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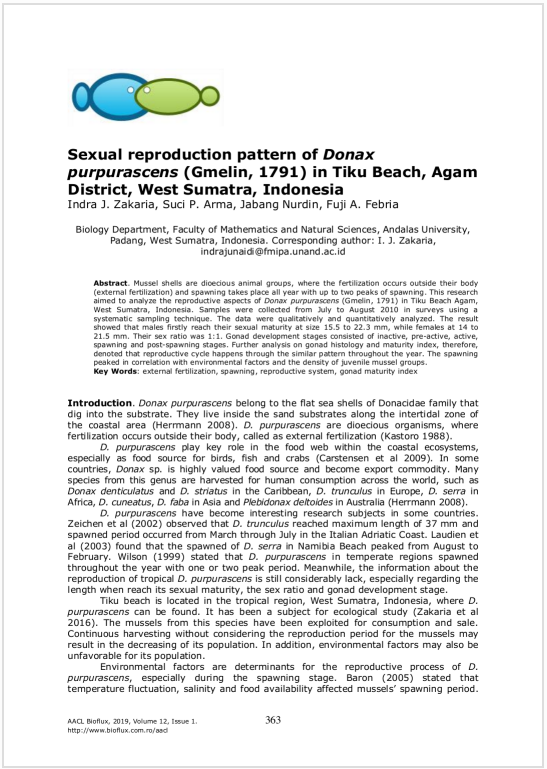

Sexual reproduction pattern of *Donax purpurascens* (Gmelin, 1791) in Tiku Beach, Agam District, West Sumatra, Indonesia

Indra J. Zakaria, Suci P. Arma, Jabang Nurdin, Fuji A. Febria

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Abstract. Mussel shells are dioecious animal groups, where the fertilization occurs outside their body (external fertilization) and spawning takes place all year with up to two peaks of spawning. This research aimed to analyze the reproductive aspects of *Donax purpurascens* (Gmelin, 1791) in Tiku Beach Agam, West Sumatra, Indonesia. Samples were collected from July to August 2010 in surveys using a systematic sampling technique. The data were qualitatively and quantitatively analyzed. The result showed that males firstly reach their sexual maturity at size 15.5 to 22.3 mm, while females at 14 to 21.5 mm. Their sex ratio was 1:1. Gonad development stages consisted of inactive, pre-active, active, spawning and post-spawning stages. Further analysis on gonad histology and maturity index, therefore, denoted that reproductive cycle happens through the similar pattern throughout the year. The spawning peaked in correlation with environmental factors and the density of juvenile mussel groups.
Key Words: external fertilization, spawning, reproductive system, gonad maturity index

Introduction. *Donax purpurascens* belong to the flat sea shells of Donacidae family that dig into the substrate. They live inside the sand substrates along the intertidal zone of the coastal area (Herrmann 2008). *D. purpurascens* are dioecious organisms, where fertilization occurs outside their body, called as external fertilization (Kastoro 1988).

D. purpurascens play key role in the food web within the coastal ecosystems, especially as food source for birds, fish and crabs (Carstensen et al 2009). In some countries, *Donax* sp. is highly valued food source and become export commodity. Many species from this genus are harvested for human consumption across the world, such as *Donax denticulatus* and *D. striatus* in the Caribbean, *D. trunculus* in Europe, *D. serra* in Africa, *D. cuneatus*, *D. faba* in Asia and *Plebidonax deltoides* in Australia (Herrmann 2008).

D. purpurascens have become interesting research subjects in some countries. Zeichen et al (2002) observed that *D. trunculus* reached maximum length of 37 mm and spawned period occurred from March through July in the Italian Adriatic Coast. Laudien et al (2003) found that the spawned of *D. serra* in Namibia Beach peaked from August to February. Wilson (1999) stated that *D. purpurascens* in temperate regions spawned throughout the year with one or two peak period. Meanwhile, the information about the reproduction of tropical *D. purpurascens* is still considerably lack, especially regarding the length when reach its sexual maturity, the sex ratio and gonad development stage.

Tiku beach is located in the tropical region, West Sumatra, Indonesia, where *D. purpurascens* can be found. It has been a subject for ecological study (Zakaria et al 2016). The mussels from this species have been exploited for consumption and sale. Continuous harvesting without considering the reproduction period for the mussels may result in the decreasing of its population. In addition, environmental factors may also be unfavorable for its population.

Environmental factors are determinants for the reproductive process of *D. purpurascens*, especially during the spawning stage. Baron (2005) stated that temperature fluctuation, salinity and food availability affected mussels' spawning period.

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by Jabang Nurdin

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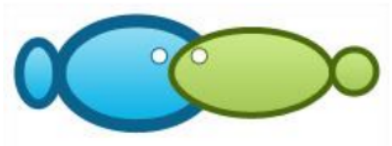

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Riascos and Urban (2002) observed that the El Nino cyclone that occurred in the Gulf of Malaga, Columbia, is related to the reproductive cycle of mussels *D. dentifer*. Considering the importance of the ecosystem where *D. purpurascens* exist for conservation and cultivation, it is necessary to do a research on the reproduction of of the species, as well as on its affecting environmental factors, in Tiku beach, Agam District, West Sumatra, Indonesia.

Material and Method

Research method. This research used survey method with systematic sampling technique. *D. purpurascens* were collected once in every two weeks from June until August 2010 in Tiku Beach, Agam District, West Sumatra, Indonesia (0°27'10 '' S and 99°58'48 '' E). Sampling was carried out at high and low tide in the intertidal zone and divided into three parts: edge, middle and precipice of mudflat. Each part was at least 10 m apart. Collected samples were taken to the laboratory, grouped by the depth of substrate, and then measured for their parameters: length, width, height and dry weight. *D. purpurascens* used for histological analysis measured 11 to 42.3 mm (n = 90). The samples were then regrouped based on the size ranges and divided into two groups. The first group was used for histological analysis, while the second one was for weighing the dried gonad. An amount of 14-16 randomly selected gonads represented each sampling period was used as histological preparation.

Data analysis (qualitative analysis). Qualitative methods were in use to determine the developmental stages of gonad using histological preparation. The development stages of gonad based on the criteria established by Herrmann et al (2010) (Table 1).

Table 1
Sex determination, length at sexual maturity and gender-based histology comparison of *Donax purpurascens*

Parameter	*Method*
Sexes determination	Results from histological preparations were grouped according sexes. Each histological preparation was determined through the presence of male or female gamete
Length at first sexual maturity	It was histologically determined by looking at the size of the oocytes and sperms. Sexual immaturity was characterized by small alveoli and undeveloped gamete
Sexes comparison (Male : Female)	All histological preparation results were separated by gender; the percentage of male and female calculated (Lefort & Clavier 1994)

Quantitative analysis. Reproduction of *D. purpurascens* can be quantitatively assessed by using several parameters, including the dry weight of the meat, visceral organs and gonads (Wilson 1987; Baron 2005). The developmental stages of gonads were quantitatively determined using gonad maturity index using the formula given by Wilson (1987):

$$\left[dGI = \frac{dGW.10^6}{H^3} \right]$$

Where:
dGI = Gonad Maturity Index
dGW = Dry Weight of *D. purpurascens* Gonad (g)
H = Shell Length of *D. purpurascens* (mm)

Result and Discussion

Sexes of D. purpurascens. Histologically, sexes of *D. purpurascens* are distinguishable through the presence of male or female gametes (Figure 1). The preparation showed that *D. purpurascens* are dioceous (has both male and female gametes). However, this research did not observe any hermaphrodite group; despite this organism has both gametes.

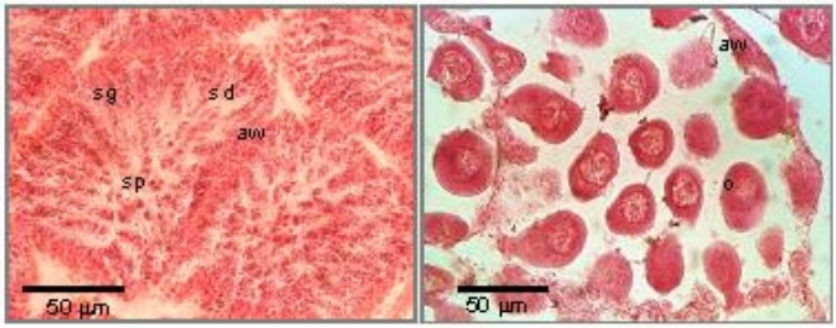

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Figure 1. Histological preparation of *Donax purpurascens*. a – male; b – female; sg – spermatogonia; sd – spermatids; sp – spermatozoa; aw - alveolar walls; o – oocyte (original).

The sexual maturity of *D. purpurascens* can be observed through the coloration, albeit it cannot distinguish its gender. From the observations on histological preparation, matured *D. purpurascens* have milky white gonadal masses, slightly yellowish or brownish and looks like granules (Figure 2).

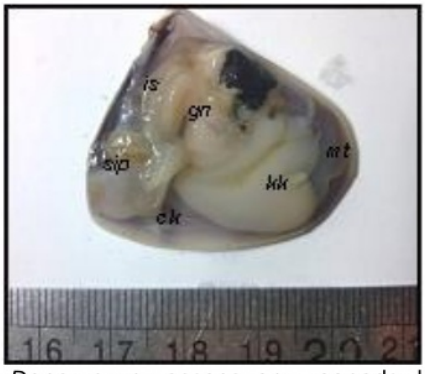

Figure 2. Sexually mature *Donax purpurascens*. gn - gonads; kk - foot; sip – siphon; ck - eggshell; is – gill; mt – mantle (original).

Length of first sexually matured D. purpurascens. Histological observation confirmed the difference between male and female when reaching sexual maturity. Males get sexual maturity at length of 15.5 to 22.3 mm (n = 11), and females at 14 to 21.5 mm (n = 9). The fully matured males were measured at length of 23 to 42.3 mm (n = 31), while female from 22.1 to 40.4 mm (n = 34). According to Nurdin (2009), males sexually mature earlier than females, resulted from the differences in growth patterns, especially on the eve of mature sex. Males grow their shell height and volume of visceral organs faster than the female.

In this research, it is known that gonad of male *D. purpurascens* is earlier mature than the female. Gil & Thome (2004) found that the gonad of males *D. hanleyanus* in

Brazilian Beach matured earlier than in females (4 and 7 mm respectively). The difference of length at first-time sexual maturity is affected by the growth pattern and size of substrate particles. Tiku beach, despite had different types of sand particle, yet dominated by fine sand substrates (Zakaria et al 2016). Herrmann et al (2010) observed populations of *D. hanleyanus* at three coastlines with different sand particle size which affect the length of shells at first sexual maturity. Santa Teresa Beach and Mar de las Pampas Beach with fine sand substrates showed early sexual maturity on males (8.61 and 12.72 mm) compared to females (9.35 and 13.21 mm). On the other hand, at Faro Querandi Beach with coarse sand substrate, the early sexual maturity occurred in females (22.44 mm) and male (22.92 mm).

Distribution of length frequency percentage between active and inactive reproductive *D. purpurascens* is shown in Figure 3, which indicates that the reproductively active individuals have size of 14-42 mm, while the ones in an active spawning period are 24-38 mm sized. Immature *D. purpurascens* were found in groups with size 14-22 mm. For the mussels in this condition is important to determine the first-time sexual maturity, as well as the first spawning. Distribution of length during transitional period is determinant for reproductive cycle of *D. purpurascens* (Nurdin 2009).

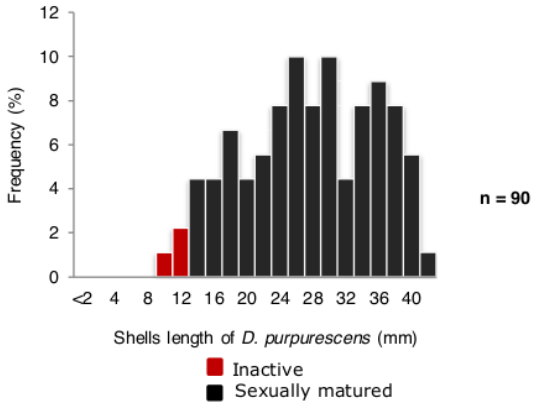

Figure 3. Shell frequency distribution of sexually mature *Donax purpurascens* at Tiku Beach, Agam District, West Sumatra, Indonesia.

The sex ratio. Distribution of length frequency percentage between males and females is shown in Figure 4. In males, the length of shell measured between 14-42 mm, while in females between 14-40 mm. There was found that the total number of female *D. purpurascens* individuals dominated over male individuals. Mzighani (2005) found that *Anadara antiquata* population in Tanzania Beach was dominated by females.

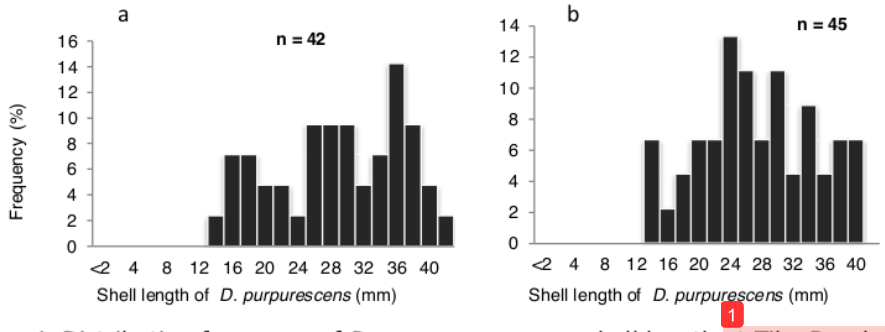

Figure 4. Distribution frequency of *Donax purpurascens* shell length at Tiku Beach, Agam District, West Sumatra, Indonesia. a – male; b - female (b).

Gonad developmental stages. Histological observation on the gonad preparations of male and female *D. purpurascens* revealed four developmental stages in their life cycle (Figure 5). Each stage was marked with several different microscopic characters (Table 2).

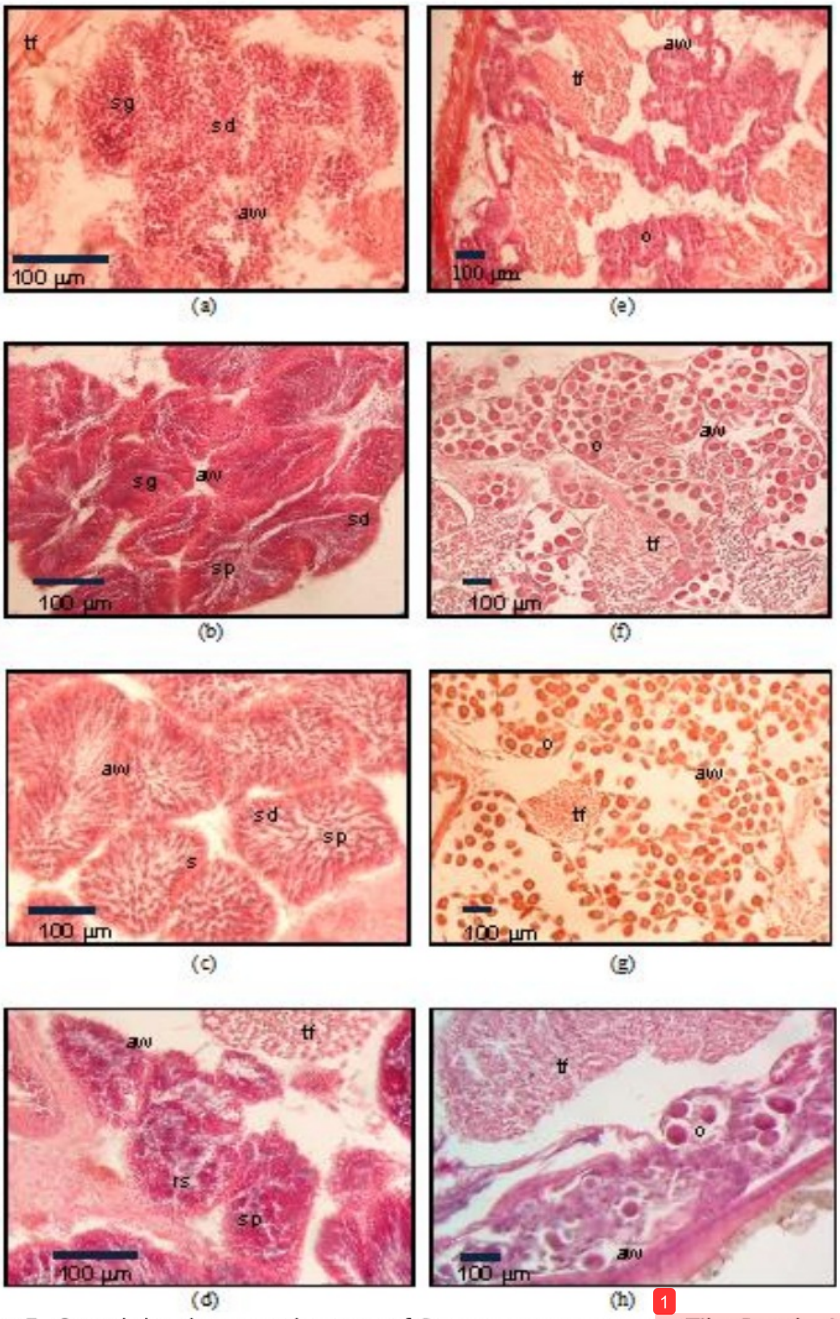

Figure 5. Gonad developmental stages of *Donax purpurascens* at Tiku Beach, Agam District, West Sumatra, Indonesia. (a-d) - males; (e-h) - females; (a,e) - pre-active; (b,f) - active; (c,g) - spawning; (d,h) - post-spawning; (sg) - spermatogonia; (sp) – spermatozoa; (sd) – spermatids; (rs) - residual spermatozoa, (aw) - alveolar walls, (o) - oocytes; (tf) connective tissue.

Table 2

Gonad developmental stages of male and female *Donax purpurascens* at Tiku Beach, Agam District, West Sumatra, Indonesia

Stages	*Description*	
	Male	*Female*
Pre-active	Spermatogonium rare and undersized. The alveolar wall unclear (Figure 5 A)	Few and small ovaries. Alveolar wall unclear. Oocytes at the early step of maturations (Figure 5 E)
Active	Number of spermatogonium increasing significantly. It advances in size. Alveolar wall clear. There are colony of spermatosites and spermatids in the spermatogonium. Number of spermatozoa still countable (Figure 5 B)	The number and size of ovaries increased. The ovary compact. Alveolar wall looks clear. Lots of oocytes stick to the alveolar wall. The numbers of free oocytes still countable (Figure 5 F)
Spawning	The size of spermatogonium was increased and full of spermatozoa. The composition of spermatocytes and spermatids decreased (Figure 5 C)	The size of oocytes in ovary got bigger, as well as its number. Alveolar walls get thicker (Figure 5 G)
Post-spawning	Spermatogonium getting smaller and weaker. The number of spermatozoa and spermatid much decreased and not uniform. The residue of spermatozoa looks clear (Figure 5 D)	The ovary got weak. Alveolar wall looks damage and unclear. The new follicles start developing to replace the old ones (Figure 5 H)

Qualitative reproductive cycle of D. purpurascens. The percentage of gonad in each developmental stage was crucial in the pattern of reproductive cycle. Gonad developmental stages fluctuated during the period of the present research (Figure 6).

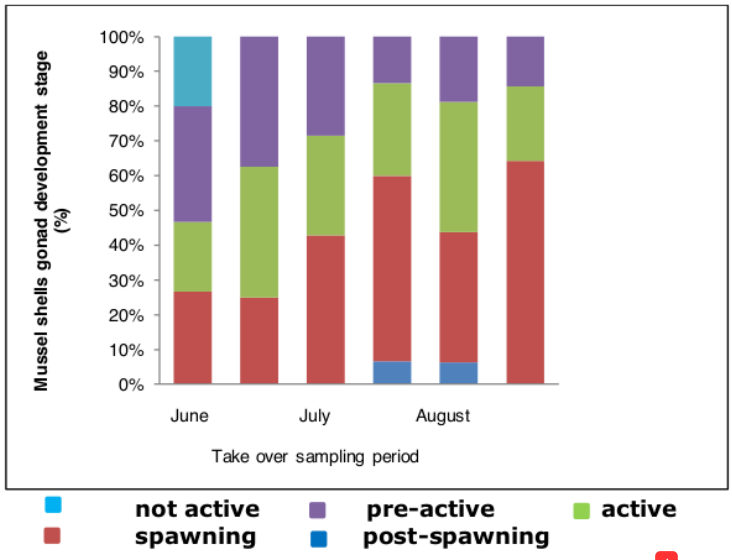

Figure 6. Gonad developmental stages of *Donax purpurascens* at the Tiku Beach, Agam District, West Sumatra, Indonesia.

The average percentage for each gonad developmental stage was as follow: not active (2.22%), pre-active (41.11%), active (28.89%), spawning (24.44%), and, post-spawning (3.33%). The non-active stage was obtained during the first sampling period in June 2010. This stage was undetected in the next sampling periods, confirmed through the observation on histological preparations.

On the pre-active stage, it ranged from 13.33-47.50% with the highest percentage occurred during the second sampling period in June and tend to decrease during the subsequent period. Active stages of sexual maturity, where gonad developmental percentage ranged 20-37.5% with the highest percentages occurred at the second sampling period in June and at the fifth sampling periods in August. The active stage started decreasing from the second sampling period of June until the fourth sampling period in July and then re-increasing in August. During the spawning stage, developed gonad ranged from 25-53.33%, with the highest percentage occurred within the sixth sampling period in August. This stage tends to increase during the whole study period. The percentage of post-spawning stage ranged from 0 to 6.67%. This stage was observed to occur twice during the sampling period (in the fourth period in July and the fifth period in August). *D. purpurascens* remained reproductively active during the post-spawning stage. Histological preparation of post-spawning stage showed the development of new follicles in either male or females, which will replace the old ones, resuming in the gametes to be constantly active.

Histological analysis on developmental stage of gonads indicated *D. purpurascens* in Tiku Beach spawn throughout the year (continuous spawned). The attendance/presence frequency for early active stages, active and spawning within the gonad developmental stages reached 100%. It is also characterized by the attachment of oocytes to the walls of alveolar follicles in females, signifying that the oocytes mature in rotational arrangement. In addition, in males, the spermatogonia were observed with several different stages of development. It means that the male gametes mature alternately (Figure 7).

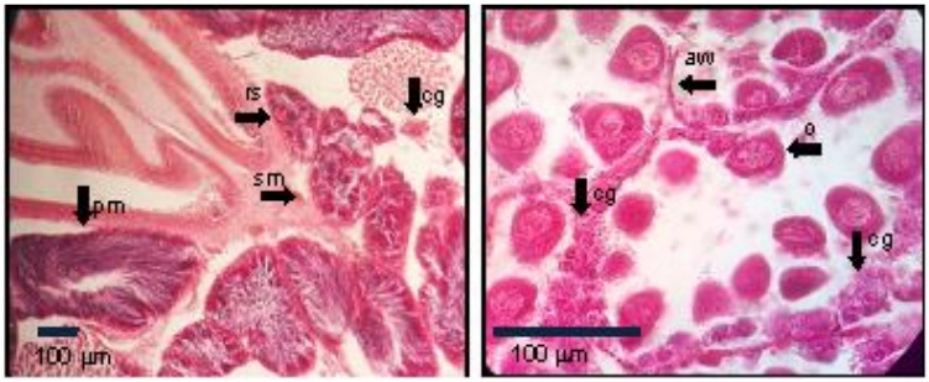

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Figure 7. Gonad development stages of *Donax purpurascens* spawning throughout the year. a – male; b – female; cg - candidate gamete cells; pm - spawning stage; sm - post-spawning stage; o - mature oocytes, aw - alveolar walls (aw), rs - residual spermatozoa.

Some *D. purpurascens* also spawn throughout the year such as *D. denticulatus* in Venezuela (Rojas 1985), *D. hanleyanus* in Brazil (Gil & Thome 2004), *D. variabilis* in South Carolina (Wilson 1999), *D. dentifer* in Columbia (Riascos & Urban 2002) and *D. trunculus* in Turkey (Deval 2009). In spite of spawning occurs throughout the year, *D. purpurascens* have spawning peaks. Unfortunately, this research failed to reveal the total number of *D. purpurascens* in spawning peak in a year. Nevertheless, the analysis on its reproductive cycle indicated one spawning peak, which occurred in August. It was marked by the finding of ample amount of juvenile mussel groups within the sampling

period. Moreover, Baron (2005) delineated that the spawning of the tropical mussels occurred once or twice a year.

Some other studies found that *D. trunculus* in the Sea of Marmara Turkey has the spawning peak in May and June (Deval 2009), while *D. denticulatus* peaks in July and December at the Punta Araya Beach Venezuela (Rojas 1985), and *D. dentifer* in March and December in Columbia (Riascos & Urban 2002). According to Gil & Thome (2004), the difference in spawning peak is affected by the fluctuation in temperature and salinity.

Quantitative reproduction cycle of D. purpurascens. Reproduction cycle of *D. purpurascens* was quantitatively determined by the fluctuation of dried gonad weight (dGW) and gonad maturity index (dGI) (Figure 8). The highest average of *D. purpurascens* dGW was 0.087±0.07 g (n = 14) within the sixth sampling period in August, while the lowest was 0.052±0.06 g (n = 12) at the first sampling period in June. Average of *D. purpurascens* dGW showed inclination during the study period (from June to August).

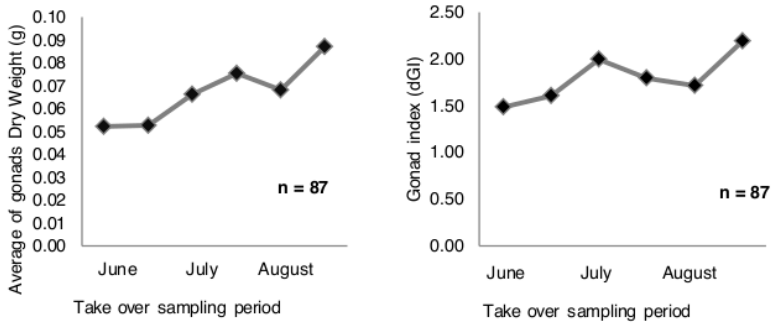

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Figure 8. Average of gonads dry weight and gonad maturation index in each sampling period at Tiku Beach, Agam District, West Sumatra, Indonesia. a - average of dried gonads weight; b - gonad maturation index.

The highest index value for *D. purpurascens* gonad maturity index (dGI) was 2.19±1.20 (n = 14) in August and the lowest was 1.48±0.99 (n = 12) in the sampling period of June. The average of both gonad dry weight and gonad maturity index indicated that the quantitative spawning peak also occurs in August. It can be observed from the fluctuation of the average dried gonad weight during this month.

Temperature and food availability are important factors in regulating the gametes maturity and spawning in bivalves (Morriconi et al 2002). Low salinity fluctuation becomes the initial parameter for accelerating the gametogenesis in *Atactodea striata*, Gafrarium pectinatum and *Anadara antiquata* in New Caledonia (Baron 2005).

Conclusions. The study on reproduction of *D. purpurascens* at Tiku Beach Agam District, West Sumatra, Indonesia can be concluded as follows:

(1) The length when *D. purpurascens* reach first sexual maturity was between 15.5 to 22.3 mm in males, and from 14 to 21.5 mm in females.
(2) Sex ratio of *D. purpurascens* was 1:1, while gonad developmental stage consisted of inactive, pre-active, active, spawning and post-spawning.
(3) *D. purpurascens* reproduction cycle and gonad maturity index showed the similar pattern that the spawning occurs throughout the year. The spawning peak correlates with environmental factors and the number of juvenile mussel group density.

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