Chapter IV

DISCUSSION

General discussion

Differences in the results of analyses of biochemical composition are mainly due to the method of obtaining samples and the method used in biochemical determination. In general practice, tissues used for biochemical studies, wet or dry should be preserved in fresh condition approximately identical to the living state by freezing them immediately in order to stop enzymatic action. If a long time lapse between collection and biochemical determinations, the frozen tissue should be dried by various methods such as had been suggested by Giese (1967).

In the modified Kjeldahl method used to determine proteins, if some of the nitrogen-containing gases escaped during digestion or if the heterocyclic rings of the aromatic amino acids failed to be ruptured and the nitrogen was not reduced to ammonia, the resulted values may be low. The value obtained correctly was called crude proteins, It was being based on an assumed average figure of amino-acid composition of protein. The non-protein nitrogen has not been determined. The major difficulty in the determination of glycogen was the inability to get quantitative precipitation of glycogen solution.

Because each of the biochemical composition was determined separately, the combined values of these biochemical composition in dry weight level (calculated from total solid) seldom come to 100%. The lowest added figure found were 57-60% and the maximum values were 95-98%. A portion of the discrepancy may be due to the presence of considerable salt in the tissue. Ash contents (mineral contents) should be determined.

It could be concluded that the proteins made up 50% or more of the tissues. This was the structural material of the cells and of many extracellular structure. By contrast, the percentage of lipid content was very low. Lipids were present as structural components of the cells composing the tissues and as storage food. Carbohydrates were most variable ranging from 0.7 to 25%. Carbohydrate, mainly as glycogen, serves as an important reserved material.

Biochemical Analyses in field Specimens.

The percentage of total solid in the mantle, non-mantle and total tissues remained constant at approximately the same level throughout the year. The protein content in the summer (during February to May) did show variations in various tissues. The mantle showed the least content of protein. During summer the mussels were developing gonads. These could easily be seen by the occurence of gametes in the mantle tissue. According to Giese (1969) the developing gonads in bivalves contained large stores of nutrient reaerves, such as carbohydrate. The decrease in protein level often caused by the increase in carbohydrate level until the gametes reproduce or spawn. The lipid contents in the mantle, non-mantle and total tissues did show variation throughout the year. Maximal values of lipids were found in the non-mantle tissue. According to Giese (1969) the lipids were usually stored in the digestive gland or visceral organ and foot in mollusc's body. As the non-mantle tissue included foot and visceral organ of the mussel, thus the lipid content was highest comparing to other tissues. The carbohydrate content showed the similar trend as the total solid.

Other than the gonad development and abundance and type of the food available, seasonal changes in biochemical composition depend on local condition such as temperature and salinity. Neamsurp (1975) reported in her thesis that the yearly temperature of the surface waters at Angsila, Cholburi, varied in the range of 25.0-30.5°C and the surface salinity range was 7.5-32.0 ppt. By the use of her data in relation to this experiment of biochemical changes in mussels, the major environmental factor affecting these changes is the salinity. Variation of salinity caused the fluctuation in the water content and the total solid due to absorption of water. According to Prosser & Brown (1961), the mussel which was an osmotic conformer would undergo volume changes as the salinity changed in order to regulate the osmotic balance within the body. Under unfavorable conditions, mussels might lose salt and gained water. Those in low salinity water may have a higher water level than those in high salinity water. The total solid and the water content did show seasonal variation in the mantle and the non-mantle tissue. The total solid were lowest in the mantle and non-mantle tissue during November and December. The water content were highest in these two months. Surface salinity at this period was low due to the heavy rain from the depression and the Bangpakong river-runoff.

Seasonal changes in the biochemical composition followed distinct patterns related to the gonad development and spawning time. There was a clear inverse relationship between the two main components, proteins and carbohydrates. Lipid contents are small and showed no marked seasonal trends. Seasonal changes in relation to the gonad development in each component were discussed as followed

Total solid

The total solid tended to increase as the gonad developed. The highest values of total solid were found during the rainy season (June to September). Gonad condition index for this period is between 2.4-3.5. Thus the increase in gonad may also increase total solid content.

Protein content

There was variation in protein level from month to month, it seemed that the reproductive cycle was not the only factor. It could also depend upon the differences in nutrient conditions or other variables in the environment which needs further study. The proteins tended to increase as the gonads developed. In mantle (germinal tissue), the protein content was highest in September during which the gonads are gravid. This is also in agreement with Giese (1969) in his study on a bivalve Tivela stultorum.

Lipid content

The lipid content was small and showed no marked seasonal variation. As the gonad developed, the lipid content decreased. The lowest value was found in September during which the gonads were gravid and some mussels have already spawned. Goddard & Martin (1964) explained that during the discharges of sexual product in molluscs, both the glycogen and fats were being depleted. This may clearly explained in this study why the lipid content was lowest during spawning period.

Carbohydrate content

Seasonal changes in the glycogen content showed a definite cycle related to gonad development and spawning time. The reserved materials were used up while the number of sex cells in the gonad increased. Thus the glycogen content was brought to its minimum value which usually occurred immediately after apawning. There was a sharp drop in mantle tissue (germinal tissue) during September when spawning occurred. This corresponded to Quayle's (1969) work on the Pacific oysters in British Columbia. He reported that as spawning started in early summer,glycogen content declined due to it being used up. Galtsoff (1964) reported similar changes in glycogen content in the mantle and labial palps of the oyster. Glycogen content was the reserved material. During the rapid proliferation of sex cells the reserved supply was used and by the end of the reproductive cycle the glycogen content was at a minimum. The mantle was reduced from a thick heavy layer to a thin transparent membrane. Soon after spawning the oysters began to form and stored glycogen again. In <u>Tivela stultorum</u>, Giese (1966) reported that the gonad showed the least carbohydrate storage when mature gametes were present, suggesting a massive conversion of carbohydrates into the gamete tissue.

There was a clear inverse relationship between proteins and carbohydrates. The relations were determined as carbohydrates: proteins ratio. This tended to show seasonal variation in relation to gonad development. The carbohydrates: proteins ratio tended to increase toward the month of September. The proteins were highest when gametes were produced in large number. At this time carbohydrates and lipids reached minimum level, suggesting their conversion into the material of the gametes. Comparing to the carbohydrates: proteins ratio, the pattern for the lipids: proteins was the opposite and there was no marked seasonal change.

Biochemical variations were not only due to seasons and gonad development, but probably due to growth. The total solid, protein content and carbohydrate content seemed to increase with growth. But the lipids declined as the animal became larger in size. Holland and Spencer(1973) suggested that neutral lipid was the major energy reserve of the larvae and young spat of oyster. This was in contrast to the adult in which polysaccharides served as a major energy reserve. This was possibly true in Mytilus viridis. Further study in the biochemical composition of the larvae and young spat in Mytilus viridis is needed to give more evidence to this suggestion.

Galtsoft (1964) suggested that seasonal changes in biochemical composition in oyster was also related to the abundance and type of food available and the intensity of feeding. But it could be pointed out that nutritional state of mussel in this experiment was not clearly related to the biochemical composition. The stomachs were mostly in the full class showing the same intensity of feeding every month. Relative abundance (%) of plankton in stomach contents were given as preliminary study showing the three major groups of plankton ingested as food. The present knowledge of the nutritional value of algae was applied to animal nutrition. Parsons, Stephens & Strickland (1961) analysed eleven species of algae for protein, carbohydrate and lipid. He found that the protein content in Chaetoceros sp and Coscinodiscus sp. was 35 % and 17 % respectively. The values were given in percentage dry weight of cells. The carbohydrate content for these two species mentioned were 6.6 % and 4.1% respectively. The fat content were 6.9 % and 1.8 %. The biochemical composition in the three major groups of plankton ingested by the mussel should be conducted. Further experiment should be taken into the biochemical composition in mussels fed by centrics diatom, pennates diatom and dinoflagellates.

Biochemical Analyses in Laboratory Specimens

In comparison to the field specimens of the same size, the protein and carbohydrate contents in the laboratory specimens tended to be somewhat lower. There was a greater reduction in carbohydrates than proteins. Giese (1969) suggested that starvation of animals resulted in depletion of food reserves. Although the amount of food available approximate to the maintenance requirement of laboratory specimens, the food levels were presumably lower than those in the field. Bayne & Thompson (1970) kept the common mussel, *Mytilus edulis* for a long period in the laboratory. According to the nutritive and temperature stress, the carbohydrates and proteins were lost from the body of the mussel. There was a more rapid reduction of carbohydrates than that of proteins.