Biometric Relationship Between Growth Pattern and Biological Reproduction of a Living Fossil Benthic Brachiopods (*Lingula anatina* Lamarck, 1801) in Aceh Northern Shore

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Abstract

Brachiopods are a group of ancient marine invertebrates that are still found today, so they are known as "living-fossil". The purpose of this study was to determine reproductive biology through sex ratio approach, gonad maturity stages, body mass weight and growth pattern of L. anatina through size distribution approach, length-weight relationship, and condition factors. This research was conducted from April to December 2021, at the Aceh northern shore. The method used in this research is purposive sampling. The results showed that based on the morphological characteristics, only one species of brachiopods from the class Lingulata was found, namely Lingula anatina Lamarck, 1801. This species has an unequal sex ratio. Analysis of Body Mass Weight (BMW) and Gonado Somatic Index (GSI) shows an inverse relationship where the BMW value is lower than the GSI value. The condition factor of L. anatina also fluctuates every month, this is presumably due to availability of food in habitat. Gonad maturity stages of L. anatina at the locations of Syiah Kuala and Alue Naga were in spawning conditions (stages IV), especially for female L. anatina. These results indicate that the reproductive quality of L. anatina at the Aceh northern shore was maintained due to minimal exploitation from the surrounding community. Furthermore, the biometric (length – weight relationship) of L. anatina in the Aceh northern shore showing an isometric and negative allometric pattern. Basically, allometric growth is temporary, for example due to changes related to gonadal maturity, while isometric growth is a continuous change that is proportional.

Keywords: *Aceh northern shore, Brachiopods, baree, lamp shells, Lingula anatina*

Introduction

Lingula anatina Lamarck, 1801 is a species of the family Lingulidae phylum Brachiopoda which is a part of marine fauna in the Cambrian period (Ambarwati *et al.,* 2021). This biota is commonly known by several local names in Aceh, namely baree and biree. Its long existence since ancient times until now has resulted in the spread of Brachiopods, including Lingulidae, to be very wide. This species is able to live from intertidal regions to depths of more than 5000 m (Emig *et al.,* 2013) and it is geographically widely distributed across the Indo-West Pacific region, Asia, Australia, Europe, Africa (Yang *et al*., 2013), Ratnagiri Maharashtra mangrove area, India (Sundaram and Deshmuskh, 2011), mangrove in China (Printrakoon and Kamlung-ek, 2014), and even Shirotnoe Priob'e in Siberia (Smirnova *et al*., 2015).

L. anatina's ability to "live for a long time" has earned this species the nickname "living-fossil" because of its very slow evolution (Emig *et al*., 2013). This is a unique feature of *L. anatina* because it is suspected that ecologically this species plays a role in determining environmental changes. This is because Lingulidae is also classified as a sessile benthos, which are species with limited movement so that they are easily exposed to the surrounding environmental conditions (Zhang *et al.,* 2014).

Furthermore, in the economic aspect, *L. anatina* can be consumed by humans. This is clearly seen in the preliminary study which shows that *L. anatina* in the Ujung Pancu and Syiah Kuala areas are the livelihood targets of the local community (Octavina *et al*., 2021a). Direct human exploitation of this species will then lead to a decline in the lamp shells population. The pressure on lamp shells

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resources in Aceh northern shore indirectly affects the reproductive biology of lamp shells (Octavina *et al*., 2021a; 2021b; 2021c). Previous research has also been conducted reporting the recent discovery of lamp shells in Lubuk Damar, Aceh Tamiang (Darmarini *et al.,* 2017). In addition, research has also been carried out on the density and distribution of lamp shells on the North Coast of Aceh (Agustina *et al.,* 2019). Research on the relationship between the length and weight of lamp shells and population structure on the Aceh northern shore (Octavina *et al.,* 2021a; 2021b) as well as on the population structure of *L. anatina* in Alue Naga waters (Octavina *et al*., 2021c) have also been conducted. Considering that *L. anatina* is an infauna, the effect of pollution cannot be separated from its ability as a bioindicator of the marine environment, therefore research on microplastic pollution on *L. anatina* has been carried out by Akkajit *et al*. (2023).

Based on several studies conducted on *L. anatina*, it has not been fully carried out on the Aceh northern shore. Reproductive biology aspect of this biota is also quite interesting to study. The purpose of this research is determined reproductive biology through sex ratio approach, gonad maturity stages, body mass weight and growth pattern of *L. anatina* through size distribution approach, length-weight relationship, and condition factors in Aceh northern shore.

Materials and Methods

Sampling were conducted at several shores representing Aceh northern shore namely Alue Naga, Kuala Cangkoi, Syiah Kuala, and Ujung Pancu (Figure 1.). Direct sampling was done based on *L. anatina* air holes with depths ranging from 30-50 cm with a hole width of 1-2 cm when low tide. Collection of *L. anatina* was done on a quadratic transect on top of soil that has *L. anatina* air holes on them, digging every hole in the quadratic transect using a machete or PVC pipe (Figure 2.). Subsequently, samples were taken for environmental physio-chemical parameters such as pH (MQuant pH 0-14 Universal Indicator), temperature (thermometer 110 ^{0}C), salinity (Hand-refractometer 0-18% Brix, incl. ATC), and sediment (soil texture, Wentworth's methods) at the research site.

Body morphometric measurements of *L. anatina* were carried out using a digital caliper with an accuracy of 0.01 mm, following the Ricker method (1970) on the characteristics of shell length (LS), shell width (WS), Foot Length (LF), Total Length (LT), shell thickness (TS), shell weight Mass (SWM), body weight Mass (BWM), and gonadal weight (GW) (Figure 3).

The procedure for measuring body weight mass (BWM) and gonadal weight (GW) was performed by dissecting lamp shells using surgical instruments to remove them from their mantles. Then the lamp shells flesh is separated from the mantle to be weighed one at a time using an Ohaus scale which has an accuracy of 0.001 g. To measure the weight of the gonads, the gonads of the lamp shells were first separated from the flesh and then the gonads were weighed one at a time using an Ohaus scale.

Furthermore, to assess the gonad maturity stages, the lamp shells gonads were separated from the meat and then the lantern clam's gonad maturity level was examined using an Olympus microscope (CX 33 Trino) with 10 x 10 and 10 x 40 magnification. Assessment of gonad maturity stages was done using a visual approach. The level of gonad maturity can be observed from the gonads that have been removed from the body by looking at visual indicators such as the shape of the gonads, the size of the gonads, the color of the gonads, and the softness of the gonads (Hukom *et al.,* 2006). The gonad maturity stages visual approach assessment here was done based on Khairiyah *et al.,* 2019).

Data analysis

Length Distribution. Size distribution was used to determine the group size of *L. anatina*. The size distribution group can be determined by measuring several parameters including calculating the minimum and maximum values for all data, calculating the number of size classes, calculating the data range, and calculating the width of the classes separated using the Bhattacharya method in the FAO-ICLARM Fish Stock Assessment Tools (FiSAT) II program (Sparre and Venema, 1999).

Condition factor. The relative condition factor is calculated by an equation (Effendie, 1997), which is $K = W / (a L^b)$. Where, K is the relative condition factor; W is body weight (g); L is body length; a is intercept, and b is slope.

Length-Weight Relationship. Information on length-weight is very important for proper fisheries management, length-weight data is also used in physiological and productivity investigations. The growth pattern of *L. anatina* can be determined by the relationship between shell length and body weight (Effendie, 1997) as follows: $W = a L^b$, where, W is body weight (g); L is total length (mm); a is intercept and b is slope. The criteria of length-weight relationship indicates that b= 3 means isometric relationship; if b> 3 means positive allometric relationship, and if b< 3 means negative allometric relationship.

Sex ratio**.** The sex ratio (F:M) was calculated by comparing the number of male lamp shells and female lamp shells in the lamp shells samples and

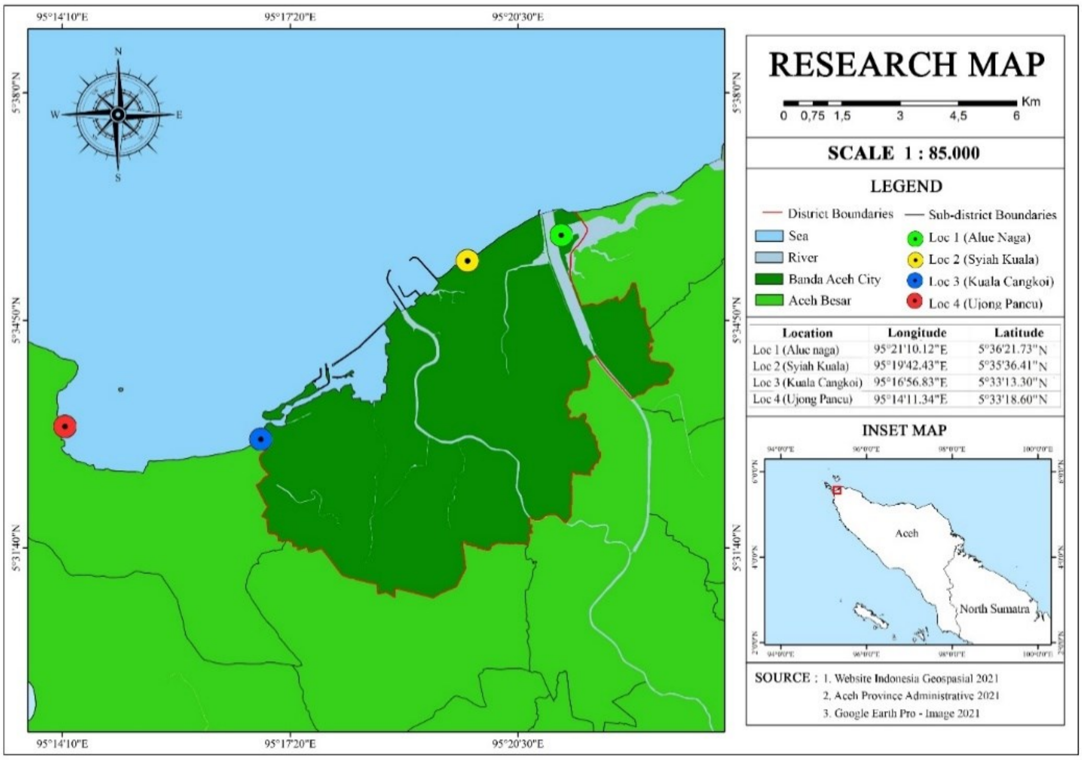

Figure 1. Aceh northern shore

Figure 2. *Lingula anatina* air holes

then multiplying by 100 %. (Effendie, 1997). In this sex ratio analysis, statistical tests were carried out, namely the Chi-square test and the p-value. The hypothesis used is that H_0 states that there is no relationship between *L. anatina* shell size and sex, while the H' hypothesis states that there is a relationship between *L. anatina* shell size and sex. The confidence interval used is 0.05 or 5%. Then GSI dan BMI were also analyzed by equation Effendie (1997) and Qurani *et al.* (2020).

Result and Discussion

The results of the study on the length distribution of *L. anatina* that was conducted by measuring the length of the shell, the length of the pedicle, the thickness of the body, and the body weight showed that there are about 6-7 class interval ranges (Figure 4.). The average body length of *L. anatina* overall is 35.69 mm where the highest overall average length was recorded from the Syiah

Kuala Beach which is 45.65 mm, while at Kuala Cangkoi the average body length is 32.92 mm, at Ujung Pancu the average length was 32.16 mm, and at Alue Naga the average body length was 32.06 mm (Table 1.). In general, based on the overall size distribution from the beaches on the northern shore of, it was found that the largest class interval was in the 31-35 mm class range (99 individuals). Based on the research that has been carried out at 4 locations in the northern coast of Aceh, there are differences in the number of *L. anatina* individuals that were sampled, namely 58 individuals from Alue Naga, 60 individuals from Syiah Kuala, 55 individuals from Ujung Pancu, and 160 individuals from Kuala Cangkoi. Growth in *L. anatina* can be measured based on several factors including size distribution, size parameters, length and weight, and condition factors (Fujii *et al.,* 2019).

Analysis of *L. anatina* size groups using the Bhattacharya method resulted in different cohorts for each size group at each location. However, there was no visible recruitment in the observed cohorts (Figure 5.). The size distribution of *L. anatina* in the Aceh northern shore shows the longest overall size of 35.69 mm, which is in accordance with the size range measured by previous research (Octavina *et al.,* 2021c). Samples collected from Ujung Pancu had the lowest class interval frequency, this is presumably because the people around Ujung Pancu use a lot of *L. anatina* for personal consumption or sale, which is in accordance with a statement that stated how the people around Ujung Pancu used *L. anatina* a lot for consumption and to be sold for their daily needs (Octavina *et al.,* 2021c). Kuala Cangkoi was the location that yielded the most samples of *L. anatina* with a total of 140 individuals.

Based on the average body weight and average length of *L. anatina* found in Kuala Cangkoi, their size was almost the same as in other areas, as previous research (Octavina *et al.,* 2021c) showed that the growth in length and weight of *L. anatina* in the Alue Naga area had a size range of 43.9 – 48.6 mm. The high number of *L. anatina* found in the Kuala Cangkoi area is suspected because it is still rare for people here to take or use *L. anatina*. Based on the researcher's direct observation, there were still a low number of people around Kuala Cangkoi who knew *L. anatina*. If seen in Figure 4, the Syiah Kuala location does not have individuals within the lower size class intervals, this was seen when the research was conducted that at the Syiah Kuala location there were many *L. anatina* which had long pedicles.

Based on the overall length weight relationship of the sampled *L. anatina*, it was found that the body length of this species is more dominant than their body weight, indicating negative allometric relationship (Figure 6.). This is supported by statements from previous research (Samanta *et al.,* 2014) which said that the flattened and elongated body shape of *L. anatina* caused the shell length to grow faster than the growth of their body weight. However, at the Syiah Kuala location, it was found that the growth pattern of *L. anatina* in this location to be isometric. The result recorded from the Syiah

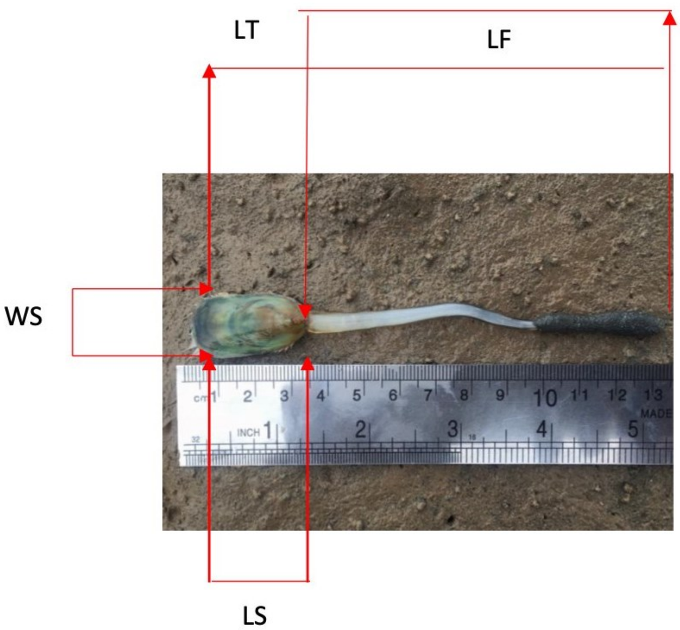

Figure 3. Morphometric of *Lingula anatina*

Table 1. Morphometric characteristics of *Lingula anatina* from Aceh northern shore

Meristic character	Location			
	Kuala Cangkoi	Ujung Pancu	Syiah Kuala	Alue Naga
Shell Length	32.93±2.74a	32.17±4.71a	45.66±3.55 b	32.07±7.87a
Shell Width	15.04±1.86a	14.57±1.87a	20.69±2.36 b	14.44±3.09a
Shell Thickness	6.00±0.66b	5.52±1.01a	8.71±1.04c	5.90±1.61ab
Pedicle length	51.21±8.84b	50.28±11.20b	89.57±19.99c	38.83±12.86a
Total length	84.14±10.51b	82.4514.64b	133.74±24.33c	70.90±19.47a

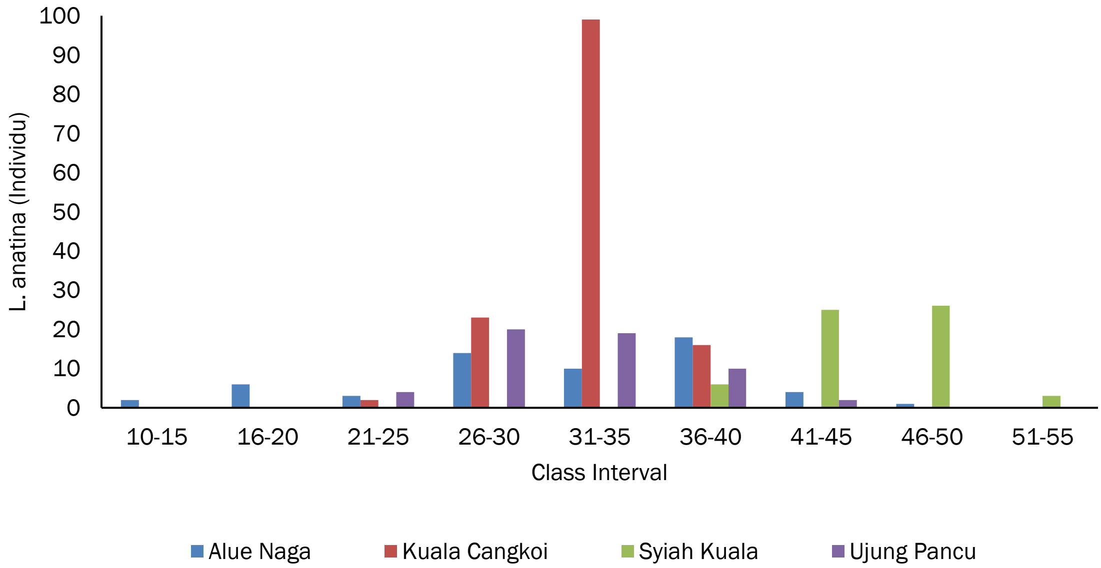

Figure 4. Length class of *Lingula anatina* in Aceh northern shore

Kuala beach were different from the other 3 locations as well as from several studies on the growth pattern of *L. anatina* (Zhang *et al.,* 2021) which shows that the phylum Brachiopoda since the Cambrian period has an allometric growth pattern.

Other research (Octavina *et al.,* 2021c) also showed that the growth pattern of *L. anatina* was negative allometric. Differences in growth patterns in each location can be influenced by environmental or ecological conditions in the waters where these animals grow and develop (Jaiswar and Kulkarni, 2002). Factors that cause b= 3 may be due to suitable and good habitat factors, such as more space resulting in increased availability of food and vegetation that can support a good growth condition (Ngor *et al*., 2018). Isometric conditions in *L. anatina* could also be caused by an improved ecosystem condition as well as improved population conditions and a balanced environment that results in an increase in the length and weight balance (Sousa *et al.,* 2020).

The length-weight relationship can also be used to determine the condition factor of *L. anatina.* Condition factors gives a measure on the level of fatness of *L. anatina* in their habitat. The condition factor is also an environmental parameter of whether or not their habitat is in accordance with the organism's tolerance level. The highest condition factor value of *L. anatina* sampled from the Aceh northern coast was highest at the Alue Naga sampling location, but this is value did not differ much from what was recorded at the other stations. This shows that overall, the condition of *L. anatina* in the waters of the Aceh northern coast could be classified as less flat or fat. Small shellfish generally has a high condition factor value (Sinaga, 2018), so it can be concluded that the condition of *L. anatina* in the North Aceh waters was quite fat because they had a fairly low condition factor value. The condition factor values obtained reflect a fairly good environment for *L. anatina*, this is in accordance with the statement (Silaban *et al.,* 2021) which states when the value of the condition factor of an organism is low, it reflects good environmental conditions for the organism. This is also supported by Sinaga (2018) which states that when the value of conditions is above 1, it indicates that the actual weight is greater than the predictive weight, therefore the conditions in the Aceh northern coast are still quite good for *L. anatina*.

L. anatina on the Aceh northern shore was dominated by female individuals, while at the Alue Naga sampling location the population of *L. anatina* was dominated by male individuals. Based on samples taken from the north coast of Aceh, it showed that the sex frequency ratio of all *L. anatina* was unbalanced (1:0.88) (Table 2.). This is in line with the results of this study another (Khairiyah *et al.,* 2019) which states that the sex ratio in animals is generally balanced, namely 1:1 (males and females). In general, in gonochoristic cases, the cause of the ratio of male or female sex differences in Brachiopods arises due to morphological assistance of Brachiopod sex, while for Brachiopods it is limited to visual methods both macroscopically and histologically (Emig, 2008). This is also problematic because the classification of *L. anatina* into male or female is difficult to distinguish macroscopically. Although the sex of the majority of Brachiopods is not externally identifiable, the female to male ratio for brachiopods is generally found to be gonochoristic (Hirose and Endo, 2021).

Most of the samples of *L. anatina* from the Aceh northern shore were at stage IV of gonadal maturity, as evidenced by the results found at the fourth location after being observed under a microscope (Table 1. and Figure 7.). This is presumably because the peak of gonadal maturity in lantern shells occurs in the April-July period (Bahtiar, 2017). Overall, the sex ratio of *L. anatina* was unbalanced, where during the study the number of males was always lower than the females (except Alue Naga). This disappointment has no effect on the size of the shell with the number of males and females.

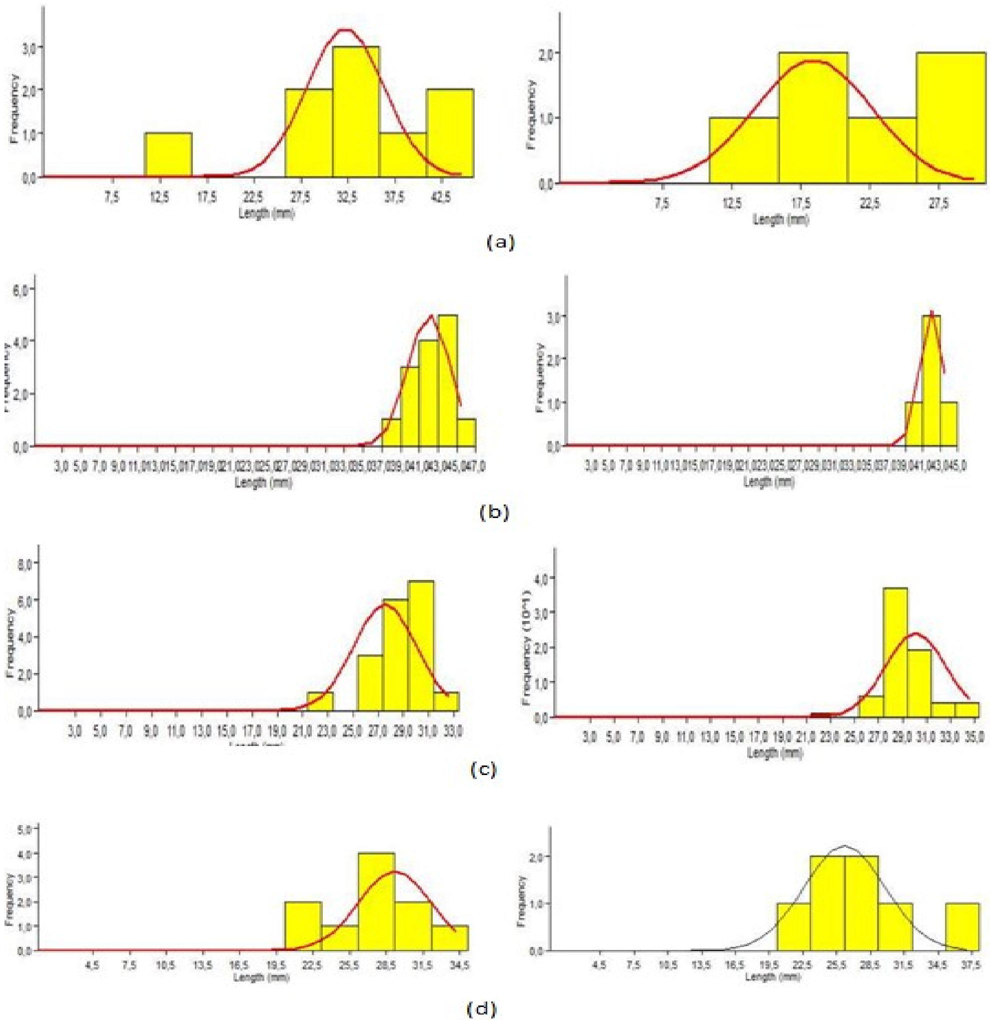

Figure 5. Length class and cohort of *Lingula anatina* in Aceh northern shore: a) Alue Naga, b) Syiah Kuala, c) Kuala Cangkoi, d) Ujung Pancu.

Table 2. Sex ratio with shell size of *Lingula anatine*

Location	Sex		Sex ratio (F:M, %)	$\chi 2$	P-value
	Female	Male			
Ujung Pancu	31	24	1:0,77 (56,36:43,63)	1 (χ2cal < χ2tab)	0,5
Kuala Cangkoi	71	69	1:0,97 (20,71:49,28)		
Syiah Kuala	37	23	1:0,62 (61,66:38,33)		
Alue Naga	27	31	1:1,48 (46,55:53,44)		

Table 3. Gonad Somatic Index of *Lingula anatine*

Sex	Location			
	Kuala Cangkoi	Ujung Pancu	Syiah Kuala	Alue Naga
Female	3.27±1.33	3.97±1.35	5.32±0.67	5.35±1.14
Male	3.04±1.14	3.59±1.51	5.24±0.46	4.67±1.19

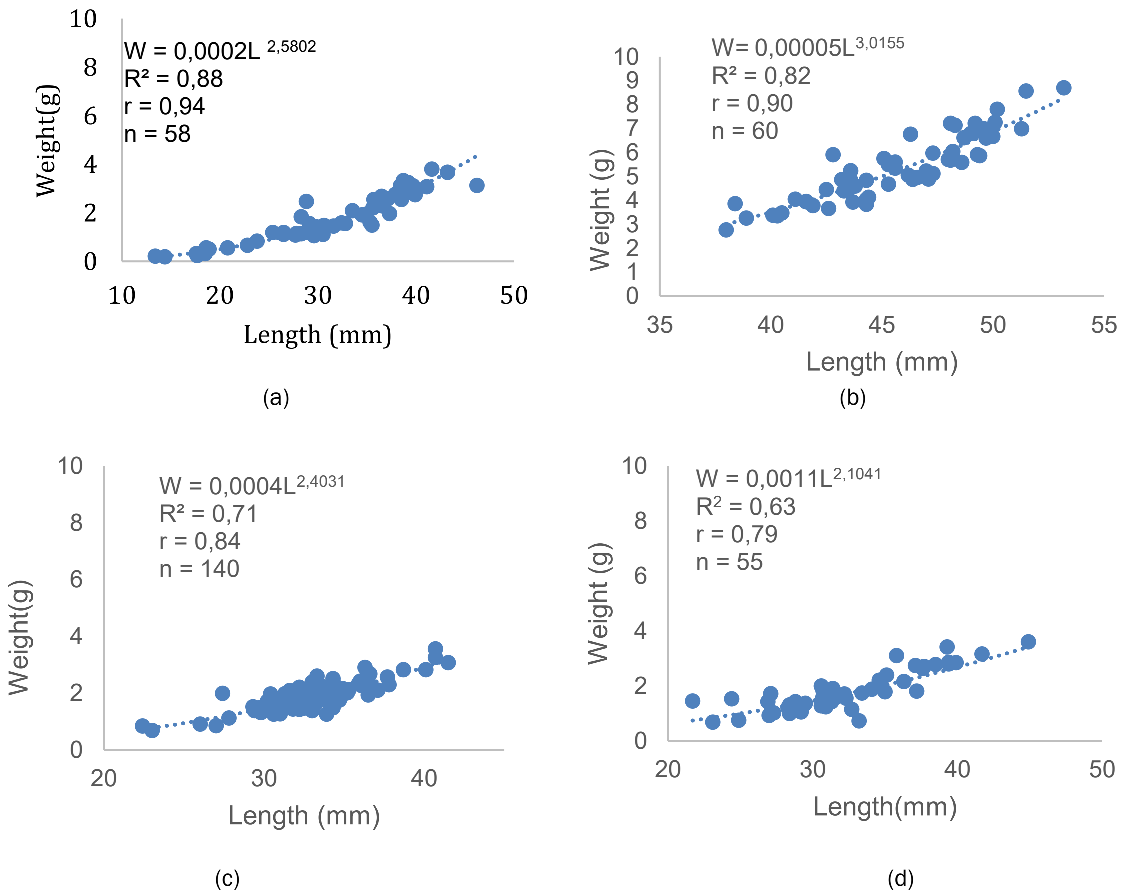

Figure 6. Length-Weight relationships of *Lingula. anatina* in Aceh Northern Coast: a) Alue Naga, b) Syiah Kuala, c) Kuala Cangkoi, d) Ujung Pancu.

This is clearly seen from Table 2 where the P>0.05 and the $\chi 2$ (Chi-square) test value is 1, where the χ2cal < χ2tab, i.e. the null hypothesis (0) is accepted. Shell size does not indicate the sex of a biota (Fujii *et al.,* 2019).

The GSI values obtained from the *L. anatina* samples showed that the average GSI values of males and females sampled at the Syiah Kuala and Alue Naga locations had an average value of > 5%, indicating that at both this location it was suspected

Stage	Stadium	Sex	
		Male	Female
Phase I	Early develop	-	-
Phase II	Gonads develop		
	Gonad maturity		
Phase IV	Spawning		

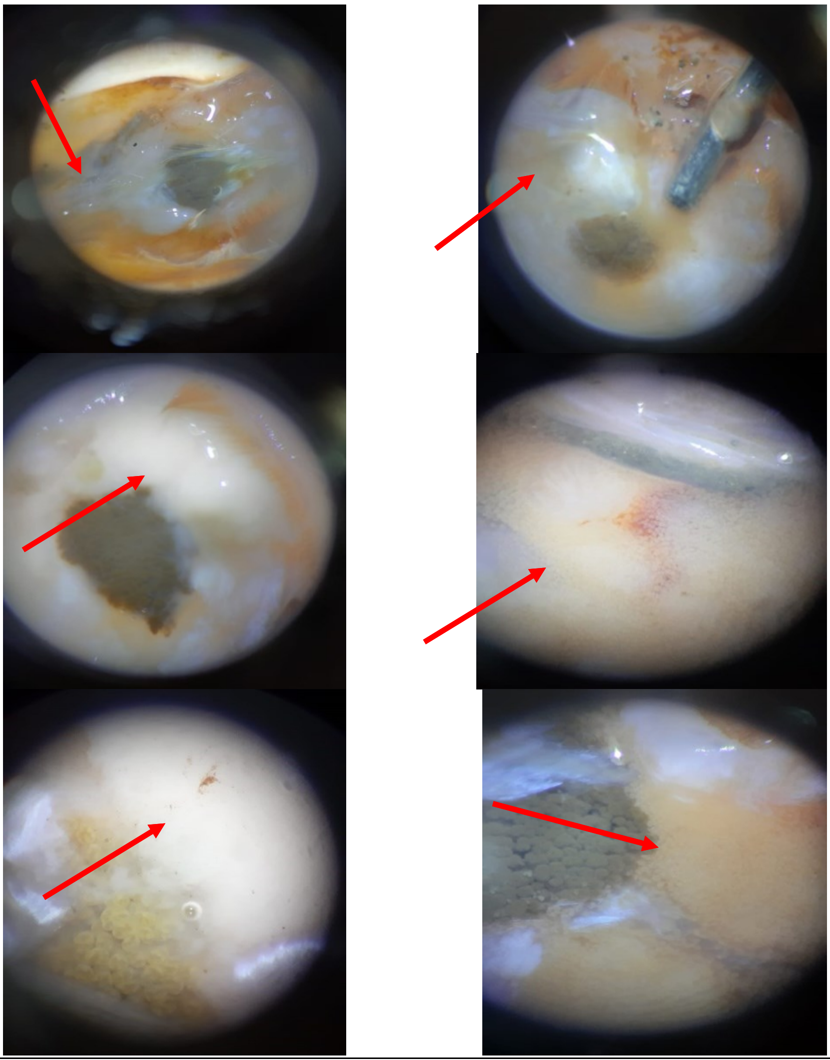

Figure 7. characteristic of *L. anatina* gonadal

that they were in the spawning phase. However, at Ujung Pancu and Kuala Cangkoi locations the samples had an average GSI value of <4%, this shows that in these locations that are also close together, the lamp shells populations have a high exploitation (catch) value and are often seen being taken by the surrounding community. As a result, lamp shells samples taken at the Ujung Pancu and Kuala Cangkoi locations were generally smaller in size than the samples taken at the Syiah Kuala and Alue Naga.

It shows that the GSI values for males and females ranged from 3.04-5.24 for the male lamp shells and 3.27-5.35 for the female lamp shells, respectively (Table 3.). Changes in GSI are closely related to gonadal development and egg growth, where the bigger the GSI value, the higher the gonad-maturity stage will be, because the gonads will get bigger and heavier as the GSI increases (Pratiwi *et al.,* 2019). A high GSI value also indicates the occurrence of spawning because the gonads have matured (Widowati *et al.,* 2002). While a low GSI value indicates that the individuals have already spawned or has not experienced spawning. Gonads that are categorized as mature will give increased volume and weight values, thereby increasing the GSI value (Pratiwi *et al.,* 2019). In mollusks, this GSI value is a good indicator for observing the activity and development of gonads (Lubet, 2016). An increase in the index value can be interpreted as the beginning of gonadal maturity and a drastic decrease in the index value indicates spawning has just occurred (Sereflisan and Gokce, 2016).

Both male and female *L. anatina* show a relatively uniform pattern when initiating gonadal maturation and spawning. This relationship is taken from the comparison between the weight of the flesh and the total weight of the lamp shells based on the length class. BMI can describe the obesity condition of shellfish. BMI for lamp shells is neither uniform nor variable, as it can be influenced by environmental factors, food availability, and biology (Qurani *et al.,* 2020). Energy allocation from somatic growth to reproductive tissue forms an inverse relationship. Somatic growth is expressed in the BMI, while the gonad-maturity stages is a sign to distinguish gonadal maturity based on gonadal weight, and is naturally related to body size and weight. At the time of sampling, the peaks of GSI and BMI values fluctuated in Alue Naga. When the GSI increases, the BMI will decrease, this is presumably because the lamp shells were sampled during the spawning month. At the time of spawning, somatic growth experienced a significant decrease due to the transfer of energy for somatic.

Conclusion

L. anatina at the Aceh northern shore has a faster increase in length compared to its weight when it is young (not yet spawning) and some have a balanced increase in length and weight when they are adults. The GSI values of *L. anatina* males and females at the Syiah Kuala and Alue Naga locations had an average value of > 5%, indicating that at both this location it was suspected that they were in the spawning phase. It was also found that the ratio of males and females of *L. anatina* was also unbalanced.

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