

## Article

# Effect of Substratum Structural Complexity of Coral Seedlings on the Settlement and Post-Settlement Survivorship of Coral Settlers

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**Abstract:** The substratum structure is critical for facilitating settlement and increasing the survivorship of coral settlers. However, knowledge about its structural complexity is largely lacking. In this study, we examined the effect of complexity on the settlement and post-settlement survivorship of coral settlers using four types of structures: groove, using a CSD (Coral Settlement Device, 4.5 cm  $\phi$   $\times$  2.5 cm H, top-shaped ceramic); flat, using a CP (Ceramic Plate, 29.5 cm L  $\times$  3.1 cm W  $\times$  0.9 cm H, unglazed ceramic plate); linear, using a CN (Coral Net, mesh size 19 mm, biodegradable plastic net); and wrinkle, using a SS (Scallop Shell, 11.0 cm in shell length). The complexity was obtained from the ratio of the surface area to the vertically projected area of the substratum. The substratum sets were installed in the coral reef around the Ryukyu Islands every May from 2012 to 2014. After about 2 or 6 months of spawning, a certain number of substratum types were sampled, and the number of coral spats that settled on them was counted by taxa classified into *Acropora*, *Pocilloporidae*, *Millepora*, and Others. The larval settlement rate in the first set of samples and the survivorship of coral spats in the second set of samples were estimated. The mean settlement rate was, in order, the CSD; SS; CN; and CP, and the mean survivorship was, in order, the CSD; CP; SS; and CN, over three years. A positive correlation was found between the structural complexity, mean settlement rate, and mean survivorship. Our results show that the structural complexity of coral seedlings affects the settlement of coral larvae and the survivorship of coral spats.

**Keywords:** substratum; structural complexity; sexual propagation; larval settlement; survivorship; coral seedling



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## 1. Introduction

Coral reefs have been declining globally in recent years. In total, 19% of the world's coral reefs have already deteriorated, and more than 60% are under serious threat [1]. The causes include increased environmental loads due to human activities, such as sediments and nutrients in zooxanthellate corals (hereinafter referred to as corals); predation by crown-of-thorns starfish; and coral diseases. Coral bleaching due to rising water temperatures caused by global warming also poses a long-term threat to coral reefs [2]. These impacts have put coral reefs in a potential extinction crisis in one-third of the world [3].

It is important to remove anthropogenic impacts to allow for the recovery of degraded coral reefs. However, as noted by Rinkevich (2005) [4], if the reefs have declined beyond their innate ability for recovery, then coral outplanting is a potentially significant method of reef restoration. Coral outplanting contributes to the recovery of ecosystems, the expansion of larval sources, and the rehabilitation of marine habitats by artificially restoring coral communities in areas where coral reefs cannot recover under their own power [5]. Due to rising water temperatures and ocean acidification caused by global warming, there is also concern about the survival of outplanted coral [6]. However, coral reefs that have

suffered fewer anthropogenic effects are known to exhibit better resilience against large-scale bleaching [7]. It has also been reported that zooxanthellae with heat tolerance [8] and fluorescent proteins in coral tissue prevent bleaching [9]. Therefore, it is expected that outplanting coral seedlings, leveraged by these findings, will be an effective measure for coral reef restoration [10].

Attempts to restore coral communities using outplanting have been conducted in Japan since the 1990s by affixing fragments produced by donor colonies. However, this method runs the risk of collecting a large amount of existing coral, and due to its low genetic diversity, a larval supply created through reproduction cannot be expected [11]. Therefore, since 2002, we have been developing a seedling production technique using sexual reproduction that causes coral larvae to settle and grow on artificial substrata. The most important goals in the production of juvenile polyps are making the coral larvae settle on as many substrata as possible and ensuring high survivorship in the settled coral spats. The structure of the substratum affects them significantly, but knowledge about its complexity is largely lacking. Therefore, we examined the effect of substratum structural complexity on the coral settlement rate and post-settlement survivorship using four types of structures: groove, flat, linear, and wrinkle.

This paper was partially created using the data of a project on coral reef conservation and restoration conducted from 2010 to 2016 by the Okinawa Prefecture.

## 2. Materials and Methods

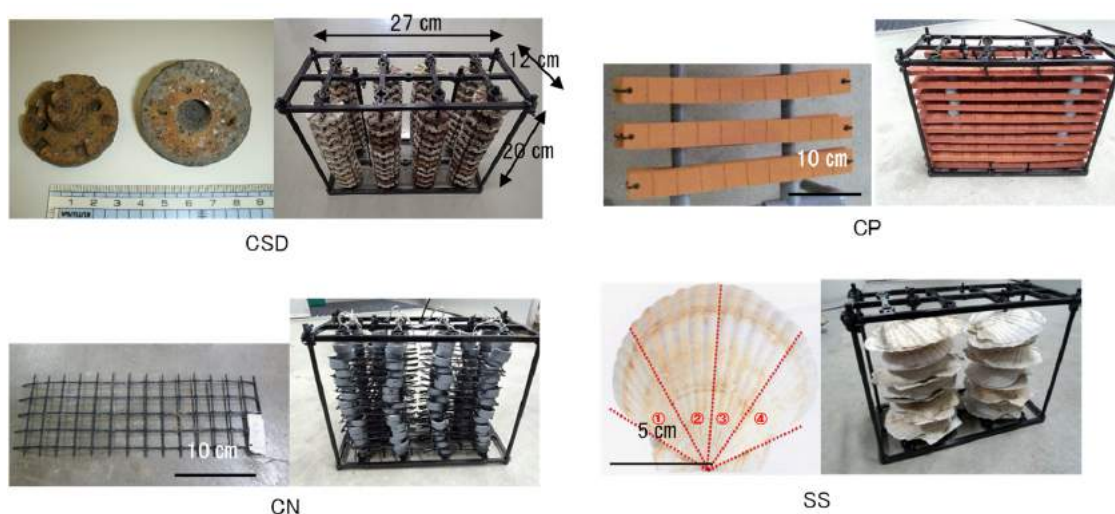
### 2.1. Substratum Used for the Study

Specifications of the substrata are shown in Table 1 and Figure 1.

**Table 1.** Specifications of the substrata.

| Structure | Name                         | Shape | Material                       | Size (cm)                                 | Projected Area (cm <sup>2</sup> ) | Surface Area (cm <sup>2</sup> ) |
|-----------|------------------------------|-------|--------------------------------|-------------------------------------------|-----------------------------------|---------------------------------|
| Groove    | Coral Settlement Device: CSD | Top   | Ceramic including steel slug   | $4.5\phi \times 2.5\text{ h}^1$           | 15.9                              | 60.0                            |
| Flat      | Ceramic Plate: CP            | Plate | Ceramic                        | $29.5^2 \times 3.1 \times 0.9\text{ t}^3$ | 91.5                              | 241.6                           |
| Linear    | Coral Net: CN                | Net   | Biodegradable plastic          | $8.7 \times 28.4^4$ , mesh: 1.9           | 44.9                              | 89.8                            |
| Wrinkle   | Scallop Shell: SS            | Shell | <i>Mizuhopecten yessoensis</i> | $11.0\phi^5$                              | 94.5                              | 188.9                           |

<sup>1</sup> Height. <sup>2</sup> 26.0 in 2013 and 2014 experiments. <sup>3</sup> Thick. <sup>4</sup> 9.5 in 2013 experiment. <sup>5</sup> Average of 10 sets.



**Figure 1.** Four types of substrata with different structures used for the study. Individual substratum shown on left side was combined into a set on right side.

- CSD (Coral Settlement Device):

A top-shaped ceramic (brown color) with a diameter of 4.5 cm and a height of 2.5 cm containing about 50% steel slag. Radial grooves on the underside facilitate the settlement of coral larvae. The surface has rich, minute irregularities. A bundle of 11 CSDs connecting a lower leg with an upper hole was loaded into a plastic case [12]. Eight bundles with 88 CSDs were loaded into one case, which was fixed horizontally on a stainless steel frame.

- CP (Ceramic Plate):

An unglazed ceramic (brown color) with a length of 29.5 cm, a width of 3.1 cm, and a thickness of 0.9 cm. Its surface has rich, minute irregularities. Note that the length of the plate installed in 2013 and 2014 was 26.0 cm. There is a groove for fragmentation with a depth of about 2 mm at intervals of about 3 cm on one side (about 2 cm for installations in 2013 and 2014), which can be divided in the field at the time of outplanting for seedlings. In 2012, 2 plates were made into one set, and 9 sets for 18 plates were fixed vertically on the frame. In 2013 and 2014, 2 sets of 20 plates were loaded in the same plastic case as the CSD and fixed vertically on the frame.

- CN (Coral Net):

A hard plastic mesh base with a mesh size of 19 mm, composed of biodegradable material. The surface shape is glossy and smooth. In 2012, two nets measuring 30 cm × 60 cm were fixed horizontally on the frame. In 2013, 15 nets measuring 10 cm × 10 cm were stacked to form a set, and 2 sets of 30 nets were horizontally mounted on the same case and fixed to the frame.

- SS (Scallop Shell):

The central parts of several pairs of the bivalve *Mizuhopecten yessoensis* with lengths of 10–12 cm (11.0 cm on average), were skewered by an iron rod. Two sets of 28 shells in 14 pairs, for a total of 56 shells, were fixed vertically on the frame in 2012. In 2013, two sets of 12 shells in 6 pairs, for a total of 24 shells, were installed in the same case and fixed vertically on the frame.

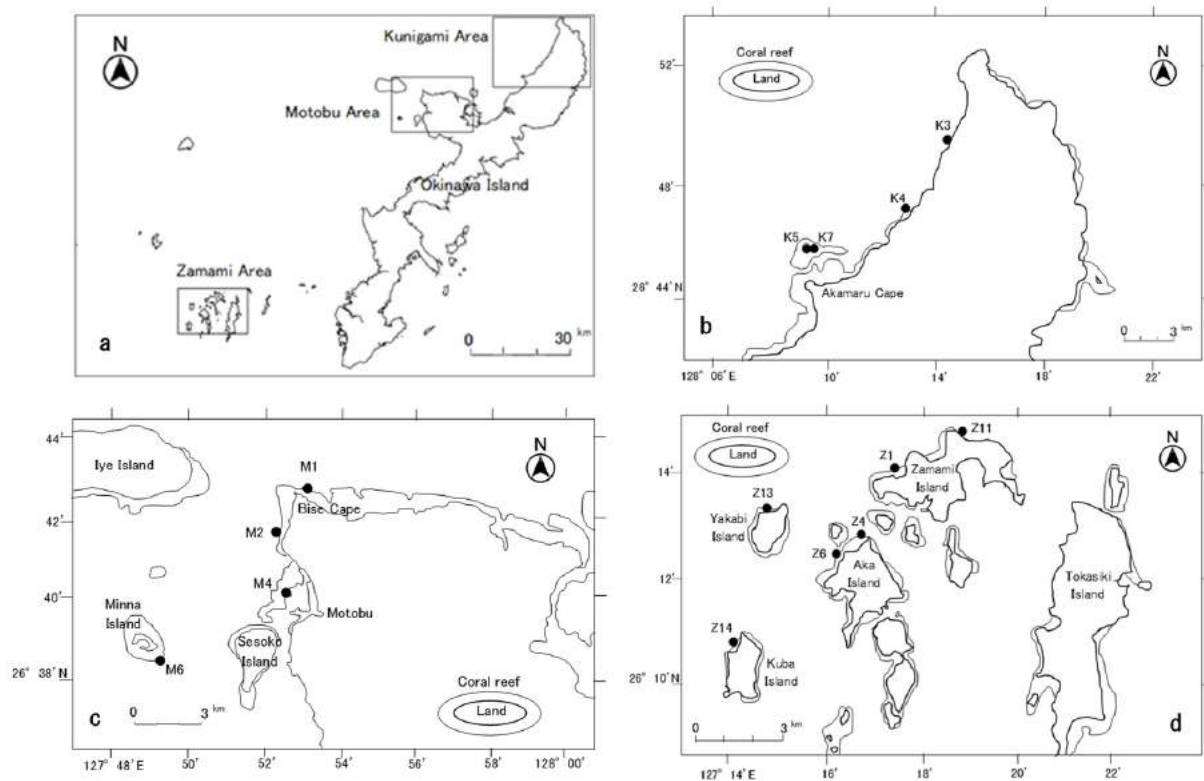
## 2.2. Calculating Structural Complexity of the Substratum

Complexity was calculated with the following equation using the vertically projected area and surface area of each substratum shown in Table 1. Substrate surface roughness is known to physically affect planktonic larval settlement [13]. The ratio of the roughness spacing to the roughness height of the substrate has been suggested as a theoretical value for the roughness index [14]. In this study, we applied this theory to the substratum used and showed the complexity as the ratio of the surface area in a certain area of the substratum.

$$\text{Complexity} = \text{surface area} / \text{vertical projected area} \times 100$$

## 2.3. Study Sites

Based on the results of previous surveys for coral distribution, we selected three high-cover areas covered with *Acropora*, which predominantly occurs in the Ryukyu Islands. Furthermore, we surveyed the distribution of juvenile coral at each site to confirm the recruitment of *Acropora*. The number of deployment sites was 4 in the Kunigami area, 4 in the Motobu area, and 6 in the Zamami area. Those in fringe and patch reefs were located at a depth of about 1 to 6 m (Figure 2, Table 2).



**Figure 2.** Study sites. (a) Overview; (b) Kunigami area; (c) Motobu area; (d) Zamami area.

#### 2.4. Deployment and Sampling of the Substratum

*Acropora* is dominant in the Kerama Archipelago of Okinawa, and it spawns annually, starting around the full moon in May up to June [15], and once a year, it does so simultaneously [16]. The settlement rate reaches a maximum of 80% or more on the 8th day after spawning begins [7]. On Ishigaki Island in southern Ryukyu, more than 80% of *Acropora* settle 5 to 8 days after spawning [17]. Based on these data, we considered the fastest settlement period of *Acropora* to be 5 days after spawning. Coral settlement is inhibited by microalgae and favored by crustose coralline algae [18,19]. Since the adhesion of crustose coralline algae to the window of the underwater observatory in central Japan is noticeable around the 14th day after cleaning the glass [20], the substratum was installed at least 9 days before the full moon. There is a view that the earlier the installation time, the better. However, if the installation period is too long, the sessile of the microalgae will increase, meaning that frequent cleaning must occur. The substrata were installed around the full moon on 4 June 2012, 24 June 2013, and 13 June 2014, as shown in Table 2.

In 2012, a stainless steel frame (60 cm × 60 cm) equipped with the substratum cases was installed by sticking iron stakes through its four corners so that the frame bottom was fixed 30–50 cm above the seabed. In 2013 and 2014, the frames were fixed to a steel pipe frame assembled on the seabed. Regarding the set angle of the substrata, it is known that vertical installation can obtain more settlement in shallow places with abundant light because this avoids the influence of sedimentation [21]. Therefore, the CPs and SSs with flat shapes were installed to make a wide, vertical surface. After installation, in every period between July and August (about 2 months after spawning) and between December and January (about 6 months after spawning), a certain number of substrata were sampled to measure the coral settlers (Table 2).

**Table 2.** Numbers of the substrata deployed and sampled at the study sites from 2012 to 2014. Deployment was carried out every year before the full moon in June. CSD was installed in 3 cases in 2012, 8 cases in 2013, and 20 cases in 2014. CP was installed in 1 case in 2012, 8 cases in 2013, and 18 cases in 2014. CN was installed in 1 case in 2012 and 8 cases in 2013. SS was installed in 1 case in 2012 and 8 cases in 2013. The substrata were sampled twice, about 2 months and 6 months after spawning. Sampling was performed on the sea bottom with a bundle (11 pieces) for CSD, plate for CP, face for SS, and pair (2 shells) for SS from each case.

| Year | Area     | St. | Depth (m) | Deployment       |      |     |     |     | First Sampling     |     |    |      |    | Second Sampling |     |    |      |    |
|------|----------|-----|-----------|------------------|------|-----|-----|-----|--------------------|-----|----|------|----|-----------------|-----|----|------|----|
|      |          |     |           | Period           | CSD  | CP  | CN  | SS  | Period             | CSD | CP | CN * | SS | Period          | CSD | CP | CN * | SS |
| 2012 | Kunigami | K3  | 4.8       | May 24<br>to 25  | 264  | 18  | 2   | 56  | August 30          | 22  | 2  | 2    | 4  | December 14     | ND  | ND | ND   | ND |
|      |          | K4  | 4.9       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | K5  | 5.3       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | 22  | ND | ND   | ND |
|      |          | K7  | 5.3       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      | Motobu   | M1  | 4.2       | May 25<br>to 26  | 264  | 18  | 2   | 56  | August 15          | 22  | 2  | 2    | 4  | December 4      | ND  | ND | ND   | ND |
|      |          | M2  | 5.9       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | M4  | 1.1       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | 22  | ND | ND   | ND |
|      |          | M6  | 3.9       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | 22  | ND | ND   | ND |
|      | Zamami   | Z1  | 5.3       | May 15<br>to 16  | 264  | 18  | 2   | 56  | August 13<br>to 14 | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | Z4  | 3.9       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | Z6  | 4.8       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | Z11 | 5.3       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | Z13 | 4.2       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
|      |          | Z14 | 5.4       |                  | 264  | 18  | 2   | 56  |                    | 22  | 2  | 2    | 4  |                 | ND  | ND | ND   | ND |
| 2013 | Kunigami | K5  | 5.3       | May 17, 21 to 23 | 704  | 160 | 240 | 192 | July 26            | 88  | 20 | 30   | 24 | December 23     | 88  | 20 | 30   | 24 |
|      | Zamami   | Z6  | 4.8       | May 27, 29 to 30 | 704  | 160 | 240 | 192 | July 16            | 88  | 20 | 30   | 24 | December 3      | 88  | 20 | 30   | 24 |
| 2014 | Kunigami | K7  | 5.3       | April 25         | 1760 | 360 | –   | –   | August 21          | 264 | 20 |      |    | 6 January 2015  | ND  | ND |      |    |
|      | Motobu   | M4  | 1.1       | May 1            | 1760 | 360 | –   | –   | August 30          | 264 | 20 |      |    | December 3      | 264 | 20 |      |    |

\* Sampling was performed in 8.7 cm × 28.4 cm units in 2012. ND: Lost in a typhoon. –: No deployment due to acquisition difficulty.

### 2.5. Measurement of Coral Settlers

After sampling, the substrata were divided into units to compare them as each out-planting unit: 1 piece of CSD; 10 pieces of CP in 2012 and 13 pieces in 2013 and 2014; a piece 8.7 cm × 9.5 cm of CN; and 4 pieces of SS; see Figure 1. Coral settlers on the units were classified into four types (*Acropora*, Pocilloporidae, *Millepora*, and Others), and we recorded the numbers with a loupe or stereomicroscope. In the first sampling, the number of settlers was measured; then, the settlement rate, defined as seedling collection rate in this study, was calculated ( $=\text{number of units settled}/\text{number of units installed} \times 100$ ). In seedling production, the number of substrata containing at least one coral is important [22]. In the second sampling, the same measurement as in the first sampling was performed, and the survivorship was calculated ( $=\text{number of settlers in the second sampling}/\text{number of settlers in the first sampling} \times 100$ ). Since Pocilloporidae coral spawns every month except in winter [23], the number of settlers in the second sampling likely included colonies spawned after the first sampling, having grown for about 4 months. *Pocillopora damicornis* was reared downward with a net to prevent grazing on the seabed after settlement in a tank, and it had a mean maximum diameter of 5 mm from 13 to 21 weeks after settlement [24]. Therefore, among the number of Pocilloporidae settlers in the second sampling, those with a diameter of 5 mm or less were excluded.

## 3. Results

### 3.1. Structural Complexity of the Substratum

The complexity of each substratum was measured. The CSD had the highest complexity at 3.77, followed by the CP at 2.65, and there was no difference between the CN (2.00) and the SS (2.00) (Table 3). Because the CSD had a three-dimensional structure and radial grooves formed on the underside, its complexity was high; on the other hand, the CP, CN, and SS had almost flat shapes.

**Table 3.** Structural complexity of the substrata.

| Substratum | Complexity                                                  |
|------------|-------------------------------------------------------------|
| CSD        | 3.77                                                        |
| CP         | 2.65 <sup>1</sup> (2.64 in 2012, and 2.65 in 2013 and 2014) |
| CN         | 2.00 <sup>2</sup> (2.00 in 2012 and 1.99 in 2013)           |
| SS         | 2.00                                                        |

<sup>1</sup> An average of 2.64 in the 2012 experiment and 2.65 in the 2013 and 2014 experiments. <sup>2</sup> An average of 2.00 in the 2012 experiment and 1.99 in the 2013 experiment.

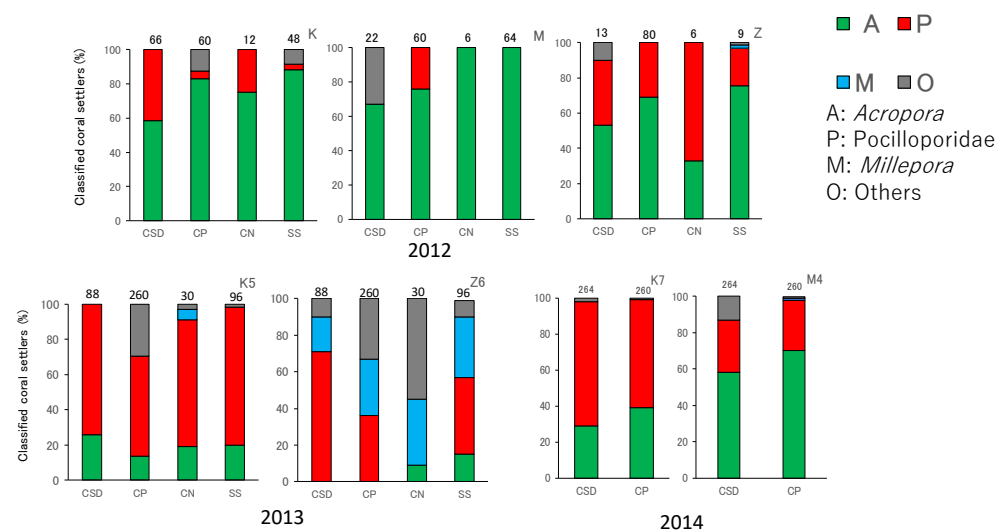
### 3.2. Settlement Rate

Table 4 shows the mean settlement rate for each study area in 2012. In the Kunigami area, the mean settlement rate order was the SS (28.3%) and CP (26.3%), followed by the CSD (13.6%) and CN (8.5%). The mean rate in the Motobu area was also the SS (17.3%), CP (13.8%), CSD (4.6%), and CN (4.3%), in that order. In the Zamami area, although the mean rate of the SS was as high as 38.5%, the order of the others was different compared with other areas: CSD (19.7%), CP (9.2%), and CN (8.3%). Even in an average of three areas, the SS was higher at 28.0%, followed by the CP (16.4%) and CSD (12.6%), and the CN was the lowest at 7.0%. In the settlement ratio of coral taxa settled on the substrata, we found that *Acropora* prevailed by over 50% in all substrata except in the CN in the Zamami area (Figure 3). In 2013, the settlement rates recorded at site K5 in the Kunigami area showed that the CSD (78.4%) and CN (73.3%) were higher than the SS (47.9%) and CP (45.0%). At site Z6 in the Zamami area, the CN was also higher (33.3%), followed by the SS (28.1%), CP (25.4%), and CSD (19.3%). The highest average for the two areas was the CN (53.3%), followed by the CSD (48.9%), SS (38.0%), and CP (35.2%) (Table 4). In the proportion of coral settlers by taxa, the ratio of *Acropora* was small in both areas. Although the ratio at K5 in the Kunigami area was higher than at site Z6 in the Zamami area, the ratio of *Acropora* was less than 30% in all substrata. Pocilloporidae dominated all substrata by 57% to 78%

at site K5 in the Kunigami area. However, it only appeared at a rate of 71.0% in the CSD at site Z6 in the Zamami area; there were no dominant coral taxa in the other substrata (Figure 3). The 2014 settlement rate was 64.8% in the CSD and 48.1% in the CP at site K7 in the Kunigami area, and it was 36.0% in the CSD and 24.6% in the CP at site M4 in the Motobu area. The CSD was clearly higher than the CP in both areas, and the average in both areas was 50.4% in the CSD and 36.4% in the CP (Table 4). In the proportion of coral settlers by taxa, Pocilloporidae appeared at a rate of 60%, greater than *Acropora* in both the CSD and CP in the Kunigami area. However, at site M4 in the Motobu area, *Acropora* dominated in both the CSD (58.1%) and CP (70.3%) (Figure 3).

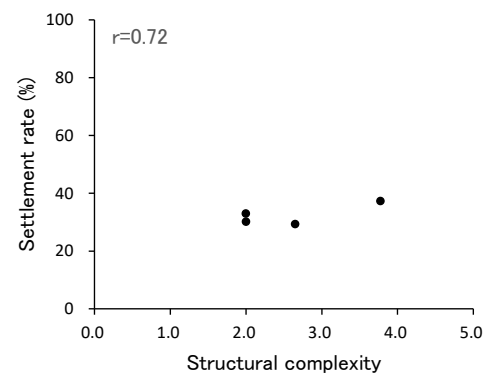
**Table 4.** Mean settlement rate and standard deviation (%) of the substrata in the first sampling.

| Year      | Area      | Substratum  |             |             |             |
|-----------|-----------|-------------|-------------|-------------|-------------|
|           |           | CSD         | CP          | CN          | SS          |
| 2012      | Kunigami  | 13.6        | 26.3        | 8.5         | 28.3        |
|           | Motobu    | 4.6         | 13.8        | 4.3         | 17.3        |
|           | Zamami    | 19.7        | 9.2         | 8.3         | 38.5        |
|           | Mean      | 12.6        | 16.4        | 7.0         | 28.0        |
| 2013      | Kunigami  | 78.4        | 45.0        | 73.3        | 47.9        |
|           | Zamami    | 19.3        | 25.4        | 33.3        | 28.1        |
|           | Mean      | 48.9        | 35.2        | 53.3        | 38.0        |
| 2014      | Kunigami  | 64.8        | 48.1        |             |             |
|           | Motobu    | 36.0        | 24.6        |             |             |
|           | Mean      | 50.4        | 36.4        |             |             |
| 2012–2014 | Mean ± sd | 37.3 ± 27.8 | 29.3 ± 14.6 | 30.2 ± 29.1 | 33.0 ± 11.6 |



**Figure 3.** Percentage of coral settlers classified by taxa in the substrata in first sampling. Values in the data bars are the number of analyses.

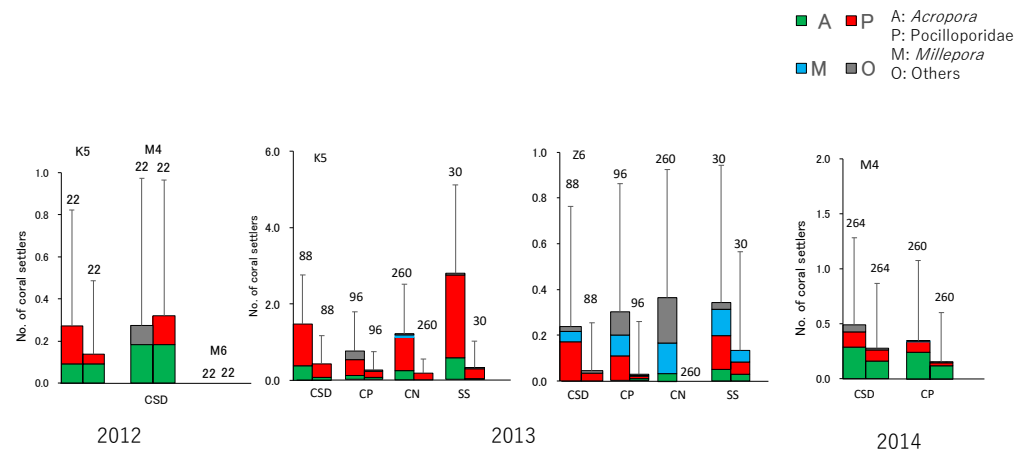
The mean settlement rate after 3 years was estimated to be the highest on the CSD (37.3%), followed by the SS (33.0%), CN (30.2%), and CP (29.3%) by considering the data for all sampling years together (Table 4). The coral taxa settlement ratio showed significant spatiotemporal variation, and the coral taxa scarcely revealed any tendency in settling on the substrata. A correlation between the complexity and settlement rate was so low that it showed no 5% significance level ( $p = 0.27$ ) (Figure 4).



**Figure 4.** Correlation between settlement rate and structural complexity in the substrata.

### 3.3. Survivorship

The number of coral settlers in the CSD in the first and second samplings in 2012 is shown in Figure 5. The survivorship was 50.0% at site K5 in the Kunigami area and 100% at site M4 in the Motobu area, although there was an increase in the number of settlers. No settlers were observed at site M6 in the Motobu area in either the first or second samplings. The mean survivorship for both areas is estimated at 75.0% (Table 5). For survivorship measured by taxa, the number of *Acropora* settlers did not change in either site. Pocilloporidae showed a decrease at site K5 in the Kunigami area, and it was replaced by other species at site M4 in the Motobu area.



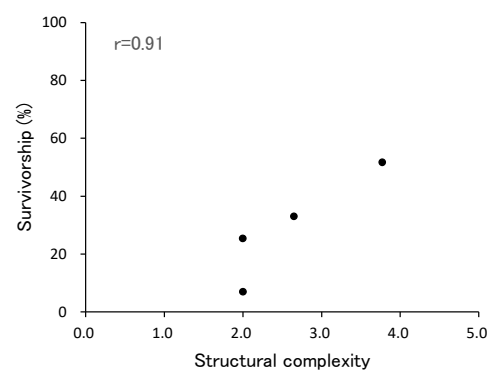
**Figure 5.** Change in number and standard deviation of coral settlers on the substrata between the first and second samplings based on taxa. The data bar set comprises the first sampling on the left and the second on the right. The values of the data bars indicate the number of samples.

**Table 5.** Post-settlement survivorship and standard deviation (%) in the substrata.

| Year      | Area          | Substratum      |                 |      |      |
|-----------|---------------|-----------------|-----------------|------|------|
|           |               | CSD             | CP              | CN   | SS   |
| 2012      | Kunigami      | 50.0            |                 |      |      |
|           | Motobu        | 116.7           |                 |      |      |
|           | Mean          | 75.0            |                 |      |      |
| 2013      | Kunigami      | 28.3            | 32.7            | 13.9 | 10.9 |
|           | Zamami        | 18.9            | 9.0             | 0.0  | 39.8 |
|           | Mean          | 23.6            | 20.9            | 7.0  | 25.4 |
| 2014      | Motobu        | 56.6            | 45.1            |      |      |
| 2012–2014 | Mean $\pm$ sd | 51.7 $\pm$ 26.0 | 33.0 $\pm$ 17.1 | 7.0  | 25.4 |

The number of coral settlers on each substratum in the first and second samplings in 2013 is shown in Figure 5. The survivorship was 32.7% in the CP, 28.3% in the CSD, 13.9% in the CN, and 10.9% in the SS at site K5 in the Kunigami area; in the Zamami area, the order was SS (39.8%), CSD (18.9%), CP (9.0%), and CN (0.0%). The mean survivorship in both areas was 25.4% in the SS, 23.6% in the CSD, 20.9% in the CP, and 7.0% in the CN (Table 5). Based on coral taxa, the survivorship of Pocilloporidae tended to be higher than that of *Acropora* at site K5 in the Kunigami area, and this taxon showed higher survivorship in the SS at site Z6 in the Zamami area. The number of coral settlers on each substratum in the first and second samplings in 2014 is shown in Figure 5. The survivorship was 56.6% in the CSD and 45.1% in the CP at site M4 in the Motobu area. No data were acquired at site K7 in the Kunigami area due to the loss of the substrata because of typhoons (Table 5). Based on coral taxa, *Acropora* did not change, as its highest value was 57.1%, and Pocilloporidae increased from 28.6% to 35.7% in the CSD. In the CP, *Acropora* also showed the highest rate, increasing from 71.4% to 75.0%, but Pocilloporidae decreased from 28.6% to 18.8%.

The mean survivorship of settlers 6 months after spawning in all areas from 2012 to 2014 was 51.7% in the CSD, the highest, followed by 33.0% in the CP, 25.4% in the SS, and 7.0% in the CN (Table 5). A correlation between the complexity and the survivorship was found (Figure 6), but there was no 5% significance level ( $p = 0.09$ ). The coral taxa survivorship on the substrata showed few tendencies.



**Figure 6.** Correlation between post-settlement survivorship and structural complexity in the substrata.

## 4. Discussion

### 4.1. Effect of the Structural Complexity of the Substratum on the Settlement Rate

It is well known that coral larvae selectively settle on calcareous substrates in the field [25]. Based on this mechanism, it is believed that substances and biofilms derived from crustose coralline algae play a role in signaling larval settlement [26]. Since we installed substrata in the same areas at the same times, it was suggested that smothering the substratum material by crustose coralline algae in each area proceed in the same way. Therefore, we believe the structure of each substratum was the main factor in the differences between the coral larval settlements. Coral larvae prefer cryptic microhabitats and the undersides of complicated substrata [27]. Although biofilms are important as attractive factors for settlement, it has been pointed out that the structure of the substratum is more effective [28]. We found a trend that the settlement rate increased as complexity increased (Figure 4); therefore, a complicated structure is significant for larvae settlement. The CSD did not show a high settlement rate despite the high complexity. This may be because the grooves on the underside (4 mm wide) were not adapted to the size of the larvae. They can detect micro-seafloor features smaller than their length (approximately 200  $\mu\text{m}$ ) and settle on them [29]. Despite the low complexity, the SS showed a higher settlement rate than those of the CN and CP, possibly due to the micro-wrinkle structure, which is not reflected in the complexity of the shell surface.

There have been several studies on the relationship between coral settlement and the structure of the substrate. One study found that two coral species settled selectively in the

grooves of tiles with a depth and width of 2 mm [19]. Another study compared the number of coral settlers found between grooved tiles and flat tiles, showing that the number of settlers in the grooved tiles was one digit higher than in the flat tiles [30]. In addition, as a result of placing a coral skeleton on the seabed and counting the number of settlers 4 months after spawning, another study found a correlation between the number of settlers and the irregularity of the skeleton surface [21]. These studies suggest that the complexity of the substrate plays a significant role in the settlement of coral larvae.

#### 4.2. Effect of the Structural Complexity of the Substratum on the Survivorship

It is well known that the mortality of juvenile corals settled on the sea bottom is extremely high [7]. After 7–9 months, the survivorship of broadcast-spawning coral species settled on horizontally installed tiles was less than 2.8% on the east coast of Australia [31]. In Amakusa, western Japan, after 3–10 months, the survivorship of coral spats settled on tiles set horizontally was less than 12% [32]. The coral spats on these tiles are not only affected by sediments and algae but are also grazed by benthic invertebrates such as sea urchins [25,33]. On the reef bottom of the Red Sea, juvenile coral mortality after one year was found to be 27 to 33% [34]. The post-settlement mortality of juvenile coral in the first year is 30–99%, which is the most critical period in the coral life cycle [10]. Since predation by fish and invertebrates is a major factor in the post-settlement survivorship of *Acropora*, the complexity of the settlement base structure is important for reducing predation pressure. It has been reported that the complexity of the settlement base structure, which provides cryptic microhabitats and refuge, reduces the grazing pressure of predators and greatly contributes to the improvement of juvenile coral survivorship [30,33]. Therefore, at all sites for 3 years, we examined the relationship between the complexity of substrata and mean survivorship after 6 months of settling (Table 5). As a result, we found that complicated seedling substratum structures contribute to increased post-settlement survivorship (Figure 6). Although there was no difference in complexity between the SS and CN, a significant difference in survivorship was found. This is likely due to the micro-wrinkle structure of the shell surface, which was not reflected in the complexity.

Concerning the effect of complex settlement base structures on decreases in survivorship due to predation, the survivorship of coral settlers was up to 12% on tiles with micro-crevices on the surface, installed on the seabed for one year, compared with 0% for tiles without micro-crevices [35]. The survivorship of coral settlers after 2 years on tiles with projections on the surface was higher in the most closely spaced projection bases compared with other bases. This showed the effect of preventing benthic invertebrates such as sea urchins and snails from grazing [36]. After 29 days, the survivorship rate of the two species of coral juveniles attached to tiles with large and small grooves was higher than that of juveniles attached to exposed substrate, regardless of the size of the groove. It has been reported that this is likely due to differences in predation among coral-eating fish [37].

Sedimentation and algal thriving also affect juvenile coral survivorship, in addition to predation pressure. It is known that the survivorship of early coral settlers on artificial substrate is lowest on the upper surface and highest on the undersurface. This is because the undersurface is less affected by sedimentation and algal overgrowth [24,38]. Since the CSD was installed horizontally, many larvae settled in the groove on the undersurface so that the effect of sedimentation and algal growth can be avoided [39], which is also a factor in high survivorship.

## 5. Conclusions

We showed that the complexity of the seedling substratum is a critical factor in creating adequate coral seedlings with high larval settlement and post-settlement survivorship, although the small number of cases did not show any statistical significance. More studies can resolve this point. This study also showed that the CSD not only has a high settlement function but also a high survival function because of the structure's greater complexity. We continue further study on the settlement rate using substratum with different micro-

features to clarify a more adequate material composition for larval settlement. The shape of the sampling units, the number of sampling units per frame, and how the number and distribution of units within each frame might alter the hydrodynamic conditions around the frame. Because we have qualitatively obtained knowledge that increasing the space between the CSD bundles, cases, and frames improves hydrodynamic conditions and the settlement rate, we continue to study to obtain quantitative data. Those results will help develop coral restoration techniques using sexual reproduction. Studies on the production of thermal-tolerant coral seedlings are also desirable to prevent degradation due to coral bleaching.

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## References

1. International Coral Reef Initiative. Available online: [https://www.icriforum.org/wp-content/uploads/2019/12/ICRIGM28-Resolution\\_C2A\\_FFA.pdf](https://www.icriforum.org/wp-content/uploads/2019/12/ICRIGM28-Resolution_C2A_FFA.pdf) (accessed on 25 May 2021).
2. Hughes, T.P.; Andersen, K.D.; Connolly, S.R.; Heron, S.F.; Kerry, J.T.; Lough, J.M.; Baird, A.H.; Baum, J.K.; Berumen, M.L.; Bridge, T.C.; et al. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* **2018**, *359*, 80–83. [[CrossRef](#)] [[PubMed](#)]
3. Carpenter, K.E.; Abrar, M.; Aeby, G.; Aronson, R.B.; Banks, S.; Bruckner, A.; Chiriboga, A.; Cortés, J.; Delbeek, J.C.; DeVantier, L.; et al. One-third of reef building corals face elevated extinction risk from climate change and local impacts. *Science* **2008**, *321*, 560–563. [[CrossRef](#)] [[PubMed](#)]
4. Rinkevich, B. Conservation of coral reefs through active restoration measures: Recent approaches and last decade progress. *Environ. Sci. Technol.* **2005**, *39*, 4333–4342. [[CrossRef](#)] [[PubMed](#)]
5. Fujiwara, S.; Omori, M. *Restoration Technique*; Coral Reefs of Japan, Japanese Coral Reef Society and Ministry of the Environment, Ed.; Ministry of the Environment: Tokyo, Japan, 2004; pp. 142–151.
6. Hoegh-Guldberg, O.; Mumby, P.J.; Hooten, A.J.; Steneck, R.S.; Greenfield, P.; Gomez, E.; Harvell, C.D.; Sale, P.F.; Edwards, A.J.; Caldeira, K.; et al. Coral reefs under rapid climate change and ocean acidification. *Science* **2007**, *318*, 1737–1742. [[CrossRef](#)]
7. Edwards, A.J. (Ed.) *Reef Rehabilitation Manual*; Coral Reef Targeted Research & Capacity Building for Management Program: St. Lucia, Australia, 2010; ii+166p.
8. Baker, A.C.; Starger, C.J.; McClanahan, T.R.; Glynn, P.W. Coral's adaptive response to climate change. *Nature* **2004**, *430*, 741. [[CrossRef](#)]
9. Salih, A.; Larkum, A.; Cox, G.; Kühl, M.; Hoegh-Guldberg, O. Fluorescent pigments in corals are photoprotective. *Nature* **2000**, *408*, 850–853. [[CrossRef](#)]
10. Randall, C.J.; Negri, A.P.; Quigley, K.M.; Foster, T.; Ricardo, G.F.; Webster, N.S.; Bay, L.K.; Harrison, P.L.; Babcock, R.C.; Heyward, A.J. Sexual production of corals for reef restoration in the Anthropocene. *Mar. Ecol. Prog. Ser.* **2020**, *635*, 203–232. [[CrossRef](#)]
11. Omori, M. Coral restoration research and technical developments: What we have learned so far. *Mar. Biol. Res.* **2019**, *15*, 377–409. [[CrossRef](#)]
12. Okamoto, M.; Nojima, S.; Fujiwara, S.; Furushima, Y. Development of ceramic settlement devices for coral reef restoration using in situ sexual reproduction of corals. *Fish. Sci.* **2008**, *74*, 1245–1253. [[CrossRef](#)]
13. Mullineaux, L.S.; Butman, C.A. Initial contact, exploration and attachment of barnacle (*Balanus amphitrite*) cyprids settling in flow. *Mar. Biol.* **1991**, *110*, 93–103. [[CrossRef](#)]
14. Quinn, N.P.; Ackerman, J.D. The effect of bottom roughness on scalar transport in aquatic ecosystems: Implications for reproduction and recruitment in the benthos. *J. Theor. Biol.* **2015**, *369*, 59–66. [[CrossRef](#)] [[PubMed](#)]
15. Hayashibara, T.; Shimoike, K.; Kimura, T.; Hosaka, S.; Heyward, S.; Harrison, P.; Kudo, K.; Omori, M. Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Mar. Ecol. Prog. Ser.* **1993**, *101*, 253–262. [[CrossRef](#)]

16. Hayashibara, T.; Iwao, K.; Omori, M. Induction and control of spawning in Okinawan staghorn corals. *Coral Reefs* **2004**, *23*, 406–409. [[CrossRef](#)]
17. Suzuki, G.; Arakaki, S.; Hayashibara, T. Rapid in situ settlement following spawning by *Acropora* corals at Ishigaki, southern Japan. *Mar. Ecol. Prog. Ser.* **2011**, *421*, 131–138. [[CrossRef](#)]
18. Vermeij, M.J.A.; Sandin, S.A. density-dependent settlement and mortality structure the earliest life phase of a coral population. *Ecology* **2008**, *89*, 1994–2004. [[CrossRef](#)] [[PubMed](#)]
19. Peterson, D.; Laterveer, M.; Schuhmacher, H. Innovative substrate tiles to spatially control larval settlement in coral culture. *Mar. Biol.* **2005**, *146*, 937–942. [[CrossRef](#)]
20. Misaki, H.; (Kushimoto Marine Park Center, Wakayama, Japan). Personal communication, 2020.
21. Carleton, J.H.; Sammarco, P.W. Effects of substratum irregularity on success of coral settlement: Quantification by comparative geomorphological techniques. *Bull. Mar. Sci.* **1987**, *40*, 85–98.
22. Guest, J.R.; Baria, M.V.; Gomez, E.D.; Heyward, A.J.; Edwards, A.J. Closing the circle: Is it feasible to rehabilitate reefs with sexually propagated corals? *Coral Reefs* **2014**, *33*, 45–55. [[CrossRef](#)]
23. Yamazato, K.; Suwardi, E.; Sultana, S. Reproductive cycle of brooding corals at high latitude. *J. Jpn. Coral Reef Soc.* **2008**, *10*, 1–11, (In Japanese with English abstract). [[CrossRef](#)]
24. Sato, M. Mortality and growth of juvenile coral *Pocillopora damicornis* (Linnaeus). *Coral Reefs* **1985**, *4*, 27–33. [[CrossRef](#)]
25. Schuhmacher, H. On the condition accompanying the first settlement of corals on artificial reefs with special reference to the influence of grazing sea urchins (Eilat, Red Sea). In Proceedings of the Second International Symposium on Coral Reefs, Brisbane, Australia, 22 June–2 July 1974; Volume 1, pp. 257–267.
26. Tebben, J.; Motti, C.A.; Siboni, N.; Tapiolas, D.M.; Negri, A.P.; Schupp, P.J.; Kitamura, M.; Hatta, M.; Steinberg, P.D.; Harder, T. Chemical mediation of coral larval settlement by crustose coralline algae. *Sci. Rep.* **2015**, *5*, 10803. [[CrossRef](#)] [[PubMed](#)]
27. Harrison, P.L.; Wallace, C.C. Reproduction, dispersal and recruitment of scleractinian corals, ch. 7. In *Coral Reef Ecosystems, Ecosystems of the World*; Dubinsky, Z., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1990; Volume 25, pp. 133–207.
28. Whalan, S.; Wahab, M.A.A.; Sprungala, S.; Poole, A.; de Nys, R. Larval settlement: The role of surface topography for sessile coral reef invertebrates. *PLoS ONE* **2015**, *10*, e0117675. [[CrossRef](#)] [[PubMed](#)]
29. Levenstein, M.A.; Marhaver, K.L.; Quinlan, Z.A.; Tholen, H.M.; Tichy, L.; Yus, J.; Lightcap, I.; Kelly, L.W.; Juarez, G.; Vermeij, M.J.A.; et al. Composite substrates reveal inorganic material cues for coral larval settlement. *ACS Sustain. Chem. Eng.* **2022**, *10*, 3960–3971. [[CrossRef](#)]
30. Nozawa, Y.; Tanaka, K.; Reimer, J.D. Reconsideration of the surface structure of settlement plates used in coral recruitment studies. *Zool. Stud.* **2011**, *50*, 53–60.
31. Wilson, J.; Harrison, P. Post-settlement mortality and growth of newly settled reef corals in a subtropical environment. *Coral Reefs* **2005**, *24*, 418–421. [[CrossRef](#)]
32. Nozawa, Y.; Tokeshi, M.; Nojima, S. Reproduction and recruitment of scleractinian corals in a high-latitude coral community, Amakusa, southwestern Japan. *Mar. Biol.* **2006**, *149*, 1047–1058. [[CrossRef](#)]
33. Sammarco, P.W. *Diadema* and its relationship to coral spat mortality: Grazing, competition, and biological disturbance. *J. Exp. Mar. Biol. Ecol.* **1980**, *45*, 245–272. [[CrossRef](#)]
34. Glassom, D.; Chadwick, N.E. Recruitment, growth and mortality of juvenile corals at Eilat, northern Red Sea. *Mar. Ecol. Prog. Ser.* **2006**, *318*, 111–122. [[CrossRef](#)]
35. Nozawa, Y. Micro-crevice structure enhances coral spat survivorship. *J. Exp. Mar. Biol. Ecol.* **2008**, *367*, 127–130. [[CrossRef](#)]
36. Nozawa, Y. Effective size of refugia for coral spat survival. *J. Exp. Mar. Biol. Ecol.* **2012**, *413*, 145–149. [[CrossRef](#)]
37. Gallagher, C.; Doropoulos, C. Spatial refugia mediate juvenile coral survival during coral-predator interactions. *Coral Reefs* **2017**, *36*, 51–61. [[CrossRef](#)]
38. Babcock, R.; Mundy, C. Coral recruitment: Consequences of settlement choice for early growth and survivorship in two scleractinians. *J. Exp. Mar. Biol. Ecol.* **1996**, *206*, 179–201. [[CrossRef](#)]
39. Okamoto, M.; Yap, M.; Roeroe, A.K.; Nojima, S.; Oyamada, K.; Fujiwara, S.; Iwata, I. In situ growth and mortality of juvenile *Acropora* over 2 years following mass spawning in Sekisei Lagoon, Okinawa (24° N). *Fish. Sci.* **2010**, *76*, 343–353. [[CrossRef](#)]

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