Seasonal Variation in Biochemical Composition of Black Clam (*Villorita cyprinoides)* in Cochin Estuary with Special Emphasis on the Impact of Thanneermukkom Bund

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Abstract: ***Comparatively higher protein, total carbohydrate and caloric values were observed in tissues of organisms collected from Zone B to that of Zone A (P<0.001). Carbohydrate: Protein ratio was almost steady throughout the study period and on comparison it was observed that there was not much significant difference between two zones at 5% level. Protein, Carbohydrate, lipid and Caloric value decreased during January and June-July at both the zones. In the case of lipid, there was not much significant difference between zones A and B at 5% level. Though there was not much significant difference between zones A and B at 5% level, it was noticed that Zone A has higher annual average of percentage of moisture than Zone B. In all the class ranges at both zones, after July and December there was a sharp decrease in G;S index ; Zone B showed a high annual average than Zone A. Clams seen at Zone B showed comparatively higher % of edibility and condition index than that of at Zone A. All the biochemical parameters and indexes were varying with respect to annual reproductive cycle. Salinity of the ambient water and nature of the substratum were found to be having some influence on the biochemical composition of the clam. It is concluded that organisms living at Zone B showed better biochemical composition than those at Zone A.***

Keywords: Protein, Carbohydrate, Lipid, G:S Index, Caloric value. Percentage of Moisture

1. Introduction

Clams are perhaps the most widely distributed and utilized aquatic molluscs providing protein rich food in much greater abundance than mussels and oysters. They are economically important either as food or as industrial raw material. The factors such as salinity, temperature, silt and water quality of the habitat are believed to affect the health and biochemical conditions of the shellfish (Galtsoff *et al*., 1947; Korringa and Postma, 1957). An understanding of physiological mechanism of an animal presupposes knowledge of chemical constituents within the body. Such an investigation is also essential for the understanding of the balance that exists between the animal and its environment. In economically important animal group such as clams, knowledge of chemical composition of the body is important not only for the evaluation of their utility for food or other purposes, but also for a more balanced utilization of the resources. Seasonal variations in the biochemical composition should also be studied in order to have an idea about the efficient means of culture and conservation.

Quality of the meat of clams has been known to undergo seasonal variation as they pass through various physiological phases such as maturation, spawning and recovering (Bayne, 1976, Gabbot, 1983). Information on these aspects of shellfish biology is important, as the preference of the consumer always lies on the fatty ones and, knowledge of the period when the shellfish is in its best condition is essential to determine the seasons at which shellfish could be harvested with advantage. Condition index can serve as an indication of the recent physiological history of the animal since it reflects an estimate of the stored energy over and above that require for the animal's maintenance and physiological activity.

Though some information is available on the chemical and biological composition of clams in Indian waters, detailed and systematic information on the biochemical composition of these clams particularly *Villorita Cyprinoides* (plate 2) are lacking. A critical study from the nutritional point of view does not appear to have been made even though many processing industries producing clam meat products are set up. *Villorita cyprinoides* popularly known as black clam is an economically important estuarine bivalve found along the south west coast of India especially in the backwaters of Kerala. This study is aimed to evaluate variations of biochemical composition of the clam in different seasons and different ecological backgrounds.

2. Materials and Methods

2.1 Study Area

To evaluate the impact of Thanneermukkom bund on the growth, survival and biochemical composition of clams two sampling sites in Cochin estuary were selected, one site was select at south of Thanneermukkom bund (Zone A)(Plate I) and one was at north of bund (Zone B). Regular fortnightly sampling was carried out from zones A and B for a period of two and half years (November 2013 to April 2016).

Sampling procedure

Clams were collected from the natural bed during early morning hours and they were kept in water with aeration for 48 hours to get rid of the debris and food materials present inside the body. Then clams were cut open to collect soft body parts for the biochemical analysis.

Hydrographic parameters

Among different hydrographic parameters temperature was measured by ordinary thermometer, salinity by Mohr's titration method (Strickland and Parsons, 1968) and sediment texture by the method proposed by Carver (1971).

Other parameters

Estimation of protein was carried out based on Lowry's method (Lowry *et al*., 1951); Phenol – Sulphuric acid method proposed by Kemp and Van Kitz (1954) was used to estimate carbohydrate; Lipid estimation was done by the method established by Barnes and Blackstock (1973); Gonado-Somatic Index was measured by the method standardised by Sato(1994); Percentage of Edibility was determined by the method established by Durve (1964) and Ansell *et al.* (1964) and Condition Index (Body condition Index) was estimated by a method proposed by Walne (1970). Conversion factors were made use for the determination of caloric value (for protein: 5.7, for glycogen: 4.2 and for lipid: 9.5). This was standardized by using the standard procedure of Karzinkin and Tarkovskaya (1960). To estimate moisture content of the meat (whole clams and component organs), flesh was weighed immediately on removal from shell and after dried in a hot air oven at 800C. The difference between the dry weight and fresh weight expressed as percentage of moisture. Gonado - Somatic Index, Percentage of edibility and condition Index were examined using standard procedure.

3. Results

Annual average protein concentration during '2013-'14, at Zone A and B was 35.82% and 52.81% respectively, whereas during 14-15 it was 43.58% and 48.48% and in 15–16 it was 57.58% and 67.67% in the respective order. Throughout the study period ('2013-'14, 2014 – '15 and 2015-'16) there was a sharp decrease in protein during January and June-July at both zones (Graph. 1). At Zone A protein showed a significant positive correlation with percentage of moisture and a significant negative correlation with percentage of dry meat, condition index and G : S index, whereas at Zone B protein exhibited a significant positive correlation with percentage of shell weight and a significant negative correlation with percentage of edibility, percentage of dry meat, G : S index and condition index. Compared with Zone A, Zone B reported the maximum protein concentration in all respects. A higher annual average of total carbohydrate was observed in Zone B than Zone A during the study period (2013-'14, 2014 – '15 and 2015-'16) ($P<0.001$) (Graph. 2). At Zone A the total carbohydrate varied from 5.16 % to 38.64 % and at Zone B it was between 7.74 % and 41.45 %. At Zone A carbohydrate showed significant positive correlation with caloric value, percentage of edibility, percentage of dry meat and condition index, and Zone B showed significant positive correlation with percentage of edibility, G : S index, dry meat weight, condition index and a significant negative correlation with percentage of moisture. Carbohydrate : Protein ratio showed almost steady state throughout the year (Graph 5) and there was no much difference between two zones at 5% level. There is an increase in the C : P ratio during June-July and a decrease during March to May at both zones. At Zone A the concentration of lipid in the tissues varied between 0.40 % and 11.99 % and at Zone B it was between 0.64 % and 8.88 % and Zone A showed higher concentration than Zone B. At Zone A, lipid has a significant positive correlation with percentage of edibility, G : S index, condition index and percentage of dry meat weight and a significant negative correlation with percentage of moisture. During December-January in whole body (Graph 3) there was a steady decrease in the concentration of lipid at both zones. Clams at Zone B had higher caloric value than Zone A ($P<0.001$). In whole body there was a sharp decrease in caloric value during May as well as January onwards (Graph 4). Though there was no significant difference between zones A and B at 5% level, it was noted that Zone A has higher annual average of percentage of moisture than Zone B (Graph.6), but in the case of seasonal average at both zones pre-monsoon showed the maximum percentage of moisture and monsoon minimum. At Zone A there was a gradual decrease in percentage of moisture from April to July and September to November, whereas at Zone B it was from August to Novembers and January to March. Percentage of moisture showed a significant negative correlation with percentage of edibility, C: P ratio and condition Index at Zone A & B. Though there was no significant difference between zones A and B at 5% level, as per annual average values of Gonado–Somatic index, Zone A has high values than Zone B. Besides this at zones A and B, after July and December there was a sharp decrease in G;S index. The maximum percentage of edibility of clams in Zone A was during September (14.1 %), whereas the minimum recorded was 5.72 % during April, but at Zone B the maximum was 14,02 % during July and a minimum of 7.71 % during February and monsoon has high seasonal average values and pre–monsoon has low at zones A and B (Graph.7**)**. There was significant difference between zones A and B ($P<0.05$) and Zone B showed higher percentage of edibility than Zone A. The maximum value for condition index was 103.41 during November at Zone A and 119.62 at Zone B during October, whereas the minimum at Zone A and B were during April and the observations were 38.12 and 44.25 respectively (Graph.8). It is noted that comparatively higher percentage dry meat weight was noted at Zone B (Graph.9). Evaluating the shell weight it varied from 69.82 % during January to 75.93 % during August at Zone A, but at Zone B it varied from 68.34 % in July to 79.51 % during April (Graph.10)**.** Temperature varied between 33.8 ^{0} C and 27.1 ^{0} C at zone A and 33.4 ^{0} C and 26.1 ^{0} C at zone B (Graph.11), Zone A has comparatively low average temperature than zone B especially during pre-monsoon (Closed period of Thaneermukkom bund). Zone B showed high salinity fluctuation when compared to zone A ($P>0.001$) especially during the closed period of bund. Salinity varied from 0 ppt to 8.54 ppt at zone A and 0 ppt to 10.18 ppt at zone B (Graph.12). At zone A, from January to April there was an increase in the percentage of silt and clay and corresponding decrease in sand (Graph. 13, Graph. 14 & Graph. 15) and at zone B the sediment texture was dominated with sand (Plate 3).

4. Discussions

Organisms store reserve energy in the form of lipid, glycogen or protein.In eulamellibranchs, the tissue of the visceral mass represents the main site for storage of reserves (Ansell, 1974 a). Effect of these variables; age, reproductive cycle, seasons and conditions of the environment affect the biochemical composition of the bivalves (Salih, 1975, Taylor and Venn, 1979). Seasonal changes in the flesh weight and biochemical composition are the characteristics of seasonal activities of bivalves, which result from storage, utilization of food reserves, growth and reproductive processes (Ansell 1972; Dare and Edwards 1975).

It is evident from the results obtained that the percentage of protein was higher at Zone B than that of Zone A. At Zone B the prevalent sediment texture was sand whereas at Zone A it was sand and silt (during the closure of bund). Thick sandy-muddy bottom and nutrient conditions are suitable for clams (Durve and George 1973). According to Peddicord (1977) sandy bottom is the favourable environment for clam growth. During the period of closure of bund, clay and silt start to accumulate over the clam bed at Zone A, the stress produced by the accumulation of silt and clay over the clam bed may be the major reason for the low protein concentration at Zone A than Zone B. Mc Lachlan and Lombard (1980) in *Turbo sarmaticus* and Appukuttan and Aravindan (1995) in *Paphia malabarica* indicated that protein reserves are used by molluscs during stress condition. High values of protein content during the ripe stage was to be expected as protein constitutes the major organic component of bivalve oocytes (Holland, 1978, Yan *et.al*, 2010a, Yan *et.al*, 2010b and Ke.*et.al*.2012), this fact substantiates the present observation i.e., in *Villorita cyprinoides* there was an increase in concentration of protein during April-May and November-December. Though a decrease in concentration was observed during the second spawning also (December- January), it was comparatively low with regard to major spawning. The same result was obtained by Nagabhushanam and Deshmukh (1974) in *Meretrix meretrix*, Nagabhushanam and Talikhedkar (1977 b) in *Donax cuneatus*, Jaybal and Kalyani (1986 a) in *Meretrix meretrix*, Balasubramanyan and Natarajan (1988 a, b) in *Meretrix casta*, Maqbool (1993) in *Marcia Opima and* Hamdani and. Soltani-Mazouni(2011). It was evident that in V.*cyrpinoides* the stage of reabsorption of residual gametes of previous spawning, sexually intermediate resting period and gametogenic states show an increasing trend of protein concentration, owing to the accumulation of nutrients. Maqbool (1993) reported similar observation in *Marcia opima* of Kayamkulam waters. Utilization of somatic tissue protein reserves during gametogenesis has been reported in *Tapes philippinarum* by Adachi (1979) and in *Tapes decussates* by Behninger and Lucas (1984). According to various authors protein would show a decrease in relation to spawning and would follow reverse pattern with respect to carbohydrate (Lubet, 1959; Gabbot, 1975; Pieters *et al.*, 1980), it coincides with present observation in *Villorita cyprinoides*.

In bivalves (Eble, 1969) glycogen is largely stored in vesicular (Leydig) cells, which are distributed close to active metabolic tissue like developing gonad; The carbohydrate level *in Villorita cyprinoides* increased during gametogenic period and then it showed decreasing trend with the advancement of gametogenesis and a low level during mature condition. It is probable that carbohydrate is being converted to lipid and protein reserve of the gametes in clams; Giese *et al*. (1967) in Tivela stultorum, Nagabhushanam and Deshmukh (1974) in *Meretrix meretrix*, Nagabhushanam and Talikhedkar (1977 b) in *Donax cuneatus*, Lakshmanan and Nambisan (1980) in *Villorita cyprinoides* var. *cochinensis.* and Katticaran (1988) in *Sunetta scripta*. At Zone A comparatively low carbohydrate concentration was noted during pre-monsoon, this period coincides with the closure of the bund and gradual settlement of silt and clay over the clam bed in thick layer. According to Ansell (1973); Nagabhushanam and Talikhedkar, (1977 a) ; Salih, (1979), decrease in the glycogen content under stressed period could be attributed to the utilization of carbohydrate reserves during anaerobiosis under unfavourable condition.

Lipid stands third in percentage of composition of *Villorita cyprinoides*, soft tissue having seasonal as well as location wise variation in lipid composition. Comparatively low values were detected during spawning period and high values during the period just prior to spawning. Similar changes in relation to the reproductive cycle were observed in *Abra alba* (Ansell, 1974 a), in *Meretrix meretrix* (Nagabhushanam and Deshmukh 1974), in *Villorita cyprinoides* var. *cochinensis* (Nair and Shynamma 1975), in *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977 a), in *Donax trunculus* (Ansell *et al.*,1980), in *Meretrix meretrix* (Jaybal and Kalyani, 1986 a) and in *Marcia opima* (Maqbool 1993). Results have shown that there was an accumulation of lipid during the period just after spawning till next gametogenesis started. Lubet (1959) found that lipid accumulation coincides with the end of reproductive phase and beginning of sexual resting phase. In adult bivalves lipids are stored mainly in gonads and constitute the main component of reproductive material (Gabbott, 1975 and Davis and Wilson, 1983). At zones A and B there was a significant negative correlation between lipid and water content in clam meat. These observations are in agreement with those of Joshi and Bal (1965) in *Katelysia marmorata*, Giese (1969) in *Tivela stultorum*, Ansell (1974 a, c, d) in *Abra alba*, *Chlamys septemradiata* and *Nucula sulcata*, Salih (1979) in *Meretrix casta*, Appukuttan and Aravindan (1995) in *Paphia malabarica*. Higher caloric values were observed just before spawning at both zones (higher values during April-May and November-December at both stations). Similar observation was made by Ansell (1972, 1974 d) in *Donax vittatus* and *Nucula sulcata* and Salih (1975) in *M.casta*.

Villorita cyprinoides at Zone B has low % of moisture than Zone A, especially in pre- monsoon months, this effect may be due to the presence of saline mixed water at Zone B (where salinity will increase rapidly during the closure of bund with respect to Zone A). An inverse relationship between salinity and percentage of moisture has noted in the present study, a rise in moisture level with a decrease in ambient salinity was reported in *Meretrix meretrix* (Deshmukh, 1972) and in *Katelysia opima* (Nagabhushanam and Mane, 1975 a), in *Marcia opima* (Maqubool, 1993). According to Nagabhushanam and Deshmukh (1974) in *Meretrix meretrix*, Ansell (1974 b, d) in *Lima trians* and *Nucula sulcata*, Nagabhushanam and Talikhedkar (1977 a) in *Donax cuneatus* and George (1998) in *Donax incarnates* water content in soft tissue increased with decrease in salinity during the monsoon season which might be due to the loss of salt and gain of water. Salih (1977) in *Meretrix casta* concluded that in tropical waters the variation in percentage of moisture seems to be controlled by monsoon or salinity than by the sexual conditions of the clam.

An inverse relationship between water content and organic content (carbohydrate and lipid) was observed in *Villorita cyprinoides*, seasonal variation in water content and its inverse relationship with other organic constituents have reported by Ansell (1972) in *Donax vittatus*, Salih (1977) in *Meretrix casta*, Maqbool (1993) in *Marcia opima* and George (1998) in *Donax incarnates*. Higher G : S Index during December-January and May-June was noted in the present study; (higher values coincide with the mature stages of gonad); Sato (1994) observed comparatively higher G : S Index during mature phase in *Phacosoma japonicum* and Ranjeeth (1997) in *Villorita cyprinoides* var. *cochinensis*. Percentage of edibility was reasonably high, just before or at the beginning of the spawning and it was low immediately on completion of spawning, similar observation was done by Durve, (1964 b) in *Meretrix casta*, Alagarswami, (1966) in *Donax faba* and Narasimham, (1988) in *Anadara rhombea* and Durve ,(1964 b) and Rao, (1988) in *Meretrix casta*.

The factors such as salinity, dissolved oxygen, phosphates, organic contents, silt and nature of the bottom are believed to affect the health and condition of shellfish (Galtsoff et al., 1947**;** Korringa and Postama, 1957, Delgado *et.al*.2004, Serdar and Lök. 2009, Li *et.al*, 2009, Li *et.al*,2011)). Present study pointed out that Zone B has high condition index than Zone A, in other words organisms in Zone B are fatter than Zone A. This may be due to the sandy sediment prevalent in those areas. According to Durve and George (1973), thick sandy – muddy bottom and nutrient conditions are suitable for growth of *Meretrix casta*. Tenore *et al.* (1968) found that condition index of *Rangia cuneata* was higher in sandy substrata than in mud and a high organic content in the mud was associated with even lower condition index, this fact substantiate the present observation that, during the period of closure of bund Zone A showed lower condition index than other seasons; during that time muddy deposition takes place due to the prevention of water current. Condition index is related to the overall metabolic resources of an animal to the conditions imposed by its environment (Peddicord, 1977). In Zone B there was a low condition index during pre-monsoon period, which coincides with high saline variation due to the intrusion of saline water into that area and the organism has to make use of extra energy for the osmoregulation to cope with the variation in ambient salinity. Bedford and Anderson (1972) assumed *Rangia cuneata* would require metabolic energy for the osmoregulation of the species during low saline fluctuation. The ratio of wet tissue weight to dry tissue weight followed a pattern exactly opposite to that of condition index; indicating that when condition index was high, a greater portion of the tissue weight was solid and the tissues of clams with low condition index contained primarily water Galtsoff (1964) and Peddicord (1977).

From these observations it is concluded that, biochemical composition of the clam in the estuary is directly or indirectly influenced by the presence of bund. So closing and opening of bund should be regulated in such a manner that, which affect the clam bed in least adverse level.

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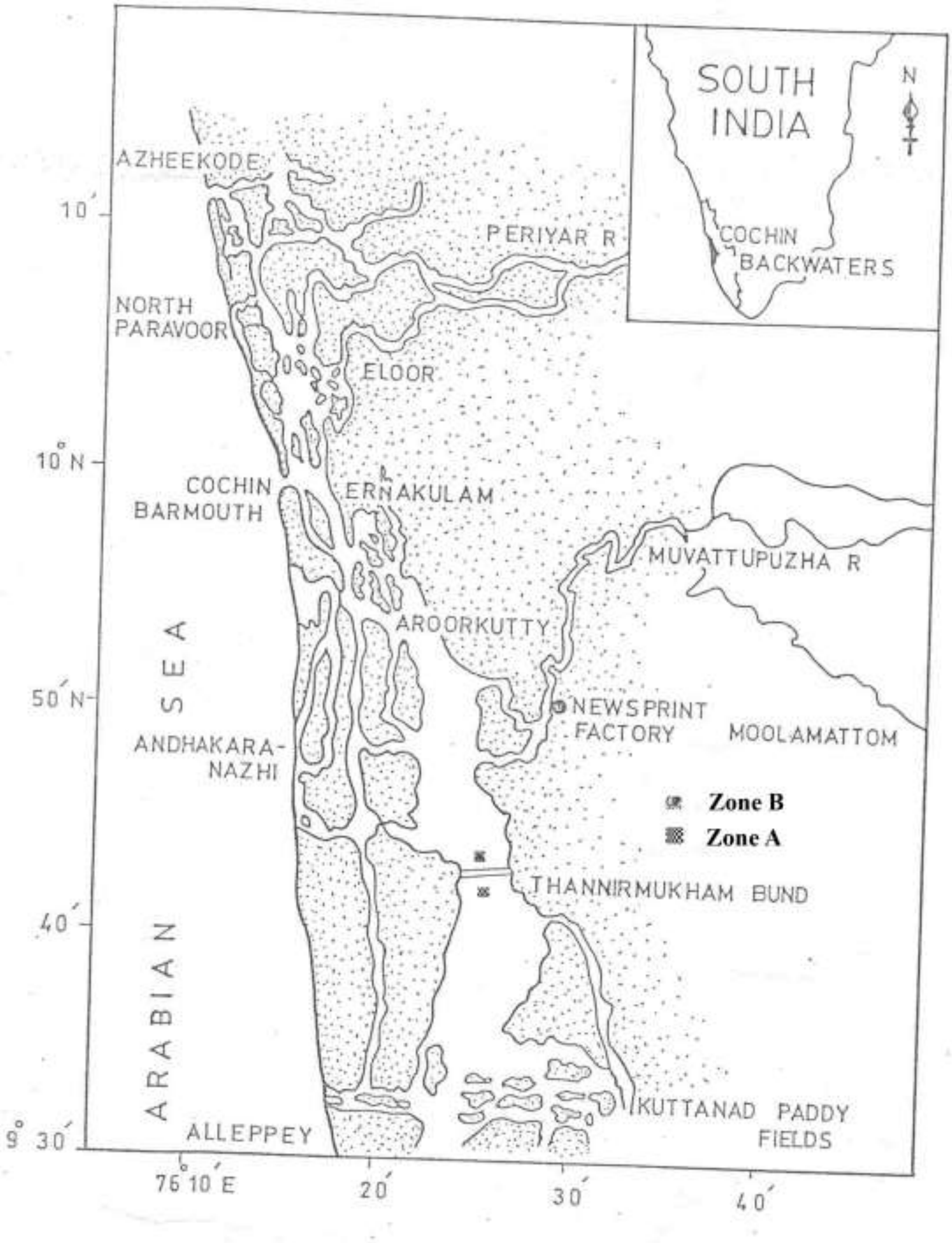
SOUTH
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ERNAKULAM
MUVATTUPUZHA R
AROORKUTTY
NEWSPRINT
FACTORY
MOOLAMATTOM
ANDHAKARA-
NAZHI
Zone B
Zone A
THANNIRMUKHAM BUND
ARABIAN SEA
KUTTANAD PADDY
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9° 30′
76° 10′ E
20′
30′
40′

plate 1

PLATE 2

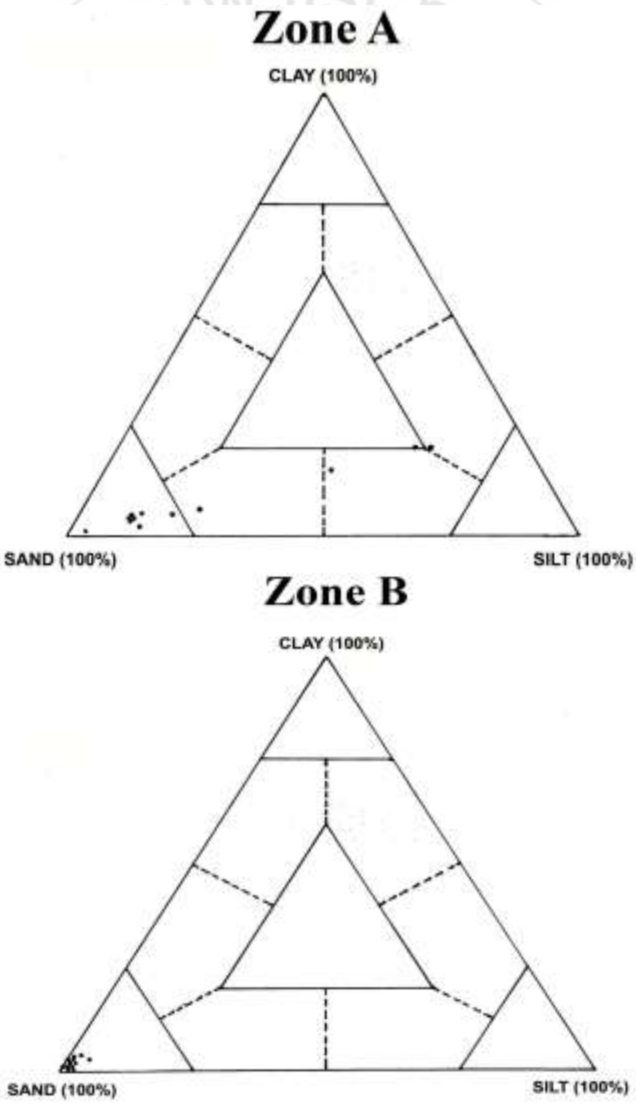
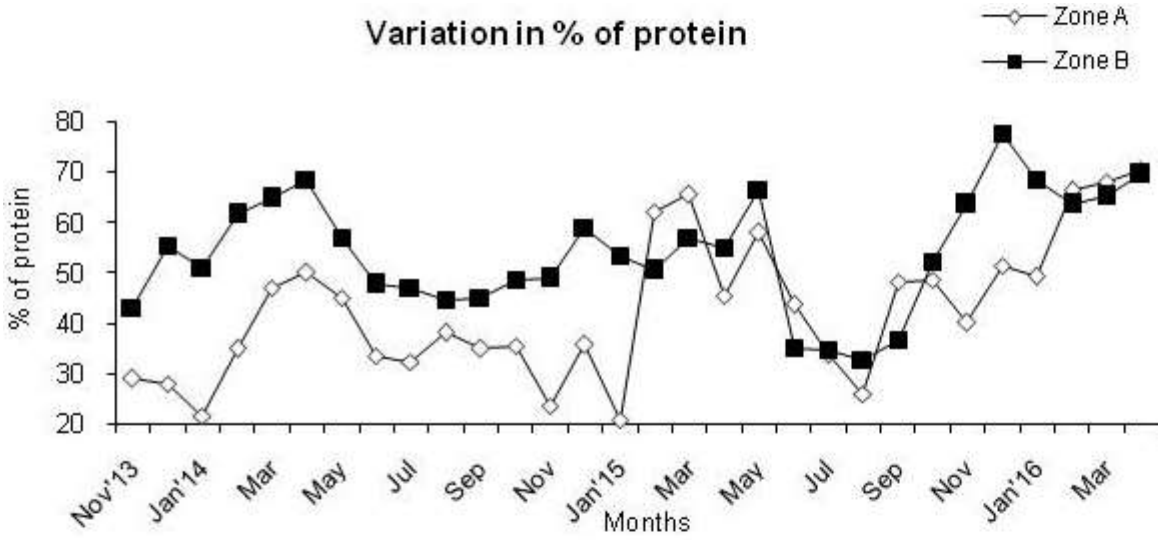
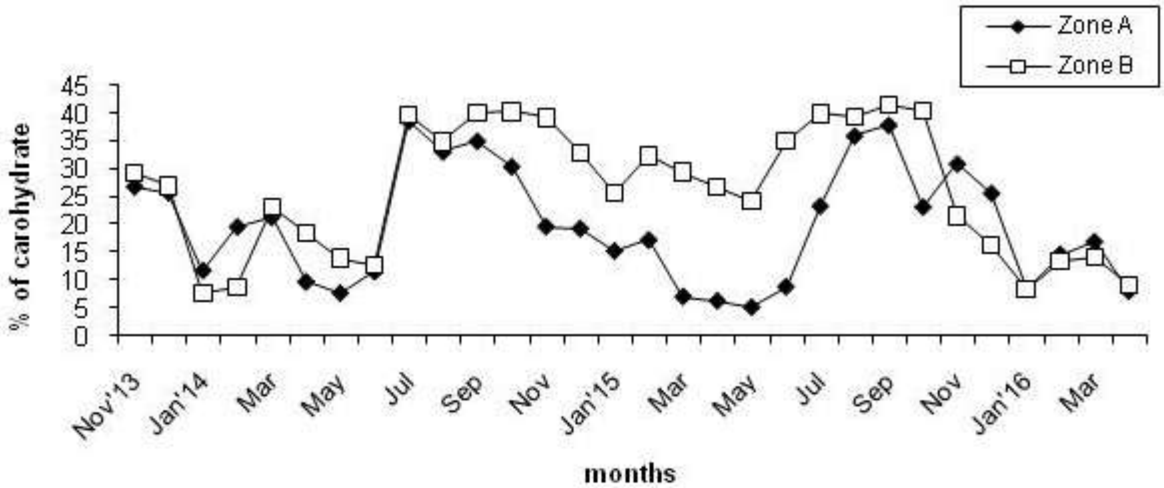


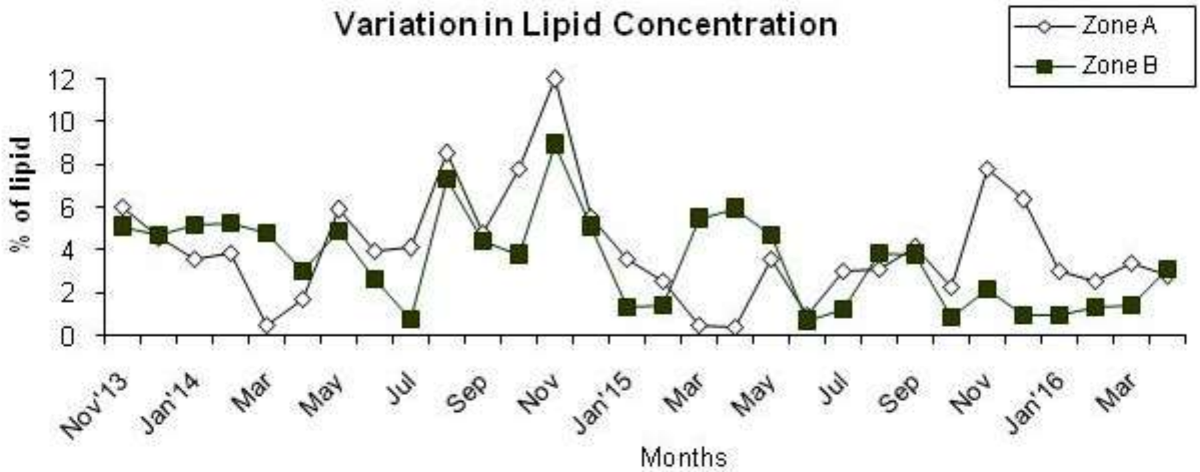
PLATE 3



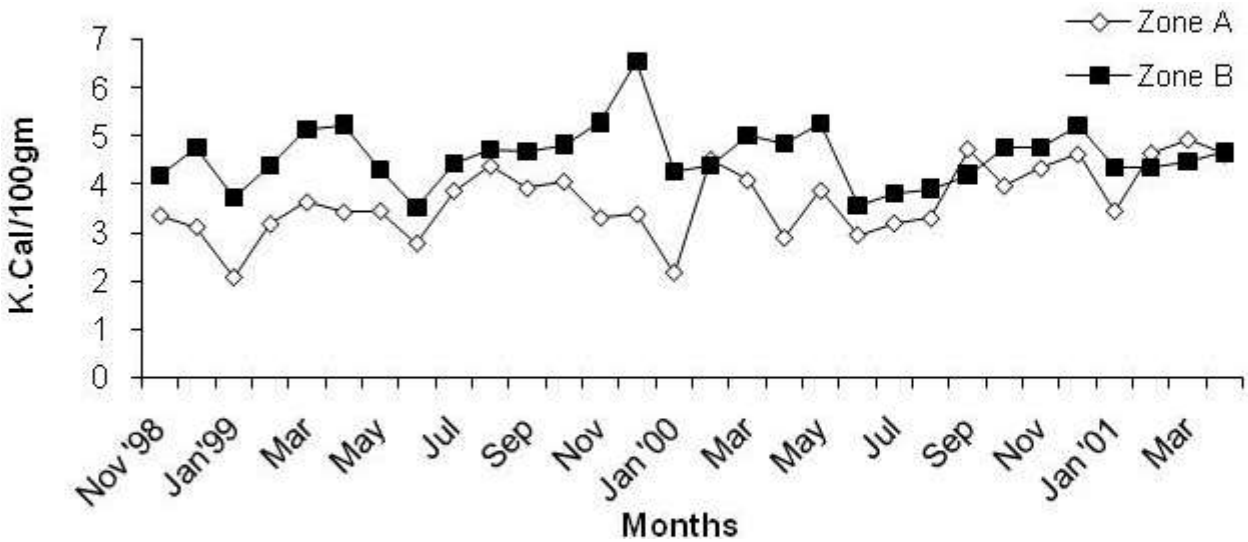
Graph 1: Variation in protein concentration



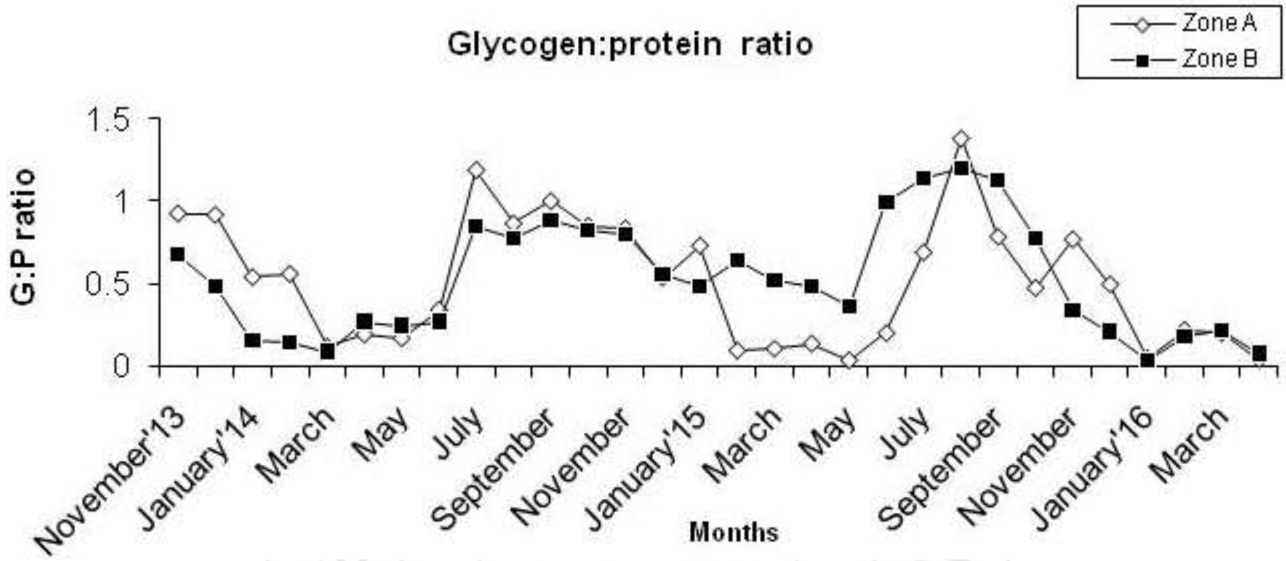
Graph 2: Variation in Carbohydrate concentration



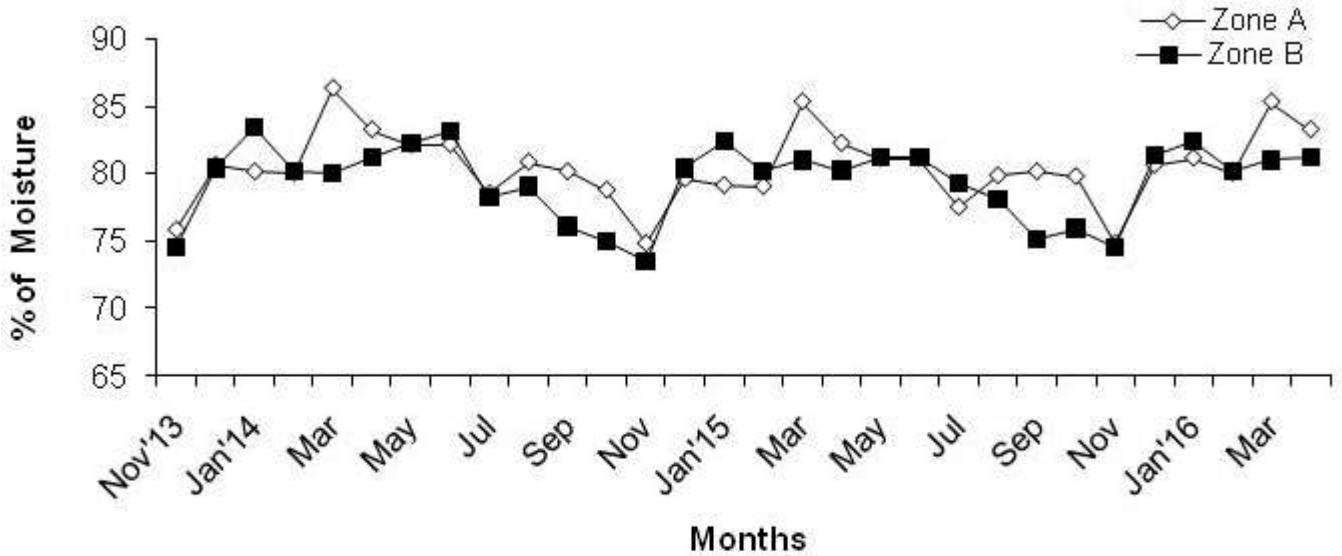
Graph 3: Variation in lipid concentration



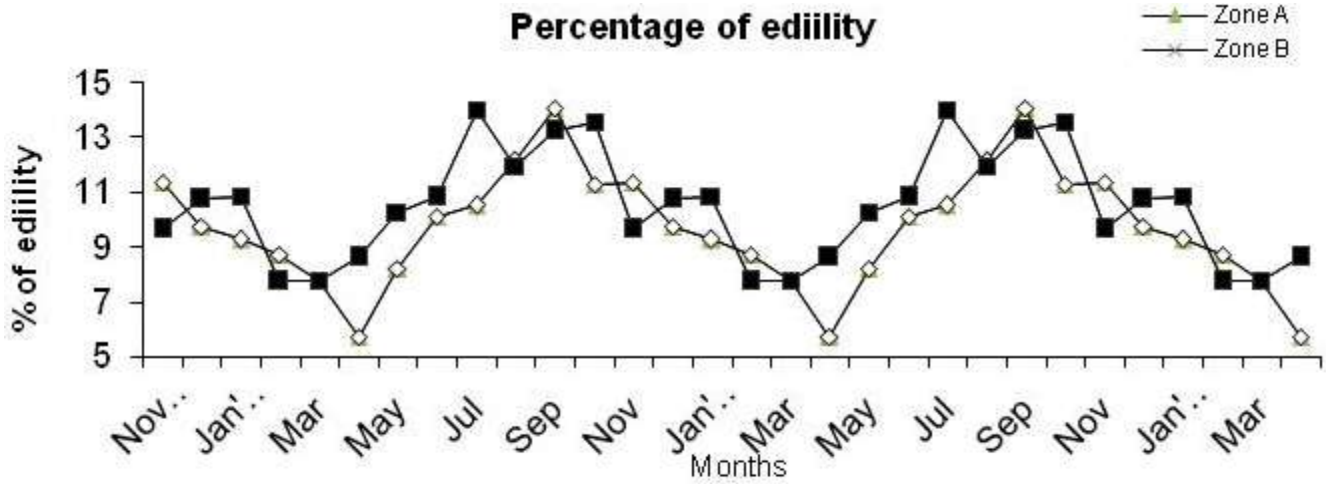
Graph 4: Variation in caloric value



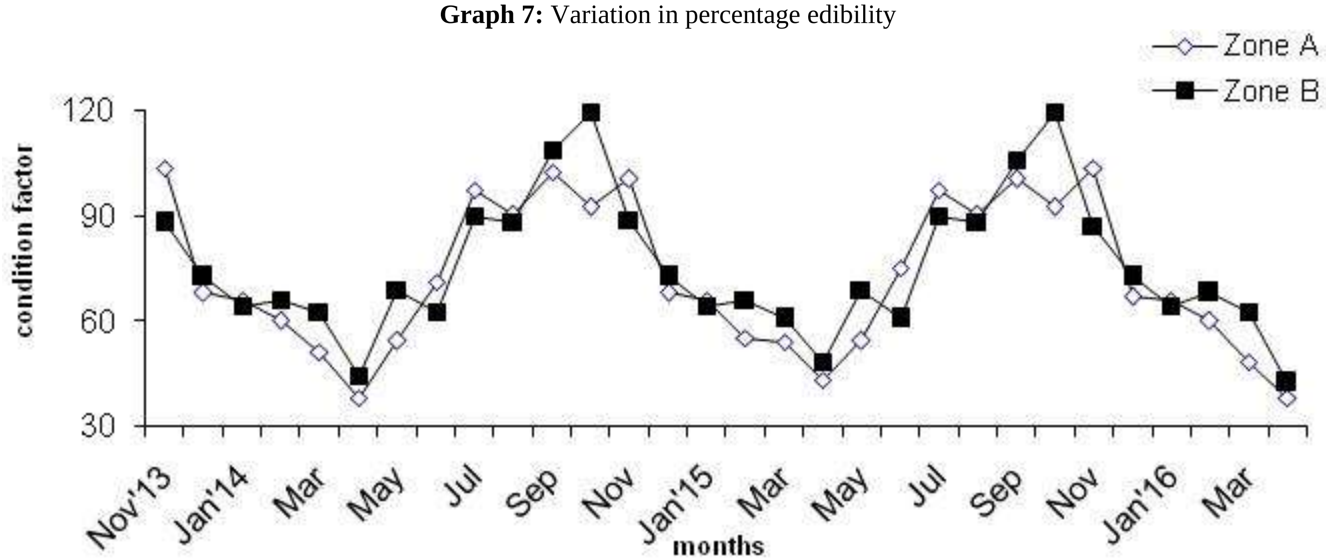
Graph 5: Variation in carbohydrate to protein ratio



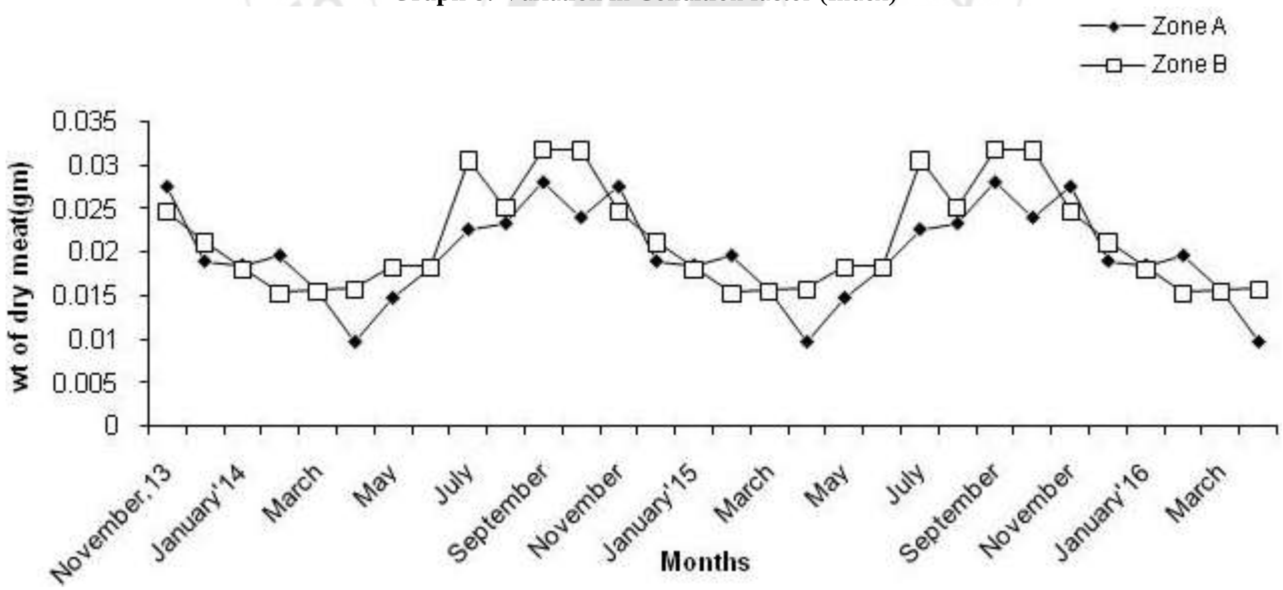
Graph 6: Variation in percentage of moisture in clam meat



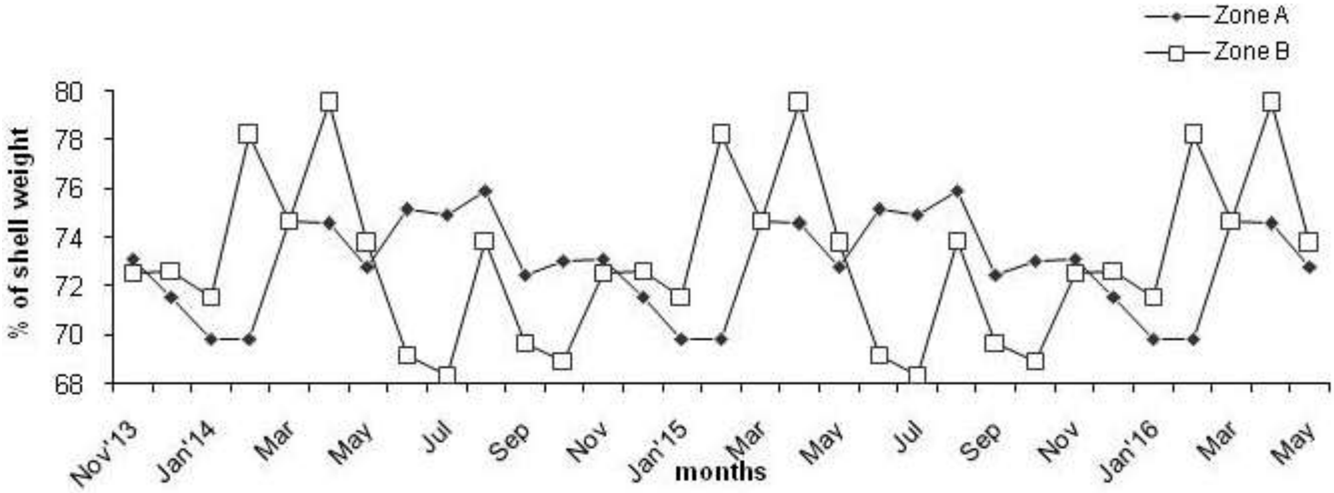
Graph 7: Variation in percentage edibility



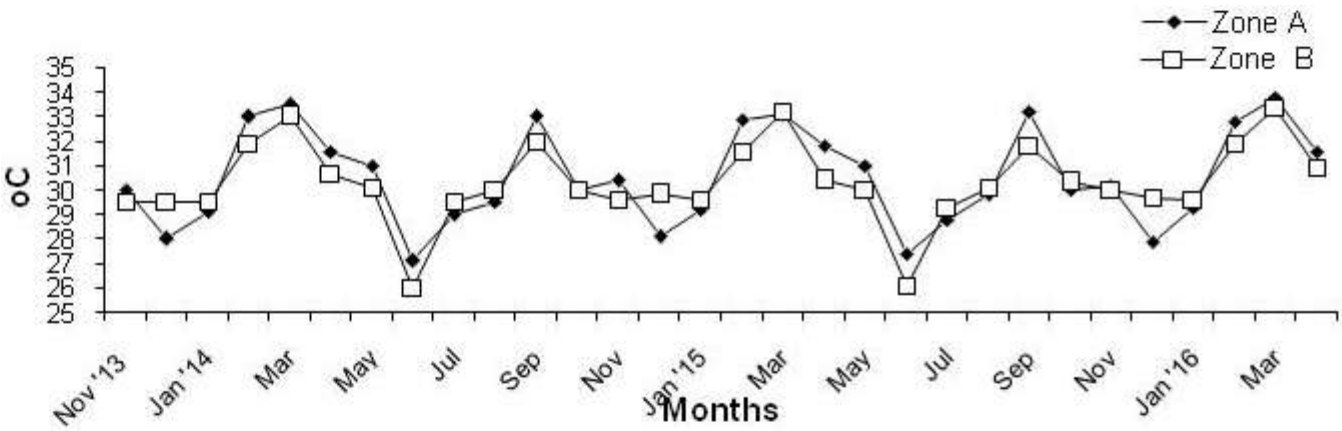
Graph 8: Variation in Condition factor (Index)



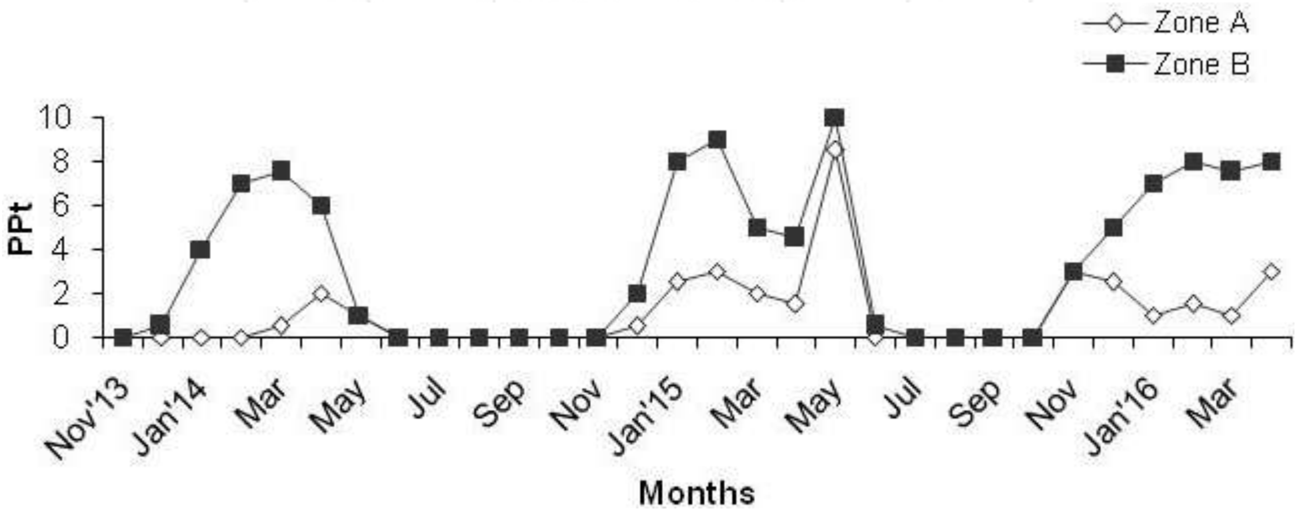
Graph 9: Variation in the percentage of dry meat weight.



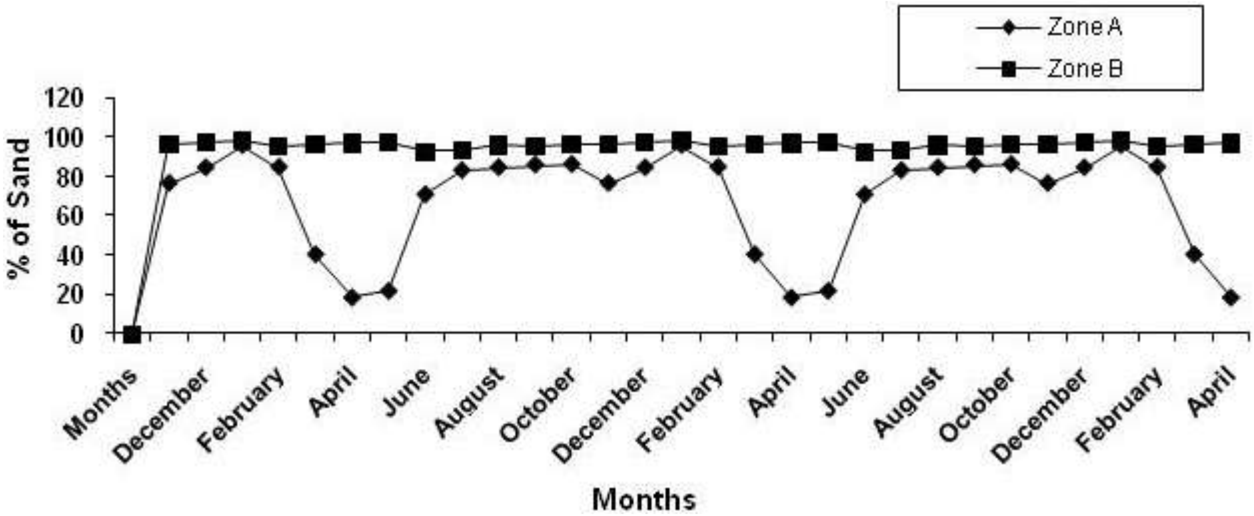
Graph 10: Variation in percentage of shell weight to total weight of clam



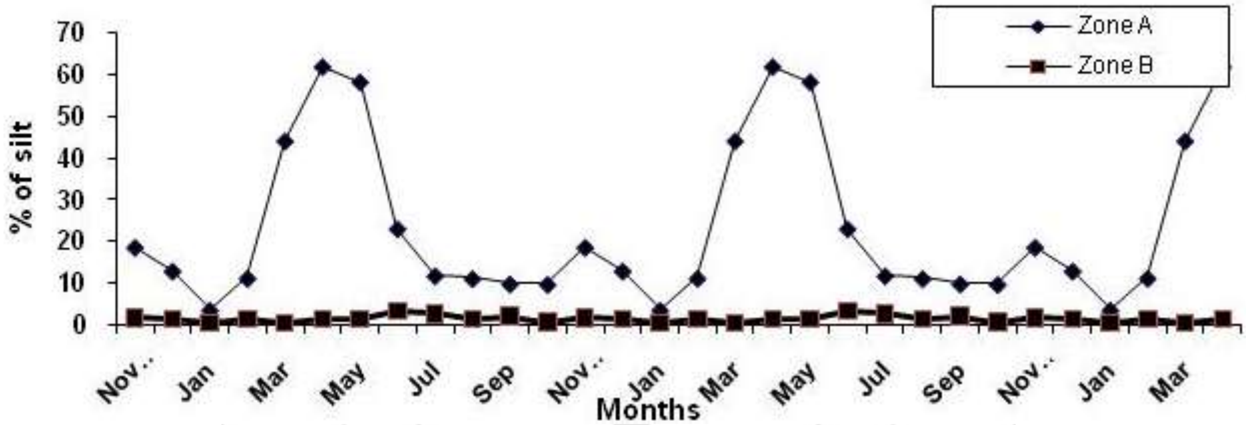
Graph 11: Monthly variation in temperature



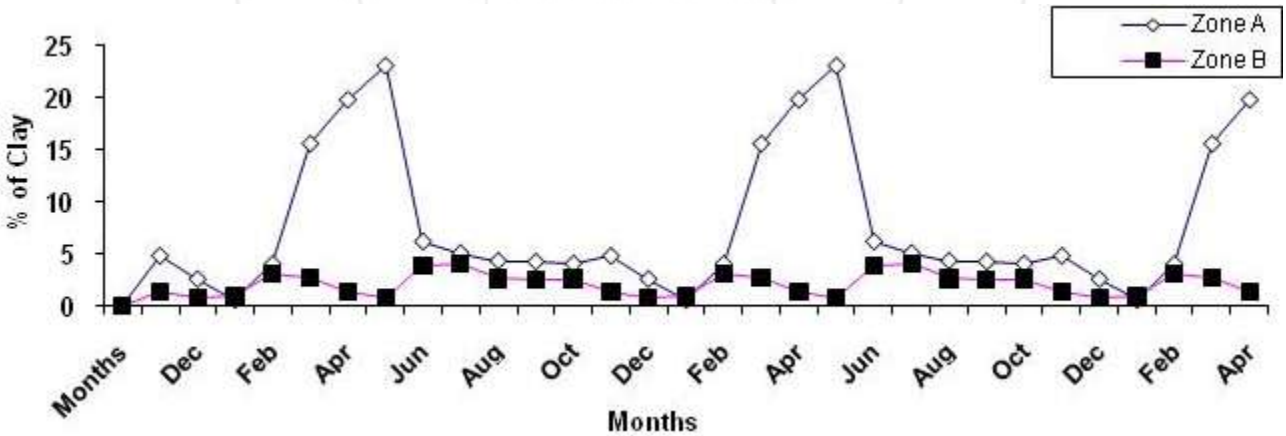
Graph 12: Monthly variation in salinity



Graph 13: Monthly variation in % of Sand



Graph 14: Monthly variation in % of Silt



Graph 15: Monthly variation in % of Clay