3rd IBOC International Biology Conference 2016

Biodiversity and biotechnology for human welfare

proceeding

Rectorate Building of ITS - October 15, 2016

Biology Department
Faculty of Science
Institut Teknologi Sepuluh Nopember
Surabaya - Indonesia

ISBN: 978-979-97316-5-4
July 2017

biologi ITS

PROCEEDING OF

3rd INTERNATIONAL BIOLOGY CONFERENCE – 2016

Biodiversity and Biotechnology for Human Welfare

3rd IBOC International Biology Conference 2016

Biodiversity and biotechnology for human welfare

October 15th, 2016
Seminar Room – Rectorate Building
Institut Teknologi Sepuluh Nopember
Surabaya – Indonesia

Organized by
Biology Department, Institut Teknologi Sepuluh Nopember, Surabaya – Indonesia

Proceeding of 3rd International Biology Conference (IBOC) 2016: Biodiversity and Biotechnology for Human Welfare

ISBN: 978-979-97316-5-4

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Preface

Biodiversity of Indonesia has been acknowledged as one of the richest among other countries in the world. Until recently, biodiversity is still a hot topic and issue to be disseminated not only in scientific meeting, but also as a core of major subject that should be introduced in the early education in Indonesia. In addition to biodiversity, biotechnology is also a discipline of life science that has been implemented for future used of human welfare. For this reason, we still use a theme "Biodiversity and biotechnology for human welfare" for our third biannual series conference.
This biannual meeting is devoted for creating networks among our department and several institutions that have a similar biological science in implementing that theme. It has been known world environment's encounters problems because of natural and anthropogenic impacts that effects on human being. Some examples like global warming, reducing biodiversity and extinction of vulnerable organisms, vanishing environmental water, and land - air quality, are examples that has a serious impact human welfare. Through this meeting we would like to achieve many personal contacts, ideas, biological-environmental problem solve sharing and fruitful discussions in order to save the earth together. Therefore, we are really grateful and thankful that the participants who are interested to joint with are from the Philippine, Thailand, Singapore, Korea, Italia, and Indonesia.

The committee of 3rd International Biology Conference (IBOC), Biology Department Institut Teknologi Sepuluh November has received 95 articles and furthermore 43 full papers has been published in the American Institute of Physics (AIP) Scopus indexed conference proceeding of 3rd IBOC volume 1854, after reviewing process (http://aip.scitation.org/toc/apc/1854/1?size=20&expanded=1854). These 43 papers cover subject fields of Biomass and Bioenergy; Environmental Science and Ecology; Animal Science; Agricultural and Natural products. The rest of reviewed articles, which are 19 articles are published in this volume proceeding of 3rd IBOC

We would like also to thank to Surabaya-Indonesia to the Mathematics and Natural Sciences Faculty, for supporting the conference. The big remarkable applause is also going to our students who are giving their excellent hands for keeping the conference running on schedule. Last but not least, we have a big hope that a real excellent networking in the future may arise from this event.

Thank you and best regards.

Surabaya, July 15th 2017

Head of Biology Department
Dr. Dewi Hidayati

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Iwenda Bella Subagio, Edwin Setiawan, Sucipto Hariyanto, and Bambang Irawan

Citation: AIP Conference Proceedings **1854**, 020034 (2017); doi: 10.1063/1.4985425
View online: http://dx.doi.org/10.1063/1.4985425
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Published by the American Institute of Physics

Spicule Size Variation in *Xestospongia testudinaria* Lamarck, 1815 at Probolinggo-Situbondo Coastal

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Abstract. *Xestospongia testudinaria* Lamarck, 1815 is a marine sponge that become a main constituent in reef ecosystems at northern waters Probolinggo-Situbondo. This barrel sponge species possesses an oxea type of spicule that varies in dimensions (length and width) in concordance to condition and location of habitat. The experiment aimed to understand how spicules condition of this sponge reacted to environment variables. Sponges' specimen were taken by SCUBA equipment in 6-7 m, 10-11 m, and 14-15 m depths in addition to four different localities and three different part of sponges' body (upper, middle and basal parts). Environmental variables data were also retrieved (salinity, water clarity, temperature, dissolve silica, and depth) in each locations. Results confirmed that oxea spicule size either in length or width dimensions in four locations (Batu Lawang coral cluster [BL], Karang Mayit coral cluster [KM], Paiton coral cluster [PT], and Takat Palapa [TP]) relatively increased toward depth. Likewise, the size of spicules in the TP relatively longer than three other locations. In contrast, spicules oxea in PT relatively wider than three other locations. Salinity gave negative impact to spicules length, while depth gave positive impact. Depth, water clarity, dissolve silica, and temperature gave negative effect to spicules width while salinity gave positive impact.

INTRODUCTION

Porifera or sponges is one of the metazoan phylum that constitute coral reefs, sea grass or mangrove ecosystem. This phylum has spicules as one of its morphological characters. Spicules are an important part of the skeletal material of porifera, along with collagen and spongin fibers.[1] The size and type of sponge spicules indicate the base character used in the sponges taxonomy. Sponge spicules morphology, size and composition vary depending on each group taxa. Nevertheless, these macro and micro morphological characters are inconsistent. Particularly, the proportion of spicule is not static among individuals in different population and even individuals within a population.[2] Based on several studies in sub-tropical waters, sea sponge is able to adapt the proportion of morphological characters based on environmental conditions (ecological factor or morphological plasticity).[3-5]

There are effects of bathymetric gradient against length and width of spicules proportion in *Petrosia ficiformis* (Petrosiidae - Demospongiae) and *Cliona azzaroliae* (Clionaidae – Demospongiae) explained that.[6] Changes in the proportion size spicules are also recognized in *Cliona celata* (Clionaidae – Demospongiae).[7] In addition, in *Halichondria semitubulosa* (Halichondriidae – Demospongiae) water temperature and dissolved silica content cause on the spiculogenesis process and furthermore, on the morphological spicules.[8] Several studies have also shown that seasonal changes in the number of spicules caused by the increased of inorganic content.[8, 9]

Since several of those studies explain that variation in spicule morphologies as an adaptive response to the prevailing environmental condition, however, all those studies were conducted in subtropical areas.[7, 8, 9] For this reason, effect of ecological factors waters on the morphology in sea sponge spicules in tropical waters are needed to be explored due to lacking publicity on it. In addition, it is necessary to do a study on that issue for a comparison to

Proceeding of International Biology Conference 2016
AIP Conf. Proc. 1854, 020034-1–020034-13; doi: 10.1063/1.4985425
Published by AIP Publishing. 978-0-7354-1528-7/$30.00

the results of subtropical waters. Finally, main variable factors that have an important role in influencing morphological spicules of sponges in the tropical region are going to be resolved.

MATERIAL AND METHODS

Xestospongia testudinaria Lamarck, 1815

Specimens of *X. testudinaria* Lamarck, 1815 were studied from four sites on Probolinggo-Situbondo coast, specifically at Batu Lawang (BL), Karang Mayit (KM), Takat Palapa (TP) and Paiton (PT) cluster reef. *X. testudinaria* is big barrel-shaped sponge, like-a-stone consistency, with lammellae morphotype in all location. Some individual become micro habitat of *Synaptula lamperti*.

Study Sites

Pasir Putih Beach located north of the Probolinggo and Situbondo city, East Java, Indonesia (Fig. 1). Extends from the west (7° 41'S, 113° 49'T) to east (7° 40'S, 113° 51'T) along 3.7 km. Pasir Putih Beach is a tourist attraction, as well as fishing activities location, or ponds. While Paiton located at 30 km west Pasir Putih Beach (7° 42'S, 113° 35'T), Situbondo, precisely in the District Paiton, Probolinggo, East Java. The location is adjacent to the Paiton power plant managed by PT. Java-Bali power plant where the waste water turbine has a relatively high temperature. The location chosen because it has the accessibility that allows to take samples and have distinct environmental characteristics as well as the habitat from species of sponges *X. testudinaria* (Petrosiidae Demospongiae).[9]

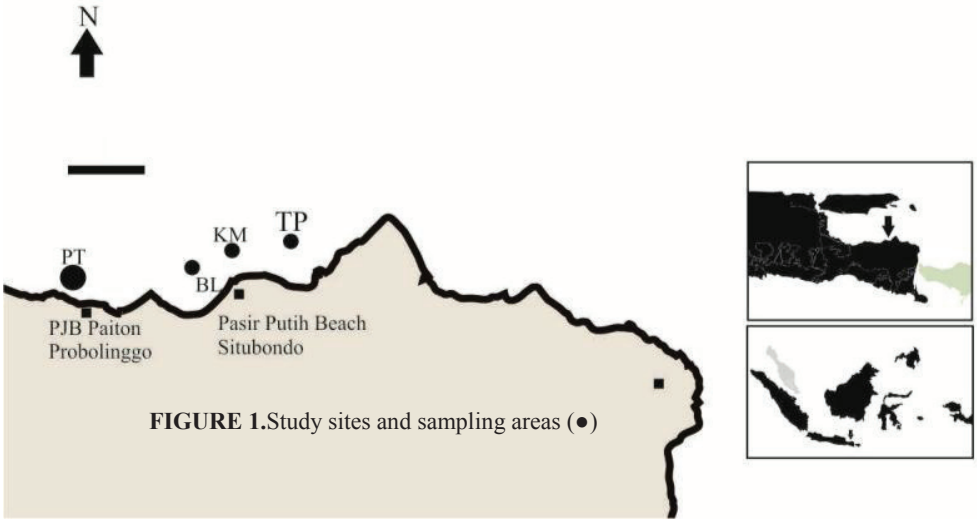


FIGURE 1. Study sites and sampling areas (●)

Sampling Protocol

Core samples were taken at Batu Lawang (BL), Karang Mayit (KM), Takat Palapa (TP) and Paiton (PT) cluster reef (Fig. 1). Sampling was done using SCUBA equipment with random sampling method in each predetermined depth (± 6-7, ± 10-12 and ±15-16 m at KM and BL, ± 6-7 and ±14-15 m at PT, as well as ±10-11 m and ±14-15 m at TP). In KM and BL, individual X. *testudinaria* be divided into three categories based on the size of the body height (<0.5 m, 0.5 to 1 m, and ≥1 m) (Fig. 2). Then, in each size category sponge per body height taken at three (3) parts of the body, osculum upper wall (a), middle of the sponge body (b) and basal area of the sponge (c) (Fig. 2). At the location of Paiton (PT) and a cluster of reefs Takat Palapa (TP), the specimen is taken not by size category, but taken random at each depth. Taken piece of five individual *X. testudinaria* Lamarck, 1815 from each depth and each size category using dive knives. Each individual cut to the approximate diameter of 1 cm. Pieces of the body were put into the sample bottle. Then, after the specimen was brought to the surface, the specimens were fixed with 90% alcohol. Specimen photographs were taken using a Canon G15 with hosting under water. The camera was attached to a fixed frame allowing the camera-to-sponge distance to be kept constant for all photographs.

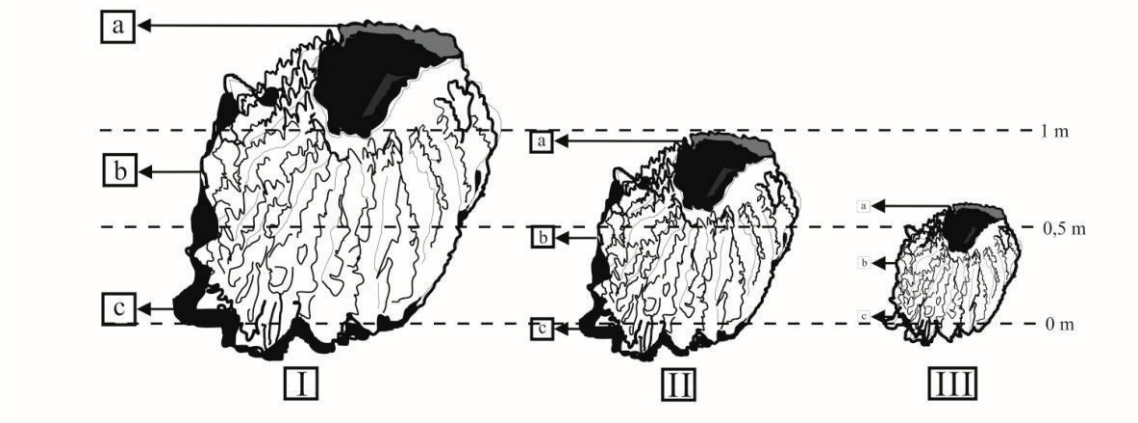


FIGURE 2. Size categories and part of the specimen which taken for the study.

Environmental variables data were measured including water temperature, depth, water clarity, salinity, dissolved silica along with the water (DSi). Water temperature measured by a mercury thermometer (°C). Water transparency at each depth is measured by measuring the visibility in the water using the unit meter (m). While salinity is measured in units ‰ by the Hand Saline Refractometer. The depth of the sea is measured using the Depth Gauge contained in the SCUBA instrument. While measuring the content of silica ($Si0_2$) was conducted in the LSPro Laboratory, BBTPPI, and Surabaya East Java.

Sample Preparation and Analysists

For morphometric analysis of spicule size, sponge samples were cleaned of tissue by boiling them in Chlorine solution and washed repeatedly in distilled water. Samples were agitated, pipetted onto microscope slides and viewed under a compound microscope. An ocular micrometre was used to measure the length and width 25 random spicules. Three different slides for each sponge sample were taken. For morphometric analysis of the spicule size, sponge samples were cleaned of tissue by boiling them in Chlorine solution and washed repeatedly in distilled water. Samples were agitated, pipetted onto microscope slides and viewed under a compound microscope. An ocular micrometre was used to measure the length and width 25 random spicules. Three different slides for each sponge sample were taken.

Statistics

The results from this study generally was average size of ‘oxea’ spicule dimensions (length and width). Spicules size data displayed using bar charts to see the different of spicule proportion between body sections, locations and depths. Then evaluated the normality using 1-samples Kolmogorov-Smirnov statistical test. After the data known to

distributed normally, then continued with parametric test to compare the size of the spicules. Comparison test used One-Way ANOVA with a significance level (α) of 0.05 and then continued with Duncan test to see any significant difference between the three-dimensional body sections *X. testudinaria*, between the depth and location of the water. For the size of spicules in PT and TP, that only has two depth, we used Independent-Samples T-test. Then, the data are modelled using multiple linear regression analysis to determine which environmental variables that most influence on the size of the oxea spicules.

RESULTS

Coral reef position in Probolinggo-Situbondo coastal varied, depending on the coast contour of each location. Pasir Putih Beach include Batu Lawang (BL) and Karang Mayit (KM) coral cluster has ranging from 4 to 20 m depth. Paiton (PT) coral cluster ranging from 5 m to 13 m depth, whereas, Takat Palapa (TP) coral cluster has 5 m to 18 m depth. Each location has average physical and chemical conditions, respectively. Therefore, each location has an environment variable with average conditions different to another location (Table 1).

TABLE 1. Average of environmental conditions at each location.

Parameter	Location and Depth (m)										
	BL			KM			TP			PT	
	7	10	15	7	10	15	7	10	15	10	12
Water Clarity(m)	7	6	4	6	5	3	4	3	1	4	3
Salinity (‰)	30	31	32	30	31	33	30	31	32	32	33
DSi (mg/L)	0.25	0.166	0.25	0.27	0.166	0.25	0.25	0.21	0.25	0.25	<0.12
Temperature	29.5	28	27.5	29	28.5	27.5	30	29	28.5	29	28.5

Spicule of *X. testudinaria* Lamarck 1815, in Probolinggo-Situbondo Coastal.

Spicule of *X. testudinaria* in north coast Probolinggo-Situbondo generally was megascleres tangential type or curved spicules *oxea* with no microscleres (Fig. 3). Based on measurements of spicules, the size of spicules known in each size category of individuals in the BL ('size minimum - **on average** - the maximum size') 130 - **348**.37 - 490.30µm x 2.2 - **17.24** - 30.20 µm. Meanwhile, size dimensions of the reef KM ranged from 146.6 - **353.49** - 468.5µm x 5.2 - **17.69** - 28.3 µm. Oxea spicules in BL has relatively longer size dimension, while KM has a relatively wider size (Tab.1).

Spicules size dimension of BL coral cluster

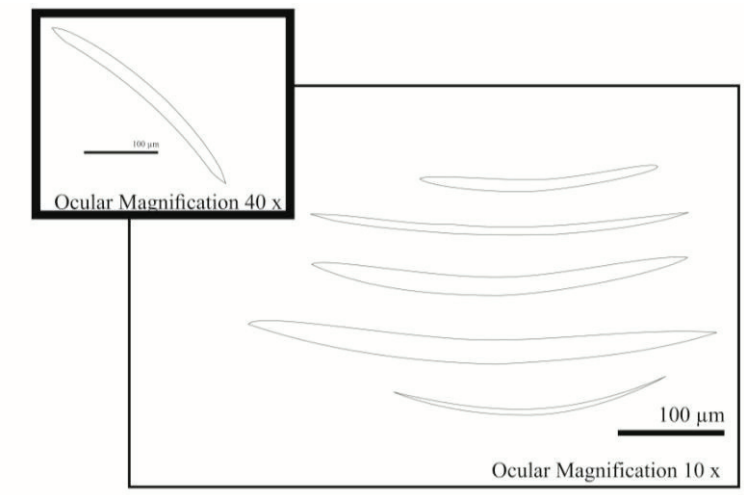


FIGURE 3. Illustrations of oxea spicules variation found in individuals *X. testudinaria* in Probolinggo-Situbondo coastal.

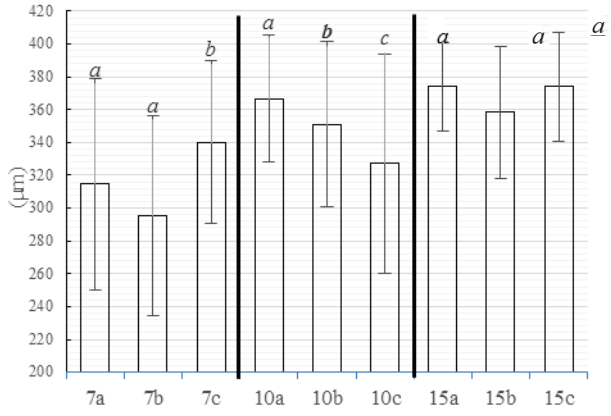
TABLE 2. Spicule morphometric parameter in BL and KM samples.

BL	< 0.5 m Length		< 0.5 m Width		0.5 - 1 m Length		0.5 - 1 m Width		> 1 m Length		> 1 m Width	
B/K	*r* (µm)	SD (±)	*r* (µm)	SD (±)	*r* (µm)	SD (±)	*R* (µm)	SD (±)	*r* (µm)	SD (±)	*R* (µm)	SD (±)
7a	314.72	64.34	13.42	4.58	345.31	60.20	15.69	2.75	-	-	-	-
-7b	295.24	61.13	12.19	5.01	348.41	61.05	15.56	4.59	-	-	-	-
7c	340.23	49.54	14.90	5.90	342.34	49.77	16.86	2.85	-	-	-	-
10a	366.79	38.99	18.50	1.52	347.48	55.85	17.97	1.50	361.28	50.54	18.68	2.68
10b	351.18	50.32	18.84	2.95	351.34	54.84	17.88	3.18	340.81	73.10	17.56	4.04
10c	327.01	66.85	16.78	4.48	333.39	64.46	16.93	2.83	343.95	54.80	17.92	4.00
15a	373.88	26.64	19.29	1.23	396.89	24.54	20.34	1.89	385.03	61.90	18.96	2.24
15b	358.46	40.05	18.77	2.33	381.98	35.38	19.74	1.63	389.45	64.12	20.44	3.38
15c	373.93	33.04	17.69	4.33	361.01	30.36	17.70	2.08	377.33	39.54	18.71	2.16
KM	< 0.5 m Length		< 0.5 m Width		0.5 - 1 m Length		0.5 - 1 m Width		> 1 m Length		> 1 m Width	
B/K	*r* (µm)	SD (±)	*r* (µm)	SD (±)	*r* (µm)	SD (±)	*r* (µm)	SD (±)	*r* (µm)	SD (±)	*r* (µm)	SD (±)
7a	341.47	46.10	16.91	3.00	356.90	36.28	16.67	1.66	364.09	37.34	16.95	1.97
7b	349.63	45.32	16.85	3.33	353.59	35.78	18.05	1.00	362.21	34.67	16.52	1.28
7c	333.79	59.76	16.98	2.72	326.17	54.76	18.20	2.01	359.47	61.53	16.61	1.90
10a	367.07	34.88	18.26	1.81	377.23	29.72	18.41	1.36	-	-	-	-
10b	357.00	42.04	17.86	1.49	351.86	49.04	18.53	1.82	-	-	-	-
10c	351.49	45.93	18.25	2.48	300.88	68.92	17.27	2.24	-	-	-	-
15a	377.87	23.09	19.41	2.03	370.90	34.08	18.90	1.33	355.55	42.61	18.60	2.33
15b	356.03	50.80	17.43	2.36	352.27	47.21	18.04	1.94	368.61	41.97	18.61	2.04
15c	333.65	66.91	15.62	3.97	355.84	44.98	17.69	2.56	363.46	50.14	18.18	1.73

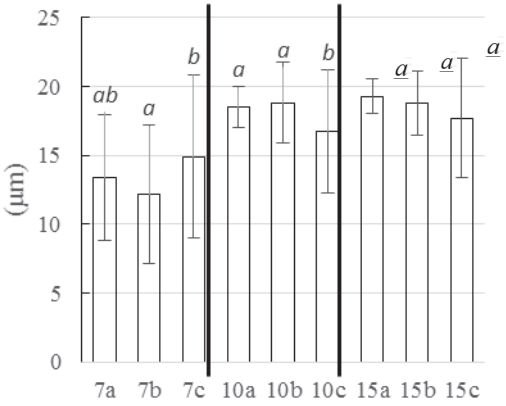
Sponge with height category 0.5-1 m high has relatively no significant difference between individual body parts at all depths. Specimens at 6-7 m depth; length $F = 0.282 P = 0.755$ width $F = 4.202$ $P = 0.016$, at 10 -11 m depth; length $F = 1.955$ $P = 0.144$ width $F = 1.955$ $P = 0.144$, at 15-16 m depth; length $F = 8.778$ $P < 0.001$ width $F = 13.557$ $P = < 0.001$. There was significant difference between basal part compared to the size of spicules oxea upper and middle part of the body. That condition occurs in all the parameters (width and length) of oxea spicules at 15-16 m depth (Fig 4A2-4B2).

Specimens with > 1 m high category at 10-11 m depth, has relative not differ significantly length spicule size between the parts of the body (*F = 2.508 P = 0.084*) while the width size did not differ between the three part of body (*F = 1.843 P = 0.161*). Similar to 10-11 m depth, dimensions of oxea spicules at 15-16 m depth, also not significantly different at length (*F = 0.297 P = 0.744*) and relative did not differ significantly on the width (*P = 3.105 F = 0.051*) (Fig 4A3-4B3).

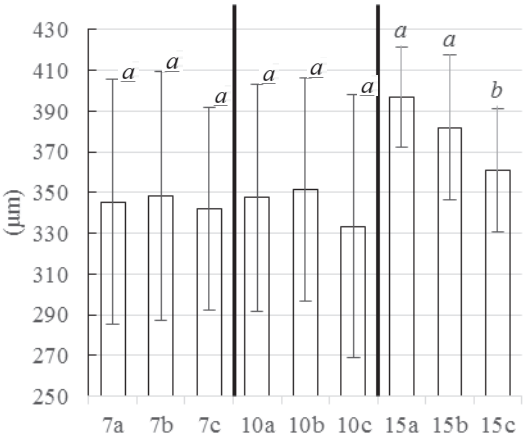
Oxea spicules dimensions of each body parts at 6-7 m depth for category of sponge<0.5 m high had a significant difference in the length (*F = 7.391 P = 0.001*) and width (*F = 3.425 P = 0.0035*) spicules. Significant size difference seen in the basal area of the sponge compared to the top and centre body part of sponge. At 10 -11 m depth, significant difference was seen in the length (*F = 14.140 P <0.001*) among the three parts of the body sponge. Width size differed significantly between the basal part of the sponge with 2 other body parts (*F = 11.802 P <0.001*). Meanwhile, at 15-16 m depth, there's no significant differed for length (*F = 1.752 P = 0.181*) and width (*F = 1.946 P = 0.150*) of the spicules (Fig 4A1-4A2).



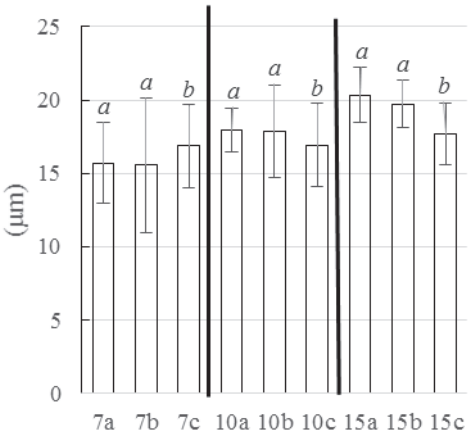
A1



B1



A2



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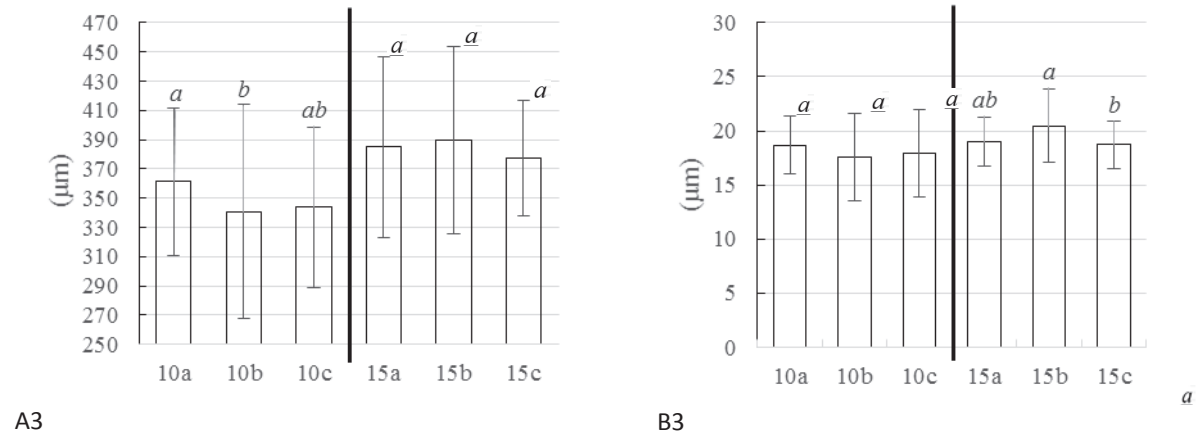


FIGURE 4. Length (A) and width (B) of spicules OXEA in every part of the body *X. testudinaria* at BL. (a) upper (b) middle (c) basal. Depth (7) (10) (15) m. The superscript letter (*a, b, c*) show similarity between parts by Duncan statistical-test. The analysis tested between body parts in each depth. (1) specimen < 0.5 m high, category (2) specimen 0.5-1 m high, category (3) specimen > 1 m high, category.

Spicules Size Dimension of KM Coral Cluster

At 6 -7 m depth KM, oxea spicules dimensions between body parts *X. testudinaria* <0.5 m high category did not differ significantly either on the length parameter (*F = 1.820 P = 0.164*) and width (*F = 0, 36 P = 0.965*). Similarly, the individual at 10-11 m depth. The length or width respectively - were no significant differences (*F = 1.839 P = 0.163, F = 0.678 P = 509*). Meanwhile, at 15-16 m depth, two parameters (length and width) alike - each has a different size significantly between parts (upper, middle and basal) (F = 9.662 P <0.001, F = 21.138 P <0.001) (Fig 5A1-5B1).

On specimens 0.5 – 1 m high category have different sizes significantly on the length (*F = 3.813 P = 0.27*) and width (*F = 6.837 P = 0.002*). Something similar happened to measure the dimensions of oxea spicules at 10-11 m depth. Parameter length and width respectively - were significantly different in size between the basal part of the upper and middle body parts (*F = 14.107 P< 0.001, F = 3.594 P = 0.033*). At of 15-16 m depth, length of oxea spicules has relatively no significant different size between parts of the body (*F = 2.709 P = 0.070*). While the width parameter oxea spicules statistically significant difference between spicules upper body parts with other parts (*F = 4.829 P = 0.009*) (Fig 5A2-5B2).

In contrast to the previous categories that have significant differences spicules OXEA dimensions between parts of the body, the sponge with high category> 1 m dimensions spicules OXEA did not differ significantly between the parts of the body. At a depth of 6-7 m length and width parameters spicules OXEA did not differ statistically significant (F = 0.064 P = 0.938, F = 0.434 P = 0.650). At a depth of 15-16 m, parameter length of spicules OXEA has no significant difference between the sponge body part (F = 1.066 P = 0.347). Similarly, the width parameter statistical OXEA spicules have different sizes between the sponge body part (F = 0.702 P = 0.497) (Fig 5A3-5B3).

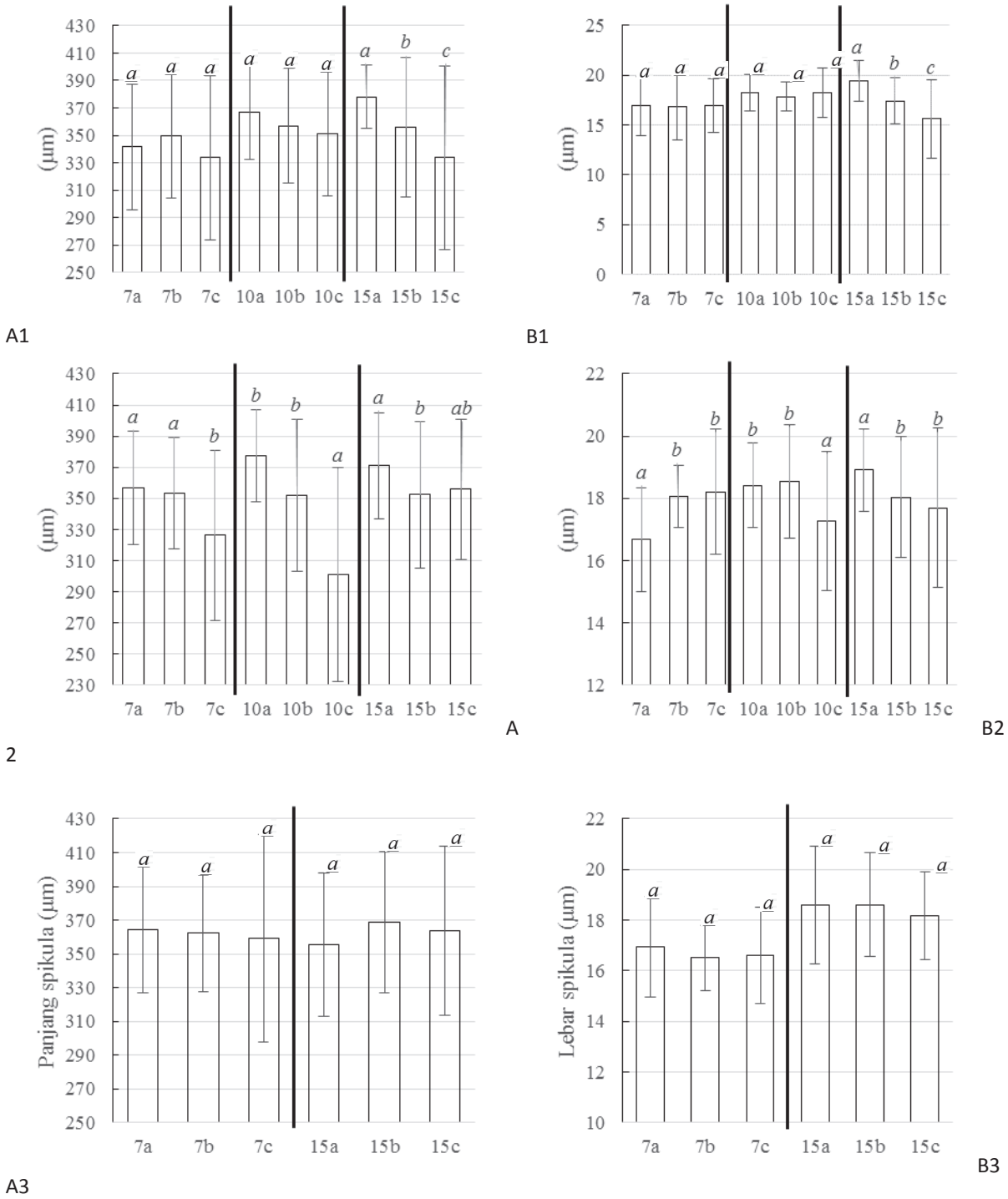
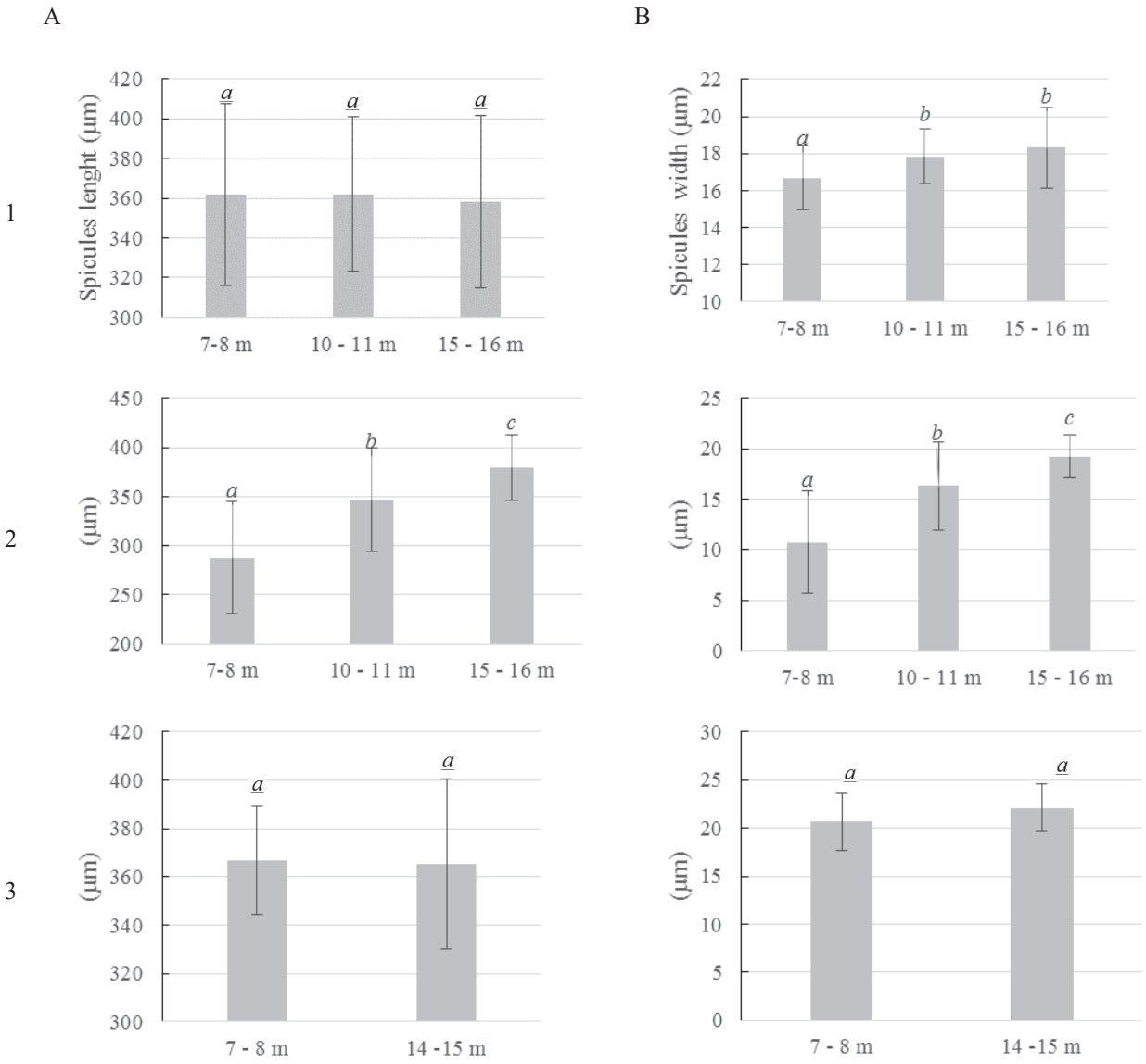


FIGURE 5. Length (A) and width (B) of spicules OXEA in every part of the body *X. testudinaria* at KM. (a) upper (b) middle (c) basal. Depth (7) (10) (15) m. The superscript letter (*a, b, c*) show similarity between parts by Duncan statistical-test. The analysis tested between body parts in each depth. (1) specimen < 0.5 m high, category (2) specimen 0.5-1 m high, category (3) specimen > 1 m high, category.

Spicule Size at Different Depths

Generally, length or width of oxea spicule at different depths of four study site have average size that does not vary. At KM at 7-8 m depth, dimension spicules has spicule size 168.90-**424.30**-424.30 x 12,10-**16.69**-20.01 µm. Whereas 10-11 m depth has spicules size 179-**361.96**- 426.2 x 13.6-**17.85**-21 µm and at 15-16 m depth has spicule size 247.3-**358.60**-427.7 x 8.9-**18.32**-23.5 µm. At a depth of 7-8 m the BL site has spicule size dimension 188.50-**287.91**-420.10 x 3-**10.76**-21.10 µm. Whereas 10-11 m depth has spicules dimensions size 184, 4-**347.21**-398.2 x 5.1-**16.33**-25.8 µm and 15-16 m depth has spicule size dimensions 246.2-**379.96**-432.6 x 13.3-**19.24**-24.5 µm. PT has a different condition than the previous locations. PT dimensions spicules on depth 7-8 m ranges from 308.5-**366.73**-400.4 x 15.3-**20.68**-29.20 µm and at depth 14-15 m ranges from 209.1-**365.3**-432.2 x 18.1-**22.12**-30.5 µm. Spicule size relatively similar to the spicule length of KM and BL, but relatively has wider width than the previous locations. Longest spicules size dimensions located at TP, ranges 338.1-**386.16**-499 x 16.9-**20.49**-24.9 µm at 10-11 m depth. Whereas at 14-15 m depth, spicules size dimensions ranges 338.1-**402.45**-13.1 x 499-**20.21**-38.3 µm.



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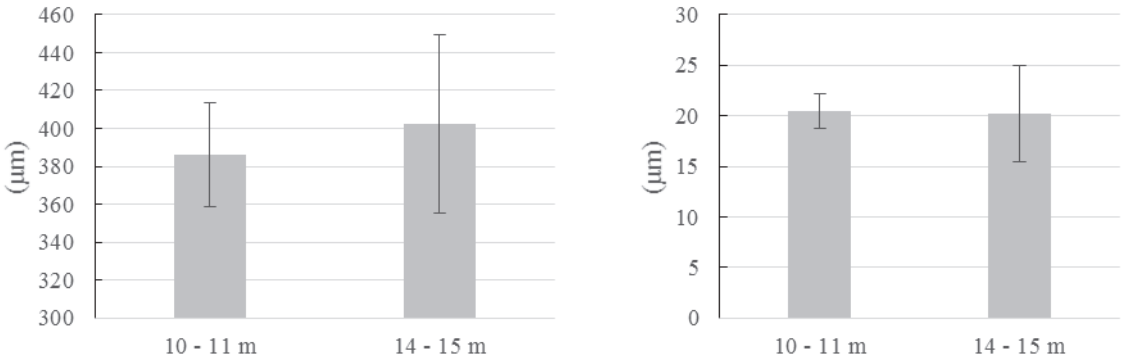

FIGURE 6. Dimensions (A = Length and B = Width) spicules *X. testudinaria* at 4 different locations (1 = KM, 2 = BL, 3 = PT, 4 = TP). The superscript letter (*a, b, c*) show similarity between parts by Duncan statistical-test

Results of ANOVA One Way to notice any difference in the length and width of the depth the water, showing the site KM there is no significant difference in the variable length between depth the water (*F = 0.153, P = 0.858*), while significant in the width of spicules(*F = 52.478, P 0 < 0.001*). Duncan test at KM site showed no significant difference in the size of the width spicules found at 7-8 m depth compared with 10-11 m and 15-16 m depth. Whereas inside BL, variable length and width of the each depths have significantly different values (length [*F = 67.445, P <0.001*] wide [*F = 82.919, P <0.001*]). In contrast to the site PT which both depths between length and width are not significantly different (length [*F = 2.585, P = 0.112*] and width [*F = 0.600, F = 0.441*]). Whereas, condition of the size of spicules at TP site has significant difference length between depths, while not significant for the width variable (length [*F = 10.349, P = 0.002*] and width [*P = 3.495, F = 0.066]*) (Fig. 6).

Spicule Size at Different Locations

Location TP has a size megascleres OXEA is longer than in other locations. As for the width size, spicules in PT wider than other locations. *X. testudinaria* contained in locations BL and KM has a length and width dimensions almost uniform, and smaller than locations PT and TP. There is a significant difference in the length (*F = 13.711, P <0.001*) and width (*F = 64.189, P <0.001*) oxea spicules of *X. testudinaria* between each locations (Fig. 7).

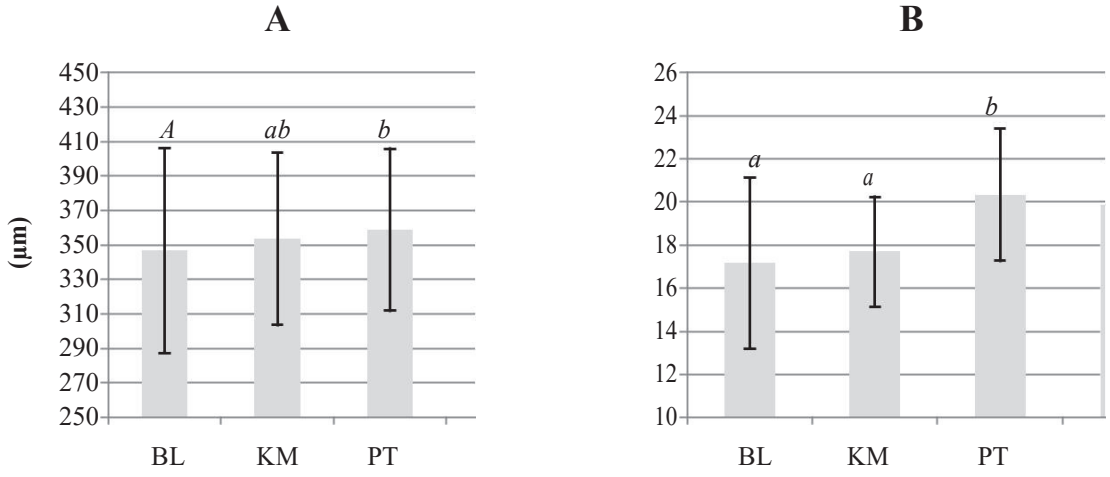

FIGURE 7. Oxea spicule size of *Xestospongia testudinaria* in different location. (A) Length (B) Width. The superscript letter (*a, b, c*) show similarity between parts by Duncan statistical-test

Environmental Factors that Affect the Size of Spicules

Based on the partial linear regression test that has been done, temperature, dissolved silica content, and water clarity have not significant outputs coefficients to the length variable (Sig Temperature = 0.126, DSi = 0.458, water clarity = 0.241). Meanwhile, salinity (x1) and depth (x2) were having significant value and is used in an actual regression test (Sig Salinity = 0.006, depth = 0.008). Correlation Coefficient (R) = 0.202, which shows that the correlation between the two (depth and salinity) to the length parameter is very weak.[10] While the adjusted R2 value obtained was 0,037 (3.7%) with a SEE value of 46.73. These values indicate levels of influence from depth and salinity to length of spicules. Based on the values obtained, it can be said that the value of the length of oxea spicules more influenced by others variables than salinity and depth. Simultaneous test and partial (individual parameter) also get the Sig. ≤ 0.001 so the model and depth and salinity significantly affect the length of spicules.

TABLE 3. Linear regression model on length and width of oxea spicule

No	Variable of Oxea Spicule	Linear Regression Model
1	Length	y = 668,696 -11,909x1 + 66,639x2
2	Width	y = 32,706 +0,463y1 – 0,440y2 – 0,588y3-7,665y4-1,166y5

For partial regression tests performed on the width variable, depth (Sig. 0,012), dissolved silica content (DSi) (Sig. 0,008) and water clarity (Sig. 0.008) have a significant value. Correlation Coefficient (R) obtained by correlation of 0.572, which means independent and dependent variables classified as strong.[10] The value of adjusted R2 of 0.321 (32.1%) with a very small SEE of 2.44 (high accuracy). Simultaneous test is conducted to get the significant value (Sig. <.001). As for DSi and only partial test and water clarity generate significant value (Sig. DSi = 0.008, Brightness <0.001). Variables that have a positive correlation with the width of oxea spicules only salinity [y1]. Other variables (depths [y2], temperature [y3], DSi [y4], and brightness [y5]) has a negative correlation with the size of width of spicules

DISCUSSION

Spicule composition of *X. testudinaria* in Probolinggo-Situbondo coastal that are represented by the four study sites are oxea megascleres without any type of ornaments spicule (microscleres). These results are appropriate with studies conducted, which mentions the dominant form of spicules *X. testudinaria* in Pecaron Pier, Situbondo area is the type of diactinal oxea spicules (monoaxonal spicules with similar two ends and tapered at both ends).[9]

X. testudinaria spicules dimensions in Probolinggo-Situbondo coastal have relatively larger size than neotype of *X. testudinaria* coming from Cape Denison, Queensland with specimen code BMNH 1881.10.21.266. Neotype spicules dimensions is 174.95-**277.17**-331.71 x 8.34-**14.67**-21.65 µm.[11]Oxea spicules dimensions of *X. testudinaria* in Probolinggo-Situbondo coast approaching the morphological characters of its sympatric species holotype (*Xestospongia bergquistia*) in the Pasir Putih coast, Situbondo. *X. bergquistia* (holotype code QM G25018, which has oxea spicules size 303.28-**352.29**-378.08 x 4.96-**11.54**-16.26 µm. Whereas, oxea spicules size data obtained in accordance with the character of the genus Xestospongia de Laubenfels (1930) is 254-**308**-400 x 16 -**21**-30 µm.[12]The difference size of the spicules sponge morphology in one species may occur such as research which shows the dimensions of spicules on the species of sponge *Callyspongia* spp across different locations. [13] With obtained issue, identification of sponges species using the morphology of spicules could become more difficult and uncertain, given the size of spicules very varies.

Oxea spicules size between parts of the body of *X. testudinaria* at all the study site stated that generally there is a difference in size between the spicule of the parts of the body sponge. This is less appropriate to which states that the genus Xestospongia spicules characterized by a uniform size oxea (single size oxea spicule) without any gradation size. [12] However, [12]also says that there are also variations in the size of oxea spicules though it cannot be categorized in tiers size of spicules. The size variation over to the age level, where the very-small size oxea could be possible are young or immature spicule.

Besides the immature spicule, the diverse-size spicule can also be due to the influence of habitat conditions.[3-5]Depth is one of the important variables to the life of aquatic organisms particularly sponges. Our study proved that some of the locations have significantly different size of oxea spicule between depths.[6, 14]states that there is an anomaly in Spongillidae (freshwater sponge) that live in deep waters. In his research, known that there are increasing the length of the spicules alongside with increasing depth. It also revealed by [6]that mentioned species of marine

sponges *Petrosia ficiformis* (Petrosiidae - Demospongiae) and 'boring sponges' *Cliona azzaroliae* (Clionaidae - Demospongiae) getting statistically significant increase in spicule length alongside the water depth. Similarly, *C. azzaroliae* which also has a length difference significantly on the type of oxea spicules but not significantly different in his tylostyle spicules.[6] The significant differences result also supports [6] that mentioned the depth of influence must've against variations in the size of spicules on the species of freshwater sponges *Ephydatia fluviatilis* (Spongillidae - Demospongiae). Deeper location, significantly generate the size of the spicules longer and wider, it is possible because the deep waters tend to be minimal disturbance, both physical and chemical changes in variables or predation. And also, the deeper waters have a relatively stronger currents than the water near the surface so obtained more likely is longer and thick spicules [15, 16], although many similar studies with results opposite to those results.[6]

In this study, the location showed a significant difference statistically of spicules dimensions than the body part or depth. From the results obtained, we drew an understanding that habitat factors affecting the morphology even the physiology of marine sponges. In the different habitats, growth form (external) and the spicule size, density and also spongin proportion (internal) of a species is likely to have big differences between them, adjusting the variable of chemical physical condition each habitat [6]. [17]in his research on *Tetilla* sp (Tetillidae - Demospongiae) obtain significant differences between macro (external) and micro (internal) morphology of the sponges. *Tetilla* sp. obtained from different location with varies condition of environmental variables. The result of the study was supported by [6] by comparing the spicules *Cliona celata* (Clionaidae - Demospongiae) shallow waters that represented by sub tidal cave with quiet current and low light exposure habitat character; tidal pool with 30-80 cm depth which have high turbulence at the highest tide characteristics; the exposure area waves in 3-5 m depth and high-intensity light exposure area; and the last remaining habitat is 30 m depth waters that not directly exposed by waves and light compared to another shallow water habitat. Differences location of the morphological form of macro or micro species of sea sponge *Tettila* sp.

Changes in the micro-morphology size of the sea sponge is dependent upon habitat environment conditions either temporal or spatial. One of temporal changes caused by the seasons that affected the physical and chemical conditions of the waters. Season changes will certainly affect the water temperature. In this study, based on regression-test, temperatures not effected to dimensions size of the oxea spicules. In contrasts to [7]that mentions the trend changes in the size of spicules positively correlated to the trend of temperature variable [7], where, the spiculogenesis process will occur when the temperature conditions of water at conditions relatively high. [18]

Meanwhile, based on regression-test DSi positively correlated with the size of the width of spicules, it supports the notion that dissolved silica variable affected the process of spiculogenesis that occurred in the sponge, where the higher concentration of silica, greater the size of spicules sponge would be. [7] Silica uptake processes on marine sponges was influenced by water temperature conditions. In addition to affecting the consumption of oxygen, water pumping activity, and physiological processes such as growth and reproduction of marine sponge phases[19], the temperature also affected the polymerization process silica in the marine sponge. [20] state that temperature had no significant relationship to the length of 'achantostyle' and 'style' spicules on the *Clathria prolifera* (Microcionidae - Demospongiae). While the width of spicules *C. prolifera* have increased significantly to changes in water temperature. With the results obtained, it can be deduced that the polymerization process silica is more efficient at lower temperatures than high temperatures.[20] Growth (increase in length and width) spicules are directly related to the growth of axial filament that will turn into axial canal to the formation of alongside the deposition of silica on the axial canal. Thick-size spicules production at lower temperatures give a hypothetical that silica particles deposition on the polymerase region becomes more efficient. It means the possibility of axial canal losses the silica when deposited will be reduced.

Salinity had a negative impact, on the length and width of spicules. This result contrast to [21] which states that salinity along DSi has a positive correlation with the proportion of skeletal *Halichondria panicea* (Halichondriidae - Demospongiae). Salinity in previous study, believed to be supporting the physiological processes in the body sponge. Sponge with low-salinity stress will be disturbed its physiological processes, that impact on spiculogenesis processes in the sponges.[21] Not many studies that mention the correlation between water clarity to size dimensions of spicules. Indirectly, waters clarity will reflected the level of waters sedimentation. In this study, clarity negatively impact the size of the width of spicules. Theoretically, sediment input changes on aquatic ecosystems have an effect on aquatic organisms in various aspects. In the case of sea sponge that rely on the suspension of the water that goes into the aquiferous system, macro-particle sediment will effected to the filtering process of organic material,[22] and even lead to blockage of water absorption through oscules (pores) or a system of canals in sea sponge.[23]The canal system blockage will cause interference with the filtration material feeding system, because flagellates of choanocyte cells be disrupted due to sediment particles that get into choanocyte chambers.[23] Sedimentation also

affected the rate of respiration of sponges. The higher sedimentation rate, smaller oxygen consumption.[24] While the influence of sedimentation of the morphology of spicules is still not very clearly understanding. [25]states that the combination of the location with high current speed and high sedimentation rate will produced longer spicules than location with low current speed and sedimentation. When sponges exposed to higher sedimentary stress conditions, possible sponge spicules need to produce a more robust structure spicules that provides greater support for not ideal conditions habitat. Sponge with a stronger structure spicule is maintained and minimize the possibility of the sponge broken buried by sediments.[16]

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