Transplantation Tests of Precious Coral Fragments Using Small-sized Artificial Substratum

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Since the Roman era, precious corals have been used to make ornaments worldwide, and their demand has recently increased. As a basic study for artificial cultivation, we transplanted *Corallium japonicum* fragments. In 2016 and 2017, 132 fragments approximately 3–5 cm in length were attached to small-sized artificial substratums using marine epoxy on land. These artificial substratums, acting as transplant substrates, were then transported and sunk to a depth approximately 100 m off the coast of Otsuki Town and Tosashimizu City, Kochi Prefecture, where precious corals once flourished. From six months to three years post-submersion, we successfully recovered the transplanted substrates and found a total of 107 fragments (81%). We confirmed that

106 of these fragments were alive 177 to 936 days after transplantation. Although we could not measure growth rates due to the initial damage caused by the transplantation, we observed growth of coenenchyme tissues, new polyps and new branches in the 104 surviving fragments. This result suggests there is great potential to artificially multiply precious corals, which could aid in the development of a sustainable precious coral industry.

Key words: Coralliidae, *Corallium japonicum*, mesophotic zone, Kochi, Japan.

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BACKGROUND

Precious corals have a long history of use and have been excavated from ancient sites as far back as 30,000 years ago (Liverino 1986; Kosuge 1987; Smith et al. 2007). *Corallium rubrum* (Linnaeus, 1758) is a precious coral species imported to Japan via the Silk Road. These precious coral bead products were worn by Emperor Shomu in 725 AD, which is stored at the Shosoin Temple in Nara Prefecture (Suzuki 1999). Their use as jewelry and ornaments continues to this day, making them culturally very important. Currently, 42 species of three genera in the family Coralliidae are known (WoRMS 2021), seven of which are valuable for ornamental use. Among them, three species [*Corallium japonicum* (Kishinouye, 1903), *Pleurocorallium konojoi* (Kishinouye, 1903), and *Pleurocorallium elatius* (Ridley, 1882)] are mainly used for ornaments and accessories in Japan. *Corallium japonicum* from Kochi Prefecture in Japan is very popular due to its deep red color, and trades at an average price up to ten times higher than other species of precious corals (Shiraishi 2018).

Kochi Prefecture is the birthplace of commercial precious coral fisheries in Japan (Kosuge 1993) and is still the main production area of precious corals today. According to Nakanishi (2010), in 2005, 58% of Japan’s total precious coral catch was obtained from Kochi Prefecture. Since traditional trolling is used in the precious coral fishery in Kochi Prefecture, living tips of precious corals have been caught occasionally and small ones less than 7 cm in length are released from the viewpoint of resource conservation.

Precious corals belong to the phylum Cnidaria and mainly include those belonging to the family Coralliidae (Octocorallia: Alcyonacea) (Nonaka and Muzik 2010; Tsounis et al. 2010; Tu et al. 2012 2015a b 2016). They are colonial organisms that clone themselves by asexual reproduction and sexual reproduction by eggs and sperm (Nonaka et al. 2015). Therefore, it can be assumed that a broken part of a colony fixed to a substrate can grow and stabilize itself by spreading its coenenchyme over the substrate.

Scleractinian corals, which inhabit the shallow waters, have been transplanted worldwide, totaling over 100,000 colonies (Rinkevich 2014). In contrast, *C. japonicum* inhabits the mesophotic zones, which are extremely difficult to survey and study, and there are few cases of transplantation. Only Nagamune (1918) and Kuno (1922) have conducted transplantation experiments of precious corals in Japan. There are few descriptions of these experiments, and the survival rate and growth of precious corals have not been sufficiently examined because of their low collection rate. Understanding the survival rate and growth of transplanted precious corals is important for determining optimal transplantation methods and sites. Therefore, in this study, we used branch tips of precious corals provided by fishers to examine their survival and growth rates, which will be useful for the future conservation of the resource.

MATERIALS AND METHODS

Securing/storing donors of precious coral for transplantation

Branch tips of *C. japonicum* caught off Cape Ashizuri, Kochi Prefecture, were provided by precious coral fishers. On the fishers' ship, branch tips of the precious corals were placed in a 500 ml round sample bottle with surface seawater. They were subsequently transported to the Kuroshio Biological Research Foundation laboratory using a cooler box to prevent the water temperature from increasing. The water temperature was not adjusted at this time. The precious corals were then transferred to a temperature-controlled aquarium and kept at 18°C, which was the estimated water temperature at a depth of 100 m off the coast of Cape Ashizuri, Kochi Prefecture, until transplantation. Feed was prepared by grinding dried samples (mollusks, arthropods, and algae) using an electric mill and mortar, and the precious corals were fed once a day.

Preparation of transplantation fragments

Healthy donor colonies living in the aquarium were selected a few days before the transplantation, and the fragments were prepared by cutting them into 3–5 cm lengths using a nipper which had been sterilized with 70% ethanol to prevent bacterial contamination on the cut surface (Fig. 1A, B). Donor health was determined visually, and healthy donors were considered those with no discoloration or significant loss of the coenenchyme. Transplantation fragments were individually assigned an identification number and were photographed before and after transplantation. To stabilize their adhesion to transplant substrates, the lower part of the fragments was covered with a submersible epoxy bond (Submersible Bond E380, Konishi Co., Japan). The curing was timed to ensure that it was completed by the day of transplantation.

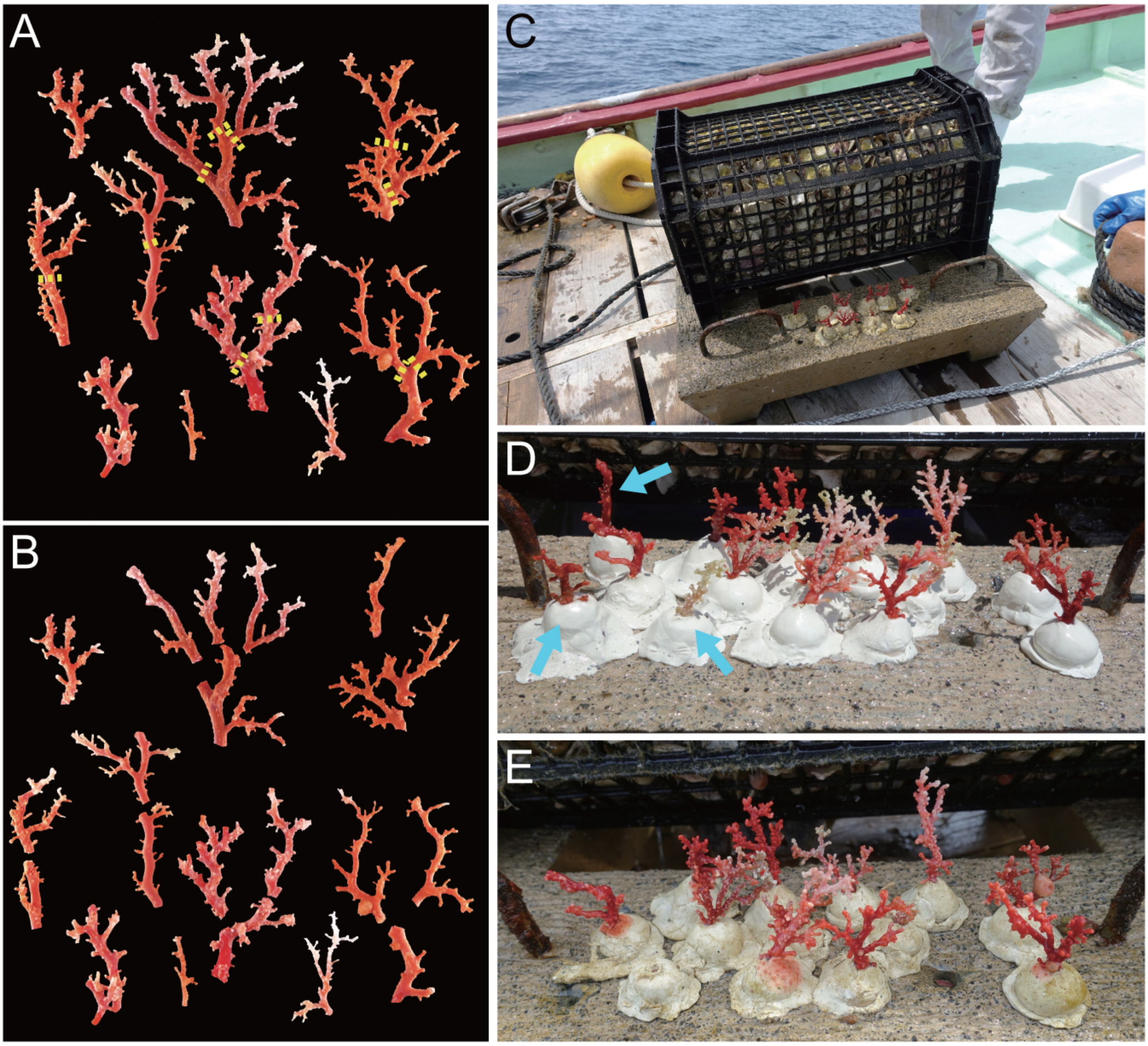

Fig. 1. Precious coral colonies used for transplantation. A: Before cutting. The broken yellow line shows the separation line. B: After cutting. C: Precious coral fragments were transplanted on the concrete part of substrate (Kaiso-kun). D: Pre-release substrate. Blue arrows show fragments detached along with epoxy mount. E: Recovered substrate. C–E is based on Koido and Toshino 2022.

Installation of transplanted substrates

On the day of transplantation, fragments were stored in a cooler box with rearing water approximately 1 h before the release. Small artificial substratums ("Kaisou-kun", Ocean Construction Co., Japan) were used as substrates (Fig. 1C). The artificial substratums are 60 cm in length, 55 cm in width, 45 cm in height, and weigh 60 kg. Onboard the vessel used to transport the fragments to the test site, the lower portions of the transplantation fragments were coated with a new submersible epoxy bond and inserted into the holes drilled on the concrete part of the artificial substratums (Fig. 1D, E). We transplanted 5 to 15 fragments onto each artificial substratum. Two transplanted substrates were connected by a 200 m-long rope, which was a key tool for substrate collection. These connected substrates were dropped simultaneously from two boats. Precious corals inhabit rock and bedrock substrates at depths of 50–320 m (*C. japonicum*: 75–300 m, *P. konojoi* and *P. elatius*: 50–320 m) (Seki 1991; Hasegawa et al. 2012; Cannas et al. 2019). Therefore, we sunk the substrates to a depth of 100–115 m in waters close to their typical habitat, where fishing for precious coral is now prohibited (Fig. 2A, B). The substrates were released five times from 2016 to 2017 (Table 1).

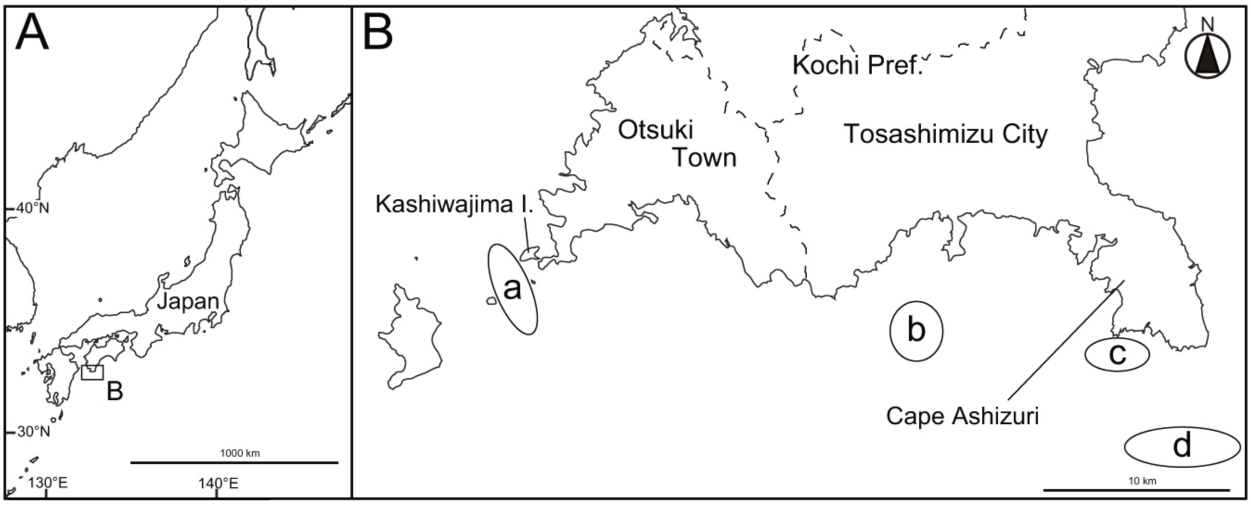

Fig. 2. Index map (A) and release points of transplanted substrates in southwest Kochi Prefecture (B). a and b: this study, c and d: Nagamune (1918), and Kuno (1922). respectively.

To clarify a part of the living conditions of precious corals, a water temperature data logger (HOBO TIDBIT V2®, Onset Computer Corp., USA) was attached to the transplanted substrates. Seawater temperature off the coast of Otsuki Town was measured every 2 h from July 26, 2016, to July 1, 2017 (Code B-1), and from February 26, 2017, to June 2, 2018 (Code C-2), for a total of 801 days. Seawater temperature was also measured off the coast of Tosashimizu City every hour from June 9, 2017, to June 7, 2018 (Code D), for a total of 363 days.

Table 1. Release and collection dates of transplanted substrates

Code	Release date	Recovery date	Period (days)	Release locality*	No. of substrates	No. of fragments
A	01/28/2016	07/23/2016	177	a	1	5
B-1	07/26/2016	02/26/2017	215	a	2	9, 8
B-2	07/26/2016	07/01/2017	340	a	1	8
C-1	02/26/2017	01/20/2018	328	a	3	12, 12, 12
C-2	02/26/2017	06/02/2018	461	a	1	12
D	06/09/2017	06/07/2018	363	b	3	15, 15, 12
E	07/01/2017	01/22/2020	936	a	1	12
Total					12	132

* Shown in figure 3B.

Collection method

Transplant locations were recorded with Global Positioning System loggers (GPSMAP 64csx, Garmin Ltd., USA), and transplantation substrates were collected using a hook. The twelve substrates were collected in seven times between 2016 and 2020 (Table 1). After successful substrate recovery, planted-fragments were first photographed as a whole (Fig. 1E) and then immediately removed from the substrates and stored in a cooler box with seawater. The substrates were then transported back to the laboratory. The identification numbers of the collected transplantation fragments were confirmed, and these transplanted fragments were photographed individually to compare colony size, branch growth, polyp formation, and coenenchyme elongation before and after the transplantation.

RESULTS

We released a total of 132 fragments of precious coral colonies in five release events and collected them at seven instances, from 177 to 936 d after the transplantation (Table 2). Between days 177 and 936 after transplantation, we successfully collected 107 of 132 transplanted fragments at a recovery rate of 81.1%. However, 25 specimens (18.9%) fell off the epoxy mount or fell off from the substrate with the entire mount (Table 2), and most of the collected specimens were partly broken, based on the comparison between pre-release and collected specimens (Figs. 1D, E, and

3A). Since the installation and collection of the transplanted substrates were performed manually, transplanted fragments and their mounts may have been damaged due to shock. The collection rope may be another factor damaging transplanted corals. Because many transplanted fragments were partly lost, comparing the weight before and after transplantation is not significant. We only identified whether the transplanted fragments were alive or dead on the basis of the coenenchyme, polyp formation and/or new branching. Of the specimens collected, 106 of 107 (99.1%) were alive after the installation. The comparison of the 106 living precious coral fragments with photographs taken prior to the transplantation shows thickening and elongation of the coenenchyme or polyp formation in 103 fragments (97.2%), as well as elongation of branches and formation of new branches in 73 fragments (71.6%) (Table 2, Figs. 3A, 4). The dead colony was the fragment installed off the coast of Tosashimizu City from June 9, 2017, to June 7, 2018. It likely died shortly after the transplantation, as the collected fragment did not appear to have spread coenenchyme on the substrate and did not exhibit any branch growth (Fig. 3B). However, the cause of death is unknown because it looked healthy without any coenenchyme decay prior to release.

Table 2. Results of transplantation tests in relation to collection and survival number of transplanted precious corals. Based on Koido and Toshino 2022

Code	Period (days)	No. of fragments	No. of recovery	No. of alive	No. of dead	No. of growth fragments	
						Coenenchyme	Branches
A	177	5	5	5	0	5	NA
B-1	215	17	17	17	0	17	13
B-2	340	8	8	8	0	8	4
C-1	328	36	30	30	0	28	21
C-2	461	12	11	11	0	10	3
D	363	42	27	26	1	26	26
E	936	12	9	9	0	9	6
Total (rate %)		132	107 (81.1%)	106 (99.1%)	1 (0.9%)	103 (97.2%)	73 (71.6%)

NA: Not analyzed.

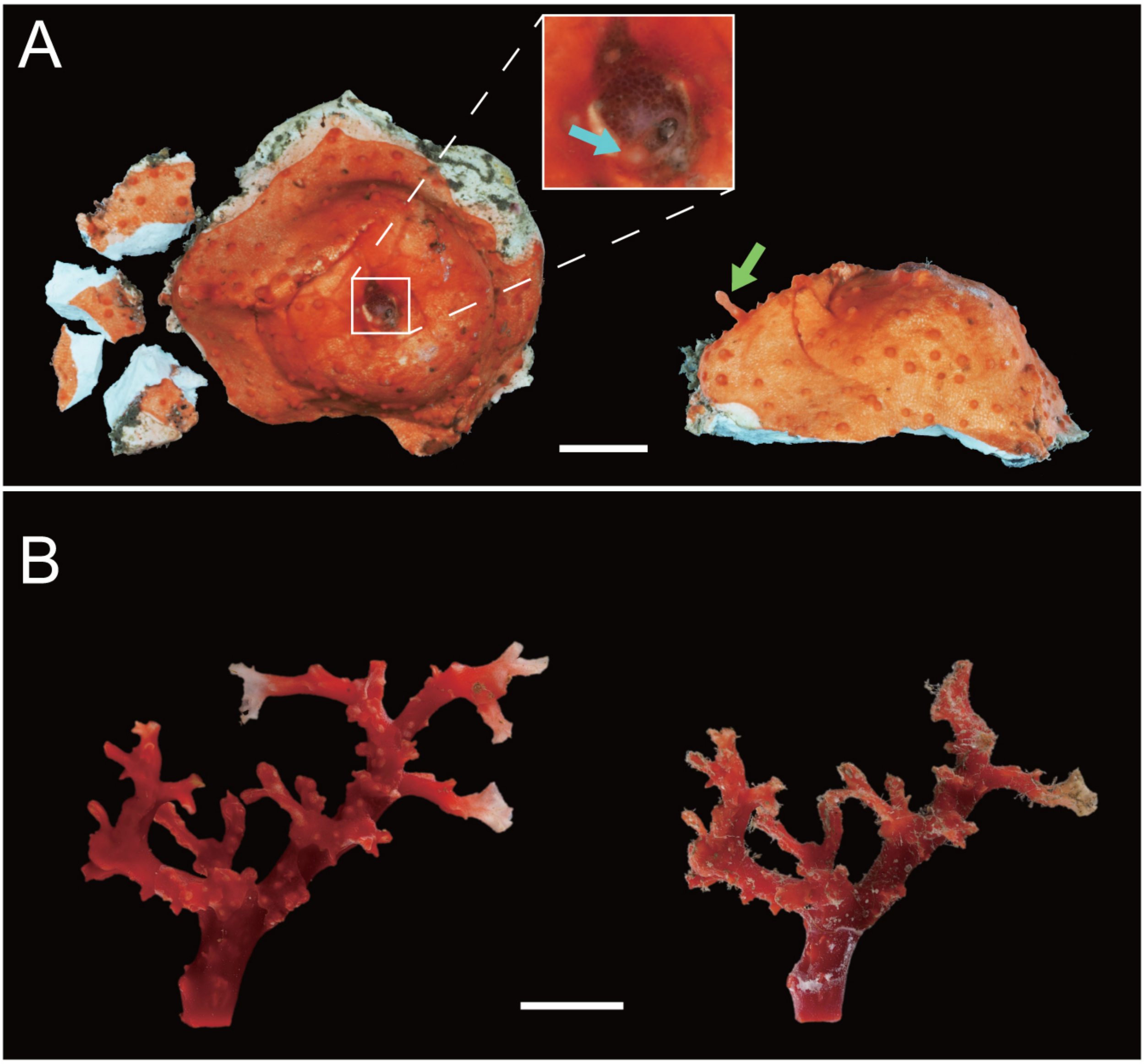

Fig. 3. An example of living broken and dead fragments. A: Coenenchyme had spread over the mount with the formation of many tiny polyps. Blue arrow: cross section was covered with coenenchyme, and a polyp had formed. Green arrow: Newly growing branch. B: Comparison of the dead specimen. Left: Before the release (Jun. 9, 2017). Right: After the collection (Jun. 7, 2018). Scale bars: 10 mm.

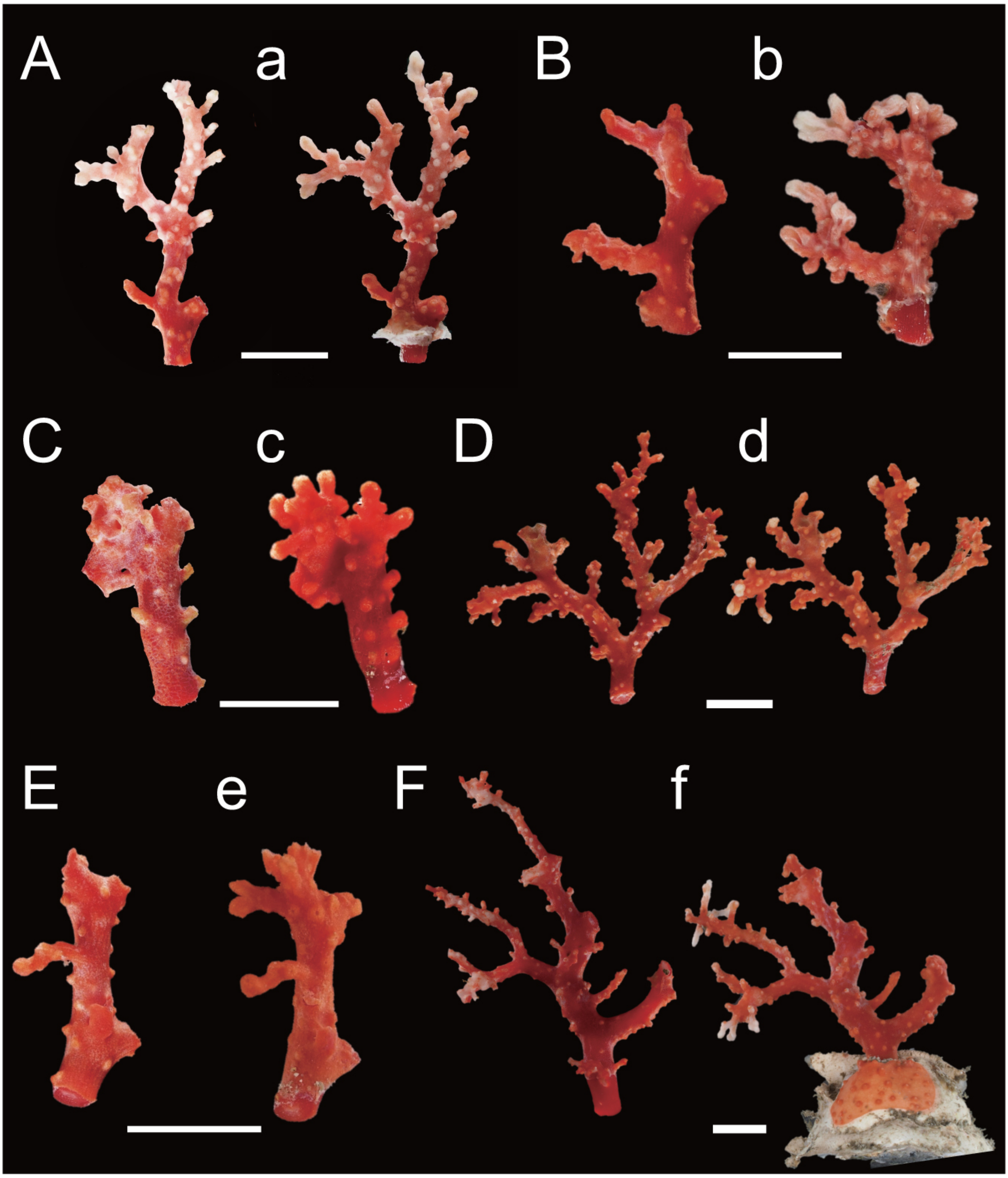

Fig. 4. Examples of the coral fragments showing substantial growth. Coenenchyme and branch growth, cross-section repair, and polyp increase were confirmed. Left side with capital letters: Before the release. Right side with small letters: After the collection. A and a: 215 days (Jul. 26, 2016, to Feb. 26, 2017), B and b: 328 days (Feb. 26, 2017, to Jan. 20, 2018), C and c: 340 days (Jul. 26, 2016, to Jul. 1, 2017), D and d: 363 days (Jun. 9, 2017, to Jun. 7, 2018), E and e: 461 days (Feb. 26, 2017, to Jun. 2, 2018), F and f: 936 days (Jul. 1, 2017, to Jan. 22, 2020). Scale bars: 10 mm. A–B is based on Koido and Toshino 2022.

The monthly average water temperatures at the transplantation test sites are shown in figure 5. The minimum water temperatures were 13.8°C off the coast of Otsuki Town on March 12, 2017, and 12.3°C off the coast of Tosashimizu City on February 18, 2018. The maximum water temperatures were 20.3°C off the coast of Otsuki Town on December 4, 2016, and 19.8°C off the coast of Tosashimizu City on November 20, 2017. The average annual water temperatures during the measurement periods were 16.1°C (July 26, 2016, to July 1, 2017) and 16.6°C (February 26, 2017, to June 2, 2018) off the coast of Otsuki Town and 15.6°C off the coast of Tosashimizu City (Fig. 5).

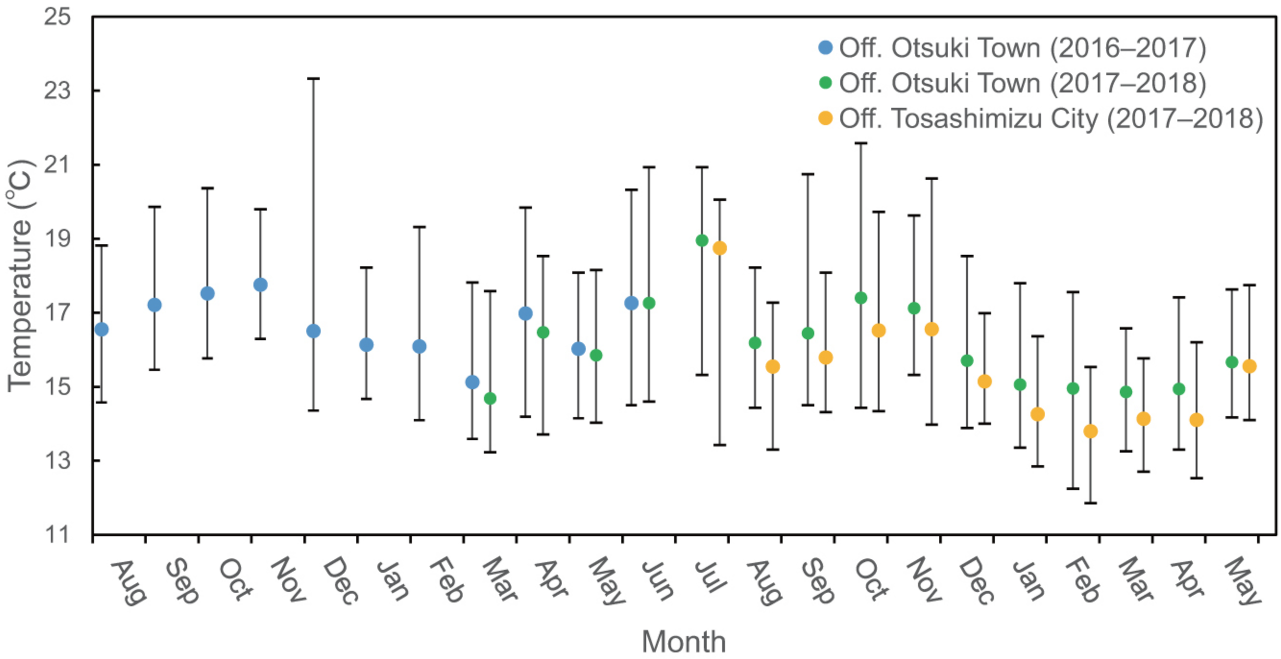

Fig. 5. Monthly average of water temperatures off Otsuki Town and off Tosashimizu City from Aug. 2016 through May 31, 2018. Logger data collected every 1 or 2 h were used to calculate the average monthly temperatures. Bars indicate maximum or minimum water temperatures.

DISCUSSION

In the present study, we installed artificial substratums transplanted with branch tips of precious corals, at a depth of approximately 100 m off the coasts of Otsuki Town and Tosashimizu City. The area was previously used for precious coral fishing but is now a prohibited area and therefore suitable for precious coral growth. In fact, of the specimens collected, 99.1% of the fragments were alive, of which 97.2% thickening and elongation of the coenenchyme or polyp formation, 71.6% showed elongation of branches and formation of new branches. The results suggest that transplanted branch tips can grow on an artificial substrate in natural marine conditions. In Japan, Nagamune (1918) and Kuno (1922) conducted transplantation experiments on precious corals. In these experiments, living fragments were inserted into holes drilled on coastal boulder stones and fixed by mortar cement. The boulder stone was set with a hook for release and collection. Nagamune (1918) transplanted fragments of *P. konojoi* and *C. japonicum* and installed them in shallow (36 m depth) and deep (91 m depth) waters off the coast of Tosashimizu City. In total, 54 fragments were released into the shallow area and most of them were not recovered. Only one dead fragment was collected. In the deep area, 138 fragments of precious corals (detailed species unknown) were transplanted, and two fragments of *P. konojoi* and one fragment of *C. japonicum* were collected alive. Although all three collected fragments were partly broken, their growth was vigorous as the coenenchyme had spread over the substrate (Table 3).

Table 3. A summary of past precious coral transplantation experiments. Based on Koido and Toshino 2022

References	Period (days)	Depth (m)	No. of fragments	No. of recovery	No. of dead
Nagamune 1918	51–339*	36	54	1 (2%)	1
	341–356*	91	138	3 (2%)	0
Kuno 1922	548	102	NR	2	0
	549			1	0
	212	102	NR	1	0
	186			1	0

*: There is no accurate data on the release and collection dates. Therefore, we displayed the maximum and minimum number of days during this period. NR: Not Reported.

In a similar transplantation experiment conducted by Kuno (1922), precious corals fragments were installed at a depth of 102 m off the coast of Tosashimizu City, although the total number of transplanted precious coral colonies was unknown. Five fragments of *P. konojoi* and/or *C. japonicum* were collected alive. The growth of *C. japonicum* was not confirmed due to the short transplantation period of half a year (Table 3). The collection rate of the transplantation fragments was very low in both experiments (Nagamune 1918; Kuno 1922). Of the 21 substrates recovered, only 9 specimens were collected. This suggests the weakness of fixing materials, which, at the time, was mortar. In this study, 25 of the 132 fragments fell off the epoxy mount or fell off from the substrate with the entire mount. Because epoxy mounts are faster drying and more viscous than mortar, resulting in a lower percentage of fragments dropping out compared to previous studies. In addition, when transplanting without consideration of retrieval, the ropes for recovery are not attached, so it is expected that fragments will be less likely to fall off or be damaged.

Precious coral fragments installed near a suitable habitat at a depth of 100 m had a high survival rate. This suggests that depth is an important factor for precious coral survival in relation to light intensity, wave agitation, and seawater temperature. In a recent transplantation experiment on corals inhabiting the deep waters off the coast of California, USA, Boch et al. (2019) transplanted five fragments of *Corallium* sp., all of which were alive and in good health one year after transplantation. However, although the same method was used for transplanting various Octocorallia species [*Keratoisis* sp., *Isidella tentaculum* Etnoyer, 2008, *Paragorgia arborea* (Linnaeus, 1758), and *Sibogagorgia cauliflora* Herrera, Baco & Sánchez, 2010], their survival rates after one year of transplantation ranged from 0% to 50%, except for *Swiftia kofoidi* (Nutting, 1909) for which the survival rate was 80% to 100% (Boch et al. 2019). These results indicate that the deep-sea Octocorallia have different adaptabilities depending on the species. Transplantation experiments have also been conducted on the Mediterranean Sea precious coral *C. rubrum* (Weinberg 1979; Montero-Serra et al. 2018). It inhabits a wide range of depths (5–1016 m) but is commonly found at depths of 30–200 m (Cannas et al. 2019; Abbiati et al. 1993; Knittweis et al. 2016), and its transplantation experiments have been conducted by scuba diving in relatively shallow areas with a depth of 15–35 m. Montero-Serra et al. (2018) showed that the survival rate of *C. rubrum* four years after transplantation was significantly high at 99.1%, and similarly, the genus *Corallium*, to which *C. japonicum* belongs, has a significantly high survival rate after transplantation, suggesting that this species tends to be transplanted successfully.

CONCLUSIONS

The present study revealed that *C. japonicum,* a kind of precious coral, has a high survival rate after artificial transplantation. In this study, the precious corals were kept in an aquarium at

18°C, which is estimated as the water temperature at a depth of 100 m off the coast of Cape Ashizuri, Kochi Prefecture, until transplanted. This prediction of water temperature did not deviate significantly from the water temperatures at the two release points, and was within the annual range of water temperatures (Fig. 5). In addition, we transplanted precious corals to a depth of about 100 m, in an area that was previously used for precious coral fishing. These factors could contribute to their high survival rates. Other transplantation tests mentioned above also suggest a high survival rate for some Octocorallia species when they are attached to appropriate substrates and installed in environments similar to their preferred habitat. We plan to downscale the transplantation substrate, which will enable fishers to transplant precious corals from their boats with ease. This new tool will promote production and resource conservation, leading to the sustainable development of the precious coral industry.

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Competing interests: TK, ST, FK, SN, NY, and TM, declare that there are no conflicts of interest.

Availability of data and materials: All data generated and analyzed during this study are included in this published article.

Consent for publication: Not applicable.

Ethics approval consent to participate: Precious coral samples were legally collected between 2016 and 2020 under Kochi Prefecture's precious coral fishing permission number (413).

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