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REPRODUCTIVE BIOLOGY OF THE FRESHWATER CLAM POKEA (*Batissa violacea* VAR. *celebensis*, VON MARTEN 1897) (Bivalvia: Corbiculidae) IN THE POHARA RIVER, SOUTHEAST SULAWESI, INDONESIA

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ABSTRACT

The freshwater clam, locally known as Pokea, (*Batissa violacea* var *celebensis*, von Martens 1897; Bivalvia: Corbulidae) is a popular and widely consumed food in Kendari, Southeast Sulawesi. Despite its popularity, basic information required for conservation managenment, such as reproductive biology, is lacking. This study aims to examine the reproductive biology of the clam obtained from the Pohara River, Kendari. Pokea samples were collected monthly from February 2012 to January 2013. We recorded parameters of reproductive biology, including sex ratio, stage of gonadal maturity, gonadosomatic index (GSI), fecundity, and size of the first mature gonad from each sample. Data were analysed using chi-square test and linear regression in the package Sigma Plot v.6.0. Pokea population in the Pohara River was male-biased. The population spawns throughout the year and the peak spawning season was August-September. Mature gonad was found at small shell size (indicating early sexual maturity). Food availability might have influenced the gonadal development in Pokea. This baseline information is very relevant for conservation practices of Pokea population in the Pohara River.

Keywords: spawning, freshwater, clams, gonadal maturity

INTRODUCTION

The freshwater clam, locally known as Pokea, (*Batissa violacea* var *celebensis*, von Martens 1897, Bivalvia: Corbulidae) is a popular and widely consumed food in Kendari, Southeast Sulawesi (Figure 1). Geographically, the genus *Batissa* is widely distributed in the western and southern Pacific (from Malaysia, Philippines, Papua New Guinea, Western Australia to Fiji) (Dudgeon and Morton, 1989). According to Sastrapradja (1977), *B. violacea* was reported to be distributed in Southeast Asia and Northern Australia. In Indonesia, this species occurs in some of the big islands, including Sumatra (Putri, 2005), Java (Sastrapradja 1977), Papua (Djajasasmita, 1977) and Sulawesi (Kusnoto, 1953). In Southeast Sulawesi, the clams can be found in some of the big rivers in the Southeast Peninsular, such as the Pohara River, the Lasolo River, the Roraya River and the Laeya River (Bahtiar, 2005). Ecologically, this species occupies the substrate surface or lives beneath the substrate in the river estuary (Sastrapradja, 1977; Djajasasmita, 1977; Bahtiar, 2007a). Pokea can be found in a variety of sediment types, from gravel to clay (Bahtiar, 2007b; Bahtiar et al, 2008). This species lives in small and big size colonies (Bahtiar, 2007a). The clams were believed to have different reproductive characteristics with other species of freshwater bivalves. This is because individuals of the clams are unisexual, while other species are mostly hermaphrodite.

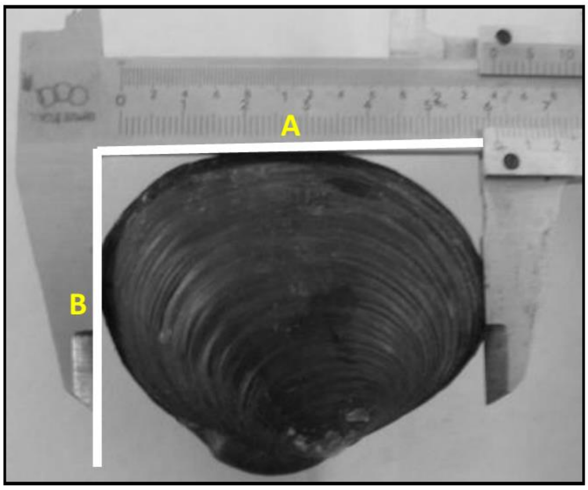

Figure 1. The freshwater clam Pokea (*Batissa violacea* var *celebensis*). A: width, B: length.

In the Pohara River, the Pokea populations have been declining, very likely due to a combination of uncontrolled harvesting and sand mining in its habitat. This is indicated from the declining trend of both the number and size of pokea sampled. In addition, local extinction has been reported in the mining areas. This situation urges conservation management applied on this species (Bahtiar *et al*., 2012). Despite its popularity for consumption, yet declining populations, basic information required for effective conservation management, such as reproductive biology, is lacking (Bahtiar, 2012). Accurate information on the reproductive biology of this species is very relevant for several conservation practices, for example determining harvesting season and determining minimum clam size for sustainable harvesting (Cantillaneza et al, 2005). This research aims to examine the aspects of reproductive biology of the Pokea clam in the Pohara River, Southeast Sulawesi.

MATERIAL AND METHODS

Sample and data collection

Pokea samples were collected monthly in the estuary region of the Pohara River ($03^{o}58^{’}551^{’’}$ S dan $122^{o}23^{’}556$’’E), from February 2012 to January 2013 (Figure 2). At the first week of each month, a total of 120 individual clams were collected, then separated based on the sex. Male individuals have milky white colored gonad, while female ones have brown colored gonad. We recorded parameters of reproductive biology, including sex ratio, fecundity, stage of gonadal maturity, gonado somatic index, and size of the first mature gonad from each samples.

Gonadal development was divided into 5 levels: inactive (stage I), early development (stage II), final development (stage III), mature (stage IV), spawning/post-spawning (stage V). The gonadal development was examined based on the gonad morphology and histology (Bahtiar, 2012). We measured gonado somatic index (GSI) based on Illanes *et al* (1985) and Wolff (1987). Fecundity was assessed based on gravimetric method, using egg weight, total gonad weight, and body weight (Effendi, 2002). Size of the first mature gonad was screened from all class sizes (Arocha and Barios, 2009). We also recorded phytoplankton abundance at the sampling sites. All laboratory works were conducted in the Faculty of Fisheries, Haluoleo University.

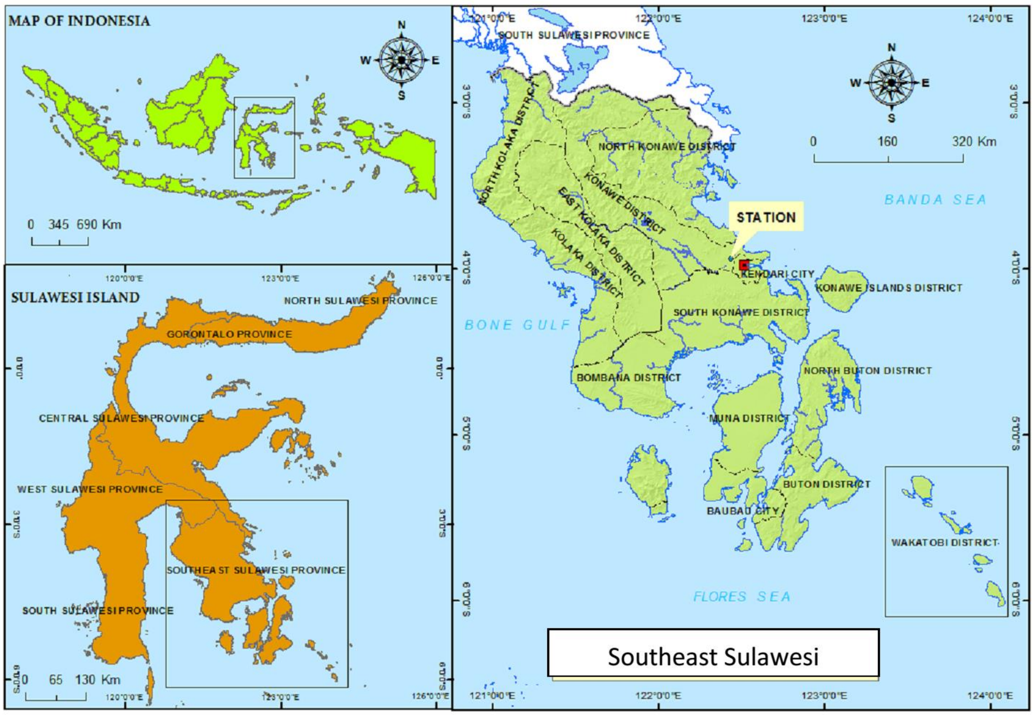

Figure 2. Sampling site in the River Pohara, Southeast Sulawesi

Data analysis

The significance of sex ratio was determined using test Chi-square (X^2) (Mzighani, 2005), using the following formula:

$$X^2 = \sum_{i=1}^{n} \frac{(Oi - ei)^2}{Oi}$$

Description :

Oi = The number of males and females observed at *i*

ei = The expected number of males and females at *i*

Gonado somatic index (GSI) was calculated using the formula described by Sastry (1979):

$$\text{GSI} = \frac{\text{Bg}}{\text{Bt}} \text{ x } 100\,\%$$

Description :

GSI = gonado somatic index

Bg = gonad weight (grams)

Bt = body weight including gonad (grams)

Pokea's fecundity was calculated based on the gravimetric method (Effendi, 2002), using the following formula:

$$N = n * \frac{G}{g}$$

Description :

N = fecundity number

n = number of eggs from sampled gonads

G = total gonad weight

g = weight of the sampled gonads

The relationship between fecundity and the shell width was analysed using regression analysis (Steel and Torie, 1981). The probability of mature gonad was calculated using nonlinear regression functions with a logarithmic curve (Arocha and Barios, 2009), as detailed in the following formula:

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

Description :

Y = probability of mature gonad (%)

e = exponential function

a = intercept

b = slope

x = width at i (cm)

The relationship between the GSI and phytoplankton abundance was analyzed using Pearson correlation. All statistical analysis was done using Sigma Plot v.6.0.

RESULTS AND DISCUSSION

Sex ratio

The number of male individuals were higher than that of female individuals at each sampling month (Table 1), with an average male/female ratio of 65/35. The sex ratio is significantly male biased, indicated from the Chi-square test (p-value =0.0003). Our finding that the sex ratio is male-biased conforms previous studies by Bahtiar (2005) and Bahtiar (2012). However, sex ratio in bivalve population is not always male-biased, but varies with different species. For example, the clam *Gari elongata* population exhibited a slightly female-biased sex ratio (Nabuap and Campos, 2006). The male-biased sex ratio might be the consequence of the fact that (i) Pokea reproduces by external fertilization, and (ii) male Pokeas are generally smaller in size than females. In external fertilization, the sperms are subjected to greater mortality in the aquatic environment. Therefore, to increase the probability of fertilization success, the number of male individuals should be higher than that of female individuals (Bahtiar, 2012).

Table 1. Sex ratio of the freshwater clam Pokea

Month	Male (%)	Female (%)
March	61.9718	38.0282
April	60.2829	39.7171
May	58.4479	41.5521
June	63.6258	36.3742
July	63.9573	36.0427
Augusts	61.7827	38.2173
September	64.3243	35.6757
October	63.7524	36.2476
November	71.9048	28.0952
December	69.6457	30.3543
January	67.659	32.341
February	72.4041	27.5959
Total	65.0591	34.9409

As dieocious organisms, Pokea shows sex differentiation even at the juvenile stage (shell width < 1.5cm), that can be observed from the gonad color. Male individuals have milky white colored gonad, while female ones have brown colored gonad. The color intensity (yellow) on the female gonad represents the egg density, as also demonstrated by some of the other freshwater species, such as *Pisidium reticulatum* (Korniushin and Glaubrecht. 2005)

Stage of gonadal maturity

We observed that the male and female Pokea showed simultaneous and parallel stages of gonadal maturity. Although some individuals already initiated gonadal maturity in March, both male and female Pokea reached the peak of gonadal maturity at the same time which was in August – September. Furthermore, spawning events were observed almost in each month, but it peaked in August to September. A profound inactive phase occured in November, following the peak period of spawning (Figure 3).

Figure 3. Stage of gonadal development in male and female Pokea.

Gonadal development represents reproductive processes that occur until the spawning event. The processes were divided into five stages, from inactive phase to the spawning phase. The parallel gonadal stages of both male and female Pokea observed in this study confirms previous studies by Bahtiar (2012). Furthermore, the peak periods of spawning events were also similar. We also found partial spawning based on the changes in the egg diameter in each observation. Bahtiar (2012) reported that the egg diameter at gonadal stage IV consisted of two size groups, with median value of 21.60 µm and 159.16 µm. At the post-spawning stage (V), three size groups were reported, with median value of 22.80 µm, 172.06 µm and 262.82 µm (Figure 4). This condition implies that the eggs reached maturity at different periods, indicating that the Pokea spawns throughout the year. Therefore, Pokea can be classified into partial/multiple spawner, although there is a peak period of spawning. Partial/multiple spawner is not uncommon in bivalves, for example the clam *Venus verrucosa* that spawns all-year long, however the spawning event peaks in August. Some populations spawn in December (Tirado *et al.* 2003), or the clam *Donax trunculus* showing two peak spawning periods (March and August) with gametogenesis cycle beginning in late November and ending at the end of August (Gaspar *et al.*, 1999).

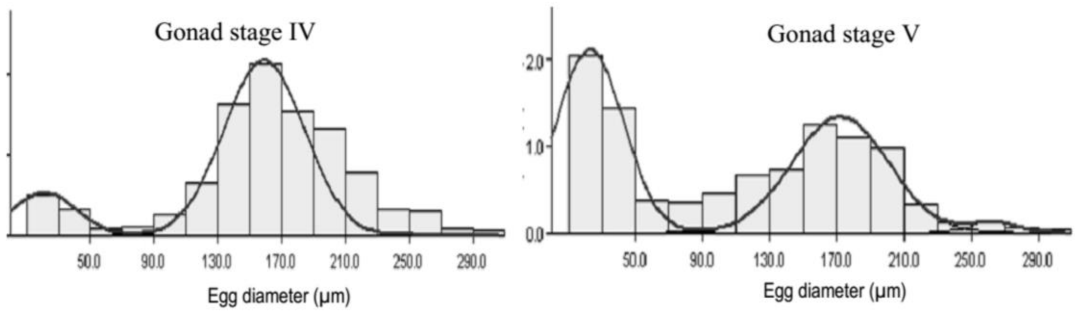

Figure 4. Bhattacharya analysis on the distribution of egg diameter on gonadal stage IV and V (modified from Bahtiar, 2012).

Gonado somatic index (GSI)

Both male and female Pokea showed similar temporal patterns of GSI. Averaged GSI ranged from 1.46 to 15.69, and peaked in July. After the peak of spawning event (August-September), the GSI value declined (Figure 5).

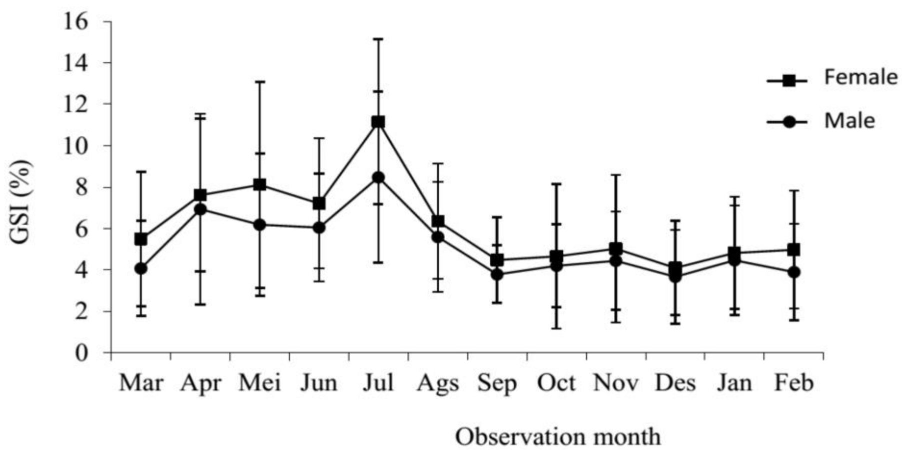

Figure 5. Averaged GSI of male and female Pokea at each observation month.

GSI indicates annual reproductive cycle and it links with the stages of gonadal maturity. Therefore, there was concordance of temporal pattern between GSI and gonadal stages, in which GSI peaked in July, followed by the peak of spawning event in August-September. A previous study by Bahtiar (2012) also reported a similar pattern. Reproductive cycles in bivalves varies with different species. For example, *Corbicula australis* spawns at the end of September and October (Bryne, 2000), while *Corbicula fluminea* spawns twice a year in early May or June and late

September (Mouthon, 2001). There is possibly an intraspecific variation in the reproductive cycle related with different locations. However, we cannot find evidences of this scenario as no other studies havereported the reproductive biology of Pokea elsewhere.

Gonadal maturity can be examined from changes of egg diameter in each month, as egg size represents reproductive growth in the gonad (Haggerty *et al.*, 2005). When compared with the GSI value, changes in egg diameter showed a similar temporal pattern. This is because the GSI value is determined by egg size (Bahtiar, 2012) (Figure 6). Similar pattern was also reported in other species *Megalonaias nervosa* showing the number and size of oosit increased at the end of June (mature gonad) until the end of September (spawning), then a decreased in early October (inactive phase) (Haggerty *et al.*, 2005).

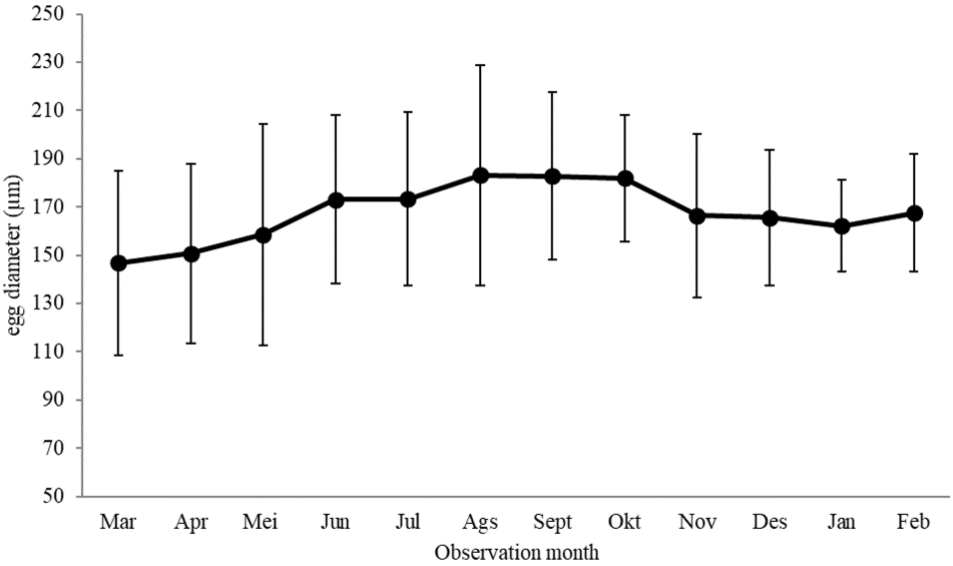

Figure 6. Egg diameter of mature gonad in Pokea (Bahtiar, 2012).

Fecundity

The relationship between fecundity (F) with the median class of shell width (L) was determined from a simple regression equation, F=36815*L-81706 with a determination coefficient (R) = 0.97. An individual with a minimal width of 2.97 cm contained a total 4652 eggs, while another one with a width of 5.93 cm contained 130465 eggs (Figure 7). Numbers of eggs found in this study were relatively smaller compared with a previous study by Bahtiar (2012) which ranged from 4950 - 1,007,384. The small number of eggs observed in this study might be related with environmental disturbances resulted from the sand mining activities. The disturbances might have triggered the clams to mature earlier and consequently resulted in the number of eggs numbers decrease. Compared with other species, the egg number recorded in this study was much lower. For example, the unionid mussels (Unionidae) can contain 200,000 to 17,000,000 eggs (Vaughn, 2005) and the macker mussel *Actinonaias ligamentina* contains 80,616 to 1,561,224 eggs (Moles and Layzer, 2008).

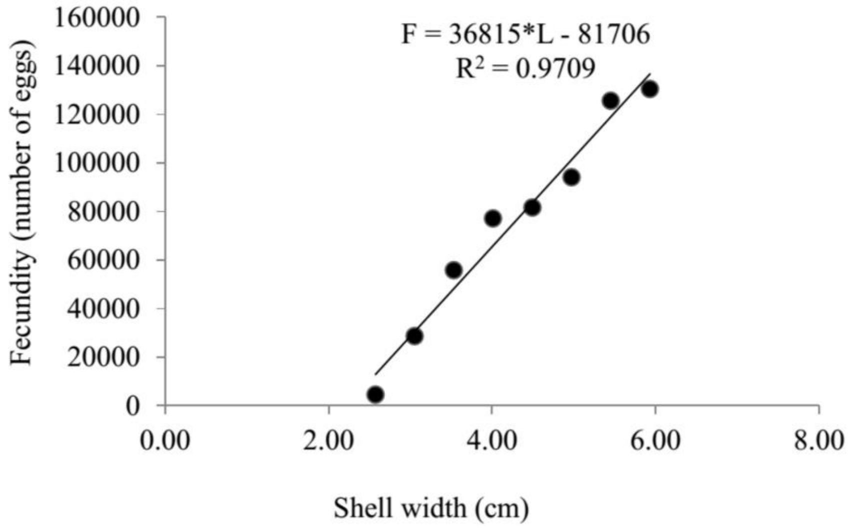

Figure 7. Linear relationship between fecundity and shell width in Pokea.

Size of the first mature gonad

Male Pokea reached the first mature gonad at a slightly smaller size than female Pokea. The first mature gonad in male individuals was found at a width of 1 cm, while in females it was at a width of 1.15 cm. The probability of 50% mature gonad was observed at a width of 2.95 cm and 3.05 cm, in male and female Pokea respectively (Figure 8). This finding is in contrast with previous study by Bahtiar (2012) that observed the first mature gonad in male and female individuals at a bigger shell size (male=2.10 cm and female=2.50 cm). Our finding was also contrast with other species of freshwater bivalves generally having infinitive shell growth. For example, the first mature gonad in *Gari elongata* was reported at 4.54 and 4.48 cm in male and female individuals, respectively (Nabuap and Campos, 2006). The smaller shell size indicates that Pokea reaches sexual maturity at a younger age. This might be related with high environmental disturbances in their habitat. To ensure the population persists, amid the environmental disturbances, Pokea's reproductive strategy is to sexually mature at a younger age, so that it can reproduce earlier. This strategy is not uncommon in bivalves (Heino and Kaitala, 1996), for example in *Corbicula australis* (Sousa *et al.*, 2008). Based on its reproductive strategy, Pokea is classified as r-selected species, characterized as spawning throughout the year, early sexual maturity, high fecundity, and rapid growth (Bone and Marshal, 1982). R-selected species is commonly found in less stable environment, such as tide subjected estuary, where Pokea can be found.

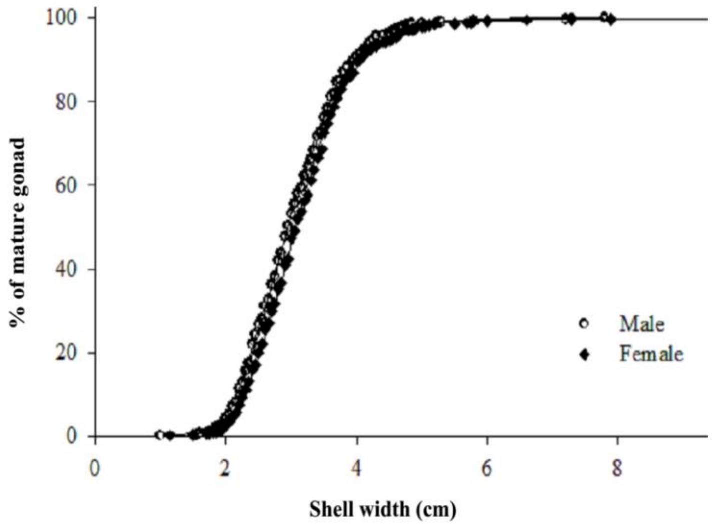

Figure 8. Size of the first mature gonad in male and female Pokea

Relationship between reproductive parameters and environmental factors

Simple line plots showed a concordance between the reproductive performance of Pokea (represented by GSI) and environmental conditions (represented by phytoplankton abundance). This pattern was further examined in a simple linear regression showing that the relationship has a coefficient of determination $R^2 = 69.05$ % (Figure 9). As Pokea feeds on phytoplankton, the increase of phytoplankton abundance in July might be related with gonadal maturity in Pokea, as it reaches spawning peak in August. Environment condition can influence bivalve reproductive performance. The importance role of food availability in triggering sexual maturity has been well documented, particularly in bivalve farming. For example, Gosling (2002) reported the increased GSI in *Argopecten irradians* was stimulated by an external factor that increased availability of food (represented by organic matter). Nabuab and Campos (2006) reported that increased of GSI in *Gari elongata* occurred following the increased of food supply (as represented by organic matter). Other environmental factors, such as pH, temperature, organic matter, and seston, might also be important (Bahtiar, 2012).

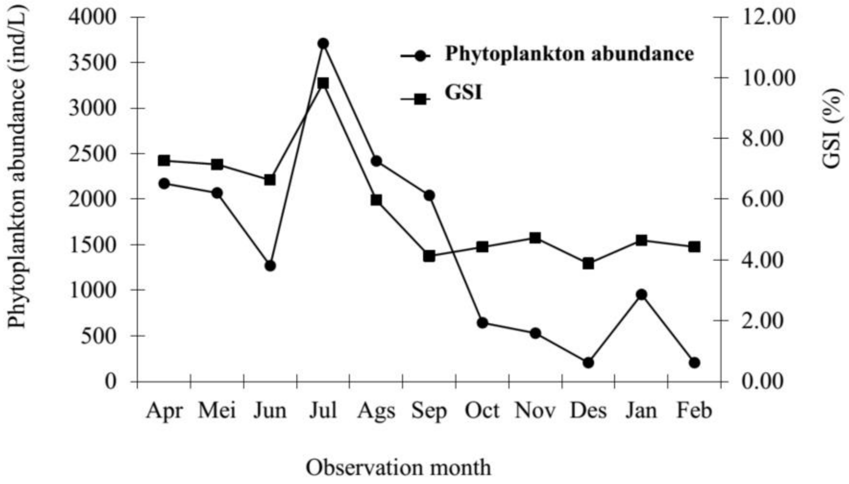

Figure 9. Relationship between GSI and phytoplankton abundance.

CONCLUSION

We reported that Poke population in the Pohara River was male-biased. The population spawns throughout the year and the peak spawning season was August-September. Mature gonad was found at small shell size (indicating early sexual maturity). Food availability might have influenced the gonadal development in Pokea. This baseline information is very relevant for conservation practices of Pokea population in the Pohara River.

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