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**To:** Munawar Khalil khalil@unimal.ac.id

WY

Ref.: Ms. No. OSJO-D-20-00110

Biometric relationship of Anadara granosa (Bivalvia: Arcidae) from the northern region of the Strait of Malacca  
Ocean Science Journal

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# Ocean Science Journal

## Biometric relationship of *Anadara granosa* (Bivalvia: Arcidae) from the northern region of the Strait of Malacca --Manuscript Draft--

<b>Manuscript Number:</b>	OSJO-D-20-00110
<b>Full Title:</b>	Biometric relationship of <i>Anadara granosa</i> (Bivalvia: Arcidae) from the northern region of the Strait of Malacca
<b>Article Type:</b>	Article
<b>Keywords:</b>	blood cockle; bivalvia; growth model; Malacca Strait; morphometric
<b>Abstract:</b>	<p>The study on the growth pattern of blood cockle <i>Anadara granosa</i> focused on the aspects of biometric prints on the shell, which aimed to predict the growth of <i>A. granosa</i> population in the northern region of Malacca straits. The local sample populations of the cockle were collected in three different intertidal areas called Lhokseumawe and Banda Aceh in Indonesia and Pulau Pinang in Malaysia. The biometric analysis showed that the length-weight relationship model of <i>A. granosa</i> populations in this region indicated that the cockle population generally had a negative allometric growth pattern (<math>b &lt; 3</math>) or shell length is more dominant compare to shell weight. Therefore, the result showed that the growth performance of <i>A. granosa</i> was not ideal, where the <math>b</math> value (the coefficient of biometric relationship) was highest recorded in Lhokseumawe, followed by Banda Aceh and Pulau Pinang. The value of coefficient <math>b</math> could be affected by various factors such as environmental conditions, adaptation and dietary patterns. Cluster analysis displayed that the population of <i>A. granosa</i> from the northern region of the Strait of Malacca was divided into two clusters, which were <i>A. granosa</i> from the northern Strait of Malacca (Banda Aceh and Lhokseumawe in Indonesia) and population of <i>A. granosa</i> from the western Strait of Malacca (Pulau Pinang in Malaysia). The Factors that might cause the differences in the biometric component of both clusters were geographical level on the source of population and the locality of the environmental parameter.</p>

## 1 Abstract

2 The study on the growth pattern of blood cockle *Anadara granosa* focused on the aspects of  
3 biometric prints on the shell, which aimed to predict the growth of *A. granosa* population in  
4 the northern region of Malacca straits. The local sample populations of the cockle were  
5 collected in three different intertidal areas called Lhokseumawe and Banda Aceh in Indonesia  
6 and Pulau Pinang in Malaysia. The biometric analysis showed that the length-weight  
7 relationship model of *A. granosa* populations in this region indicated that the cockle population  
8 generally had a negative allometric growth pattern ( $b < 3$ ) or shell length is more dominant  
9 compare to shell weight. Therefore, the result showed that the growth performance of *A.*  
10 *granosa* was not ideal, where the *b* value (the coefficient of biometric relationship) was highest  
11 recorded in Lhokseumawe, followed by Banda Aceh and Pulau Pinang. The value of coefficient  
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13 patterns. Cluster analysis displayed that the population of *A. granosa* from the northern region  
14 of the Strait of Malacca was divided into two clusters, which were *A. granosa* from the northern  
15 Strait of Malacca (Banda Aceh and Lhokseumawe in Indonesia) and population of *A. granosa*  
16 from the western Strait of Malacca (Pulau Pinang in Malaysia). The Factors that might cause  
17 the differences in the biometric component of both clusters were geographical level on the  
18 source of population and the locality of the environmental parameter.

19  
20 **Keywords:** blood cockle; bivalvia; growth model; Malacca Strait; morphometric

21 **1. Introduction**

22 *Anadara granosa* is one of the important fishery commodities in several areas of  
23 Southeast Asia. This species has been cultivated in countries such as Malaysia and Thailand  
24 due to limited natural stocks. However, this species in Indonesia still harvested directly from  
25 nature (Broom 1983; Khalil et al. 2017). Nevertheless, annual harvested-cockle data designate  
26 a reduction in natural stocks in the last decade. The huge demand for this species as a protein  
27 source marks the natural stocks significantly diminished. This condition is possibly caused by  
28 **insufficient** controlling of wild cockle population stock. Therefore, the management of this  
29 species is required for the sustainability of this important species. Comprehensive information  
30 on biometric (morphometric relationship pattern of the species) is necessary to predict the  
31 annual recruitment, as well as to interpret growth, mortality, reproductive biology and survival  
32 data in the marine culture of species (Kim et al. 2006; Peharda et al. 2007; Pinn et al. 2005;  
33 Zelditch et al. 2004).

34 Length-weight is an important variable to compare among growth, physiological  
35 processes and environmental factors that affect the lives of aquatic organisms (Hemachandra  
36 and Thippeswamy, 2008). Growth of bivalves can be defined as the increase of the length size  
37 of the shell and body fweight (body mass) that have also been used extensively as the  
38 corresponding parameters to assess the growth of them (Bailey and Green 1988; Bayne and  
39 Worrall 1980; Garton and Haag 1991; Smit at al. 1992). Measuring the length and weight of  
40 aquatic species is used to evaluate the growth patterns of this species qualitatively. Such  
41 relationships are expressed via the data distribution of length and weight of the shell. These  
42 data also represent the ratio of the addition of an animal's body size by period. Length and  
43 weight relationships have several purposes, namely (1) for measuring weight and length ratio  
44 of a species to the weight-length in Taxa class (Anderson and Neumann 1996; Shine 1990),  
45 and (2) for age (Pauly 1983).

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46 The length and weight relationship also allow the life history and morphological  
47 differences to be identified between species and among populations of different habitats and  
48 areas (Beukema and Meehan 1985; Gaspar et al. 2002; Holopainen and Hansk 1986; Morton  
49 1985; Peters 1985). This study aimed to analyze the biometric relationship of *A. granosa* by  
50 using a morphometric relationship and dendrogram analysis of specimens collected from the  
51 northern region of the Strait of Malacca.

## 52 **2. Materials and Methods**

### 53 *2.1 Samples collection*

54 The specimens of *A. granosa* (120 specimens/month) were collected monthly from June  
55 2009 till September 2010 from the muddy natural habitat in Banda Aceh (5°32'34.67"N -  
56 95°17'2.54"E), Lhokseumawe (05°09'35.3"N - 097°08'29.4"E) in Aceh Province, Indonesia  
57 and Pulau Pinang (5°16'9.66"N - 100°23'27.37"E) in Malaysia (Fig. 1). The total number of  
58 specimens sampled was 1,920, with cockle sizes ranged from 38–71 mm in length. The  
59 sampling areas were characterized by muddy substrate surrounding by mangroves patch, no  
60 wave action and high in salinity. The specimens were collected at a depth of 5-30 cm and  
61 salinity ranged from 10-33 ppt. The live specimens were collected manually with the aid of a  
62 harrow during the low tide period. After collecting, the specimens were stowed in isotherm  
63 containers and directly transferred to the laboratory.

64 *Insert figure 1.*

65 In the laboratory, the samples were cleaned from mud and organisms attached to the  
66 shell. The recorded biometric values (Fig. 2) were the results of the morphological  
67 measurement of blood cockle collected from the sampling sites. The data were taken including  
68 shell length, shell thickness, cockle height, the weight of fresh tissue, wet cockle weight and  
69 sex category. The measurement instrument of length and width of the cockle was by using a  
70 digital vernier caliper with an accuracy of 0.1 mm and cockle weight tissue was weighed using

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71 a digital weighing scale (in grams). The length was defined as the maximum shell length  
72 (measured from the posterior margin to the anterior margin of cockle); the thickness was  
73 measured on inflating position, which was from the most protruding part on the top of cockle  
74 to the most protruding part on the bottom of cockle. The height was measured from the highest  
75 ventral margin of the cockle towards the dorsal margin of the cockle.

76 *Insert figure 2.*

## 77 2.2 *Morphometric relationship*

78 The morphometric ratio of *A. granosa* between length : height, length : thickness and  
79 height: thickness analyzed using the following formula:

$$80 a = L / H, a = L / C \text{ and } a = H / C$$

81 Where: L = shell length, H = shell height, C = shell thickness, a = index (coefficient),

82 The growth pattern of cockle was able to be designated through a relationship of shell  
83 length and cockle body weight (wet weight), which analyzed through the equation relationship  
84 of power regression (Ricker, 1975). From the analysis results, then it was informed that if the  
85 growth rate of cockle length balanced with the cockle weight or in the mathematical expression  
86  $b = 3$ , then it was said that the cockle growth was isometric. Whereas if  $b \neq 3$ , it was called  
87 allometric which means the growing of cockle length imbalanced with the weight. To test  
88 whether the values of the constants were  $b = 3$  or  $b \neq 3$  (isometric or allometric), a statistical  
89 test was performed through a statistical *t*-test. The equation above applied both to the whole  
90 cockle and by sex. Based on statistical *t*-test, the hypothesis used was:

91  $H_0: b = 3$ , shell length and cockle weight relationship was isometric

92  $H_1: b \neq 3$  mean shell length and cockle weight relationship was allometric (namely: positive  
93 allometry), if  $b > 3$  meant that the growth of cockle weight was faster than the growth of shell  
94 length (namely: negative allometry) and if  $b < 3$  mean the growth of shell length was faster  
95 than the cockle weight).

96 2.3 *Statistical Analysis*

97 The raw data obtained was collected and put into a package of Microsoft Excel 2011  
98 software Macintosh version to be processed and analyzed. Statistical analysis of Co-Variant  
99 Analysis was used to determine significant differences in the values obtained in each collected  
100 group data. The determining factor used in this study was the population differences in the  
101 three different regions. Therefore, the coefficients *a* and *b* analyzed by observing at the growth  
102 differences in population and sex differences in the sample (male, female, or neutral). The  
103 statistical test was continued by the post hoc test to ensure which factor significantly differed  
104 in one particular parameter.

105 The parametric statistical test of Co-Variant Analysis was analyzed by using the  
106 package of SPSS (Statistical Package for the Social Science) software release 23.0 Macintosh  
107 version. The relationships existed both of two variables: the relationship between the *b*  
108 coefficient (relationship of shell length and cockle weight) and environmental factors in the  
109 sampling area would be analyzed by this test. Hypothesis testing was then performed on the  
110 sample parameters to test ~~whether the correlation was significant or not significant~~ at the level  
111 of 95% ( $P = 0.05$ ). The statistical *t*-test was used to find significant differences in the pattern  
112 of change in the *b* coefficient that was the growing nexus of shell length and cockle weight of  
113 each sampling area.

114 Cluster analysis through the dendrogram diagram was designed to clarify the  
115 relationship between each biometric component (Ramesha and Thippeswamy 2009) on the  
116 blood cockle ~~so it could~~ show the relationship between the biometric components of *A. granosa*  
117 from the northern region of the Strait of Malacca. Cluster analysis was processed by using  
118 ~~SPSS (Statistical Package for the Social Science) software release 23.0 of the Macintosh~~  
119 ~~version.~~

120

121 **3. Results**

122 *3.1 Equality a and b coefficients from different populations*

123 The biometric studies of *A. granosa* (from June 2009-September 2010) from the  
124 northern region of Straits of Malacca had involved 1920 individuals in total, consisted of 756  
125 males, 974 females, 190 neuters. The statistical analysis showed that *A. granosa* shell height :  
126 shell length relationship from three sampling areas showed dissimilar in coefficient *a*, but there  
127 was similar to the coefficient *b*. Furthermore, The relationship of shell thickness : shell length  
128 of *A. granosa* populations showed an identical in coefficient *a* and *b* between populations. In  
129 contrast, The relationship of shell thickness : shell height coefficient (*a* and *b*) values in term  
130 of their allometric equations showed differences between populations (Table 1).

131 *Insert Table 1*

132 *3.2 Morphometric coefficients model from different populations*

133 *A. granosa* weight and shell length relationship analysis showed that there are no  
134 differences in coefficient *a* and *b* value between populations, comprised between sexes (Table  
135 2). A statistical test was performed on the coefficient *b* through *t*-test indicating the coefficient  
136  $b < 3$  or negative allometry in all *A. granosa* populations. This condition showed that the growth  
137 of cockle shell length was faster or more dominant compared to the growth of cockle weight.  
138 A further test of the hypothesis also showed that  $H_0$  was rejected ( $P < 0.05$ ) and means the  
139 growth rate of shell length and cockle weight overall was imbalanced.

140 *Insert Table 2*

141 *3.3 Environmental parameter*

142 Seasonal variations of environmental parameters in the sampling areas are informed in  
143 Table 3. Water temperature, salinity and phytoplankton concentration fluctuated significantly  
144 compared to other environmental parameters during the study period.

145 *Insert Table 3.*



## 146 4. Discussion

### 147 4.1. Biometric relationship model of *Anadara granosa*

148 Biometric data analysis of *A. granosa* from the northern region of the Strait of Malacca  
149 had shown that in general, the cockle growth model was negative allometry, in which the  
150 growth of shell length was more dominant than the growth of cockle weight. The growth model  
151 generated from the three sampling sites showed that the value of the  $b$  coefficient was less than  
152 3 ( $b < 3$ ). The balance value of the  $b$  coefficient generally has a range between 2.4 to 4.5 (Wilbur  
153 and Owen 1964) and when the  $b$  value is equal to 3 ( $b = 3$ ) the relationship of shell length and  
154 cockle weight is isometric (Carlander 1969). In this study, the  $b$  coefficient differs in a  
155 population or when compared to other populations. The cockle population from Lhokseumawe  
156 had a higher  $b$  coefficient ( $b = 2.7629 \pm 0.3894$ ) compared to the cockle population from Banda  
157 Aceh ( $b = 2.6178 \pm 0.2095$ ) and the cockle population from Pinang ( $b = 2.2018 \pm 0.5866$ ). In the  
158 sexes level, a similar condition was presented, whereas both male and female *A. granosa* from  
159 Lhokseumawe had highest  $b$  coefficient (male  $b = 2.7713 \pm 0.567$ , female  $2.7559 \pm 0.3838$ )  
160 compared to other sampling locations (Banda Aceh male  $b = 2.6306 \pm 0.2831$ , female  $b =$   
161  $2.5695 \pm 0.3368$ ; Pulau Pinang male  $b = 2.0043 \pm 0.889$ , female  $b = 2.3697 \pm 0.7607$ ). These  
162 conditions indicated that the cockle growth rate in Lhokseumawe was more appropriate or  
163 suitable compared to the other two sampling areas. The  $b$  coefficient value of biometric  
164 relationships is characteristically compared between dimensional growth of related or similar  
165 species in various geographical areas (Ramesha and Sophia 2015).

166 The contrast conditions could be seen on the cockle found from the Pulau Pinang  
167 sampling area, where the value of the  $b$  coefficient was lower than the range value of the  $b$   
168 coefficient 2.4-4.5 that described by Wilbur and Owen (1964), causing the shell length to  
169 become imprecise. It was faster than the increase of the body volume of the cockle, causing the  
170 cockle to be unhealthy. Water quality-analyzed showed that this condition was impacted by the

171 **highest** fluctuation in the environmental condition in cockle habitats. **Other, the environment**  
172 quality was unsuitable for cockle life due to **increased pollution** that exceeded the standards  
173 rate for marine life (Table 3). ~~At a high level, it was recognized that the nutrient could~~  
174 ~~potentially be toxic to the cockle.~~ Environmental aspects have been recognized as the main  
175 factor that effects shell development in bivalves. The shell size and shape are affected by the  
176 variation of ambient environmental constraints (Wilbur and Owen 1964; Seed 1968).

177 Furthermore, the variety of growth patterns of *A. granosa* highly correlates to factors  
178 of food availability, temperature, salinity, pollution materials and reproductive activities  
179 (Broom 1982; **Day and Fleming, 1992; Tarr, 1995**). **The main factor, which expected to affect**  
180 **changes in the value of the *b* coefficient of cockle on the sampling area, was the change of the**  
181 **concentration value of phytoplankton.** Pearson correlation test showed the opposite condition  
182 that the *b* coefficient had a strong correlation to the density of phytoplankton in Banda Aceh ( $r$   
183 = 0.766). While the density of the phytoplankton factor showed a moderate correlation with  
184 the *b* coefficient for cockle from Lhokseumawe ( $r = 0.532$ ) and Pulau Pinang ( $r = 0.579$ ). The  
185 density of phytoplankton was expected as a limiting factor for growth activity, where the  
186 phytoplankton was used as an energy source for the growth process of shell length and cockle  
187 weight. The supply of food sources is considered as an important factor for sustainable growth  
188 (Seed and Suchanek 1992; Widdows and Johnson 1988).

189 Changes in the fluctuation of the *b* coefficient were also expected to have a relationship  
190 with the reproduction period. Sudden changes in the value of the *b* coefficient meant that there  
191 was a rapid change in the cockle weight tissue due to a few biomass of cockle. Weight reduction  
192 of the cockle volume could be caused by the reproduction process, such as gamete production  
193 process and gonad or gamete process that were in a state of inactivity. In bivalve animals, the  
194 gonadal growth and the results of the gonadal maturation process can increase the mass density  
195 of tissue as well as causes an increase in the weight of tissue overall. Exchange of the value of

196 the *b* coefficient also indicates the beginning of the activities of gonadal maturation and growth  
197 in bivalve animals (Hemachandra and Thippeswamy 2008; Hickman and Illingworth 1980).

198 The total cockle weight described as the total of shell weight, including the weight of  
199 cockle meat. In *A. granosa*, the shell weight was generally heavier than the meat weight. When  
200 the shell size increased, then the overall weight of the cockle also increased linearly. However,  
201 the samples analyzed from the northern region of the Strait of Malacca showed no significant  
202 weight despite the shell size increased. This condition was assumed to be the result of the  
203 increased volume of cockle meat that did not grow or develop linearly, causing the shell length  
204 was not in line with the cockle weight overall.

205 The growth pattern was not always fixed for species. Differences in growth models  
206 could be found on the same or different species, among sex, indifferent or the same locations  
207 and in different seasons. The difference in latitudinal gradient is also related to the shell size,  
208 reproduction level and reproductive cycle in bivalves (Kanazawa and Sato 2008; Mirzaei et al.  
209 2017) as well as the growth pattern models of cockle in these three sampling areas. Cockles  
210 from Banda Aceh and Lhokseumawe had morphological differences compared to the cockles  
211 from **Pinang**. *A. granosa* from Banda Aceh and Lhokseumawe had special shell features which  
212 were thicker and wider compared than *A. granosa* cockles from Pinang. The other studies on  
213 the relationship in length and weight on some *Anadara* species had demonstrated diversity and  
214 differences in growth patterns (Table 4).

215 *Insert table 4*

216 The value of the *b* coefficient on the relationship of shell length and cockle weight noted  
217 in this study was lower than the recorded one in other species in the same family, namely  
218 *Tegilarca granosa* ( $b = 2.82$ ) from Korean waters. Another difference was also found in their  
219 relationship model, namely *Scapharca* from Korean water, which had positive allometry and  
220 isometric relationships, while *A. granosa* from the northern region of the Strait of Malacca was

221 negative allometry. Different growth patterns at different latitudes might be caused by the  
222 influence of environmental factors, changes in the composition of the food and competition  
223 between individuals that were locality in habitat.

#### 224 4.2. Relationships of the biometric component of *Anadara granosa*

225 Analysis of the relationship of each biometric parameter of *A. granosa* through  
226 dendrogram or classification methods (hierarchy) was used to analysis the growth patterns of  
227 the three *A. granosa* populations in the northern region of the Strait of Malacca. Dendrogram  
228 diagram was designed to clarify the relationship of each biometric component in blood cockles.  
229 Fig. 3 showed the relationship between the biometric component of *A. granosa*. Through this  
230 analysis, the degree of dissimilarity between generated clusters had shown. The higher the  
231 value of the generated scale, the more distance the relationship of the biometric component in  
232 a cluster with another cluster. Dendrogram analysis showed that cluster A had two sub-clusters  
233 namely A1 represented the biometric component of *A. granosa* from Lhokseumawe and A2  
234 represented the biometric component of *A. granosa* from Banda Aceh which was separated on  
235 a scale of 16. B-cluster was a hierarchy cluster of biometric components of *A. granosa* from  
236 Pinang. The B-cluster was separated by A on a scale of 25. The higher recorded scale means  
237 the dissimilarity elements forming the component were larger. It showed the growth pattern  
238 based on the biometric parameter was very different from the high level of inequality among  
239 the clusters (population) and sub-clusters (subpopulations). Gaspar et al. (2002, 2001) and Popa  
240 et al. (2010) state that the population can be explained in particular by the growth characteristics  
241 through the measurement of biometric morphology.

242 *Insert fig. 3.*

243 The factor that might cause differences in biometric components between *A. granosa*  
244 populations from A and B cluster was the geographical differences of the population source to  
245 give effect on the growth pattern of the cockle. The basic source of cockle populations of A

246 cluster was different from the B cluster population, namely *A. granosa* populations of cluster  
247 A cluster came from the northern region of Sumatra island while B cluster came from the  
248 eastern region of Sumatra or the western region of Peninsular Malaysia. A sub-clusters, they  
249 were also known to have differences in growth patterns of the biometric component; they even  
250 had close proximity. *A. granosa* cockles of A1 and A2 sub-cluster was expected to come from  
251 the same source of population. Differences in the pattern of biometric components were  
252 possible because of the differences in environmental factors that were locality in nature,  
253 affecting the growth patterns. Differences range of salinity (Carmichael et al. 2004; Schöne et  
254 al. 2003), temperature (Goodwin et al. 2001; Jones et al. 1989; Kennish and Olsson 1975;  
255 Pilditch and Grant 1999; Schöne et al. 2002) and density of phytoplankton (Alunno-Bruscia et  
256 al. 2001; Carmichael et al. 2004; Grant 1996; Lorrain et al. 2000; Miyaji et al. 2007) are  
257 expected to play a role in determining the growth pattern of cockle in three sampling areas.

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396 **Captions for figures**

397 **Fig. 1.** *Anadara grannosa* sampling location in the northern region of the Strait of Malacca

398 (*Insert after line 63, page 3*)

399 **Fig. 2.** Biometric of *Anadara grannosa*

400 (*Insert after line 75, page 4*)

401 **Fig. 3.** Dendrogram parameter of *Anadara granosa* biometric relationship from the northern

402 region of Strait of Malacca

403 (*Insert after line 241, page 10*)

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405 **Captions for table**

406 **Table 1.** Equality a and b coefficients in the biometric model from different populations

407 (*Insert after line 130, page 6*)

408 **Table 1.** Equality a and b coefficients in the biometric model from different populations

409 (*Insert after line 139, page 6*)

410 **Table 3.** Ranges of the seasonal environmental parameter at the sampling areas

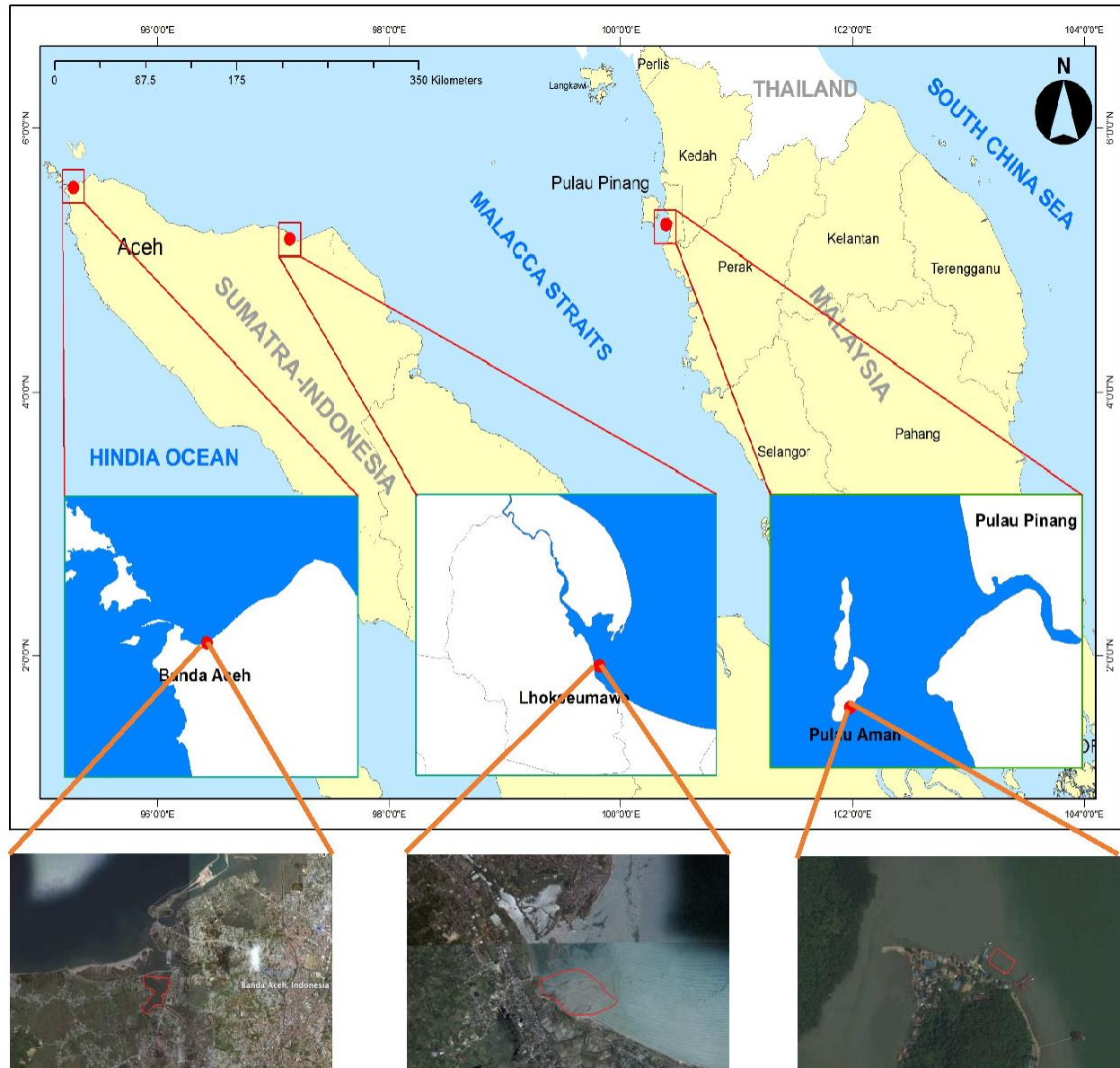
411 (average±st.dev).

412 (*Insert after line 144, page 6*)

413 **Table 4.** The length-weight relationship model for Archidae.

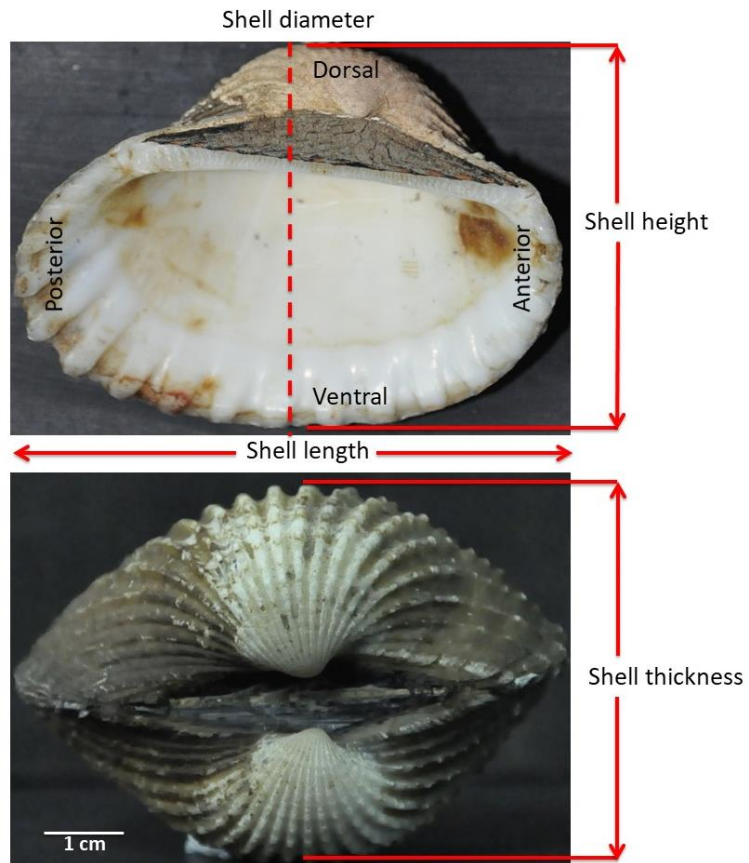
414 (*Insert after line 214, page 9*)

Figure 1

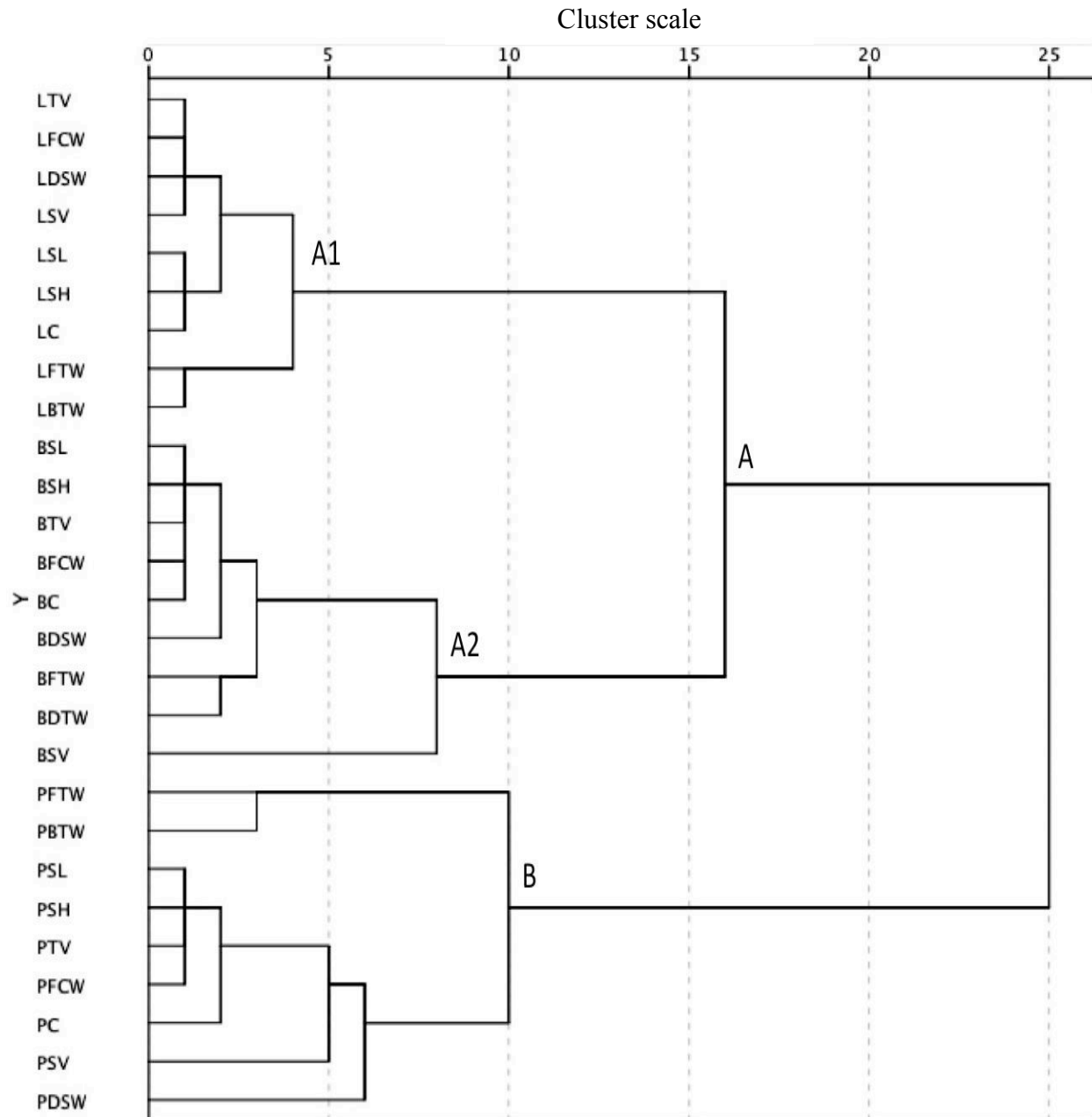


**Fig. 1.** *Anadara grannosa* sampling location in the northern region of the Strait of Malacca

Figure 2



**Fig. 2.** Biometric of *Anadara grannosa*



**Figure 3.** Dendrogram parameter of *Anadara granosa* biometric relationship from the northern region of Strait of Malacca

(A: Cluster of *Anadara granosa* population A; A1 and A2: Sub-cluster of *Anadara granosa* population A; B : Cluster of *Anadara granosa* population B).

(BSL: Banda Aceh *Anadara granosa* shell length; BSH: Banda Aceh *Anadara granosa* shell height; BC: Banda Aceh *Anadara granosa* shell thickness; BTV: Banda Aceh *Anadara granosa* total volume; BSV: Banda Aceh *Anadara granosa* shell volume; BFCW: Banda Aceh *Anadara granosa* flesh weight; BFTW: Banda Aceh *Anadara granosa* tissue weight; BDSW: Banda Aceh *Anadara granosa* dry shell weight; BDTW : Banda Aceh *Anadara granosa* dry tissue weight; LSL: Lhokseumawe *Anadara granosa*

shell length; LSH: Lhokseumawe *Anadara granosa* shell height; LC: Lhokseumawe *Anadara granosa* shell thickness; LTV: Lhokseumawe *Anadara granosa* total volume; LSV: Lhokseumawe *Anadara granosa* shell volume; LFCW: Lhokseumawe *Anadara granosa* flesh weight; LFTW: Lhokseumawe *Anadara granosa* tissue weight; LDSW: Lhokseumawe *Anadara granosa* dry shell weight; LDTW: Lhokseumawe *Anadara granosa* dry tissue weight; PSL: Pulau Pinang *Anadara granosa* shell length; PSH: Pulau Pinang *Anadara granosa* shell height; PC: Pulau Pinang *Anadara granosa* shell thickness; PTV: Pulau Pinang *Anadara granosa* total volume; PSV: Pulau Pinang *Anadara granosa* shell volume; PFCW: Pulau Pinang *Anadara granosa* flesh weight; PFTW: Pulau Pinang *Anadara granosa* tissue weight; PDSW: Pulau Pinang *Anadara granosa* dry shell weight; PDTW: Pulau Pinang *Anadara granosa* dry tissue weight).

Table 1. Equality of coefficients  $a$  and  $b$  in allometric model from populations

Model	Banda Aceh		Lhokseumawe		Pulau Pinang		ANCOVA's F, df, p	
	$a \pm SEa$	$b \pm SEb$	$a \pm SEa$	$b \pm SEb$	$a \pm SEa$	$b \pm SEb$	$a$	$b$
SH= $a$ *SL <sup>b</sup>	1.662±4.031	0.765±0.088	7.487±8,107	0.650±0.125	10.832±5.863	0.502±0.146	7.155, 1, 0.011	3.220, 1, 0.080
ST= $a$ *SL <sup>b</sup>	1.820±4.640	0.646±0.103	5.902±8.240	0.574±0.139	13.458±5.418	0.362±0.142	0.827, 1, 0.368	1.099, 1, 0.301
ST= $a$ *SH <sup>b</sup>	0.924±3.313	0.831±0.089	3.101±7.191	0.797±0.165	3.283±3.661	0.796±0,127	11.850, 1, 0.001	16.408, 1, 0.00



Table 1. Equality  $a$  and  $b$  coefficients in the biometric model from different populations

Model $W=a*L^b$	Banda Aceh		Lhokseumawe		Pulau Pinang		ANCOVA's F, df, p	
	$a \pm SEa$	$b \pm SEb$	$a \pm SEa$	$b \pm SEb$	$a \pm SEa$	$b \pm SEb$	$a$	$b$
Whole sample	0.0016±0.0025	2.6178±0.2095	0.0015±0.0328	2.7629±0.3894	0.0061±1.4319	2.2018±0.5866	0.510, 1, 0.479	2.752, 1, 0.105
Males	0.0015±0.0079	2.6306±0.2831	0.0008±0.1961	2.7713±0.567	0.0127±2.9646	2.0043±0.889	0.002, 1, 0.961	0.332, 1, 0.568
Females	0.0019±0.0069	2.5695±0.3368	0.0009±0,0447	2.7559±0.3838	0.0033±2.5539	2.3697±0.7607	0.135, 1, 0.715	0.023, 1, 0.881
ANCOVA's F, df, p	1.570, 1, 0.217	4.386, 1, 0.042	2.966, 1, 0.092	2.878, 1, 0.097	2.985, 1, 0.091	0.324, 1, 0.572	-	-

**Table 2.** The length-weight relationship model for Archidae.

Family/Species	n	Allometric models	R <sup>2</sup>	Relationship model	location	Reference
<b><i>Archidae</i></b>						
<i>Scapharca broughtonii</i>	88	$W=0.000073L^{3.31}$	0.943	(+) Allometric	Korea	Park and Oh, 2002
<i>Scapharca subcrenata</i>	114	$W=0.0004L^{2.97}$	0.935	Isometric	Korea	Park and Oh, 2003
<i>Tegilarca granosa</i>	377	$W=0.00068L^{2.82}$	0.96	(-) Allometric	Korea	Park and Oh, 2004
<i>Anadara granosa</i>	640	$W=0.0016L^{2.618}$	0.884	(-) Allometric	Banda Aceh, Indonesia	Current research
<i>Anadara granosa</i>	640	$W=0.009L^{2.763}$	0.924	(-) Allometric	Lhokseu mawe, Indonesia	Current research
<i>Anadara granosa</i>	640	$W=0.061L^{2.202}$	0.735	(-) Allometric	Pulau Pinang, Malaysia	Current research

Table 3

**Table 3.** Ranges of the seasonal environmental parameter at the sampling areas (average±st.dev).

<i>Environmental parameter</i>	June 2009	July 2009	August 2009	September 2009	November 2009	October 2010	December 2009	January 2010	February 2010	March 2010	April 2010	May 2010	June 2010	July 2010	August 2010	September 2010	Average±st.dev
<i>Temperature (°C)</i>																	
Banda Aceh																	
Minimum	26.32	24.27	24.29	25.43	26.90	25.00	25.39	25.79	22.96	22.84	25.47	21.87	25.90	22.82	25.27	26.00	24.78±1.98
Maximum	30.97	30.44	30.45	29.88	32.61	31.93	31.95	32.48	32.93	32.10	31.40	31.03	30.93	32.11	30.98	30.38	31.41±1.37
Lhokseumawe																	
Minimum	28.82	28.82	26.81	28.07	27.71	27.03	28.45	28.06	28.96	28.48	28.60	28.23	27.95	28.23	27.23	27.40	28.05±2.02
Maximum	31.08	31.71	30.06	31.17	30.87	30.33	30.81	30.68	30.75	31.10	30.90	31.65	31.27	31.39	30.84	31.17	30.99±1.28
Pulau Pinang																	
Minimum	27.23	27.52	26.65	27.10	25.87	27.93	26.05	23.68	25.61	26.74	26.37	26.29	26.67	26.74	26.65	27.37	26.53±1.94
Maximum	31.63	31.10	31.45	30.60	30.90	31.43	30.58	28.35	30.46	31.71	31.13	30.68	31.53	31.32	31.45	31.30	30.98±1.32
<i>Salinity (ppt)</i>																	
Banda Aceh	32.27	31.35	29.98	27.47	30.06	27.20	26.45	29.68	31.50	31.16	29.30	30.71	30.85	31.29	30.45	28.27	29.87±3.23
Lhokseumawe	31.00	30.97	31.16	31.20	31.03	29.07	30.94	31.16	31.46	31.84	30.57	31.26	30.90	30.81	30.65	31.27	30.95±1.04
Pulau Pinang	29.33	28.52	26.39	26.87	27.35	26.13	25.23	28.06	29.00	28.94	28.67	26.48	29.70	29.32	31.06	30.40	28.22±2.97
<i>pH</i>																	
Banda Aceh	7.65	8.02	8.03	8.17	7.80	8.02	7.91	8.17	8.08	8.06	8.02	7.97	7.74	8.27	7.94	8.23	8.01±0.17
Lhokseumawe	8.13	7.88	8.04	8.06	8.17	8.13	7.98	8.21	7.89	8.18	7.91	7.84	8.08	7.93	7.99	8.13	8.03±0.13
Pulau Pinang	8.02	7.49	7.85	8.14	8.04	8.07	7.79	8.02	7.95	7.86	8.08	8.21	7.91	7.86	7.33	7.54	7.89±0.25
<i>Dissolved oxygen (mg/L)</i>																	
Banda Aceh	6.53	6.81	6.96	6.05	5.97	6.12	6.05	5.95	6.10	5.84	6.23	5.86	6.68	5.84	6.32	5.47	6.17±0.40
Lhokseumawe	6.01	6.38	6.47	6.14	6.28	6.04	6.07	6.10	6.97	6.02	6.17	5.98	6.02	5.89	6.28	6.13	6.18±0.25
Pulau Pinang	7.20	5.20	5.20	4.90	5.13	5.21	5.09	5.29	5.20	5.64	5.87	5.39	7.67	5.64	6.29	5.87	5.67±0.78
<i>Turbidity (NTU)</i>																	
Banda Aceh	17.40	29.30	8.61	9.02	10.86	9.12	19.16	14.09	16.03	10.27	18.98	13.83	10.27	34.29	9.74	10.48	15.09±7.43
Lhokseumawe	43.20	30.50	36.50	66.90	31.60	15.18	103.00	93.67	29.13	37.30	64.92	38.95	35.30	49.98	46.90	98.30	51.33±25.24
Pulau Pinang	29.30	17.36	15.11	13.09	17.27	74.30	57.80	77.10	109.67	107.00	98.00	76.00	103.40	107.00	76.65	93.12	67.01±36.44
<i>Orthophosphate (mg/L)</i>																	
Banda Aceh	0.05	0.03	0.04	0.13	0.03	0.02	0.00	0.40	0.00	0.07	0.13	0.07	0.03	0.09	0.08	0.53	0.11±0.15
Lhokseumawe	0.05	0.01	0.02	0.07	0.01	0.01	0.01	0.70	0.00	0.04	0.06	0.04	0.04	0.08	0.07	0.01	0.08±0.17
Pulau Pinang	0.10	0.05	0.08	0.06	0.03	0.01	1.00	0.01	0.16	0.13	0.09	0.08	0.52	0.13	0.15	0.81	0.21±0.29
<i>Nitrate (mg/L)</i>																	

Banda Aceh	0.71	0.03	0.11	0.73	0.75	0.01	0.04	0.05	0.05	0.09	0.23	0.63	0.18	0.05	0.53	0.65	0.30±0.31
Lhokseumawe	0.68	0.14	0.03	0.03	0.20	0.01	0.00	0.03	0.01	0.10	0.77	0.58	0.10	0.17	0.07	0.98	0.24±0.24
Pulau Pinang	0.80	0.02	1.30	0.73	0.03	2.02	0.03	1.02	0.11	0.14	0.61	0.42	0.64	0.14	0.65	1.76	0.65±0.62
<i>Nitrite (mg/L)</i>																	
Banda Aceh	0.05	0.02	0.02	0.03	0.75	0.01	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.03	0.04	0.05	0.08±0.17
Lhokseumawe	0.03	0.03	0.04	0.04	0.05	0.03	0.44	0.03	0.43	0.02	0.08	0.07	0.02	0.08	0.03	0.04	0.09±0.14
Pulau Pinang	0.03	0.03	0.04	0.06	1.09	1.68	0.18	1.00	0.13	0.12	0.09	0.03	0.14	0.12	0.39	0.87	0.37±0.50
<i>Ammonia (mg/L)</i>																	
Banda Aceh	0.87	0.20	0.16	0.15	0.11	0.68	0.06	0.13	0.08	0.09	0.19	0.79	0.18	0.06	0.59	0.13	0.28±0.27
Lhokseumawe	0.19	0.17	0.25	0.25	0.14	0.35	0.23	0.30	0.19	0.27	0.39	0.49	0.27	0.21	0.43	0.24	0.27±0.10
Pulau Pinang	0.24	0.18	0.14	0.25	0.42	0.11	0.61	0.15	0.68	0.54	0.65	0.98	0.65	0.54	0.82	0.65	0.48±0.27
<i>Phytoplankton density (cell/L)</i>																	
Banda Aceh	1831.67	1446.67	851.67	1178.33	630.00	991.67	1201.67	385.00	1773.33	1388.33	1516.67	1785.00	1738.33	1283.33	1341.67	1108.33	1278.23±433.14
Lhokseumawe	1656.67	1365.00	711.67	2601.67	3010.00	1435.00	4001.67	1365.00	2986.67	2415.00	2333.33	2298.33	2415.00	1050.00	1003.33	2310.00	2059.90±225.33
Pulau Pinang	4340.00	4001.67	1470.00	11713.33	4340.00	4281.67	2636.67	4561.67	4235.00	5751.67	5693.33	6090.00	5728.33	5751.65	2905.00	7910.00	5088.12±937.89

## COVER LETTER FOR SUBMISSION OF REVISION MANUSCRIPT

Ocean Sciences Journal (OSJ)

### COVER LETTER FOR SUBMISSION OF MANUSCRIPT

Date: December, 5<sup>th</sup> 2020

We appreciate the opportunity to revise our manuscript. With this cover letter, we will submit the revised manuscript (Ms. No. OSJO-D-20-00110) entitled “Biometric relationship of *Anadara granosa* (Bivalvia: Arcidae) from the northern region of the Strait of Malacca” for publication in OSJ. We carefully considered the comments offered by the reviewers. We would like to thank the referees for the careful and constructive reviews. Detailed corrections have been listed below point by point, and the major revised parts are highlighted in **red** color in the revised manuscript. We want to extend our appreciation for taking the time and effort necessary to provide such insightful guidance.

Based on the comments from the referees, we have made changes to the manuscript, which are detailed below.

#### **Reply to the evaluation by the First Referee:**

We would like to express our appreciation for your incredibly thoughtful comments and constructive criticisms of our manuscript. As you will see below, we have been able to revise and improve the paper as a result of your valuable feedback. Detailed corrections have been listed point by point, and the major revised parts are highlighted in **red** color in the revised manuscript.

#### General comment:

Reviewer #1: It's a good manuscript that can contribute information related to this species, growth and environment impact. But there are certain point or method that are not clearly mention in the manuscript. There are certain term used in this paper are not standardize. Few amendments need to be made to produce a good paper. Please rephrase any highlighted phrases to be easier understand by reader. I would like to suggest the author to proofread before submission.

*Answer: The author revised the manuscript following referee comment directly in the paper. Proofread was made during revision process.*

#### Specific comment:

*Comment on manuscript and response:*

1. There are certain term used in this paper are not standardize.

*Answer: The author was revised the 'term' that suggested by reviewer in the manuscript.*

2. Please rephrase any highlighted phrases to be easier understand by reader

*Answer: The author revised the highlighted phrases that suggested by reviewer in the manuscript. e.g., The consideration that could cause variances in biometric components and thus affected *A. granosa* growth patterns between cluster A and cluster B were spatial differentiation. The source of *A. granosa* from cluster A was distinct from the cluster B, where *A. granosa* populations within cluster A came from the northern region of Sumatra island, while *A. granosa* in the cluster B originated from the western region of the Peninsular Malaysia.*

3. I would like to suggest the author to proofread before submission.

*Answer: the manuscript was checked and corrected by professional English translator in biological sciences and native speakers.*

4. Typos and other grammatical errors (suggested by reviewer) were changed directly in the manuscript (highlighted in red color)

5. Before you start with your sample collection, justify a bit about the study sites for example, three locations of the muddy natural habitat of *Anadara granosa* were selected at the Strait of Malacca. Why? then you can explain about the procedure:

*Answer: the author added: The selection of these three sampling areas was based on the geographical distribution aspect of *A. granosa* in the northern region of the Malacca Strait. Banda Aceh and Lhokseumawe sampling areas shared similar characteristics by larger coastal mudflat areas exposed during all low tides, minimum wave action, high salinity surrounding by mangroves patch and it becomes a natural habitat for *A. granosa*. Meanwhile, Pulau Pinang sampling area differentiated by larger cockle culture plots, continuously submerged underwater and composited by muddy substrate with no wave action, located bordering industrial zones and considered being disturbed by human activities.*

6. Environmental parameter did not mention in method.

*Answer: the author added subchapter 2.3.Environmental parameter measurement*

### **Reply to the evaluation by the Second Referee:**

#### General comment:

Reviewer #2: A well prepared article which findings are supported by the statistical analysis. The manuscript is acceptable.

*Answer: We would like to thank you for your time to read and respond to the manuscript and finally gave 'accepted decision' for our manuscript.*

#### Specific comment:

*No specific comment was made by second referee*

1 **Abstract**

2 The study on the growth pattern of blood cockle *Anadara granosa* focused on the aspects of  
3 biometric prints on the shell, which aimed to predict the growth of *A. granosa* population in  
4 the northern region of **Malacca Strait**. The local sample populations of the cockle were  
5 collected in three different intertidal areas called Lhokseumawe and Banda Aceh in Indonesia  
6 and Pulau Pinang in Malaysia. The biometric analysis showed that the length-weight  
7 relationship model of *A. granosa* populations in this region indicated that the cockle population  
8 generally had a negative allometric growth pattern ( $b < 3$ ) or shell length is more dominant  
9 compare to shell weight. Therefore, the result showed that the growth performance of *A.*  
10 *granosa* was not ideal, where the *b* value (the coefficient of biometric relationship) was highest  
11 recorded in Lhokseumawe, followed by Banda Aceh and Pulau Pinang. The value of coefficient  
12 *b* could be affected by various factors such as environmental conditions, adaptation, and dietary  
13 patterns. Cluster analysis displayed that the population of *A. granosa* from the northern region  
14 of the Strait of Malacca was divided into two clusters, which were *A. granosa* from the northern  
15 Strait of Malacca (Banda Aceh and Lhokseumawe in Indonesia) and population of *A. granosa*  
16 from the western Strait of Malacca (Pulau Pinang in Malaysia). The **f**actors that might cause  
17 the differences in the biometric component of both clusters were geographical level on the  
18 source of population and the locality of the environmental parameter.

19

20 **Keywords:** blood cockle; bivalvia; growth model; **Strait of Malacca**; morphometric

## 21 1. Introduction

22 *Anadara granosa* is one of the important fishery commodities in several areas of  
23 Southeast Asia. This species has been cultivated in countries such as Malaysia and Thailand  
24 due to limited natural stocks. However, this species in Indonesia still harvested directly from  
25 nature (Broom 1983; Khalil et al. 2017). Nevertheless, annual harvested-cockle data designate  
26 a reduction in natural stocks in the last decade. The huge demand for this species as a protein  
27 source marks the natural stocks significantly diminished. This condition is possibly caused by  
28 **lack in management of** controlling wild cockle population stock. Therefore, the management  
29 of this species is required for the sustainability of this important species. Comprehensive  
30 information on biometric (morphometric relationship pattern of the species) is necessary to  
31 predict the annual recruitment, as well as to interpret growth, mortality, reproductive biology,  
32 and survival data in the marine culture of species (Kim et al. 2006; Peharda et al. 2007; Pinn et  
33 al. 2005; Zelditch et al. 2004).

34 Length-weight is an essential variable for comparing growth, physiological processes,  
35 and environmental factors that affect aquatic organisms (Hemachandra and Thippeswamy,  
36 2008). Growth of bivalves can be defined as the increase of the length size of the shell and  
37 body weight (body mass) that have also been used extensively as the corresponding parameters  
38 to assess the growth of them (Bailey and Green 1988; Bayne and Worrall 1980; Garton and  
39 Haag 1991; Smit et al. 1992). Measuring the length and weight of aquatic species is used to  
40 evaluate the growth patterns of this species quantitatively. Such relationships are expressed via  
41 the data distribution of shell length and cockle weight. These data also represent the ratio of  
42 the addition of an animal's body size by period. Length and weight relationships have several  
43 purposes, namely (1) for measuring the weight and length ratio of a species to the weight-length  
44 in Taxa class (Anderson and Neumann 1996; Shine 1990), and (2) for age (Pauly 1983).



45 The length and weight relationship allows life history and morphological differences to  
46 be identified between species and populations from different habitats (Beukema and Meehan  
47 1985; Gaspar et al. 2002; Holopainen and Hansk 1986; Morton 1985; Peters 1985). This study  
48 aimed at analyzing the biometric relationship of *A. granosa* by using a morphometric  
49 relationship and dendrogram analysis of specimens collected from the northern region of the  
50 Strait of Malacca.

## 51 2. Materials and Methods

### 52 2.1 Samples collection

53 The specimens of *A. granosa* (120 specimens/month) were collected monthly from June 2009  
54 till September 2010 from the muddy natural habitat in Banda Aceh (5°32'34.67"N -  
55 95°17'2.54"E), Lhokseumawe (05°09'35.3"N - 097°08'29.4"E) in Aceh Province, Indonesia  
56 and Pulau Pinang (5°16'9.66"N - 100°23'27.37"E) in Malaysia (Fig. 1). The selection of these  
57 three sampling areas was based on the geographical distribution aspect of *A. granosa* in the  
58 northern region of the Malacca Strait. Banda Aceh and Lhokseumawe sampling areas shared  
59 similar characteristics by larger coastal mudflat areas exposed during all low tides, minimum  
60 wave action, high salinity surrounding by mangroves patch and it becomes a natural habitat for  
61 *A. granosa*. Meanwhile, Pulau Pinang sampling area differentiated by larger cockle culture  
62 plots, continuously submerged underwater and composited by muddy substrate with no wave  
63 action, located bordering industrial zones and considered being disturbed by human activities.

64 *Insert figure 1.*

65 The total number of specimens sampled was 1,920, with cockle sizes ranged from 38–  
66 71 mm in length. The specimens were collected at a depth of 5-30 cm, and salinity ranged from  
67 10-33 ppt. The live samples were collected manually with the aid of a harrow during the low  
68 tide period. After collecting, the specimens were stowed in isotherm containers and directly  
69 transferred to the laboratory. The samples were cleaned from mud and organisms attached to

70 the shell in the laboratory, reared in the aquarium, and continued with the recorded biometric  
71 values (Fig. 2), including shell length, shell thickness, cockle height, fresh tissue weight, wet  
72 cockle weight, and sex category. The measurement instrument of length and width of the cockle  
73 used a digital Vernier caliper with an accuracy of 0.1 mm, and the cockle weight tissue was  
74 weighed using a digital weighing scale (in grams). The length was defined as the maximum  
75 shell length (measured from the posterior margin to the anterior margin of the cockle); the  
76 thickness was measured on the inflating position, from the most protruding part on the top of  
77 cockle to the most protruding portion on the bottom of cockle. The height was measured from  
78 the highest ventral margin of the cockle towards the dorsal margin of the cockle.

79 *Insert figure 2.*

## 80 2.2 Morphometric relationship

81 The morphometric ratio of *A. granosa* between length : height, length : thickness and  
82 height: thickness analyzed using the following formula:

$$83 a = L / H, a = L / C \text{ and } a = H / C$$

84 Where: L = shell length, H = shell height, C = shell thickness, a = index (coefficient),

85 The growth pattern of the cockle was able to be designated through a relationship of  
86 shell length and cockle body weight (wet weight), which was analyzed through the equation of  
87 power regression (Ricker 1975). From the results, then it was informed that if the growth rate  
88 of cockle length balanced with the cockle weight or in the mathematical expression  $b = 3$ , then  
89 it was assumed that the cockle growth was isometric. Whereas if  $b \neq 3$ , it was called allometric,  
90 which means the growth of cockle length imbalanced with the weight. To test whether the  
91 constants  $b = 3$  or  $b \neq 3$  (isometric or allometric), a statistical test was performed through a  
92 statistical *t*-test. The equation above applied both to the whole cockle and by sex. Based on the  
93 statistical *t*-test, the hypothesis used were:

94  $H_0: b = 3$ , shell length and cockle weight relationship was isometric

95 H<sub>1</sub>:  $b \neq 3$  mean shell length and cockle weight relationship was allometric (namely: positive  
96 allometry), if  $b > 3$  meant that the growth of cockle weight was faster than the growth of shell  
97 length (namely: negative allometry) and if  $b < 3$  mean the growth of shell length was faster  
98 than the cockle weight).

### 99 *2.3. Environmental parameter measurement*

100 Maximum and minimum seawater temperatures were measured daily using a portable  
101 max-min thermometer device fixed in the sample area. Seawater salinity, pH, and dissolved  
102 oxygen were assessed regularly using a handheld Multiparameter Portable Meter (Hanna HI-  
103 9828) at the study site, where turbidity was measured with a turbidity meter (Turbidity meter  
104 800-ESD). Monthly analyzes of dissolved nutrients for ammonium, nitrate, nitrite, and  
105 phosphate concentrations were performed using standard methods (Brewer and Riley 1965,  
106 Grasshoff 1976; Mantoura and Woodward 1983; Kirkwood 1989; Zhang and Chi 2002).  
107 Phytoplankton samples were obtained monthly from mid-surface water by towing a plankton  
108 net (mouth diameter 0.35 m), made of bolting silk (No. 30, mesh size: 48  $\mu$ M) for 30 minutes  
109 and preserved using Lugol's solution. Phytoplankton cell density was measured monthly using  
110 a hemocytometer and a compound microscope following the Martinez et al. (1975) protocol.

### 111 *2.4 Statistical analysis*

112 The raw data obtained was collected and put into a package of Microsoft Excel 2011  
113 software Macintosh version to be processed and analyzed. Statistical analysis of Co-Variant  
114 Analysis was used to determine significant differences in the values obtained in each collected  
115 group data. The determining factor used in this study was the population differences in the  
116 three different regions. Therefore, the coefficients  $a$  and  $b$  were analyzed by observing the  
117 growth differences in population and sex differences in the sample (male, female, or neutral).  
118 The statistical test was continued by the post hoc test to ensure which factor significantly  
119 differed in one particular parameter.

120 The parametric statistical test of Co-Variant analysis was analyzed by using the package  
121 of SPSS (Statistical Package for the Social Science) software release 23.0 Macintosh version.  
122 The relationships existed between two variables: the relationship between the  $b$  coefficient  
123 (relationship of shell length and cockle weight) and environmental factors in the sampling area.  
124 Hypothesis testing was then performed on the sample parameters to test **the significance of**  
125 **correlation** at the level of 95% ( $P = 0.05$ ). The statistical  $t$ -test was used to find significant  
126 differences in the pattern of change in the  $b$  coefficient that was the growing nexus of shell  
127 length and cockle weight of each sampling area.

128 Cluster analysis through the dendrogram diagram was designed to clarify the  
129 relationship between each biometric component (Ramesha and Thippeswamy 2009) on *A.*  
130 *granosa* from the northern region of the Strait of Malacca. Cluster analysis was processed by  
131 using SPSS (Statistical Package for the Social Science).

### 132 **3. Results**

#### 133 *3.1 Equality $a$ and $b$ coefficients from different populations*

134 The biometric studies of *A. granosa* (from June 2009-September 2010) from the  
135 northern region of the **Strait of Malacca involving 1920 individuals consisted** of 756 males,  
136 974 females, and 190 neuters. The statistical analysis showed that *A. granosa* shell height :  
137 shell length relationship from three sampling areas showed dissimilar coefficient  $a$ , but there  
138 was similar to the coefficient  $b$ . Furthermore, **the** relationship of shell thickness : shell length  
139 of *A. granosa* populations showed an identical in coefficient  $a$  and  $b$  between populations. In  
140 contrast, **the** relationship of shell thickness : shell height coefficient ( $a$  and  $b$ ) values in terms  
141 of their allometric equations showed differences between populations (Table 1).

142 *Insert Table 1*

#### 143 *3.2 Morphometric coefficients model from different populations*

144 *A. granosa* weight and shell length relationship analysis showed no differences in  
145 coefficient  $a$  and  $b$  value between populations (Table 2). A statistical test was performed on  
146 the coefficient  $b$  through  $t$ -test indicating the coefficient  $b < 3$  or negative allometry in all *A.*  
147 *granosa* populations. This condition showed that the growth of cockle shell length was faster  
148 or more dominant compared to the growth of cockle weight. A further test of the hypothesis  
149 also showed that  $H_0$  was rejected ( $P < 0.05$ ) and means the growth rate of shell length and cockle  
150 weight overall was imbalanced.

151 *Insert Table 2*

### 152 3.3 *Environmental parameter*

153 Seasonal variations of environmental parameters in the sampling areas are informed in  
154 Table 3. Water temperature, salinity, and phytoplankton concentration fluctuated significantly  
155 compared to other environmental parameters during the study period.

156 *Insert Table 3.*

## 157 4. Discussion

### 158 4.1. *Biometric relationship model of Anadara granosa*

159 Biometric data analysis of *A. granosa* from the northern region of the Strait of Malacca  
160 showed that the cockle growth model was negative allometry. The growth of shell length was  
161 more dominant than the growth of cockle weight. The growth model generated from the three  
162 sampling sites showed that the value of the  $b$  coefficient was less than 3 ( $b < 3$ ). The balance  
163 value of the  $b$  coefficient generally has a range between 2.4 to 4.5 (Wilbur and Owen 1964),  
164 and when the  $b$  value is equal to 3 ( $b = 3$ ), the relationship of shell length and cockle weight is  
165 isometric (Carlander 1969). In this study, the  $b$  coefficient differs in a population or when  
166 compared to other populations. The cockle population from Lhokseumawe had a higher  $b$   
167 coefficient ( $b = 2.7629 \pm 0.3894$ ) compared to the cockle population from Banda Aceh ( $b =$   
168  $2.6178 \pm 0.2095$ ) and the cockle population from Pulau Pinang ( $b = 2.2018 \pm 0.5866$ ). In the sexes

169 level, a similar condition was presented, whereas both male and female *A. granosa* from  
170 Lhokseumawe had the highest  $b$  coefficient (male  $b = 2.7713 \pm 0.567$ , female  $2.7559 \pm 0.3838$ )  
171 compared to other sampling locations (Banda Aceh male  $b = 2.6306 \pm 0.2831$ , female  $b =$   
172  $2.5695 \pm 0.3368$ ; Pulau Pinang male  $b = 2.0043 \pm 0.889$ , female  $b = 2.3697 \pm 0.7607$ ). These  
173 conditions indicated that the cockle growth rate in Lhokseumawe was more appropriate or  
174 suitable compared to the other two sampling areas. The  $b$  coefficient value of biometric  
175 relationships is characteristically compared between dimensional growth of related or similar  
176 species in various geographical areas (Ramesha and Sophia 2015).

177         The contrast conditions could be seen on the cockle found from the Pulau Pinang area,  
178 where the value of the  $b$  coefficient was lower than the range value of the  $b$  coefficient for most  
179 bivalves 2.4-4.5 that was described by Wilbur and Owen (1964), causing the shell length  
180 against cockle weight becomes imprecise. Shell length was growth faster than the increase of  
181 the cockle body volume, affecting the cockle to be unhealthy. Factors such as the reproductive  
182 biology (Rueda and Urban 1998), physical and biological variables of habitat (Seed 1968,  
183 Thorarinsdittir and Johannesson 1996) are recognize affected the growth and be able to change  
184 the allometry relationship between the shell length and the cockle weight in bivalvia. Water  
185 quality analysis showed that this condition due to the fluctuation in the environmental condition  
186 in cockle habitats. Furthermore, the environment quality was unsuitable for cockle growth due  
187 to an increased level of nutrients that exceeded the standards rate for marine life (Table 3).  
188 From observation, high concentration of nutrients potentially toxic to the cockle and affected  
189 cockle growth. Environmental aspects have been identified as the main factor that affects shell  
190 development in bivalves. The shell size and shape are affected by the variation of ambient  
191 environmental constraints (Wilbur and Owen 1964; Seed 1968).

192         Furthermore, the variety of growth patterns of *A. granosa* highly correlates to factors  
193 of food availability, temperature, salinity, pollution materials, and reproductive activities

194 (Broom 1982; Day and Fleming, 1992; Tarr, 1995). The phytoplankton concentration changes  
195 were expected as the primary regulator of the fluctuation of the  $b$  coefficient from all sampling  
196 areas. The Pearson correlation test showed the opposite condition: the  $b$  coefficient strongly  
197 correlated to phytoplankton density in Banda Aceh ( $r = 0.766$ ). In comparison, the  
198 phytoplankton density showed a moderate correlation with the  $b$  coefficient for cockle from  
199 Lhokseumawe ( $r = 0.532$ ) and Pulau Pinang ( $r = 0.579$ ). The phytoplankton density was  
200 expected as a limiting factor for growth activity. The phytoplankton was used as an energy  
201 source for the growth process of shell length and cockle weight. The supply of food sources is  
202 considered an essential factor for sustainable growth (Seed and Suchanek 1992; Widdows and  
203 Johnson 1988).

204 Changes in the  $b$  coefficient fluctuation were also expected to have a relationship with  
205 the reproduction period. Sudden changes in the value of the  $b$  coefficient meant that there was  
206 a rapid change in the cockle weight tissue due to a few biomass of cockle. Weight reduction of  
207 the cockle volume could be caused by the reproduction process, such as the gamete production  
208 process and gonad or gamete process in a state of inactivity. In bivalve animals, gonadal growth  
209 and gonadal maturation process results increase the tissue mass density and increase tissue  
210 weight. Exchange of the value of the  $b$  coefficient indicates the beginning of the activities of  
211 gonadal maturation and growth in bivalve animals (Hemachandra and Thippeswamy 2008;  
212 Hickman and Illingworth 1980).

213 The total cockle weight is described as the total shell weight, including the weight of  
214 cockle meat. In *A. granosa*, the shell weight was generally heavier than the meat weight. When  
215 the shell size increased, then the overall weight of the cockle also increased linearly. However,  
216 the analysis of the samples showed no significant weight despite the shell size increased. This  
217 condition was assumed to be the result of the increased volume of cockle meat that did not gain  
218 or develop linearly, causing the shell length growth was not in line with the cockle weight.

219 The growth pattern was not always fixed for species. Differences in growth models  
220 could be found in the same or different species, among sex, indifferent or the same locations  
221 and in different seasons. The difference in latitudinal gradient is also related to the shell size,  
222 reproduction level and reproductive cycle in bivalves (Kanazawa and Sato 2008; Mirzaei et al.  
223 2017) as well as the growth pattern models of cockle in these three sampling areas. Cockles  
224 from Banda Aceh and Lhokseumawe had morphological differences compared to the cockles  
225 from Pinang. *A. granosa* from Banda Aceh and Lhokseumawe had special shell features that  
226 were thicker and wider compared to *A. granosa* cockles from Pulau Pinang. The other studies  
227 on the relationship in length and weight on some *Anadara* species had demonstrated diversity  
228 and differences in growth patterns (Table 4).

229 *Insert table 4*

230 The value of the *b* coefficient on the relationship of shell length and cockle weight noted  
231 in this study was lower than the recorded one in other species in the same family, namely  
232 *Tegilarca granosa* ( $b = 2.82$ ) from Korean waters. Another difference was also found in their  
233 relationship model, namely *Scapharca* from Korean water, which had positive allometry and  
234 isometric relationships, while *A. granosa* from the northern region of the Strait of Malacca was  
235 negative allometry. Different growth patterns at different latitudes might be caused by the  
236 influence of environmental factors, changes in the composition of the food, and competition  
237 between individuals that were locality in habitat.

#### 238 4.2. Relationships of the biometric component of *Anadara granosa*

239 Analysis of the relationship of each biometric parameter of *A. granosa* through  
240 dendrogram or classification methods (hierarchy) was used to analyze the growth patterns of  
241 the three *A. granosa* populations in the northern region of the Strait of Malacca. The  
242 dendrogram was designed to clarify the relationship of each biometric component in cockles.  
243 Fig. 3 showed the relationship between the biometric component of *A. granosa*. Through this



244 analysis, the degree of dissimilarity between generated clusters had shown. The relationship  
245 between a biometric component in one cluster compared to another biometric component in  
246 the different cluster within the dendrogram was generated by the distance of the scale, e.g., if  
247 the scale value found high, it is assumed that the distinction between two biometric components  
248 in two different clusters was correspondingly high.

249 *Insert fig. 3.*

250 Dendrogram analysis showed that cluster A had two sub-clusters; namely, A1  
251 represented the biometric component of *A. granosa* from Lhokseumawe and A2 represented  
252 the biometric component of *A. granosa* from Banda Aceh, which was separated on a scale of  
253 16. Cluster B was a hierarchy cluster of biometric components of *A. granosa* from Pulau  
254 Pinang. Cluster B was separated by cluster A on a scale of 25. The larger recorded scale means  
255 the dissimilarity elements forming the component were higher. It showed the growth pattern  
256 based on the biometric parameter was very different from the high level of inequality among  
257 the clusters (population) and sub-clusters (subpopulations). This condition was interpreted that  
258 *A. granosa* population from Lhokseumawe and Banda Aceh (Indonesia) and Pulau Pinang  
259 (Malaysia) had significant differences in shell form and growth pattern and further correlated  
260 to the biometric relationship models. Gaspar et al. (2002, 2001) and Popa et al. (2010) state  
261 that the population can be explained in particular by the growth characteristics through the  
262 measurement of biometric morphology.

263 The consideration that could cause variances in biometric components and thus affected  
264 *A. granosa* growth patterns between cluster A and cluster B were spatial differentiation. The  
265 source of *A. granosa* from cluster A was distinct from cluster B, where *A. granosa* populations  
266 within cluster A came from the northern region of Sumatra island, while *A. granosa* in the  
267 cluster B originated from the western region of Peninsular Malaysia. Sub-clusters A was also  
268 known to have differences in growth patterns of the biometric component; they even had close

269 proximity. *A. granosa* cockles of **sub-cluster A1 and A2 were** expected to come from the same  
270 source of population. Differences in the pattern of biometric components were possible because  
271 of the differences in environmental factors that were locality in nature, affecting the growth  
272 patterns. Differences range of salinity (Carmichael et al. 2004; Schöne et al. 2003), temperature  
273 (Goodwin et al. 2001; Jones et al. 1989; Kennish and Olsson 1975; Pilditch and Grant 1999;  
274 Schöne et al. 2002) and density of phytoplankton (Alunno-Bruscia et al. 2001; Carmichael et  
275 al. 2004; Grant 1996; Lorrain et al. 2000; Miyaji et al. 2007) are **playing a role in** determining  
276 the growth pattern of cockle in three sampling areas.

277

## 278 **Acknowledgments**

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284

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442 **Captions for figures**

443 **Fig. 1.** *Anadara grannosa* sampling location in the northern region of the Strait of Malacca

444 *(Insert after line 63, page 3)*

445 **Fig. 2.** Biometric of *Anadara grannosa*

446 *(Insert after line 78, page 4)*

447 **Fig. 3.** Dendrogram parameter of *Anadara granosa* biometric relationship from the northern  
448 region of Strait of Malacca

449 *(Insert after line 248, page 11)*

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451 **Captions for table**

452 **Table 1.** Equality a and b coefficients in the biometric model from different populations

453 *(Insert after line 141, page 6)*

454 **Table 1.** Equality a and b coefficients in the biometric model from different populations

455 *(Insert after line 150, page 7)*


456 **Table 3.** Ranges of the seasonal environmental parameter at the sampling areas

457 (average±st.dev).

458 *(Insert after line 155, page 7)*

459 **Table 4.** The length-weight relationship model for Archidae.

460 *(Insert after line 228, page 10)*

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1 **Biometric relationship of *Anadara granosa* (Bivalvia: Arcidae) from the northern region**  
2 **of the Strait of Malacca**

3 Munawar Khalil\*<sup>1,2</sup>, Riri Ezraneti<sup>1</sup>, Rachmawati Rusydi<sup>3</sup>, Zulfigar Yasin<sup>4,5</sup>, Aileen Tan Shau  
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21 **Abstract**

22 This study on the growth pattern of the blood cockle *Anadara granosa* focused on the aspects  
23 of biometric prints on the shell, which aimed to predict the growth of the *A. granosa* population  
24 in the northern region of **Malacca Strait**. The local sample populations of the cockle were  
25 collected in three different intertidal areas called Lhokseumawe and Banda Aceh in Indonesia  
26 and Pulau Pinang in Malaysia. The biometric analysis showed that the length-weight  
27 relationship model of *A. granosa* populations in this region indicated that the cockle population  
28 generally had a negative allometric growth pattern ( $b < 3$ ) or that shell length is more dominant  
29 compared to shell weight. Therefore, the result showed that the growth performance of *A.*  
30 *granosa* was not ideal, and the highest *b* value (the coefficient of biometric relationship) was  
31 recorded in Lhokseumawe, followed by Banda Aceh and Pulau Pinang. The value of the  
32 coefficient *b* could be affected by various factors such as environmental conditions, adaptation,  
33 and dietary patterns. Cluster analysis revealed that the population of *A. granosa* from the  
34 northern region of the Strait of Malacca was divided into two clusters, which were *A. granosa*  
35 from the northern Strait of Malacca (Banda Aceh and Lhokseumawe in Indonesia) and *A.*  
36 *granosa* from the western Strait of Malacca (Pulau Pinang in Malaysia). The factors that might  
37 cause the differences in the biometric component of both clusters were at the geographical level  
38 on the source of population and local environmental parameters.

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40 **Keywords:** blood cockle; bivalvia; growth model; **Malacca Strait**; morphometric

41 **1. Introduction**

42 *Anadara granosa* is one of the important fishery commodities in several areas of  
43 Southeast Asia. This species has been cultivated in countries such as Malaysia and Thailand  
44 due to limited natural stocks. However, this species in Indonesia is still harvested directly from  
45 nature (Broom 1983; Khalil et al. 2017). Nevertheless, annual harvested-cockle data reveal a  
46 reduction in natural stocks in the last decade. One of the main factors for the significant reduction  
47 in natural stocks is overharvesting due to the high demand as protein source. This condition may  
48 also be a result of the **lack in management in** controlling the wild cockle population stock.  
49 Therefore, the management of this species is required for the sustainability of this important  
50 species. Comprehensive information on biometrics (morphometric relationship pattern of the  
51 species) is necessary to predict the annual recruitment, as well as to interpret growth, mortality,  
52 reproductive biology, and survival data in the marine culture of species (Kim et al. 2006;  
53 Peharda et al. 2007; Pinn et al. 2005; Zelditch et al. 2004).

54 Length-weight is an essential variable for comparing growth, physiological processes,  
55 and environmental factors that affect aquatic organisms (Hemachandra and Thippeswamy,  
56 2008). Growth of bivalves can be defined as the increase of the length size of the shell and  
57 body weight (body mass) and these indicators have also been used extensively as corresponding  
58 parameters to assess their growth (Bailey and Green 1988; Bayne and Worrall 1980; Garton  
59 and Haag 1991; Smit et al. 1992). Measuring the length and weight of aquatic species is used  
60 to evaluate the growth patterns of species quantitatively. Such relationships are expressed via  
61 the data distribution of shell length and cockle weight. These data also represent the ratio of  
62 the addition of an animal's body size by period. Length and weight relationships have several  
63 purposes, namely (1) for measuring the weight and length ratio of a species to the weight-length  
64 in Taxa class (Anderson and Neumann 1996; Shine 1990), and (2) for age (Pauly 1983).

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67 The length and weight relationship allows life history and morphological differences to  
68 be identified between species and populations from different habitats (Beukema and Meehan  
69 1985; Gaspar et al. 2002; Holopainen and Hanski 1986). This study sought to analyze the  
70 biometric relationship of *A. granosa* by using a morphometric relationship and dendrogram  
71 analysis of specimens collected from the northern region of the Strait of Malacca.

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## 72 2. Materials and Methods

### 73 2.1 Samples collection

74 The specimens of *A. granosa* (120 specimens/month) were collected monthly from June  
75 2009 till September 2010 from the muddy natural habitat in Banda Aceh (5°32'34.67"N -  
76 95°17'2.54"E), Lhokseumawe (05°09'35.3"N - 097°08'29.4"E) in Aceh Province, Indonesia  
77 and Pulau Pinang (5°16'9.66"N - 100°23'27.37"E) in Malaysia (Fig. 1). The selection of these  
78 three sampling areas was based on the geographical distribution characteristics of *A. granosa*  
79 in the northern region of the Malacca Strait. Banda Aceh and Lhokseumawe sampling areas  
80 share similar features such as larger coastal mudflat areas exposed during all low tides,  
81 minimum wave action, high salinity waters surrounded by mangroves patches and such  
82 locations are a natural habitat for *A. granosa*. Meanwhile, Pulau Pinang sampling area is  
83 differentiated by larger cockle culture plots which were continuously submerged underwater  
84 and composited by muddy substrate with no wave action, located near industrial zones and are  
85 thought to be disturbed by human activities.

Commented [cj5]: The meaning is unclear. High salinity waters surrounded by mangrove patches? High salinity waters surrounding mangrove patches?

Commented [MOU6R5]: high salinity waters surrounded by mangroves patches

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Commented [cj7]: The plots are continuously submerged underwater or the sampling area is continuously submerged underwater?

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Commented [MOU8R7]: plots which were continuously submerged underwater

86 *Insert figure 1.*

87 The total number of specimens sampled was 1,920, with cockle sizes ranging from 38–  
88 71 mm in length. The specimens were collected at a depth of 5-30 cm, and salinity ranged from  
89 10-33 ppt. The live samples were collected manually with the aid of a harrow during the low  
90 tide period. After collection, the specimens were stored in isotherm containers and directly  
91 transferred to the laboratory. The samples were cleaned and mud and organisms attached to the

94 shell were removed in the laboratory, then reared in the aquarium where biometric values were  
95 continuously recorded (Fig. 2), including shell length, shell thickness, cockle height, fresh  
96 tissue weight, wet cockle weight, and sex category. The measurement of length and width of  
97 the cockle was taken with a digital Vernier caliper with an accuracy of 0.1 mm, and the cockle  
98 weight tissue was weighed using a digital weighing scale (in grams). The length was defined  
99 as the maximum shell length (measured from the posterior margin to the anterior margin of the  
100 cockle); the thickness was measured on the inflating position, from the most protruding part on  
101 the top of cockle to the most protruding portion on the bottom of cockle. The height was  
102 measured from the highest ventral margin of the cockle towards the dorsal margin of the cockle.

103 *Insert figure 2.*

## 104 2.2 Morphometric relationship

105 The morphometric ratio of *A. granosa* between length: height, length: thickness and  
106 height: thickness was analyzed using the following formula:

$$107 a = L / H, a = L / C \text{ and } a = H / C$$

108 Where: L = shell length, H = shell height, C = shell thickness, a = index (coefficient),

109 The growth pattern of the cockle was able to be designated through the relationship of  
110 shell length and cockle body weight (wet weight), which was analyzed through the equation of  
111 power regression (Ricker 1975). From the results it could be determined if the growth rate of cockle  
112 length balanced with the cockle weight or the ratio (between length and weight) could be formulated in  
113 the mathematical expression  $b = 3$ , and if so then it was assumed that the cockle growth was  
114 isometric. Whereas if  $b \neq 3$ , it was allometric, which means the growth of cockle length was  
115 not proportionate to in balance the weight. To test whether the constants were  $b = 3$  or  $b \neq 3$   
116 (isometric or allometric), a statistical test was performed through a statistical *t*-test. The  
117 equation above applied both to the whole cockle and by sex. Based on the statistical *t*-test, the  
118 hypotheses used were:

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Commented [MOU10R9]: The punctuation used to mark 'comparison' between two variables.

Commented [cj11]: The meaning is not clear. Do you mean: from the results it could be determined ..... or the ratio (between length and weight) could be formulated in the mathematical expression  $b=3$ ?

Commented [MOU12R11]: From the results it could be determined if the growth rate of cockle length balanced with the cockle weight or the ratio (between length and weight) could be formulated in the mathematical expression  $b=3$

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Commented [cj13]: The length was not in sync with the weight? The length was not proportionate to the weight?

Commented [MOU14R13]: cockle length was not proportionate to the weight?

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Commented [cj16R15]: To test whether the constants were  $b=3$  or  $b$  not equal 3? Or, to test the constants  $b=3$  or ...?

Commented [MOU17R15]: To test whether the constants were  $b = 3$  or  $b \neq 3$

120  $H_0: b = 3$ , shell length and cockle weight relationship was isometric  
121  $H_1: b \neq 3$  mean shell length and cockle weight relationship was allometric (namely: positive  
122 allometry), if  $b > 3$  meant that the growth of cockle weight was faster than the growth of shell  
123 length (namely: negative allometry) and if  $b < 3$  meant the growth of shell length was faster  
124 than the cockle weight.

### 125 2.3. Environmental parameter measurement

126 Maximum and minimum seawater temperatures were measured daily using a portable  
127 max-min thermometer device fixed in the sampling areas. Seawater salinity, pH, and dissolved  
128 oxygen were assessed regularly using a handheld Multiparameter Portable Meter (Hanna HI-  
129 9828) at the study sites, where turbidity was measured with a turbidity meter (Turbidity meter  
130 800-ESD). Monthly analyzes of dissolved nutrients for ammonium, nitrate, nitrite, and  
131 phosphate concentrations were performed using standard methods (Brewer and Riley 1965,  
132 Grasshoff 1976; Mantoura and Woodward 1983; Kirkwood 1989; Zhang and Chi 2002).  
133 Phytoplankton samples were obtained monthly from mid-surface water by towing a plankton  
134 net (mouth diameter 0.35 m) made of bolting silk (No. 30, mesh size: 48  $\mu\text{M}$ ) for 30 minutes  
135 and preserved using Lugol's solution. Phytoplankton cell density was measured monthly using  
136 a hemocytometer and a compound microscope following the Martinez et al. (1975) protocol.

### 137 2.4 Statistical analysis

138 The raw data obtained were collected and put into a package of Microsoft Excel 2011  
139 software Macintosh version to be processed and analyzed. Statistical analysis of Co-Variant  
140 Analysis was used to determine significant differences in the values obtained in each collected  
141 group data. The determining factor used in this study was the population differences in the  
142 three different regions. Therefore, the coefficients  $a$  and  $b$  were analyzed by observing the  
143 growth differences in population and sex differences in the sample (male, female, or neutral).

Commented [cj18]: Areas?

Commented [MOU19R18]: Areas

Commented [cj20]: Study sites?

Commented [MOU21R20]: Study sites

144 The statistical test was continued by the post hoc test to [determine](#) which factor significantly  
145 differed in one particular parameter.

146 The parametric statistical test of Co-Variant analysis was analyzed by using the package  
147 of SPSS (Statistical Package for the Social Science) software [version 23.0](#) Macintosh version.

148 Relationships existed between two variables: the relationship between the *b* coefficient  
149 (relationship of shell length and cockle weight) and environmental factors in the sampling area.

150 Hypothesis testing was then performed on the sample parameters to test **the significance of**  
151 **correlation** at the level of 95% ( $P = 0.05$ ). The statistical *t*-test was used to find significant  
152 differences in the pattern of change in the *b* coefficient that was the growth nexus of shell length  
153 and cockle weight of each sampling area.

154 Cluster analysis through the dendrogram diagram was designed to clarify the  
155 relationship between each biometric component (Ramesha and Thippeswamy 2009) [regarding](#)  
156 *A. granosa* from the northern region of the Strait of Malacca. Cluster analysis was processed  
157 by using SPSS (Statistical Package for the Social Science).

### 158 3. Results

#### 159 3.1 Equality *a* and *b* coefficients from different populations

160 The biometric studies of *A. granosa* (from June 2009-September 2010) from the  
161 northern region of the Strait of Malacca **involving 1920 individuals consisted** of 756 males,  
162 974 females, and 190 neuters. The statistical analysis showed that *A. granosa* shell height: shell

163 length relationship from three sampling areas **revealed a dissimilar coefficient *a*, but the**  
164 **coefficient *b* was similar.** Furthermore, **the** relationship of shell thickness: shell length of *A.*  
165 *granosa* populations showed [that the coefficients \*a\* and \*b\* were identical between](#) populations.

166 In contrast, **the** relationship of shell thickness: shell height coefficient (*a* and *b*) values in terms  
167 of their allometric equations showed differences between populations (Table 1).

168 *Insert Table 1*

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Commented [cj23]: Is this correct?

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Commented [cj25]: Is this correct? Or, showed that the coefficients *a* and *b* were identical between populations?

Commented [MOU26R25]: showed that the coefficients *a* and *b* were identical between populations

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171 3.2 *Morphometric coefficients model from different populations*

172 *A. granosa* weight and shell length relationship analysis showed no differences in  
173 coefficient *a* and *b* values between populations (Table 2). A statistical test was performed on  
174 the coefficient *b* through a *t*-test indicating the coefficient  $b < 3$  or negative allometry in all *A.*  
175 *granosa* populations. This condition showed that the growth of cockle shell length was faster  
176 or more dominant compared to the growth of cockle weight. A further test of the hypothesis  
177 also showed that  $H_0$  was rejected ( $P < 0.05$ ) and indicates that the growth rate of shell length  
178 and cockle weight was in an overall imbalance.

179 *Insert Table 2*

180 3.3 *Environmental parameter*

181 Seasonal variations of environmental parameters in the sampling areas are presented in  
182 Table 3. Water temperature, salinity, and phytoplankton concentration fluctuated significantly  
183 compared to other environmental parameters during the study period.

184 *Insert Table 3.*

185 **4. Discussion**

186 4.1. *Biometric relationship model of Anadara granosa*

187 Biometric data analysis of *A. granosa* from the northern region of the Strait of Malacca  
188 showed that the cockle growth model was negative allometry. The growth of shell length was  
189 more dominant than the growth of cockle weight. The growth model generated from the three  
190 sampling sites showed that the value of the *b* coefficient was less than 3 ( $b < 3$ ). The balance  
191 value of the *b* coefficient generally has a range between 2.4 to 4.5 (Wilbur and Owen 1964),  
192 and when the *b* value is equal to 3 ( $b = 3$ ), the relationship of shell length and cockle weight is  
193 isometric (Carlander 1969). In this study, the *b* coefficient differs within a population or when  
194 compared to other populations. The cockle population from Lhokseumawe had a higher *b*  
195 coefficient ( $b = 2.7629 \pm 0.3894$ ) compared to the cockle population from Banda Aceh ( $b =$

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Commented [cj27]: Differs in the populations sampled? Differs within a population?

Commented [MOU28R27]: *b* coefficient differs within a population

196 2.6178±0.2095) and the cockle population from Pulau Pinang ( $b = 2.2018 \pm 0.5866$ ). According  
197 to sexual orientation, a similar condition was presented, although both male and female *A.*  
198 *granosa* from Lhokseumawe had the highest  $b$  coefficient (male  $b = 2.7713 \pm 0.567$ , female  
199  $2.7559 \pm 0.3838$ ) compared to other sampling locations (Banda Aceh male  $b = 2.6306 \pm 0.2831$ ,  
200 female  $b = 2.5695 \pm 0.3368$ ; Pulau Pinang male  $b = 2.0043 \pm 0.889$ , female  $b = 2.3697 \pm 0.7607$ ).  
201 These  $b$  coefficient results indicated that the cockle growth rate in Lhokseumawe was more  
202 appropriate or suitable compared to the other two sampling areas. The  $b$  coefficient value of  
203 biometric relationships is characteristically compared between dimensional growth of related  
204 or similar species in various geographical areas (Ramesha and Sophia 2015).

205 The contrary conditions could be found among cockles from the Pulau Pinang area,  
206 where the value of the  $b$  coefficient was lower than the range value of the  $b$  coefficient for most  
207 bivalves at 2.4-4.5 that was described by Wilbur and Owen (1964), causing the cockle shell  
208 length against cockle weight showed an unbalanced condition causing the cockle shell length  
209 against cockle weight showed an unbalanced condition. Shell length growth was faster than the  
210 increase of the cockle body volume, causing the cockle to be unhealthy. Factors such as  
211 reproductive biology (Rueda and Urban 1998), and the physical and biological variables of a  
212 habitat (Seed 1968, Thorarinsdottir and Johannesson 1996) are recognized as affecting growth  
213 and can change the allometry relationship between the shell length and the cockle weight in  
214 bivalvia. Water quality analysis showed that faster increase in shell length compared to body  
215 weight was due to the fluctuation in environmental conditions in cockle habitats. Furthermore,  
216 the environmental circumstances were unsuitable for cockle growth due to an increased level  
217 of nutrients that exceeded the standard rate for marine life (Table 3). Based on observations,  
218 the high concentration of nutrients are potentially toxic to the cockles and could affect cockle  
219 growth. Environmental aspects have been identified as the main factor that affects shell

Commented [cj29]: The term sexes level is somewhat unclear. Do you mean something like: From the perspective of sex or according to sexual orientation?

Commented [MOU30R29]: According to sexual orientation

Commented [cj31]: This means that the value of the  $a$  and  $b$  coefficients were similar in all sites with regard to sex/sexual orientation or there was no difference in the values between sexes in all sites?

Commented [MOU32R31]: the  $b$  coefficient differs within a population or when compared to other populations

Commented [cj33]: You mean 'results'?  $a$  and  $b$  coefficient results indicated...?

Commented [MOU34R33]:  $b$  coefficient results indicated

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Commented [cj35]: Do you mean 'contrary' or 'contrasting'?

Commented [MOU36R35]: contrary

Commented [cj37]: The meaning is unclear. Do you mean: making the determination of the shell length in relation to the cockle weight imprecise?

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Commented [cj38]: Refers to what? Faster increase in shell length increase compared to body volume?

Commented [MOU39R38]: Faster increase in shell length compared to body weight



224 development in bivalves. The shell size and shape are affected by the variation of ambient  
225 environmental constraints (Wilbur and Owen 1964; Seed 1968).

226 Furthermore, the variety of growth patterns of *A. granosa* highly correlates to factors  
227 of food availability, temperature, salinity, pollution materials, and reproductive activities  
228 (Broom 1982; Day and Fleming, 1992; Tarr, 1995). The phytoplankton concentration changes  
229 were expected to be the primary regulator of the fluctuation of the *b* coefficient from all  
230 sampling areas. The Pearson correlation test showed the opposite circumstance: the *b*  
231 coefficient was strongly correlated to phytoplankton density in Banda Aceh ( $r = 0.766$ ). In  
232 comparison, the phytoplankton density showed a moderate correlation with the *b* coefficient  
233 for cockles from Lhokseumawe ( $r = 0.532$ ) and Pulau Pinang ( $r = 0.579$ ). The phytoplankton  
234 density was expected to be a limiting factor for growth activity. Phytoplankton are used as an  
235 energy source for the growth process of shell length and cockle weight. The supply of food  
236 sources is considered an essential factor for sustainable growth (Seed and Suchanek 1992;  
237 Widdows and Johnson 1988).

238 Changes in the *b* coefficient fluctuation were also expected to reveal a relationship with  
239 the reproduction period. Sudden changes in the value of the *b* coefficient meant that there was  
240 a rapid change in the cockle weight tissue due to meagre biomass of cockles. Weight reduction  
241 of the cockle volume could be caused by the reproduction process, such as the gamete  
242 production process and gonad or gamete process being in a state of inactivity. In bivalve  
243 animals, gonadal growth and gonadal maturation process results in increased tissue mass  
244 density and increased tissue weight. Changes in the value of the *b* coefficient indicates the  
245 beginning of the activities of gonadal maturation and growth in bivalve animals (Hemachandra  
246 and Thippeswamy 2008; Hickman and Illingworth 1980).

247 The total cockle weight is described as the total shell weight, including the weight of  
248 cockle meat. In *A. granosa*, the shell weight was generally heavier than the meat weight. When

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Commented [MOU41R40]: due to meagre biomass of cockles

Commented [cj42]: Changes in the value?

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249 the shell size increased, then the overall weight of the cockle also increased linearly. However,  
250 the analysis of the samples showed no significant weight increase despite the increased shell  
251 size. This condition was assumed to be the result of the increased volume of cockle meat that  
252 did not gain or develop linearly, causing the shell length growth was not in line with the cockle  
253 weight.

254 The growth pattern is not always fixed for species. Differences in growth models might  
255 appear in the same or different species, among sex, indifferent or the same locations and in  
256 different seasons. The difference in latitudinal gradient is also related to the shell size,  
257 reproduction level and reproductive cycle in bivalves (Kanazawa and Sato 2008; Mirzaei et al.  
258 2017) as well as the growth pattern models of cockles in these three sampling areas. Cockles  
259 from Banda Aceh and Lhokseumawe had morphological differences compared to the cockles  
260 from Pinang. *A. granosa* from Banda Aceh and Lhokseumawe had special shell features that  
261 were thicker and wider compared to *A. granosa* cockles from Pulau Pinang. Other studies on  
262 the relationship in length and weight regarding some *Anadara* species have demonstrated  
263 diversity and differences in growth patterns (Table 4).

264 *Insert table 4*

265 The value of the *b* coefficient on the relationship of shell length and cockle weight noted  
266 in this study was lower than that recorded in other species in the same family, namely *Tegilarca*  
267 *granosa* ( $b = 2.82$ ) from Korean waters. Another difference was also found in their relationship  
268 model, namely *Scapharca* from Korean water, which had positive allometry and isometric  
269 relationships, while *A. granosa* from the northern region of the Strait of Malacca displayed  
270 negative allometry. Different growth patterns at different latitudes might be caused by the  
271 influence of environmental factors, changes in the composition of the food, and competition  
272 between individuals particular to a particular habitat.

273 4.2. Relationships of the biometric component of *Anadara granosa*

Commented [cj44]: Weight difference or weight increase?

Commented [MOU45R44]: significant weight increase

Commented [cj46]: Are you talking generally or for the species sampled in this study?

Commented [MOU47R46]: generally

Commented [cj48]: See previous memo. If you are talking generally, the present tense is more appropriate.

Commented [MOU49R48]: changed to present tense

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Commented [cj50]: Do you mean these factors, changes, etc. particular to a habitat? Or are you referring to individuals that inhabit a particular habitat?

Commented [MOU51R50]: particular to a particular habitat.

274 Analysis of the relationship of each biometric parameter of *A. granosa* through  
275 dendrogram or classification methods (hierarchy) was used to assess the growth patterns of the  
276 three *A. granosa* populations in the northern region of the Strait of Malacca. The dendrogram  
277 was designed to clarify the relationship of each biometric component in cockles. Fig. 3 shows  
278 the relationship between the biometric components of *A. granosa*. Through this analysis, the  
279 degree of dissimilarity between generated clusters is shown. **The relationship between a**  
280 **biometric component in one cluster compared to another biometric component in a different**  
281 **cluster within the dendrogram was generated by the distance of the scale, e.g., if the scale value**  
282 **was found to be high, it is assumed that the distinction between two biometric components in**  
283 **two different clusters was correspondingly high.**

284 *Insert fig. 3.*

285 Dendrogram analysis showed that cluster A had two sub-clusters; namely, A1  
286 represented the biometric component of *A. granosa* from Lhokseumawe and A2 represented  
287 the biometric component of *A. granosa* from Banda Aceh, which was separated on a **scale of**  
288 **16. Cluster B** was a hierarchy cluster of biometric components of *A. granosa* from **Pulau**  
289 **Pinang. Cluster B** was separated by cluster A on a scale of 25. The larger recorded scale means  
290 the dissimilarity of elements forming the component were higher. It showed that the growth  
291 pattern based on the biometric parameter was very different from the high level of inequality  
292 among the clusters (population) and sub-clusters (subpopulations). **This condition was**  
293 **interpreted to mean that the *A. granosa* populations from Lhokseumawe and Banda Aceh**  
294 **(Indonesia) and Pulau Pinang (Malaysia) had significant differences in shell form and growth**  
295 **pattern and further correlated to the biometric relationship models.** Gaspar et al. (2002, 2001)  
296 and Popa et al. (2010) stated that the population can be explained in particular by the growth  
297 characteristics through the measurement of biometric morphology.

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298 It is thought that the factor that could cause variances in biometric components and thus  
299 affect *A. granosa* growth patterns between cluster A and cluster B is spatial differentiation. The  
300 source of *A. granosa* from cluster A was distinct from cluster B, where *A. granosa* populations  
301 within cluster A came from the northern region of Sumatra island, while *A granosa* in the  
302 cluster B originated from the western region of Peninsular Malaysia. Sub-clusters of A were  
303 also known to have differences in growth patterns of the biometric component; they even had  
304 close proximity. *A. granosa* cockles of sub-clusters A1 and A2 were expected to come from  
305 the same population source. Differences in the pattern of biometric components may have  
306 occurred because of the differences in environmental factors that were local in nature, affecting  
307 the growth patterns. Differences in the range of salinity (Carmichael et al. 2004; Schöne et al.  
308 2003), temperature (Goodwin et al. 2001; Jones et al. 1989; Kennish and Olsson 1975; Pilditch  
309 and Grant 1999; Schöne et al. 2002) and density of phytoplankton (Alunno-Bruscia et al. 2001;  
310 Carmichael et al. 2004; Grant 1996; Lorrain et al. 2000; Miyaji et al. 2007) also play a role in  
311 determining the growth pattern of cockles in the three sampling areas.

### 313 Acknowledgments

314 The authors would like to thank Marine Sciences Laboratory Universiti Sains Malaysia,  
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317 (MAQIS) Malaysia and Indonesia Fisheries Quarantine Service for their continuous support in  
318 making this project a success.

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474 **Captions for figures**

475 **Fig. 1.** *Anadara grannosa* sampling location in the northern region of the Strait of Malacca

476 *(Insert after line 85, page 4)*

477 **Fig. 2.** Biometric of *Anadara grannosa*

478 *(Insert after line 102, page 5)*

479 **Fig. 3.** Dendrogram parameter of *Anadara granosa* biometric relationship from the northern  
480 region of Strait of Malacca

481 *(Insert after line 283, page 12)*

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483 **Captions for table**

484 **Table 1.** Equality a and b coefficients in the biometric model from different populations

485 *(Insert after line 167, page 7)*

486 **Table 1.** Equality a and b coefficients in the biometric model from different populations

487 *(Insert after line 178, page 8)*

488 **Table 3.** Ranges of the seasonal environmental parameter at the sampling areas

489 *(average±st.dev).*

490 *(Insert after line 183, page 8)*

491 **Table 4.** The length-weight relationship model for Archidae.

492 *(Insert after line 263, page 11)*

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**Table 4** The length-weight relationship model for Arcidae

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M. Khalil et al.

## Article

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Relationship of ~~Anadara~~*Tegillarca*

# *granosa* (Bivalvia: Arcidae) from the Northern Region of the Strait of Malacca

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## Abstract

This study on the growth pattern of the blood cockle *Anadara tegillarca granosa* focused on the aspects of biometric prints on the shell, which aimed to predict the growth of the *AT. granosa* population in the northern region of Malacca Strait. The local sample populations of the cockle were collected in three different intertidal areas called Lhokseumawe and Banda Aceh in Indonesia and Pulau Pinang in Malaysia. The biometric analysis showed that the length–weight relationship model of *AT. granosa* populations in this region indicated that the cockle population generally had a negative allometric growth pattern ( $b < 3$ ) or that shell length is more dominant compared to shell weight. Therefore, the result showed that the growth performance of *AT. granosa* was not ideal, and the highest  $b$  value (the coefficient of biometric relationship) was recorded in Lhokseumawe, followed by Banda Aceh and Pulau Pinang. The value of the coefficient  $b$  could be affected by various factors such as environmental conditions, adaptation, and dietary patterns. Cluster analysis revealed that the population of *AT. granosa* from the northern region of the Strait of Malacca was divided into two clusters, which were *AT. granosa* from the northern Strait of Malacca (Banda Aceh and Lhokseumawe in Indonesia) and *AT. granosa* from the western Strait of Malacca (Pulau Pinang in Malaysia). The factors that might cause the differences in the biometric component of both clusters were at the geographical level on the source of population and local environmental parameters.

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## Keywords

Blood cockle

Bivalvia

Growth model

Malacca Strait

Morphometric Please insert 'Morphometric' after 'Bivalvia' .....

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# 1. Introduction

*Anadara tegillarca granosa* is one of the important fishery commodities in several areas of Southeast Asia. This species has been cultivated in countries such as Malaysia and Thailand due to limited natural stocks. However, this species in Indonesia is still harvested directly from nature (Broom 1983; Khalil et al. 2017). Nevertheless, annual harvested-cockle data reveal a reduction in natural stocks in the last decade. One of the main factors for the significant reduction in natural stocks is overharvesting due to the high demand as a protein source. This condition may also be a result of the lack in management in controlling the wild cockle population stock. Therefore, the management of this species is required for the sustainability of this important species. Comprehensive information on biometrics (morphometric relationship pattern of the species) is necessary to predict the annual recruitment, as well as to interpret growth, mortality, reproductive biology, and survival data in the marine culture of species (Kim et al. 2006; Peharda et al. 2007; Pinn et al. 2005; Zelditch et al. 2004).

Length–weight is an essential variable for comparing growth, physiological processes, and environmental factors that affect aquatic organisms (Hemachandra and Thippeswamy 2008). Growth of bivalves can be defined as the increase of the length size of the shell and body weight (body mass) and these indicators have also been used extensively as corresponding parameters to assess their growth (Bailey and Green 1988; Bayne and Worrall 1980; Garton and Haag 1991; Smit et al. 1992). Measuring the length and weight of aquatic species is used to evaluate the growth patterns of species quantitatively. Such relationships are expressed via the data distribution of shell length and cockle weight. These data also represent the ratio of the addition of an animal's body size by period. Length and weight relationships have several purposes, namely (1) for measuring the weight and length ratio of a species to the weight–length in Taxa class (Anderson and Neumann 1996; Shine and Reiss 1990), and (2) for age (Pauly 1983).

The length and weight relationship allows life history and morphological differences to be identified between species and populations from different habitats (Beukema and Meehan 1985; Gaspar et al. 2002; Holopainen and Hanski 1986). This study sought to analyze the biometric relationship of *AT. granosa* using a morphometric relationship and dendrogram analysis of specimens collected from the northern region of the Strait of Malacca.

## 2. Materials and Methods

### 2.1. Samples Collection

The specimens of *AT. granosa* (120 specimens/month) were collected monthly from June 2009 till September 2010 from the muddy natural habitat in Banda Aceh ( $5^{\circ}32'34.67''$  N– $95^{\circ}17'2.54''$  E), Lhokseumawe ( $5^{\circ}09'35.3''$  N– $97^{\circ}08'29.4''$  E) in Aceh Province, Indonesia and Pulau Pinang ( $5^{\circ}16'9.66''$  N– $100^{\circ}23'27.37''$  E) in Malaysia (Fig. 1). The selection of these three sampling areas was based on the geographical distribution characteristics of *AT. granosa* in the northern region of the Malacca Strait. Banda Aceh and Lhokseumawe sampling areas share similar features such as larger coastal mudflat areas exposed during all low tides, minimum wave action, high salinity waters surrounded by mangroves patches and such locations are a natural habitat for *AT. granosa*. Meanwhile, Pulau Pinang sampling area is differentiated by larger cockle culture plots which were continuously submerged underwater and composited by muddy substrate with no wave action, located near industrial zones and are thought to be disturbed by human activities.

**Fig. 1** Please change the Map with the new one in attached file .....

~~Anadara~~*Tegillarca granosa* sampling location in the northern region of the Strait of Malacca



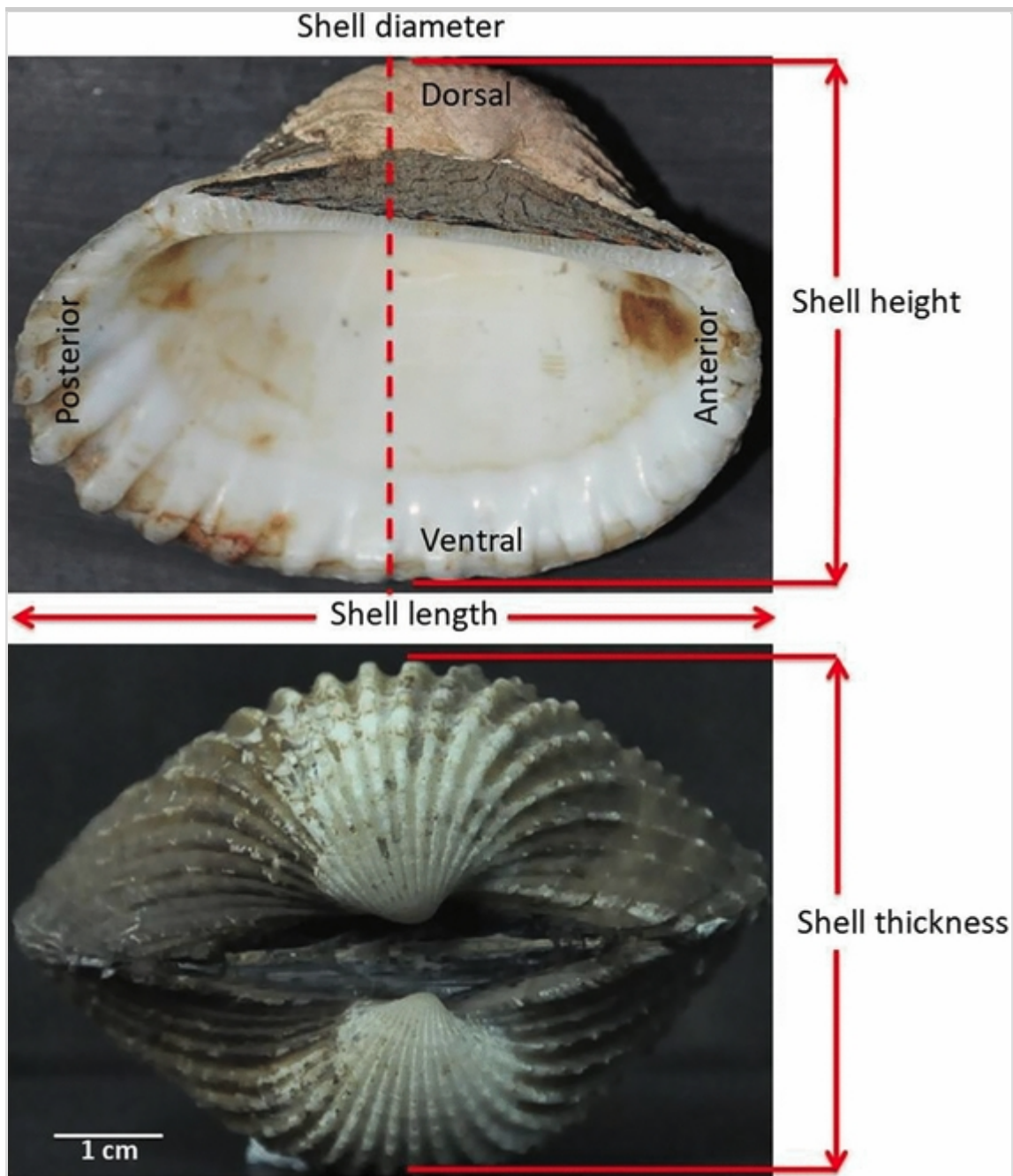


The total number of specimens sampled was 1920, with cockle sizes ranging from 38 to 71 mm in length. The specimens were collected at a depth of 5–30 cm, and salinity ranged from 10 to 33 ppt. The live samples were collected manually with the aid of a harrow during the low tide period. After collection, the specimens were stored in isotherm containers and directly transferred to the laboratory. The samples were cleaned ~~and~~ from mud and organisms attached to the shell were removed in the laboratory, then reared in the aquarium where biometric values were continuously recorded (Fig. 2),

including shell length, shell thickness, cockle height, fresh tissue weight, wet cockle weight, and sex category. The measurement of length and width of the cockle was taken with a digital Vernier caliper with an accuracy of 0.1 mm, and the cockle weight tissue was weighed using a digital weighing scale (in grams). The length was defined as the maximum shell length (measured from the posterior margin to the anterior margin of the cockle); the thickness was measured on the inflating position, from the most protruding part on the top of **the** cockle to the most protruding portion on the bottom of **the** cockle. The height was measured from the highest ventral margin of the cockle towards the dorsal margin of the cockle.

**Fig. 2**

Biometric of ~~Anadara~~*Tegillarca granosa*



## 2.2. Morphometric Relationship

The morphometric ratio of *AT. granosa* between length:height, length:thickness and height:thickness was analyzed using the following formula:

$$a = L/H, a = L/C \text{ and } a = H/C,$$

where  $L$  is the shell length,  $H$  is the shell height,  $C$  is the shell thickness, and  $a$  is the index (coefficient).

The growth pattern of the cockle was able to be designated through the relationship of shell length and cockle body weight (wet weight), which was analyzed through the equation of power regression (Ricker 1975). From the results, it could be determined if the growth rate of cockle length balanced with the cockle weight or the ratio (between length and weight) could be formulated in the mathematical expression  $b = 3$ , and if so then it was assumed that the cockle growth was isometric. Whereas if  $b \neq 3$ , it was allometric, which means the growth of cockle length was not proportionate to the weight. To test whether the constants were  $b = 3$  or  $b \neq 3$  (isometric or allometric), a statistical test was performed through a statistical  $t$  test. The equation above applied both to the whole cockle and by sex. Based on the statistical  $t$  test, the hypotheses used were:

$H_0$ :  $b = 3$ , shell length and cockle weight relationship was isometric.

$H_1$ :  $b \neq 3$  mean shell length and cockle weight relationship was allometric (namely: positive allometry), if  $b > 3$  meant that the growth of cockle weight was faster than the growth of shell length (namely: negative allometry) and if  $b < 3$  meant the growth of shell length was faster than the cockle weight.

### 2.3. Environmental Parameter Measurement

Maximum and minimum seawater temperatures were measured daily using a portable max–min thermometer ~~device~~ fixed in the sampling areas. Seawater salinity, pH, and dissolved oxygen were assessed regularly using a handheld Multiparameter Portable Meter (Hanna HI-9828) at the study sites, where turbidity was measured with a turbidity meter (Turbidity meter 800-ESD). Monthly analyses of dissolved nutrients for ammonium, nitrate, nitrite, and phosphate concentrations were performed using standard methods (Brewer and Riley 1965; Grasshoff 1976; Mantoura and Woodward 1983; Kirkwood 1989; Zhang and Chi 2002). Phytoplankton samples were obtained monthly from mid-surface water by towing a plankton net (mouth diameter 0.35 m) made of bolting silk (No. 30, mesh size:

48  $\mu\text{M}$ ) for 30 min and preserved using Lugol's solution. Phytoplankton cell density was measured monthly using a hemocytometer and a compound microscope following the Martinez et al. (1975) protocol.

## 2.4. Statistical Analysis

The raw data obtained were collected and put into a package of Microsoft Excel 2011 software Macintosh version to be processed and analyzed. Statistical analysis of Co-Variant **Analysis** was used to determine significant differences in the values obtained in each collected group data. The determining factor used in this study was the population differences in the three different regions. Therefore, the coefficients  $a$  and  $b$  were analyzed by observing the growth differences in population and sex differences in the sample (male, female, or neutral). The statistical test was continued by the post hoc test to determine which factor significantly differed in one particular parameter.

The parametric statistical test of Co-Variant analysis was analyzed using the package of SPSS (Statistical Package for the Social Science) software version 23.0 Macintosh version. Relationships existed between two variables: the relationship between the **bb** coefficient (relationship of shell length and cockle weight) and environmental factors in the sampling area. Hypothesis testing was then performed on the sample parameters to test the significance of correlation at the level of 95% ( $P = 0.05$ ). The statistical  $t$  test was used to find significant differences in the pattern of change in the **bb** coefficient that was the growth nexus of shell length and cockle weight of each sampling area.

Cluster analysis through the dendrogram diagram was designed to clarify the relationship between each biometric component (Ramesha and Thippeswamy 2009) regarding *AT. granosa* from the northern region of the Strait of Malacca. Cluster analysis was processed using SPSS **software** (~~Statistical Package for the Social Science~~).

### 3. Results

#### 3.1. Equality $a$ and $b$ Coefficients from Different Populations

The biometric studies of *AT. granosa* (from June 2009 to September 2010) from the northern region of the Strait of Malacca involving 1920 individuals consisted of 756 males, 974 females, and 190 neuters. The statistical analysis showed that *AT. granosa* shell height:shell length relationship from three sampling areas revealed a dissimilar coefficient  $a$ , but the coefficient  $b$  was similar.

Furthermore, the relationship of shell thickness:shell length of *AT. granosa* populations showed that the coefficients  $a$  and  $b$  were identical between populations. In contrast, the relationship of shell thickness:shell height coefficient ( $a$  and  $b$ ) values in terms of their allometric equations showed differences between populations (Table 1).

**Table 1**

Equality of coefficients  $a$  and  $b$  in allometric model from populations

Model	Banda Aceh		Lhokseumawe		Pulau Pinang		ANCOVA's $F$ $df, P$	
	$a \pm$ $SEa$	$b \pm$ $SEb$	$a \pm$ $SEa$	$b \pm$ $SEb$	$a \pm$ $SEa$	$b \pm$ $SEb$	$a$	$Bb$
SH = $a \times$ SL <sup>b</sup>	1.662 $\pm$ 4.031	0.765 $\pm$ 0.088	7.487 $\pm$ 8,107	0.650 $\pm$ 0.125	10.832 $\pm$ 5.863	0.502 $\pm$ 0.146	7.155, 1, 0.011	3.22 1, 0.08
ST = $a \times$ SL <sup>b</sup>	1.820 $\pm$ 4.640	0.646 $\pm$ 0.103	5.902 $\pm$ 8.240	0.574 $\pm$ 0.139	13.458 $\pm$ 5.418	0.362 $\pm$ 0.142	0.827, 1, 0.368	1.09 1, 0.30
ST = $a \times$ SH <sup>b</sup>	0.924 $\pm$ 3.313	0.831 $\pm$ 0.089	3.101 $\pm$ 7.191	0.797 $\pm$ 0.165	3.283 $\pm$ 3.661	0.796 $\pm$ 0,127	11.850, 1, 0.001	16.4 1, 0.

#### 3.2. Morphometric Coefficients Model from Different Populations

*AT. granosa* weight and shell length relationship analysis showed no

differences in coefficient  $a$  and  $b$  values between populations (Table 2). A statistical test was performed on the coefficient  $b$  through a  $t$  test indicating the coefficient  $b < 3$  or negative allometry in all *AT. granosa* populations. This condition showed that the growth of cockle shell length was faster or more dominant compared to the growth of cockle weight. A further test of the hypothesis also showed that  $H_0$  was rejected ( $P < 0.05$ ) and indicates that the growth rate of shell length and cockle weight was in an overall imbalance.

**Table 2**

Equality  $a$  and  $b$  coefficients in the biometric model from different populations

Model $W = a \times L^b$	Banda Aceh		Lhokseumawe		Pulau Pinang		AN $F, df, P$
	$a \pm$ $SEa$	$b \pm$ $SEb$	$a \pm$ $SEa$	$b \pm$ $SEb$	$a \pm$ $SEa$	$b \pm$ $SE$ <i>is not italic</i>	
Whole sample	0.0016 $\pm$ 0.0025	2.6178 $\pm$ 0.2095	0.0015 $\pm$ 0.0328	2.7629 $\pm$ 0.3894	0.0061 $\pm$ 1.4319	2.2018 $\pm$ 0.5866	0.51 1, 0.47
Males	0.0015 $\pm$ 0.0079	2.6306 $\pm$ 0.2831	0.0008 $\pm$ 0.1961	2.7713 $\pm$ 0.567	0.0127 $\pm$ 2.9646	2.0043 $\pm$ 0.889	0.00 1, 0.96
Females	0.0019 $\pm$ 0.0069	2.5695 $\pm$ 0.3368	0.0009 $\pm$ 0,0447	2.7559 $\pm$ 0.3838	0.0033 $\pm$ 2.5539	2.3697 $\pm$ 0.7607	0.13 1, 0.71
ANCOVA's $F, df, P$	1.570, 1, 0.217	4.386, 1, 0.042	2.966, 1, 0.092	2.878, 1, 0.097	2.985, 1, 0.091	0.324, 1, 0.572	—

### 3.3. Environmental Parameter

Seasonal variations of environmental parameters in the sampling areas are presented in Table 3. Water temperature, salinity, and phytoplankton **density concentration** fluctuated significantly compared to other environmental parameters during the study period.

**Table 3**

Ranges of the seasonal environmental parameter at the sampling areas (average

<b>Environmental parameter</b>	<b>June 2009</b>	<b>July 2009</b>	<b>August 2009</b>	<b>September 2009</b>	<b>November 2009</b>
<i>Temperature (°C)</i>					
Banda Aceh					
Minimum	26.32	24.27	24.29	25.43	26.90
Maximum	30.97	30.44	30.45	29.88	32.61
Lhokseumawe					
Minimum	28.82	28.82	26.81	28.07	27.71
Maximum	31.08	31.71	30.06	31.17	30.87
Pulau Pinang					
Minimum	27.23	27.52	26.65	27.10	25.87
Maximum	31.63	31.10	31.45	30.60	30.90
<i>Salinity (ppt)</i>					
Banda Aceh	32.27	31.35	29.98	27.47	30.06
Lhokseumawe	31.00	30.97	31.16	31.20	31.03
Pulau Pinang	29.33	28.52	26.39	26.87	27.35
<i>pH</i>					
Banda Aceh	7.65	8.02	8.03	8.17	7.80
Lhokseumawe	8.13	7.88	8.04	8.06	8.17
Pulau Pinang	8.02	7.49	7.85	8.14	8.04



<b>Environmental parameter</b>	<b>June 2009</b>	<b>July 2009</b>	<b>August 2009</b>	<b>September 2009</b>	<b>November 2009</b>
<i>Dissolved oxygen (mg/L)</i>					
Banda Aceh	6.53	6.81	6.96	6.05	5.97
Lhokseumawe	6.01	6.38	6.47	6.14	6.28
Pulau Pinang	7.20	5.20	5.20	4.90	5.13
<i>Turbidity (NTU)</i>					
Banda Aceh	17.40	29.30	8.61	9.02	10.86
Lhokseumawe	43.20	30.50	36.50	66.90	31.60
Pulau Pinang	29.30	17.36	15.11	13.09	17.27
<i>Orthophospate (mg/L)</i>					
Banda Aceh	0.05	0.03	0.04	0.13	0.03
Lhokseumawe	0.05	0.01	0.02	0.07	0.01
Pulau Pinang	0.10	0.05	0.08	0.06	0.03
<i>Nitrate (mg/L)</i>					
Banda Aceh	0.71	0.03	0.11	0.73	0.75
Lhokseumawe	0.68	0.14	0.03	0.03	0.20
Pulau Pinang	0.80	0.02	1.30	0.73	0.03
<i>Nitrite (mg/L)</i>					
Banda Aceh	0.05	0.02	0.02	0.03	0.75
Lhokseumawe	0.03	0.03	0.04	0.04	0.05

Environmental parameter	June 2009	July 2009	August 2009	September 2009	November 2009
Pulau Pinang	0.03	0.03	0.04	0.06	1.09
<i>Ammonia (mg/L)</i>					
Banda Aceh	0.87	0.20	0.16	0.15	0.11
Lhokseumawe	0.19	0.17	0.25	0.25	0.14
Pulau Pinang	0.24	0.18	0.14	0.25	0.42
<i>Phytoplankton density (cell/L)</i>					
Banda Aceh	1831.67	1446.67	851.67	1178.33	630.00
Lhokseumawe	1656.67	1365.00	711.67	2601.67	3010.00
Pulau Pinang	4340.00	4001.67	1470.00	11,713.33	4340.00

## 4. Discussion

### 4.1. Biometric Relationship Model of *Anadara tegillarca granosa*

Biometric data analysis of *AT. granosa* from the northern region of the Strait of Malacca showed that the cockle growth model was negative allometry. The growth of shell length was more dominant than the growth of cockle weight. The growth model generated from the three sampling sites showed that the value of the  $b$  coefficient was less than 3 ( $b < 3$ ). The balance value of the  $b$  coefficient generally has a range between 2.4 and 4.5 (Wilbur and Owen 1964), and when the  $b$  value is equal to 3 ( $b = 3$ ), the relationship of shell length and cockle weight is isometric (Carlander 1969). In this study, the  $b$  coefficient differs within a population or when compared to other populations. The cockle population from Lhokseumawe had a higher  $b$  coefficient ( $b = 2.7629 \pm 0.3894$ ) compared to the cockle

population from Banda Aceh ( $b = 2.6178 \pm 0.2095$ ) and the cockle population from Pulau Pinang ( $b = 2.2018 \pm 0.5866$ ). According to sexual orientation, a similar condition was presented, although both male and female *AT. granosa* from Lhokseumawe had the highest  $b$  coefficient (male  $b = 2.7713 \pm 0.567$ , female  $2.7559 \pm 0.3838$ ) compared to other sampling locations (Banda Aceh male  $b = 2.6306 \pm 0.2831$ , female  $b = 2.5695 \pm 0.3368$ ; Pulau Pinang male  $b = 2.0043 \pm 0.889$ , female  $b = 2.3697 \pm 0.7607$ ). These  $b$  coefficient results indicated that the cockle growth rate in Lhokseumawe was more appropriate or suitable compared to the other two sampling areas. The  $b$  coefficient value of biometric relationships is characteristically compared between dimensional growth of related or similar species in various geographical areas (Ramesha and Sophia 2015).

The contrary conditions could be found among cockles from the Pulau Pinang area, where the value of the  $b$  coefficient was lower than the range value of the  $b$  coefficient for most bivalves at 2.4–4.5 that was described by Wilbur and Owen (1964), causing the cockle shell length against cockle weight showed an unbalanced condition causing the cockle shell length against cockle weight showed an unbalanced condition. Shell length growth was faster than the increase of the cockle body volume, causing the cockle to be unhealthy. Factors such as reproductive biology (Rueda and Urban 1998), and the physical and biological variables of a habitat (Seed 1968; Thorarinsdottir and Johannesson 1996) are recognized as affecting growth and can change the allometry relationship between the shell length and the cockle weight in bivalvia. Water quality analysis showed that a faster increase in shell length compared to body weight was due to the fluctuation in environmental conditions in cockle habitats. Furthermore, the environmental circumstances were unsuitable for cockle growth due to an increased level of nutrients that exceeded the standard rate for marine life (Table 3). Based on observations, the high concentration of nutrients is potentially toxic to the cockles and could affect cockle growth. Environmental aspects have been identified as the main factor that affects shell development in bivalves. The shell size and shape are

affected by the variation of ambient environmental constraints (Wilbur and Owen 1964; Seed 1968).

Furthermore, the variety of growth patterns of *AT. granosa* highly correlates to factors of food availability, temperature, salinity, pollution materials, and reproductive activities (Broom 1982; Day and Fleming 1992; Tarr 1995). The phytoplankton density concentration changes were expected to be the primary regulator of the fluctuation of the  $b$  coefficient from all sampling areas. The Pearson correlation test showed the opposite circumstance: the  $b$  coefficient was strongly correlated to phytoplankton density in Banda Aceh ( $r = 0.766$ ). In comparison, the phytoplankton density showed a moderate correlation with the  $b$  coefficient for cockles from Lhokseumawe ( $r = 0.532$ ) and Pulau Pinang ( $r = 0.579$ ). The phytoplankton density was expected to be a limiting factor for growth activity. Phytoplankton are used as an energy source for the growth process of shell length and cockle weight. The supply of food sources is considered an essential factor for sustainable growth (Seed and Suchanek 1992; Widdows and Johnson 1988).

Changes in the  $b$  coefficient fluctuation were also expected to reveal a relationship with the reproduction period. Sudden changes in the value of the  $b$  coefficient meant that there was a rapid change in the cockle weight tissue due to the meagre biomass of cockles. Weight reduction of the cockle volume could be caused by the reproduction process, such as the gamete production process and gonad or gamete process being in a state of inactivity. In bivalve animals, gonadal growth and gonadal maturation process results in increased tissue mass density and increased tissue weight. Changes in the value of the  $b$  coefficient indicate the beginning of the activities of gonadal maturation and growth in bivalve animals (Hemachandra and Thippeswamy 2008; Hickman and Illingworth 1980).

The total cockle weight is described as the total shell weight, including the weight of cockle meat. In *AT. granosa*, the shell weight

was generally heavier than the meat weight. When the shell size increased, then the overall weight of the cockle also increased linearly. However, the analysis of the samples showed no significant weight increase despite the increased shell size. This condition was assumed to be the result of the increased volume of cockle meat that did not gain or develop linearly, causing the shell length growth was not in line with the cockle weight.

The growth pattern is not always fixed for species. Differences in growth models might appear in the same or different species, among sex, indifferent or the same locations and in different seasons. The difference in latitudinal gradient is also related to the shell size, reproduction level and reproductive cycle in bivalves (Kanazawa and Sato 2008; Mirzaei et al. 2017) as well as the growth pattern models of cockles in these three sampling areas. Cockles from Banda Aceh and Lhokseumawe had morphological differences compared to the cockles from Pulau Pinang. *AT. granosa* from Banda Aceh and Lhokseumawe had special shell features that were thicker and wider compared to *AT. granosa* cockles from Pulau Pinang. Other studies on the relationship in length and weight regarding some *Anadara Tegillarca* species have demonstrated diversity and differences in growth patterns (Table 4).

**Table 4**

~~xxxx~~ The length-weight relationship model for Arcidae

Family/species	<i>n</i>	Allometric models	<i>R</i> <sup>2</sup>	Relationship model	Location
<i>Archidae</i>					
<i>Scapharca broughttonii</i>	88	$W = 0.000073L^{3.31}$	0.943	(+) Allometric	Korea
<i>Scapharca subcrenata</i>	114	$W = 0.0004L^{2.97}$	0.935	Isometric	Korea
<i>Tegillarca granosa</i>	377	$W = 0.00068L^{2.82}$	0.960	(-) Allometric	Korea
<i>Anadara Tegillarca granosa</i>	640	$W = 0.0016L^{2.618}$	0.884	(-) Allometric	Banda A Indonesi

Family/species	<i>n</i>	Allometric models	$R^2$	Relationship model	Location
<del>Anadara</del> <i>Tegillarca granosa</i>	640	$W = 0.009L^{2.763}$	0.924	(-) Allometric	Lhokseu Indonesi
<del>Anadara</del> <i>Tegillarca granosa</i>	640	$W = 0.061L^{2.202}$	0.735	(-) Allometric	Pulau Pi Malaysi

AQ3

AQ4

The value of the  $b$  coefficient on the relationship of shell length and cockle weight noted in this study was lower than that recorded in other species in the same family, namely *Tegillarca granosa* ( $b = 2.82$ ) from Korean waters. Another difference was also found in their relationship model, namely ~~Scapharea~~ *Scapharca* from Korean water, which had positive allometry and isometric relationships, while *AT. granosa* from the northern region of the Strait of Malacca displayed negative allometry. Different growth patterns at different latitudes might be caused by the influence of environmental factors, changes in the composition of the food, and competition between individuals ~~particular to~~ in a particular habitat.

AQ5

## 4.2. Relationships of the Biometric Component of *Anadara granosa*

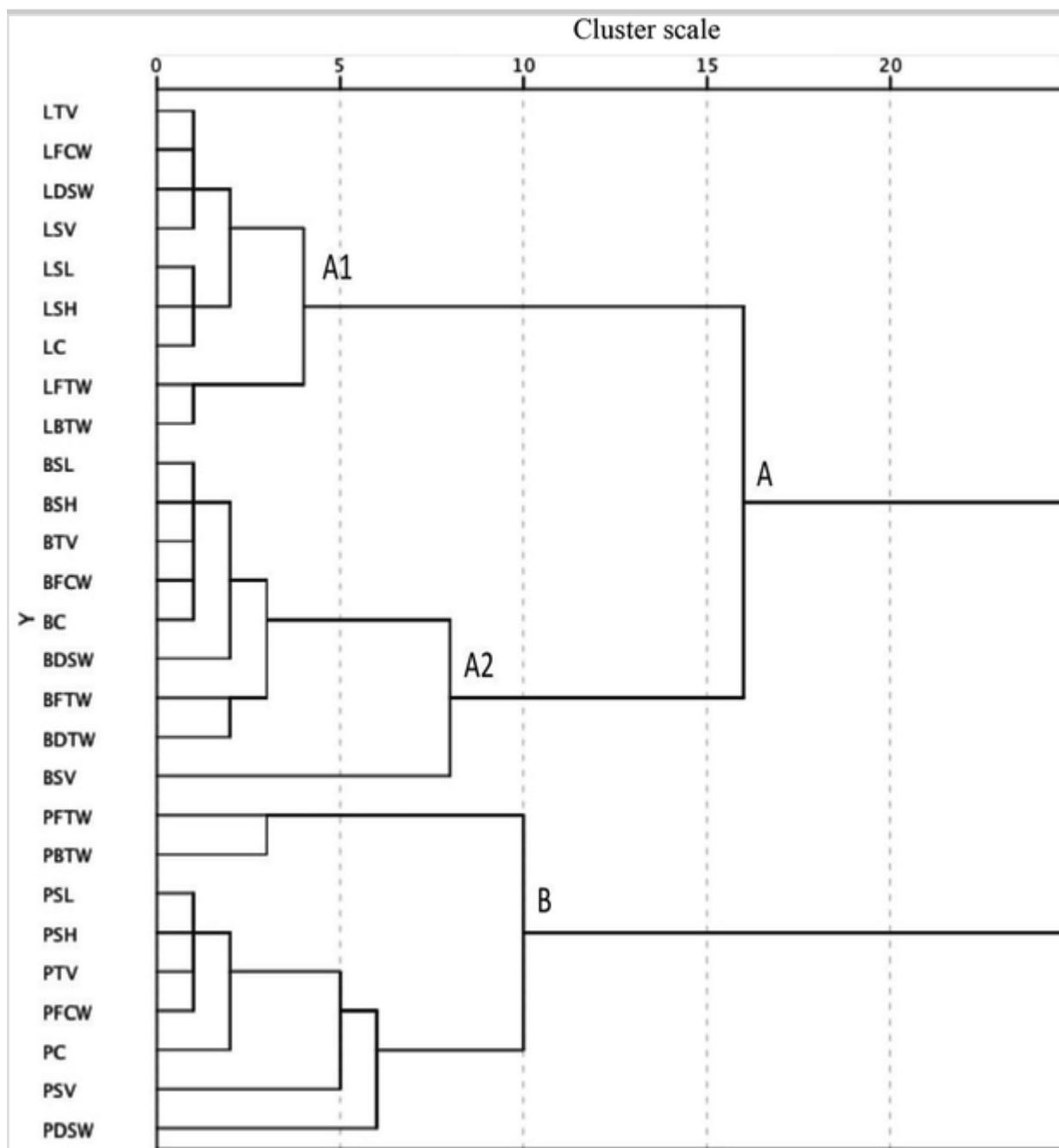
Analysis of the relationship of each biometric parameter of *AT. granosa* through dendrogram or classification methods (hierarchy) was used to assess the growth patterns of the three *AT. granosa* populations in the northern region of the Strait of Malacca. The dendrogram was designed to clarify the relationship of each biometric component in cockles. Figure 3 shows the relationship between the biometric components of *AT. granosa*. Through this analysis, the degree of dissimilarity between generated clusters is shown. The relationship between a biometric component in one cluster compared to another biometric component in a different cluster within the dendrogram was generated by the distance of the

scale, e.g., if the scale value was found to be high, it is assumed that the distinction between two biometric components in two different clusters was correspondingly high.

### Fig. 3

Dendrogram parameter of *Anadara Tegillarca granosa* biometric relationship from the northern region of the Strait of Malacca. (aA cluster of *Anadara Tegillarca granosa* population A; A1 and A2: sub-cluster of *Anadara Tegillarca granosa* population A; bB cluster of *Anadara Tegillarca granosa* population B). (BSL Banda Aceh *Anadara Tegillarca granosa* shell length, BSH Banda Aceh *Anadara Tegillarca granosa* shell height, BC Banda Aceh *Anadara Tegillarca granosa* shell thickness, BTV Banda Aceh *Anadara Tegillarca granosa* total volume, BSV Banda Aceh *Anadara Tegillarca granosa* shell volume, BFCW Banda Aceh *Anadara Tegillarca granosa* flesh weight, BFTW Banda Aceh *Anadara Tegillarca granosa* tissue weight, BDSW Banda Aceh *Anadara Tegillarca granosa* dry shell weight, BDTW Banda Aceh *Anadara Tegillarca granosa* dry tissue weight, LSL Lhokseumawe *Anadara Tegillarca granosa* shell length, LSH Lhokseumawe *Anadara Tegillarca granosa* shell height, LC Lhokseumawe *Anadara Tegillarca granosa* shell thickness, LTV Lhokseumawe *Anadara Tegillarca granosa* total volume, LSV Lhokseumawe *Anadara Tegillarca granosa* shell volume, LFCW Lhokseumawe *Anadara Tegillarca granosa* flesh weight, LFTW Lhokseumawe *Anadara Tegillarca granosa* tissue weight, LDSW Lhokseumawe *Anadara Tegillarca granosa* dry shell weight, LDTW Lhokseumawe *Anadara Tegillarca granosa* dry tissue weight, PSL Pulau Pinang *Anadara Tegillarca granosa* shell length, PSH Pulau Pinang *Anadara Tegillarca granosa* shell height, PC Pulau Pinang *Anadara Tegillarca granosa* shell thickness, PTV Pulau Pinang *Anadara Tegillarca granosa* total volume, PSV Pulau Pinang *Anadara Tegillarca granosa* shell volume, PFCW Pulau Pinang *Anadara Tegillarca granosa* flesh weight, PFTW Pulau Pinang *Anadara Tegillarca granosa* tissue weight, PDSW Pulau Pinang *Anadara Tegillarca granosa* dry shell weight, PDTW Pulau Pinang

*Anadara Tegillarca granosa* dry tissue weight).



Dendrogram analysis showed that cluster A had two sub-clusters; namely, A1 represented the biometric component of *A.T. granosa* from Lhokseumawe and A2 represented the biometric component of *A.T. granosa* from Banda Aceh, which was separated on a scale of 16. Cluster B was a hierarchy cluster of biometric components of *A.T. granosa* from Pulau Pinang. Cluster B was separated by cluster A on a scale of 25. The larger recorded scale means the dissimilarity of elements forming the component were higher. It showed that the



growth pattern based on the biometric parameter was very different from the high level of inequality among the clusters (population) and sub-clusters (subpopulations). This condition was interpreted to mean that the *AT. granosa* populations from Lhokseumawe and Banda Aceh (Indonesia) and Pulau Pinang (Malaysia) had significant differences in shell form and growth pattern and further correlated to the biometric relationship models. Gaspar et al. (2002, 2001) and Popa et al. (2010) stated that the population can be explained in particular by the growth characteristics through the measurement of biometric morphology.

It is thought that the factor that could cause variances in biometric components; and thus, affect *AT. granosa* growth patterns between cluster A and cluster B is spatial differentiation. The source of *AT. granosa* from cluster A was distinct from cluster B, where *AT. granosa* populations within cluster A came from the northern region of Sumatra island, while *AT. granosa* in the cluster B originated from the western region of Peninsular Malaysia. Sub-clusters of A were also known to have differences in growth patterns of the biometric component; they even had close proximity. *AT. granosa* cockles of sub-clusters A1 and A2 were expected to come from the same population source. Differences in the pattern of biometric components may have occurred because of the differences in environmental factors that were local in nature, affecting the growth patterns. Differences in the range of salinity (Carmichael et al. 2004; Schöne et al. 2003), temperature (Goodwin et al. 2001; Jones et al. 1989; Kennish and Olsson 1975; Pilditch and Grant 1999; Schöne et al. 2002) and density of phytoplankton (Alunno-Bruscia et al. 2001; Carmichael et al. 2004; Grant 1996; Lorrain et al. 2000; Miyaji et al. 2007) also play a role in determining the growth pattern of cockles in the three sampling areas.

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