Sustainable large-scale coral restoration by establishing "artificial spawning hotspots"

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Abstract: Coral reefs have degraded over time and, recently, severe bleaching events caused further damage to coral communities worldwide from 2015 to 2017. Because coral reefs are an important habitat for coastal fish and invertebrates in tropical coasts, the decline of coral reefs also results in decreased fishery productivity. Therefore, for sustainable use of these resources, it is important to conserve coral communities that act as fishing grounds and fish nurseries. Coral transplantation is known as an effective restoration method. However, large-scale transplantation requires a great deal of labor and the transplanted corals are vulnerable to extermination by only a single disturbance such as bleaching or an outbreak of crown-of-thorns starfish (COTS). Hence, enhanced annual coral larval recruitment is needed for sustainable large-scale restoration.

Two key factors are crucial for sustainable coral restoration by enhancing reproduction. The first factor is establishment and maintenance of "artificial spawning hotspots" that consist of densely populated conspecific adult colonies. The second factor is improvement of early life survivability by collecting eggs and sperms at spawning and rearing larvae until settlement. For "artificial spawning hotspots," safeguards are required against predation by COTS and the use of shading is desirable against bleaching during the high seawater temperature season. In addition, a special gamete and larval collector, termed "larval cradle", was developed to consistently perform bundle collection, fertilization, and larval rearing. Eventually, it is suggested that a set of simple methods and techniques are used for sustainable large-scale coral restoration.

Key words: larval supply, Acropora, fish nursery, larval cradle, artificial substrate

Current Coral Reefs Status and Crisis

Coral reefs are large biogenic structures that develop in shallow waters in tropical and subtropical regions. Most of the modern coral reefs have formed atop stacks of dead corals created several thousand years ago (Braithwaite *et al.*, 2000; Kayanne *et al.*, 2002; Hongo and Kayanne, 2009). Recently, several disturbance factors frequently endanger coral reefs (De'ath *et al.*, 2012). One of those factors is coral bleaching. Corals have symbiotic microalgae, called "zooxanthellae", which largely contribute to coral growth by providing nutrients obtained by photosynthesis to the host coral (Tanaka *et al.*, 2018). This symbiotic relationship can collapse due to high

seawater temperature where corals lose symbiotic algae (Fujise *et al.*, 2014). Such corals become white in color and this phenomenon is called "bleaching." Some researchers have described bleaching of corals as an adaptive behavior to environmental change, known as "adaptive bleaching" (Kinzie *et al.*, 2001). In any case, if the ambient temperature is 1°C higher than normal for longer than 1 month, many corals die after bleaching. Some taxonomic groups such as genus *Acropora*, *Montipora*, and *Seriatopora* are susceptible to bleaching (Marshall and Baird, 2000), though these groups still dominate most of the Indo-Pacific reefs. Global warming may cause an increase in seawater temperature and some predictions forecast that the frequency of large-scale bleaching

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events will be higher than in past centuries.

Another crisis is outbreak of crown of thorns starfish (COTS) (Moran et al., 1992). COTS is a large starfish armed with poisonous spines that predates corals, especially Acropora and Montipora species (Pratchett et al., 2009). Generally, the population density of COTS is 1-2 individuals per 100 m². Once an outbreak occurs, the density increases by 100 times, and several hectares of corals can be eaten up within a few months (Suzuki et al., 2012). Factors that cause a COTS outbreak are not clear, but it is probable that high nutrients contribute to outbreaks because COTS larval survival was drastically improved by increased phytoplankton density (i.e. the prey of COTS larvae) that resulted from high nutrient concentrations in laboratory experiments (nutrient hypothesis) (Fabricius et al., 2010).

The third crisis is physical damage to corals caused by large typhoons (cyclones or hurricanes). Some corals that have weak skeletal structures, such as branching corals, are easily and severely damaged by large typhoons that typically occur once every several years.

It is important to understand the extent of anthropogenic impacts on coral reef destruction. For bleaching, if the cause of global warming is the accumulation of greenhouse gases such as CO₂ primarily discharged by human activities, bleaching is thus a consequence of anthropogenic activities. However, it is difficult to distinguish the roles between global warming and the natural cycle of the global environment (e.g. the frequency of El Niño) on the occurrence of bleaching (Claar et al., 2018). In addition, it is probable that eutrophication and/or increase of bacteria in the water column accelerate damage to corals during bleaching (Fabricius et al., 2013). For outbreak of COTS, if the nutrient hypothesis as stated above is true, the frequency of COTS outbreak is expected to increase due to anthropogenic impact. Similarly, anthropogenic impacts may indirectly increase the size of typhoons because global warming may be a contributing cause (Balaguru et al., 2016). Although the extent of anthropogenic impacts on these crises remains poorly understood, coral populations are seriously damaged by a higher frequency of disturbances, especially in the long term, because they decrease

coral reproductive output.

As mentioned above, most disturbances have large impacts on specific taxa such as *Acropora*. Although *Acropora* species are fast growing corals, it still takes at least 3 years (5 years on average) to grow to a mature size. Furthermore, the older the corals grow, the more eggs they spawn that contribute to larval supply. If most corals die from each disturbance within 10 years, robust reproduction (*i.e.* adequate larval supply) lasts only a few years and this could also gradually reduce coral populations.

Impact on Fisheries Resources

Fisheries resources in coral reefs have been a primary source of protein for people in tropical regions (Burke et al., 2011). Many fish and invertebrates directly or indirectly depend on coral communities. For example, juveniles of one of the dominant groupers, Epinephelus ongus, selectively settle in the bottle-brush coral (Acropora species) habitat (Nanami et al., 2013). Most parrotfishes utilize the space among coral branches (mainly branching Acropora species) as a sleeping bed and coral recruitment is enhanced by fish grazing (Russ et al., 2015). Some cuttlefish lay eggs in the space among coral branches (mainly branching Porites species). In addition, small fish such as damselfish and cardinalfish live associated with corals, which feed large fish such as groupers (Shpigel and Fishelson, 1989; Nakai et al., 2001). Importantly, recent studies suggested that organic matter produced by symbiotic algae through photosynthesis contributed to the whole coral reef ecosystem as primary production. Therefore, it is highly probable that the decrease of corals is linked to the loss of fisheries resources (Wilson et al., 2006). In terms of reproduction, "spawning aggregation" is one of the representative behaviors in the reproduction for some reef fishes such as groupers (Domeier and Colin, 1997; Nanami et al, 2017). Considering that such behavior could also be affected by the decrease of corals, the loss of fisheries resources is likely very serious with long-term consequences.

Previous Studies on Coral Restoration

Corals reproduce sexually and asexually. "Fragmentation" is a process of asexual reproduction, in which some branches of corals regrow as a new colony by adhering to the seafloor once they snapped off. Corals have various methods of sexual reproduction including mass spawning (both gonochoric and hermaphroditic) and larval brooding (Baird *et al.*, 2009). Corals produce swimming planula larvae that settle on the seafloor and metamorphose to juvenile corals.

One of the simplest methods for coral restoration is transplantation of coral fragments that can be accomplished using different techniques such as direct attachment of coral fragments (fallen branches are more effective) onto the seafloor, or attachment on artificial plates in aquaria followed by fixation on the seafloor. The first report of the coral fragment attachment method was from Guam in the 1970s (Birkeland *et al.*, 1979) and this method has advanced each year since. In Okinawa, a total of 100,000 or more fragments were transplanted to approximately 3 hectares of damaged reef over a 6-year period from 2011 to 2016 (Okinawa Prefectural Government, 2017).

The next step to the transplantation is seedling production by sexual reproduction. Coral communities made by transplantation of fragments are likely to have low genetic diversity because most are clones of parental strains. Clones cannot reproduce sexually in most coral species. In addition, generally availability of parent strains is limited. In contrast, we can produce many seedlings (*i.e.* genets) by collecting and fertilizing gametes, rearing larvae, and settling them on substratum.

One of the most efficient methods for gamete collection is through collection of the "slick". In tropical regions, approximately 100 coral species simultaneously spawn gametes on the same night in a "mass spawning" (Harrison *et al.*, 1984). Substantial numbers of fertilized and unfertilized eggs are gathered at the sea surface the following morning; this is called a "coral slick." By collecting these slicks and rearing them, many larvae are obtained at once. Recently, a large-scale slick collection method was developed using a large vessel (Doropoulos *et al.*,

2019). However, it is possible that natural slicks may not form or be detected due to inclement weather conditions. In addition, natural spawning and fertilization may decrease after disturbances because these events reduce the population density of adult corals.

Methods for outplanting are very important because all seedlings (also called fragments) are required to be outplanted on the seafloor. Random outplanting to copy natural coral communities may be manageable on a small scale, similar to gardening. However, for large-scale restoration, there is risk that all the required cost and effort will be wasted by a single disturbance. Therefore, the key to restoration is the concept called "artificial spawning hotspots" (Zayasu and Suzuki, 2019).

Artificial Spawning Hotspots

The basic ideas for sustainable large-scale coral restoration are: 1) creating a high density of mature colonies of a single species to enhance fertilization and collection of gametes, and 2) sustaining larval supply by protecting these high-density populations.

First, selection of the target species is important for establishment of artificial spawning hotspots. Approximately 800 coral species with different reproductive characteristics are known in the world; some are broadcast spawners and some are broadcast (Baird *et al.*, 2009). Also, within the broadcast spawners, some are gonochoric and some are hermaphroditic.

The target corals for artificial spawning hotspots are *Acropora* species, a representative genus of hermaphroditic broadcast spawners. *Acropora* is a dominant group in Indo-Pacific reefs and one of the most diverse genera, which forms various habitats with different colony morphologies among species (Wallace, 1999). Within *Acropora*, the branching species are an especially important target, as stated above, in that they contribute to fisheries resources. Because the coral polyps of branching coral distribute less densely than those of tabular and corymbose species (Suzuki unpubl. data), the number of eggs spawned each year is lower, suggesting that their larval supply is also small. It takes longer for these species to recover through natural recruitment

from local extinction due to severe disturbance. Hence, the efficacy of artificial spawning hotspots would be higher for the branching *Acropora* species.

Indeed, the number of *Acropora* recruits is clearly different among reef habitats. From the results of long-term monitoring of *Acropora* recruits around Ishigaki Island, the number of recruits was extraordinarily high in 2014 in the reef slope after the local extinction in 2011 due to a COTS outbreak. The coral cover visually increased from 2017 where the cohort grew to a visible size (Suzuki unpubl. data). However, there was not high recruitment in the lagoon area over a 10-year period and no coral recovery was found (Suzuki unpubl. data). Artificial spawning hotspots should be established in such places where natural recruitment is always low.

In the concept of artificial spawning hotspots, it is necessary to complete seedling production (from gamete collection to larval settlement on artificial substrate) in the sea, which led to the development of the "larval cradle" (Suzuki et al., 2020). Larval rearing techniques using aquaria have already been developed using fertilized eggs collected in the field from adult corals or reared in a tank (Omori et al., 2004); however, there were no methods for completing the entire process in the field. Acropora corals release egg-sperm bundles and burst open at the sea surface, then the gametes are fertilized with the gametes released from other colonies (i.e. almost no self-fertilization) (Willis et al., 1997). Utilizing this characteristic, a device that catches released bundles with a large net and holds them until they are competent larvae (called the larval cradle) was invented.

A completed version of the larval cradle consisted of a cylinder with a diameter of 1.7-m and a height of 4.25 m made of 30 µm mesh nylon net (Fig. 1a), which achieved the collection and rearing of several million coral larvae with more than 90% fertilization and survival rates. The size of the larval cradle was

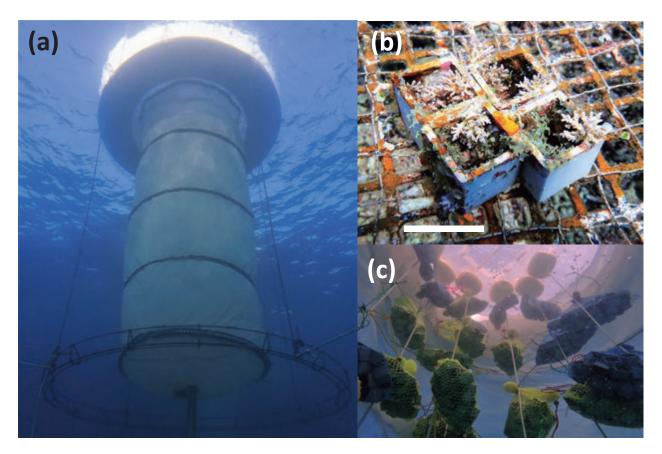


Fig. 1. (a) Setting of the larval cradle with a dedicated float. Size of the cylindrical cradle was 1.7-m diameter and 4-m height below sea level (0.25-m height above sea level). (b) Artificial substrate of short square tube (*i.e.* square hollow section, SHS) for Acropora settlers. Scale bar represents 4 cm. (c) Method of placing the SHSs into the cradle (SHSs were put in net bags and hung from an EVA float).

designed to be easy to handle for a small group of people (e.g. fishermen or leisure divers) while ensuring the production of a significant number of larvae. A larger sized larval cradle was not preferred because it would break more easily during stormy weather.

Next, the larvae produced by the larval cradle should be settled on substrates. Post-settlement mortality of coral juveniles is very high. This mortality is primarily caused by unintentional predation by fish grazing on epibenthic fauna and algae (Baria et al., 2010). To prevent fish grazing on coral settlers while keeping moderate light intensity, a lattice-shaped plate was contrived that resulted in good survival of coral juveniles (Suzuki et al., 2011). The short square tube (i.e. square hollow section, SHS) substrate was further developed from the lattice-shaped plate (Fig. 1b). The SHS substrate was like a single cell of the lattice-shaped plate, which allows us to outplant easily after settled juvenile corals grow up. By hanging the SHSs into the cradle from a buoy (Fig. 1c), completion of the settlement process in the sea could be achieved. Settlement of Acropora larvae at a moderate density on SHS was also attained by contriving the number of SHSs and the timing and method of placing them into the cradle (Suzuki et al., 2020). The survival rate of the settled corals on SHS was usually 10-20% in the suitable environment (3-15 m in depth, moderate current, little sediment, etc.), although it varied due to the environmental conditions (Suzuki et al., 2013).

Methods for protection of adult corals from disturbances, the second factor for creating artificial spawning hotspots, have also been developed (Suzuki et al., unpubl. data). Bleaching and COTS outbreak are the most serious disturbances for adult Acropora corals, and, therefore, the processes of these disturbances were focused for the development of protection methods. One of the available countermeasures against bleaching is shading of corals. It is known that seawater temperature rise of approximately 1°C causes photoinhibition in the photosynthetic process and generates excess reactive oxygen species that obstruct the symbiosis with zooxantellate algae (Warner et al., 1999). Thus, shading corals during high temperature periods could reduce the mortality

by buffering photoinhibition (Coelho *et al.*, 2017). In addition, moving the corals to deeper locations could also reduce light intensity. Another candidate countermeasure against bleaching is reducing starvation of the corals. It is highly probable that the direct cause of death for bleaching corals is starvation by losing nutritional supplementation from symbiotic algae (Borell and Bischof, 2008). Hence, if any nutrition could be artificially supplied during bleaching, mass mortality events may be avoided.

A countermeasure that addresses COTS outbreaks is easier relative to countermeasures that address bleaching. Because COTS cannot climb on thin rods, 50-cm bottom-raised racks with, reinforcing steel rods, is sufficient to prevent COTS damage. In fact, only corals on bottom-raised racks survived the COTS outbreak in 2010–2011 in the Urasoko Bay, Ishigaki Island, where 99% of *Acropora* corals within the bay were eaten by COTS (Suzuki unpubl. data).

By using these countermeasures for corals in artificial spawning hotspots, the larval supply from the hotspots could be several hundred times higher than that of a wild *Acropora* population experiencing the same environmental conditions (Fig. 2). Calculation of the total larval supply in a longer span showed an even larger gap between the hotspot and wild population. That is, the larval supply from the hotspots could be stable annually for a long period, while the gradual deterioration of wild populations is predicted over 50-100 years (IPCC, 2018).

There may be concern with species diversity of target corals for restoration. However, only 5-10 branching Acropora species dominate the lagoon area in Indo-Pacific reefs, covering more than 50% of the total coral area, even though more than 100 coral species were recorded in the area. In other words, the larval supply from the artificial spawning hotspots of 5-10 Acropora species is comparable to that from the wild coral community in terms of species diversity. In addition, the phase shift from corals to macroalgae in a damaged reef is considered to delay the recovering of coral communities after disturbances (Kuffner et al., 2006; Bozec et al., 2019). Rapid restoration of dominant species could be effective to avoid or reverse such a phase shift to macroalgae.

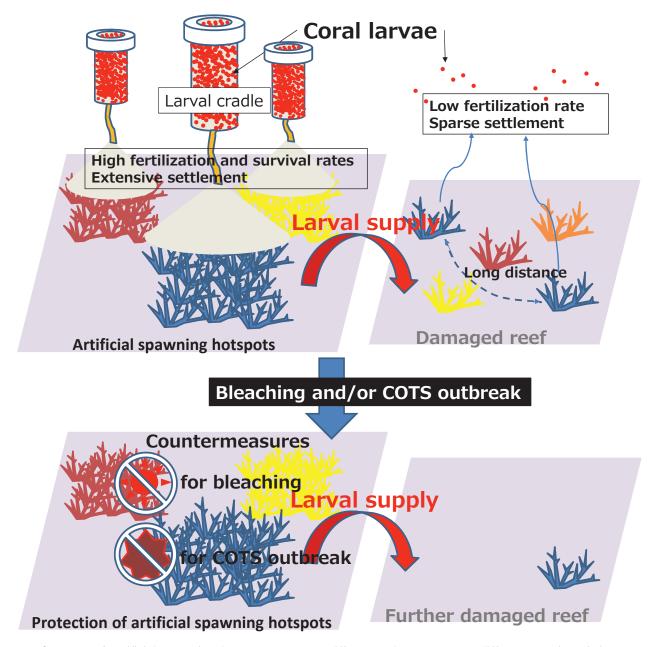


Fig. 2. Image of artificial spawning hotspots concept. Different colors represent different species of *Acropora* corals.

Direct Larval Seeding

Direct seeding of mass larvae acquired from larval cradles or natural slicks would be a shortcut to large-scale coral restoration (Heyward *et al.*, 2002). However, at present, direct larval seeding on the seafloor remains impractical due to low settlement and survival rates of the seeded larvae (*e.g.* Edwards *et al.*, 2015). In the Philippines, approximately 0.4 million larvae were directly seeded to several tens of m² of damaged reef and 2.3 colonies per

m² was found 3 years after the seeding (dela Cruz and Harrison, 2017). An enclosing net was used to prevent dispersal of the larval seeding in that study; however, it is difficult to apply this method on a larger scale (*e.g.* more than 1 hectare). Ultimately, the direct seeding of an overwhelming number of larvae with high density that is comparable to natural recruitment would be effective in large scale restoration even if the larvae were seeded without an enclosing net. Studies to determine suitable seeding methods, including the necessary minimum

density, are currently in progress. In addition, there are some places where larval seeding has no effect. In the sandy bottom, covering most of the lagoon, few settlement substrata such as rock reefs are available. It is expected that outplanting or seeding of corals settled on the artificial plates is more effective in such places (Chamberland *et al.*, 2017).

In conclusion, the methods and techniques reviewed in this paper can be effectively used for gamete collection, larval rearing, seedling production, direct larval seeding, maintenance of adult corals for larval supply, and protection of adult corals from disturbances in the field (*i.e.* without land facilities). Using these simple methods together shows promise to successfully achieve large-scale coral restoration.

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Annotated Bibliography of Key Works

(1) Zayasu Y., and Suzuki G., 2019: Comparisons of population density and genetic diversity in artificial and wild populations of an arborescent coral, *Acropora yongei*: implications for the efficacy of "artificial spawning hotspots". *Restor. Ecol.*, 27, 440–446.

The authors assessed population density and genetic diversity of a wild, arborescent coral, *Acropora yongei*, and compared these parameters with those of an artificially established *A. yongei* population in the field. The population density of wild arborescent corals was only 0.27% of that in the artificial population, even in a high-coverage area. Genetic diversity was also low in the wild population compared with the artificial population, and approximately 10% of all wild colonies were clones. Based on these results, the larval supply in the artificial population was estimated to be at least 1,400 times higher than that in wild *A. yongei* populations for the same area of adult population.

(2) Suzuki G., Yamashita H., Kai S., Hayashibara T., Suzuki K., Iehisa Y., Okada W., Ando W., and Komori T., 2013: Early uptake of specific symbionts enhances the post-settlement survival of *Acropora* corals. *Mar. Ecol. Prog. Ser.*, **494**, 149–158.

The authors tested the hypothesis that early acquisition of symbionts enhances post-settlement survival. Symbiotic and aposymbiotic *Acropora* larvae were prepared in the laboratory and settled

on experimental plates in the field. The survival of settlers was monitored for 15 months, and the results showed that more larval-stage settlers harbouring symbionts survived than those without. The higher survival rate of 'early uptake' corals was more pronounced on shaded plates. These results suggest that the early uptake of specific symbionts enhances post-settlement survival in dark places such as reef crevices, which are sites commonly settled by coral larvae.

(3) Suzuki G., Kai S., Yamashita H., Suzuki K., Iehisa Y., and Hayashibara T., 2011: Narrower grid structure of artificial reef enhances initial survival of in situ settled coral. *Mar. Pollut. Bul.*, **62**, 2803–2812.

The authors demonstrated through field experiments that the design of artificial grid plates may influence the initial survival of *Acropora* corals, with narrower grids being the most effective. In fact, grid plates with a 2.5-cm mesh presented the highest recorded survival rate (14%) at 6 months after settlement (representing approximately 50 corals per 0.25 m² of plate). This was the first study where such high survival rates, matching those of cultures under aquarium conditions, were obtained in the field without using additional protective measures, such as guard nets against fish grazing after seeding. Therefore, their results provide a foundation for establishing new and effective coral restoration techniques for larval seeding.