Development of active restoration methodologies for coral reefs using asexual reproduction in Okinawa, Japan

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Abstract The Coral Reef Preservation and Rehabilitation Project by the Okinawa Prefectural Government, Japan, investigated since 2011 the possibility of large-scale reef restoration with asexually derived corals. The research also sought to establish standard methodologies of active coral reef restoration in Okinawa. During three years of consecutive transplantation from 2012 to 2014, about 31,000 non-nursery-farmed and nursery-farmed corals were out-planted, resulting in gradual refinement of the technique. Small coral colonies collected from neighboring natural reefs were raised as donor corals on top of iron poles 50 cm above the seafloor. Fragments taken from those donor corals were subsequently attached to substrata and transplanted to back-reef moats off Onna Village, Okinawa Island (approximately 26°26'50"N, 127°47'40"E). In 2012 and 2013, 15 and 12 acroporid species, respectively, were transplanted without nursery farming. Whereas in 2014, transplantation focused on six species that appeared tolerant to elevated temperatures and were subject to a period of nursery farming. The survival rate at one year post transplantation improved with each annual cohort, rising from 31.8% for the 2012 transplants to 65.2% for the 2013 cohort, and to 84.7% for the 2014 cohort. More than 70% of out-planted corals in 2014 survived two years after transplantation. The average annual growth rate of the corals transplanted in 2012 and 2013 was 3.4 cm and 5.6 cm in the geometric mean diameter (GMD), respectively, but was 10.9 cm for the six species out-planted in 2014. Proper choice of species with respect to the restoration site, size of fragments, and the processes of nursery farming and out-planting are decisive factors for the success of transplantation. Genomic DNA analysis of farmed and natural populations of *Acropora tenuis* suggested that a substantial proportion of the farmed donor colonies were clones and that propagation of *A. tenuis* in nature mainly takes place sexually and not asexually. Nearly 2,000 Yen (20 US$) per colony was required for asexually-derived fragment farming, out-planting, and monitoring

Keywords: coral reefs, restoration, *Acropora*, Okinawa, out-planting

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Introduction

Coral reefs worldwide continue to degrade due to multiple anthropogenic threats and persisting global environmental impacts (De'ath et al. 2012, Hoegh-Guldberg et al. 2007). Despite many alerts, approximately 40% of the global coral reef system has been lost over the last four decades (Bruno and Selig 2007, Burke et al. 2011). Almost all traditional conservation management measures have been insufficient. Although techniques for the active restoration of coral reefs using coral transplantation have been developed for the last three decades, effectiveness of restoration has not been fully validated at realistic scales that support a recovery of ecosystem services (Edwards 2010). Indeed, reluctance continues to exist towards the active restoration of coral reefs as the area that can be restored by transplantation is limited in comparison to the

worldwide scale of reef degradation. However, as out-planted corals can reproduce to produce larvae, they may lead to natural recruitment enhancement where recovery is slow or limited. Therefore, the out-planting process has become more acceptable as an active management tool (e.g. Horoszowski-Friedman et al. 2011).

The Coral Reef Preservation and Rehabilitation Project by the Department of Environmental Affairs of the Okinawa Prefectural Government, Japan, attempted to validate the possibility of large-scale reef restoration and to establish standard methodologies of active coral reef restoration in Okinawa. Experimental out-planting of a total of 100,000 corals in 3 ha of degraded reefs was planned. Since 2011, 75,000 asexually-derived corals were out-planted in 1.75 ha off Onna Village, Okinawa Island. In the present paper, we describe the gradual development of the methodologies and outcome during the initial three years.

Materials and methods

Coral colonies smaller than 5 cm, collected from neighboring natural reefs within 3 km northeastward and southwestward, and 1 km northwest and southeastward from donor farms, were cultured as donor corals in the back-reef moat off Onna Village (approximately 26°26’50”N, 127°47’40”E) (Fig. 1). Some large corals were additionally fragmented and reared as donor corals. They were kept on top of iron poles 50 cm above the seafloor at a depth of 2-3 m (Fig. 2). This farming technique using iron poles, reduced the mortality of corals resulting from mobile sand and rubble and limited predation by coral-eating invertebrates. Average growth and survivorship of *Acropora tenuis* on the top of poles increased 225% and 146%, respectively, over those on the seafloor. Flow velocity on the top of poles was higher than that on the seafloor. Average amounts of silt sedimentation and turbidity were reduced to 50% and 32%, respectively, when compared to values measured at the seafloor (Fishery Agency of Japan 2012, Higa and Omori 2014).

When the donor colonies grew to approximately 30 cm in diameter, 25–30% of a colony was pruned with a hammer and chisel, and 20-30 small fragments were prepared for out-planting. Subsequently, they were attached to substrata made of Mug White (a patented soil hardening agent, Fujimori and Kobari, 2000) using stainless steel wire. The substrata were plate

type (length 60 mm × width 30 mm × thickness 9 mm) in 2012 and 2013, but were changed to cylinder type (length 80 mm × diameter 28 mm) in 2014. All coral fragments were kept in seawater tanks in a terrestrial aquaculture facility, for approximately 1 week to allow for bonding to the substrata.

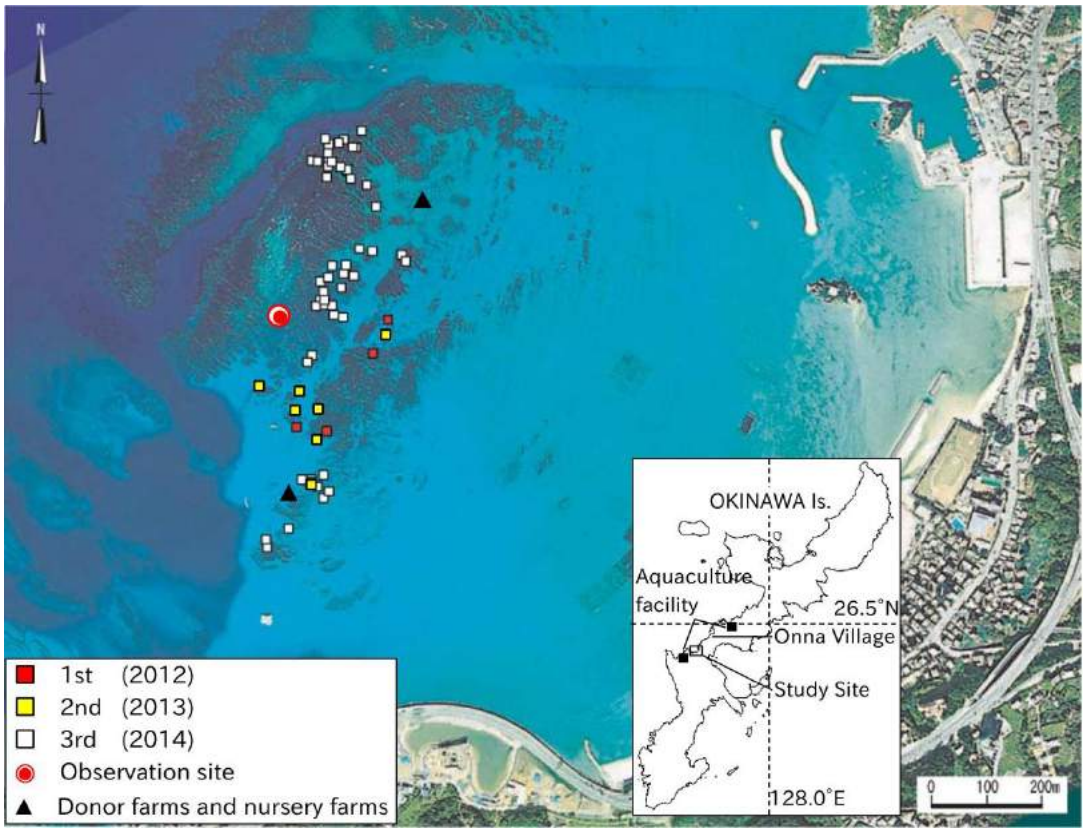

Fig. 1 Locations of donor farms and nursery farms (indicated together), coral out-planting site and observation site Station 1 off Onna Village, Okinawa. Location of terrestrial aquaculture facility on land is also indicated

Currently, over 20,000 donor colonies of 54 species are being farmed at the donor farms. To determine approximately how many clonal colonies were included among the donor corals, 31 colonies of *Acropora tenuis* from about 2,800 colonies in the farms were randomly chosen and their fragments smaller than 5 cm were collected. Subsequently, a genomic DNA analysis was performed using universal primers of *Acropora* corals for 14 microsatellite loci (Shinzato et al. 2014). At the same time, analysis was done for 10 natural colonies of the same species collected from neighboring waters.

In order to determine if self-fertilization occurs among clones of *A. tenuis* in the donor farms, gametes from 3 clonal colonies were collected once they had spawned synchronously in separate containers in the laboratory in June 2014. Sperm and eggs were carefully separated to prevent cross-contamination (Willis et al. 1997). The sperm were kept separately, and the eggs were washed with seawater to remove sperm. Then fertilization were attempted between separated gametes from 3 clones with 300 eggs and a sperm concentration of 10^5 per ml at 28.0-28.5 ℃. After 30 minutes of mixing sperm and eggs, 200 eggs were examined 4 hours later.

Fig. 2 Rearing of donor colonies at donor farms off Onna Village, Okinawa

In 2012 and 2013 the corals attached to the substrata were out-planted without nursery farming, whereas in 2014 they were farmed for more than three months in fixed nurseries in shallow back-reef moats at a depth of 2–3 m before transplantation (Fig. 1). One nursery frame was 3 m (length) × 1 m (width), standing approximately 40 cm above the seafloor. Nearly 400 coral fragments attached to the substrata were set on each frame (Fig. 3).

All corals were out-planted in the outer part of the moats (depth 2–3 m) off Onna Village (Fig. 1) where acroporid corals flourished before a catastrophic coral bleaching event in 1998 due to a strong ENSO event, which may have been connected to global temperature rise. At present, some scleractinian colonies (e.g. *Porites lutea* and *P. cylindrica*) are distributed in the inner part of the moat.

Fig. 3 Farming of coral fragments in underwater nurseries

The sea temperature and salinity were measured continuously at observation site Station 1 near the out-planting area using JFE Advantech's measure (Compact-CT ACT-HR) from December 23, 2011 to March 23, 2014 and from June 20, 2013 to March 23, 2014, respectively. Other environmental water and sediment parameters at 2.3 m depth at Station 1 were measured five times during the period from December 27, 2011 to December 12, 2013 with Japan's standard analytic methods (Japanese Industrial Standards).

In the out-planting area from January to March 2012, 700 corals from 15 species were transplanted (Table 1). This was followed by the addition of 124 corals from eight species in July 2012. The 2013 out-planting was done between August 2012 and February 2013 with 10,440 corals from 12 species. Holes were drilled in the surface of the reef using a pneumatic drill, and

Table 1 Periods of transplantation in 2012, 2013, and 2014 with species and number of corals transplanted in each year. The exact number of corals for each species was not correctly counted in 2012 (except for July 2012) and 2013

Out-planting	2012		2013	2014
Period	1/2012-3/2012	7/2012	8/2012-2/2013	11/2013-3/2014
Acropora cerealis	○			100
A. cytherea	○		○	700
A. digitifera	○		○	2,900
A. divaricata	○		○	100
A. donei	○		○	2,400
A. florida	○		○	
A. formosa		1		
A. gemmifera	○		○	
A. hyacinthus	○	1	○	5,200
A. intermedia		14	○	
A. microclados		3		100
A. microphthalma	○		○	
A. nana	○	1		
A. nasuta	○	22	○	100
A. secale	○	1		
A. selago	○		○	
A. tenuis	○	81	○	5,000
A. valenciennesi				3,400
A. valida	○			
A. verweyi		1		
No. of species	15	8	12	10
No. of corals out-planted	700	124	10,440	20,000

the area around each hole was cleaned before transplantation. Then, each coral attached to a substrate was plugged into the hole using an anchor bolt. Except for the fragments transplanted

in July, 2012, the exact number of fragments for each species could not be counted correctly due to logistical difficulties.

Some corals bleached in August to September 2013 when the sea temperature had risen to 29.8℃ in August. For the 2014 transplantation, six species of *Acropora* i.e. *A. cytherea, A. digitifera, A. donei, A. hyacinthus, A. tenuis,* and *A. valenciennesi* that mostly did not bleach in 2013 and were considered to be relatively more tolerant to elevated temperatures by experienced local workers were selected, and a total of 20,000 corals that were comprised mainly of those six species were transplanted during the cooler season between November 2013 and March 2014. In 2014, in order to reduce labor, the cylindrical substrata were simply wedged into holes without using an anchor bolt.

Based on work by Omori et al. (pers. comm.) on dispersion effects of eggs and sperm after spawning, it was decided that for enhancement of fertilization from out-planted corals, corals without clones should be out-planted within a distance shorter than 2 m where high fertilization rates can be expected. The density of the transplanted corals was 20 corals per m^2 in 2012 and 2013 as we considered that dense transplantation may reduce the damage caused by predation from fish. However, as the corals required sufficient space for growth to avoid intraspecific or interspecific disturbances, the 2014 corals were out-planted 60 cm apart (2.8 corals per m^2). A number of squares were set in the restoration area. Each square (14 m × 14 m) was divided into 4 frames for 4 different species, and 100 corals were out-planted in each frame (6 m × 6 m) (Fig. 4). The fragments were carefully arranged to minimize the possibility of two clonal corals being placed adjacent to each other. Corals were checked at least monthly from March to December to allow extermination of coral-eating crown-of-thorns starfish and the gastropod *Drupella fragum*.

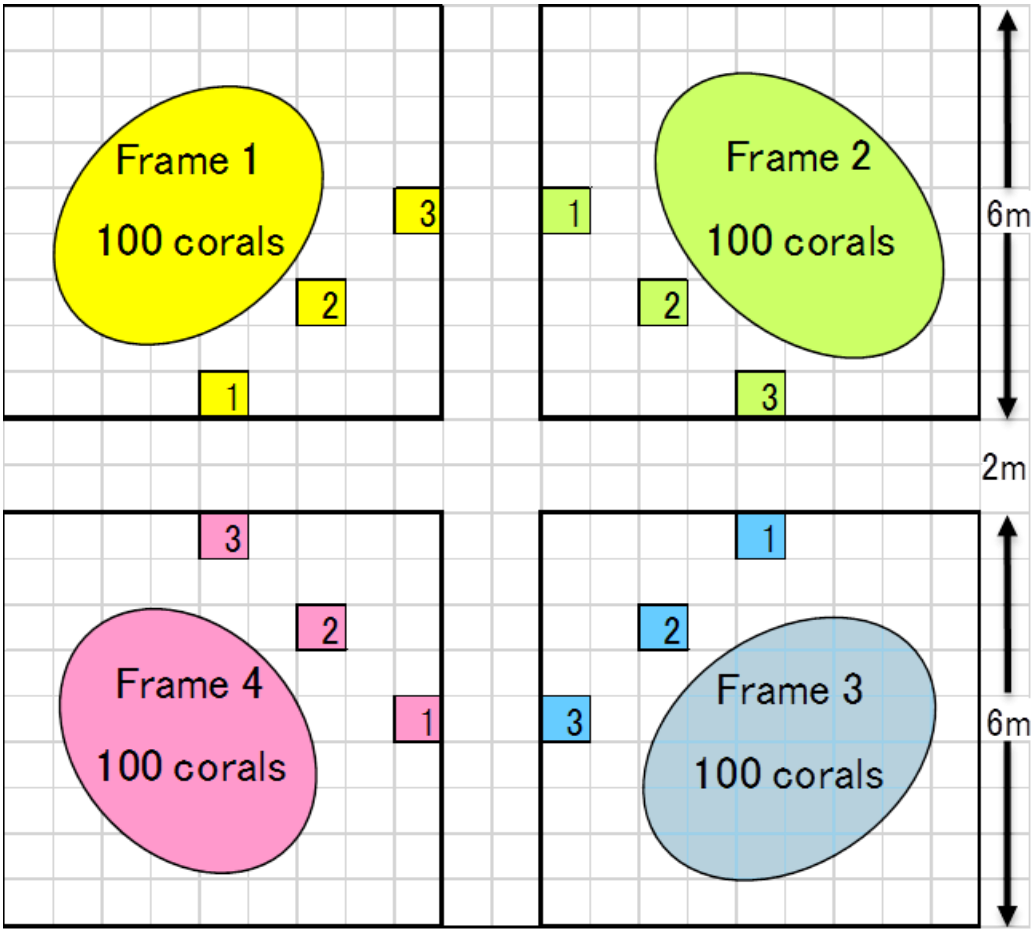

Fig. 4 Arrangement of out-planting. A square (14 m × 14 m) was divided into 4 frames (6 m × 6 m) for 4 different species and 100 corals were out-planted in the distance of 60 cm in each frame. There are nine gaps of 60 cm between 10 corals on line and two gaps of 30 cm outside of the outermost corals. Three corals in each frame were set as a reference for measurement

The size of each coral was estimated through photographs, using a 50 cm × 50 cm frame and an underwater camera with GPS function. The size was expressed by calculating the geometric mean diameter (GMD.): GMD = $\sqrt{d1 \times d2}$ where d1 is the maximum diameter and d2 is the maximum perpendicular diameter.

The average size (GMD) of corals transplanted in 2012 and 2013 were 3.7 ± 0.7 cm (measured one month after transplantation) and 5.5 cm ± 1.7 cm (eight months after transplantation), respectively. As smaller fragments were easily damaged and killed by moving

sand and rubble during rough weather and by the nibbling of fish, the size of the corals was increased for the 2014 out-planting to an average of 8.2 cm ± 3.1 cm. In addition, in 2014 the corals were transplanted to higher positions on the reef where the bottom was rough, avoiding the lower parts of the seafloor where the surface was smooth. The lower, smooth part was considered to be easily abraded by the moving sediments. Transplantation continued in 2016, and the total number of out-planted corals will reach 100,000 in 3 ha by the end of this project.

Survivorship and growth of 70 same corals (irrespective of species) were monitored for the corals transplanted in 2012, whereas for the 2013 out-planting, 508 randomly selected corals (irrespective of species) in 100 frames were measured. The monitoring was carried out 1–3 times in the first year after transplantation; it was then continued 1-3 times per year. For the six species of corals transplanted in 2014, three same corals in each frame (354 colonies in all) were monitored 4 times during 2 years after transplantation.

Results

The temperature at Station 1 varied between 19.2℃ in March and 29.8℃ in August; salinity varied between 35.5 in December and 31.1 in August (Table 2). Water flow varied from 0-4.0 cm per sec. and horizontal transparency was greater than 20 m in the area. Total nitrogen in the water varied between 0.08 and 0.17 mg per L, whereas total phosphate was less than 0.006 mg per L. Other environmental parameters measured at DL-2.3 m at Station 1 are also shown in Table 2.

The 31 colonies of *A. tenuis* in donor-farms represented only 7 different genotypes (Higa et al. 2017 for supplementary data). In contrast, no clonal colonies were identified in the samples

Table 2 Variation of temperature, salinity, and qualities of water and sediment at Station 1 on the seafloor at DL-2.3 m. Temperature and salinity were measured every 10 minutes; other items were measured five times during the indicated period. SPSS means content of Suspended Particles in Sea Sediment

Water	Measurements	Range
Temperature (℃)	12/2011 to 3/2014	19.2-29.8
Salinity	6/2013 to 3/2014	31.1-35.5
pH	12/2011 to 12/2013	8.2-8.3
COD (mg/L)	12/2011 to 12/2013	0.7-1.1
Suspended solids（mg/L)	12/2011 to 12/2013	<1
Total Nitrogen (mg/L)	12/2011 to 12/2013	0.08-0.17
Total Phosphate (mg/L)	12/2011 to 12/2013	0.003-0.006
n-Hexane extracts (mg/L)	12/2011 to 12/2013	<0.6
Dissolved Oxygen (mg/L)	12/2011 to 12/2013	6.2-6.9
Total Coliform (MPN/100mL)	12/2011 to 12/2013	5-130
Chlorophyll-a（μg/L)	12/2011 to 12/2013	0.10-0.27
Sediment		
COD (mg/L) for sediment	12/2011 to 12/2013	1.3-1.7
Water content (%)	12/2011 to 12/2013	18.0-40.0
Ignition loss (%)	12/2011 to 12/2013	3.9-4.2
Sulfide (mg/g)	12/2011 to 12/2013	<0.01
SPSS (kg/m3)	12/2011 to 12/2013	1.4-1.8

from natural colonies from widely separated neighboring waters. Fertilization among 3 clonal colonies of *A. tenuis* was always unsuccessful (0%).

The survival rate of the corals out-planted in 2012 was not favorable. Only 31.8% of colonies survived one year after transplantation and only 17.5% remained after 3 years. For the 2013 out-planting, the survival rates were higher at 65.2% and 49.2% after l and 3 years, respectively. The survival rate of the 2014 corals further increased to 84.7% after 1 year and over 70% after 2 years, respectively (Fig. 5).

Monthly mortality rate of the 2012 corals varied greatly, with the highest value recorded between March and July, 2013 (12.9%) and second highest between September and October, 2012 (10.3%) (Table 3). In the 2013 corals, mortality varied between 0.6 and 8.8% with the highest rate in March to July, 2013 (8.8%). The death of corals in these periods was mainly

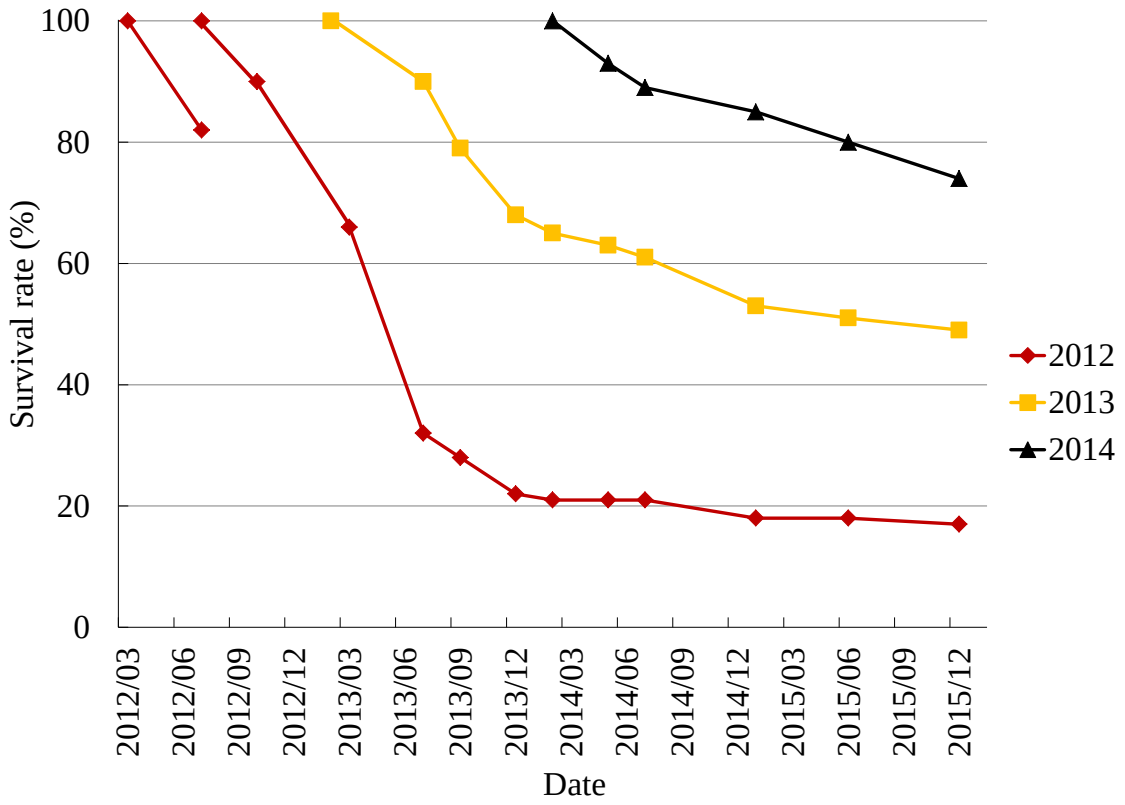

Fig. 5 Variation of survival rates of the corals transplanted in 2012, 2013, and 2014

Table 3 Variation of monthly mortality rates of the corals transplanted in 2012, 2013, and 2014

Period	2012	2013	2014
Mar-July 2012	4.4		
Sept-Oct, 2012	10.3		
Oct 2012-Mar 2013	5.3		
Mar-July 2013	12.9	8.8	
July-Sept 2013	0.9	6.6	
Sept-Dec 2013	6.5	4.6	
Dec, 2013-Feb, 2014	2.8	2.2	
Feb-May 2014	3.4	1.2	2.3
May-July 2014	6.8	1.5	2.3
July 2014-Jan, 2015	4.2	2.3	0.8
Jan-June 2015	0	1.1	1.1
June-Dec 2015	0.5	0.6	1.4
Dec 2015-May 2016	4.2	0.6	2.8

caused by the moving seabed sediments due to waves caused by large typhoon system Jelawat in September 2012 and a rapidly developed low pressure in July 2013. The mortality rates of the corals out-planted in 2014 were consistently less than 2.3%.

In the corals transplanted in 2012, three of 9 colonies of *A. tenuis* that attained 15-20 cm GMD matured sexually. Twelve out of 20 colonies of 20-30 cm and all colonies greater than 30 cm had gamete bundles in June 2015. Spawning of *A. tenuis* was confirmed on June 26, 2015. Observations were not made for other species.

The average annual growth rate (cm GMD) of the corals transplanted in 2012 and 2013 were 3.4 cm and 5.6 cm, respectively. That of the six coral species transplanted in 2014 was 10.9 cm, being much higher than the corals transplanted in the previous years (Fig. 6). Growth of *A. cytherea* after May 2015 was particularly notable. The size of these six coral species after 3 years was estimated to be 18.2-46.0 cm (Table 4).

Estimates of set-up and operational costs needed for the reef restoration in 2014 were as follows: 5,500 Yen for sampling and farming of one donor coral (275 Yen per fragment); 400 Yen for nursery farming, 300 Yen for substratum and handling, and 400 Yen for out-planting for one coral, respectively. The cost for monitoring and maintenance of the corals after transplantation was 200 Yen per colony. Thus, a total of 2,000 Yen (20 US$) per colony was required for farming, out-planting, and monitoring of each asexually-derived colony.

Discussion

The “transplantation of corals” can be divided into 4 categories: 1) direct transplantation of fragments clipped from natural corals or “corals of opportunity” without nursery farming; 2) transplantation of nursery-farmed, asexually-propagated corals; 3) transplantation of nursery-farmed, sexually propagated corals; and 4) translocation of the entire coral colonies to rescue the ones that would otherwise be destroyed or severely damaged by coastal and/or underwater development.

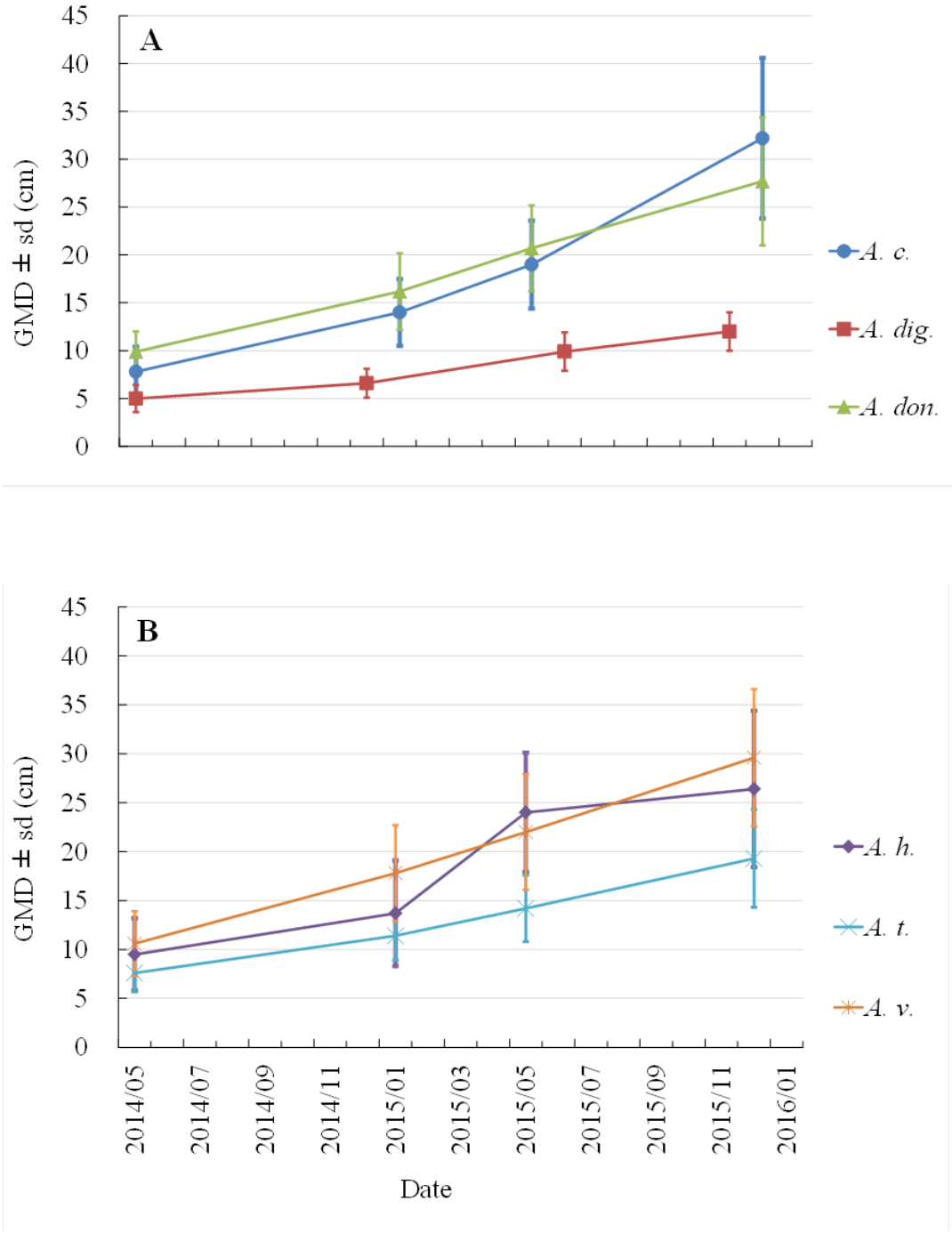

Fig. 6 Growth of six coral species (average size, cm GMD ± standard deviation) transplanted in 2014. Number of same coral colonies monitored is indicated in parenthesis. A. *A.c., Acropora cytherea* (12); *A. dig., A. digitifera* (102); *A.don., A. donei* (87): B. *A. h., A. hyacinthus* (42)*; A. t., A. tenuis* (51)*; A.v., A. valenciennesi* (60). Dates of measurement: *A. dig*., 2014/5/25, 2014/12/27, 2015/6/2, and 2015/11/6. Other species, 2014/5/1, 2015/1/11, 2015/5/26, and 2015/12/31

Table 4 Annual growth rate (cm GMD) of six species out-planted in 2014 and their estimated size (cm GMD) 3 years after transplantation. *A.c., Acropora cytherea; A. dig., A. digitifera, A.don., A. donei; A. h., A. hyacinthus; A. t., A. tenuis; A.v., A. valenciennesi*

Period \ Species	*A. c.*	*A. dig**	*A. don.*	*A. h.*	*A. t.*	*A. v.*	Mean
Number of corals	12	102	87	42	51	60	
5/2014 to 1/2015	8.9	2.6	9.0	6.0	5.4	10.3	7.0
1/2015 to 5/2015	13.5	7.8	12.2	27.8	7.6	11.4	13.4
5/2015 to 12/2015	22.0	4.8	11.7	4.0	8.5	12.7	10.6
Average annual growth rate	14.8	5.1	11.0	12.6	7.2	11.4	10.9
Estimated GMD, 3 years after transplantation	46.0	18.2	38.2	42.1	26.1	40.2	26.7
*A. dig**: Dates of measurement, 2014/5/25, 2015/12/27, 2015/6/2, and 2015/11/6							
Other species: 2014/5/1, 2015/1/11, 2015/5/26, and 2015/12/31							

"Direct transplantation" is simple and inexpensive. Thus, it is a suitable method for local communities to actively participate in reef restoration (e.g., dela Cruz et al. 2014). But corals grow more slowly and have higher mortality rates than normal, as reallocation of energy after transplantation is considerable (Omori, pers. comm.).

In the case of "Transplantation of nursery-farmed, asexually-propagated corals" (e.g. Rinkevich 2000, Shafir et al. 2006), coral fragments are generally collected from corals on reefs. This causes collateral damage to the donors. If fragments are raised from only a few donors, they may have low genetic diversity, and may lead to lower fertilization rates among corals when they became mature. Thus, they may reduce the long-term benefits of reef restoration. In this method, mortality rate may also increase due to detachment of the fragments from the artificial substrate. In order to keep genetic diversity high large number of donor corals could be cultured in a donor farm, as employed in the present study.

"Transplantation of nursery-farmed, sexually-propagated coral products" (e.g. Omori and Iwao 2014) requires more labor and techniques than the above method, and hence is more expensive. However, it does not cause damage to natural reefs and can produce large number of corals with high genetic diversity. Increased genetic diversity may enable corals to respond in a variety of ways to bleaching, disease or other stressors, reducing the risk of complete loss.

Unstable survivorship of the juvenile corals after settling on the artificial substrate must be overcome by further research (Omori pers. comm.).

These two techniques of transplantation comprise two phases, i.e., 1. propagation and nursery phase and 2. transplantation phase. The first phase has been developed considerably, although there is still much work needed. The second phase includes problems such as the selection of species and sites of transplantation, size of fragment, time of out-planting, design to maintain genetic diversity, species combination, and landscape manipulations, which is in its infancy and needs further study (Rinkevich 2014).

We cannot expect coral recruitment derived from transplants in poor environmental conditions where chronic stressors are not reduced. Sites for transplantation must be chosen carefully. In the present out-planting, the site was chosen based on examining environmental parameters, the history of the reef area and eye witness information from local inhabitants. The corals were placed on higher positions of the reef where the structure is rough with finer scale complexity. Proper choice of species with respect to the restoration site and processes of nursery farming and out-planting are clearly decisive factors for successful coral transplantation. Six *Acropora* species that were considered to be comparatively tolerant to elevated sea temperatures were selected in 2014. To gain higher survival rates, the size of the nursery farmed coral fragments should be approximately 8 cm. Maintenance of the fragments in fixed nurseries for over 3 months strengthened their attachment to substrata and therefore increased survivorship.

The methodologies developed in the present study comprise of the following: 1) finding suitable species and locations for out-planting; 2) farming of donor corals; 3) genomic DNA analysis of donor colonies; 4) nursery farming of coral fragments; 5) out-planting design for each species to minimize the possibility of out-planting clonal colonies adjacent to each other; 6) the combination of various species in a plot to maximize fertilization rate and genetic diversity; and 7) long-term monitoring.

No clonal colonies were identified in the 10 samples of *A. tenuis* from natural colonies from widely separated neighboring waters. It may suggest that the propagation of *A. tenuis* mainly takes place sexually and not asexually in nature. This is supported by Zayasu et al. (2016) who

collected fragments from 298 colonies of *A. tenuis* within 3 ha each at 15 locations across the Nansei Islands including Okinawa Island, and found no clonal colony among them.

Cross-fertilization of clones is mostly unsuccessful (Heyward and Babcock 1986, Willis et al. 1997, present study). Different genotypes should be interspersed when out-planting and fragments of two clonal colonies adjacent to each other should be minimized. Designing transplantation sites comprising many different genotypes has also been proposed by Johnson et al. (2011). To provide the highest fertilization rate, the distance between colonies may be arranged within 2 m (Omori et al. pers. comm.) and more than 6 colonies of different genotypes should participate to ensure high genetic diversity (Iwao et al. 2014). Genetic diversity and future resilience among fragmented colonies are important. Accurate evaluations of population structure and genetic diversity using the present microsatellite markers technique will be valuable for selection of genotypes that may be tolerant to environmental change.

Currently, it is estimated that over 100,000 corals of at least 86 nursery-farmed species have been out-planted worldwide (Rinkevich 2014). However, the condition of corals after transplantation was monitored mostly for one year or less. Only a few reefs were monitored for over 3 years until the out-planted corals spawned. According to limited records, survival rate of transplanted corals around Okinawa Island is mostly lower than 40% at 3 years after transplantation. We propose a survival rate of over 40% at 3 years after out-planting as a reasonable performance target for active coral reef restoration. In the case of *A. tenuis*, for which the growth rate was estimated in the present study, the colonies initiate spawning and their coverage would increase by over 4.3 fold compared to the initial area at 3 years after transplantation.

Acknowledgements

This study was conducted as a part of the Coral Reef Preservation and Rehabilitation Project by Okinawa Prefectural Government, Japan. The authors express high gratitude to the Department of Environmental Affairs of the Okinawa Prefectural Government, which presided over the project, and to all who assisted with its progress. We would like to thank P. Harrison and two anonymous reviewers for the English language review and helpful comments.

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