# Seasonal changes of reproductive cycle and proximate compositions in *Cerithidea obtusa* from Ca Mau mangrove forest, Vietnam

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**Abstract-** *The present study investigated seasonal changes in gonad development and proximate compositions of the mangrove snail, Cerithidea obtusa in the mangrove forest of Ca Mau province, Mekong Delta of Vietnam. Our findings showed that gametogenesis of C. obtusa occurred year around, however, the major spawning period lasted from September to November. The highest spawning synchrony was observed in November with 43% collected individuals in spawning stage. The results also showed that in mangrove snails, proteins are the major component (~60%) followed by carbohydrates (~25%) and then lipids (~5%). Proteins could be the major energy resource to support growth and reproductive cycle of this species. Results from this study provide detail information on the biology of mangrove snails and it could be useful for resource management, bio-conservation and sustainable aquaculture in mangrove forests of Vietnam.*

***Index Terms***- *Cerithidea obtusa*, gonad index, proximate composition, reproductive cycle

## I. INTRODUCTION

Mangrove snail, *Cerithidea obtusa* is common species in the mangrove ecosystem in Asian-Pacific region (Ellison *et al*., 1999; Tan and Chou, 2000). This snail species plays an important role in food web inside mangrove forest because they feed on benthic diatoms and the detritus that original from the mangrove leaf litter decompositions (Bouillon *et al*., 2002). Several studies have been conducted on *Cerithidea* genus, such as oxygen consumption (Houlihan, 1979), feeding behaviour (Bouillon *et al*., 2002) and lipid and fatty acids of *C. obtusa* (Misra *et al*., 1986). Studying on the reproduction, Suwanjarat and Klepal (2001) described the ultrastructural characteristics of euspermatogenesis and euspermatozoa of *Cerithidea obtusa* but not reproductive cycle. The snail is a highly priced in domestic market of Vietnam with around 5-7 USD/kg, and annual landings in Vietnam rely mainly on its wild fishery and partially from aquaculture. Until now, there is no report about the production of this snail species in Vietnam, however, Chau *et al*. (2009) reported that the wild fishery dropped dramatically. Authors suggested that water quality decreased, mangrove destruction and over-exploitation are the main reasons to be responsible for the decline. Several studies have reported that population decreases in marine mollusk in coastal areas are often associated with overfishing and environmental problems (Peterson, 2002; Gangloff *et al*., 2008; Ren *et al*., 2008). To protect the spawning bloodstocks and to improve the recruitment of the wild population, fishing is often limited or suspended for a certain period based on the annual reproductive cycle of the species.

On aquaculture aspect, mangrove snail has been cultivated inside mangrove systems in Ca Mau province, Mekong Delta of Vietnam since 1990s (Chau *et al*., 2009). In the intensive culture system or co-culture with blood cockles, juvenile snails have been released at the stocking density above 30 ind./$m^2$ and the required seed amount at 1000-1500 kg/ha (Chau *et al*., 2009; Thao *et al*., 2011). Therefore, seed requirements are increasing year by year and the population size of this species has been decreased over two past decades. It is necessary to understand biology, especially reproductive characteristics of *Cerithidea obtusa* for the sake of resource management, biodiversity conservation and sustainable aquaculture. The objectives of this research are to: (1) investigate the reproductive cycle; and (2) determine the seasonal variation of proximate compositions in body tissues of *C. obtusa* in mangrove forests of Ca Mau province, Mekong Delta of Vietnam. Based on the findings of annual reproductive cycle, we would like to recommend the period of limited fishing for *C. cerithidea* in studied area in order to ensure the population recruitment.

## II. MATERIAL AND METHODS

### Snail sampling

This research was conducted in Ca Mau province in the Mekong Delta of Vietnam, located from 8°30’ to 9°10’N, 104°80’ to 105°5’E with 107 and 147 km of east and west coastline, respectively. With its dense network of canals, creeks and rivers, mangroves, this province is considered a very important area for forestry, fisheries and agriculture (Fig. 1).

Samples of *Cerithidea obtusa* were collected from April 2007 to March 2008. A sample set comprised at least 100 individuals, collected randomly from all tidal levels, kept alive and transferred to the laboratory.


Fig.1: Map showing the location of Ca Mau province and sampling site ()

### Histological preparation

At the laboratory, after recording the shell height and width, tissues of 30 snails were weighed. After drying at 60°C for 24 hours, snail tissues were ground and then the biochemical components (organic matter, protein, carbohydrate, lipid, ash) were analyzed on pooled sample of 30 snails from each sampling site per month (AOAC, 2001). Another 30 adult snails were used to determine the reproductive cycle of the population by histological method (Howard *et al*., 2004). Transverse cut was made in the middle of body part where gonad present, and a 3 mm thick section extracted and fixed in Davidson fixative. Tissue samples were then processed and embedded in paraffin. Serial sections, 4 µm thick, were obtained with a rotary microtome and stained with Harris' hematoxylin and eosin Y. Histological slides were observed under microscope to grade the developmental stages of snail gonads.

Based upon microscopic examination of histology, gonad development of *C. obtusa* was categorized into different stages as described by Horiguchi *et al*. (2006) with some modifications. Gonad development of each female snail was scored as the following: undifferentiated stage (0); initial development (1); developing stage (2); ripe stage (3) and spawning (4). Male gonadal development was also staged 0 to 4, however the initial development stage was not considered.

### Environmental conditions

Organic matter of mudflat, humidity, water temperature, water salinity and pH were recorded at each sampling to figure the variation of environmental conditions.

## III. RESULTS

### Seasonal variation of environmental conditions

Humidity was stable from May to October (~80%) during rainy season (Fig. 2). High variation of humidity occurred from November to April with the highest value in November (90.4%) and the lowest value in February (69.1%).

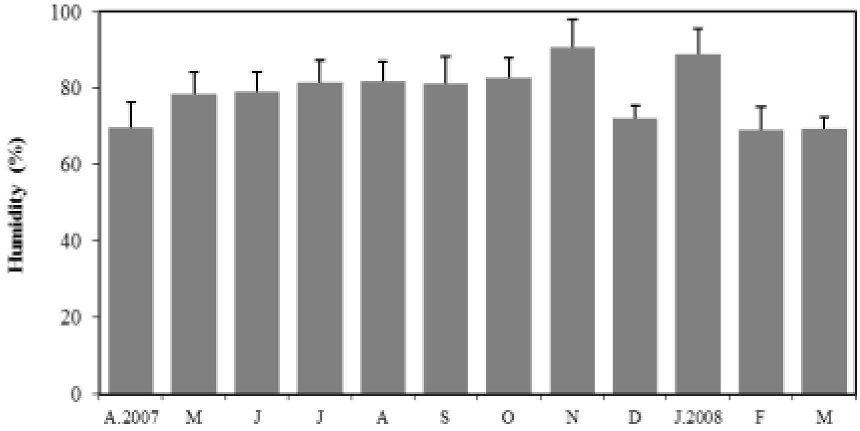


Fig. 2: Variation of humidity (%) during sampling period

Monthly mean temperature is presented in Fig. 3. Morning temperature varied from 23.5°C (January) to 26.3°C (April). Temperature in the afternoon showed low values in November and January (~29.5°C) and highest in April (33.0°C). In general, morning temperature was high from April to June (early rainy season) and low from November to February (early dry season).

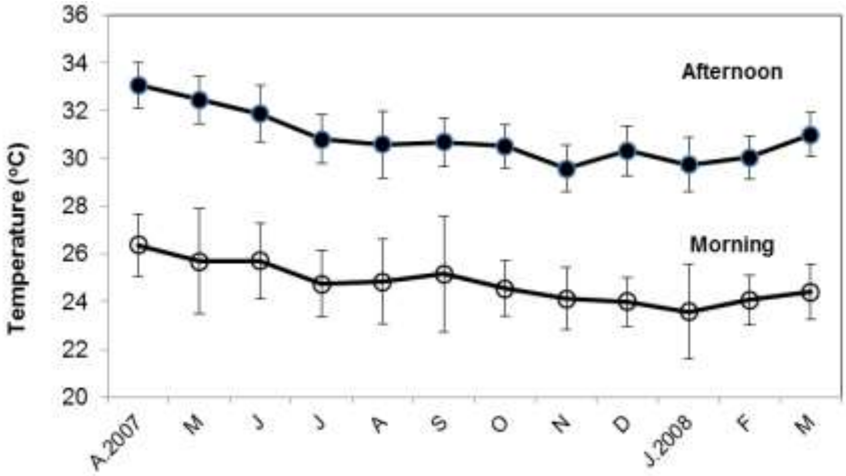


Fig.3: Variation of water temperature (°C) during sampling period

Water pH decreased from April to July, remained high in August-September, dropped abruptly and reached minimum in October (6.4). From November onward, pH decreased steadily to 6.9 in March. Highest salinity was recorded in May (32.3 ppt). From June to December, salinity decreased steadily and reached minimum value at 25.4ppt. From January onwards, salinity fluctuated around 29 ppt and increased to 31.4 ppt in April. There is no clear relationship between rainy period (April – November) and levels of pH and salinity at sampling sites. Probably, the variation of pH and salinity was affected by high tidal level and flooded period that occur annually from August to November (Fig. 4).

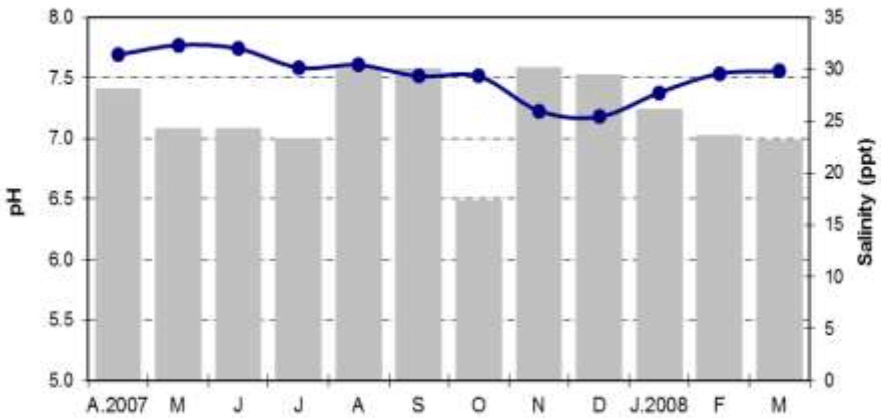


Fig. 4: Variation of pH (bar) and salinity (line) during sampling period

Total organic matter (TOM, %) showed high level from December to March (Fig. 5). TOM was low from June to November. Highest and lowest value was recorded in April (18.9%) and in August (9.1%), respectively. In general, TOM was low from May to November corresponding to rainy season in Mekong Delta.

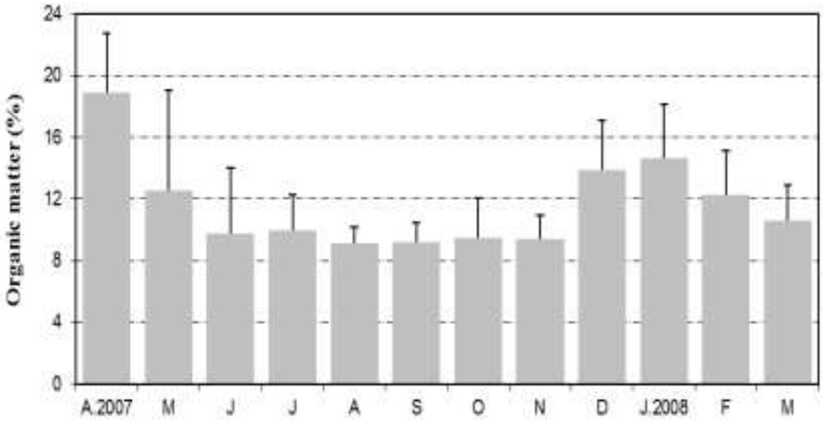


Fig. 5: Variation of total organic matter (%) of sediment during sampling period

**Reproductive cycle of *Cerithidea obtusa***

Table 1 showed biometric data of samples used for histological analysis. Mean shell height of snails varied from 36.3 to 42.2 mm, total weight from 3.7 to 6.1 g/snail and body mass varied 1.2 – 2.0 g/snail. Ratio between wet meat weight and total weight of snails was lowest in April (26.2%), highest in July (40.6%) and not relating to shell height of snails.

Table 1. Mean shell height (mm), total weight (g), meat weight (g), ratio of meat weight to total weight (%) and gonad index of individuals used for histological analysis.

| Months | SH | Wtt | Wm | Wm/Wtt | GI |
|---|---|---|---|---|---|
| Apr.2007 | 40.4 ±0.7 | 5.7 ±0.5 | 1.5 ±0.2 | 26.2 ±1.5 | 1.6 ±0.4 |
| May | 42.2 ±0.1 | 6.1 ±0.5 | 2.0 ±0.0 | 32.8 ±2.7 | 1.6 ±0.2 |
| Jun | 40.2 ±0.8 | 5.2 ±0.2 | 1.7 ±0.0 | 33.0 ±2.3 | 1.4 ±0.2 |
| Jul | 39.3 ±0.4 | 4.7 ±0.6 | 1.9 ±0.2 | 40.6 ±0.7 | 1.7 ±0.0 |
| Aug | 39.6 ±0.4 | 4.7 ±0.5 | 1.7 ±0.2 | 39.0 ±1.7 | 2.0 ±0.1 |
| Sep | 37.4 ±0.9 | 3.7 ±0.3 | 1.2 ±0.0 | 33.3 ±3.6 | 2.3 ±0.1 |
| Oct | 39.6 ±1.8 | 4.6 ±0.7 | 1.6 ±0.3 | 35.1 ±1.7 | 2.3 ±0.1 |
| Nov | 38.3 ±1.1 | 4.7 ±0.8 | 1.4 ±0.4 | 30.3 ±3.4 | 2.9 ±0.3 |
| Dec | 36.3 ±2.2 | 3.7 ±0.9 | 1.2 ±0.4 | 32.7 ±3.0 | 1.4 ±0.3 |
| Jan.2008 | 37.5 ±2.3 | 4.2 ±0.8 | 1.4 ±0.3 | 35.0 ±2.4 | 1.2 ±0.4 |
| Feb | 38.1 ±2.3 | 4.7 ±1.1 | 1.6 ±0.6 | 34.3 ±5.0 | 1.3 ±0.1 |
| Mar | 38.0 ±3.2 | 4.6 ±0.8 | 1.7 ±0.4 | 37.6 ±2.6 | 1.3 ±0.1 |

*SH, shell height (mm); Wtt, total weight (g); Wm, meat weight (g), Wm/Wtt, ratio of meat weight to total weight (%); GI, gonad index.*

Photomicrographs of various developmental stages of male and female snails are presented in Fig. 6 and Fig. 7. In undifferent stage, little or no gonad tissue was visible, and the gender of snails was not distinguished in this period. During developing stage, the number of follicles increased and they became expanded in male. In females, egg capsule glands appeared and the primary oocytes located on the wall of digestive gland. As developing stage progressed, the follicles continued to expand and coalesce. Some mature gametes were observed in male, however in female gonad, oocytes increased in size and became more spherical. During ripe stage, mature oocytes moved to the center of the lumen while male gonad showing free mature sperm filling the follicles. In spawning male, the follicles were almost filled with spermatozoa. Female gonad showed different size class of oocytes, some lumen already empty.

Table 2. Classification of female *Cerithidea obtusa* gonad developmental stages (0-4) with numerical score (0-4)

| Developmental stages | Numerical score | Histological description |
|---|---|---|
| Sexually undifferentiated | 0 | No egg capsule gland present. No gonad tissue visible. |
| Initial development | 1 | Egg capsule gland present. Little or no primary oocyte. |
| Developing | 2 | Egg capsule gland present. Increasing numbers and size of accumulating oocytes. |
| Mature | 3 | No or little egg capsule gland present. Most mature oocytes have round shape and similar size. |
| Spawning | 4 | No egg capsule gland present. Reduced numbers of oocytes. |

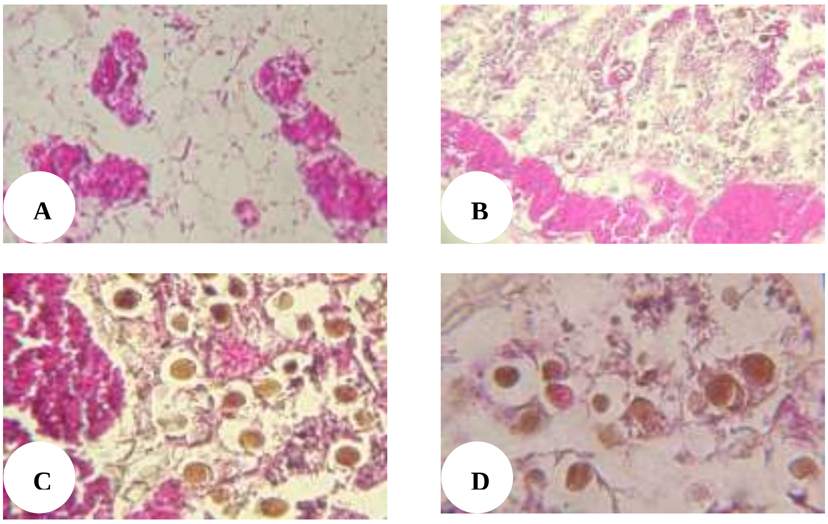


Fig. 6: Gonad development in female *Cerithidea obtusa*. A. Initial development; B. Developing stage; C. Ripe stage; D. Spawning stage

Table 3. Classification of male *Cerithidea obtusa* gonad developmental stages (0-4)

| Developmental stage | Numerical score | Histological description |
|---|---|---|
| Sexually undifferentiated | 0 | No gonad tissue visible. |
| Developing | 2 | Increasing numbers and size of follicles; spermatocytes present. |
| Ripe | 3 | Little connective tissue remaining; most spermatozoon has thread shape and concentrated at the center of follicle. |
| Spawning | 4 | Reduced numbers of gametes. Follicle wall is thin and testis volume is small. |

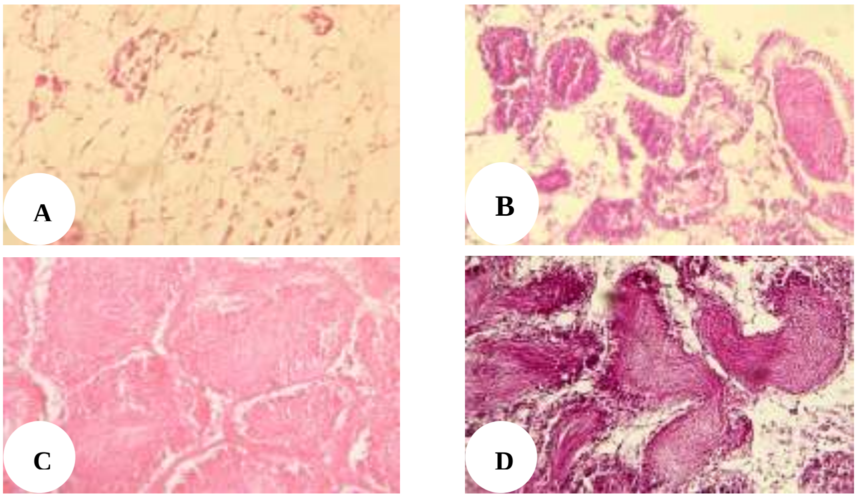


Fig. 7: Gonad development in male mangrove snail *Cerithidea obtusa*. (*A. Sexually undifferentiated stage; B. Developing stage; C. Ripe stage; D. Spawning stage*)

Fig 8. shows changes in mean GI of snails at different sampling sites. Observation of the histological slides indicated that gametogenesis of snails occurred almost year around. Mean GI increased quickly from June to October when the salinity and temperature decreased. In November, mean GI of snails showed that most snails were ready for spawning or in partial spawning. Relatively lower GI observed in December onward suggested that major spawning of snails occurred from September to November.

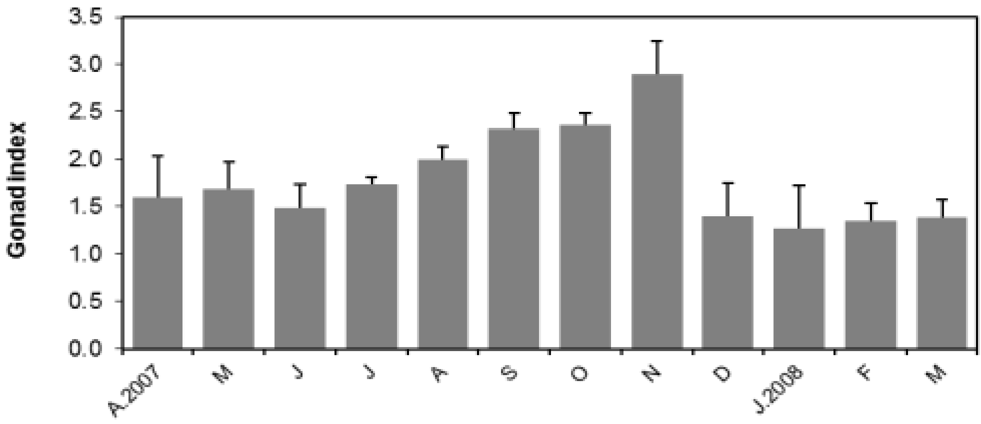


Fig. 8: Seasonal variation of gonad index of *Cerithidea obtusa* during sampling period

Monthly changes in percent composition of various gonad development stages were summarized in Table 4. The data showed that spawning more synchronous in November (43.3%) compare to those in September or October (20.0 – 23.3%). Gonad developing snails were dominant during January to July, however mature individuals were only abundant from August to October.

Table 4. Percentage of snails at each reproductive stage during sampling period (%)

| Gonad stages | Sampling period | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Apr.2007 | May | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. | Dec | Jan.2008 | Feb | Mar. |
| Indifferent | 23.3 | 20.0 | 22.5 | 15.0 | 20.0 | 16.7 | 6.7 | 3.3 | 26.7 | 23.3 | 16.7 | 16.7 |
| Developing | 55.0 | 53.3 | 60.0 | 65.0 | 31.7 | 28.3 | 30.0 | 31.7 | 55.0 | 66.7 | 70.0 | 68.3 |
| Ripe | 16.7 | 18.3 | 7.5 | 10.0 | 35.0 | 35.0 | 40.0 | 21.7 | 15.0 | 10.0 | 11.7 | 15.0 |
| Spawning | 5.0 | 8.3 | 10.0 | 10.0 | 13.3 | 20.0 | 23.3 | 43.3 | 3.3 | 0.0 | 1.7 | 0.0 |

**Changes of proximate compositions in snail tissues**

Protein content in mangrove snail decreased gradually from April to September (63.3 to 54.4%). It increased slightly in October (58.2%) and then decreased again to February (52.0%). From June to September, variation of protein level seemed to be opposite with gonad index in mangrove snails. High values of protein in October (58.2%) and November (55.6%), probably link to the reproduction of this species, especially females.

Table 5. Mean shell height (mm), total weight (g), meat weight (g), ratio of meat weight/total weight (%) and gonad index of individuals used for biochemical analysis.

| **Months** | **SH** | **Wtt** | **Wm** | **Wm/Wtt** | **Dw** | **Dw/Wtt** |
|---|---|---|---|---|---|---|
| Apr.2007 | 34.3 ±5.3 | 3.7 ±1.3 | 0.9 ±0.3 | 23.8 ±0.0 | 0.3 ±0.0 | 7.3 ±0.1 |
| May | 38.1 ±2.1 | 4.6 ±0.6 | 1.4 ±0.2 | 31.3 ±1.4 | 0.3 ±0.0 | 8.4 ±1.0 |
| Jun | 41.7 ±1.7 | 5.5 ±0.0 | 1.7 ±0.1 | 29.1 ±0.9 | 0.4 ±0.1 | 7.8 ±1.7 |
| Jul | 38.5 ±0.8 | 4.6 ±0.2 | 1.8 ±0.0 | 40.4 ±2.2 | 0.2 ±0.0 | 5.4 ±1.2 |
| Aug | 39.3 ± 0.9 | 4.7 ±0.7 | 1.8 ±0.3 | 38.6 ±3.1 | 0.3 ±0.0 | 7.7 ±0.8 |
| Sep | 36.5 ±3.8 | 3.7 ±1.0 | 1.2 ±0.2 | 33.7 ±2.5 | 0.3 ±0.1 | 8.1 ±1.5 |
| Oct | 39.2 ±2.2 | 4.5 ±0.7 | 1.6 ±0.3 | 35.9 ±2.4 | 0.3 ±0.1 | 7.9 ±1.0 |
| Nov | 39.1 ±0.0 | 4.9 ±0.4 | 1.5 ±0.2 | 29.6 ±3.0 | 0.3 ±0.0 | 6.9 ±1.7 |
| Dec | 34.9 ±2.0 | 3.9 ±0.4 | 1.3 ±0.2 | 34.4 ±4.8 | 0.4 ±0.0 | 11.3 ±1.9 |
| Jan.2008 | 37.9 ±1.4 | 4.7 ±0.5 | 1.7 ±0.3 | 34.2 ±2.6 | 0.4 ±0.0 | 9.1 ±0.6 |
| Feb | 34.6 ±2.1 | 4.1 ±0.9 | 1.4±0.5 | 33.2 ±3.4 | 0.3 ±0.1 | 9.0 ±0.8 |
| Mar | 34.5 ±6.9 | 3.8 ±1.4 | 1.5 ±0.6 | 38.4 ±5.1 | 0.3 ±0.1 | 8.2 ±1.9 |

*SH: shell height (mm); Wtt: total weight (g); Wm: meat weight (g), Wm/Wtt: ratio of meat weight to total weight (%); GI: gonad index.*

Lipid level was high in May, July and February with major peak in July (11.3 %). Lipid showed high variation from April to July. After major spawning peak, lipid level was lowest in November (5.9 %) and varied slightly from late to early of the year. From July to November, variation of lipid level seemed to be opposite with gonad index in mangrove snails. It indicated certain link between this energy resource and the gametogenesis of mangrove snails.

Among proximate compositions, Carbohydrates showed high variation during year cycle. It increased steadily from April (13.0 %) to September (29.9 %) and then fluctuated from November onward. Carbohydrates showed limited link to gametogenesis during June to September and the relationship is unclear for other months of the year.

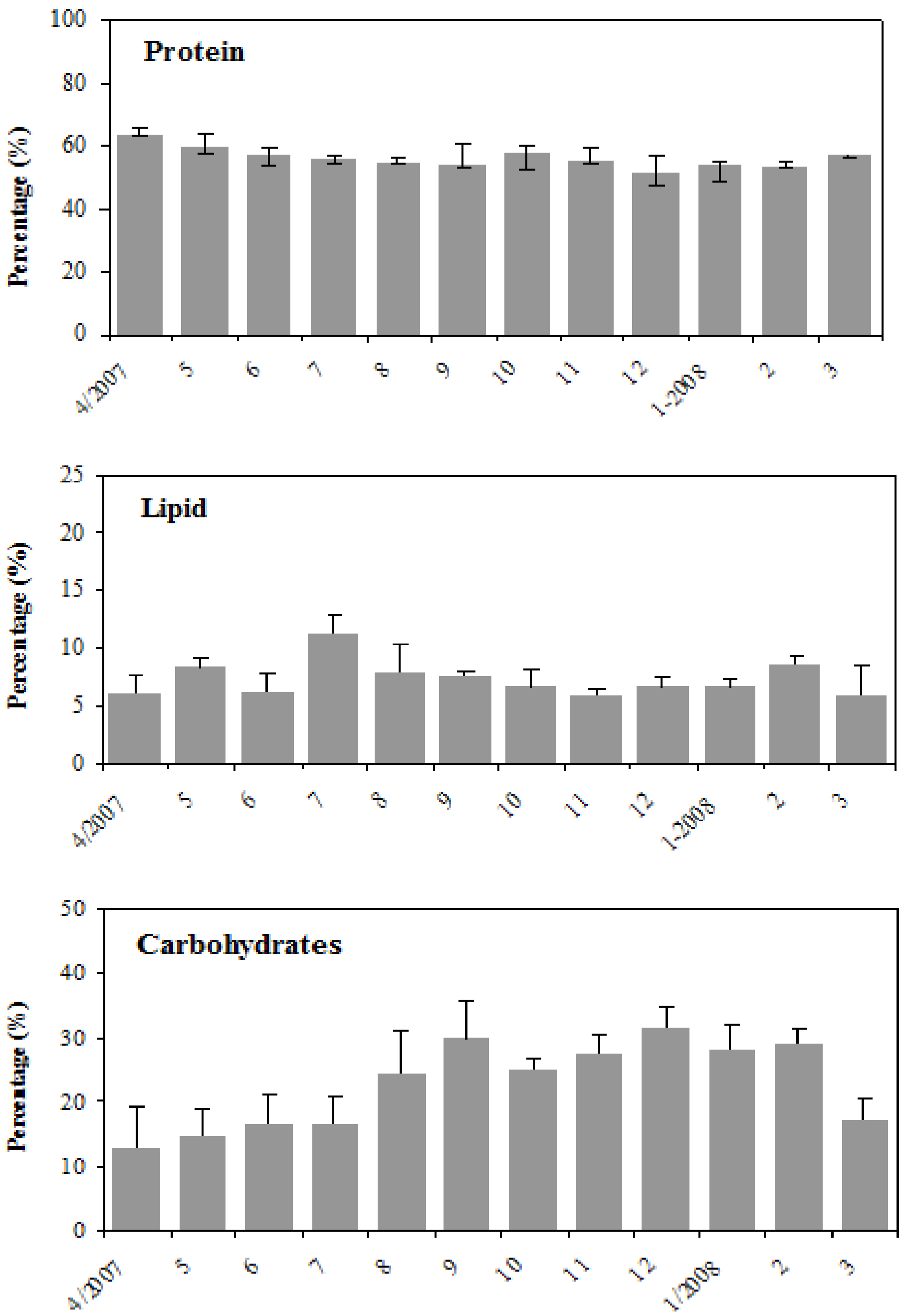


Fig. 9: Change of protein, lipid and carbohydrates during sampling period

## IV. DISCUSSION

Simpson (1978) determined the relationships between organic carbon content, total dry weight and shell length in small euryhaline gastropod *Potamopyrgus jenkinsi* living in different combinations of salinity and temperature. The author found that differences in organic carbon content were found in relation to weight at different growth temperatures. Our findings showed that total organic matter (TOM) seemed to be related to protein content of mud snails than other proximate compositions such as lipid or carbohydrates. In addition, Christensen *et al*. (2001) used stable isotope technique to analyse gut contents of several snail species in mangrove forests and reported that *C. obtusa* was selective feeder on diatoms and organic sediments.

In bivalve mollusc, Gabbott (1976) indicated that the natural gametogenic cycle is closely linked to cycles of glycogen storage and subsequent de novo synthesis of lipid during vitellogenesis. However, in mangrove snails proteins are the major component (around 60%) followed by carbohydrates (about 25%). It is suggested that protein could be the major energy resource to support growth and reproductive cycle of mangrove snails. Thao *et al*. (2008) analysed the proximate components of adult mangrove snails after 120 cultured days and showed that protein levels (around 40%) were higher than carbohydrates (about 25%) or lipids (less than 12%). Gomot (1998) found that the percentage of protein in artificially reared or wild *Helix* snails varied between 55-59.3%. Author also reported the percentage of total sugars is 23.7% and lowest lipid levels in *H. lucorum* and *H. pomatia* (1.9 – 3.1%). In snails produced egg capsules, Martinez *et al*. (2008) observed that during intracapsular life of muricid snail *Chorus giganteus,* protein was high (around 35% organic matter) followed by lipid (about 12%). Except for remarkable increase in July and minor increases in May or February, lipid levels remain almost unchanged in other months of the year. Carbohydrates showed two peaks before and after major spawning which has been suggested as a secondary energy source to support the gametogenesis in mangrove snails.

*Cerithidea obtusa* develops a thickened outer lip at the shell apertures upon reaching sexual maturity as description in other species of *Cerithidea* genus (Houbrick, 1984, Vemerij, 1993). *Cerithidea scalariformis* is dioecious, females being slightly larger than males (Houbrick, 1984). According to this author, females produce masses of eggs, which are laid in long strings (~51mm) on the bottom sediments and plant materials, undergo direct development, and hatch as juvenile snails. During sampling period, we could not see any egg string in the field. However, the histological observations showed the present of egg capsule gland and there were not different developmental stages of oocytes. Those results suggested that this species did not release separated eggs into the environment. In addition, we observed abundant individuals which bearing both mature oocytes and spermatozoa in gonad during spawning peak from September to November. Previous studies suggested *C. scalariformis* is aphallate and after matching, the sperm will migrate to the seminal receptacle where eggs fertilization takes place. Probably, mangrove snail *C. obtusa* is also possessing this character.

Our findings showed that gametogenesis of *C. obtusa* occurred year around with major spawning from September to November. Smith and Ruiz (2004) reported that snail *C. scalariformis* deposited egg strings in the field from late September through November when hatchlings were also found. However, Bagarinao and Lantin-Olaguer (2000) found that *C. cingulata* in shrimp ponds reproduced the whole year with a peak in March-September. In Japan, Harumi *et al*. (2001) observed mating behavior of snail *C. rhizophorarum* in July and August. It is suggested that species of *Cerithidea* genus is able to shift its life history (e.g. lifespan, growth, maturation rate) with current environmental conditions as findings from *Thais kiosquiformis* (Koch and Wolff , 1996) or *C. scalariformis* (Smith and Ruiz, 2004).

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