Original Article

Recruitment of crustose coralline algae on tiles material for monitoring coral larvae settlement's consolidators at Nature Reserve Pulau Sempu, East Java, Indonesia

Recrutamento de algas coralinas crustosas em azulejos para monitorar consolidadores de assentamentos de larvas de coral na Reserva Natural Pulau Sempu, Java Oriental, Indonésia

Guntur[a]* , O.M. Luthfi[a,b] and M. A. Asadi[a,b]

[a]Department of Marine Science, University of Brawijaya, Jl. Veteran Malang, East Java Indonesia
[b]Research Group Coastal Resilience and Climate Change – CoRECT,University of Brawijaya, Jl. Veteran Malang, East Java, Indonesia

Abstract

Crustose coralline algae (Corallinophycideae) are red algae that produced calcium carbonate and are well recognized as foundation species in the epipelagic zone of the marine ecosystem. These algae induced settlement juvenile of coral by released chemical cues from bacterial communities on the surface of their colonies. Their extracellular calcium carbonate also can stabilize reef structure that influencing many invertebrate attaches and growth in the seabed. Crustose coralline algae (CCA) have obtained attention because of their distribution and health compromise to increasing seawater temperature, ocean acidification, and pollutant. As a cryptic species in the ecosystem, the presence of CCA recruit sometimes doesn't have attention, especially on their capability to occupy the empty space. This study aimed to document coverage and number of CCA recruit in two different recruitment tile's material. The highest CCA percentage of the cover was showed inside surface than others surface in all stations. Light intensity and low sedimentation were suggested as a key factor of success of high coverage. Overall, station higher CCA recruits have shown from Tiga Warna. Low sedimentation and protection from aerial exposure became the main reason for it. No significant difference number of CCA recruits between marble and sandstone in this study. Successful CCA recruitment in this study can give a wide picture that natural recruitment of coral and other reef biodiversity in Southern Malang might be will succeed because of the abundance of coralline algae that support their life history stage.

Keywords: CCA, settlement, recruitment, Pulau Sempu, coral reefs.

Resumo

As algas coralinas crustosas (Corallinophycideae) são algas vermelhas que produzem carbonato de cálcio e são bem reconhecidas como espécies de base na zona epipelágica do ecossistema marinho. Essas algas induziram o assentamento juvenil de coral por meio de estímulos químicos liberados por comunidades bacterianas na superfície de suas colônias. Seu carbonato de cálcio extracelular também pode estabilizar a estrutura do recife que influencia muitos invertebrados anexados e crescimento no fundo do mar. As algas coralinas crustosas (CCA) têm obtido atenção devido à sua distribuição e comprometimento da saúde com o aumento da temperatura da água do mar, acidificação dos oceanos e poluentes. Como uma espécie enigmática no ecossistema, a presença de recrutamento CCA às vezes não recebe atenção, especialmente em sua capacidade de ocupar o espaço vazio. Este estudo teve como objetivo documentar a cobertura e o número de recrutamento de CCA em dois materiais de recrutamento diferentes. A maior porcentagem de CCA da cobertura foi mostrada na superfície interna do que nas outras superfícies em todas as estações. A intensidade da luz e a baixa sedimentação foram sugeridas como um fator-chave para o sucesso da alta cobertura. De modo geral, recrutas de CCA mais graduados mostraram-se em Tiga Warna. A baixa sedimentação e a proteção contra a exposição aérea se tornaram a principal razão para isso. Não houve diferença significativa no número de recrutas CCA entre mármore e arenito neste estudo. O recrutamento bem-sucedido de CCA neste estudo pode dar uma imagem ampla de que o recrutamento natural de corais e outros recifes da biodiversidade no sul de Malang será bem-sucedido por causa da abundância de algas coralinas que sustentam seu estágio de história de vida.

Palavras-chave: CCA, assentamento, recrutamento, Pulau Sempu, recifes de coral.

1. Introduction

Crustose coralline algae (CCA) are terms to support the amount of red calcifying algae and nowadays become the most complex community of rhodophytes in seawater environments. These algae are wide range geographical distribution that can be found from low to high latitude and from intertidal to depth area. In alive condition, these algae have pink-reddish color and categories as a marine plant (Phylum: Rhodophyta, Class: Florideophyceae, Subclass: Corallinophycidae, Order: Corallinales, Family: Corallinaceae) that attached on the substrate with rootlike called as holdfast (Littler and Littler, 2013; Nash et al., 2019). In the early description, the CCA divide into generic names because of their morphologies differences, a flat form knowing as Lithophyllum and a branched form as Lithothamnium (Littler and Littler, 2013). McCoy and Kamenos (2015) have a different term for flat and branching form that referred as non-articulate (non-genicular) and articulate (genicular) respectively. Last report the Corallinaceae has 30 genera and more than 500 species (Dean et al., 2015).

CCA is one of the most abundant species to inhabit hard substrates in the shallow marine zone. The success of the wide distribution of CCA is triggered by several factors, for instance, light, temperature, nutrient, current, and depth (Steneck, 1985; Nash and Adey, 2017). High cover of CCA has been confirmed from the marine ecosystem in the world Indonesia (Verheij, 1993), GBR Australia (Dean et al., 2015), Indian Ocean (Caragnano et al., 2014), Mediterranean (Zelli et al., 2020), Japan (Yoshioka et al., 2020), Caribbean (Johnson et al., 2019), Arctic (Williams et al., 2020), New Caledonia (Aeby et al., 2016), and Hawaii (Vermeij et al., 2011). Many researchers revealed that CCA has an important role contributing to reef communities such as cementing process of reef framework against the strong current, wave, and storm. Cementing is important to reduce bio-erosion due to herbivore action and physical erosion of reef to maintains reef complexity. CCA also valuable to coral recruitment as they capability to guide coral planulae to find a suitable substrate for them. Through chemical cues (glycoglycerolipids and polysaccharides) coral planulae induce on the surface of CCA (Tebben et al., 2015). To compete on space among macroalgae, CCA release substance that be agent for antifouling compounds resulted in positive recruitment on coral (Vermeij et al., 2011). They cast their thalli to inhibit macroalgal spores attach to their surface (Keats et al., 1997). The increase of CO_2 in the atmosphere leads to global climate change that effects on decreasing pH of seawater. CCA species will become the first coralline alga affected by lower pH because they deposited $MgCO_3$, which is about 20% more solvent than aragonite. In recent work, CCA has become more be considered as a bioindicator of seawater chemistry change (McCoy and Kamenos, 2015; Muth et al., 2020).

Benthic organisms such as coral have a planktonic phase that swimming passively on seawater column while relies on chemical cues for finding suitable substrate to settle. For spawner scleractinian coral fertilization between eggs and sperm will occur in the water column for a few days. Larvae then explore the substrate beneath seawater for several days and if any signal from suitable substrates they will attach to it and metamorphose as single polyps (Harrison and Ward, 2001). The larval settlement of scleractinian coral is triggered by CCA and a thin layer of microbe on the substrate (biofilms). Planula larvae of scleractinian, *Goniastrea retiformis* and *Stylaraea punctata*, have a preference on CCA covering substrate (Golbuu and Richmond, 2007). Larvae of Acropora also have been induced by CCA for settlement (Ritson-Williams et al., 2014; Whitman et al., 2020) and in Caribbean CCA also facilitates settlement of *Agaricia humilis* and 3 species of Acropora of Great Barrier Reef also experience the similar condition. Biofilms have been known as an inducer of coral planulae, for instance, *Acropora microphthalma* larvae prefer to settle on eight-week-old biofilms while planula of coral *Pocillopora damicornis* prefers on dense biofilms substrate (Tran and Hadfield, 2011; Siboni et al., 2020).

Tiles are a common use for coral recruitment information and many effects of difference of tile have been widely documented (Kennedy et al., 2017). And less information on tile usage for other benthic organisms such as coralline algae. The information about CCA recruitment is very important to measure reef growth in a certain area and monitor reef health. Knowing the basic biology process of recruitment of CCA will further encourage our perception of other reef engineers such as coral or scleractinian (Jorissen et al., 2020; Mallela et al., 2017). This study aims to record CCA percentage on two different types of tiles and comparing of percentage CCA on different and to know CCA population characteristics using their mean crust size.

2. Materials and Methods

2.1. Site selection

The study was conducted on the reef flat area of Nature Reserve Pulau Sempu, Malang, East Java, Indonesia (8°26'28.18LS; 112°40'58.33BT) which focusing on Sempu strait, where almost coral grows well in this location (Rosdianto et al., 2020). Three stations have been decided as the location to deploy of tiles for CCA settlement, two in the adjacent of Pulau Sempu which has a local name as Teluk Semut (08°26'20,8'' S; 112°40'52,5'' E) and Waru-Waru (8°25'49.57 S; 112°41'33.70 E). One station is adjacent to Java Island or 500 m in front of Teluk Semut, it calls as Tiga Warna (8°26'22.48 S; 112°40'39.65 E) (Figure 1). Although these stations are in a marine protected area some limited local tourists still visit these areas for water sport activities. And some fishermen still anchoring their ships for maintaining and park for logistic supply from Pondok Dadap (Sendang Biru) harbor (indicated black dot in Figure 1). According to Luthfi et al. (2015), in a day is about 300 fish-ships passed in this area.

2.2. CCA settlement tiles

To monitor the number of CCA settlements in Nature Reserve Pulau Sempu were used different types of settlement plates they were sandstone (Palimanan Stone) and marble stone (Marmo Stone) which each has 10 x 10 cm (Figure 2b). In the center of stone tiles was bored using an electric drill
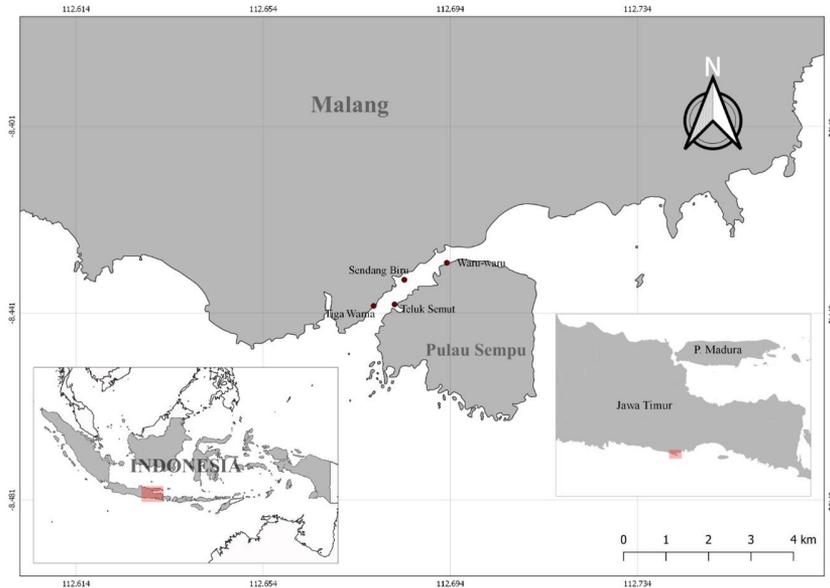
Figure 1. Location of field sites in Natural Reserve Pulau Sempu, Indonesia.
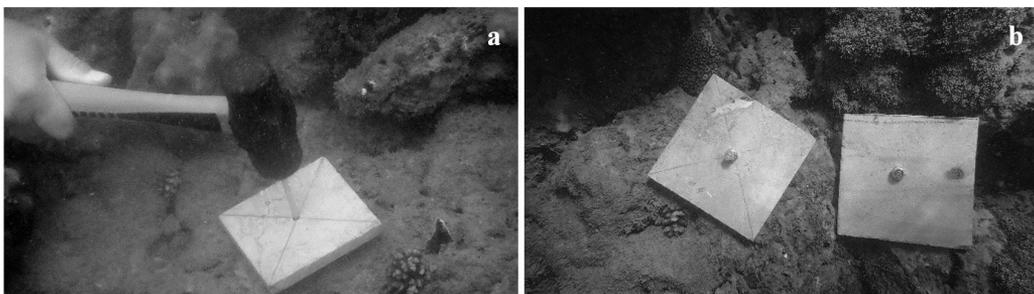

Figure 2. Settlement tiles fixed using 10 cm stainless nails into a substrate (a) Marmo Stone (MM); (b) Palimanan Stone (PL).

(Bull BL 1811) with drill bit size 10. Through these drilled holes a stainless-steel rod (nail) anchored into hard substratum around of living coral reef by 8 cm inserted. A nut, size 10, set between the head of rod and stone made it a space that easier to pull the rode during retrieval. 15 blocks (1 block consists of sandstone and marble stone tiles) of CCA settlements anchored in each station which each block separated 1-2 m (Edmunds et al., 2014). All of the blocks were deployed in the middle of July 2020 and retrieved in the middle of October 2020 (3 months). These three months were north east monsoon (dry season) in Java which made sea water very clear due to minimize of river run off from terrestrial and also predicted as main reproductive of reef in this area.

2.3. Percent cover calculation

Settlement tiles then dried naturally under the sunlight for two days and photographed on all sides (Figure 3). Each colony of CCA was then delineated and calculated percent cover by ImageJ (NIH, USA) (Reich et al., 2017). 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 (Cruz and Harrison, 2017; Oliver et al., 2011). The Formula 1 as below:

$$CP = \frac{\Sigma a}{A} 100\% \tag{1}$$

Where: CP is coral cover percentage A; A = Area, and Σa: total area of CCA's colonies

2.4. Statistical analysis

The densities and cover of coral recruits were compared between types of settlement plates. ANOVAs and t-test were used to test the significant difference of types of settlement plates to densities and coverage of CCA. Data were normalized by transforming it using log (n + 1) for fulfilled variance analysis. SPSS ver. 19 was used for statistical analysis of all data (Kennedy et al.).

3. Results

3.1. CCA percent cover

The mean percentage cover of CCA on Palimanan Stone (PL) at station 1 (Waru-Waru), station 2 (Teluk Semut), and
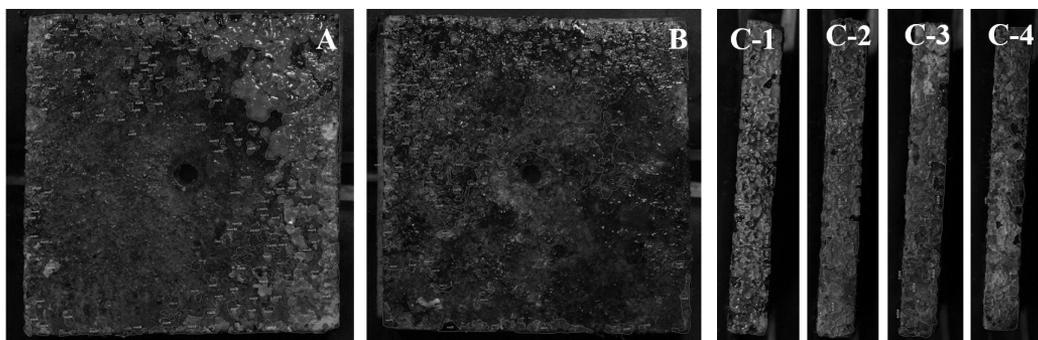
Figure 3. Representation of marble settlement tiles from station Tiga Warna 10 x 10 cm. (A) upward surface; (B) downward surface; (C)1-4 side surface number 1 to 4. Coralline algae are seen on delineated yellow lines that cover almost the surface of tiles.
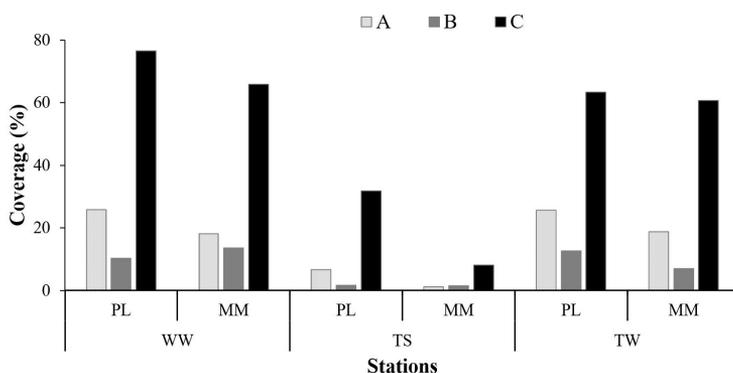

Figure 4. The percentage of CCA settlement cover at all research stations. Most CCA recruits were cover 3 different sides of settlement tiles (A) upward surface; (B) downward surface; (C) sides surface. WW = Waru-Waru; TS = Teluk Semut; TW= Tiga Warna.
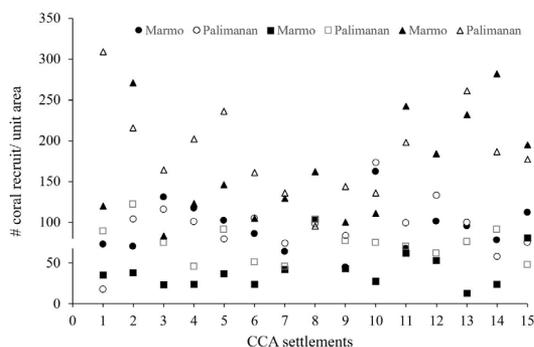

Figure 5. The total number of CCA recruits at all stations, full and blank circle, rectangle, and triangle represent from stations 1, 2, and 3 respectively. The total unit of the surface area is 240 cm² in each settlement tile.

station 3 (Tiga Warna) were 37.5% ± 20 SE, 13.4% ± 9.3 SE, and 33.8% ± 15.2 SE. The coverage on the other stone (Marmo Stone/ MM) was slightly lower 32.5% ± 16.7 SE, 3.58% ± 2.3 SE, and 28.8% ± 16.3 SE respectively). Overall, the highest trend of CCA coverage was found in Waru-Waru (WW) and the lowest one was found in Teluk Semut (TS) (Figure 4). As resumed in Figure 2, that CCA recruits are attached on all sides of settlement tiles, upward, downward, and side surface. Domination CCA cover of tile sides surface (C) was found from both of Marmo and Palimanan in all stations (Figure 4). At this side the percent coverage of CCA almost three and six times higher than A and B sides. In average palimanan stone were more attached by algae than marmo stone (Figure 5).

3.2. Distribution and number of CCA recruit

The total number CCA recruit adhered at settlement tiles was 9,866 colonies after three months of deployment. The number CCA recruits at Tiga Warna was higher than others, more than half of CCA recruits reported from this station (5,291 colonies) (Table 1). The mean number of CCA recruits per tile was 110. CCA recruits seemed to have a preference on settlement orientation where upward and side surface were higher occupied by them (Table 1). According to of statistical process there was no strong correlation between the number CCA recruits at Palimanan stone and Marmo stone (p-value = 0.057 or p > 0.05).

4. Discussion

Calcification on CCA quite different from other invertebrates such as scleractinian coral. In coral, the skeleton consists of aragonite form while in CCA mineralizes magnesium calcite (Nash et al., 2019). Deposit of Mg-Ca was on their cell wall and made their thallus became hard.

Table 1. Composition and total of CCA recruits in three different orientations (upward, downward, and side stated as A, B, and C respectively) at three stations which n=15 block or 30 settlement tile per station. The unit is colony.

Stations	Marmo			Palimanan			Total
	A	B	C	A	B	C	
Waru-Waru	575	522	308	671	521	225	2,822
Teluk Semut	150	79	402	396	128	598	1,753
Tiga Warna	734	769	982	749	976	1081	5,291

The red pigment that was called phycobiliproteins caused these species to have red or pink color (Mogstad and Johnsen, 2017). In this study, almost all the CCA recruits had thin and thick thallus. The thin crust was more dominated in all side surfaces of settlement tiles than the thick one. *Pneophyllum conicum,* for instance, is one of the thin crust of CCA that has a complete reproductive structure in their thallus after 14 days of settlement that causes these species to become dominant species in the southwest Atlantic (Mariath et al., 2013). Abiotic factors such as sedimentation rate also influenced of CCA cover in the new substrate which the result of this study showed that Teluk Semut has more turbid area than Waru-Waru and Tiga Warna. In this case, might be a high sedimentation rate will reduce the growth of CCA. The high accumulation of sediment, more than 10 mg/cm^2/day, can reduce irradiance and inhibit the photosynthesis process of CCA. Furthermore, sediment can cover the surface of CCA and create anoxic condition and decrease the recruitment process (Harrington et al., 2005; Mariath et al., 2013).

The highest coral cover percentage was found from the side surface of settlement tiles and followed by an upward position (Figure 4). CCA is dependent on light, only view of CCA reported growth and distributed limited from the non-euphotic zone. For instance, distribution of the deepest rhodoliths that was found in 295 m off San Salvador Seamount of Bahama, survive with dim light about 8.10^{-2} µmol photons/m^2/s[1], less diverse comparing with high sunlight irradiance in the upper-intertidal area (Littler and Littler, 2013). Photosynthesis is an important process of CCA growth or calcification. Carbon dioxide and bicarbonate uptake from seawater elevate pH in the internal body of CCA leading to aragonite precipitation in thallus surface (Larkum et al., 2003; Nash et al., 2019). Side and the upward surface was received full of sunlight up to 2,300 µmol photons/m^2/s[1] that can ensure of growth rate CCA rapidly (Kim et al., 2013). Furthermore, these positions will receive more nutrients and give a chance CCA faster-growing horizontally along with a rose of fertility (Littler and Littler, 2013).

As all stations are located in the nearshore of coral reef ecosystem, threats such as sedimentation from runoff of rivers also anthropogenic activity. In this study we didn't measure the sediment rate in each location however we suggested that less sedimentation effect on the side surface of settlement tiles increasing the number of CCA recruit survival. 90 degree of shape of side tile to the sea bottom had pulled sediment particle into sea floor because gravity force. Similar to in sessile invertebrates, coral, that smothering sediment can inhibit the photosynthesis process and increasing bacterial infection that allows triggering juvenile mortality (Moeller et al., 2017). Other research also showed that sediment deposition could reduce the number of CCA coverage in New Zealand and GBR (Chew et al., 2013; Harrington et al., 2005). All side of settlement tiles has a 90-degree angle that reduces number sediment on their surface. And the third reason for the high coverage of the side surface of the settlement tile might be because of the influence of mathematics calculation that surface area of each settlement side only 10 cm^2, however in upward and downward of settlement tile the area was 100 cm^2. As simple mathematic formula stated that the smaller denominator will affect on the high number of results.

After 3 months deployed, CCA settlement tiles were occupied by 9,866 colonies which overlapping each other. In this study, the term of single CCA colony is occupying space by hard substrate from thallus of coralline algae growing laterally margin of the substrate (forming disk-like) which separated with others (McCoy and Kamenos, 2015; Mariath et al., 2013). And because the CCA horizontally growth is very slow around 0-10 mm/year (McCoy and Kamenos, 2015), and our investigation only conducted on 3 months after deployed so fused and overlapping of CCA has been counted as one colony for simplifying of calculation.

Initially, un-calcified spore released from the reproductive organ of CCA (conceptacles) then attach to substratum used glycosaminoglycans (GAGs) or mucopolysaccharides as a single cell and proliferate forming germinative disk to the certain pattern (Bradassi et al., 2013). Cementation occurred below the thallus (hypobasal) to secure tissue calcification and cell division. The next step was developed by hypothallial filaments and thallus growth. A normal thallus is considered when it had a regular shape which it divisions are followed by a square-angled pattern (Bradassi et al., 2013). The success CCA recruit in Tiga Warna was suggested on the influence of environmental factors. Firstly, all settlement tiles in this location are still immersed in seawater although on the lowest tide condition. CCA being able to survive in most threatened but they straightforwardly bleach when exposed to seawater (Tâmega and Figueiredo, 2019). Others two station, Waru-Waru and Teluk Semut, always exposed during low tide so that number of CCA recruit lower than Tiga Warna. Intertidal is a harsh area not only quick environmental change such as temperature and wave action but also highly competitive of the benthic organism. CCA sometimes needs herbivorous fish to remove epiphytes and other algae competitors from their surface area. Some epiphytes such as seaweed and alga turf

increasing the stress of CCA by surface cell-layer shedding (Littler and Littler, 2013).

CCA became a secondary reef builder since 150 million years ago and can any colonize any substrate and distributed on wide range habitat, from tropical to the arctic area (Williams et al., 2020; Muth et al., 2020; Halfar et al., 2013). CCA can live in a wide range of light, temperature and carbon dioxide (McCoy and Kamenos, 2015). In this study, we used two types of material that composed settlement tile, marble, and sandstone. The statistical result showed no significant number of coral recruit at both materials. No information from the previous study about the effect different material of CCA settlement attracts CCA recruits. Edmunds et al. (2014) researched several different material types of refugee coral recruitment such as terracotta, plastic, marble, and coral, in the Mediterranean and Pacific area. After 12 months deployed all material fouled by CCA heavily. This flexibility of CCA habitat in line with their key role to bind sand and rubble, fill cracks, reduce reef erosion and consolidate reef that considered as a natural glue to hold coral reef together.

5. Conclusions

In summary, habitat selection and recognition of CCA were influenced by several factors such as sediment, light, tides, and pH. Type of settlement materials was no overall effect on crustose coralline algae recruits' preference in this study, it proved by no significance difference number of colonies attached to different settlement tile. Knowing this fact can ensure that CCA coverage in Nature Reserve Pulau Sempu is good and in the future can attract more coral juveniles in this area.

Acknowledgements

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