual activity would be beneficial.

Controlling devices utilizing microchips are being used to try and increase the numbers and likelihood of sexual reproduction (Borneman, 2001). Various devices are employed to automatically monitor and regulate tank temperatures, water flow, and water quality. Pump currents are automatically lowered at night and could potentially be used to simulate tides. Surge devices are commonly employed to create natural and 'plankton friendly' flow to closed systems (Adey and Loveland, 1998; Borneman, 1998b; Carlson, 1996).

Table 9. Commercially available captive-bred and captive-grown algae and marine plants.

<i>Caulerpa racemosa</i>	<i>Neomeris annulata</i>	<i>Acetabularia</i> sp.
<i>C. mexicana</i>	<i>Sargassum</i> spp.	<i>Ochtodes</i> spp.
<i>C. taxifolia</i>	<i>Dictyota</i> spp.	<i>Penicillus</i> spp.
<i>C. sertularioides</i>	<i>Halimeda</i> spp.	<i>Ulvaria</i> spp.
<i>C. brachypus</i>	<i>Halitilon</i> spp.	<i>Gracilaria</i> spp.
<i>C. paspaloides</i>	<i>Halymenia</i> spp.	<i>Chaetomorpha</i> spp.
<i>C. prolifera</i>	<i>Codium</i> spp.	<i>Enteromorpha flexuosa</i>
<i>Ulva fasciata</i>	<i>Derbesia</i> spp.	<i>Amphiroa</i> sp.
<i>Peyssonnelia</i> sp.	<i>Mesophyllum</i> spp.	<i>Hydrolithon</i> spp.
<i>Thalassia testudinum</i>		

Temperatures can be regulated to approximate seasonal cycles. Furthermore, 'moonlight' using blue or white low-wattage incandescent bulbs is being employed to simulate lunar cycles. There are also several electronic controlling devices that are used to control and dim both fluorescent and metal halide lighting. These controllers have programs built into them that simulate various cloud covers, and some have averaged meteorological data for various reefs included in their programs. Handheld lux meters have proved valuable in establishing both day and night irradiance levels. Such advances, together with the increasing numbers of spawning reports as more aquaria become stable over long terms, make it likely that sexual reproduction will be a more regular occurrence in years to come. Inexpensive larval collectors are already available for removing planula into settlement and rearing tanks free of predation (Hernandez and Lindquist, 1999; Wilkerson, 1998). Other technologies that may be used for faster coral culture, such as larval fusion, electrolytic mineral accretion, oriented translocation, grafting, zooxanthellae inoculation, and induced injury were summarized by Borneman (2000). The Breeder's Registry is one organization created with the purpose of assimilating and distributing reports and aiding with the attempts of spawning and raising marine invertebrates and fishes. A list of species that have been reported to have spawned or bred in captivity is available through their publications (Brown, 1999).

CONCLUSION

Despite the potential negative impact of the marine aquaria trade (Lowrie and Borneman, 1999), advances and observations resulting from captive husbandry of marine organisms can be used as a conservation tool in reef restoration (Wheeler, 1996; Yates and Carlson, 1992; Carlson, 1999). In a 1994 paper to the American Association of Zoological Parks and Aquaria (AAZPA), Carlson noted several questions and issues to be addressed in this area. Surprisingly, they have been answered. There has indeed been a shift in a preference toward faster-growing, easily propagated corals such as *Acropora* spp., though their availability and survival is less than ideal in many cases. However, captive-bred colonies of the same species, once acclimated to captivity, have proved to be very adaptive and hardy. This is true to the extent that *Acropora* is, by far, the most commonly available captive-raised genus in both number and type. The ability to raise corals in private aquaria has proved to be a valuable source of specimens for trading or populating other aquaria on a local level (Tulloch, 1999). Not only have public aquaria begun disseminating information on coral culture (Carlson, 1994, 1999), but classes and workshops on propagation

have also become common at aquarium societies, in hobby publications, Internet forums, and conferences. Some propagation facilities even hold regular classes as part of their education program (Headlee, 1998; Lidster, pers. comm.). The success of asexual propagation methods could easily allow for a drastic reduction of wild collected corals, although there is still some resistance due to the total variety and value per size of collected vs captive-grown corals.

During the 1990s, reports indicate that Indonesia provided about 92–95% of the world trade in live corals and that the U.S. was purchasing 80–98% of them (Bentley, 1998; O'Shoup and Gaski, 1995). For 1999, the Indonesian Scientific Authority for CITES recommended a quota of over 1,000,000 pieces of live coral, up from 1998 (Lilley, pers. comm.). Fiji and the Solomon Islands have, over the past few years, become major exporting sources for live coral reef species, with an estimated 687 t of live corals being exported from all nations in 1997 (Green and Shirley, 1999). Fiji has become one of the largest wild-collected, coral-exporting nations. The Solomon Islands, by contrast, are providing a large percentage of maricultured corals. Trade in live corals, primarily for the U.S. aquarium industry, has been increasing dramatically over the past 10 yrs (Green and Shirley, 1999). Many of the more common species in trade are comparatively rare, slow growing, or have a low potential for survival (Lowrie and Borneman, 1999). Studies to assess the impact of collection on coral reefs are few, and there is a lack of data to show if recruitment and regrowth can keep up with current collection (Lovell, 1999). Current harvesting methods for wild colonies are typically invasive and destructive as the entire colonies are collected with some reef substrate attachment. By contrast, corals collected for mariculture or captive propagation are typically fragments or buds of existing colonies leaving the parent colony intact, with a single colony grown from a fragment able to be grown out and produce many hundreds or thousands of propagules within a few years (Bowden-Kerby, pers. comm.). It is unknown whether increases in coral culture would produce a significant increase in the demand for harvesting operations, but it is likely that necessary harvest for the parent stock would represent but a small fraction of the number of colonies currently being collected, even with increasing demand in global markets. Because the parent colonies are typically left intact in mariculture or captive propagation, the genetic diversity and species composition of harvested reefs remains unaffected. While corals grown by asexual means are of the same genotype and could decrease the diversity of the gene pool of that region, cultured coral species from various regions and with various genotypes could be reintroduced to actually increase the diversity or number of damaged, declining, or impoverished reef areas. Monitoring and record keeping of the species' location and genotype would provide proper data for replenishment use. Native peoples currently involved in wild collection could be employed in sustainable and long term farming operations. The higher prices commanded by smaller cultured corals also indicate that profits would be comparable or greater than those currently provided by wild collection. Demand for cultured corals already outstrips availability, with a great majority of aquarists indicating a preference for captive grown or maricultured species (Lowrie and Borneman, 1999). Because this is a developing industry, the costs associated with starting commercial operations for coral farming are yet to be adequately documented (Lidster, pers. comm.). However, the costs to grow corals in aquaria are quite low, consisting primarily of the electricity used to run pumps and lighting.

Some commercial facilities, such as Tropicorium in Romulus, Michigan, have 10,000 stony coral fragments and 5000 corallimorphs consistently available (Perrin, pers. comm.).

C-Quest hatchery has the ability to produce 1,000,000 fish yr⁻¹ (Wilkerson, 1997). Other facilities are equally impressive in terms of their output per square foot (Lidster, pers. comm.; Curry, 1998; Thiel, 1997). These efforts, and others, can be utilized to provide large numbers and a continuous source of coral fragments and other species for use in reef replenishment projects from existing stock in the trade and from those sustainably collected wild fragments and colonies. Moreover, cultured corals can be used for scientific studies in many areas that do not depend on in situ measurements. Facilities could be utilized to specifically address various coral populations of damaged reefs, and the species propagated to an adequate number and size within 2–4 mo. In addition, there are numerous monitoring and ‘eco-tourism’ type programs that utilize the tourist and SCUBA-diving community to aid in conservation efforts. To have a captive propagation area nearby to reefs under stress could allow for the members of such dive groups to simply ‘plant’ a coral on their dive using methods outlined here, much like reforestation efforts. Such a program would be self-rewarding, and divers could return to visit their site and check on the status of ‘their’ corals. Furthermore, the constant addition of species would negate many of the problems inherent to reef transplantation efforts: namely, a lack of funds and/or manpower to accomplish the effort, and losses due to predation and natural catastrophe. Finally, it could be helpful for those involved with reef replenishment projects to contact various public and private aquaria groups and solicit their aid in providing specimens (Carlson, 1994). Most aquarium clubs and aquarist communities have provisions for education and conservation as part of their mission, and frequently lack a means to allow for their contribution in this area.

The primary obstacle towards successful reef replenishment is in establishing species in numbers great enough to survive various influences. While the production of captive species cannot completely eliminate natural factors that influence overall survivability, the species being raised are hardier, can be produced in large amounts, and can be introduced without concomitant loss of populations from other areas. The labor and time required to collect and transport species from one reef area to another can be significantly reduced. Finally, the possibilities of introducing non-native species, pathogens, or disease is significantly reduced or eliminated by monitoring the grow-out procedure. If coupled with other programs and groups, reef replenishment utilizing captive-grown species could become a highly constructive tool for re-establishing viable reef populations in areas which have been damaged or disturbed.

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