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REPRODUCTIVE BIOLOGY OF FRESHWATER CLAM POKEA (*Batissa violacea* VAR. *celebensis*, VON MARTEN 1897) (Bivalvia: Corbiculidae) IN POHARA RIVER, KENDARI, SOUTHEAST SULAWESI PROVINCE, 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 management, such as reproductive biology, is lacking. Hence, this study aims to examine the reproductive biology of the clam obtained from Pohara River, Kendari, Southeast Sulawesi Province, Indonesia. Pokea samples were collected monthly from February 2012 to January 2013. Its reproductive biology, including sex ratio, stage of gonadal maturity, gonadosomatic index (GSI), fecundity, and size of the first mature gonad from each sample were recorded. Data were analyzed using chi-square test and linear regression in the package Sigma Plot v.6.0. Pokea population in Pohara River was male-biased. The population spawns throughout the year and the peak spawning season was in August-September. Mature gonads were found at small shell size (indicating early sexual maturity). This gonadal development in Pokea that might have been influenced by food availability, is a very relevant baseline information for the conservation of Pokea population in the Pohara River.

Keywords: clams, freshwater, gonadal maturity, spawning

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 (Fig. 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 & Morton 1989). *B. violacea* was known to be distributed in Southeast Asia and Northern Australia (Sastrapradja 1977). 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 are found in some of the big rivers in the Southeast Peninsular, such as Pohara River, Lasolo River, Roraya River and 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 clam species lives in small and big colonies (Bahtiar 2007a). These clams were believed to have different reproductive characteristics from other species of freshwater bivalves. This is because individuals of the clams are unisexual, while other species are mostly hermaphrodite.

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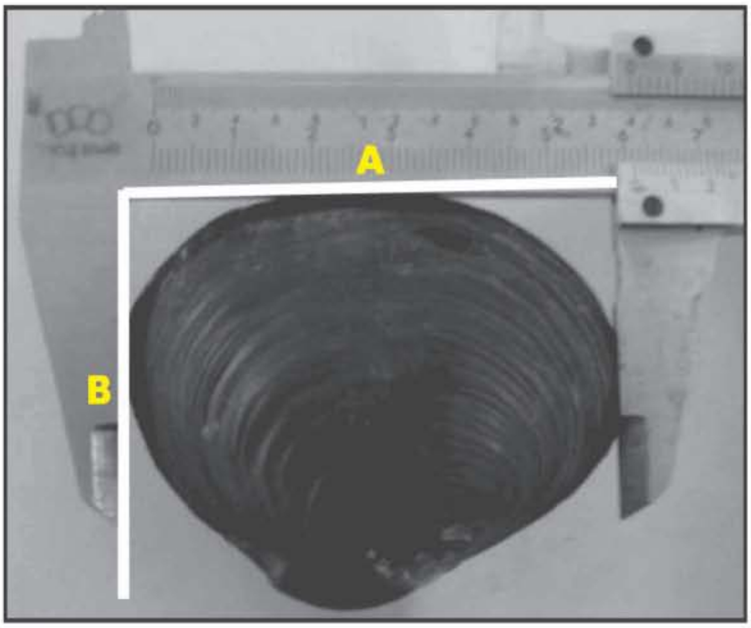

Figure 1 The freshwater clam Pokea (*Batissa violacea* var *celebensis*)
Notes: A: width; B: length.

Very likely due to a combination of uncontrolled harvesting and sand mining in its habitat, the Pokea populations in Pohara River have been declining. This is manifested by the declining trend of both the number and size of the sampled pokea. In addition, localized extinction has been reported in the mining areas. This situation calls for some form of conservation management intervention of this species (Bahtiar *et al.* 2012). Despite its high demand for consumption, coupled with its declining populations, some 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 critically relevant for several conservation practices, particularly in determining the harvesting season and minimum clam size for sustainable harvesting (Cantillaneza *et al.* 2005). Hence, this research aims to examine the reproductive biology of the Pokea clam in Pohara River, Kendari, Southeast Sulawesi.

MATERIAL AND METHODS

Sample and Data Collection

Pokea samples were collected monthly from the estuary of Pohara River (03°58'551" S and 122°23'556" E), from February 2012 to January 2013 (Fig. 2). During the first week of each month, a total of 120 individual clams were collected, then separated based on the gender. Male individuals have milky white colored gonad, while female ones have brown colored gonad. The recorded parameters for reproductive biology, included 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). This gonadal development was examined based on the gonad morphology and histology (Bahtiar 2012). The gonado somatic index (GSI) was measured based on Illanes *et al.* (1985) and Wolff (1987) methods. 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 & Barios 2009). The phytoplankton abundance was also recorded at the sampling sites. All laboratory works were conducted at the Faculty of Fisheries, Haluoleo University, Kendari, Southeast Sulawesi Province, Indonesia.

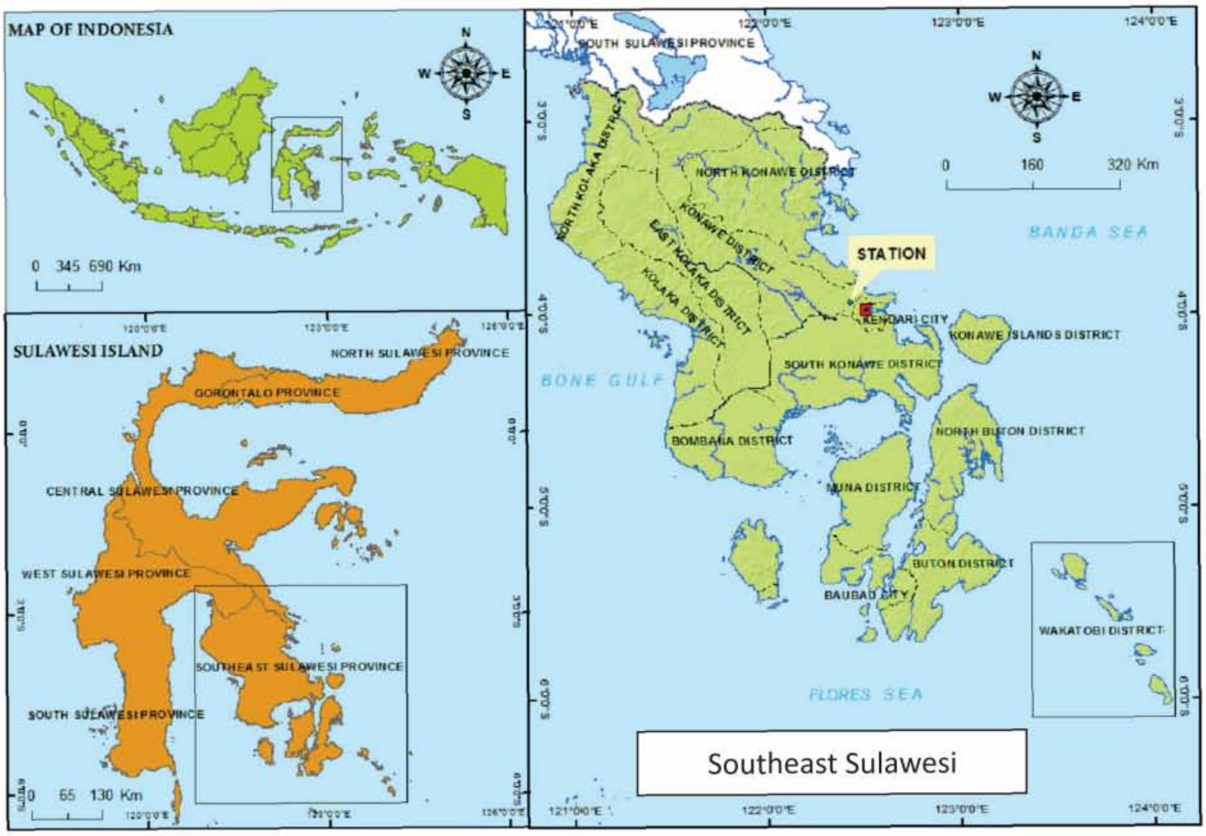

Figure 2 Map of the sampling sites at Pohara River, Kendari, Southeast Sulawesi Province

Data Analysis

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

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

where:

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):

$$GSI = \frac{Bg}{Bt} \times 100\ \%$$

where:

GSI = gonado somatic index

Bg = gonad weight (g)

Bt = body weight including gonad (g)

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

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

where:

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 analyzed using regression analysis (Steel & Torrie 1981). The probability of mature gonad was calculated using nonlinear regression functions with a logarithmic curve (Arocha & Barios 2009), using the following formula:

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

where:

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 the Pearson correlation. All statistical analyses were done using Sigma Plot v.6.0.

RESULTS AND DISCUSSION

Sex Ratio

The number of male individual clams was higher than that of the female individuals at each sampling month, at an average male/female ratio of 65/35 (Table 1). The sex ratio was significantly male biased, indicated by the Chi-square test (P value = 0.0003). This male-biased sex ratio conforms with 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 & Campos 2006). The male-biased sex ratio might be the consequence of the fact that (i) Pokea reproduces by external fertilization, and (ii) males of Pokea 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 a successful fertilization, the number of male individuals should be higher than that of female individuals (Bahtiar 2012).

Gonadal development represents the 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 confirmed the studies of Bahtiar (2012). Furthermore, the peak periods of spawning events were also similar. Partial spawning was also observed based on the changes in the egg diameter. Egg diameters at gonadal stage IV consisted of two size groups, with median values of 21.60 μm and 159.16 μm (Bahtiar 2012). 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 (Fig. 4). This condition implied that the eggs reached maturity at different periods, indicating that the Pokea spawns throughout the year. Therefore, Pokea can be classified as a partial/multiple spawner, although it has a peak period of spawning. Partial/multiple spawner is common in bivalves; for example the clam *Venus verrucosa* 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* shows two peaks of spawning periods (March and August) with gametogenesis cycle starts in late November and finishes by the end of August (Gaspar *et al.* 1999).

Table 1 Sex ratio of the freshwater clam Pokea

Month	Male (%)	Female (%)
March 2012	61.9718	38.0282
April 2012	60.2829	39.7171
May 2012	58.4479	41.5521
June 2012	63.6258	36.3742
July 2012	63.9573	36.0427
August 2012	61.7827	38.2173
September 2012	64.3243	35.6757
October 2012	63.7524	36.2476
November 2012	71.9048	28.0952
December 2012	69.6457	30.3543
January 2013	67.659	32.341
February 2013	72.4041	27.5959
Total	65.0591	34.9409

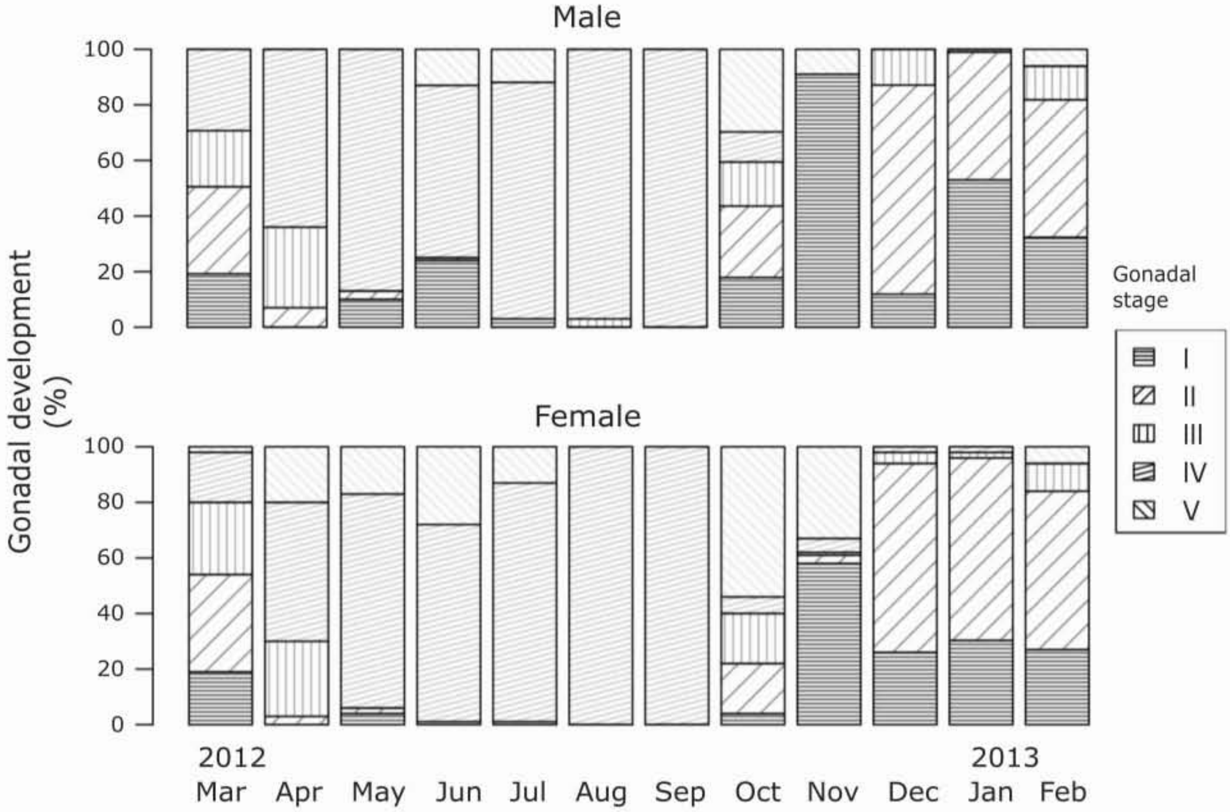

Figure 3 Stages of gonadal development in male and female Pokea
Notes: Stage I (inactive); early Stage II (development); Stage III (final development); Stage IV (mature); and Stage V (spawning/post-spawning).

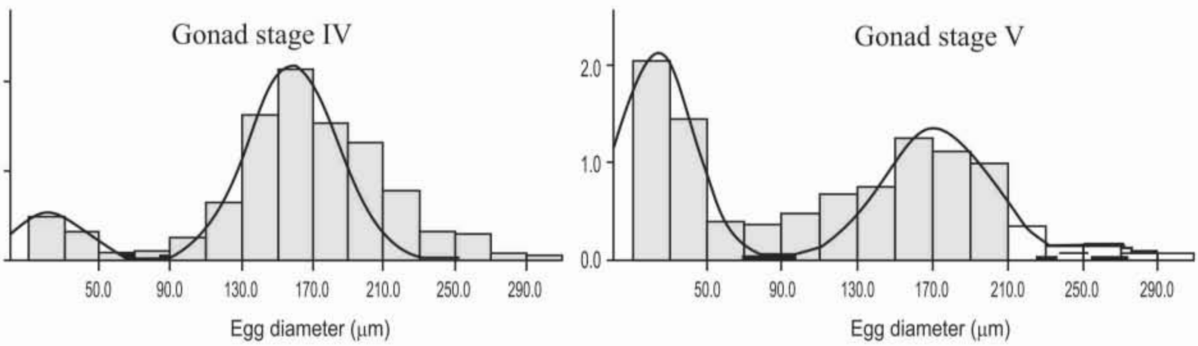

Figure 4 Bhattacharya analysis on the distribution of egg diameter on gonadal stage IV and V (modified from Bahtiar 2012)

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 in July, the GSI value declined in August-September 2012 (Fig. 5).

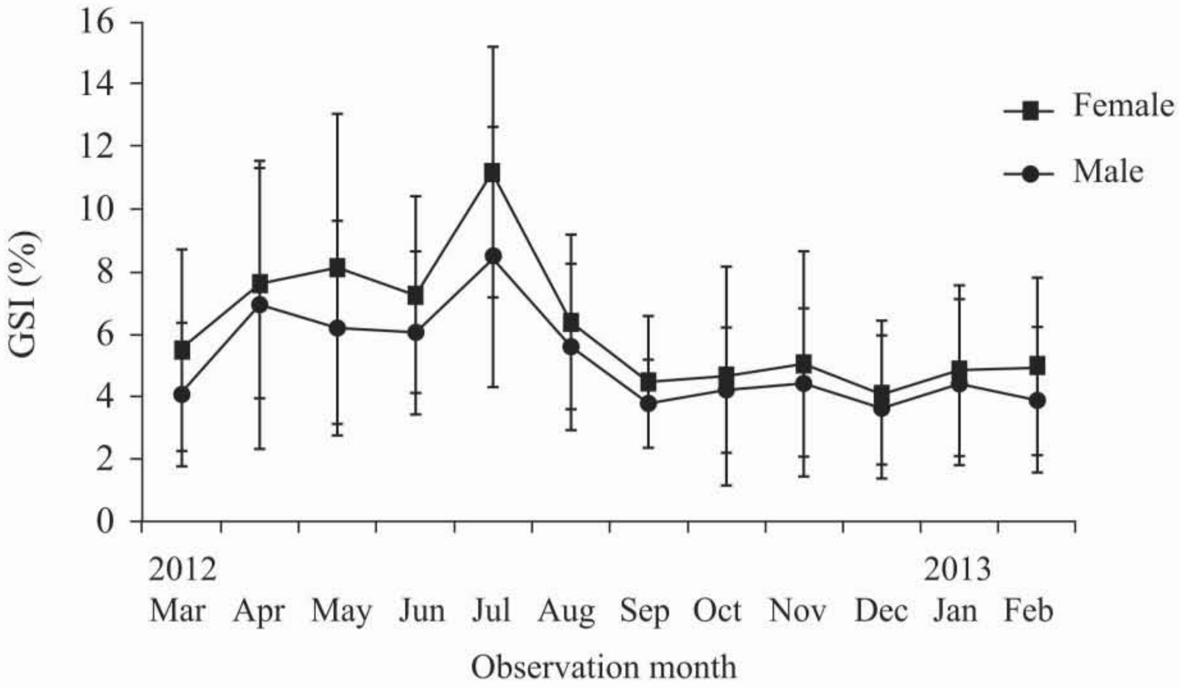

Figure 5 Mean GSI of male and female Pokea in each observation month

GSI indicates annual reproductive cycle and it links with the stages of gonadal maturity. Therefore, a concordance of temporal pattern existed between GSI and gonadal stages, in which GSI peaked in July, followed by the peak of spawning event in August-September. 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). This intraspecific variation in the reproductive cycle is probably related with different geographical locations. However, this is not conclusive as evidences or studies on the reproductive biology of Pokea are still lacking.

Gonadal maturity is determined based on egg diameter changes in each month, as egg size represents reproductive growth in the gonad (Haggerty *et al.* 2005). When the GSI values were compared, changes in egg diameter showed a similar temporal pattern. This is because the GSI value is determined by egg size (Bahtiar 2012) (Fig. 6). A similar pattern was also observed in the 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 decreased in early October (inactive phase) (Haggerty *et al.* 2005).

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 shell width of 2.97 cm contained a total of 4652 eggs, while another one with a width of 5.93 cm contained 130,465 eggs (Fig. 7). Numbers of eggs found in this study were relatively smaller compared with the findings of Bahtiar (2012) ranging from 4,950 to 1,007,384. The small number of eggs observed in this study might be a consequence of the environmental disturbances resulted from the sand mining activities. The disturbances might have triggered the early maturity of the clams resulting in the decreased number of eggs. Compared with other species, the egg number recorded in this study was much lower. For example, the unionid mussels (Unionidae) contained 200,000 to 17,000,000 eggs (Vaughn 2005) and the macker mussel *Actinonaias ligamentina* contained 80,616 to 1,561,224 eggs (Moles & Layzer 2008).

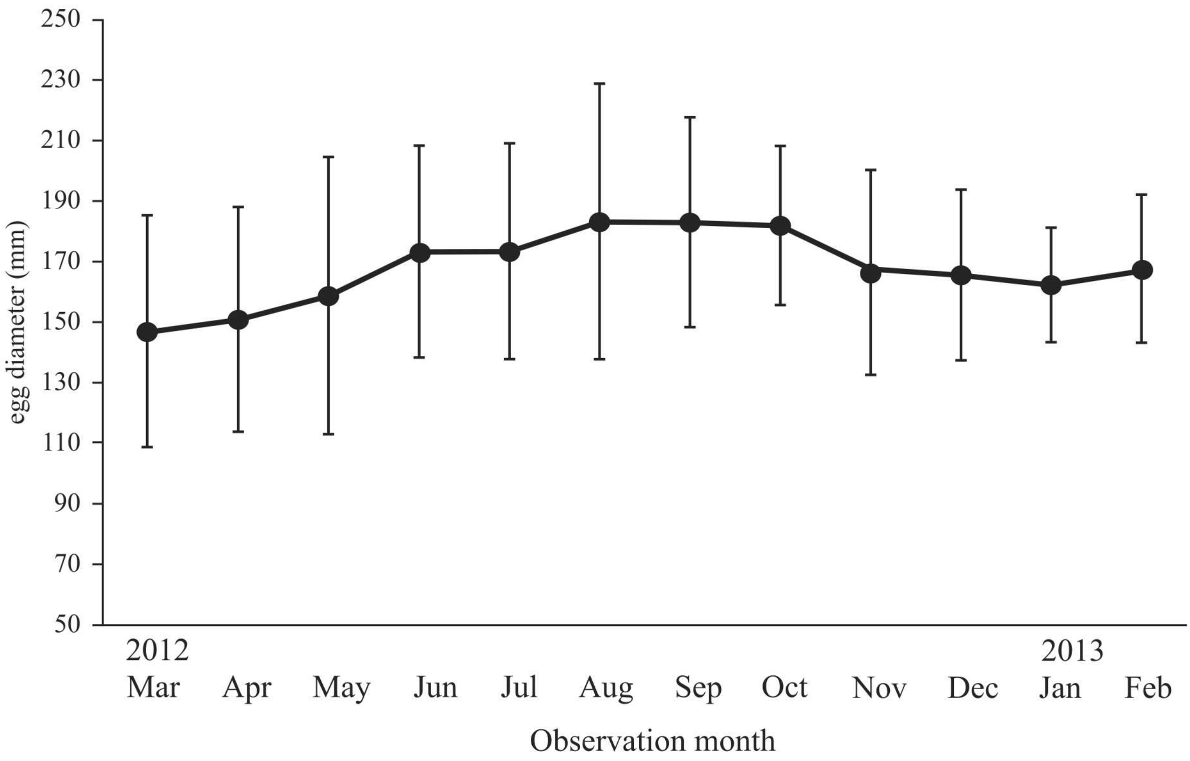

Figure 6 Egg diameter of mature gonad in Pokea (Bahtiar 2012)

Size of the First Mature Gonad

The first mature gonad was produced by the male Pokea at a slightly younger age (as shown by its smaller shell size) than the female Pokea. It was found at a shell 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 (Fig. 8). This finding is contrary to 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). This finding is also in contrast with other species of freshwater bivalves generally having infinitive shell growth. The first mature gonad in *Gari elongata* was reported at 4.54 and 4.48 cm in male and female individuals, respectively (Nabuap & Campos 2006). The smaller shell size indicated that Pokea has reached sexual maturity at a younger age, a condition that might be related with the 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 *Corbicula australis* (Sousa *et al.* 2008; Heino & Kaitala 1996). 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 & Marshal 1982). R-selected species are commonly found in less stable environment, such as in tide subjected estuary, where Pokea is found.

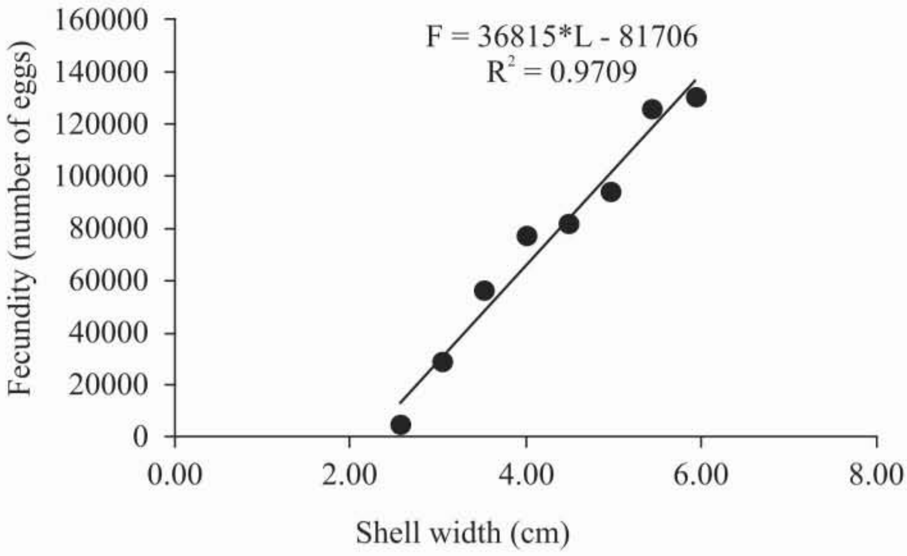

Figure 7 Linear relationship between fecundity and shell width in Pokea

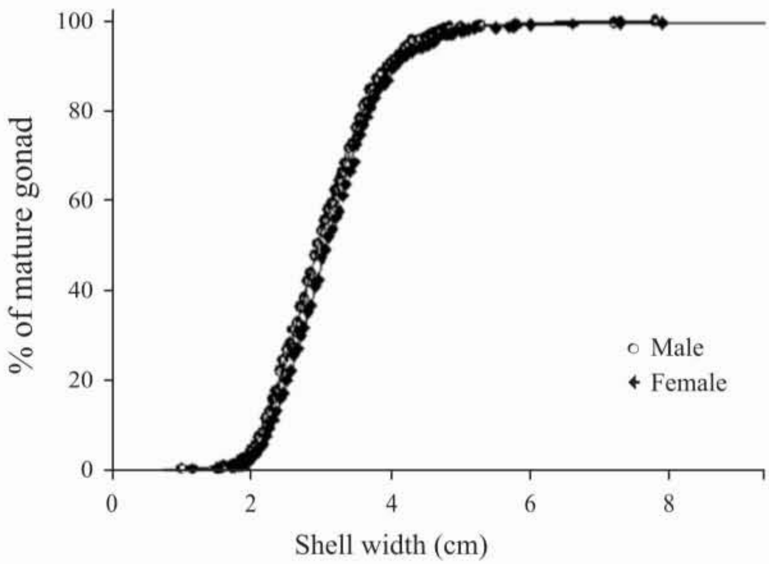

Figure 8 Size of the first mature gonad in male and female Pokea

Relationship between the Reproductive Parameters and Environmental Factors

Simple line plots showed a concordance between the reproductive performance of Pokea (GSI) and environmental conditions (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% (Fig. 9). As Pokea feeds on phytoplankton, the increase of phytoplankton abundance in July might be related with gonadal maturity in Pokea, as it reached a spawning peak in August-September. Hence, environmental condition has influenced the bivalve reproductive performance. The important role of food availability in triggering sexual maturity has been well documented, particularly in bivalve farming. The increased GSI in *Argopecten irradians* was stimulated by an external factor that increased availability of food in terms of organic matter (Gosling 2002). The increase of GSI in *Gari elongata* occurred following the increased of food supply, again in terms of organic matter (Nabuab & Campos 2006). Other environmental factors, such as pH, temperature, organic matter, and seston, might also be important (Bahtiar 2012).

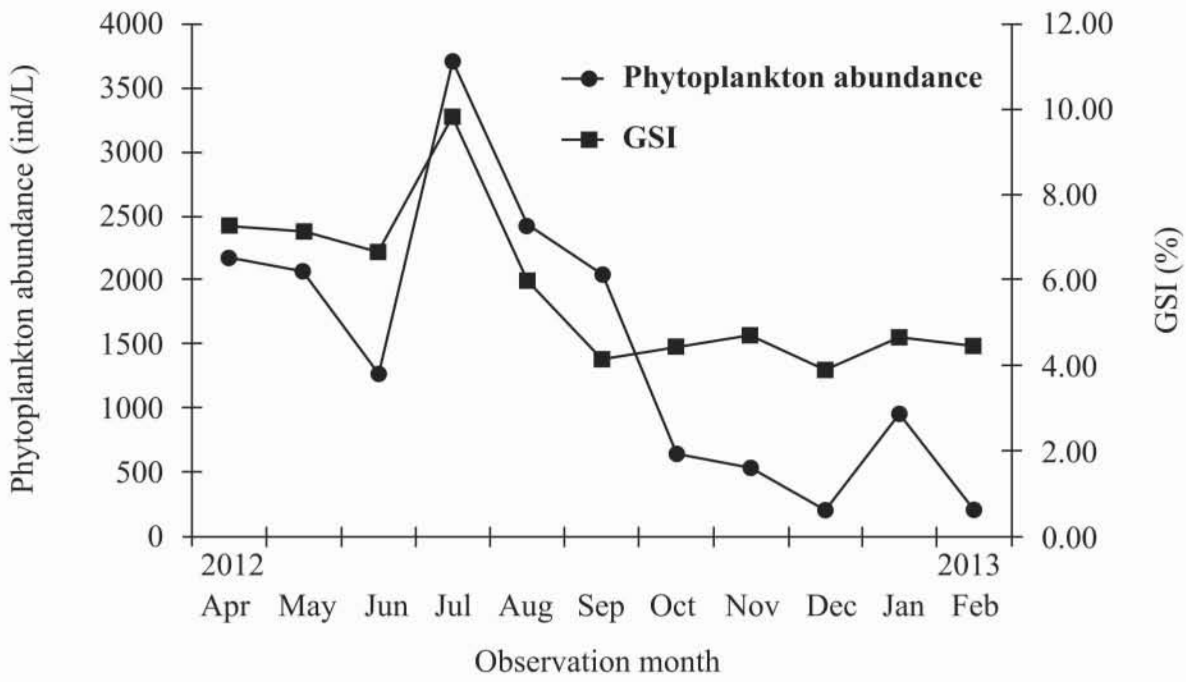

Figure 9 Relationship between GSI and phytoplankton abundance

CONCLUSION

The Pokea (*Batissa violacea* var *celebensis*) population in Pohara River was male-biased. The population spawned throughout the year and the peak spawning season was in 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 is a very relevant baseline information for the conservation management of Pokea population in the Pohara River.

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