Some Aspects of the Reproduction in the Elongate Sunset Clam, *Gari elongata* (Lamarck 1818) from Banate Bay Area, West Central Philippines

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Date submitted: January 27, 2006; Date accepted: October 13, 2006

ABSTRACT

The elongate sunset clam, *Gari (Gabraeus) elongata* (Lamarck 1818) is one of the commercially important invertebrates in Panay Island. The reproductive biology of this species from the Banate Bay Area, Panay Island, West Central Philippines was studied over one annual cycle. Elongate sunset clams were gathered monthly every last quarter of the lunar phase from September 2004 to August 2005.The shell length at first maturation, the sex ratio and the spawning periodicity were determined. The different stages of the reproductive cycle were characterized by histological examination. Seven distinguishable gonad stages were determined in both male and female clams namely, immature, early and late developing, mature, partially spawned, redeveloping and spent. Indeterminate gonads were also noted. *Gari elongata* is a dioecious species but in a few specimens hermaphroditism was observed. It exhibits no sexual dimorphism. The male to female ratio is 1:1.04. Sexual maturity is attained at 45.40 mm SL in the male and 44.80 mm SL in the female. It is therefore recommended that clams smaller than these sizes should not be collected to allow recruitment and proper sustainability of the resource. Based on the GSI and Index of Sexual Maturity elongate sunset clams have a protracted or continuous breeding period. Sexual activity is highest during the wet season while gamete development occurred during the preceding dry season. The latter is supported by higher phytoplankton densities.

Key words: Gari (Gabraeus) elongata (Lamarck 1818), reproductive biology, gonad stages, dioecious, protracted breeding season, phytoplankton density

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INTRODUCTION

The elongate sunset clam, *Gari elongata* (Fig. 1) is a mollusk that belongs to the Family Psammobiidae of Class Pelecypoda. It is found in muddy-sand bottoms, from littoral to sublittoral to a depth of 30 m (FAO 1998). In the Banate Bay area it is locally known as "bayuyan" and it thrives in non-vegetated rice paddy irrigation canals and riverine tributaries, which are less than one meter in depth. The occurrence of this organism in these freshwater systems indicates possible upstream transport and consequent adaptations of this species to lower salinities. Elongate sunset clam shows a burrowing habit, whereby the posterior end is sunk into the substrate and the anterior end exposed in the overlying water (del Norte-Campos 2004a).

Figure 1. The elongate sunset clam, *Gari elongata*.

G. elongata is considered as one of the commercially important invertebrates in Panay (del Norte-Campos, et al. 2000). Based on interviews with the locals in the Banate Bay area, the species used to be common in the past. The reason for the dwindling occurrence of this species is not clear at the present. However, proper management of the wild populations as well as artificial propagation of the species can mitigate this problem. Such efforts however, require more information on the species' reproductive biology.

The studies that have been done on *G. elongata* focused on some aspects of its distribution and population biology (del Norte-Campos 2004a) and physiology (del Norte-Campos 2004b). These papers reported that: *G. elongata* has a marine ancestry and has been able to acclimatize to a freshwater habitat; has a fast growth rate which is typical of a tropical species; and has the potential as a biofilter in fresh - to brackishwater polyculture set-ups.

Preliminary results on the reproductive biology of *G. elongata* based solely on the gonadosomatic index (GSI) of the species showed two spawning peaks: a minor spawning between May and June and a major spawning between December and January (del Norte-Campos 2004a). However, it is important that histological assessment of the gonads must also be integrated since the estimation of gonadosomatic indices in bivalves often presents a problem. This is because most species of this group have fused gonads (Urban and Riascos 2002). On the other hand, the close association between the reproductive and non-reproductive tissues may be functionally significant (Mackie 1984). Furthermore, the quantity and quality of the available food are among the environmental factors with the most influence over reproductive processes (Stead, et al. 2002).

Knowledge of the reproductive biology of the *G. elongata* would help interpret its growth pattern and population biology as well as help ensure a steady supply of broodstock and fertilizable eggs to help formulate rational management measures.

This study aims to investigate some aspects of the reproductive biology of the elongate sunset clam, *G. elongata* in the Banate Bay Area, West Central Philippines. The specific objectives of the study are: to determine the sex ratio, describe the various gonad stages, determine the minimum size at sexual maturity, and determine the annual spawning periodicity of the species and relate it to phytoplankton occurrence.

MATERIALS AND METHODS

Collection of samples was conducted in Barangay Bugnay, Barotac Viejo, Iloilo (Fig. 2). Clams were collected from an irrigation canal which leads to the

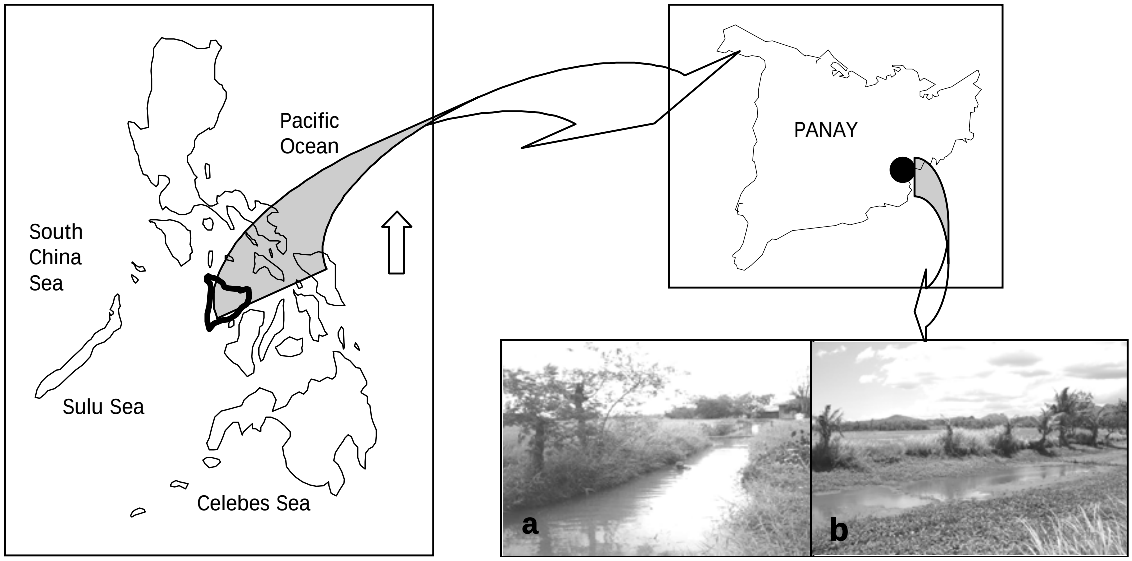

Figure 2-a/b. Site of collection of the clam *Gari elongata* : a. irrigation canal (depth ~0.5m), b. pond (depth <0.5m) in Barotac Viejo, Iloilo, Panay Island, Philippines (modified from del Norte Campos 2004a).

rice fields (Fig. 2a) from September 2004 to February 2005 and June to August 2005. However, when the canal became dried up during the summer months (March to May 2005), collection was conducted in a nearby pond 300 meters away from the irrigation canal (Fig. 2b).

Water in the irrigation canal and in the pond is clearly freshwater. Water flow was moderate but allows the stirring of the soft substrates since both collection sites are shallow (~ 0.5 m in depth), thus resulting in turbid water conditions. However, the pond is closed so that no water movement or water discharge from the river occurs. Vegetation is present at the banks of both collection sites.

Fifty clams were collected monthly at every last quarter of the lunar phase. After collection, the clams were packed with ice for transport to the laboratory. During each sampling, salinity measurements were taken using a refractometer. Phytoplankton was sampled by filtering 15 liters of water with a phytoplankton net with a mesh size of 25mm. Phytoplankton samples were afterwards preserved in 10% formalin.

In the laboratory, the clams were measured, weighed and dissected. Total lengths were measured to the nearest 0.01 mm using a vernier caliper. Total weight (TW), viscera weight (VW), and gonad weight (GW) of each individual were taken to the nearest 0.01 g using a digital electronic balance. The middle portion of the gonads was dissected and used for histological processing. Gonads were preserved in Bouin's solution and processed histologically. Samples were then dehydrated using decreasing concentration of ethanol solutions and embedded in paraffin.The fixed, paraffinized gonads were cut at the transversely at seven µm with a microtome and mounted on slides using standard techniques. Tissues were then rehydrated using serially increasing ethanol solutions, stained with Delafield's hematoxylin and counter-stained with eosin (Humason 1972).

Determination of sexes of the individuals was done based on the prepared slides examined under a compound microscope. Monthly and overall sex ratios were computed after sexes have been determined. A Chi-square test was used to check the hypothesis that males and females are equally represented in the samples.

Size at sexual maturity by sex was determined based on the smallest shell length (SL) of the clam staged with a ripe or mature gonad.

Gonads were classified into the following stages of maturity: immature, late and early developing, mature (ripe), redeveloping, partially-spawned and spent, based on their predominant appearance under the microscope, and using comparisons with related literature (e.g. Ledesma-Fernandez and del Norte-Campos, 2004; Juhel et al. 2003; del Norte, 1988). Monthly frequencies of gonad stages were plotted against time.

The quantitative Gonadosomatic Index (GSI %) of each individual was computed using the equation GSI (%) = GW/VW x 100 (Sastry, 1979). An index of maturity (= qualitative GSI) was also computed by assigning values to the gonad stages, as follows: 1 = immature, 2 = developing, 3 = ripe, 2 = partially spawned, 2 = redeveloping, and 1 = spent. These values are so designated to indicate the degree of deviation from the highest possible gonad stage of maturity (del Norte 1988). Mean values of both gonad indices were plotted against time to assess the pattern of reproductive maturity.

The volume of the phytoplankton to be examined was standardized to 20 ml before identification of organisms was done. Fifty random grids of the Sedgewick Rafter (SR) were evaluated and based on this and on the standardized 20 ml volume a raising factor (RF) was calculated, as follows:

RF = total # of grids in the SR/
# of grids analyzed * 20

Three replicates were analyzed. Phytoplankton organisms in each replicate were identified to the lowest level possible based on literature (Gran & Angst 1931; Shirota 1966). Mean densities in no. of individuals L - 1 were plotted against time and computed according to the following equation:

Mean density = RF * (mean count) /
total volume filtered

RESULTS AND DISCUSSION

Morphology, Sex Ratio and Size at Sexual Maturity

G. elongata is a dioecious species without sexual dimorphism. Both male and female gonads are cream in color regardless of stage. Thus, it is not possible to characterize its gonads macroscopically. In both sexes, the gonad is intertwined with visceral mass which is composed of the stomach and the intestinal loop.

Five individuals exhibited hermaphroditism (Fig. 3a) constituting only a small percentage of the population (0.83%). Hermaphrodites were mostly observed during the month of June. During this month, the irrigation canal was re-irrigated causing a shift from the dry to wet environment of the canal. This unnatural condition of the environment might have cause hermaphroditism of the clams. Genetic sex determination is very unstable in bivalves and can be modified by changes in

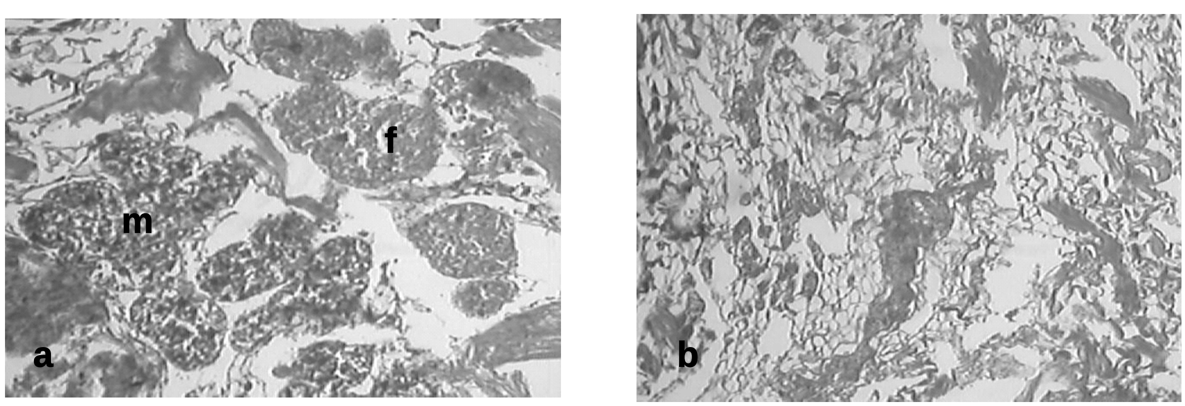

Figure 3a-b. a. Gonad of hermaphroditic *G. elongata* showing male (m) and female (f) gametes in separate follicles (Magnification 100x), b. Gonad of indeterminate *G. elongata*.

environmental or nutritional conditions (Baron 1992). In addition, some indeterminate (2.37%) gonads were also noted. This gonad stage shows no gamete development or proliferation (Fig. 3b). In *Tagelus (Tagelus) dombeii* and *Semele solida* from lower intertidal zone of south-central Chile, undetermined stage were found only at the same time that recovery stage was predominant, which suggests that the former stage is only an advanced state of the latter (Stead, et al. 2002).

Hermaphrodites and indeterminate individuals were excluded in the computation of sex ratio. Based on the Chi square test, the relatively same proportion (1M: 1.04F) of male to female indicates that both sexes are equally represented in the population. In gonochoristic or dioecious bivalves, the ratio of the sexes is approximately 1:1, with females often slightly more numerous than males (Mackie 1984) and thereby, ensuring the higher number of eggs to be fertilized by the sperm.

Albeit in lesser quantity, smaller individuals (< 40 mm) were also present in the monthly samples and these individuals were all found to have non-mature gonads. However, it is possible to sex these smaller individuals because of the presence of developing gametes. Sexual maturity is attained at 45.40 mm shell length in male and 44.80 mm shell length in female. This result supports the findings of del Norte-Campos (2004a) which showed that the elongate sunset clam matures at about 40 mm. Since external sex dimorphism does not exist for this species, 45 mm is thus suggested as the minimum size for both sexes of the clams that should be collected to allow recruitment of new individuals and ensure sustainability of this species.

Table 1. Gonad development of the various stages of the testicular and ovarian regions of *Gari elongata*

Stage	Male	Female
1. Immature	Rounded tubules with dispersed spermatogonia within the acinus, some line the edges	Acini filled with follicle cells, presence of oogonia within the follicle cells.
2. Early developing	Small and rounded tubules. Spermatogonia start to develop at the edges of the acinus.	Actively developing with oogonia at different stages of development. Acini still filled with follicle cells.
3. Late developing	Tubules start to expand. Smaller cells at the center and larger cells lining the edges. Spermatozoa with tail start to proliferate at the center.	Oocytes are still attached to the edges but starting to fill the follicles. Follicle cells start to disappear giving more spaces for developing oocytes.
4. Ripe	Tubules are elongated and filled with mature sperm.	Follicles are elongated. Free oocytes at the center of the acini, mature oocytes with prominent nucleus and nucleolus.
5. Partially spawned	Lumen shows streaky appearance of streaming sperms. Spermatozoa still occupy the lumina but have numerous gaps, may have few spermatocytes or spermatids	Spacious or empty acini, remaining mature oocytes at some acini.
6. Redeveloping	Connective tissue present. Triangle-shaped acini. Hemocytes present in the tubules and outside generally surrounding residual spermatozoa.	Connective tissues present. Oocytes at different stages are present. Some follicles are empty.
7. Spent	Tubules contain few or no residual spermatogenic cells at the wall.	Empty follicles. May contain residual oocytes at the connective tissues.

Gonad Stages

Microscopic examination of the gonads of the clams showed seven stages of development described in Table1 : immature, early and late developing, mature, partially spawned, redeveloping, and spent for females (Figs 4a-g) and for males (Figs 5a-g).

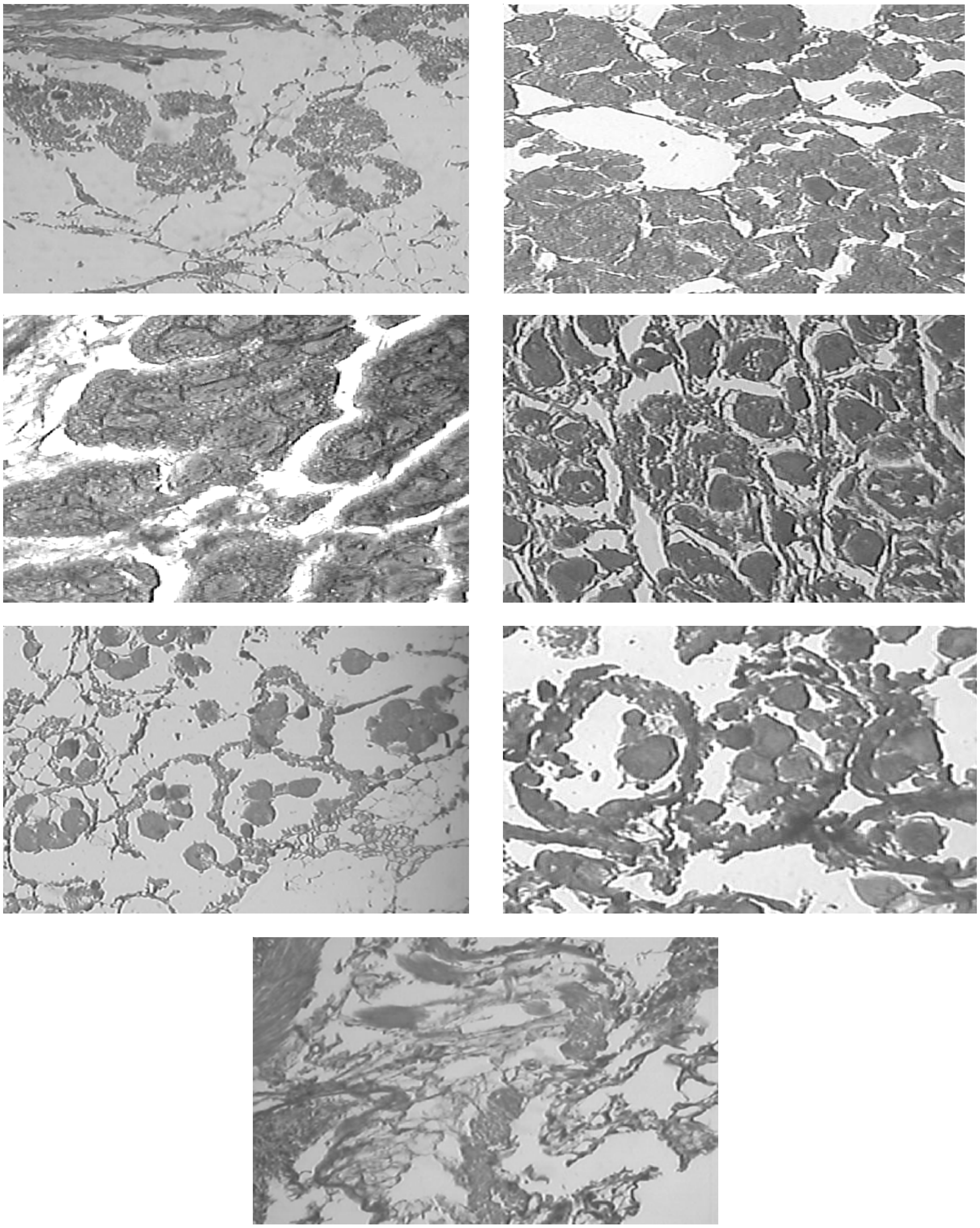

Figure 4a-g. Gonad stages of development of female *Gari elongata* (Magnification 100x). a. immature, b. early developing, c. late developing, d. mature, e. partially spawned, f. redeveloping, g. spent.

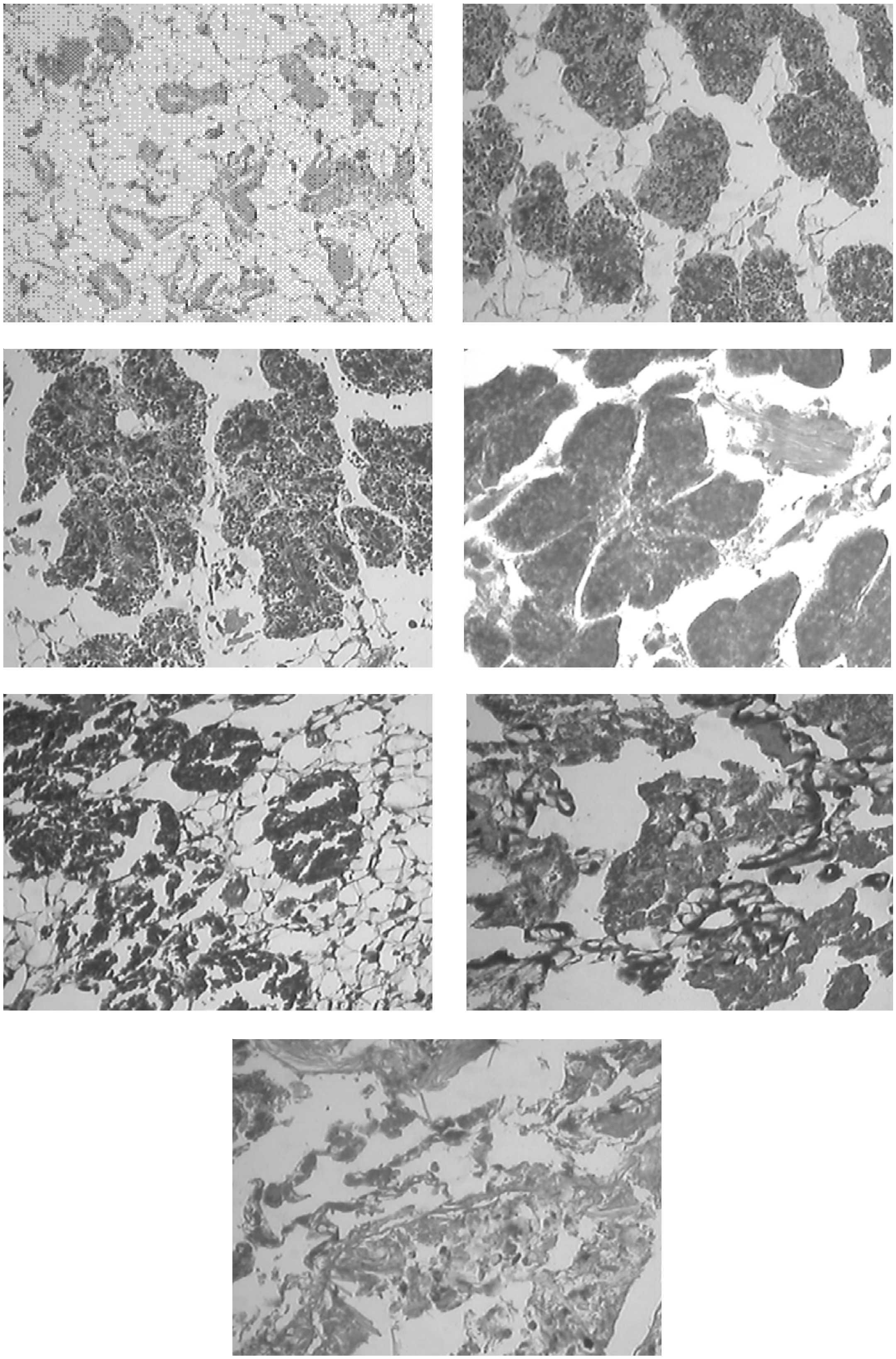

Figure 5a-g. Gonad stages of development of male *Gari elongata* (Magnification 100x). a. immature, b. early developing, c. late developing, d. mature, e. partially spawned, f. redeveloping, g. spent.

Gonad Indices

The GSI values of male and female clams were both high (26.74 - 37.92% for male and 27.91 - 37.24% for female) and there was only a very minor fluctuation shown in both sexes over an annual cycle (Figs. 6-7). The Index of sexual maturity also showed the same trend with values ranging from 1.41-2.74 for male and 1.46 - 2.39 for female (Figs. 8-9). The pattern of quantitative (GSI %) and qualitative GSI (Index of Sexual Maturity) within the sampling period thus indicate that there are gametogenic activity and spawning all throughout the year.

The high GSI values computed for this species can be partly explained by its inherent anatomy, i.e. close association of the gonad and the digestive structures. At the same time, the pattern of the gonad indices indicates that this species is reproductively active continuously during the year which is typical of tropical mollusks (Baron, 1992).

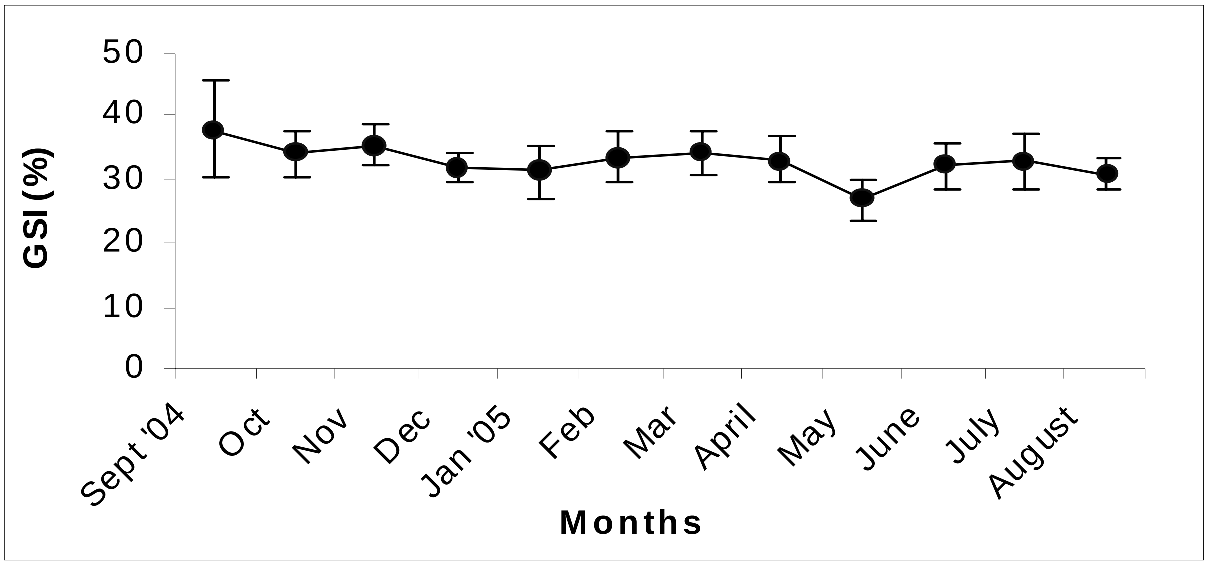

Figure 6. Gonadosomatic index (GSI) of male (n=257) Gari elongata collected from September 2004 to August 2005 in Brgy. Bugnay, Barotac Viejo.

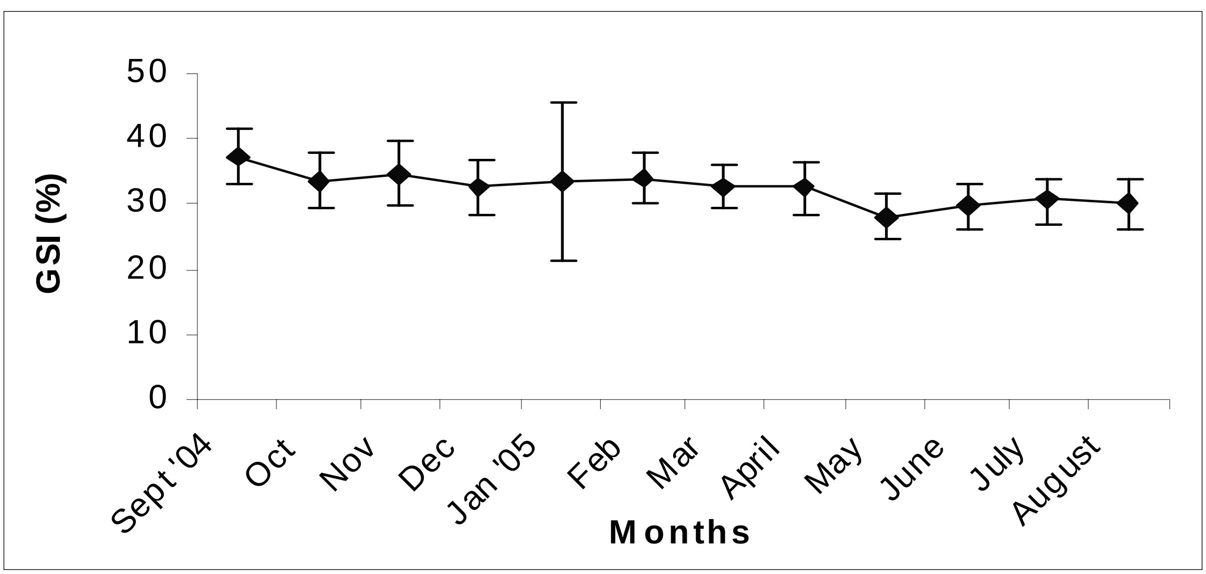

Figure 7. Gonadosomatic index (GSI) of female (n=272) *Gari elongata* collected from September 2004 to August 2005 in Brgy. Bugnay, Barotac Viejo.

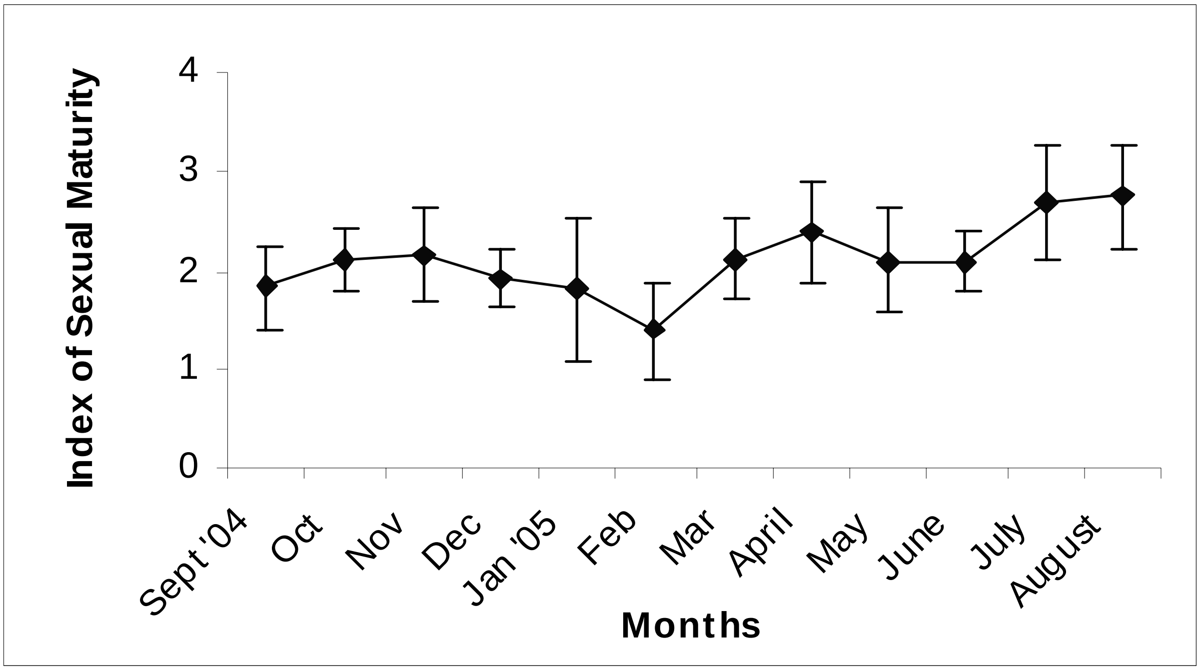

Figure 8. Gonad sexual maturity index (qualitative GSI) of male (n=257) *Gari elongata* collected from September to August 2005 in Brgy. Bugnay, Barotac Viejo, Iloilo.

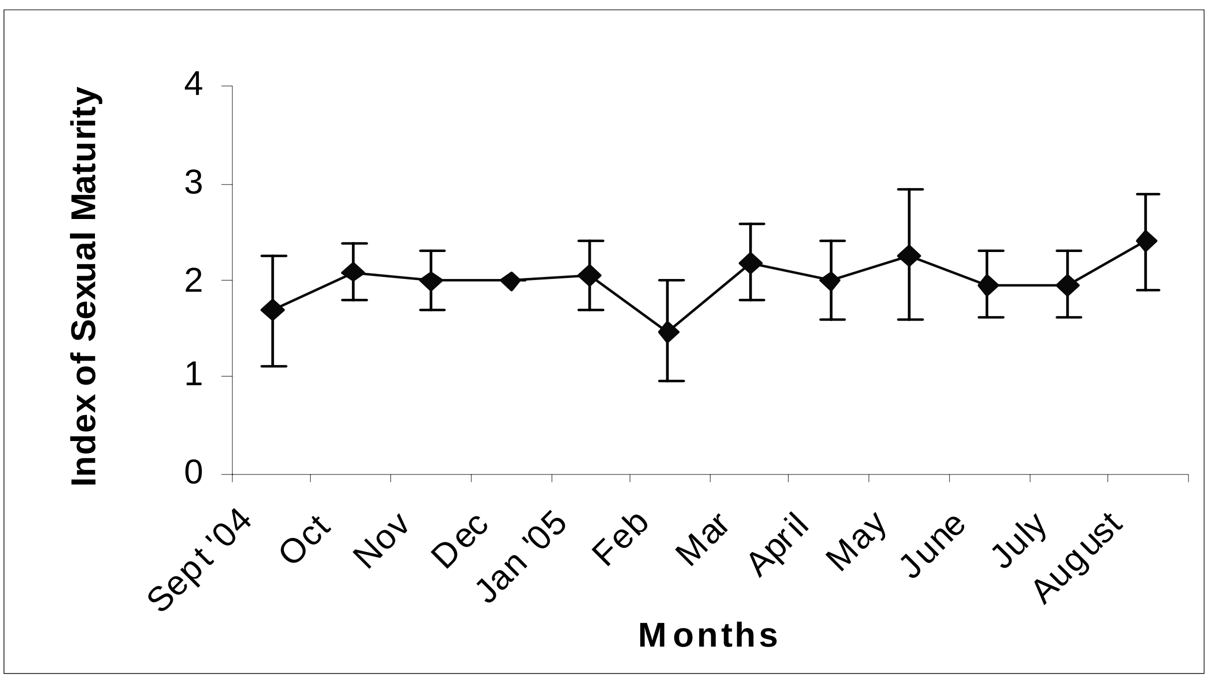

Figure 9. Gonad sexual maturity index (qualitative GSI) of female (n=272) Gari elongata collected from September to August 2005 in Brgy. Bugnay, Barotac Viejo, Iloilo.

Gonad Development

Gonad development in both male and female clams showed that gonads are active all throughout the year and only few spent stages were observed (Figs. 10-11). This observation supports the patterns of GSI and Index of Sexual Maturity which indicate that this species has a protracted breeding season. The very few spent or resting stages imply that gonad development is persistent all throughout the year. This is further supported by the co-occurrence of the various sexual development stages within the sampled monthly population. The freshwater clam, *Corbicula australis*, studied in southeast Australia also exhibits this aseasonal pattern of gametogenesis (Byrne, et al. 2000). Despite this pattern however, reproduction in *G. elongata* is highest during the wet season (May-October) as shown by the higher frequency of mature stages during these months. This trend is both exhibited by both male and female clams. Spawning individuals can further be observed in all months except in February in female and in February and July in males. The highest frequency of spawning individuals is observed in November for the females and in June for the males (Figs. 10-11), which are also comparable to the spawning peaks of this species based on GSI by del Norte-Campos 2004a.

The high frequency of mature gonads in *G. elongata* coincided with the southwest monsoon. It was also observed for the ark shell, *Scapharca inequivalvis*, a species that thrives on intertidal areas of Banate Bay (Ledesma-Fernandez and del Norte-Campos 2004). Southwest monsoon causes continuous rains lasting for weeks during the months of June to September which bring higher riverine inputs and cause stirring of the substrates that increase organic matter in the canal.

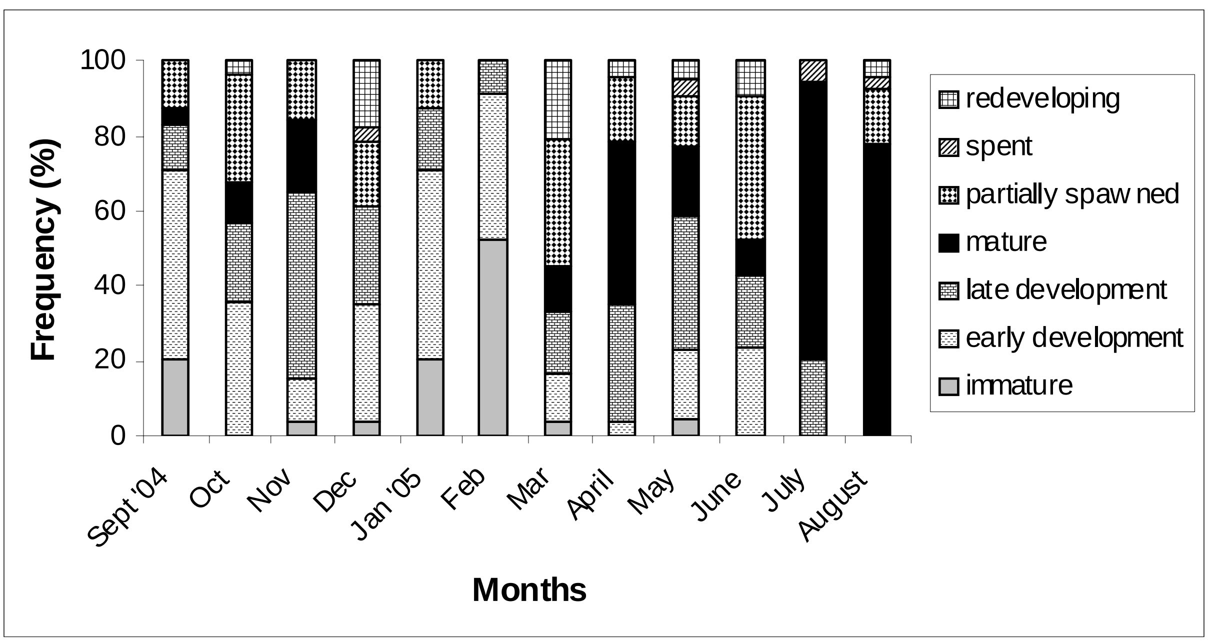

Figure 10. Frequency distribution of gonad stages in male (n=257) *Gari elongata* collected from September to August 2005 in Brgy. Bugnay, Barotac Viejo, Iloilo.

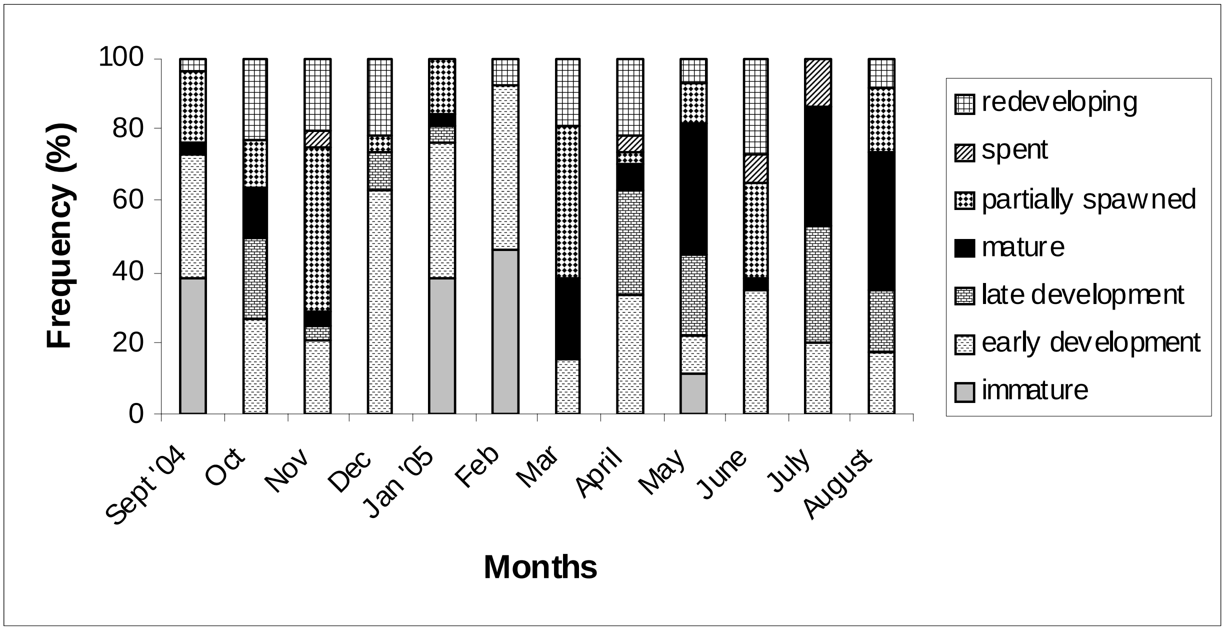

Figure 11. Frequency distribution of gonad stages in female (n=272) *Gari elongata* collected from September to August 2005 in Brgy. Bugnay, Barotac Viejo, Iloilo.

High proportions of immature, developing and redeveloping individuals are observed during dry months which are also during high mean densities of phytoplankton were observed (Fig. 12). Energy derived by the clams from the phytoplankton was utilized in gamete proliferation and growth. This can be evidenced by marked accumulation of gametes as indicated by the high frequency of developing and redeveloping gonads during this season resulting in the increase in frequency of mature individuals as the wet season approaches.

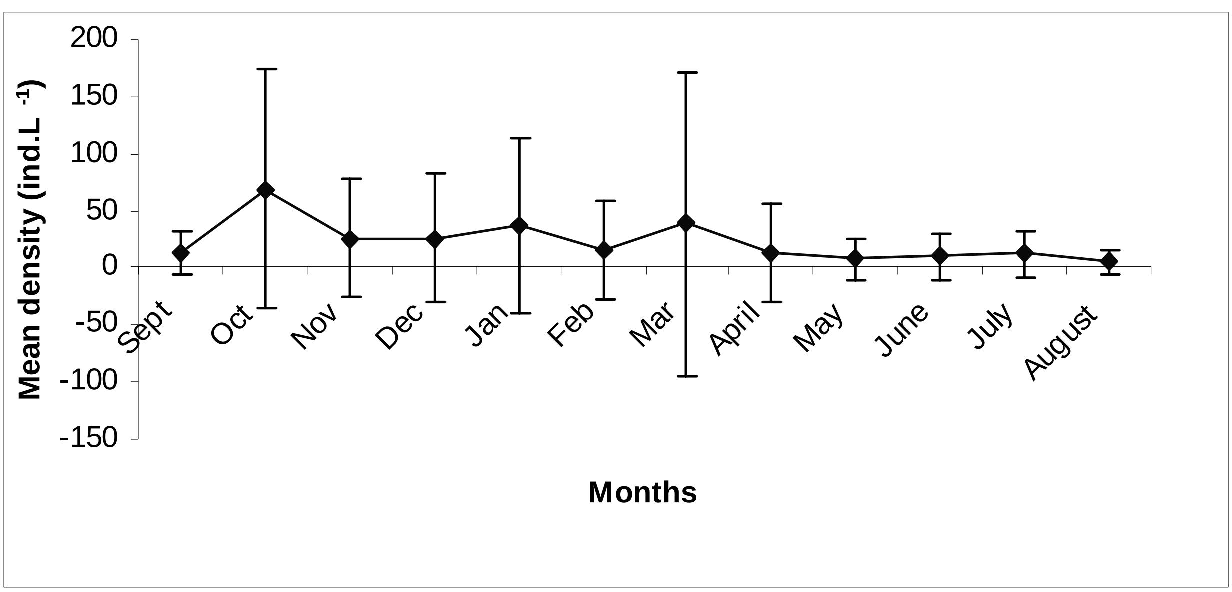

Figure 12. Mean densities of phytoplankton sampled from September 2004 to August 2005 in Brgy. Bugnay, Barotac Viejo, Iloilo.

Phytoplankton

Overall phytoplankton mean density plotted against time is shown in Figure 12. Mean density from September 2004 to August 2005 was 20 ind.L^{-1}. Higher mean phytoplankton densities were observed during dry months (October - April). Maximum mean density is attained in October with 69 ind.L^{-1}. Increased mean densities of phytoplankton during this season can be attributed to the optimum utilization of sunlight energy for photosynthesis. Alternatively, mean densities decreased towards the wet season.

CONCLUSIONS

G. elongata is a dioecious species and attains sexual maturity at 45.40 mm SL in the male and 44.80 mm SL in female. Since this species exhibit no external dimorphism, 45 mm is the recommended minimum size to be harvested in order to allow adequate recruitment and ensure sustainability of the species. Male to female sex ratio is 1.0: 1.04 and the Chi- Square test showed that both sexes are equally represented in the population.

Seven distinguishable gonad stages based on histological examination were determined in male and female clams: immature, early and late developing, mature, partially spawned, redeveloping and spent. Indeterminate and hermaphrodite gonads were also observed although in lesser quantities and mostly in the month of June.

Based on the gonadosomatic index (GSI), index of sexual maturity, and frequency distribution of gonad stages, *G. elongata* has a continuous breeding season. Despite the protracted breeding season, highest gonad activity is observed during the wet season while gamete development occurs during the preceding dry season. The latter is supported by higher phytoplankton densities.

RECOMMENDATION

Since sampling was done in two different sites, a study on the reproductive biology of the same species from both irrigation canal and pond is recommended to evaluate the reproduction of *G. elongata* from these two sites.

ACKNOWLEDGEMENTS

This study was funded by UPV in-house project. Initial sampling assistance was provided by Mae Angeline Tajolosa and monthly sampling assistance by the Sulartin family in Brgy. Bugnay, Barotac, Viejo, Iloilo. To TATAY Frank Sulartin, to you I dedicate the completion of this paper.

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