Enhancing coral larval supply and seedling production using a special bundle collection system “coral larval cradle” for large-scale coral restoration

Go Suzuki1,2, Wataru Okada3, Yoko Yasutake3, Hidekazu Yamamoto3, Iwao Tanita1, Hiroshi Yamashita1, Takeshi Hayashibara1, Toshiaki Komatsu4, Toru Kanyama5, Masahito Inoue6, Masashi Yamazaki6

Larval recruitment is essential for sustaining coral communities and a fundamental tool in some interventions for reef restoration. To improve larval supply and post-settlement survival in sexually assisted coral restoration efforts, an integrated in situ collector system, the larval cradle, was designed to collect spawned gametes then culture the resulting larvae until settled on artificial substrates. The final design of the larval cradle was cylindrical, a nylon mesh structure with a volume of 9 m³, suspended in the sea and extending vertically toward the seabed. We found three key design features that improved the efficiency of the apparatus: (1) an open area of sea surface and mesh size of less than 100 μm produced high fertilization and optimal survival (>90%), (2) a special skirt-shaped net (3 m in diameter) with a connection hose for attaching the cradle to collect bundles from many adult colonies over a wide area and at various depths, and (3) adding short square tube pieces, called square hollow sections, as a substrate for enhancing larval settlement and survival, to a larval cradle at 4 days after spawning was optimal for uniform settlement. This system allowed not only the collection of several million eggs, but also subsequent production of several thousand settled juvenile corals, without land facilities. Our design achieved several hundred times higher survival for early life stages of Acropora tenuis compared to nature.

Key words: Acropora, artificial reef, coral larvae collector, recruitment

Implications for Practice

- Several million coral larvae can be produced in situ (without land facilities) for direct seeding and seedling production.
- As many as several thousand coral seedlings can be produced from a single larval cradle.
- Corals attached to the artificial substrate are easy to outplant after 1 year.

Introduction

Coral reef degradation has become increasingly widespread in recent years (Gardner et al. 2003; Pandolfi et al. 2003; De'ath et al. 2012), escalating significantly due to pandemic bleaching events during 2015–2017 that caused severe damage to coral communities worldwide (Hughes et al. 2017, 2018; Muko et al. 2019). Conservation of these coral communities, especially branching corals, which function as fishing grounds and fish nurseries is crucial for ensuring the sustainable utilization of fisheries resources along tropical coasts (Nanami et al. 2013; Wilson et al. 2016). Over the last decade, the role of active coral restoration in coral reef management has escalated worldwide (Rinkevich 2008; Chamberland et al. 2017; Pollock et al. 2017; Doropoulos et al. 2019a; Boström-Einarsson et al. 2020). While coral transplantation has been demonstrated to be an effective coral reef restoration method (Birkeland et al. 1979; Harriott & Fisk 1988; Edwards & Clark 1999; Soong & Chen 2003; Omori et al. 2016), large-scale transplantation is very labor intensive. Furthermore, transplanted corals are very vulnerable to even a single disturbance, such as a bleaching event.

Author contributions: GS conceptualized the study; GS, WO, HidY designed the research; MI, MY planned the entire project; TK managed the project; GS, WO, YY, IT, HirY, TH, TK performed the experiments; GS, WO, TT analyzed the data; GS prepared the manuscript.

1Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, 148 Fukai-Ota, Ishigaki, Okinawa, Japan
2Address correspondence to G. Suzuki, email gosuzu@fra.affrc.go.jp
3ECOH Corporation, 2-6-4, Kitaueno, Taitou-ku, Tokyo, Japan
4Kokusai Kogyo Co., Ltd, 2 Rokubancho, Chiyoda-ku, Tokyo, Japan
5Fisheries Infrastructure Development Center, 2-14-5 Tsukiji, Chuou-ku, Tokyo, Japan
6Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries, 1-2-1 Kasumigaseki, Chiyoda-ku, Tokyo, Japan

© 2020 The Authors. Restoration Ecology published by Wiley Periodicals, Inc. on behalf of Society for Ecological Restoration. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

doi: 10.1111/rec.13178

Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.13178/supinfo
event or outbreak of crown-of-thorns starfish (COTS). In addition, severe disturbances not only affect existing coral cover, but also future coral recruitment because the reduction in adult (i.e. mature) corals tends to result in reduction in larval supply (Hughes et al. 2019). In particular, some areas that have suffered a marked reduction in reproductive adults on their source reefs show impacts to coral recruitment (Suzuki unpublished data). Consequently, enhancing annual larval recruitment, which could be facilitated by amplifying coral reproduction, may be useful for sustaining coral restoration efforts, especially in areas that are chronically recruitment-limited and/or in which larval retention on the natal reef is high (Zayasu & Suzuki 2019).

Amplifying coral reproduction can be approached by considering two key factors. First, “artificial spawning hotspots” comprising densely populated conspecific adult colonies that can be protected from disturbances should be established to prevent disruptions to annual spawning (Zayasu & Suzuki 2019). Second, larval survival could be improved by collecting eggs and sperm at the time of spawning and then rearing larvae until they settle (Heyward et al. 2002; Omori et al. 2004; Doropoulos et al. 2012). In this study, we demonstrate a package of novel techniques and methods that facilitate gamete collection, larval rearing, and settlement on a substrate. These techniques reduce initial mortality in corals by ensuring high fertilization rates, larval survival, and settlement on artificial substrates.

Scleractinian corals, especially the broadcast spawners, produce substantial numbers of larvae at annual synchronous mass spawning events (Harrison et al. 1984; Richmond 1997). Methods focused on Acropora species are particularly important because they are most susceptible to both bleaching and COTS outbreaks (Marshall & Baird 2000; Loya et al. 2001; Pratchett et al. 2009), and belong to one of the most well-represented spawners in the Indo-Pacific reefs. Protocols for rearing larvae of these corals have already been developed in aquaria after collecting egg–sperm bundles in the field or in aquaria (Guest et al. 2014; Omori & Iwao 2014; Pollock et al. 2017), and the process is straightforward if natural sea water is available (e.g. Nakamura et al. 2011; dela Cruz & Harrison 2017). However, rearing substantial quantities of larvae (e.g. more than 10 million) is difficult and costly in land-based facilities. For example, dela Cruz and Harrison (2017) collected 30 colonies of mature corals from which they produced 410,670 larvae using land-based facilities, using them to seed 96 m² of reef. If this larval seeding method was scaled up to 1 ha, more than 40,000,000 larvae would be required. Although the grounds for estimation is described later, several hundred or more adult colonies are needed for gathering tens of millions of larvae, which make it difficult in land-based facilities. To overcome this issue, we developed a special gamete bundle collection system that can be used for larval rearing in the field. The idea of larval collection from slicks (i.e. clusters of fertilized and unfertilized eggs that appear on the surface of the sea the morning following mass spawning) has already been suggested by Heyward et al. (2002) (see also Heyward et al. 1999; Omori et al. 2004, 2007; Doropoulos et al. 2012). However, there are some problems associated with collecting larvae from natural slicks. First, the natural slicks rarely contain the target species for restoration. In Okinawa, many natural slicks of mostly Acropora eggs formed the morning after mass coral spawning (Suzuki et al. 2011). Several species of tabular and corymbose Acropora corals (those corals abundant on shallow reefs) dominated these slicks, whereas branching Acropora species that are important for fish nurseries constituted less than 5% of all recruits (Suzuki et al. 2008). If a shallow reef slope habitat was the target area for restoration, natural slicks would be useful as larval source. However, restoration is more in demand in the places where natural recruitment is scarce, such as lagoon habitats, and branching Acropora corals dominate such places on Indo-Pacific reefs. Considering that larval supply from branching coral is relatively low, it is efficient to collect larvae artificially rather than depending on natural slicks. Second, because the slicks contain numerous unfertilized eggs, larval culture from slicks is less efficient than that from aquaria (G. Suzuki, personal observation). Third, it is possible that natural slicks may not form or be detected due to inclement weather conditions. Moreover, natural spawning and fertilization may decrease after disturbances such as bleaching because these events reduce the population density of adult corals. Accordingly, we concluded that methods for obtaining coral larvae directly from the densely arranged adult colonies of target species, similar to farms (i.e. man-made spawning hotspots), are more efficient for restoration. However, very few trials involving the practical application of larval collection devices have been performed using adult colonies in the field, including spawning, fertilization, and larval development (i.e. not using the larvae from slicks). In addition, the larval collection is not a goal in itself. Rather, what is needed to perform large-scale coral restoration is an integrated system of improved larval collection, settlement, post-settlement survival, and enlarged artificial spawning hotspots. In this study, we therefore sought to test and develop, over several annual spawning seasons, field-based systems to collect and steadily produce large quantities of larvae (i.e. about 10 million).

Acropora corals release eggs and sperm in capsules called bundles, which float to the surface due to the buoyancy conferred by the oil content of the egg (Harrison & Wallace 1990). By floating to the surface in this way, the coral colonies increase their chances of fertilization. We therefore designed a large net system that collects these bundles and contains them at the surface, facilitating fertilization and larval rearing; we call this rearing device a “larval cradle.”

Mass producing coral larvae in a larval cradle can satisfy two major aims. The first is that direct larval seeding of damaged reefs will be possible. As stated above, hundreds of millions of larvae will be required for large-scale (i.e. more than 1 ha) restoration by direct larval seeding. To obtain these numbers of larvae, hundreds of millions of eggs are needed. To provide these, several hundred to a thousand gravid colonies would be needed. Although a huge pool (100–1,000 m²) could hold all gametes from those mature corals, such a huge structure on the seabed would be easily broken by wind and waves, or too costly to make it durable. Consequently, the “larval cradle” was developed at a manageable size that can be constructed by a small group (i.e. two or three persons). Such cradles can be moved to target areas for restoration, and coral larvae can be easily released from a cradle.

Our other aim was seedling production using an artificial settlement substrate within the cradle. It is well known that the initial
mortality of *Acropora* settlers is very high, especially during the first 6 months after settlement (Wilson & Harrison 2005; Penin et al. 2011; Suzuki et al. 2018a, 2018b). We considered that appropriate settlement substrates could enhance post-settlement survival of coral settlers. In this study, therefore, we also devised a new settlement substratum for outplanting and then tested methods for optimizing larval settlement within a larval cradle.

By integrating all of the elements mentioned above, we aimed to complete a sequential operating system for the mass production of coral larvae and seedlings in situ, and to upscale coral restoration under various situations.

**Methods**

**Design of the Larval Cradle**

A series of design trials began in 2012, and a single example of each type of construction was tested. The trials continued over 4 years, allowing six designs to be evaluated. A final cylindrical design with a 30-μm-mesh net was tested three times in 2014 and 2015. In designing the larval cradle, particular care was taken to optimize the materials used to construct the section at the sea surface (i.e. upper part of the larval cradle) because the fertilized eggs frequently come into contact with this section during the early stages of embryo development.

The initial prototype was cuboid (Fig. 1A), and preliminary experiments were conducted for comparison of variable mesh sizes (0–263 μm) for the walls in 2012 and 2013 (see Supplement S1). In the final design, the cradle was cylindrical in shape, measuring 1.7 m in diameter and 4.25 m in height, and made using net with a mesh of 30 μm (Fig. 1B). A large plastic zip was installed on the top and bottom of the device so that coral larvae could be placed in, or removed from, the device (Fig. 1C & 1D). The cuboid design was changed to cylindrical because a cylindrical form could weaken the pressure from currents and waves, and many eggs and larvae gathered and died in the corners of the surface layer of the cuboid one. In further experiments, two cylindrical cradles with a mesh of 30 μm were prepared in 2015.

**Dedicated Float for the Larval Cradle: the “Surface Collar”**

Although we used EVA buoys to suspend the device in earlier experiments (Fig. 1A & 1B), the larval cradle was easily damaged when a boat came alongside. To better facilitate the observations of coral larvae and the placement of settlement substrate, we developed a durable dedicated float. This float is doughnut-shaped, composed of fiber-glass reinforced plastic (FRP), has an outer diameter of 2.8 m (inner diameter: 1.8 m), and a height of 0.25 m, making it sufficiently large to totally cover the top of the device (Fig. 1C). The advantage of this surface collar is that workers can safely approach the cradle by mooring a boat to the float and can stand on the float itself for work.

![Figure 1](image-url)
Development of Bundle Collection Apparatus

To mass produce larvae in a cradle requires the collection of egg–sperm bundles. Initially, the bundles were collected by covering the coral with the larval cradle itself. However, this method not only limits the depth available for bundle collection to 4 m (i.e. the height of the larval cradle), but also the area available for collection to approximately 2.3 m² (i.e. the base area of the larval cradle). To collect the bundles from a wide range of depths and areas, a conical, skirt-shaped net was developed and deployed in May 2018 (Fig. 2A). The base diameter of this conical net is 3 m, and there is a hole at the vertex of the net to which a suction hose with an inner diameter of 75 mm can be connected. The opposite end of the suction hose is connected to the base of the larval cradle, which has a 10-cm-diameter hole at its center (Fig. 2B). The part where the suction hose connects to the base of the larval cradle was not fixed to avoid any tension caused by tides and currents to damage the cradle. Empty 1-L PET bottles were attached on the top and middle of the suction hose to act as buoys for keeping the hose perpendicular (Fig. 2C). Some colonies of *Acropora tenuis* spawned under the conical net and the number of larvae inside the larval cradle was counted at 3 days after spawning using a Kitahara quantitative plankton net.

In addition, we tried to collect bundles from multiple conical nets to extend the area available for bundle collection by a single larval cradle. Four conical nets were prepared and positioned directly under the cradle (center, 0 m), as well as at distances of 4 and 8 m from the center net (Fig. 2D). To compare the efficiency of bundle capture among these nets, the number of bundles passing through the vertex of the nets was recorded at 2-second intervals using a GoPro camera (HERO6, GoPro Inc., San Mateo, CA, U.S.A.).

Gamete Collection and Larval Rearing Within the Cradle

The experiments were conducted in Urasoko Bay, Ishigaki Island, Japan (24.458°N, 124.221°E). At the beginning, the gamete collections within the cradle were tested in 2012 and 2013 using the initial prototype (cuboid). Details of these design trials are described in Supplement S1. In 2014, because of problems in estimating the precise number of eggs within the larval cradle in previous years, *A. tenuis* bundles were collected in an aquarium and the number of eggs counted in the laboratory. First, we collected 20 adult mature colonies on 9 May from Nagura Bay (24.390°N, 124.121°E) and placed them in a 1,500-L FRP tank with running seawater for several days. On
the night of spawning (19 May), all gametes were dipped out with a 0.5-L plastic cup and put into a 100-L polycarbonate tank. After all the gametes had been collected, the volume of seawater in the tank was topped up to 80 L. Three replicate samples of collected gametes were taken using a 50-mL glass beaker (they were well mixed before each sampling) and the number of eggs in each sample was counted under a stereomicroscope to estimate the density of eggs in the tank. Approximately 3 million fertilized eggs were moved to five plastic buckets with sealing lids (20 L, TOSRON Co., Ltd., Ishioka, Japan), carried by boat, and placed into a new cylindrical larval cradle approximately 0.5 hours after fertilization. The number of surviving larvae was estimated at 4 days old. Larval sampling was conducted by taking five replicate samples using a 20-L plastic bucket after mixing water within the device; a bucket was used because the larval density was too high to estimate using a quantitative plankton net (0.2 m diameter, 100 μm mesh). To estimate the larval density, each of the five samples was diluted 20-fold and the number of larvae was counted under a stereomicroscope. The total number of larvae surviving within the larval cradle was calculated based on the average density of the five replicate samples. Additional experiments were conducted in 2015 using the same protocol as in 2014.

To statistically clarify the relationship between the mesh size and the larval collecting potential, 4 years of data were pooled and subjected to regression analysis. The mesh size of larval cradle was treated as an explanatory variable, and the survival rate of 4-day-old larvae was treated as an explained variable. All statistical analyses in this study were conducted using the free software R ver.3.1.2 (R Core Team, 2016).

Settlement Experiment Using Artificial Plates

Lattice-shaped plates have been demonstrated to improve post-settlement survival by preventing fish from grazing directly on coral spats, while also providing suitable environmental conditions for coral growth, such as promoting access to light and preventing sedimentation (Suzuki et al. 2011). The lattice structure is optimal for Acropora juveniles (10–20% survival at 1 year after settlement on average), probably because this structure gives protection for the approximately 2 years that Acropora juveniles took to grow to 3-cm diameter.

In 2014 and 2015, we conducted some preliminary experiments on larval settlement within the larval cradle using the lattice-shaped plates (Supplement S1). If the lattice can be cut and divided into cells (i.e. replicate pieces), settling coral larvae on the cells in a cradle and outplanting after rearing can easily
allow production of large numbers of seedlings. In an attempt to reduce costs in 2016, the substrate was changed and short square tubes, called square hollow sections (SHS), were tested. Two types of SHS were produced in 2017; one was composed of polyvinyl chloride (pSHS) and the other was composed of mortar (magnesium oxide powder) mixed with coral sand (mSHS) (Fig. 3A & 3B). Size was slightly different between pSHS (4-cm outside, 3.5-cm inside, 4-cm height) and mSHS (3.5-cm outside, 3-cm inside, 3.5-cm height). Only inside walls were available for settlement because all SHSs were conditioned for 1 month under seawater in close contact with each other to form a biofilm to enhance coral settlement (Webster et al. 2004) (so that the biofilm only formed on the inside walls) (Fig. 3C). The settlement and survival rates were compared between the two substrate types. The SHS were kept in a net bag with a mesh size of 2 cm and hung from a buoy to stabilize the position of the bag within the device (Fig. 3D). All SHS were retrieved 2 days after they were placed into the cradle, and the number of settlers was recorded under a stereomicroscope. In addition, the number of surviving corals was directly counted underwater by divers at 6 months after settlement.

We calculated two parameters for productivity: one was larval productivity, the percent of larvae settled on substrates within the cradle, the other was seedling productivity, the percent of substrates with at least one live coral juvenile.

An analysis of variance (ANOVA) was used to statistically compare the number of settlers among three factors; the vertical depths, the number of plates per net bag, and the substrate materials. One-way ANOVA was conducted for each factor using R ver.3.1.2 software because the experiment for each factor was independently done. In the event that a significant difference was found, multiple comparisons were then performed using Tukey’s HSD test.

### Table 1. Estimates of total number of larvae obtained using the cylindrical larval cradles constructed of 30-μm-mesh nets. Total number of larvae (mean ± SD) was calculated from the larval density within the cradle.

| Year | 0-day-old | 4-day-old | Estimated survival rate (%) |
|------|-----------|-----------|----------------------------|
| 2014 | 2,938,666 ± 36,950 | 2,912,086 ± 318,678 | 99.1 |
| 2015 | 1,170,400 ± 56,580 | 1,242,700 ± 202,300 | ~100 |
|      | 300,960 ± 14,549 | 370,932 ± 178,346 | ~100 |

### Vertical Larval Distribution within Larval Cradle

To understand the relationship between the timing of placing the plates and the larval distribution in the cradle, in 2018, the vertical distribution of Acropora larvae within the cradle was estimated. Larval sampling was conducted by sampling 1 L of water using a Kitahara-type water sampler (Nakano et al. 2018) at five depths (0, 1, 2, 3, and 4 m), three times a day for 4 days (i.e. at 17, 24, 37, 48, 61, 65, 72, and 85 hours after spawning). In addition, at 3 days after fertilization, the total number of larvae within the cradle was estimated using a Kitahara quantitative plankton net (0.225-m diameter, 0.46-m filtration diameter, 100-μm mesh) (RIGO, Cat.No.5504-A).

### Results

#### Fertilization Success and Larval Survival Rate

In the preliminary experiments, the number of surviving larvae within the cradle differed markedly between nets with a mesh size of 108 and 263 μm; 60% of the larvae survived to 4 days old in the 108-μm net, while only 2.7% survived in the 263-μm net (Table S1). Embryonic cleavage had occurred in both nets, but most of the embryos in the 263-μm net separated into each blastomere at the morula stage. For the fertility, more than 80% of eggs were fertilized, even in the 263-μm net.

Comparing fertilization rate and larval survival in the 30-μm net, the 108-μm net, and the vinyl sheet in the following year (2013), revealed that both were highest in the 30-μm net (finest mesh) (Table S1). As the estimated survival rate for the larvae exceeded 100% in the 30-μm net, it is clear that estimates of egg numbers at the time of spawning were not accurate (standard deviation of the samples reached 67% of the average) (Table S1). We observed that many eggs and larvae gathered and died in the corners of surface layer of the cuboid cradles in 2013, although the numbers of dead larvae in the corners could not be estimated.

### Table 2. Bundle collection rate using multiple conical nets. Analyzed photos mean that was recorded at 2-second intervals from the start (19:30) to the end (20:50) of spawning. Unfocused images were excluded from the analysis. Bundle photos mean the images containing bundles. Standardized bundle passing rate is no. of bundle photos/no. of analyzed photos (the results was standardized by weight of adult colony). In 4 m⁻¹, numerous unforced images were obtained during peak spawning due to divers’ bubbles affecting camera focus.

| Distance | No. of Analyzed Photos | No. of Bundle Photos | Weight of Adult Colonies (kg) | Standardized Bundle Passing Rate |
|----------|------------------------|----------------------|-----------------------------|---------------------------------|
| 0 m (center) | 2,209 | 925 | 5 | 0.84 |
| 4 m⁻¹ | 579 | 49 | 5 | 0.17 |
| 4 m⁻² | 1,953 | 1,221 | 10 | 0.63 |
| 8 m | 1,386 | 107 | 5 | 0.15 |
When the eggs were counted in an aquarium and placed into the cylindrical cradle approximately 0.5 hours after fertilization, the results revealed that the survival rate at 4 days old was 99.1% in the 30-μm net in 2014, and mathematically more than 100% in 2015 (Table 1), suggesting that very few, if any, larvae died in the 30-μm cradle.

The results of the design trials suggested that a smaller mesh size was associated with a higher survival rate (regression analysis of the relationship between mesh size [explanatory variable] and larval survival rate [explained variable] using 4 years of pooled data [adjusted $r^2 = 0.865, F = 39.35, p < 0.01$]) (Fig. S1).

### Bundle Collection Area

We first performed bundle collection using a conical net in 2018 and succeeded in collecting approximately 2 million larvae (Fig. 1D) from 20 colonies of *A. tenuis* (10 kg wet weight) on a 4 m² rack. Next, we compared the bundle collection potential of conical nets positioned at different distances from the cradle (Fig. 2D). We successfully demonstrated the collection of bundles from at least 4 m from the cradle by the bundle passing rate (Table 2). However, the number of bundles from each conical net could not be estimated, as the images used to enumerate the bundles were unfocused.

### Settlement Experiment for Seedling Production

When plates were positioned at different depth layers in the cradle before spawning in the preliminary experiment, larval settlement on the plates tended to occur in the upper layer (87.4% of the settled larvae) (ANOVA, $F = 61.95, p < 0.001$) (Fig. 4A). When the plates were placed into the cradle at 4 days after spawning in the following year, larval settlement in the upper layer was moderated (Fig. 4B). However, settlement still tended to be concentrated in the upper layers of the cradle (0.2 and 0.6 m) (ANOVA, $F = 10.79, p < 0.001$). In addition, the number of plates in the net bag did not influence the number of settlers per plate (ANOVA, $F = 1.458, p = 0.122$) (Fig. 4C). For the development of the SHS substrate, we confirmed that the density of settlers did not differ between the lattice-shaped plate and SHS, when net bags containing both types of substrate were placed into the cradle (Tables 3, S2). The development of surface biofilms was comparable because the preparation time for all plates of both types was 1 month. Furthermore, a comparison of settlement and survival rates between pSHS and mSHS over half a year revealed that there were no significant differences between the materials. At 6 months after settlement the survival rate on each substrate type was 23.8 and 28.5%, respectively (Table 3).

Larval productivity (i.e. % larvae within the cradle that settled on substrates) was 10–38% in the 4-year experiments from 2014 to 2017, and the average larval productivity was approximately 20% on average (Table 3, S2).

Seeding productivity (% substrates with >1 live coral) was 62–75% at 1 year in the 2 × 2-cell plate and pSHS in 2015 and 2016 experiments (Tables 3, S2). It was 100% at 1 year in the 8 × 8-cell plate, probably because the size of the plate was larger than others.

Low seeding productivity (44–47% at 6 months) was recorded in 2017 associated with low settlement density.

More than half of the larvae within the cradle (70%) were concentrated at the surface (0 m) 14 hours after spawning (Fig. 5). The larval concentration at the surface then decreased gradually 24 hours after spawning. A relatively uniform distribution of
larvae was then maintained until 65 hours after spawning. By the third night (72 hours after spawning), many larvae (38%) were found in the bottom layer (4 m). The average wind speed was 3.8 m/second (maximum was 7.8 m/second) and the wave height was less than 0.5 m during this period. Three days after spawning, approximately 3.4 million larvae were in the cradle.

Discussion
In this study, we developed an integrated system for both collecting coral gamete bundles and rearing *Acropora* coral larvae in the sea. Using this system allows at least several million coral larvae to be produced without land facilities. Those mass-produced larvae could be used for direct seeding to damaged reefs. Alternatively, as many as several thousand (at most 10,000) coral seedlings on substrates for outplanting, could be produced from a single larval cradle. We consider that this system could be useful for realizing large-scale coral restoration.

Potential for Gamete Collection and Larval Production
Maximizing the number of colonies available for larval collection is difficult because adult colonies cannot be stacked above each other. In this study, we therefore developed a network of conical nets which enabled us to extend the area over which bundles could be collected, to as far as 4 m away from the cradle. By employing this modification, bundles could be collected from at least five conical nets (i.e. approximately 35 m²) at theoretically any depth.

While few studies have been published on measuring the fertility of *Acropora* colonies in situ, the number of oocytes was estimated to be $10^7$–$10^8$ for every 1,000 cm² colony in the six *Acropora* species with different morphologies: *A. hyacinthus*, *A. cytherea*, *A. nasuta*, *A. spathulata*, *A. cf. digitifera*, and *A. humilis* (Álvarez-Noriega et al. 2016). For the species used in this study, $23.76 \times 10^8$ eggs were spawned from a 30-m-diameter adult colony of *A. tenuis* (Kitada 2002). In our study, we calculated the relationship between the number of eggs and the weight of an adult colony in water and concluded that approximately $20 \times 10^4$ eggs are spawned from 1 kg of mature *A. tenuis* (approximately five to six 30 × 10 cm fragments). By extrapolating from these results, it is theoretically possible that 20 million eggs could be captured by a single cradle. Twenty million larvae, if possible, would be too many to produce coral seedlings within a cradle; however, more larvae might be required for direct larval seeding. For example, this could be sufficient to seed a 2-ha area of damaged reef with larvae at a density of 1,000 larvae/m², although further studies need to be conducted to estimate the effective number of larvae for seeding especially in terms of post-settlement survival (Edwards et al. 2015; Suzuki et al. 2018a, 2018b).

Optimal Materials for Larval Cradle Construction
Nylon netting was best suited as a material for constructing the cradle and a smaller mesh size was associated with higher fertility and larval survival rate, probably because developing embryos were more prone to fragmentation in the coarser net (Heyward & Negri 2012). Netting with a mesh of 30 μm was the most inexpensive and finest mesh size that was commercially available (i.e. the netting was not custom-made or proprietary). It is considered that nets with a mesh size of <30 μm would not be necessary because a survival rate of almost 100% was obtained for 4-day-old larvae using the 30-μm net. The reason for the low survival rates of larvae in a cradle constructed of vinyl sheeting was not identified, but many larvae

### Table 3. Settlement and survival rates of *Acropora* corals on the square hollow section (SHS) substrate. PVC, polyvinyl chloride.

| Material       | Cell Size (cm) | Surface Area (cm²) | N  | Year | Settlers ± SD (cm²) | Larval Productivity ± SD (%) | Survival Rate (%) 6 Months After Settlement | At settlement | Half year | 1 yr |
|----------------|----------------|-------------------|----|------|---------------------|-------------------------------|-------------------------------------------|---------------|-----------|------|
| PVC            | 4              | 56                | 96 | 2016 | 1.03 ± 0.44         | 18.8 ± 0.3                    | 11.6                                      | 100           | 86.7      | 66   |
|                | 4              | 56                | 800| 2017 | 0.07 ± 0.05         | 10 ± 0.2                     | 23.8                                      | 93.5          | 44.4      | —    |
| Coral sand     | 3.5            | 42                | 300| 2017 | 0.09 ± 0.08         | 28.5                         | 86.2                                      | 47.5          | —         | —    |

Figure 5. Changes in vertical distribution of larvae in larval cradle over time (M = morning, D = daytime, E = evening). Each bar represents the % larvae in each depth layer within the cradle in each sampling period.
died while attaching themselves onto the vinyl sheet on the morning following spawning.

**Estimating the Number of Cultured Larvae**

Estimating the number of collected bundles (i.e., eggs) within the cradle was relatively difficult. To ensure an equitable distribution of bundles among cradles, we tried to ensure that the total size of mature colonies positioned under each cradle was the same. However, some colonies did not spawn simultaneously, even though they had mature eggs, and the number of bundles spawned at night differed among cradles. In addition, the variance in egg numbers among replicate samples was large, even though the eggs were sampled after mixing the water within the cradle; these differences likely arose because the eggs become concentrated at the surface due to their high buoyancy. Estimating the number of eggs in the cradles was further complicated by the gradation in egg density observed with depth. To overcome these issues, in 2014, the number of eggs was counted before they were placed into the cradle. This enabled us to show that the 30-μm net was the most effective for ensuring a high fertility and survival rate (i.e., 99%) of 4-day-old larvae. We are now planning to develop methods of direct larval seeding using the conical nets to build on the previous trials by Heyward et al. (2002) and de la Cruz and Harrison (2017).

**Mass Production of Coral Seedlings**

Although the larvae retained by the cradle could be used for direct seeding, we sought to optimize the mass production of seedlings by settling them on artificial substrates to enhance post-settlement survival. First, when we tested different settling methods within the cradle, we found that coral larvae settled evenly on the substrates at all depths when substrates were placed into the cradle 4 days after spawning, rather than before spawning. This might be because the larvae tend to accumulate near the surface during the first 2 days after fertilization, and that they then settle on the available substrate immediately after acquiring competency 3 days post-fertilization. A similar change in the vertical distribution of coral larvae was reported in a previous study (Omori et al. 2007). However, only 10–30% of the larval cohort was settled on the substrates at 6 days after spawning in this study (seawater temperature was 26°C), although Heyward and Negri (2010) reported that 60–70% of larvae acquired competency by 6 days old at a temperature of 26°C or more. The substrate was suspended within the cradle by placing it into a net bag (with as many as twenty 2 × 2-cell plates [total surface area: 3,840 cm²] per bag) and then allowing the larvae to settle evenly on the substrates. In the case of the SHS substrate, unbiased settlement was confirmed on as many as 50 plates in a net bag (total surface area: 2,100 cm²). To maximize settlement, a total of 7,348 SHS were placed into a single cradle in 2017 and larval settlement was found to have occurred on 83.8% of the SHS substrate cells. Based on these findings, we estimated that a maximum of 10,000 seedlings could be produced in a single larval cradle. Alternatively, other artificial substrates could be placed into the larval cradle. However, the optimal species for them may be different from those for the SHS.

We sought to determine if seedlings could be reared in the sea without any additional labor after settlement. We demonstrated that the post-settlement survival at 6 months after settlement on lattice plates and SHS was 20% if a bottom-raised rack was used. In other words, settling 10 larvae per plate would be sufficient to support more than one juvenile coral on the plate after 6 months. The cost of pSHS was one-tenth that of the lattice plate, and half of the mSHS substrate in this study. However, due to pollution concerns, the material used for the artificial plate should not be plastic if the mass-produced corals are eventually to be outplanted. When we compared the survival rate of the corals between the pSHS and mSHS substrates over a 6-month period, no significant differences were observed, suggesting that the mSHS substrate is suitable for outplanting.

**Integrated System for Large-Scale Coral Restoration**

By combining the larval cradle and the mSHS, we expect to be able to promulgate coral seedlings in the field with a higher rate of survival than that observed in nature. Although it is difficult to estimate the initial survival rate of *Acropora* corals in nature, it has been estimated, based on the egg-to-settler ratio per square meter, to be less than 1% for the larval phase; that is, more than 10⁶ eggs are spawned (Kitada 2002 and this study), but fewer than 10³ larvae are considered to settle (e.g., Fisk & Harriott 1990), probably due to fertilization failure, predation, and dissipation. In addition, several reports have estimated that the survival rate after settlement is less than 1%, mainly because of incidental predation during the post-settlement phase (Penin et al. 2011; Edmunds et al. 2015). In contrast, the rate of survival obtained using the larval cradle was nearly 100% for the larval phase (but the larval productivity was 20% on average), and that obtained with the SHS substrate was more than 20% for the post-settlement phase. Thus, the potential number of corals that could be supplied per unit area could be improved 400-fold using these techniques. Of course, there is not enough information to assess how consistent performance will be if this method is applied at larger scales or with other species. In addition, the remaining challenge for sustainable coral restoration is to establish an artificial spawning hotspot (Zayas & Suzuki 2019) that can serve as a source of coral larvae. A combination of larval cradle system and artificial spawning hotspots could therefore be an effective countermeasure against disturbances such as bleaching. Also, genetic diversity of seedlings used for artificial spawning hotspots is important for keeping high levels of fertility (Miller et al. 2018; Baums et al. 2019). Based on these premises, we aim to realize a level of artificial mass larval supply that is comparable to that of a healthy wild population. We also expect that artificial spawning hotspots could become super larval sources superior to wild corals in the long term (several decades), if the hotspots can be protected from bleaching and COTS outbreaks. In other words, the number of coral larvae (or seedlings) supplied from artificial spawning hotspots through the larval cradle could be fairly stable over the next 50 years, while a wild population would supply enough larvae...
only in some years in the same period, because of bleaching and COTS outbreaks damaging the adult corals periodically with each disturbance reducing the larval supply drastically at least over 3–5 years (until the new recruits grow to maturity).

**Acknowledgments**

This study formed part of the Coral Reef Restoration and Conservation under Severe Environmental Conditions project of the Fisheries Agency, Japan. We thank S. Kai, Y. Fujikura, S. Tashiro, M. Kitano, S. Iwamura, and C. Shinzato for assistance with experiments. The sampling of corals was permitted by the Okinawa Prefectural Government for research purposes (Permit No. 23-47, 24-54, 25-49, 26-68, 27-73, 28-76, 29-74, 31-1).

**LITERATURE CITED**

Álvarez-Noriega M, Baird AH, Dornelas M, Madin JS, Cumbo VR, Connolly SR (2016) Fecundity and the demographic strategies of coral morphologies. Ecology 97:3485–3493

Baums I, Baker A, Davies S, Grottoli A, Kenkel C, Kitchen S, et al. (2019) Considerations for maximizing the adaptive potential of restored coral populations in the western Atlantic. Ecological Applications 29:e01978

Birkeland C, Randall RH, Grimm G (1979) Three methods of coral transplantation for the purpose of reestablishing a coral community in the thermal effluent area at the Tunguissuin power plant. University of Guam Marine Laboratory, Technical Report 60:1–24

Boström-Emarsson L, Babcock RC, Bayraktarov E, Cecarelli D, Cook N, Ferse SCA, et al. (2020) Coral restoration – a systematic review of current methods, successes, failures and future directions. PLoS One 15:e0226631

Chamberland VF, Petersen D, Guest JR, Petersen U, Brittsan M, Vermeij MJA (2017) New seedling approach reduces costs and time to outplant sexually propagated corals for reef restoration. Scientific Reports 7:18076

dela Cruz DW, Harrison PL (2017) Enhanced larval supply and recruitment can replenish reef corals on degraded reefs. Scientific Reports 7:13985

De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proceedings of the National Academy of Sciences of the United States of America 109:17995–17999

Doropoulos C, Elzinga J, ter Hofstede R, van Koningsveld M, Babcock RC (2019a) Optimizing industrial-scale coral reef restoration: comparing harvesting wild coral spawn slicks and transplanting gravid adult colonies. Restoration Ecology 27:758–767

Doropoulos C, Vons F, Elzinga J, ter Hofstede R, Salee K, van Koningsveld M, Babcock RC (2019b) Testing industrial-scale coral restoration techniques: harvesting and culturing wild coral-spawn slicks. Frontiers in Marine Science 6:658

Edmunds PJ, Steneck R, Albright R, Carpenter RC, Chui APY, Fan T-Y, et al. (2015) Geographic variation in long-term trajectories of change in coral recruitment: a global-to-local perspective. Marine and Freshwater Research 66:609–622

Edwards AJ, Clark S (1999) Coral transplantation: a useful management tool or misguided meddling? Marine Pollution Bulletin 37:474–487

Edwards AJ, Guest JR, Heyward AJ, Villanueva RD, Barya MV, Bollozos ISF, Golbou Y (2015) Direct seeding of mass-cultured coral larvae is not an effective option for reef rehabilitation. Marine Ecology Progress Series 525:105–116

Fisk DA, Harriott VJ (1990) Spatial and temporal variation in coral recruitment on the Great Barrier Reef: implications for dispersal hypotheses. Marine Biology 107:485–490

Gardner TA, Côté IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958–960

Guest JR, Baria MV, Gomez ED, Heyward AJ, Edwards AJ (2014) Closing the circle: is it feasible to rehabilitate reefs with sexually propagated corals? Coral Reefs 33:45–55

Harriott VJ, Fisk DA (1988) Coral transplantation as a reef management option. Pages 375–379. In: Proceedings of the 6th International Coral Reef Symposium. Vol. 2, Australia

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass spawning in tropical reef corals. Science 223:1186–1189

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. Pages 133–207. In: Dubinsky Z (ed) Ecosystems of the world, coral reefs. Elsevier Science, Amsterdam

Heyward AJ, Negri AP (2010) Plasticity of larval pre-competency in response to temperature: observations on multiple broadcast spawning coral species. Coral Reefs 29:631–636

Heyward AJ, Negri AP (2012) Turbulence, cleavage, and the naked embryo: a case for coral clones. Science 335:1064

Heyward AJ, Rees M, Smith LD (1999) Coral spawning slicks harnessed for large-scale coral culture. Pages 188–189. In: Program and Abstracts, International Conference on Scientific Aspects of Coral Reef Assessment, Monitoring, and Restoration. National Coral Reef Institute. Nova Southeastern University, FL

Heyward AJ, Smith LD, Rees M, Field SN (2002) Enhancement of coral recruitment by in situ mass culture of coral larvae. Marine Ecology Progress Series 230:113–118

Hughes TP, Perry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, et al. (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373–377

Hughes TP, Perry JT, Baird AH, Connolly SR, Chase TJ, Dietzel A, et al. (2019) Global warming impairs stock-recruitment dynamics of corals. Nature 568:387–390

Hughes TP, Perry JT, Simpson T (2018) Large-scale bleaching of corals on the Great Barrier Reef. Ecology 99:501

Kituda H (2002) Fecundity of Acropora tenuis at Akajima Island. Midorishishi 13:26–29 (in Japanese)

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woensik R (2001) Coral bleaching: the winners and the losers. Ecological Letters 4:122–131

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155–163

Miller MW, Baums IB, Pausch RE, Bright AJ, Cameron CM, Williams DE, Moffitt ZJ, Woodley CM (2018) Clonal structure and variable fertilization success in Florida Keys broadcast-spawning corals. Coral Reefs 37:239–249

Muko S, Suzuki G, Saito M, Nakamura T, Nadaoka K (2019) Transitions in coral communities over 17 years in the Sekisei Lagoon and adjacent reef areas in Okinawa, Japan. Ecological Research 34:524–534

Nakamura R, Ando W, Yamamoto H, Kitano M, Sato A, Nakamura M, Kayanne H, Omori M (2011) Corals mass-cultured from eggs and transplanted as juveniles to their native, remote coral reef. Marine Ecology Progress Series 436:161–168

Nakano T, Obata H, Katayama K, Ozawa S, Matsuanga H (2018) Water sampling. G201EN:001-019. In: Guideline of oceanographic observation. Vol 2. The Oceanographic Society of Japan, Tokyo

Nanami A, Sato T, Takeke T, Teruya K, Sano Y (2013) Microhabitat association in white-streaked grouper Epinephelus ongus: importance of Acropora spp. Marine Biology 160:1511–1517

Omori M, Aota T, Watamaki A, Taniguchi H (2004) Development of coral reef restoration method by mass culture, transportation and settlement of coral larvae. Proceedings of Palau Coral Reef Conference 1:31–38

Omori M, Higa Y, Shinzato C, Zayasu Y, Niigata T, Nakamura R, Yokokura A, Janadou S (2016) Development of active restoration methodologies for coral reefs using asexual reproduction in Okinawa, Japan. Pages 369–387. In: Proceedings of the 13th International Coral Reef Symposium, Honolulu, HI

Omori M, Iwao K (2014) Methods of farming sexually propagated corals and outplanting for coral reef rehabilitation; with list of references for coral reef restoration; with list of references for coral reef monitoring, and restoration. G201EN:001-019. In: Guideline of oceanographic observation.
rehabilitation through active restoration measure. Akajima Marine Science Laboratory, Okinawa, Japan
Omori M, Shibata S, Yokokawa M, Aota T, Iwao K (2007) Survivorship and vertical distribution of coral embryos and planula larvae in floating rearing ponds. Galaxea 8:77–81
Pandolfi JM, Bradbury Roger H, Sala Enric, Hughes Terence P, Bjorndal Karen A, Cooke Richard G, et al. (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301:955–958
Penin L, Michonneau F, Carroll A, Adjeroud M (2011) Effects of predators and grazers exclusion on early post-settlement coral mortality. Hydrobiologia 663:259–264
Pollock FJ, Katz SM, van de Water JA, Davies SW, Hein M, Torda G, et al. (2017) Coral larvae for restoration and research: a large-scale method for rearing Acropora millepora larvae, inducing settlement, and establishing symbiosis. PeerJ 5:e3732. https://doi.org/10.7717/peerj.3732
Pratchett MS, Schenka TJ, Baine M, Symns C, Baird AH (2009) Selective coral mortality associated with outbreaks of Acanthaster planci L. in Bootless Bay, Papua New Guinea. Marine Environmental Research 67:230–236
Richmond RH (1997) Reproduction and recruitment in corals: critical links in the persistence of reefs. Pages 175–197. In: Birkeland CE (ed) Life and death of coral reefs. Chapman & Hall, New York
Rinkevich B (2008) Management of coral reefs: we have gone wrong when neglecting active reef restoration. Marine Pollution Bulletin 56:1821–1824
Soong K, Chen T (2003) Coral transplantation: regeneration and growth of Acropora fragments in a nursery. Restoration Ecology 11:62–71
Suzuki G, Hayashi T, Shirayama Y, Fukami, H (2008) Evidence of species-specific habitat selectivity of Acropora corals based on identification of new recruits by two molecular markers. Marine Ecology Progress Series 355:149–159
Suzuki G, Kai S, Fujikura Y, Yamashita H (2018b) Post-settlement survivorship of artificially supplied Acropora coral larvae in the Sekisei Lagoon. Marine Ecology Progress Series 603:105–115
Suzuki G, Kai S, Yamashita H, Suzuki K, Iehisa Y, Hayashibara T (2011) Narrower grid structure of artificial reef enhances initial survival of in situ settled coral. Marine Pollution Bulletin 62:2803–2812
Suzuki G, Okada W, Yasutake Y, Kai S, Fujikura Y, Tanita I, et al. (2018a) Interspecific differences in the post-settlement survival of Acropora corals under a common garden experiment. Fisheries Science 84:849–856
Webster NS, Smith LD, Heyward AJ, Watts JEM, Webb RI, Blackall LL, Negri AP (2004) Metamorphosis of a scleractinian coral in response to microbial biofilms. Applied and Environmental Microbiology 70:1213–1221
Wilson SK, Depczynski M, Fulton CJ, Holmes TH, Radford BT, Tinkler P (2016) Influence of nursery microhabitats on the future abundance of a coral reef fish. Proceedings of the Royal Society B 283:20160903
Wilson J, Harrison P (2005) Post-settlement mortality and growth of newly settled reef corals in a subtropical environment. Coral Reefs 24:418–421
Zayasu Y, 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”. Restoration Ecology 27:440–446

Supporting Information
The following information may be found in the online version of this article:

Supplement S1. Preliminary experiments on materials used for enclosure of the larval cradle.

Table S1. Estimates of fertility and total number of larvae obtained using larval cradle-constructed of nets with different mesh sizes and vinyl.

Table S2. Settlement and survival rates of Acroporacorals on the lattice-shaped substrates.

Figure S1. Relationship between the survival rate of coral larvae at 4 days old and the mesh size of the cradle wall.

Coordinating Editor: Alasdair Edwards

Received: 23 October, 2019; First decision: 20 November, 2019; Revised: 15 April, 2020; Accepted: 15 April, 2020