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Reproductive Biology of Pokea Clams (*Batissa violacea var. celebensis*, von Martens 1897) at Langkumbe River, Southeast Sulawesi, Indonesia

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Abstract: Pokea clams (*Batissa violacea*) are bivalves that possess different reproductive patterns depending on the location. However, their reproductive pattern in Langkumbe River (Southeast Sulawesi, Indonesia) is unknown. This study aims to determine the sex ratio, gonad maturity level (GML), gonadosomatic index (GSI), fecundity and size of the first gonad maturity of the clams. The samples were collected randomly using iron baskets in all parts of the river and were combined together. The sex ratio, GML, GSI and size at first maturity were calculated using a standard formula, each of which was analyzed using the Chi-Square test, quantitative descriptive analysis as well as simple and non-linear regression. The results showed that the clams were gonochoric with more males than females. Male clams were relatively smaller and ranged from 3.28-3.75 cm in size. Furthermore, gonadal early and late development occurred in February through March and final gonad maturation occurred in April. Gonadal maturation and spawning occurred over a long period from March to October with a pattern of 1 peak spawning marked by an increase in the GSI value in the particular month. The GSI values in males and females were relatively the same, i.e., 36.47±16.79 and 34.80±17.71 as well as 18.37±9.46 and 18.57±8.86, respectively. Male pokea clams matured earlier than female with sizes of 1.4 cm or 0.23% and 2 cm or 0.50%, respectively. The reproductive potential of pokea clams in the Langkumbe River is high.

Keywords: *Batissa violacea, Langkumbe River, reproduction, Southeast Sulawesi.*

1. Introduction

Batissa violacea var. *celebensis* von Martens 1897 (Kusnoto, 1953) is a species of bivalves that has important economic potential and can be found distributed in several large rivers in Southeast Sulawesi among them: Pohara River (Bahtiar et al., 2015), Lasolo River (Bahtiar et al., 2016), Langkumbe River (Alkadri et al., 2018) and Laeya River (Bahtiar et al., 2022a; Bahtiar et al., 2023a). The local community people named this species pokea or keha (Bahtiar et al., 2021). Clams of the same genus (*Batissa*) are widely distributed and found in the western (Sumatera) to the eastern part of Indonesia (Papua), the Philippine and the southern Pacific (Fiji Islands) (Kusnoto, 1953; Layugan et al., 2013; Mayor et al., 2016).

Ecologically, these clams live immersed in a substrate that can be found from the texture of the substrate of mud, clay, sand and gravel. These clams are relatively distributed in groups and are only found in the estuary area, namely in the tidal area farthest upstream until the meeting of fresh water and seawater towards the sea (Bahtiar et al., 2012b). Similar to other clams, this species plays an important role in the ecosystems of rivers. These clams are able to reduce the turbidity of waters through their filter feeder mechanism. This shellfish is an environmental architect capable of producing and stabilizing the bottom substrate of the water so that it can be useful for the life of other organisms at the bottom of the water (Vaughn et al., 2008). They are also economically viable, providing the community with resources to establish food businesses (Bahtiar et al., 2014a, 2012a, 2023b; Layugan et al., 2013; Mayor & Ancog, 2016) due to their highly nutritious and efficacious nature in healing several types of diseases (Bahtiar et al., 2014b; Yeni, 2012). Pokea clams also provide a source of living for many and are frequently being sold in local markets in Kendari City.

Batissa clams are overexploited in several locations including the Pohara River (Bahtiar et al., 2012b) as well as Lasolo River (Bahtiar, 2017), Laeya River, Langkumbe River (Bahtiar et al., 2022a); and have become an endangered species in the Philippines' Cagayan River (Layugan et al., 2013; Mayor et al., 2016). The catch/production of pokea shellfish by fishermen can reach 155 tons in the Pohara River (Bahtiar, 2012), 1500-1750 tons in the Lasolo River (Bahtiar et al., 2016) and 3.40-24.77 kg/trip/boat in Cagayan River, Northern Philippines (Mayor & Ancog, 2016). Overexploitation potentially leads to a decline in the quality (low condition index and b value) and quantity (the number and size of clams are getting smaller) of pokea clams (Bahtiar, 2012; Bahtiar et al., 2015, 2016, 2022a) as well as severely disturbs their reproduction pattern or cycle in nature. Clams in areas with high ecological stress adopt reproductive strategies to maintain their existence in nature (Bone & Marshall, 1982) such as prompting early gonad maturity. However, depending on their specific habitats, pokea clams can have different environments that shape food consumption and

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regulate their activities (Bahtiar et al., 2021)

Existing studies on reproduction cycles and activity related to pokea were limited and often restricted to areas within Indonesia. Previous studies have been conducted in various locations such as the Pohara River (Bahtiar et al., 2021), Batang Anai River, Padang West Sumatra (Puteri, 2005) and Lasolo River (Bahtiar, 2017). Still, sufficient report on reproduction of the clams in Langkumbe River is yet to be identified and established. This information is crucial to guide in managing clam resources in the future (Bahtiar et al., 2022b; Camilo et al., 2017). Consequently, investigations on the reproduction biology of pokea clams in the river are needed. This study aims to determine several parameters reproduction biology of pokea clams in Langkumbe River, Southeast Sulawesi, specifically on the analysis of sex ratio, Gonad Maturity Level (GML), Gonadosomatic Index (GSI) and size at first gonadal maturity.

2. Materials and Methods

This study was carried out in Langkumbe River, Southeast Sulawesi, Indonesia, for one year from February 2017 to January 2018 at the location of 04°21'58.1" S and 122°29'54.0" W to 04°22'24.6" S and 122°29'54.9" W (Figure 1). Pokea clam samples were collected randomly and periodically every month in all parts of the river waters using iron baskets. A total of 10 batches were later brought to the laboratory and separated by sex, identified by the color of the gonads. Male gonads are milky white, while female is brown. Furthermore, the reproductive parameters of pokea clams observed include sex ratio, GML, GSI, fecundity and size at first gonad maturity. The number of samples observed to determine sex ratio was all the clams caught at all observation points. In contrast, GML, GSI, fecundity and size at first maturity of gonads were observed from 120 individuals from each sex.

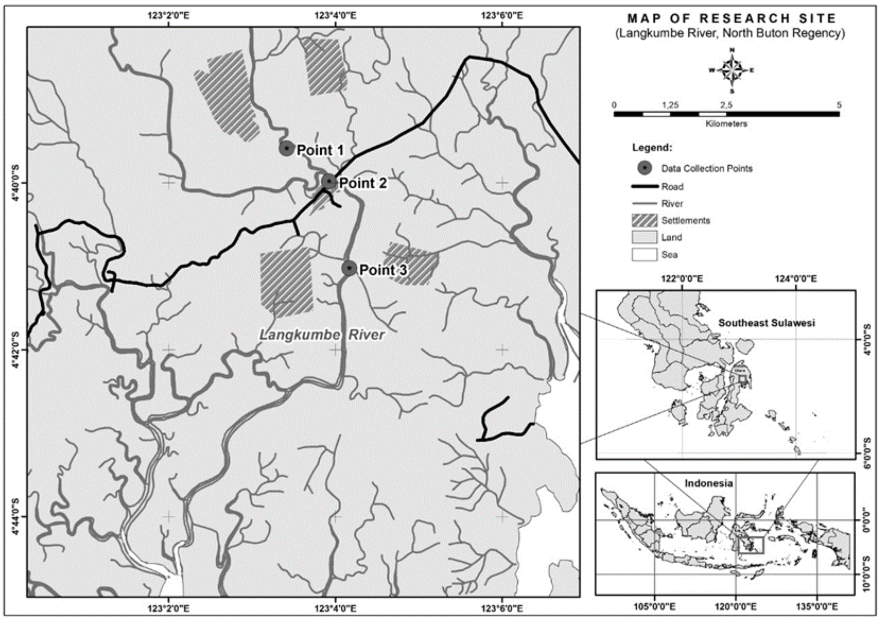

Figure 1. Map of study sites on Langkumbe River, Southeast Sulawesi.

The sex ratio was determined by calculating the ratio of the number of males and females in each month. Gonad development was observed using a microscope with a magnification of 40x10. The clams were subsequently categorized into 5 (five) different levels based on their stage of development: inactive, preparatory/early, late, mature and spawning/post-spawning. Each sample was grouped into the respective category based on the gonad morphology as observed using a microscope with histology preparation (Bahtiar et al., 2021). Furthermore, GSI observation was carried out by weighing the body and gonad weight (Bahtiar, 2017), while size at first gonad maturity was assessed by measuring the widths of all clams with mature gonads (Bahtiar et al., 2023c). Water samples were analyzed for their quality at the Laboratory of the Faculty of Fisheries and Marine Science, Halu Oleo University. The samples were taken at the same time as the pokea clams, while the quality aspects observed were the total organic and sediment organic matter and chlorophyll-a content.

The sex ratio of male to female pokea was determined by Chi-square test (χ^2) (Mzighani, 2005).

The GSI value (gonadosomatic index) was calculated using the formula described by (Bahtiar, 2017; Bahtiar et al., 2024):

$$GSI = \frac{Wg}{Wb} \times 100\ \% \qquad (1)$$

Descriptions:

GSI = gonadosomatic Index
Wg = weight of gonad (g)
Wt = weight of body including Gonad (g)

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The 50% probability of gonad maturity was determined with the non-linear regression analysis on the logistic curve (Bahtiar et al., 2023c; Mzighani, 2005) using the Sigma plot 6.0 software as shown in the following equation:

$$Y = \frac{a}{1 + e^{\frac{-x + xo}{b}}} \quad (2)$$

Descriptions:
Y = probability of pokea with mature gonad (%)
e = exponential constant
a = intercept
b = slope
x, xo = i-th width (cm)

3. Results and Discussion

Sex Ratio

Pokea shells were observed to be dominatingly gonochorism with both male and female sexes found in each month. A relatively greater trend of gonochorism was found in February and later decreased up until January. Females were smaller in proportion in February and increased onwards until October. In general, males had the larger ratios to females ranging from 34.51-71.16% and 19.04-45.98%, respectively. The χ^2 test obtained a P-value of 0.23 (p >0.05) indicating that the male to female ratio was close to 1:1, or relatively the same. Gonochorism was found in the smallest clams with a size range of 3.28-3.75 cm. Moreover, males were dominant up to the size range of 3.28-3.75 cm, above which females became generally more numerous (Figure 2). The male and female reproductive organs of pokea clams were found to be separated (dioecious) (Bahtiar, 2012; Bahtiar et al., 2021) and there was no indication of micro or true hermaphroditic nature compared to several other freshwater clams including Corbicula fluminea (Sousa et al., 2008). Pokea clams were primarily gonochoritic at a size below 1.4 cm and progressively smaller as the shells increased. Gonochorism is common in several types of shellfish including Potomida littoralis (Şereflişan et al., 2013), Chambardia rubens (Morad et al., 2018) and Atrina maura (Gongora-Gomez et al., 2016). Based on the results, it was dominant in February and was still detected every month. This condition is indicative of successful spawning and recruitment into the population implied by the dominance of young clams present at the river beds. In some species of shellfish, an equal sex ratio of 1:1 is common but this might vary depending on the conditions. This deviation was showcased in different sex ratios found in various clam colonies or population and aquatic condition. The numbers of male and female pokea found in Langkumbe River were not significantly different although males were generally larger in number. This is not the case in several rivers in Southeast Sulawesi with high fishing pressure where immoderate or significant disproportionality was found between males and females, with the former dominating the latter as in Lasolo (Bahtiar, 2017) and Pohara (Bahtiar et al., 2021). Equal sex ratio of male and female clams was shown in studies by Mayor et al. (2016) and Layugan et al. (2013), Mya arenaria (Cross et al., 2012), Cerastoderma glaucum (Tarnowska et al., 2012), Potomida littoralis (Şereflişan et al., 2013), Choromytilus chorus (Ruiz-Velásquez et al., 2017), Megapitaria squalida (Álvarez-Dagnino et al., 2017), Mytella guyanensis (Camilo et al., 2017) and Donax trunculus (Hamdani et al., 2020).

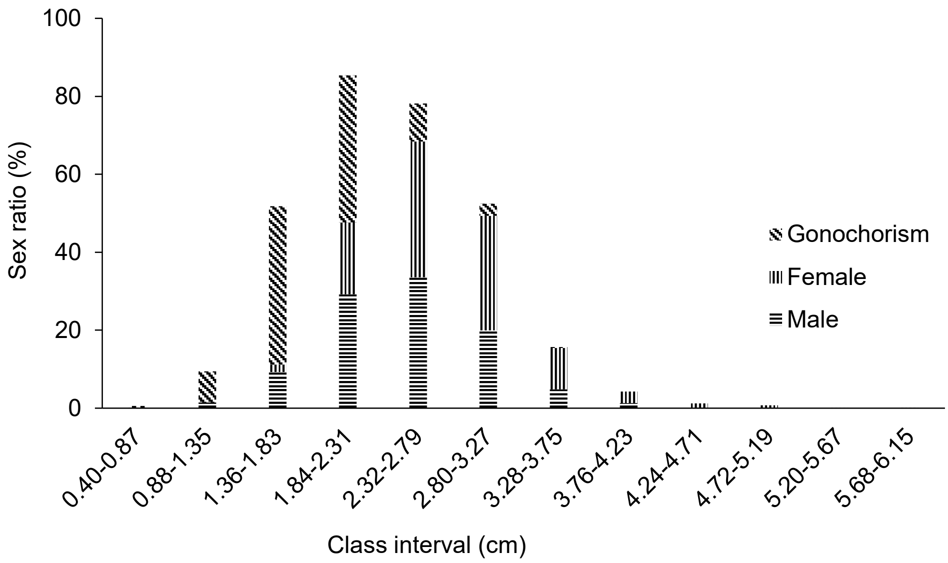

Figure 2. Sex ratio of pokea clams in Langkumbe River.

Gonad Maturity Level (GML) and Gonadosomatic Index (GSI)

The inactive phase of male and female clams occurred in February, followed by the early gonad development in March and continued to advance into the late phase in April. Furthermore, the gonad maturation and spawning took place over a long time, peaking in September and later in October. Lastly, the pokea shells experienced an inactive phase from November to January, although some were found to spawn continuously (Figure 3). The

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GSI of male and female pokea found during this study were relatively similar with values ranging from 36.47±16.79 and 34.80±17.71 to 18.37±9.46 and 18.57±86, respectively. Changes in the GSI values of both sexes were simultaneous during the peak in August and declined sharply in October (Figure 4). The water quality at the sampling sites varied temporally in the course of this study. The TOM (total organic matter) measured was within the range of 0.67-9.62 mgL^{-1} which was higher at the beginning of this study and decreased from May to the end. Sediment organic matter (SOM) observed in the water samples ranged from 0.42-4.51%. Similar to TOM, SOM was higher at the beginning, but decreased from June to September. Chlorophyll-a was measured at 0.42 to 2.93, which was relatively low in the middle of this study (August) and high at the beginning and end (Figure 5). The results showed that male and female pokea clams underwent a relatively synchronous process of gonadal maturation each month. The reproductive cycle was consistent with gonad development activity/process occurring in early February, peak reproduction in September, and long spawning in October-January. Moreover, local spawning was also found monthly in small proportions. The continuous reproductive cycle over a long period implies that pokea clams in the river observed had relatively high productivity. This was indicated by: 1) the large range in the GSI value observed suggesting that spawning occurred every month and its increase at certain times indicates a peak of maturity, while a drastic decline implies spawning (Şereflişan et al., 2013), 2) recruitment of the gonochorism phase found almost in the observation period suggests an early phase of the clam life cycle. The reproductive pattern of pokea clams in Langkumbe River was relatively the same as those found in Pohara (Bahtiar et al., 2021) and Lasolo (Bahtiar, 2017). The clams experience gonadal development in February, peak maturity and spawning in July-October, while partial spawning every month was only found in one other place namely Pohara River. It is hypothesized that the differences in reproductive patterns among these rivers were due to the conditions of sufficient food supply in Langkumbe and Pohara Rivers, which allowed the clams to reproduce in the year (Bahtiar, 2012). The gonad maturation in these rivers was relatively the same as several other shellfish in the tropical region with a long spawning period, specifically in the year, including Iphigenia brasiliana (Silva et al., 2013), Tagelus plebeuis (Ceuta & Boehs, 2012), Mytella guyanensis (Camilo et al., 2017) and Geloina expansa (Bahtiar et al., 2023c). The spawning pattern observed in this study is commonly found in the tropics when the availability of food is sufficient, thereby providing prime environmental conditions for reproduction in the year (Bahtiar et al., 2023c; Boukadida et al., 2019). Furthermore, water quality and food availability play an important role in the early development of gonadal maturity as well as during peak spawning until the shellfish larvae are released (Álvarez-Dagnino et al., 2017; Boukadida et al., 2019). Improvements in water quality aspects such as the value of total organic matter and chlorophyll-a content were followed by gonad maturation and prominent release of larvae, specifically in February. Additionally, pokea clams had sufficient nutritional intake that increased energy reserves for the reproductive process. This condition was also observed in Pohara River which was adequately supplied with nutrients as marked by an increase in the amount of food due to the high value of sedimentary organic matter originating from the Aopa Swamp that entered during the rainy season, thereby promoting peak development and maturity (Bahtiar et al., 2021). It can be inferred that gonadal maturation and early spawning were triggered by an increase in the amount of organic matter as the food source of pokea clams. Food availability accompanied by gonadal development was also found in Gari elongate with gonad maturity taking place during the rainy season (Nabuab & Norte-Campos, 2006). Similarly, food availability influences larvae spawning of Megapitaria squalida (Álvarez-Dagnino et al., 2017). Other environmental factors such as temperature and salinity also play major roles in gonad maturation and spawning (Hafsaoui et al., 2016; Hashizume et al., 2012; Khalil, 2013; Razek et al., 2014). Differences in the development of early gonadal maturity and the end of spawning period, including the release of glochidia larvae in Pseudanodonta complanata were found to be affected by changes in temperature (Ram et al., 2012; Yanovych, 2015). This was similarly reported in Paphia malabarica with changes in salinity (Thomas, 2013).

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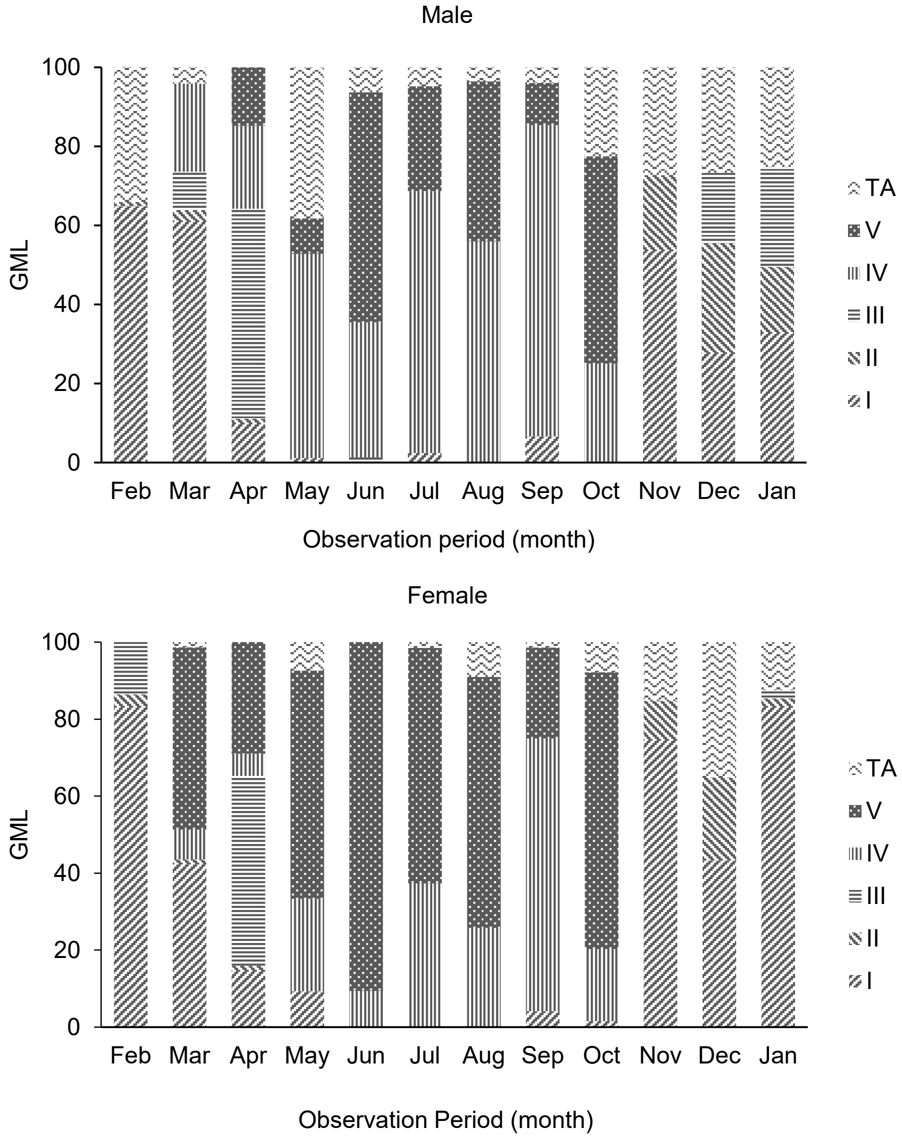

Figure 3. Gonadal maturity level of pokea clams in Langkumbe River. Description: TA = Inactive Phase.

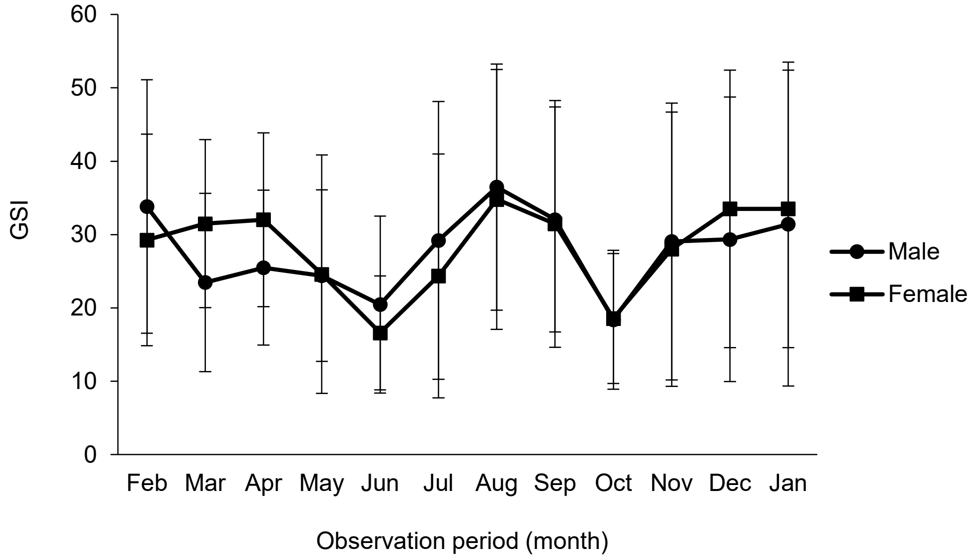

Figure 4. Average GSI values of male and female pokea during this study.

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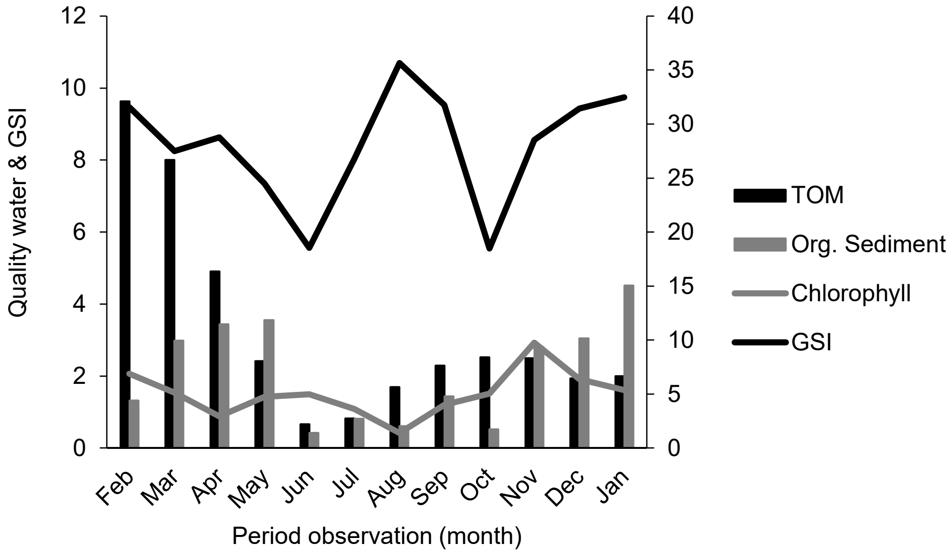

Figure 5. Langkumbe River water quality over the study period. Descriptions: TOM = total organic matter, SOM = sedimentary organic matter.

Temporarily, the reproduction pattern of pokea clams observed was not significantly different from other shellfish. Maturity in both males and females in several shellfish developed almost proportionally with the tendency to experience multiple spawning or group synchronous spawner (Sousa et al., 2008). Gonadal maturity was observed in Mya arenaria before spawning occurred in March-October (Cross et al., 2012). Potomida littoralis initiates gonad maturity in January, with a peak in May and spawning occurring in June (Şereflişan et al., 2013). The development of Paphia malabarica entails gonad maturity in March, peak maturity in September-October, and partial spawning until February (Thomas, 2013). Anodontidae bivalves of the species Pseudanodonta complanata spawns in June-August (Yanovych, 2015) which is very different from Anodontites trapezoidal cultivated in ponds and produce eggs in the season (Silva-Souza & Guardia-Felipi, 2014). Different cycles were demonstrated in freshwater clams such as Geloina erosa (Sarong et al., 2015) which showed non-concurrent spawning patterns at a specific time (asynchronous spawner).

Size at First Gonad Maturity

The non-linear regression analysis on the sigmoid pattern obtained P = 0.0001, which is α = 0.05, and a coefficient of determination (R^2) in males and females ranging from 99.88-99.96%. This means that the model established between gonad maturity and width of the pokea clams showed an appropriate relationship. The width of males with matured gonads was smaller than females at 1.4 cm and 2 cm, with a chance of gonad maturity amounting to 0.23% and 0.50%, respectively (Figure 6). Pokea clams experienced early gonadal maturation at a small size of 1.4-2 cm namely ±1/4 of the maximum length, with a quicker rate in males than females. Early gonad maturity was also found in Pohara (Bahtiar et al., 2021) and Lasolo (Bahtiar, 2017) rivers with values of 2.1-2.5 cm and 1.75-2.15 cm, respectively, all occurring at a small size. Other clams also exhibit early spawning at a small size including Paphia malabarica (1.5-2 cm) in Kerala and the West Coast of India (Thomas, 2013), and Cerastoderma glaucum (12-14 mm) on the coast of Tunisia (Derbali et al., 2009). In contrast, some species initiate gonad maturity at a size significantly larger than pokea clams such as Gari elongate and Geloina expansa with early maturation of male and female gonads at widths of 45.4 and 44.8 mm (Nabuab & Norte-Campos, 2006) and 30-52 mm (Bahtiar et al., 2023c), respectively, as well as Anadara antiquata (Mzighani, 2005). Variations in the size of clams with mature gonads can occur within the same and different species (Darrigran et al., 1999). Additionally, those in the tropical region tend to have earlier gonad maturity than others in the subtropics with sizes reaching 60% (Sato, 1994) of their asymptotic length compared to the 30-35% of the clams in the tropics. Optimum temperature and availability of food in the year trigger clams to reach gonad maturity earlier (Galimany et al., 2015). Alternatively, rapid maturity can also be caused by high environmental stress due to fishing activities. This condition is a manifestation of the strategy developed by living things to maintain the sustainability of their populations in nature. Pokea clams in Langkumbe as well as other rivers (Lasolo and Pohara) in Southeast Sulawesi, where overexploitation along with low water quality and high TSS are present, also have the proclivity to adopt such strategy when the two conditions occur. A reproductive strategy namely r-type selection pattern can be developed by initiating gonad maturity at a smaller size with multiple reproduction over a year as prompted by the less stable and unfavorable water conditions to maintain population sustainability (Bone & Marshall, 1982). This study is limited because the main cause of early initiation of gonadal maturity is not known. The following factors assumed to cause this includes: 1) adequate food availability, 2) fishing pressure or a combination of both, or 3) genetic tendency of pokea clams to have a small size when gonads mature. However, there are no previous studies that confirm these assumptions.

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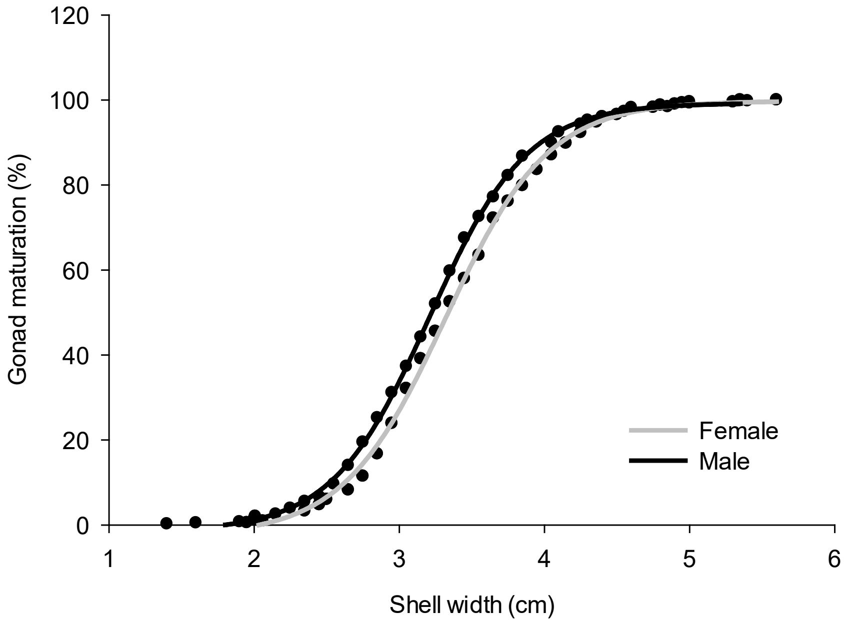

Figure 6. Size at first gonad maturity of male and female pokea.

4. Conclusion

Pokea clams in the Langkumbe river have high reproductive potential, namely: a balanced sex ratio. Although there is a tendency for certain sizes to be dominated by certain sexes, long spawning followed by partial spawning that occurs every time, small size at early gonad maturity, and water quality and food availability are sufficient to support reproduction in nature so that they can balance the fishing pressure that occurred.

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