REPRODUCTIVE CYCLE AND BIOCHEMICAL CHANGES IN THE GONADS OF THE FRESHWATER CRAB, *BARYTELPHUSA CUNICULARIS* (WESTWOOD, 1836)

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ABSTRACT

The annual breeding cycle of the crab, *Barytelphusa cunicularis*, was determined by measuring monthly the size of the gonad relative to the size of the animal. The peak of gonad size appeared in rainy season (June to September) showing the maximum breeding activity during these months. Histological observations of the monthly gonads revealed that the ripening of the gonads started during March and extended upto May. By June the gonads of most of the individuals attained full maturity and spawning began. Spawning terminated by the end of September and the gonads entered a quiescent period.

Monthly changes in the boichemical constituents of gonads were studied for one year to determine their variability in the course of the reproductive cycle. During the ripening of the gonads, glycogen, fat and protein contents were high. During spawning period all values fell considerably reaching the minimum values at the end of spawning season. During the maturation of the gonads glycogen, fat and protein contents were increased.

INTRODUCTION

The reproductive cycles and the sexual periodicities of invertebrates belonging to different parts of the world have been studied extensively by many workers (Benett and Giese, 1955; Farmanfarmaian *et al.*, 1958; *Giese*, 1959; Giese *et al.*, 1964; Knudsen, 1964; Boolootian, 1965, Rahaman, 1966, 1967; Griffin, 1968; Chandran, 1968; Lawrence and Giese, 1969; Webber and Giese, 1969 and Pillay and Nair, 1970). Boolootian *et al.*, (1959) investigated the effect of environmental factors on the reproductive cycles of brachyuran and anomuran crabs of Monterey bay. Giese (1959) worked on the reproductive cycles of some of the west coast invertebrates (U.S.A.) including echinoderms, molluscs and crustaceans. Maturity and breeding of the pacific edible crab, *Cancer magister*, of Northern British Columbia were investigated by Butler (1960). Lowe (1961) studied the influence of environmental factors on the female reproductive cycle of the crayfish, *Cambarus shufeldti*.

Studies on the biochemical changes in relation to reproductive cycle in invertebrates have been investigated by some workers. Seasonal variation in different organic constituents of oysters had been shown by Rusell (1923), Okazaki and Kobayashi (1929), Sekine *et al.*, (1930), Tully (1936), Humphrey (1941), Venkatraman and Chari (1951) and Durve and Bal (1960). Pearse and Giese (1966) have worked on the biochemical changes in relation to reproductive cycle in gastropod, *Neobuccinum eatoni* and in bivalve, *Limatula hodgsoni*. Giese (1969) in his review paper gave a complete account of the biochemical composition of different tissues in relation to reproductive cycle in a number of molluscs. Recently, Webber (1970) studied the biochemical changes in different organs of *Haliotis cracheroidii* in relation to reproductive cycle.

In India some work has been done on the reproductive cycle and breeding behaviour of the marine crustaceans. Subramanyam (1963) described the annual reproductive cycle of the prawn, *Penaeus indicus*. Rahman (1967) studied the reproductive and nutritional cycles of the crab, *Portunus pelagicus*. Similar studies were also carried out on another marine crab, *Charybdis variegata* by Chandran (1968). Recently, Pillay and Nair (1970) have investigated the reproductive cycle and breeding behaviour of three decapod crustaceans from South Coast of India.

When compared to the investigations on marine decapod crustaceans, studies on the reproductive cycle and breeding behaviour of the freshwater crabs of India have received very little attention. Mecan (1937) described the life history of the freshwater crab, *Paratelphusa guerini*. Chacko and Thyagarajan (1952) studied the breeding season of the crab, *Paratelphusa jacquemontii* while Ali (1955) described the breeding behaviour of the crab, *Paratelphusa guerini*. Less attention has been paid on the biochemical changes in relation to reproductive cycle in decapod crustace in India, especially on freshwater crabs. George and Patel (1956) were first to study the seasonal variation in the fat content of liver and gonad in marine and freshwater decapods.

Since little work has been done on the reproductive biology of the freshwater crabs in India, the present study was undertaken on *Barytelphusa cunicularis* with a view to investigate (1) the annual reproductive cycle by estimating monthly gonad index and (2) changes in the chemical composition of the gonads.

Material and Methods

The adult specimens of freshwater crab, *Barytelphusa cunicularis*, were collected from the Kham river near Aurangabad for a period of one year (June 1969 to May 1970). The crabs were maintained in glass troughs containing sufficient water. Specimens that were not in healthy conditions as well as those that had just moulted were not used for analysis. Sufficient time was given for the animals to acclimatize to the laboratory conditions before being sacrificed.

Animals were fed in laboratory with small dead animals and decaying leaves. For histological studies gonads and other parts of the reproductive system were fixed in Bouin's fluid. The tissues were dehydrated in alcohol, embedded in paraffin wax (M.P. 60-62° C.) and were sectioned at 6 to 8 μ. The sections were stained with Delafield's haematoxylin and eosin.

Several methods have been used to determine the reproductive cycle in marine invertebrates; among these the most suitable one appears to be the measurement of the gonad index (Giese, 1959). As the reproductive system showed much variations during different seasons of the year, the gonads were removed and weighed accurately upto milligram. The gonad index was calculated according to the formula given by Farmanfarmaian *et al.*, (1958) whereby the weight of the gonad is divided by wet weight of the animal and multiplied by 100.

Thus the gonad index was calculated as

$$\frac{\text{Wet weight of gonad}}{\text{Wet weight of animal}} \times 100$$

The mean value of the indices for 20 males and 20 females were calculated for every month.

The biochemical changes were recorded in the gonads for a period of one year. For estimation of glycogen the method recommended by Kemp *et al.*, (1954) was employed using Engel's colorimeter. The amount of glycogen was calculated by multiplying the glucose value by the factor 0.927. The fat content was extracted from a dried tissue in Soxhlet Apparatus and the percentage of fat was estimated. The nitrogen was estimated by Micro-Kjeldhal method (Hawk *et al.*, 1954). The amount of total protein was calculated by multiplying the nitrogen value by 6.25. All the results were expressed in percentage of the sample on dry weight basis.

Results

The monthly mean values of the gonad indices were determined and plotted in Fig. 1. From the figure it was found that the testis and ovary entered a period of growth in the month of March and attained maximum size in the month of August. The gonad index of the males ranged from 0.0760 to 0.179 and that of females from 0.0272 to 0.163. The variations are marked and the high gonad index was noticed only in the month of August both in male and female.

From the above study it is possible to conclude that the breeding activity of the species as indicated by gonad index appears to be pronounced from June to September reaching a highest peak in August. From October to February the gonads entered a resting period and attained minimum size. In breeding

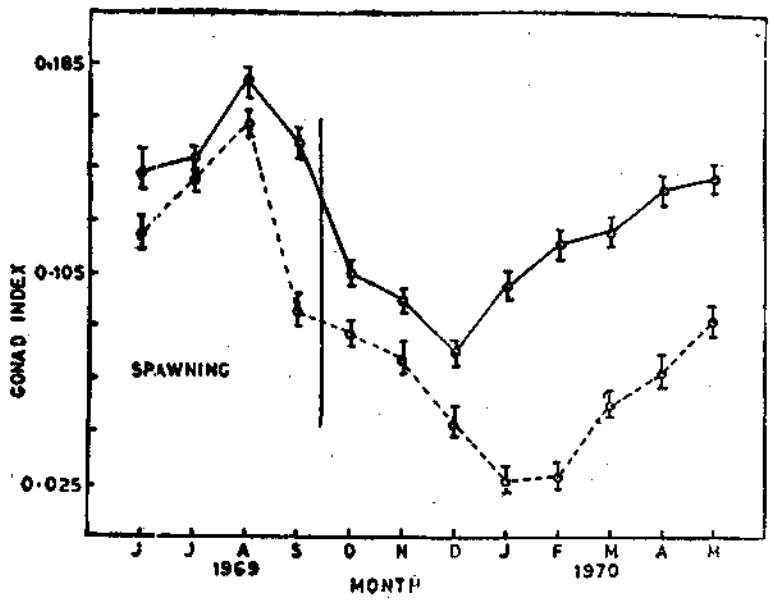

FIG. 1. Seasonal variation in gonad index of the crab, *Barytelphusa cunicularis* from June 1969 to May 1970.
——————— Male
— — — — — Female

season lobes of the testis and ovary measure about 3.0 to 3.5 mm and 4.0 to 4.5 mm in size respectively. The histological studies of the testes and ovaries throughout the year showed active sperms and ripe eggs from June to September. The biochemical changes which have been followed substantiate the above inference.

Biochemical changes:

Glycogen

By conducting monthly analysis the effect of seasonal changes on the glycogen content in the gonads of *Barytelphusa* was determined. The results are presented in the Fig. 2. The glycogen content in the case of testis ranged from 0.463 to 0.898 mg % whereas in ovary it ranged from 0.435 to 0.752 mg %. From the figure it may be concluded that there is a definite alteration of glycogen content in gonads during different periods of the year, especially in breeding season. There was a pronounced fall in the glycogen content during the spawning (June to September). The glycogen content was minimum in both males and females at the end of spawning season. During the resting stage (October — February) there is a little consistency in the glycogen content of the gonad. During the ripening period of the gonad (March to May) the percentage of glycogen was increased. The highest value was noticed in the month of May in both male and female gonads.

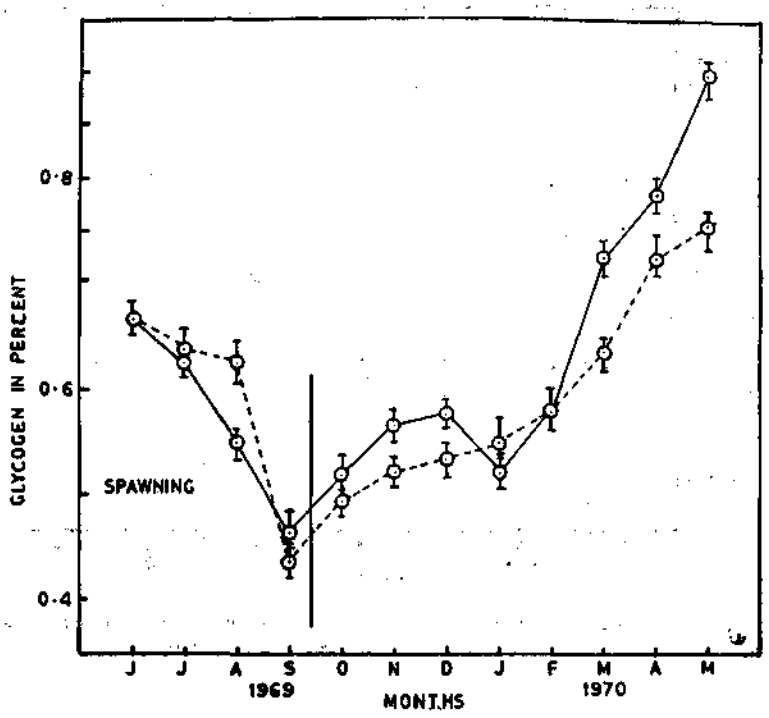

FIG. 2. Seasonal variation in the total glycogen content in the gonad of *Barytelphusa cunicularis* from June 1969 to May 1970.
——————— Male
— — — — — Female

Fat

Monthly analysis of total fat content of the gonad was carried out from June 1969 to May 1970. The results are presented in the Fig. 3. From the figure it was seen that the total fat content in the testes and ovaries varied from 20.4 to 33.3% and 12.5 to 37.5% respectively. The maximum fat content was observed in the ovaries in the month of May and June and in testes it was highest in the month of June only. A steady increase of fat content in the gonads was noticed during the maturation of the gonads (March to June). During the spawning period the fat content in both the sexes decreased.

Protein

Along with the glycogen and fat, analysis of total protein content in the gonad was estimated for alternate months of the year (June 1969 to April 1970). The results are presented in the Figs. 4 and 5. From the figures it was seen that the protein percentage was highest in the month of April. From June onwards the percentage of protein decreased and the lowest was recorded in December.

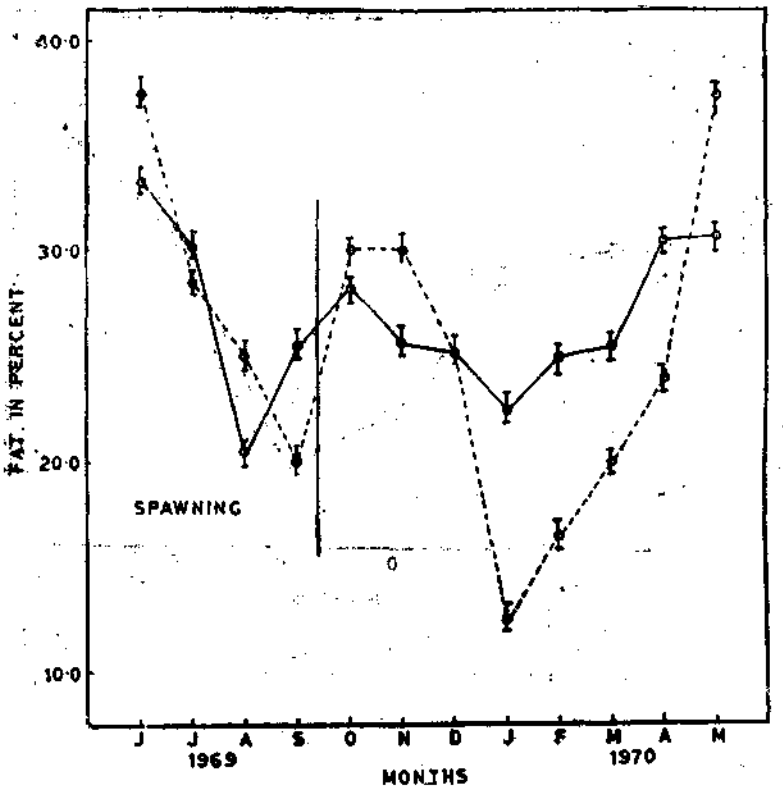

FIG. 3. Seasonal variation in the total fat content in gonad of *Barytelphusa cunicularis* from June 1969 to May 1970.
——— Male
— — — — Female

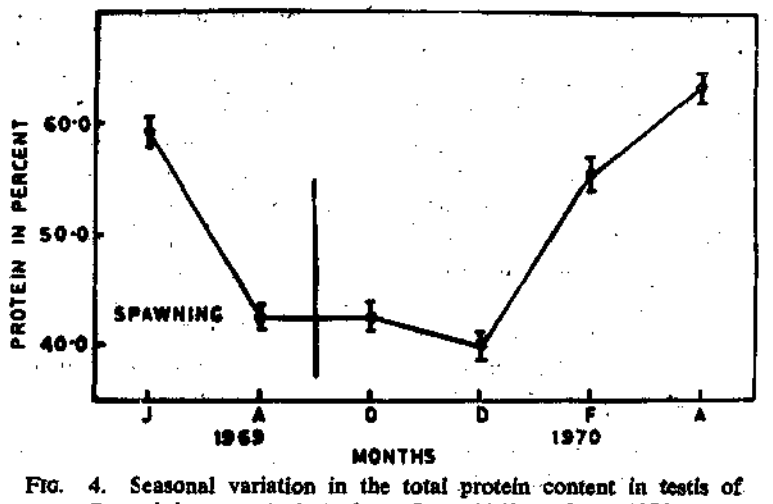

FIG. 4. Seasonal variation in the total protein content in testis of *Barytelphusa cunicularis* from June 1969 to May 1970.

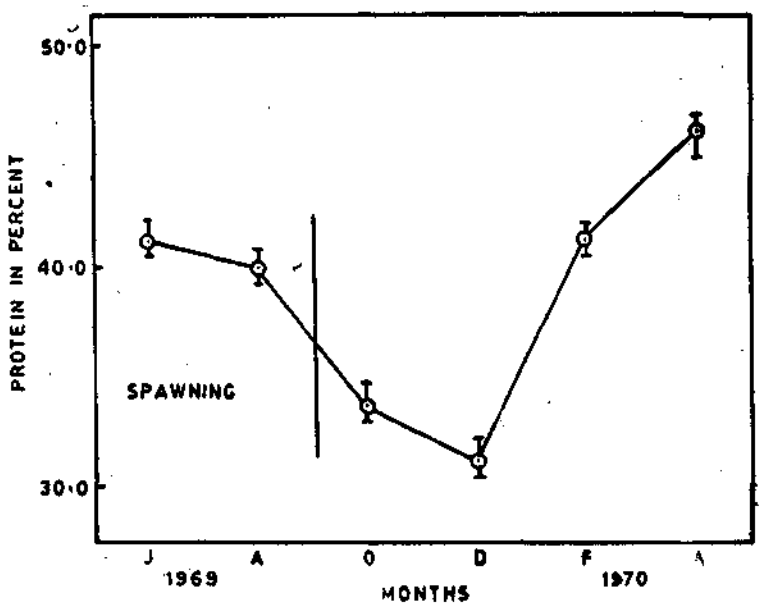

FIG. 5. Seasonal variation in the total protein content in ovary of *Barytelphusa cunicularis* from June 1969 to May 1970.

DISCUSSION

The life cycle of an animal is usually so timed by some environmental variable or variables that the young are produced at a period favourable for their survival (Giese, 1959). The crab, *Barytelphusa cunicularis*, is found to breed during the months of June to September. The reproductive activity of the animal is appeared to be dependent on rainfall. The maximum rainfall occurs in Marathwada region from June to September (Fig. 6). The seasonal breeding habits of *Barytelphusa* may be a direct result of seasonal rainfall. Since the fluctuations in the development of the reproductive system are correlated with the breeding season, it is certain that the reproductive activity depends much on the rainfall. Similar results were obtained by Ali (1955) while working on another freshwater crab, *Paratelphusa guerini*, of Bombay. Mecan (1937) mentioned that breeding in *Paratelphusa guerini* of Salsette Island, usually takes place much earlier than spring season. He found that the females were appeared to be predominant in fertile localities after the break of rainfall and majority of the females were laden with young ones. Chacko and Thyagarajan (1952) observed that *Paratelphusa jacquemontii* of Peninsular India generally breeds from February to April when water level is low and flow of water is reduced.

Histological section of the gonad at monthly interval of *Barytelphusa* showed oocytes of many sizes and spermatogenic cells in various stages including

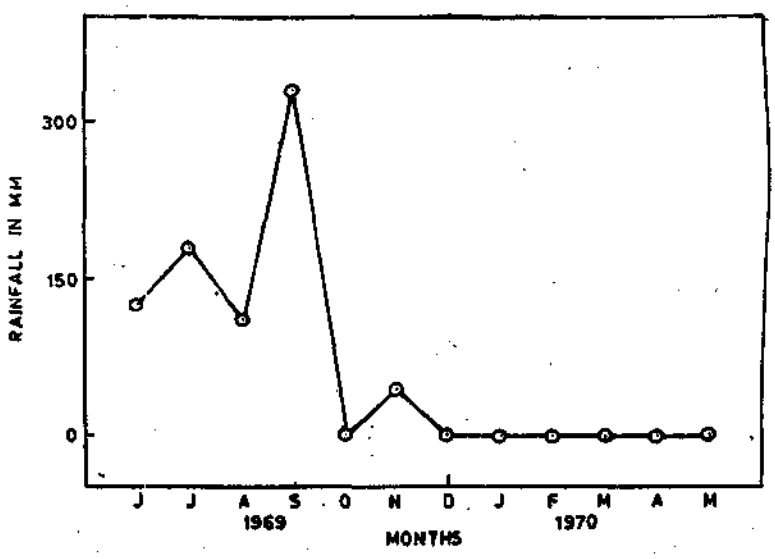

FIG. 6. Graphic representation of changes in the rainfall from June 1969 to May 1970.

spermatozoa only during the months of March, April and May. Full grown ova and spermatozoa were generally noted in gonads during the breeding season. After spawning the size of the gonad was decreased and developing oocytes and spermatogenic cells were found to be of normal size.

The annual reproductive cycle of marine invertebrates were determined by different methods such as observations of spawning, occurrence of larvae, the percentage of ovigerous females against time, appearance of ripe gametes in gonads and the brooding of eggs. The gonad index as a function of breeding cycle of marine invertebrates was first described by Benett and Giese (1955). The gonad index is the ratio of gonad size to body weight and considered to be a measure of the average state of the reproductive population (Giese, 1959). Graphic representation of the annual reproductive cycle is possible when the gonad index is plotted against time. The ratio between the gonad size and body weight bears a relation to gonad development and gonad maturity during different times of the year. This method has been used in molluscs and echinoderms (Giese, 1959) and in crustaceans (Subrahmanyam, 1963; Rahaman, 1967; Chandran, 1968 and Pillay and Nair, 1970).

On the basis of monthly gonad index it was possible to state that the fluctuations observed in the gonad indices of the crab, *Barytelphusa*, represented true seasonal fluctuations. From Table 1 it was concluded that *Barytelphusa* breed from June to September as large number of berried females were found during these months. This view was further strengthened by histological observation of the monthly gonads.

TABLE 1. *Breeding behaviour of* Barytelphusa cunicularis

Year and Month		Percentage of berried females
1969	June	20.0
	July	25.0
	August	14.5
	September	12.5
	October	—
	November	—
	December	—
1970	January	—
	February	—
	March	—
	April	—
	May	—

Stephenson (1934) found that the species inhabiting the tropical waters exhibit several types of breeding cycles i.e. (1) continuous breeders around the year, (2) discontinuous breeding in relation to lunar phases, (3) two spawning periods and (4) one single breeding season. Such different types of breeding cycles have been observed by Paul (1942) in sessile fauna of the Madras harbour. The breeding season of the crabs from the same locality varies widely. Boolootian *et al.*, (1959) studied the reproductive cycle in three species of the crab, *Pachygrapsus crassipes, Emerita analoga* and *Hemigrapsus nudus* and found that these crabs show distinct annual cycles, the first and third showed maximum breeding in summer while the second showed maximum breeding in winter. The females of *Charybdis variegata* of Tondi region on the east coast of India have two distinct peaks in the gonad index during March and September. It is, therefore, concluded that this species is a biannual breeder (Chandran, 1968). Similar studies on the other brachyurans revealed that they are either annual or continuous breeders. It is of interest to note that the blue swimming crab, *Portunus pelagicus,* from the same locality is a continuous breeder (Rahaman, 1967). Similarly, *Neptunus sanguinolentus* from west coast of India at slightly higher latitude was regarded as annual breeder (Menon, 1952). Thus it was found that marine invertebrates showed wide variety of breeding periodicities inspite of taxonomic affinity and similarity in geographical distribution. Breeding is thought to be correlated with the availability of food for the larvae. Boolootian (1965) while working on the crab, *P. crassipes,* found that during the reproductive season, ovigerous females migrated towards the water, where the developing larvae on the pleopods were kept moist and aerated until they hatch, whereas during non-reproductive season males and females were equally distributed. During the reproductive season males were rarely

found in the same location as ovigerous females. A survey of the reproductive cycles in a number of west coast marine invertebrates revealed that most of them had annual reproductive cycle and that quite a large number of them breed in winter and spring (Giese, 1959).

The variability of chemical constituents of the gonads of *Barytelphusa* during the year may be just another index to confirm the spawning period of this species. Carbohydrate level probably represents storage of food (chiefly glycogen) in tissues and might be expected to vary during the reproductive period. A striking change in the glycogen level was seen during the course of reproductive cycle in testis and ovary. The level reached lowest value at the time of spawning period and after spawning the level was increased till the complete growth of the gonad. This suggests that the stored glycogen might be utilized for the formation of the reproductive elements and this counts for the decrease in the glycogen content during the breeding season. It is known that the hepatopancreas serves as a storage organ, the principal storage nutrients being fat and glycogen (Yonge, 1924). It is further suggested that the organic material may be transferred from the storage organ to gonad as the animal matures (Ferguson, 1964). Okazaki and Kobayashi (1929) while working on the Japanese oysters have stated that glycogen was at minimum level when breeding occurred. Humphrey (1941) and Hatanka (1940) also suggested that glycogen acted as a reserve food material and utilized for the formation of gonad products. Similar observations were made by Giese *et. al.* (1958) on purple sea urchin, *Strongylocentrotus purpuratus* and Durve and Bal (1960) on oyster, *Crassostrea gryphoides*. The gonads of *Kathorina tunicata* showed lowest value of glycogen during the spawning period and the level rose again after spawning (Giese, 1969). It would be thus clear from the foregoing account that the glycogen shows seasonal changes in its percentage in association with the sexual and metabolic activity.

The total fat content was increased in testis and ovary during the ripening of the gonads. The highest value noted was 33.3% in testis and 37.5% in ovary. During spawning period the percentage of fat was decreased. Thiele (1959) determined the total fat content of *Helix* and found only slight seasonal variation. It would appear probable that as the gonad grew it received fat from the other parts of the body and accumulated in developing gonads. The fat appeared to be one of the most important sources of the energy metabolism during breeding season. With greater catabolism of fat during the spawning period, the fat content was lowered to its minimum value. Giese (1969) while studying the reproductive cycle of *Katharina tunicata,* found the accumulation of lipids in the ovary during the formation of ova preceding spawning and the lipid level remained essentially the same from month to month in mantle, testis and gut. In another study on *Katharina* (Lawrence and Giese, 1969), it was found that the partition of lipid between the neutral lipids and phospholipids was about the same in the ovary whenever examined during the reproductive

cycle, but the relative amount of phospholipids increased with increase in size of the testis while neutral lipid fraction declined. This shows increased storage of phospholipids with increase in size of the testis. Results of the present study on *Barytelphusa* are in agreement with the findings reported by Giese *et. al.* (1958) in *Strongylocentrotus purpuratus*, Greenfield *et. al.* (1958) in three species of echinoderms, *Pisaster ochraceus, Pisaster giganteus* and *Strongylocentrotus franciscanus*. Durve and Bal (1960) in oyster *Grassostrea gryphoides*, Pearse and Giese (1966) in *Sterechinus neumayeri* and Webber (1970) in *Haliotis cracheroidii*.

The protein level in the gonads during alternate month of the year (1969 — 1970) was studied in *Barytelphusa*. In the ovary and testis protein level showed marked variation during the reproductive cycle. The level was highest in April when the gonads were in developing condition. The highest percentage of protein in the testis was 63.10 mg whereas in ovary it was 46.50 mg. The protein level declined to minimum level during the spawning period. The lowest value noted in October for the testis was 40.50 mg and for ovary 31.25 mg. From February onwards the level of protein was increased till the complete maturation of gonad. Giese *et. al.* (1958) while working on the organic productivity in the reproductive cycle of the sea urchin, *Strongylocentrotus purpuratus*, observed the increase of protein and RNA with the maturation of ovary while increase of DNA proportionally was less. In testis protein and DNA increased more while RNA increased less than the increase in the total bulk. The present observation regarding the protein level in *Barytelphusa* confirms the findings of Durve and Bal (1960) in oyster, *Crassostrea graphoides* and Giese (1969) in *Katharina tunicata*. Greenfield *et. al.* (1958) in three species of echinoderms and Webber (1970) in *Haliotis* found that the protein level did not show much variation during the reproductive cycle.

From the study of the seasonal variation in the chemical composition of the gonad of *Barytelphusa*, it appears that there are three distinct periods of variations i.e.: (1) from March to May when there is a rapid development of gonad, (2) from June to September, which is the spawning season and (3) from October to February, the period of resting phase. These three periods are characterised by the variations in the chemical composition.

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