Methods of farming sexually propagated corals and outplanting for coral reef rehabilitation; with list of references for coral reef rehabilitation through active restoration measure

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Ⅰ. English edition

1. Introduction

Restoration of coral reefs through methods such as culture and transplanting of reef-building corals (hereafter they refer corals) are still in the developmental stage. In contrast to land-based silviculture practices, the lack of state-of-the-art restoration protocols for reef rehabilitation impedes the development of technical and ecological disciplines. The lessons learned from our efforts to refine the coral farming technique at Akajima Marine Science Laboratory (AMSL) have been described in the study by Hatta et al. (2004) and Guest et al. (2010). However, these manuals do not include culturing juvenile corals and outplanting techniques (see Column 8). More than 100 *Acropora* colonies reared from eggs and outplanted at AMSL grew to >15–30 cm in diameter after 4–5 years and they are spawning every year, indicating that this culturing technique could be used to assist in coral reef rehabilitation. The present overview illustrates the entire practice of reef rehabilitation using sexually propagated corals based on our studies (Fig. 1).

Corals have two different reproductive strategies that are important to their cultivation such as broadcast spawning and brooding. Corals may also be either hermaphroditic or gonochoristic. Broadcasting species release gametes (eggs and sperm) into the water column for external fertilization followed by larval development, whereas brooding species fertilize within the polyp and fully formed larvae (called planulae) are released during spawning. Broadcast spawning can be highly synchronized within species. Broadcasters usually spawn only once each year, whereas many brooders can release planulae during consecutive months. The majority of coral studied for cultivation to date are hermaphroditic broadcast spawners including Acroporidae and Faviidae species, which occupy approximately 63% of all coral species. The remaining species are gonochoric broadcasters such as Poritidae and Fungiidae (approxi-

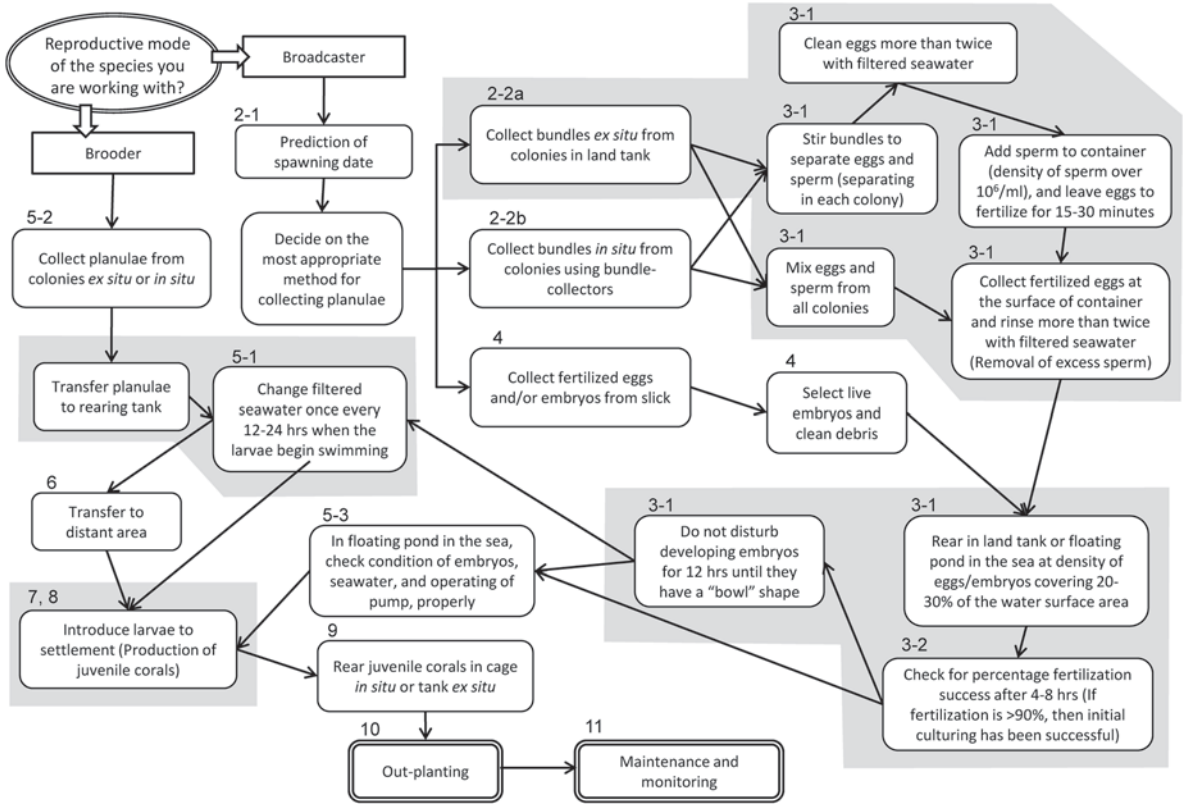

Fig. 1. Flowchart of the techniques for reef restoration with outplanting of sexually propagated corals. Numbers on the shoulder of box mean article in the text; shadow areas indicate *ex situ* operations

mately 22%), and hermachroditic or gonochoric brooders such as Pocilloporiidae (approximately 15% in total) (Guest et al. 2010).

Corals of the genus *Acropora* are highly diverse and abundant in the western Pacific Ocean, including Okinawa. They are broadcast spawners, and as their growth rate is higher than other scleractinian taxa, they are considered to be the most important genus for reef rehabilitation. Many species of *Acropora* participate in synchronous mass spawning and release millions of eggs. However, the initial loss of embryos and larvae is very high and many die before and after settlement. Synchronous spawning allows for the collection of gametes in the sea or in a container on land, fertilized, and the larvae used for coral farming. Alternatively, the embryos and larvae may be collected while they are floating on the sea surface. The larvae settle on substrate and metamorphose to juvenile corals in due course. The methods for farming and outplanting in the present study mainly consider acroporid species. When the method concerns other species, each of the species names are mentioned individually.

Three approaches are currently available to farm corals for active reef rehabilitation. The first approach is to enhance coral recruitment on artificial substrates by stocking (reseeding) planula larvae and hoping that the corals will grow well in the sea. Bundle[1)] collection→fertilization (or sampling of slicks[2)])→rearing of planula larvae→stocking (reseeding) the larvae on substrates in the sea using mesh enclosure→growth of corals.

The second is that natural settlement of coral larvae from the sea is simply encouraged using artificial substrates on coral reefs.

The third approach is to enhance mass culture of juvenile corals in a nursery followed by outplanting the juveniles on degraded reefs. Bundle collection→fertilization in aquaria on land (or sampling of slicks)→rearing of planula larvae→induction of larval settlement onto artificial substrate→farming juvenile colony→outplanting

The former two approaches are easy and do not require a long-term effort to maintain and farm the corals. However, loss of settled larvae is considerable, as the majority of them usually die because of natural causes in the sea. At present, we consider that these approaches are still not favored as a reef rehabilitation technique because of the lack of evidence for a positive long-term effect, although various artificial substrata with specially designed structures are being devised to reduce mortality.

2. Sampling of gametes

2–1. Prediction of spawning

Simultaneous mass spawning of coral, mainly *Acropora,* can be seen around the time of the full moon in May near the Yaeyama Islands of the Ryukyu Archipelago and in June at the Kerama Islands, including Akajima Island, and Okinawa Island (Hayashibara et al. 1993, Heyward et al. 1987) (Fig. 2). Spawning may be delayed by 1 month or split into 2 months depending on the lunar cycle, water temperature, and sexual maturity.

Reference to past spawning records at each location is recommended to predict the exact timing of spawning. Observation of the colonies during the spawning period from 3 days before to 7 days after the full moon is recommended. The eggs in coral polyp change from white or cream to a pastel color, often pink or orange, as the spawning date approaches (Fig. 3). Thus, examining gonad maturity by break-

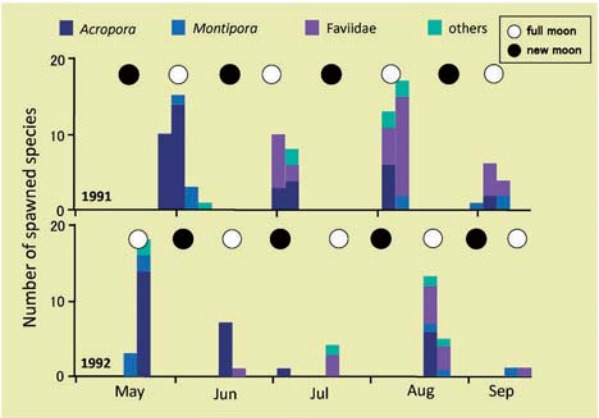

Fig. 2. Pattern of coral spawning at Akajima Island (Number of coral species spawned during each spring, middle, and neap tide periods) (after Hayashibara et al. 1993)

1) Bundle: Eggs and sperm of hermaphroditic species are usually bundied together as buoyant packages called bundles at the time of release from the polyp.

2) Slick: Aggregates of eggs and embryos floating on the sea surface.

Fig. 3. Coral gonads immediately before spawning

Fig. 4. Bundles appearing at the mouth of the coral

Fig. 5. Coral spawning

ing a branch from a colony is a useful indicator of the upcoming spawn. As the density of coral colonies has decreased recently, spawning may be delayed or split into 2 days depending on the location and even at an island. On the night of spawning gamete bundles appear in the mouth of the polyps 1–1.5 h before they are released (Fig. 4). Spawning of some corals such as Faviidae may be difficult to predict because appearance of the bundle is not definite. Fish around the coral may swim excitedly around spawning time and this behavior may be an additional indication of spawning. Spawning times vary depending on the hours of sunlight. Some species including *Acropora tenuis* spawn immediately after sunset (19:30–20:30 h), and many other species spawn 2:30–3:30 h after sunset (peak from 21:30–22:30 h) at Akajima (Fig. 5, see Fukami et al. 2003). Although density of their distribution is not large, a few species may spawn between these time zones, while the others may spawn in August or around the occurrence of the new moon.

2–2. Sampling of bundles immediately after spawning

Each acroporid polyp releases bundles. The bundles rise to the sea surface because of the buoyancy of the eggs. They subsequently dissociate and meet the gametes released from other colonies for fertilization.

2–2a. *Ex situ* sampling of bundles

The bundles may be collected *ex situ* after obtaining fragments (15–20 cm in length and width) from donor colonies using a hammer and chisel. These fragments are maintained in shallow seawater near a land-based aquaria for a few days before spawning. The fragments of *Acropora* are brought to the aquaria 1−2 h before the spawning is predicted, and each isolated fragment is maintained in a container (20 L). For corymbose corals such as *Acropora tenuis,* 3 colonies of approximately 30 cm in diameter, allow the collection of approximately 1×10^6 eggs. The fragments used for sampling the bundles should be transplanted back to the home sites within 2 weeks after spawning to minimize the stress exerted on the coral.

2–2b. *In situ* bundle sampling

Bundles can be easily collected by setting up a funnel-shaped device (bundle collector) underwater over the coral colony (Fig. 6). A rigid cone with stainless steel wire is used to construct the bundle collector by wrapping the

Fig. 6. Bundle collector (Photo courtesy of Dr. M. Hatta)

conical form with fine nylon mesh (≤300 μm). A transparent plastic screw cap bottle (500 mL to 1 L) is attached at the tip of the cone for easy attachment and detachment and a polystyrene foam float is used to keep the plastic bottle buoyant.

A few donor colonies are selected beforehand and are examined daily to predict spawning. When the bundles emerge, the bundle collector is placed over the colony and secured using string and nails. The bundle collector is allowed to set for a few hours before the expected spawning time. It is advisable to prepare whole procedure beforehand so that the work on the spawning night is minimized. If spawning does not occur, the bundle collector is removed shortly to avoid damage to the coral and prevent loss of the device.

Spawning may be completed within approximately 30 min. Because the bundles are concentrated in the cap bottle, it is required to detach the bottle immediately after spawning, cover the cap in water, and bring it back to the aquaria for fertilization. The eggs may die from the lack of oxygen if they are left in the bottle at a high density for a long time.

3. Maintenance of fertilized eggs

3–1. Fertilization

Because corals such as *Acropora* have physiological barriers to prevent the crossing of gametes from the same parent, multiple colonies are required for successful fertilization. Cross-fertilization with many colonies enhances the genetic and genotypic diversity of the population.

The collection of bundles from at least 3 colonies, but preferably 6 or more colonies is recommended for successful fertilization. Fertilization of gametes from 2 colonies may result in a low fertilization rate depending on the combination and status of the donor colonies. In addition, the bundles can be collected from cryptic species that are not easily distinguished based on morphology.

When the spawning is ceased, gather the bundles at the surface of container and stir the water with a spatula or hand to separate the eggs and sperm. If 6 donor colonies are available, we recommend that the colonies be divided into 2 separate containers for cross-fertilization. If bad eggs or eggs of cryptic species are contaminated, unfertilized eggs and abnormal embryos occur, and they can deteriorate rearing water and lead to mass decay of the entire egg supply during rearing.

For experimental purpose of fertilization or in case if the origin of gametes must be clarified, use the gametes from 1 fragment in a separate container by stirring bundles at the surface gently with a spatula. Collect the eggs with a pipette or plastic cup and transfer them to another container filled with fresh, filtered seawater. The eggs should be rinsed twice with fresh, filtered seawater by repeating this treatment. Sperm are collected from the opaque residual solution after collecting the eggs. Then, the rinsed eggs are mixed with the sperm from another fragment(s) from different container(s). The spatula, pipette, and cup are prepared for each container to prevent the contamination of sperm from other colonies.

Egg density should be such that only a thin layer of eggs cover the surface at the time of insemination. Sufficient sperm should be used to turn the seawater opaque (10^5–10^7/mL). Insemination should be completed within 4 h after spawning (Fig. 7). Allow fertilization to occur for 20–30 min, stirring occasionally and then skim the fertilized eggs from the surface using a glass or plastic cup. Transfer the fertilized eggs twice from 1 container (5–10 L) to

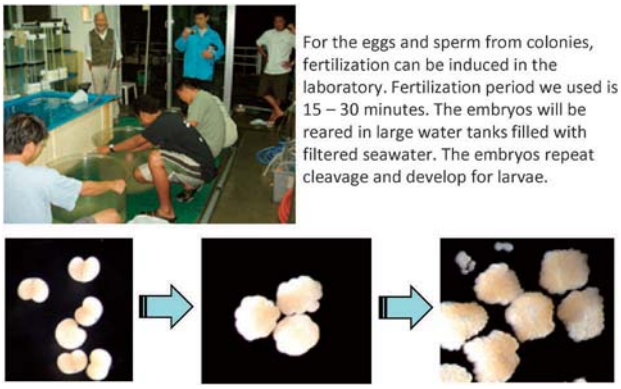

Fig. 7. Fertilization and development of embryo

another to wash away excess sperm that may cause the water quality to deteriorate. The rinsed eggs are maintained in a large rearing tank (100–500 L). Fertilized eggs should be maintained scattering thinly over 10–20% of the surface. Avoid stirring the water for 12 h after fertilization, because early development of the embryos is delicate.

3–2. Determination of the fertilization rate

The embryos develop into morulas and blastulas 4–8 h after fertilization. The fertilization ratio is measured by counting unfertilized (round in shape with no cell division) and fertilized eggs using a dissecting microscope. Under normal conditions the fertilization ratio should be $>$90%–95%.

4. Sampling of slicks after synchronous spawning

In many cases, a long narrow slick will form on the sea surface from night-time until the morning after synchronous spawning. These slicks often remain in harbors or drift onto the shore at the Ryukyu Archipelago where fringing reefs develop. When the slicks appear in a harbor or shore, they should be collected soon after spawning, as the blastomeres from the 2-cell to the morula stage can easily dissociate and die from wave impact against the pier or shore (Fig. 8).

An offshore slick can be collected by boat in the morning after spawning (Fig. 9). Slick collection is recommended, as it minimizes land-based contaminants. However, slicks may not be found because of ocean conditions.

Skim the slicks off the sea surface using a dipper and place them into a bucket with fresh seawater. The eggs and embryos that are contained in the slicks are mostly *Acropora* species, but *Montipora* and other species can also be found. Eggs and embryos of *Montipora* are distinguished from those of *Acropora* because they are smaller in size and possess zooxanthellae. The status of the embryos should be examined using a dissecting microscope before rearing. The embryos may not survive if significant dissociation of the blastomere and/or unfertilized eggs is observed. If a large quantity of foreign matter has contaminated the water, a good result could not be expected.

Fig. 8. Slicks drifting ashore inside a harbor

Fig. 9. Offshore slicks

Column 1. Spawning induction

There are a very few successful cases of artificially induced coral spawning. However, it can be accomplished with some *Acropora* and *Montipora* species using hydrogen peroxide (Fig. 10) (Hayashibara et al. 2004).

Add hydrogen peroxide (2 mM for 3 h exposure time or 5 mM for 2 h) after transferring the fragments of a mature colony from the sea to a container on land. After that, rinse the fragments to remove the hydrogen peroxide and transfer them into clean, continuous flowing seawater for spawning. Spawning by artificial induction occurs from 20:00 to 23:00 h under natural lighting, similar to natural spawning in the sea. A period of at least 9 h is needed between induction and spawning (initiation of spawning with higher probability requires 16 h). If the time is <9 h until the estimated spawning hour, induction should be postponed until the following day to secure enough time between induction and spawning. Iwao (2000) indicated that if the fragments are maintained in the light for >9 h and then in the dark by covering the container with a dark curtain, spawning occurs within 2–3.5 h.

Although fertilization of gametes and subsequent larval development has been confirmed by this method, this treatment may considerably damage the coral colonies including secretion of mucus or emission of zooxanthellae, which may lead to death. Because the degree of damage caused by hydrogen peroxide varies according to species and condition of the colonies, observing the status of the colonies during processing is recommended. The concentration of hydrogen peroxide and the length of exposure should be adjusted with the condition of the fragments for successful artificial spawning induction.

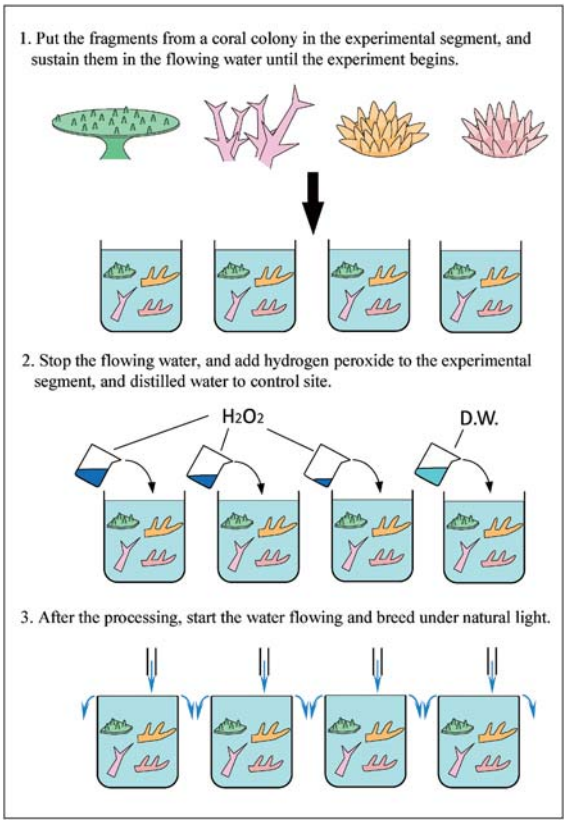

Fig. 10. Spawning induction using hydrogen peroxide (Experiment for comparison)

5. Maintenance and rearing of coral larvae

5–1. Rearing larvae in aquaria on land

Fresh seawater is very important when rearing embryos and planula larvae (Fig. 11). Twelve hours after fertilization, the seawater in the rearing tank should be changed once every 8–12 h until the larvae become competent to settle onto a substrate. During the first 2 days, as embryos are drifting to the surface, seawater in the rearing tanks can be changed easily. However, because the larvae distribute widely in the tank, changing all of the water becomes difficult. Seawater can only be changed partially. In addition, in the early stages, the larvae tend to gather at the surface edge of the rearing tanks and this causes mass mortality because of oxygen deficiency. Thus, the seawater should be mixed gently to avoid dense concentration of the larvae. Iwao devised an apparatus (trapping tank) that enables the rearing water to be replaced continuously with fresh, filtered seawater without losing the embryos and larvae from the rearing tank (Column 2).

The recommended density of larvae per rearing tank (300–500 L) is <2000 larvae/L of seawater. Divide the larvae into several rearing tanks to maintain suitable conditions and at low density without changing the water. Using eggs from bundles, Shimomura et al. (2002) successfully raised 50×10^4 larvae/ton of seawater (Fig. 12).

Fig. 11. Rearing larvae and maintenance

Fig. 12. Rearing larvae in a large water tank

When embryos and larvae collected from slicks are reared in tanks, the seawater quality may rapidly deteriorate because of the presence of foreign matter and decaying unfertilized eggs; thus, the density should be lowered. Water temperature should be maintained at $<28^{\circ}$C. Light intensity at the surface should be lowered to 70%–50% using a black shade screen when rearing outdoors.

The duration of larval settlement capability varies depending on the species and temperature. The usual period needed for the acroporid planulae to begin settling is 5.5 days at a water temperature of 26°C or 5 days at 27°C.

The density and total number of larvae raised can be estimated by counting the number of larvae in 0.1 L of seawater sampled from the rearing tank. However, it is often difficult to obtain an accurate number because the larvae are unevenly distributed on the surface during the early developmental period. In addition, larval distribution is affected by wind if a large outdoor floating pond is used. Sampling and counting are needed at least 3–5 times after gentle stirring the water to obtain reliable values.

Column 2. Rearing embryos and larvae using a flow-through trapping tank

Larval rearing in land-based aquaria is troublesome with changing water. This is particularly true in the case of large-scale rearing. We have devised a set of drainage pipes covered a large area with nylon filter mesh (60–100 μm) (Fig. 13). Fresh seawater runs from large seawater holding tank to a round trapping tank through 2 thin vinyl tubes and produces a gentle circular flow in the trapping tank to prevent dense aggregation of embryos and larvae at the surface. It is possible to rear $50–70\times10^3$ acroporid larvae using a 100 L trapping tank and save labor.

This flow-through system is also useful to rear larvae from gonochoric broadcasters and brooders that spawn eggs without bundles or release planulae over an extended period of several hours or those in which there-

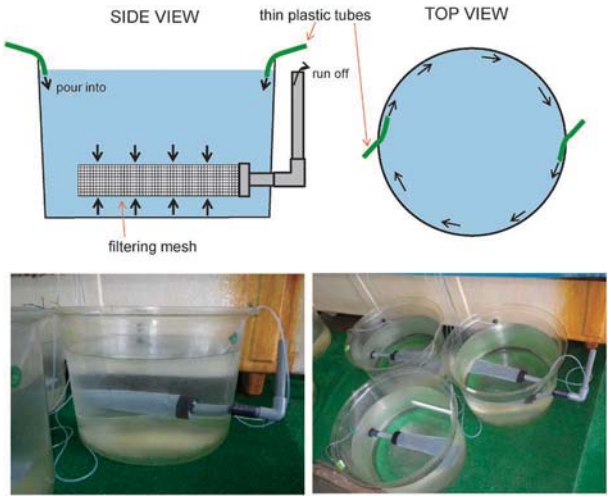

Fig. 13. Devices for rearing coral larvae in a trapping tank. Seawater is sent from a seawater holding tank to trapping tanks through thin vinyl tubes.

leasing time is unknown. When we maintained fragments of the gonochoric broadcast spawners *Fungia sctaria* and *Ctenactis echinata* in the holding tank, eggs and sperm were released and they were subsequently fertilized in the holding tank. The fertilized eggs were sent to the trapping tank and reared there until the larvae were capable of settling. In addition, we have successfully used this system for collecting planulae from blooding corals such as *Pocillopora damicornis* and *Seriatopora hystrip*.

5–2. Collecting brooding coral planulae

Brooding coral such as *Pocillopora damicornis* may release planulae several times a year, and larval rearing time is generally shorter than that for broadcast spawners because the larvae are already fully developed and ready to settle. These observations seem to be advantageous for artificial production of juvenile coral. However, because it is difficult to ascertain whether the coral is ready to release planulae, selection of adult colonies to collect larvae is difficult. A number of collectors have to be set over colonies. Large size bundle collectors similar to that shown in Fig. 6 or plankton net (mesh opening 100–200 μm) with a screw cap bottle and float are used to collect the larvae. The collector must be set over a colony tightly so that the larvae do not escape from opening. It is necessary to set an upturned funnel in the mouth of screw cap bottle so that larvae in the bottle will not escape back down into the collector (Guest et al. 2010). It is also advisable to open windows with net on the screw cap bottle to prevent stagnation inside the bottle if the collector is left overnight.

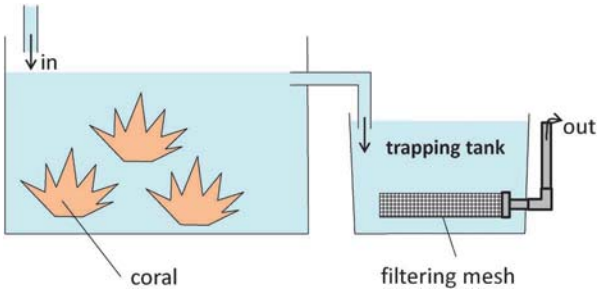

Fig. 14. Collection of larvae in a running water aquarium

Ex situ collection of planula larvae is easier than *in situ* collection. As shown in Fig. 14, donor colonies or coral fragments can be transported to a land-based flow through tank before the predicted day of planulation. The seawater in the holding tank allows the larvae to flow through an overflow pipe and be transferred to a separate collecting tank (trapping tank) that contains a mesh filter (see Column 2). The trapping tank should be periodically monitored each morning for the presence of planula larvae. Any larvae detected are collected at once and transferred to a clean container.

5–3. Mass production of larvae using a floating pond

A large number of larvae can be reared in a floating pond (Omori et al. 2004). The floating pond (2.0 m × 2.0 m, 1.0 m deep) is made of vinyl sheets used for eel aquaculture (Fig. 15). A submersible pump is used to deliver the seawater through a hose with small holes that are attached around the edge of the pond. Fresh seawater is gently sprayed against the walls to prevent the larvae from attaching to the walls of the vinyl sheet. Four screen windows are present on the sides of the pond and 1 at the bottom to allow the outflow of seawater. The screen windows should be cleaned if they become clogged with microalgae and other matter to ensure continuous flow of seawater. Omori et al. (2007) succeeded in rearing $>420 \times 10^3$ coral larvae (134 larvae/L) in each pond.

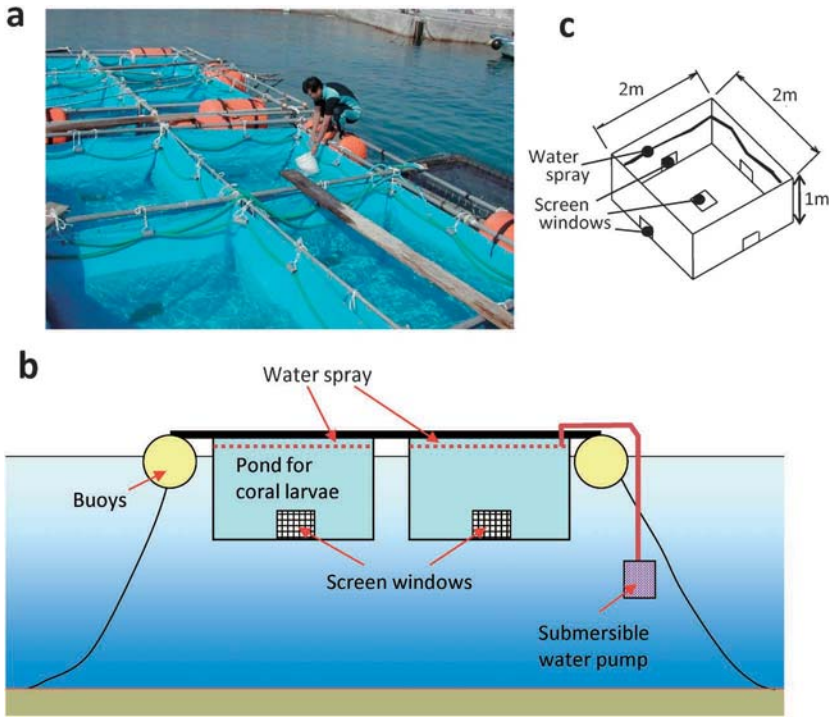

Fig. 15. Large-scale rearing of coral larvae in a floating pond (a). Sketch (b) and dimensions (c)

6. Transportation of larvae

Embryos can be transported when they attain the shape of a ball. However, the recommended time for transport is 3–4 days after fertilization when the larvae are well ciliated and move actively. At this stage, the larvae are stronger and can tolerate various stressors. A container with 2000 larvae/L can be transported to various places in Japan using a general parcel delivery service at normal temperature with survivorship >90%. Sending live larvae by air from AMSL to several public aquariums in Europe using 50-mL vials is succeeded (Petersen et al. 2005).

To reduce the stress from vibration during handing and transportation involving a long delivery time, larval density to be low in a sealed container without air space is advised. A collapsible polyethylene container (10 L or 20 L) is suitable when transporting a large number of larvae into an underwater net enclosure in order to enhance larval settlement on substrate. This type of container can be squeezed to release the larvae underwater with a SCUBA diver.

7. Induction of settlement and metamorphosis

The mechanisms of settlement and metamorphosis of hermatypic coral larvae have not been fully revealed. However, for acroporid larvae in Okinawa these processes can be induced with extracts from *Hydrolithon reinboldii*, a species of crustose coralline algae (Morse et al. 1996). The algal tissue or algal chips are added to a vessel containing the larvae in filtered seawater to induce settlement. The chips are collected by scraping the surface of the algae using a knife. They can be preserved by soaking in a rifampicin water solution (2 mg/L) followed by washing and dehydrating before freezing. If the chips remain frozen, their effect can be maintained for at least 1 year. The larvae will settle in 6–24 h after adding the thawed chips once they become competent to settle onto a substrate. In many cases, the larvae settle at the bottom of the container near the chips or sometimes on the chips. A thalloid red alga, *Peyssonnelia* sp., has a similar effect on acroporid coral (Iwao 1997). Settlement rate is 60%–90%.

Inducing settlement with coralline algal chips is easy, but collecting the algae may be difficult and preparing the chips is tedious. On

an experimental scale, the use of neuropeptide Hym-248 promotes higher, more stable induction of settlement and metamorphosis in acroporid larvae (Iwao et al. 2002).

In farming corals, we use a "conditioned" substrate that is maintained in the sea for 4–8 weeks with the surface covered in coralline algae and biofilm to enhance settlement of coral larvae (Column 4). The conditioned substrate may maintain its effect even after it is desiccated for certain period.

Column 3. Change in settlement capability of larvae

The fertilized eggs repeat cleavage and develop into planula larvae capable of metamorphosis and settlement. The duration to reach this stage varies depending on the species. Acroporid larvae take 5–5.5 days at 26°C–27°C, whereas faviid species take 2–3 days. The time taken by the larvae to maintain competency to settle also varies by species. For example, *Acropora tenuis* can settle for >20 days, but the competence becomes highest for only 1–2 days and is lowered during subsequent 2 weeks in *A. nasuta*, suggesting species-specific differences in the windows of opportunity for successful settlement and metamorphosis on the substrates (Fig. 16). The reseeding process in farming corals should be taken when larval competence peaked.

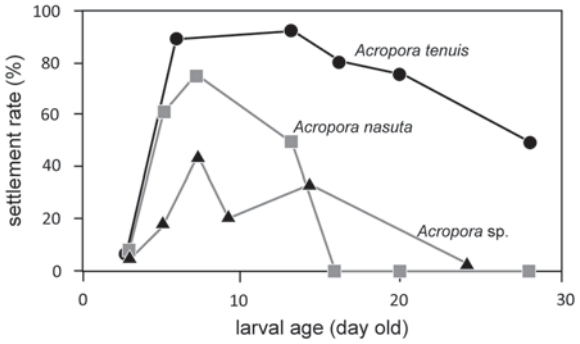

Fig. 16. Change in settlement capability of coral larvae

Column 4. Larval settlement and metamorphosis

Larval settlement and early post-settlement survival of coral explain much of the variance in the adult population. Some coral such as *Seriatopora hystrix* and *Heliopora coerulea* may settle on any material, whereas other species choose a location in response to an environmental signal from the seafloor. Settlement and metamorphosis occur consecutively after stimulation by multiple signals but they are separate phenomena. If no signals are provided, larvae remain in the water column for a considerably long period without settlement or metamorphosis even when they are competent to do so. On the other hand, if some stimulation is given, the larvae may metamorphose while suspended in the water column without settling.

Besides crustose coralline algae (Morse et al. 1988, 1996), bacteria on the coralline algae and a microbial biofilm lacking coralline algae play a role in triggering settlement and metamorphosis (Negri et al. 2002, Webster et al. 2004). A significant relationship is observed between the number of settled larvae and coralline algae covering the substrate in a natural setting (Fig. 17). Biofilms comprise complex distinct bacterial and microalgal communities, varying by time and depth. Thus, the effect could be different with species. Webster et al. (2004) found that 2-week-old biofilms induce metamorphosis of *Acropora microphthalma* in <10% of larvae, whereas the metamorphosis rate increased significantly to 41% on an 8-week-old biofilm. The settlement rate of *Favites halicora* larvae was 25% on a 2-week-old immersed substrate in the sea, whereas it increased to 68% on a 2-month-old substrate (Guest et al. 2010). In contrast, some *Porites* and *Acropora* species settle preferentially on unconditioned

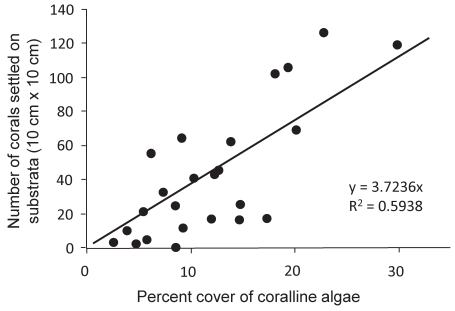

Fig. 17. Relationship between percentage cover of coralline algae and number of coral polyps settled onto the substrate (Yamaki 2010)

red substrata (Mason et al. 2007). The preferred red substrata have their spectra dominated by wavelengths 580–590 nm that is similar to the fluorescent emission peaks of coralline algae, indicating that the larvae determine settlement using light signals. (This phenomenon was not however confirmed by our experiment with *Acropora tenuis*.)

In nature, some species select a settlement location in relation to chemicals originating from coralline algae, whereas others attach to and metamorphose on biofilms. Larvae utilize a variety of environmental cues such as biological constituents and color of the substrate. This behavior appears to be a strategy of each species to optimize survivorship and growth.

8. Substrates

8–1. Quality and construction of an artificial substrate

Shells and various substrates made of concrete, slate, unglazed ceramic tile, polycarbonate, and fiberglass-reinforced plastic have been used. However, it is difficult to determine which one is best. Nondurable materials such as wood or bamboo are not recommended. A concrete substrate should be placed in the sea for some time to decrease its alkaline content. Although it is unclear whether this is because of the quality or surface structure of the substrate, it has been reported that larvae exhibit greater settlement affinity for substrate coated with calcium carbonate with electrochemical method than for unglazed ceramic tiles (Kihara et al. 2013).

Because more larvae settle inside irregular areas than on smooth surfaces, the substrates with several grids or ridges and numerous hollows are considered to be more effective for settlement. However, for induction of larval settlement biological conditioning of the surface of substrate is more important than raw material or surface structure of substrate. Before larval settlement induction, other biota (e.g., macroalgae, colonial tunicates, sponges, and sea anemones) than crustose coralline algae and biofilm should be removed by gently brushing the substrate surface.

We used a square concrete or unglazed ceramic tile (10 cm × 10 cm) as a substrate. However, the square tiles are not suitable for transportation and outplanting once juvenile corals grow on them. In addition, a considerable amount of epoxy is required to attach them to the reef. The square tiles that had nicks like a chocolate bar were devised so that they could be split into several pieces in the sea. However, juvenile corals often grew over the nicks.

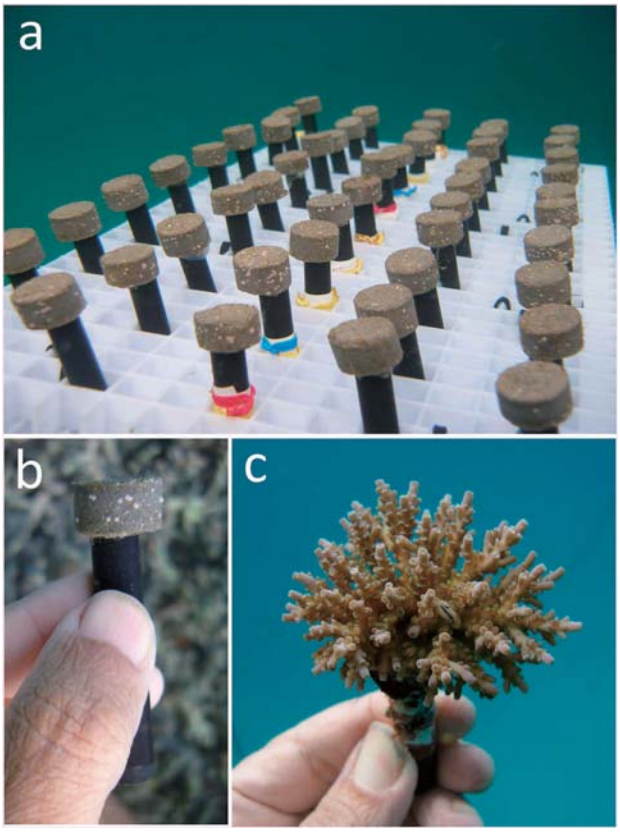

Fig. 18. Coral pegs. A number of polyps settle on head of the peg (a, b). Juvenile coral grown there (c).

A "coral peg" is currently being utilized along with the square tile, as it allows for easy transport and outplanting than the tile. The total height of the coral peg is 60 mm; the head is made of concrete cement mixed with quartz sand and is 18 mm in diameter and 10 mm in height. The leg (plastic) is 10 mm in diameter and 50 mm in height (Omori and Iwao 2009) (Fig. 18). The juvenile coral grew on the head of the coral peg. They can be transported and fixed onto a crevasse of the reef using epoxy.

Column 5. Structure of the artificial substrate

The microscale turbulence produced by

the complex shape of the substrate surface serves to retain the approaching larvae on the settlement surface. It is known empirically that coral larvae tend to settle more on the edge or a groove with approximately 1 mm wide and 1 mm deep of a substrate than its flat surface. However, the relationship between the physical movement of water and behavior of coral larvae on a complex substrate structure has not been studied in detail. On the other hand, surface irregularities reduce the danger of predation by fish and scraping by sea urchins after settlement. According to Nozawa (2008), while all juvenile corals of 3 species including *Echinophylla aspera* that settled on a plain tile surface died within the first 4 months at sea, others that settled in the small crevices of 3–4 mm deep on the substrate survived. Nozawa (2012) monitored juvenile *E. aspera* and *Favites abdita* that settled on artificial substrates with projections at 5-, 15-, and 25-mm intervals for 2 years in the sea. He found that coral survivorship on the 5-mm refugia was significantly higher than that in the 2 larger intervals.

It must remind that crevice on substrate is also excellent space for survival of algae that are competing with coral spats.

8–2. Larval settlement on the substrate

Reseeding (larval settlement on a substrate) must be accomplished at the peak period of competency. The competency should be tested twice per day by placing 10–20 larvae and coralline algae chips in a culture dish for 12 h (see text 7). Reseeding work should be prepared in advance considering that in many cases larval settlement competency is highest at half or 1 day after its capability is indicated (Column 3). Larval settlement will be completed in 1–5 days (Fig. 19).

Reseeding can be performed in aquaria on land using a tank (100–500 L). Coral larvae are released into a tank containing many substrates (Fig. 20). Settlement rate varies by timing, but usually >60% of acroporid larvae settle onto tiles within 1 day, if larval density is 100–130 larvae/L. If reseeding is accomplished outdoors, the aquaria should be shaded to reduce sunlight by 50%. If the number of spat on a substrate is lower than the expected number after 1 day, more larvae should be added on the second day. More larvae may settle onto one side of the substrate when the title is used. If larval settlement on both sides is desired, the tile should be turned over on the second day. Water in the tank is gently agitated throughout the settlement by bubbling air through the water column. The substrates should not to be hit directly by air bubbles. The water should be carefully changed during the reseeding process using a filtering apparatus to avoid losing any larvae.

Fig. 19. Juvenile corals on a conditioned substrate (right, enlarged photo)

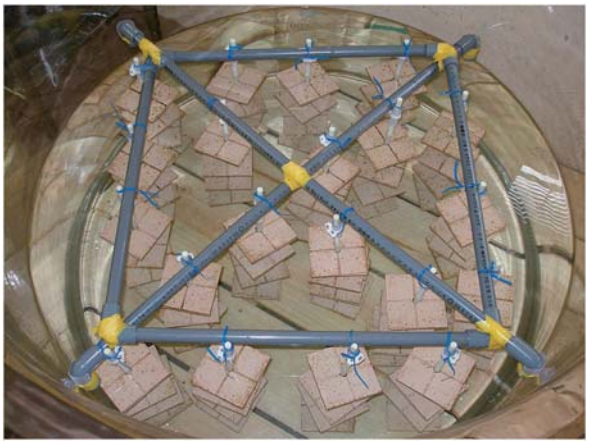

Fig. 20. Larval settlement on ceramic tiles

We recommend that the initial density should be 0.5–1.5 spats (polyps)/cm^2 on a substrate. On the one hand, if the density is very high as such that an individual spat attach to neighboring spat, it may cause significantly high mortality (>90%) because of infection during the early post-settlement period. On the other hand, coral spat will tend to form large chimeras at very high densities and this may lead to higher survival and increased growth

rate if fused colonies reach an escape size more rapidly than single spat (Puill-Stephan et al. 2012, Raymondo and Maypa 2004).

Column 6. Introduction of larvae to a substrate in the sea

It is possible to enhance settlement of larvae onto a substrate, underwater, by releasing the larvae into an enclosure using a net. AMSL and the then Tetra Co. Ltd. have constructed a nursery using a conditioned concrete block (1.3 m wide) surrounded by a seat (5.5 m long, 5.5 m wide, and 6.0 m high) at Naha Harbor, Okinawa. The concrete block was covered with fine mesh net, and approximately 1.64 million larvae cultured at Akajima were released into the enclosure by SCUBA divers (Aota et al. 2003).

Heyward et al. (2002) developed another technique using a movable floating pool containing coral larvae cultured from a slick collection that was placed over the area to be reseeded. Fresh seawater was pumped inside the floating pool to raise the water level so that the larvae were sent by hydraulic pressure to the settlement substrates in an enclosure of camping mosquito net on the seafloor through a tube connected to the underside of the pool (Fig. 21).

The results of these *in situ* reseeding experiments demonstrate that early recruitment can be significantly enhanced. However, most settled juvenile corals die because of natural causes in the sea within the first few months (e.g., Sato 1985). Thus, this method is not favored as a reef rehabilitation technique until positive evidence of a long-term effect has been demonstrated. Hayashibara (2009) devised a special substrate for settlement of the branched coral larvae and indicate possible result for this method (see also Suzuki et al. (2012)).

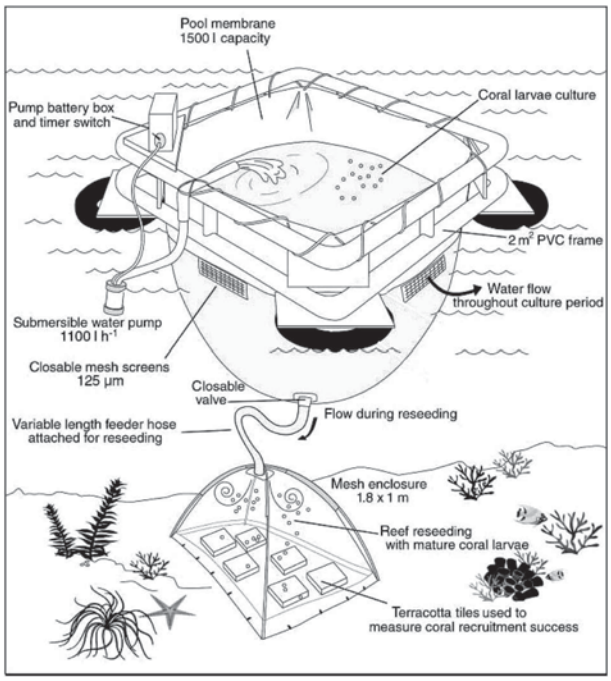

Fig. 21. An example of a method that delivers larvae to the enclosure on the seabed from a floating pool (Heyward et al. 2002)

Column 7. Acquisition of zooxanthellae

Offsprings of many coral (approximately 80% of broadcast spawners) acquire symbiotic zooxanthellae *(Symbiodinium* spp.) horizontally from the natural environment (Baird et al. 2009). Although planktonic planulae may acquire zooxanthellae, generally the coral spats begin to acquire the symbionts within 5–7 days after settlement. It is essential for them to acquire zooxanthella during this period. A failure may lead to death. Establishing symbiosis may be enhanced by introducing giant clam or coral fragments from outside to the rearing tank at the time of reseeding or immediately after settlement. This process is easy, although the effectiveness of the introduction of zooxanthella in the rearing tank for the spat's survivorship has not been distinct as yet. The coral will turn yellowish when the number of zooxanthellae in the larvae increases. The genetic type (clade) of *Symbiodinium* spp. is mostly type C in adult coral, but it is mostly type A and/or type D when coral spats are acquired (Abrego et al. 2009, Yamashita et al. 2013). Introduction of the cultured stock of planktonic type A and/or D into the tank may usher more definite results (see Suzuki et al. 2013).

9. Culturing juvenile coral

9–1. Commencement of rearing

In the sea, nibbling fish and/or coral eating gastropods, sea urchins, and amphipods reduce the number of newly settled juvenile corals. Corals are also vulnerable to sedimentation and overgrowth of algae, colonial tunicates, and bryozoans. It is recommended to rear corals in an environment where these factors that lead to mortality are nonexistent. However, there are currently no methods to eliminate these factors, unless the juvenile corals are raised on land using large flow-through seawater aquaria.

At AMSL we rear the juvenile corals on 15 pairs of ceramic tiles (30 faces, 10 cm × 10 cm each) or coral pegs that are maintained in a nursery cage (80 cm × 55 cm × 30 cm) suspended at a depth of 1.5–3.0 m from the sea surface (Fig. 22). One hundred individuals of algae-grazing juvenile top shell snails *Trochus niloticus* (5–10 mm in basal shell diameter) are reared in each cage (i.e. ca. 70 inds./m^2) (Fig. 23). Grazing trails of the juvenile top shell snails are nearly harmless to coral spats, unless density and/or size of the grazers exceed threshold (Tamura 2008). Other algae-grazing snails (We used *Clypeomorus batillariaeformis* in Palau.) may be used to algal control, but it is necessary to check if they do not scrape or dislodge the corals while creeping. Although many sea urchins are also grazers, *Diadema* should not be used, as they clash and eat the juvenile corals (Sato 2010). Sedimentation may cause newly settled coral to die. Thus, the substrate tiles are arranged vertically or obliquely in the cage.

9–2. Maintenance during rearing

The condition of juvenile corals in the nursery cages should be checked periodically and algae (top shell snails appear to avoid foliose algae), sea anemones, sponges, and colonial tunicates should be removed from the cages and substrates as much as possible using a brush and scraper. If juvenile coral are reared on land using large flow-through nursery aquaria, in addition to algae, sea anemones can be successfully removed by releasing juveniles of algae-grazing rabbitfish *Siganus* spp. juvenile, and/or butterfly fish *Chaetodon*

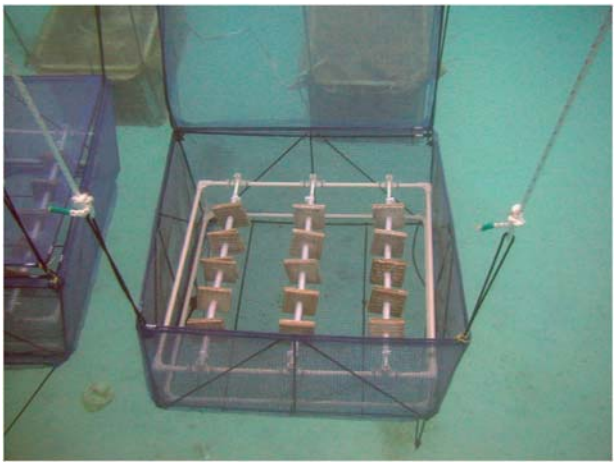

Fig. 22. Nursery cage and ceramic tiles

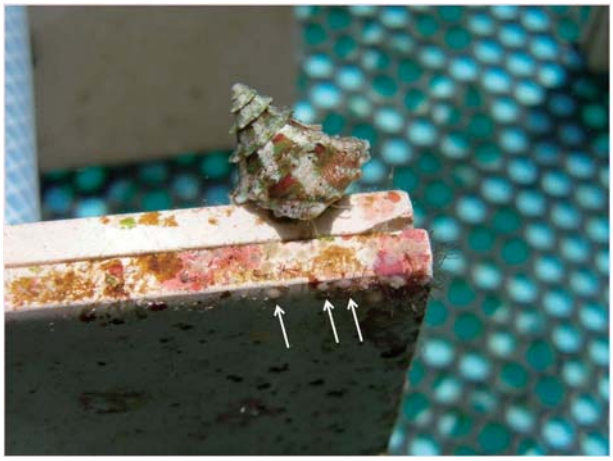

Fig. 23. Co-culture of juvenile corals (arrows) and top shell snails in a nursery cage

Fig. 24. Culture of juvenile corals in nursery cages (Photo courtesy of Mr. H. Kobayashi, Asahi Shimbun)

Fig. 25. One and half year old *Acropora tenuis* in a nursery cage

Fig. 26. Underwater outplanting operation (Photo courtesy of Mr. H. Kobayashi, Asahi Shimbun)

kleinii. Water movement is inevitably stagnated in the nursery cages causing a negative impact on coral growth. Coral species such as *Acropora digitifera* and *A. hyacinsus* require considerable water flow to sustain growth (Boch and Morse 2012). When the juvenile corals grow 1–2 cm in diameter, the mesh size of the nuresry cages may be changed or the top cover of the cage may be removed so that water flow is enhanced and algae in the nursery cages are removed by fish.

When the juvenile corals grow to a size of >5 cm in diameter, they could be outplanted (Figs. 24 and 25). This is important, as the unit for both propagation and eventual reef rehabilitation, that each substrate contains 1–3 living corals, not the total number of living corals.

10. Outplanting

The juvenile corals are outplanted with their artificial substrates onto the outcrops near their native reef (Fig. 26). If the outplanting site is far from the native site, we choose a location with physical and chemical conditions as well as a coral species composition similar to the native site. If the corals die at a prospective rehabilitation site because of an episodic disturbance, dead coral heads or coral rubble should be used to identify the site.

Smaller colonies (<2 cm) may be perished because of various factors including nibbling by fish or predation by corallivorous muricid snails. Larger colonies may reduce their size following outplanting when their branch tips are removed by fish but they survive and resume growing.

Outplanting immediately before the stormy season and/or during the year when sea temperatures are highest should be avoided to minimize risk of death. Bleaching may occur and diseases may be prevalent during the warmest months of the year. Thus, operating during cooler months and in sheltered reef environments is recommended where the outplanted coral will be protected from wave action.

The juvenile corals can be fixed to convex surfaces of the coral limestone outcrops using stainless steel wire or adhesives (refer to Okubo and Omori 2001). Marine adhesives (e.g., Konishi marine epoxy bond, Epoxyclay Aqua™, S-dine Joiner, etc.) are sufficient to secure corals on artificial substrates to the reef. Before fixing, the point of attachment should be brushed lightly with a wire brush to remove algae and sessile invertebrates on the surface. For sites such as the side of a steep outcrop or areas of high wave action, the substrate should be secured with a concrete nail or stainless steel rod in addition to the stainless steel wire and epoxy. As the movement of sand and gravel easily damages coral on a flat seabed, outplanting to such site should be avoided. The coral colonies and substrates should be firmly fixed on the side or top surface of an outcrop, at least 50 cm above the sea bed.

There should also be a distance of at least 30 cm between the outplanted colony and a nearby coral to provide ample space for future growth.

With no regard to how they were fixed, the

success of the outplanting is totally dependent on whether the coral and substrates are firmly fixed on the reefs. Therefore, it is very important to check all outplanted coral within 1 day after the operation is finished. After outplanting, the living coral gradually extends over the wire and adhesives and reaches toward the natural substrates. If outplanted colony is moved by waves, the coral tissue will not reach the natural substrates. The outplanting is considered a success when living tissue reached the outcrop. The growth rate of the living tissue varies under similar environments depending on the species. If living tissue is distant from the natural substrate, it may retard attachment of the coral tissue to the outcrop and be easier to die.

The epoxy is composed of a resin and a hardener. The chemical reaction between the 2 parts does not occur until they are mixed. The 2 parts are mixed immediately before underwater application. Because the time taken by the epoxy to remain workable and harden may differ by manufacture and water temperature, detailed and advanced planning of the underwater operation is necessary.

The effect of epoxy on marine invertebrates including the outplanted corals has not been studied in detail, because the chemical compound of each product are seldom shown. Thus, care should be taken to minimize direct contact between live coral tissue and epoxy. Although we have not encountered a negative impact of underwater adhesives on coral, the effect should be investigated further for cases in which large amounts of epoxy will be used.

The outplanting operation is the most expensive process of reef rehabilitation, because considerable time is spent to secure the coral to the reef along with the costs accompanying the use of boats and SCUBA divers. Analysis of several studies suggests that only 10–15 substrates (with corals) can be fixed per person per hour. Coral colonies grown on stick-like substrates, such as coral pegs, can be slotted into predrilled holes or natural holes of the reef with a small amount of adhesive. Thus, an operation using the coral pegs can be performed faster and cheaper than attaching flat tiles.

Column 8. Transplantation, outplanting, and translocation

The term "transplantation" is commonly used for removal of an entire coral colony or a fragment from one place to resettle in another. The operation can be divided into 4 parts as follows: 1. Direct transplantation of coral fragments that were clipped/fragmented from donor colonies and "coral of opportunity" to the reef. 2. Transplantation of nursery-grown asexually propagated fragments. 3. Transplantation of nursery-grown sexually propagated colonies. 4. Transplantation of entire coral colonies to rescue the ones that would otherwise be destroyed or severely damaged by coastal and/or underwater development. Survaival rate of nursery-reared fragments that were grown to an adequate size on substrates is higher than fragments that were clipped and/or collected in the sea and directly transplanted onto the reef (Shafir et al. 2010). We propose that the term "transplantation" be used for the direct removal of fragments onto a reef (1). The removal of nursery-reared corals (2 and 3) should be expressed by the term "outplanting". Removal of entire coral colonies or those together with a hard substrate for mitigation purposes (4) should be termed as "translocation."

Column 9. Protecting outplanted juvenile corals from nibbling by fish

We have outplanted cultured, juvenile colonies of *Acropora tenuis* (1-year-old; approximately 3 cm in diameter) on the Reef Balls™ deployed at Lukes Reef, Palau in March 2007. However, 6 months after outplanting nearly all of the coral were broken, as well as some parts of the tile substrate. The culprit was suspected to be parrotfish, although further investigation is needed. Similar damage was observed at Akajima Island with the *A. tenuis* juvenile colonies (3–4 cm in diameter) a few days after outplanting. Based on our observation, nibbling of the barred filefish *Cantherhines dumerilii* and others were thought to be the cause. Predation exclusion experiments were conducted by placing protective cages over the corals (Fig. 27). Pre-

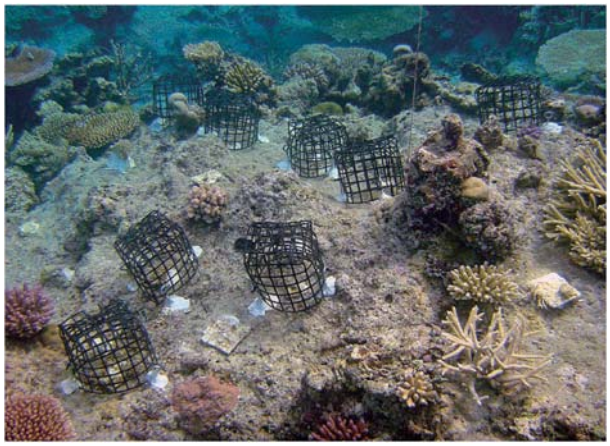

Fig. 27. Outplanting of 1-year-old juvenile colonies in protective cages

liminary results showed that nibbling/predation by fish interfered with the cages, but algae had grown on some cages. Therefore, the cages must be removed at an appropriate time (<1 year), by so doing, grazing fish may remove the algae.

11. Growth and spawning of outplanted coral colonies

When juvenile *Acropora tenuis* in mid-water nursery cages, which were of 1.5 years postfertilization, grew to approximately 6 cm in diameter, approximately 2000 colonies were outplanted with the tile substrate onto bommies at Akajima Island in December 2006. The coral grew well (89% survival 6 months after outplanting) until they were seriously damaged by waves driven by the typhoon Man-yi in July 2007. An approximate of 60 surviving colonies grew to 20–25 cm in diameter and more than seventeen colonies had spawned at 4-year-old initially (Iwao et al. 2010, Omori et al. 2008).

Up to 2013, we have outplanted >6000 juvenile corals cultured from eggs on limestone outcrops at Majanohama, Akajima Island. Most of the corals matured 4 years later. Spawning of the colonies has continued every year since 2009 (Fig. 28). This indicates that the cultured coral had grown into "a mature population" capable of producing the next generation. At present, many kinds of damselfish and butterfly fish swim in the outplanting area and gobies and crustaceans inhabit the corals, indicating that the present restoration technique has certainly enhanced the rich biodiversity of the coral reef (Fig. 29)

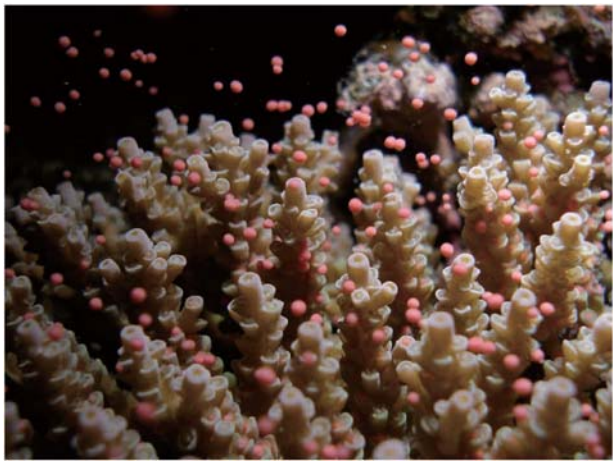

Fig. 28. Spawning of outplanted *Acropora tenuis* (5-year-old) at Akajima Island

Column 10. Cross fertilization and larval settlement rates of the outplanted corals

The merit of reef rehabilitation techniques that utilize outplanted corals cultured from sexual propagation is greater compared to that of asexually derived coral fragments, which can reduce genetic diversity of a population. Gametes from the same colony are unable to cross-fertilize in many coral species. Low genetic diversity is a concern for reef restoration when seed fragments are raised asexually from only a few donor colonies. This can lead to lower fertilization rates among seed fragments, and reducing the long-term benefits of reef restoration in particular areas. Additionally, low genetic diversity can compound the effects of increased ocean temperature and other environmental stressors, further jeopardizing the health of a reef.

We tested fertilization rates between gametes collected from 6 cultured colonies of *Acropora tenuis* (5-year-old) that were cultured from eggs and outplanted and 3 wild colonies of the same species. The tests showed that all 4 crosses, i.e., between outplanted colonies; between natural colonies; and between outplanted and natural colonies, resulted in fertilization rates >98.5%.

Fig. 29. Outplanted corals that were cultured from eggs in June 2005. The photograph was taken at Akajima Island in February 2010.

No difference in settlement rate (>95%) of the larvae (3- and 6-day-old) was observed between the wild and cultured colonies as well as in the crosses between the wild and cultured colonies (Iwao 2013). It is expected that the cultured colonies contribute to the expansion of the population into neighboring areas.

12. Cost estimate

We estimated the cost of cultivating sexually propagated coral using our experimental study in Palau in 2007. During the 1 year experiment our expenditures were as follows: Personnel cost (operational costs) for production of the juvenile colonies using 320 tile substrates and 10 nursery cages was US$20,167. The cost of equipment and consumables (set-up costs excluding SCUBA tank rental and boat transportation, but including ropes, cages, rearing containers, and epoxy bond) was US$8,639. The total cost was US$28,806 (Table 1). Assuming that 10 colonies remained on each tile, 3,200 juvenile colonies could be produced after 1 year of culture; excluding the cost for outplanting 320 substrates (US$4,795), the cost of producing 1 colony would be US$7.50 (750 JPY).

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Table 1. Set-up and operational costs for producing juvenile coral colonies using 320 tiles in 10 nursery cages and outplanting in Palau.

Activities	Detail	Staff involved (number)	Person-hours	Equipment and consurmable cost (US$)	Was SCUBA needed?	Was a boat needed?	Total operational or running costs (US$)
Sampling	Collection of donor colonies	2	4	15	2 dives	1 transport	333
	Prediction of spawning night	2	20	134	Snokeling		1,000
Rearing coral larvae	Collection of eggs and insemination	3	15	2,173			250
	Rearing of larvae	3	75				1,250
	Larval settling onto tiles	2	60	1,113			1,000
	Preconditioning of tiles in the sea	2	8		Snokeling	2 transports	667
Culturing Juvenile corals in nursery	Setting the cages	3	30	4,436	15 dives	5 transports	2,500
Maintenance of Juvenile corals in nursery	Clearing and monitoring	2	108	140	54 dives	27 transports	9,000
Outplanting	Transportation and outplanting	5	50	628	25 dives	5 transports	4,167
subtotal				8,639			20,167

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