Variation in species diversity and abundance of sponge communities near the human settlement and their bioprospect in Pramuka Island, Jakarta, Indonesia

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Abstract. Coral reefs are critical habitats in the marine environment. Human impact on corals and other associated organisms might lead to degradation. Sponges are the dominant coral-associated organism on most reefs. Sponges occupy many important habitats, hence, studying the spatial patterns of sponges is crucial. This study aimed to asses hard coral cover and investigated the variation of species diversity and abundance of sponge communities on coral reefs near the human population. *Stylissa carteri* extract is tested for cell death in colon cancer (CC) cell lines (Caco-2 and HCT-116 cells) and breast cancer (BC) cell lines. The hard coral cover was affected by human disturbances and their associated organism. The study on sponge communities was conducted on seven different sites on Pramuka Island. A total of 760 individuals from 28 species of sponges belonging to 17 families were recorded in this study. *Petrosia nigricans* and *Aaptos suberitoides* were the most abundant species. The multivariate analysis of sponges abundance and families using the Bray Curtis similarity index and non-metric multidimensional scaling (MDS) clearly showed the distribution of sponges at Pramuka. The extract of *Stylissa carteri* triggers cell death in colon and breast cancer cell lines ($p<0.01$).

Key Words: coral association, sponges diversity, human disturbances, multivariate analysis.

Introduction. The coral reef is an essential ecosystem in the marine environment (Oktarina et al 2014). However, coral-associated organisms rarely get attention (Reveillaud et al 2010). Sponges are the oldest multicellular organism that can filter dissolved organic matter (Soest et al 2012). Sponges species are also important for their commercial use (Bell et al 2015). Many natural products were made from sponges (Blunt et al 2015). Important roles of sponges on coral reefs are providing water-column productivity (Lesser & Slattery 2013), providing habitat for other species (Przeslawski et al 2015), and serving as host for the microbial community (Yang et al 2011). Although the ecological roles of particular sponges species are poorly understood on specific (Turque et al 2010), they fulfill many functional roles in marine ecosystems (Bell 2008).

Although certain species of encrusting sponges live by overgrowing on hard corals (Wang et al 2015), the presence of coral-killing sponges is still considered insignificant (Montano et al 2015). Many important habitats on coral reefs are occupied by sponges (Easson et al 2015). It is crucial to gain more information about sponges as a biotic stressor (van der Ent et al 2016). Their existence has become dominant as coral has declined (Maliao et al 2008). Human disturbances have decreased marine habitats (Törnroos et al 2013) and could enhance the sponges' population (Wang et al 2015). The human impact caused the abundance and movement of sponges to areas where they should not exist (Bell et al 2015). Determining the spatial patterns of sponges is crucial (Xavier et al 2011), as they are the source of more than 30% of the marine natural products, given a diversified array of molecules that have been found to have not only an antimicrobial action, but an anti-cancer therapeutics potential (Sipkema et al 2005; Faulkner 2002). For example, *Sipholane triterpenoid* from the Red Sea sponge *Callyspongia siphonella* has the potential to reverse the multidrug resistance (MDR) in cancer cells that are overexposed with P-glycoprotein (P-gp) (Abraham et al 2010). Marine sponges *Geodia corticostylifera*, isolated from Brazil, regulate actin cytoskeleton, which could impede the distribution and invasion of breast cancer cells (Freitas et al 2008; Paci et al 2014). Assorted marine sponges such as *Dysidea* sp., in Indonesia, and *Niphates digitalis*, in Dominican Republic, potentially inhibit transactivation of the androgen receptor and block its transcriptional activity in prostate cancer cells (Meimetis et al 2012; Sadar et al 2008). This study aimed to assess hard coral cover and to investigate the variation of biodiversity and the abundance of sponge communities near the human population in Pramuka Island, Jakarta, and also to identify the bioprospect of *Stylissa carteri* on cancer cell lines.

Material and Method

Study sites. Surveys were carried out at 10 m depth, at seven different sites on Pramuka Island. Surveys in Stations 1, 2, 6, and 7 were conducted in December 2016, while in the other sites they were conducted in April 2017 (Figure 1). Pramuka Island is part of the Kepulauan Seribu National Park, located north of Jakarta (Baum et al 2015).

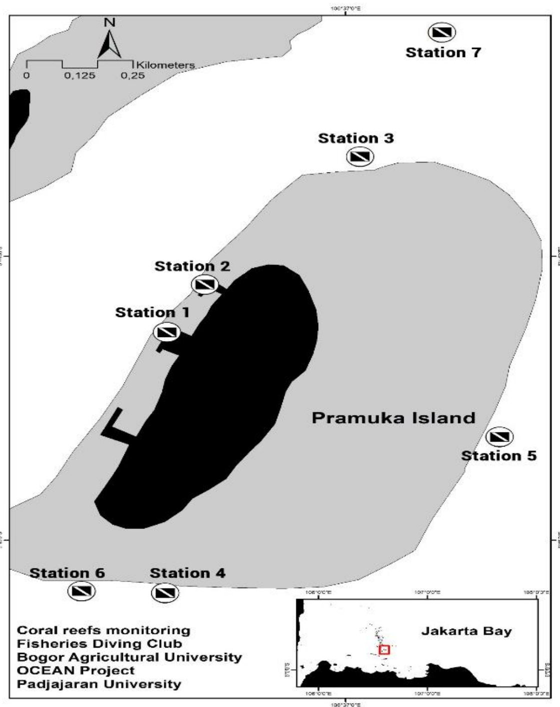

Figure 1. Map of study sites in Pramuka Island, Seribu Islands.

Data collection. Coral communities and sponges species were collected by SCUBA diving. Coral structures were observed and identified to genus at each location using three replications of 20 m point intercept transects (PIT), parallel with the shoreline (Hill & Wilkinson 2004) and separated by at least 5 m. Sponge species and their abundance were noted within 1 m on each side along, the same transects as the corals structure, using the belt transect method (English et al 1997). Species were visually identified in the field and confirmed using the World Porifera Database (Soest et al 2012).

Extraction of sponge species. Marine sponge *S. carteri* samples were cut into small sizes, then extracted using the maceration technique in ethanol solvent for 72 hours. The ethanol extract solution was collected using filter paper (Whatman), then evaporated using a rotary evaporator with a vacuum pressure (BUCHI Rotavor R-3) at 40°C. Finally, the concentrated extract was dried further using a vacuum desiccator (DURAN) to obtain a powder ethanol extract of *S. carteri*.

Cell culture and condition. The Caco-2 cell, a human colorectal adenocarcinoma, was obtained from Prof. Henning Schulze-Bergkamen (NCT, Heidelberg, Germany). Caco-2 cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA), supplemented with 10% Fetal Bovine Serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA), and they were saved in the incubator, with a controlled temperature of 37°C and 5% CO_2.

The n-hexane extract of" sarang semut" and cell treatment. The n-hexane extract of sarang semut was provided by Harold Atmaja as a member of Prof. Mieke's research group. A stock solution of 40.000 ppm was made in dimethylsulfoxide (DMSO; Sigma, USA). The stock solution was diluted to the required volume to obtain the appropriate concentration of 2 ppm and 15 ppm. The extract's small concentration was used to determine whether the formation of colonies would be inhibited with such a small concentration.

Clonogenic assay. Duplication assays were conducted: each experiment used two dishes of the same treatment and three repetitions were performed.
a. Initial handling of the cells. Cells were harvested from the flask by the trypsinization method. The whole suspension cells were placed in a 15 mL tube and centrifuged at 200G for 5 minutes. The supernatant solution was displaced, and the remaining cell pellet was mixed with a complete medium. The cells were counted using a Neubauer improved cell counting chamber and diluted at the desired cell seeding concentration. A cell number of 100, 200, and 300 cells were seeded for the control group, 2 ppm group, and 15 ppm group, respectively, on well plates.
b. Clonogenic assay setting. Cells were treated after they were attached to the well. The cells were treated or untreated (control) with an n-hexane extract of sarang semut, for three days. The Control group was treated with a complete medium containing 1% DMSO. After the treatment, the medium was replaced with a completely fresh medium without treatment, then incubated for two weeks.
c. Colony fixation and staining. After removing the medium, cells were fixed with methanol (Merck, USA) for 5 min and incubated with crystal violet (Merck, USA) for 3 min to stain the cell. Crystal violet was removed and the plate was rinsed with tap water.
d. Counting colony. In this study, the number of colonies formed was counted manually by measuring each colony using a ruler. The colony was counted if the diameter measured more than 1 mm. The formula for calculating the plating efficiency (PE) is the following (Bashari et al 2020):

$$PE\,(\%) = \frac{number\ of\ colony\ formed}{number\ of\ cells\ seeded}\ x\ 100$$

e. Area measurement. The area of the colony was calculated semi-automatically by scanning the plate, then measuring the area using the ImageJ software (NIH, USA). The formula for calculating the area per cell seeded (Bashari et al 2020):

$$Area\ per\ seed = \frac{total\ area\ of\ colony\ formed}{number\ of\ cells\ seeded}$$

Data analysis. Percentages of the hard coral cover were calculated using the formula of Wilson & Green (2009). Diversity indices of sponges were estimated using Shannon-Wiener H' on the $\log_2$ basis (Shannon 1948). Multivariate ecological analysis was conducted using the PRIMER 7 program (Kruskal 1964; Clarke & Gorley 2001) to examine the sponges assemblages' composition among site locations. A non-metric multidimensional scaling (nMDS) ordination of belt transect was constructed from a Bray-Curtis similarity matrix of square root transformed sponges abundance and species richness to visualize differences in sponges composition from the different study sites. A square root transformation was used to reduce the disparity between uncommon and abundant species by down-weighting abundant species relatively to the uncommon species (Clarke 1993). Data from this study resulted in PE (%) and area per seed (mm^2) that were analyzed using the Statistical Product and Service Solutions (SPSS; IBM, USA). To determine the association of inhibition of colony formation by each treatment group, a one-way ANOVA (Analysis of Variance) test was used with a post-hoc LSD test for plating efficiency and a Tukey test for area per seed. The statistical analysis was considered significant if the p-value <0.05.

Results and Discussion

Hard coral cover. This study indicated that coral cover on Pramuka Island did not vary significantly (ANOVA, F=0.72, p=0.64). Hard coral cover ranged from 5.83±1.67 to 26.67±16.73% (Table 1). The lowest hard coral cover was found in Station 1 site, considering that Station 1 was the island dock where all ship activities were intense. Several factors also influenced the loss of corals, such as coral mining (Haywood et al 2016), disease (Subhan et al 2011), tourism, pollution (Arini 2013), climate changes (Baumann et al 2016), unsustainable fishing, water quality, environmental condition (Putra et al 2015), anchoring, and sand mining (Amin 2009). Coral reefs are very vulnerable if human activities damage the surrounding environments (Ardiansyah et al 2013; Miller et al 2012). The highest hard coral cover was found in Station 7 site. Station 7 was located the farthest from human habitation, among the seven study sites. According to Riegl et al (2012), the coral reefs near the human habitat would have more susceptibility. Coral reefs play essential roles in the environment and their associated organism (Madduppa et al 2014). Specific sponges are associated with corals (Reveillaud et al 2011) and reef fishes (Madduppa et al 2012; Madduppa et al 2014), which seek food, shelter, and breeding on coral reefs (Madduppa et al 2012).

Table 1

The relative percentages of hard coral cover (mean ± SE) in Pramuka Island

Study sites	*Hard coral cover (% ± SE)*
Station 1	5.83±1.67
Station 2	20.83±9.61
Station 3	17.50±4.33
Station 4	20.83±7.95
Station 5	9.17±2.20
Station 6	17.50±6.29
Station 7	26.67±16.73

Sponges community structures. Reef crests in Pramuka Island are relatively similar. This coral reef condition indicates that Pramuka Island contains similar sponge communities throughout the study sites. Sponges assemblages were influenced by geomorphic features (Przeslawski et al 2015). Picoplankton distribution also indicates sponge community patterns (Pawlik et al 2013). Station 5 was the most abundant, and Station 3 had the highest species richness according to the Shannon diversity indices among the seven study sites (Table 2). The low value of coral cover in Station 5 may be the reason for the sponges high abundance. Specific sponges abundance increased as corals abundance declined (Bell et al 2015). Sponges grow relatively fast in high-nutrient reefs (de Voogd et al 2009; Hadi et al 2015). Pollution also indicates the high value of sponges abundance in Station 5. Eutrophication and pollution occurrences also increase the sponge abundance in Caribbean reefs (Mueller et al 2014). In Table 2, the ANOVA summaries and Tukey-Kramer post hoc test of significant differences were included.

Table 2

Sponges mean abundance and Shannon H' (mean ± SE) at the 7 study sites

Study sites	*Abundance (mean±SE)*	*Diversity (mean±SE)*
Station 1	20±4.41	1.37±0.19
Station 2	11±8.17	0.76±0.24
Station 3	31±13.17	2.32±0.55
Station 4	69±31.12	2.30±0.93
Station 5	82±40.17	3.30±0.69
Station 6	11±3.79	1.65±0.59
Station 7	4±2.96	1.09±0.55

A total of 682 individuals from 23 species of sponges belonging to 14 families were recorded in this study (Table 3). The highest-value taxonomically identified species were *Petrosia nigricans* and *Aaptos suberitoides* (Ismet et al 2016). *P. nigricans* from Petrosidae was the most abundant species, followed by *Aaptos suberitoides* from Suberitidae. *P. nigricans* and *Aaptos suberitoides* are the common species in Seribu Islands (de Voogd & Cleary 2008). *P. nigricans* live at 3-45 m depth; this species usually lives on reefs or sand slopes. *P. nigricans* is widely distributed in the Indo-Australian region (de Voogd & Van Soest 2002). Suparno et al (2009) conducted *P. nigricans* transplantation on Pramuka Island and found that the roles of depth influenced their growth rate. *Aaptos suberitoides* has a smooth and sometimes elevated surface with reddish black and yellow on the interior (Abdillah et al 2013), and was commonly used as source of natural products (Pham et al 2013).

Table 3

The total number of sponges species at each study site

Family Species	*St 1*	*St 2*	*St 3*	*St 4*	*St 5*	*St 6*	*St 7*
Callyspongiidae							
Callyspongia aerizusa	-	-	-	24	3	-	-
Chalinidae	-	-	-	-	-	-	-
~~*Adocia*~~ *Haliclona viola*	-	-	-	-	-	1	-
Chalinula nematifera	-	-	-	16	2	-	-
Chalinula sp.	-	-	-	-	47	-	-
Nara nematifera	1	1	-	-	-	-	5
Dysideidae							
Lamellodysidea sp.	-	-	10	-	-	-	-
Halichondriidae							
Stylissa massa	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~1~~
Irciinidae							
Ircinia ramosa	-	-	-	52	-	-	-
Microcionidae							

Family Species	St 1	St 2	St 3	St 4	St 5	St 6	St 7
Clathria (Microciona) sp.	-	-	3	1	7	-	-
Clathria (Thalysias) reinwardti	-	-	-	13	5	-	-
Clathria mima	1	-	-	-	-	5	
Petrosiidae							
Petrosia nigricans	4	2	30	23	80	5	1
Petrosia sp.	-	-	-	-	-	1	-
Xestospongia testidunaria			1				
Phloeodictyidae							
Aka sp.	22	-	-	-	-	8	4
Podospongiidae							
Diacarnus bismarckensis	-	-	7	4	9	-	-
Pseudoceratinidae							
Pseudoceratina sp.	-	-	9	51	10	-	-
Pseudoceratina verrucosa	-	-	-	-	-	3	-
Scopalinidae							
Stylissa carteri	-	-	-	-	15	-	-
Stylissa massa	-	-	-	-	-	-	1
Spongiidae							
Spongia matamata	-	-	-	-	-	2	1
Suberitidae							
Aaptos sp.	1	1	-	-	-	5	-
Aaptos suberitoides	32	28	32	22	67	3	-
Theonellidae							
Theonella sp.	-	-	-	-	-	-	1

Sponges community structures. Overall, the surveyed species were clustered into three main clusters and the sponges families into four main clusters (Figure 2). The similarity between the study sites is characterized by their habitat (Emslie et al 2010). Cluster analysis of sponges species and families from each transect level showed the separation between study sites (Figure 3). The similarity between Station 1 and 2 is explained by their geografical proximity (Figure 1).

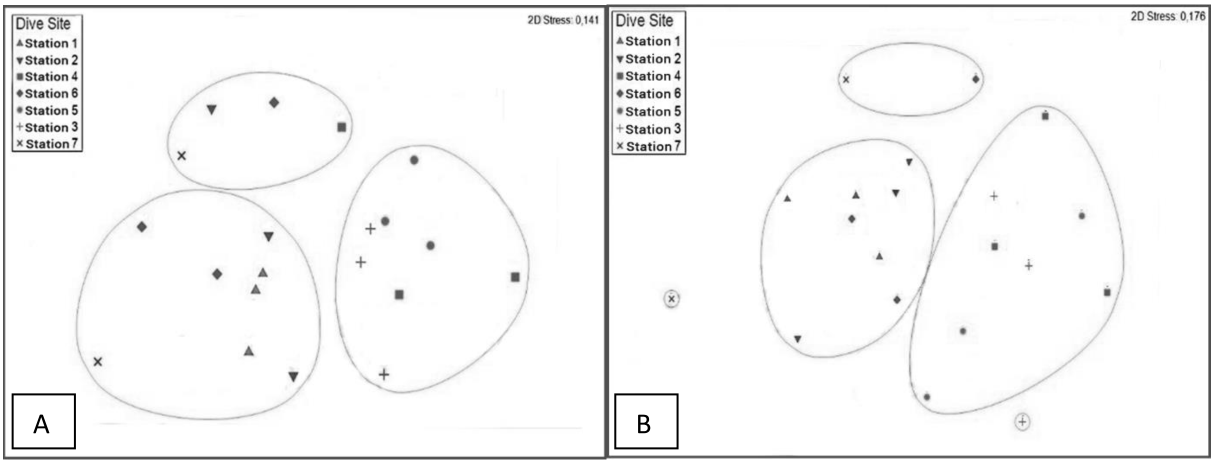

Figure 2. MDS plot of sponges communities at Pramuka Island shows a pattern of association among 23 species based on abundance (A) and 14 sponge families based on species richness (B).

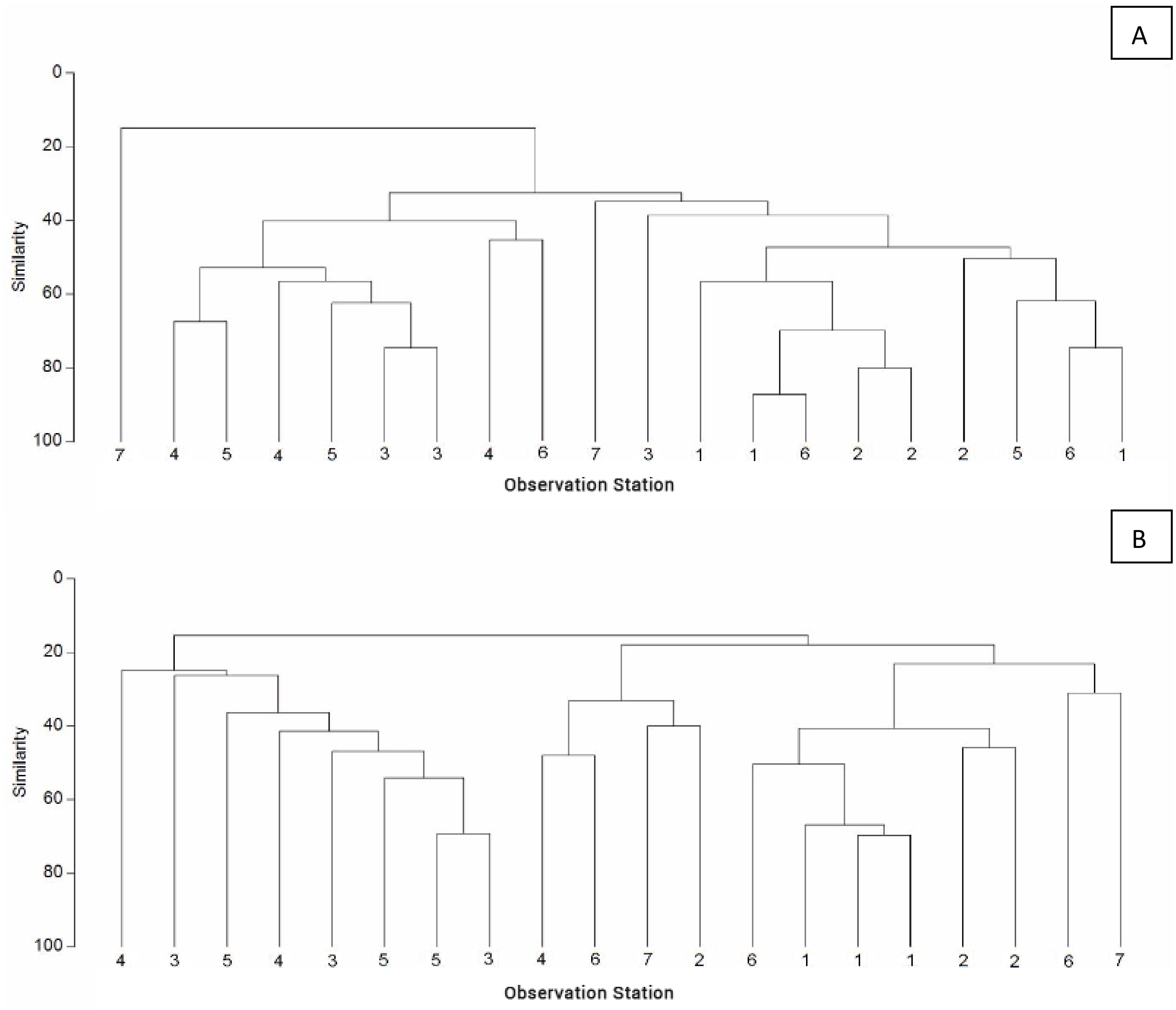

Figure 3. Cluster analysis of sponges families (A) and species (B) at each study site.

There was a unique case on Station 6 and Station 4, close one to each other, with no similarity of sponges abundance and species richness. The spatial distribution of sponges is affected by the dynamic, high-energy current environment (Berman & Bell 2010). Station 5 was located east of Pramuka Island (Figure 1). It was an open area with higher current energy than Station 1 and Station 2, located on the west side of Pramuka Island. In addition, Station 7, located on the outer side of Pramuka Island, has different sponges communities than others. However, the relationship between environmental factors and sponges is not always similar because it varies across regions (Przeslawski et al 2015). Overfishing also affected sponges communities indirectly. Loh et al (2015) have found indirect effects of overfishing on the sponges' overgrowth on Caribbean reefs.

Extract of Stylissa carteri in cell lines. Our data showed that ethanol (EtOH) extract of *Stylissa carteri* induces cell death in colon cancer (CC) cell lines (Caco-2 and HCT-116 cells), as well as in breast cancer (BC) cell lines (MCF-7 and HCC-1954 cells) (Figure 4). Interestingly, this extract's lower concentration (10 μg mL^{-1}) triggers about 50% or higher deaths in HCT-116 cells, KRAS mutated CC cells, HCC-1954 cells and HER2+ BC cells. KRAS mutation in CC and HER2 overexpression in BC correlate with a poor prognosis in CC or BC, in patients with inadequate treatment (Tovey et al 2009; Phipps et al 2013).

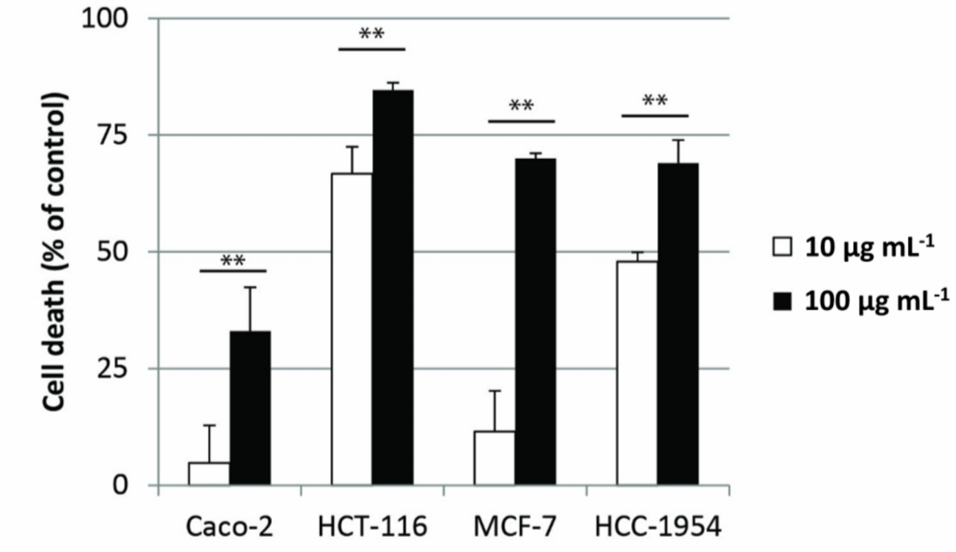

Figure 4. Ethanol (EtOH) extract of *Stylissa carteri* triggers cell death in colon and breast cancer cell lines, in a dose-dependent manner. Colon cancer cell lines (Caco-2 and HCT-116 cells) and breast cancer cell lines (MCF-7 and HCC-1954 cells) were treated with EtOH extract of Stylissa *carteri* for 72 hours, followed by cytotoxic analysis using MTT assay. DMSO was used as control. Data were presented as mean, SD from triplicate data, with a significance level of p<0.01.

Breast cancer (BC) and colon cancer (CC) are major solid tumors that cause mortality and morbidity worldwide (WHO 2008; Haggar and Boushey 2009; Ferlay et al 2010). BC is the most common cancer found among women (WHO 2008; Ferlay et al 2010), with an estimated 1.4 million new cases in 2008, representing 1 in 5 of all new cancers (Ferlay et al 2010). Colon Cancer is cancer that affect to the men and women, which mostly occur around the world (Haggar & Boushey 2009; Bashari et al 2016). Despite advanced treatment of BC and CC in the last decade, some patients failed to reach a complete remission. Therefore discovering novel anti-cancer agents is urgently needed.

Nowadays, sponges are the marine organism the most commonly candidate for cancer drug screening. Eribulin, a synthetic analog isolated from the marine sponge, has been approved by the American Food and Drug Administration (FDA) for the advanced stage of triple-negative breast cancer (Jordan et al 2005; Candida et al 2012). Furospinosulin-1, an isolated compound from *Dactylospongia elegans* from Indonesian territory, induces effects against the prostate, under hypoxic conditions (Arai et al 2010). Moreover, Papuamine, an isolated compound from sponge *Haliclona* sp., triggers synergistic anti-tumor activity in combination with doxorubicin in BC (Kanno et al 2014). Published data subjected to *Stylissa carteri* for cancer drug screening is limited. Our data demonstrated a promising anti-tumor activity of the EtOH extract of *S. carteri* in CC and BC cells. This extract induces cell death in CC and BC cells, even in more aggressive cell types, HCT-116 and HCC-1954 cells (Figure 4). We are conducting further experiments to analyze the anti-cancer activity of *S. carteri.*

Conclusions. The low abundance of the hard coral cover in Pramuka resulted from disturbances from humans and from the associated organisms to the coral reefs. The abundance of *P. nigricans* and *A. suberitoides* was higher than in other species. Sponges species and families were significantly more evenly distributed at Pramuka. *S. carteri* induces cell death in colon and breast cancer cell lines. This study could contribute to the information of the medical research throughout the world, on the availability of marine sponges in Indonesiaand their usage as medicines against the colon and breast cancers.

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Conflict of interest. The authors declare no conflict of interest.

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