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By Rosdianto Rosdianto

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Life History Strategies of Crustose Coralline Algae (Rhodophyta) as Resilience Indicator of Coral Reef at Nature Reserve Pulau Sempu

Andik Isdianto^{1,2*}, Oktiyas Muzaky Luthfi¹, Guntur¹, Muhammad Arif Asadi¹, Nuddin Harahab², Andi Kurniawan², Agus Dwi Wicaksono², Rosdianto³, Berlania Mahardika Putri¹

¹ Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Jl. Veteran, Malang 65145, Indonesia

² Doctoral Program of Environmental Studies, Graduate School, University of Brawijaya, Jl. MT Haryono 169, Malang 65145, Indonesia

³ Marine Science, East Kutai Agricultural College School (STIPER), Jl. Soekarno Hatta, Tlk. Lingga, Sangatta, East Kutai Regency, East Kalimantan 75683, Indonesia

Abstract: Crustose Coralline Algae (CCA) are known as one of the calcifying algae which have important ecological responsibility on coral reef thus attracting coral juvenile, unstable binding substrate, source of food, and creating a substrate for some invertebrates. However, the study on the life histories of these algae was very poor when compared with coral. South Malang, East Java, directly faces the Indian Ocean and has a spatial coral reef ecosystem scattered in many places, one of the Nature Reserve Pulau Sempu (NRPS). This study aimed to get information regarding CCA recruitment and their growth strategy in plate materials. We used two different tile materials (settlement plates) composed of silica and carbonate base. The research result showed that CCA adhered Palimanan stone that contained more silica. The position also defined CCA recruitment because horizontal position is more calcified than the horizontal one. This research demonstrated the availability of CCA juveniles to succeed in their recruitment process and gave hope to the coral recruitment coral in Nature Reserve Pulau Sempu put over in substrate with coated CCA.

Keywords: benthic biotas, coral reef, recruitment, settlement.

硬殼珊瑚藻 (紅藻) 作為自然保護區珊瑚礁恢復力指標的生活史策略

摘要: 硬殼珊瑚藻被稱為鈣化藻類之一，對珊瑚礁具有重要的生態責任，因此吸引珊瑚幼體、不穩定的結合底物、食物來源，並為一些無脊椎動物創造底物。然而，與珊瑚相比，對這些藻類生活史的研究非常貧乏。東爪哇的南瑪璠直接面向印度洋，擁有分佈在許多地方的空間珊瑚礁生態系統，是森普島自然保護區之一。本研究旨在獲取有關硬殼珊瑚藻招聘及其在板材中的增長策略的信息。我們使用了兩種不同的瓷磚材料（沉降板），由二氧化矽和碳酸鹽基組成。研究結果表明，硬殼珊瑚藻附著在含有更多二氧化矽的帕利馬南石上。該位置還定義了硬殼珊瑚藻招聘，因為水平位置比水平位置更鈣化。這項研究證明了硬殼珊瑚藻幼魚在其招募過程中取得成功的可用性，並為自然保護區森普島中的珊瑚招募珊瑚帶來了希望，這些珊瑚被置於塗有硬殼珊瑚藻塗層的基質中。关键词：本体社區，珊瑚礁，招体，体体，体島嶼。

关键词：底栖生物群、珊瑚礁、招募、定居。

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About the authors: Andik Isdianto, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia; Doctoral Program of Environmental Studies, Graduate School, University of Brawijaya, Malang, Indonesia; Oktiyas Muzaky Luthfi, Guntur, Muhammad Arif Asadi, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia; Nuddin Harahab, Andi Kurniawan, Agus Dwi Wicaksono, Doctoral Program of Environmental Studies, Graduate School, University of Brawijaya, Malang, Indonesia; Rosdianto, Marine Science, East Kutai Agricultural College School (STIPER), Sangatta, Indonesia; Berlania Mahardika Putri, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia

Corresponding author Andik Isdianto, andik.isdianto@ub.ac.id

1. Introduction

The condition of rising seawater temperature in the world and the increasing amount of carbon entering the waters have been predicted by researchers. Researchers have also projected the impact to the next year of 2100 with various scenarios, especially the effect on ocean acidification and increasing seawater temperature [1]. Corals worldwide have faced global bleaching many times, harming coral reef ecosystems in general [2]. Continuing pressures and threats from global or local sources will also impact the health of the corals [3].

Coral ecology experts argue that coral has a high level of resilience compared to other ecosystems. This can be proven that the ecosystem of the coral reef had after mass bleaching several times in the past, but some other experts reject this opinion [4]. Resilience is a concept that is almost 50 years old, that first time introduced by Holling [4]. Holling has used the concept of resilience to explain the return of forest ecosystems in the east of Canada due to budworm attacks. Then in the era of 1980-1990, resilience terminology was adopted on the coral reef ecosystem. This new concept inspired hundred coral researchers to explain the recovery process on the coral reef ecosystem [5]–[9]. In the early 2000s, where three years earlier there was mass coral bleaching around the world, the term coral resilience is growing, and 20 years later, the theory of coral resilience was fruitful because both theory and practice were working together along with many aspects, from molecular to social studies, that support on this theory, resulting resilience index comes out [10]–[14].

Coral reef resilience can be interpreted as the ability of the coral reef ecosystem to soak up regular disturbances or stress and accommodate change while maintaining the same function and structure [15]. Shortly, coral reef resilience should meet with two words: resist or survive and recovery. Furthermore, the concept of resilience was reviewed by [16] divided into three classes: descriptive, hybrid, and normative concept. This concept is a very broad idea that has biological to social science. In the coral reef ecosystem view, resilience can be seen in two different ways. They were coral as individuals, which means they can survive under stressors, keep growth, reproduce gamete, and reshuffle zooxanthella inside their bodies.

Furthermore, as a population where coral can repopulate (again) their colony by recruiting new individuals, maintaining biodiversity on all component positions on the food web, and sustained biomass [15]. The study [17] released indicators tools to assess coral reef resilience; at least six resilience indicators should be assessed to stated coral reef conditions. They were: resistant coral species, coral diversity, herbivore

biomass, coral disease, macroalgae cover, and recruitment of coral.

Coral recruitment is key in supporting coral reef ecosystems, and it can promote their recovery after disturbances. In another way, coral recruitment is one of basic coral reef ecosystem resilience. Coral recruitment needs a principal supporter - CCA. These algae have guided coral planulae to find suitable places and post-settlement stages [18], [19]. For instance, larvae of pocilloporids and poritids need cues from CCA to select certain substrates [18], also larvae coral of *Agaricia humilis* motile on the surface of CCA before metamorphosis [20]. Chemical cues which have resulted from CCA were identified as tetrabromopyrrole (TBP). P-producing bacteria activated varying intensity of coral larval (planula) settlement and metamorphosis [21]. Moreover, CCA also play a role of a stabilizer of substrates in the reef system and with coral as the primary carbonate source in the coral reef ecosystem [22]. Another researcher revealed that CCA constitute an energy source for many reefs biotas such as *Diadema* urchins, scariids, surgeonfish, and other invertebrates [23].

CCA are red algae that belong to the division of Rhodophyta and order of corallines. These algae have carbonate thalli with red or pink colors that are very easy to distinguish from coral [23]. These algae also have unique growth forms such as shelf-like crusts, upright branches, and thick knobby crusts. Sexual reproduction of these algae reproduces by sori that containing male and female gametes. Juvenile of these algae experience seedling and find new substrate to attach and growth encrusting for early life than grow into a frondose. For the taxonomical purpose, these algae are divided into two as geniculate and non-geniculate. Geniculate algae, in general, grows as a tree and non-calcified segment in their thalli [20], [24]. Non-geniculate algae commonly have encrusting or another form (Table 1), and all their thalli were calcified [20]. Almost CCA can attach to various substrates such as rock (epilithic), other plants (epiphytic), animals (epizoic), and other materials like glass, wood, and metals (epigenous) [25].

Corals in the South Malang area were developed on flat reefs, wherein this area was generally dominated by massive corals, encrusting corals, and branching corals [26]. Coral spread starting from Sempu Strait then spread over to Segara Anakan, Teluk Semut, and Waru-Waru, with damaged coral reef conditions. At Waru-Waru, the dominating living coral was a massive coral consisting of Faviid and Galaxea by 33%, while the percentage of corals living in Teluk Semut was quite high at 50%, dominated by Pocilloporiid and Poritid [27]. The process of coral development in the waters of Pulau Sempu is not yet clearly known from

the results of monitoring the cover of live corals revealed that that coral cover in this place was in the stagnancy range in the 25%. Furthermore, this condition was since 2008 and relatively stable or constant. Briefly, corals in the Pulau Sempu area certainly continued to grow and develop, but on the other hand, several factors might limit their development. CCA play a big role in the coral recruitment process, but there is little information regarding their early growth, colonization, and carbonate productivity in the NRPS area. This study attempted to describe the early life of CCA in different substrates, their percent cover, and the number of colonies that provided valuable data on coral resilience in conservation areas, especially in NRPS.

2. Materials and Methods

2.1. Study Site

The research was conducted in the South Malang area with three stations were selected on a reef nearby Nature Reserve Pulau Sempu (NRPS): Waru-Waru, Teluk Semut, and Tiga Warna (Fig. 1). Settlement plates installation was carried out in July 2020. Settlement plates were taken out three months later in October 2020 (short-time immersion tiles) as previous research stated that CCA colonies peak between 6 weeks to 8 months [28]. The location map of settlement plates can be found in Fig. 1.

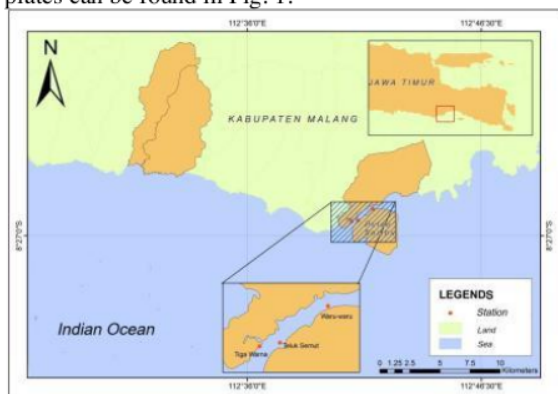


Fig. 1 Settlement plates immersion in 3 station research sites

2.2. Deployment of Settlement Plates

A total of 30 blocks of settlement plates, dimension: 10x10 cm, were placed horizontally on the surface of reef substrate at 2-5 meters depth according to the living coral was growing in the NRPS. This study used two types of settlement plates: palimanan stone (PL) and marmo stone (MM). PL and MM have different physical characters, where PL has a rougher surface composed of SiO_2 while MM has a smoother surface and is composed of CaCO_3 . One pair of PL and MM were referred to as one block, wherein each station has been installed for 15 blocks with the distance between blocks were 1-2 m (Fig. 2A). Each settlement plate has

been fixed to the bottom of reef substrate using a 10-inch concrete nail (Fig. 2B) [29], [30].

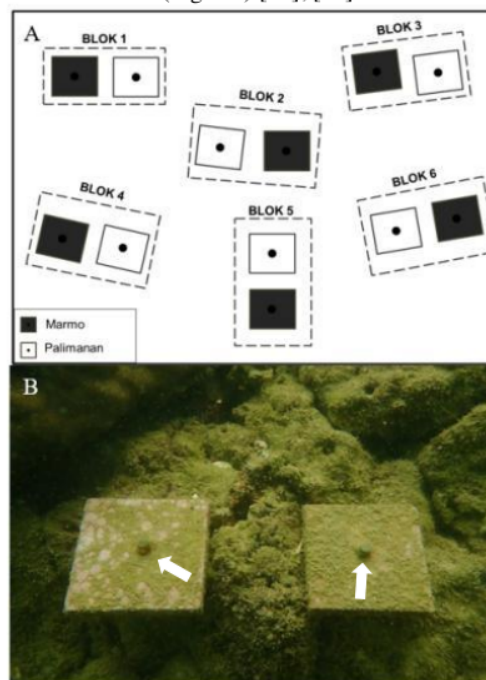


Fig. 2 2(A) A block contained one PL (white rectangle) and one MM (black rectangle), this illustration modified from [29]; (B) Settlement plates fixed using concrete nails were used on this study, see white arrow. Blok: block

2.3. Settlement Plates Collection

After three months of immersion, all settlement plates were collected and kept in zip-lock plastic each. After being photographed in the laboratories, substrates were tagged with initial code, A for the upward area, B for the downward area, and C (1-4) for the side area (Fig. 3A, B, C1, C2, C3, and C4). The tag was named like below: site_code of settlement plates_number of samples_code of orientation, for instance: WW_PL_01_A stands for Waru-Waru_Palimanan_Sample Number 01_Upward. This code was used to facilitate identifying and calculating the extent of CCA using ImageJ (NIH, USA).

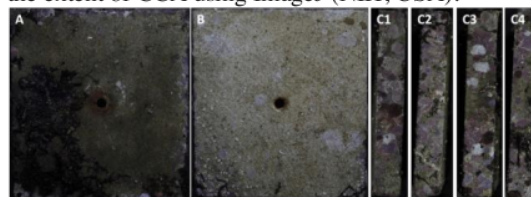


Fig. 3 Settlement plate size 10 x 10 cm has been used for research. A is the upward area, B is the downward area, and C (1-4) is the side area

2.4. CCA Growth Form Identification

CCA growth form can be described with one type of growth form or using two or more, for instance, "encrusting" to describe single growth for and "encrusting to warty", "encrusting to warty" to

fruticose” for describing two or more growth forms of CCA. One CCA species can have up to six different growth forms, and sometimes one species will be similar to another. Taxonomical morphological identification of CCA's growth form has used a USB microscope, and the growth form of CCA was followed in Table 1 [20], [24].

Table 1 Non-genic forms of algae coralline growth

No.	Life form	Description
1	<i>Unconsolidated</i>	CCA's filament separated each other
2	Encrusting	CCA growth coat the substrate
3	Warty	CCA have short branches <3 mm
4	Fruticose	CCA have cylindrical shapes and free branches >3 mm.
5	Lumpy	CCA have rounded rarely crowded branches
6	Discoid	CCA growth in free-living, rounded, and without struts in the lower part
7	Layered	CCA have flattened horizontal branches which overlay each other
8	Foliose	CCA have vertical branches like a vertical leaf in a plant
9	Ribbon-like	CCA grow like tape and without holdfast
10	Arborescent	CCA grow like a tree with holdfast as root.

2.5. Percentage of CCA Cover on Settlement Plates

Retrieved settlement plates were dried on natural sunlight for 48 h and then photographed on all sides (Fig. 3). Each colony of CCA was then delineated and calculated percent cover by ImageJ (NIH, USA) [31]. The percentage of CCA recruitment cover was calculated as the total area of CCA colonies on all sides of tiles per total area of tiles [32], [33]. The formula is as below:

$$CP = (\Sigma a/A) \times 100\% \quad (1)$$

where CP is coral cover percentage A; A - Area, and Σa - total area of CCA's colonies.

2.6. Data Analysis

Data tested by using the Mann-Whitney test compared CCA percent coverage and number of colonies trapped in substrate collectors. The decision-making provisions used in these two statistical tests are H_0 accepted for P-value > 0.05 and H_0 rejected if P-value < 0.05 [34].

3. Result and Discussion

3.1. Life Form of CCA

The type of CCA found is entirely epilithic because it is attached to rocks substrate (Fig. 4). Each tile was examined in detail to check CCA growth forms, and after checked on 90 tiles, in general, the growth forms of CCA were “encrusting, encrusting to layered and encrusting to lumpy” (Fig. 4). At the same time, the CCA colony color was variate purple, pink, orange, and

light green (Fig. 4). Some CCA is found in white color, possibly because of bleaching or it has lost its pigment. Thin and thick layer shapes of CCA were found in all research stations.

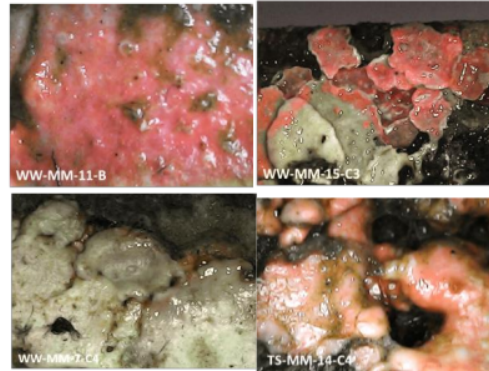


Fig. 4 Three forms of CCA growth at NRPS encrusting at Waru-Waru (WW-MM-11-B), encrusting to layered at Waru-Waru (WW-MM-15-C3), and encrusting to lumpy from Waru-Waru and Teluk Semut (WW-MM-7-C4 and TS-MM-14-C4)

Non-genic algae coralline grows relatively slowly; the horizontal growth was maximum of 0.05 mm per day [35]. Variety morphological variations observed in CCA may reflect the surrounding environmental conditions. For example, a prominent thallus can result from adaptation to the level of exposure to wave energy, such as in the lumpy form [36]. The thickness thallus of CCA indicates the result of competition in occupying space between benthic biotas. The thin crust was the early lifeform of CCA to quickly occupy as wide as possible a substrate; then, after three months, the thin layer will be covered by a thick layer [37]. CCA with a thin crust usually can be found at reef crest area with strong adherent thalli to adapt to the strong current and wave exposure. The coralline alga *Porolithon pachydermum* is an example of the common CCA in the Indo-Pacific that is dominant to the ecosystem due to its ability to adapt to the hard environment [38].

The encrusting form had characteristic sticking, crusty, and flattened, and without protrusions or branches. The form of encrusting CCA usually follows the shape of the substrate. For instance, when algae are attached to rocks, mollusk shells, seagrass leaves, or other algae, the shape of CCA will follow the contours of the host [20], [24]. Moreover, encrusting is usually the initial form of CCA growth; in the next growth, it will be possible to change it into warty, lumpy, discoid, layered, and foliose. The species that have a form of encrusting growth were *Lithophyllum stictaeforme* and *Heydrichia homalopasta* [20].

Encrusting to layered is a combination of encrusting and layer form. The stratified between these two layers was invisible because the new layer was overlapping the previous one. Encrusting to layered growth form can be found in species *Synarthrophyton patena*, *Synarthrophyton schielianum*, and *Mesophyllum*

macroblastum [20]. Encrusting to lumpy is the development from encrusting CCA with dense protrusion and close each other within a colony. This growth form can usually be found on CCA, where life is in shallow water or intertidal areas. High sunlight intensity suggested triggered this growth form. Two common species were found in this growth form were *Hydrolithon boergesenii* and *Porolithon onkodes* [38].

3.2. CCA Cover Percentage

The average percentage of CCA cover on the highest settlement plates was 35.40 ± 19.92 at Batu Palimanan (PL) located at Waru-Waru Station (WW), and the lowest was 13.25 ± 9.42 at Teluk Semut Station (TS) (Fig. 5). Meanwhile, on settlement plates marmo (MM), the highest percentage of CCA cover was found at Waru-Waru station, which was 31.10 ± 17.11 , and the lowest was at Teluk Semut station, which was 3.13 ± 2.49 (Fig. 5). Based on statistical tests, the average percentage of CCA cover at the 3 study sites had a significant difference ($P\text{-value } 0.000 < 0.05$).

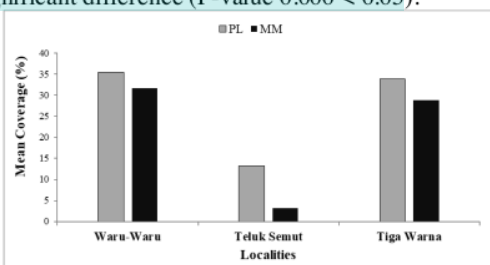


Fig. 5 Average percentage comparison of CCA cover at three stations for three months based on the tentative area (240 cm^2): PL: Palimanan; MM: Marmo

The CCA cover of PL tends to be higher than that of MM. Statistical test results showed that there was a significant difference between the two types of settlement plates used. There was still little research on the effect of material differences on CCA recruitment because, usually, similar studies have been carried out to observe recruitment in corals. However, a study conducted by [30] compared the recruitment and growth of CCA in 6 different types of materials: PVC, polycarbonate, terracotta, limestone, glass, and porcelain in the waters of the Great Barrier Reef. The comparison result stated that the glass tile was more attracted by CCA such as *Hydrolithon* spp. In this study, PL that contains more silicate material to made glass had a higher CCA cover than MM that, in first expected, could attract more reef benthos due to high carbonate inside.

The high percentage of CCA in a substrate is not decided only by the type of material, but other factors should be considered, such as availability of light, habitat, wave action, predators, and competition. Orientation of settlement tile and clarity of seawater will affect the light received of CCA [37]. Natural habitat is also known to be impacted on coverage of

CCA in tile. Research conducted on Heron island resulted in the high cover of CCA in reef slope areas with more clear water, low sedimentation, and fewer predators [30]. The wave or flow regime was suggested to determine CCA distribution and survivorship during the juvenile phase of algae in which force of the current will be affected algae's holdfast difficult to attach on substrates [39]. CCA secreted Mg-calcite as skeletons, and some reef fish and sea urchin scraped this for food or borer invertebrates inside CCA's skeleton [40]. Competition can be inter CCA which thick CCA is generally considered to be competitively superior to thinner crusts. Thin CCA will occupy the settlement on day 14, but after 112 days of deployment, the thick CCA over helmed the previous CCA [37].

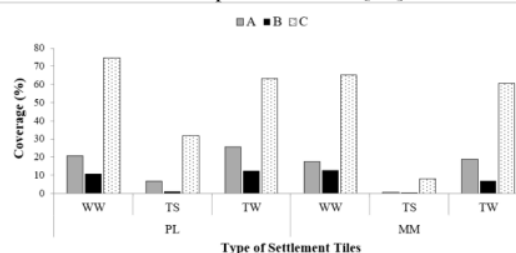


Fig. 6 The percentage of CCA cover on all sides of recruitment plates where CCA was found to have been attached to upward areas (A), downward areas (B), and sides (C). OT: Palimanan; MM: Marmo; WW: Waru-Waru; TS: Teluk Semut; TW: Tiga Warna

The highest percentage of CCA cover was on the side area (C), followed by the upward areas (A) and downward areas (B). This is suggested because it has been influenced by several factors such as the intensity of sunlight, orientation of settlement plates, and calculating the percentage of CCA cover. The upward and side area receives more light intensity that affects the fast growth of CCA because alga needs like for photosynthesis and calcification process [41]. Research conducted by [42] stated that light positively correlates with calcification and photosynthesis on two species CCA, *Hydrolithon reinboldii*, and *Lithophyllum cabiochae*.

3.3. CCA Colony

The total number of CCA colonies attached to the PL and MM settlement plates for three months was 9,866 colonies (Table 2). The highest number of CCA colonies was at Tiga Warna station, which was 5,291 colonies, and the lowest was at Teluk Semut station, which was 1,753 colonies. Based on the results of statistical tests, the comparison of the number of CCA colonies at the three study sites had a significant difference ($P\text{-value} = 0.000 < 0.05$).

Table 2 Number of CCA colonies attached to PA and settlement plates

Station	Palimanan	Marmo	Total
Waru-Waru	1.417	1.405	2.882
Teluk Semut	1.122	631	1.753
Tiga Warna	2.806	2.485	5.291

Continuation of Table 2

Total	5.345	4.521	9.866
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In this study, the term one CCA colony was defined as a thallus that occupies a space on the substrate and grows laterally to form a separate disc from the others. Furthermore, if CCA growth overlapped each other and covered the entire surface of the settlement plates, it was counted as one colony.

CCA with thick and thin crust was found attached to the settlement plates throughout the research station. The recruitment results in short-time immersion tend to be dominated by CCA with thin thallus, which shows that the dominant CCA species are those with fast growth rates. CCA calcification rate normally will be on 0.13 mg/cm²/day [43], but at the beginning of growth and supported excellent environmental conditions, the calcification rate of CCA will be faster, i.e., about 0.42 ± 0.04 mg/cm²/day [30].

Overall short-time immersion, Waru-Waru, and Tiga Warna stations had the highest number of CCA colonies. The success of CCA recruitment [23] is influenced by various factors such as settlement plate material and surface complexity, position, size, installation method, placement in the ecosystem, coral reefs, and duration of placement [30]. Environmental factors have been suspected of supporting the success of CCA recruitment at Tiga Warna and Waru-Waru Stations, both of which have high flow rates compared to other stations. Currents are thought to aid the dispersal of CCA spores in waters, whereas CCA has been known to have harmful and non-motile floating spores, which usually exhibit short dispersal distances each generation [44].

4. Conclusion

This study has provided some clues regarding one of the CCA cycles in a substrate. Juvenile CCA more widely choose Palimanan stones that contain much silica to attach to them. In addition, another important piece of information is the vertical position preferred by CCA as a place to attach. The successful attachment of CCA into different settlement plates was proved that the availability of CCA in NRPS waters still quite good and will play an important role in the succession of corals recruitment in the future.

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