Marine Biology 18, 167—198 (1973)

Observations on the Biochemical Changes in Gonads and Other Organs of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) During the Reproductive Cycle*

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Abstract

The fluctuations in biochemical constituents such as water, nitrogen, non-protein nitrogen, protein, lipid and glycogen in gonad, muscle and hepatopancreas have been followed in 3 decapod crustaceans, *Uca annulipes* (Latreille), *Portunus pelagicus* (Linnaeus) and *Metapenaeus affinis* (Milne-Edwards). The water and ash content of the entire body show no systematic fluctuation in relation to the annual reproductive cycle. The water content of the ovary diminishes as it matures. In the ovary of these crustaceans, the lipid fluctuated greatly in relation to the reproductive cycle. The maturing ovary contains more lipid than an immature or spent ovary per unit tissue weight. The changes in the biochemical constituents in the testis are not so pronounced as in the ovary, since the testicular cycle is often drawn-out and almost continuous in these crustaceans. There is an inverse relationship between water content and lipid content of the hepatopancreas; the greater the fat content, the lesser the water content. The hepatopancreas in these crustaceans is apparently a storage organ and contains much lipid and glycogen. At the height of the breeding season, when gonad production is intense, there is an indication of the mobilisation of at least a part of the lipid from hepatopancreas to gonad.

Introduction

The gonadal growth in invertebrates in the pre-spawning period is an elaborate process, involving active mobilisation and synthesis of organic substances. The development of the gonad represents a remarkable synthesis of organic material (Giese *et al.*, 1958), the gonad being the locus of intensive biochemical synthesis at the time gametes are being formed (Giese, 1959). This fact is amply borne out in marine invertebrates such as echinoderms and chitons of the west coast of America by the recent works of Giese and his collaborators (Giese *et al.*, 1958; Greenfield *et al.*, 1958; Giese and Araki, 1962; Giese *et al.*, 1964). Furthermore, it has been observed that, in the male, large amounts of nucleic acids are needed for the sperm heads and in the female much lipid and protein are mobilised for storage in the eggs. When reserves are stored in other organs preceding gametogenesis, transfer of these reserves to gonadal synthetic centres occurs at gametogenesis. The paucity of information on the biochemical changes of the gonads and food reserves, and their handling during the breeding cycle in tropical marine invertebrates, particularly crustaceans, has prompted this study, which aims at discovering the fluctuations of various biochemical constituents attending the reproductive cycles of representative crustaceans.

While some information is available on fluctuations of the biochemical constituents of the whole body and gonads in relation to reproductive cycles of invertebrates such as mussels (Daniel, 1923; Fraga, 1956), oysters (see Korringa, 1952; Tanaka and Hatana, 1952) and echinoderms (Russo, 1923, 1926; Stott, 1931), only in recent years has systematic work on the biochemical composition of gonads in relation to reproductive cycle been initiated by Giese and his coworkers (see Giese, 1959; Giese and Araki, 1962; Tucker and Giese, 1962; Giese *et al.*, 1964; Pearse and Giese, 1966). More recently, the biochemical changes attending the reproductive cycle were followed in some molluscs and echinoderms of Indian waters (Rao, 1966, 1968; Saraswathy and Nair, 1969).

From a biochemical point of view, the class Crustacea has been studied fairly extensively (Renaud, 1949; Vinogradov, 1953), but studies on biochemical changes attending the reproductive cycle have been few (George and Patel, 1956; Barnes *et al.*, 1963). Therefore, in the present study, the fluctuations in the chemical constituents of gonads, liver and muscles of the crab *Portunus pelagicus*, the prawn *Metapenaeus affinis*, and gonads and liver of the crab *Uca annulipes*, during the reproductive cycle were followed. The constituents examined during the present study were water, total nitrogen, non-protein nitrogen, protein, lipid and glycogen. An attempt has also been made to measure the organic productivity during the reproductive cycle of these crustaceans by assessing the increase in organic constituents in the gonads during their growth from unripe (or spent) to ripe condition.

* Dissertation for the award of a Doctorate Degree of the Kerala University.

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Material and Methods

For the various chemical estimations, live specimens of *Uca annulipes, Metapenaeus affinis* and *Portunus pelagicus* were brought to the laboratory from the Cochin area (latitude 9°58′ N; longitude 76°17′ E). Specimens were obtained along with those collected for gonad index studies. In most cases, material from specimens whose gonad indices had been determined was used. The gonad index value was obtained by dividing the weight of the gonad by the weight of the body, and this factor was multiplied by 100 (Pillay and Nair, 1971). Special care was taken to include only the hard-shelled crabs of the intermoult stage in all these estimations. The water content of the entire animals of *U. annulipes* and *M. affinis* was determined. Then the entire body of *U. annulipes* was ashed. In *U. annulipes,* the gonads and liver (hepatopancreas) were dissected out and used for analyses; in *P. pelagicus* and *M. affinis,* besides gonads and liver, muscle from the posterior region of the cephalothorax of the crab and muscle from the first abdominal segment of the prawn were also analysed. The gonads, liver and muscles were treated for the determination of water content, total nitrogen (TN) non-protein nitrogen (NPN), lipid and glycogen. In the figures, each point represents the average of determinations on different individuals, which constituted a sample for the month. When the gonad of a single individual was insufficient for all the determinations, identical samples were pooled for the various estimations.

Water Content

The water content of entire *Uca annulipes* and *Metapenaeus affinis* was obtained by drying the animal in an air oven at 100° to 105 °C to constant weight after determining the wet weight. The water content was calculated as the difference between the wet weight and dry weight of the animal, and this is expressed as percentage of the wet weight of the body.

For determination of the water content of gonads, muscle and liver, a few grams of these tissues were dried to constant weight in a desiccator over concentrated sulphuric acid, and the difference between the wet weight and dry weight (= the water content) has been expressed as percentage in terms of wet weight of the tissue. Since dried samples of these tissues were later used for biochemical estimations, a 10% solution of trichloracetic acid was injected into the tissue to prevent glycolysis during the process of drying (as performed by Giese *et al.*, 1958).

Ash Content

The ash content of the entire body of *Uca annulipes* was obtained by igniting the oven-dried samples in a porcelain crucible in a muffle furnace at 450° to 500 °C for about 5 h. The ignited residue thus obtained has been taken as the ash content, and expressed as percentage in terms of dry weight of the body.

Samples of tissues dried as described above, have been used for the determination of total nitrogen, non-protein nitrogen, lipid and glycogen, and all the values are expressed as percentage of dry weight of tissue. These estimations were performed in duplicate and, in some cases, in triplicate; the values presented represent the average of these determinations. In *Uca annulipes,* estimations were carried out on pooled samples of tissues, since the gonad of an individual was insufficient to make all the determinations.

Nitrogen

Total nitrogen, non-protein nitrogen and protein nitrogen in the dried tissues were estimated by the micro Kjeldahl method as outlined by Steyermark (1961). Protein value was calculated by multiplying the protein nitrogen value with a factor of 6.25, as performed by Giese *et al.* (1958).

Lipid

For lipid extraction, a micro-Soxhlet apparatus was used; ether was the solvent. Greenfield *et al.* (1958) considered the small fraction of structural lipid that was not likely to be extracted by this method to be of minor importance. Therefore, this assumption, is not accounted for in the values presented here.

Glycogen

The estimation of glycogen in the dried tissue was carried out by the method of Mendel and Hoogland (1950), later adopted by Raymont and Krishnaswamy (1960). The glucose content of the samples was calculated by measuring the transmission density at 515, using an Evelyn colorimeter with a green filter. These readings were converted to glycogen values by multiplying by a factor of 0.925.

Results

The data on the various biochemical determinations of *Uca annulipes, Metapenaeus affinis* and *Portunus pelagicus* are presented in Tables 1—26.

Uca annulipes

Water Content of the Body

The water content of the gonads was determined in each month, and 4 to 8 determinations of the water content of the entire body of the females and males at different stages of maturity have also been made (Table 1, Fig. 1, A and B).

The average values of water content of both sexes for different months do not exceed 68% of the body

Table 1. *Uca annulipes. Carapace-width range, and mean percentage of water content of entire animals, both males and females, during different months (1963/1964)*

Year	Month	Carapace-width range (mm)		Water content (%)	
		Males	Females	Males	Females
1963	November	9.8—18.0	13.2—17.2	60.5	66.5
	December	15.5—20.0	14.5—16.5	57.4	64.2
1964	January	13.2—19.5	12.2—14.5	64.0	61.7
	February	13.5—14.8	14.0	57.4	61.2
	March	17.8—20.5	13.1—13.5	57.8	58.9
	April	16.0—19.8	12.6—16.5	58.4	64.2
	May	19.2—20.0	15.5	59.1	55.6
	June	15.5—18.0	13.1—13.9	58.5	58.2
	July	17.2—20.0	13.0—14.5	60.0	62.6
	August	19.0—20.0	13.2—17.0	60.0	58.6
	September	17.0—19.9	17.0	60.1	67.7
	October	16.5—17.5	13.6—16.9	60.0	65.1

weight. This apparently low value (in terms of percentage) when compared to that of the prawn (*vide infra*) may probably be due to the semi-terrestrial habitat of these crabs, and also to their heavy exoskeleton. It is

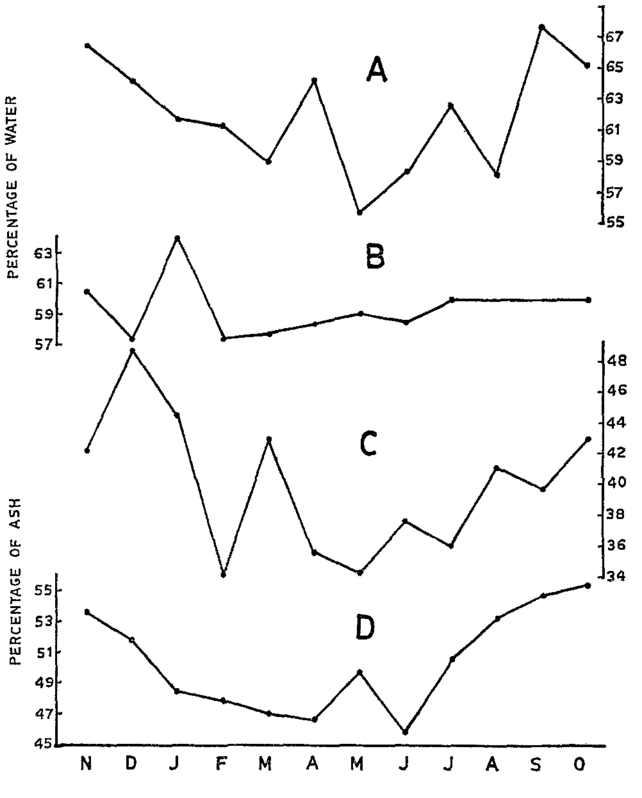

Fig. 1. *Uca annulipes.* Fluctuations in water and ash content of entire body from November, 1963 to October, 1964. (Water content, percentage of wet weight; ash content, percentage of dry weight). Each point represents average for month. Fluctuations in (A) water content in female; (B) water content in male; (C) ash content in female; (D) ash content in male

interesting to note that, in females during the breeding months, the water content is generally high. The data show that the fluctuations of water content of males are not very marked during the major part of the year and, thus, no relation apparently exists between the water content of the body and breeding activity.

It is also clear that the water content of the females is higher than that of the males. This difference may be due to the comparatively heavier skeleton of the males, especially the large chelipeds, which would considerably increase the weight of the crab and, thus, influence the percentage of water content.

Ash Content of the Body

The data for the ash content of the body of female and male *Uca annulipes* are given in Table 2 and Fig. 1 (C and D).

From the data on the ash content of the body of *Uca annulipes*, it is clear that there are variations in the ash content of the two sexes. The range of variation is greater in females. In both sexes, despite the absence of any striking correlation between the ash content of the body and breeding activity, a relation between the gonad growth and ash content of the body exists in females since, during the breeding season, an increase in the percentage of ash content is evident.

Changes in the Biochemical Composition of the Gonads During the Reproductive Cycle

Data on the determination of water content, non-protein nitrogen, protein, lipid and glycogen content of the ovary of *Uca annulipes* are summarised and presented in Tables 3 and 4 and Figs. 2 and 3.

In the ovary of *Uca annulipes*, while variations in the content of water and non-protein nitrogen do not give clear evidence of distinct correlation with gonadal

Table 2. *Uca annulipes. Carapace-width range, and mean ash content of entire animals, both males and females (1963/1964)*

Year	Month	Carapace-width range (mm)		Ash content (%)	
		Males	Females	Males	Females
1963	November	9.8—18.0	13.2—17.2	53.6	42.1
	December	15.5—20.0	14.5—16.5	51.8	48.7
1964	January	13.2—19.5	12.2—14.5	48.5	44.4
	February	13.5—14.8	14.0	47.9	34.0
	March	17.8—20.5	13.1—13.5	47.0	42.9
	April	16.0—19.8	12.6—16.5	46.6	35.5
	May	19.2—20.0	15.5	49.7	34.1
	June	15.5—18.0	13.1—13.9	45.8	37.6
	July	17.2—20.0	13.0—14.5	50.6	35.9
	August	19.0—20.0	13.2—17.0	53.3	41.0
	September	17.0—19.9	17.0	54.7	39.6
	October	16.5—17.5	13.6—16.9	55.4	42.9

Table 3. *Uca annulipes. Changes in biochemical composition of ovary (1963/1964); water content expressed as percentage wet weight, all others as percentage dry weight*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1963	November	1.88	68.9	8.41	3.50	4.90	29.6	18.18	0.66
	December	8.6	64.4	9.17	1.57	7.61	47.5	15.33	5.24
1964	January	6.44	62.9	6.86	1.68	5.19	32.4	19.67	0.40
	February	3.8	38.4	7.33	2.07	5.27	32.9	15.17	4.62
	March	3.29	50.5	6.94	1.23	5.72	35.7	15.63	3.49
	April	2.1	53.7	5.20	1.37	3.83	24.0	10.48	2.74
	May	1.64	53.8	4.59	1.57	3.02	18.9	8.57	1.63
	June	1.91	50.8	5.34	1.33	3.81	24.1	12.7	3.47
	July	2.03	52.8	8.77	1.95	6.83	42.7	16.11	2.11
	August	3.31	45.5	8.8	1.55	6.25	45.3	18.57	4.07
	September	5.36	35.8	8.70	1.90	6.8	42.5	19.58	2.40
	October	4.27	47.7	8.93	1.91	7.02	43.9	18.66	3.15
Average			52.1		1.8		34.8	15.72	2.83

Table 4. *Uca annulipes. Carapace-width range, percentage range and mean of water content of ovary and gonad index of females (1963/1964)*

Year	Month	Carapace-width range (mm)	Range of water content (%)	Mean (%)	Gonad index
1963	November	12.0—17.5	44.5—90.5	68.9	1.88
	December	12.2—15.8	46.2—83.5	64.4	8.60
1964	January	12.0—17.2	30.8—87.1	62.9	6.44
	February	9.9—15.5	48.3—68.9	58.4	3.82
	March	11.7—16.7	40.0—73.7	50.5	3.29
	April	13.2—17.5	41.7—66.6	53.7	2.13
	May	13.5—16.5	41.7—60.0	53.8	1.64
	June	13.1—15.8	45.7—60.0	50.8	1.91
	July	13.5—15.5	47.0—65.2	52.8	2.03
	August	14.5—17.0	37.4—51.2	45.5	3.31
	September	13.5—16.6	25.6—47.8	35.8	5.36
	October	14.8—15.8	34.6—58.3	47.7	4.27

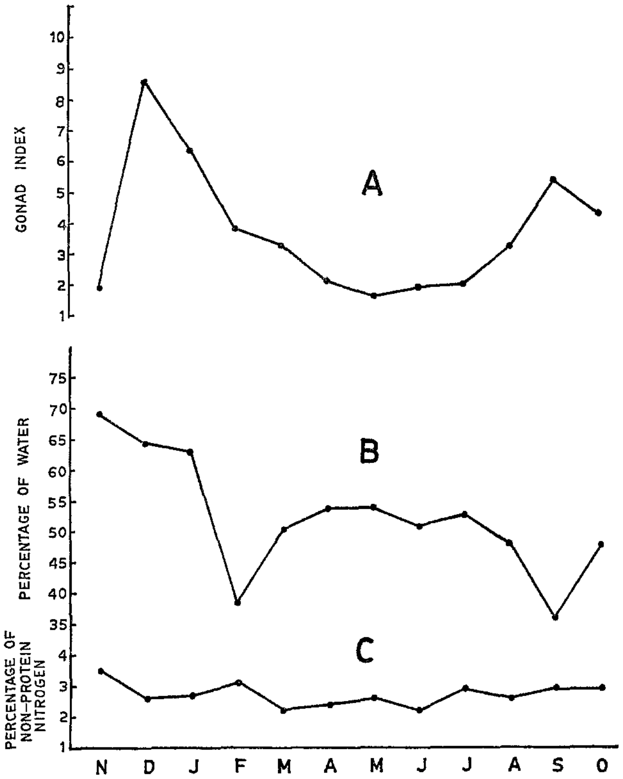

Fig. 2. *Uca annulipes.* Changes in gonad index and various biochemical constituents of ovary from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

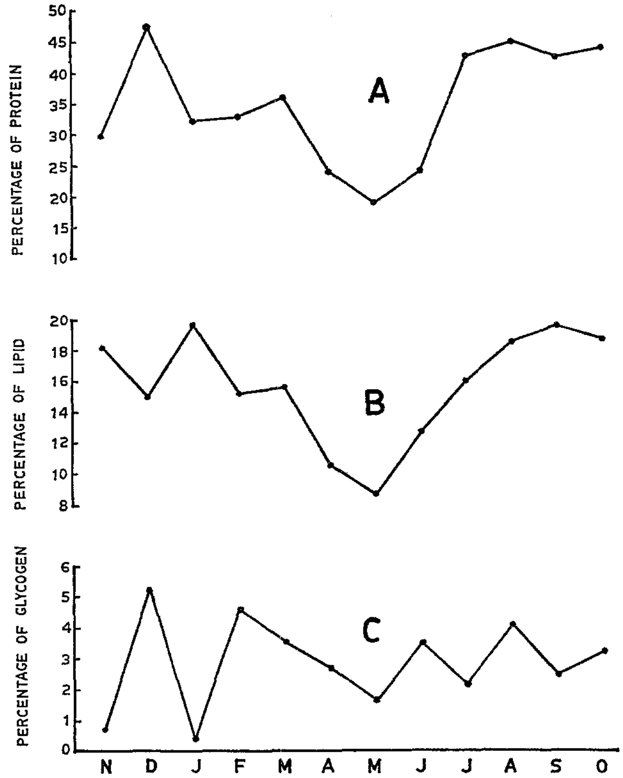

Fig. 3. *Uca annulipes.* Changes in various biochemical constituents of ovary (percentage of dry weight) from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

Table 5. *Uca annulipes. Changes in biochemical composition of testis (1963/1964); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid
1963	November	0.29	62.3	5.88	2.36	3.52	21.99	2.82
	December	0.31	59.9	11.2	4.22	6.98	43.6	5.71
1964	January	0.33	63.2	6.38	1.88	4.5	20.99	1.66
	February	0.32	63.6	9.57	2.10	7.47	46.59	10.00
	March	0.38	55.8	10.27	4.00	6.27	39.18	8.15
	April	0.23	63.5	8.97	2.51	6.46	40.38	6.54
	May	0.26	60.1	9.32	1.47	7.85	49.06	5.98
	June	0.31	57.6	7.85	3.02	4.83	30.20	3.77
	July	0.45	69.2	8.25	0.63	7.62	47.6	10.00
	August	0.30	55.4	7.28	2.10	5.18	32.39	1.43
	September	0.33	62.5	9.42	2.34	7.09	44.3	2.5
	October	0.27	57.5	12.25	2.80	9.45	59.04	9.99
Average			61.1		2.45		40.1	5.71

Table 6. *Uca annulipes. Carapace-width range, percentage range and mean of water content of testis and gonad index of males (1963/1964)*

Year	Month	Carapace-width range (mm)	Range of water content (%)	Mean (%)	Gonad index
1963	November	16.5—21.0	54.3—81.7	62.3	0.29
	December	11.0—22.2	40.0—72.7	59.9	0.31
1964	January	14.0—20.0	45.7—77.7	63.2	0.33
	February	15.8—19.5	42.9—84.6	63.6	0.32
	March	14.5—18.6	50.0—62.5	55.8	0.38
	April	15.0—18.0	56.0—80.0	63.5	0.23
	May	17.0—22.2	40.0—75.0	60.1	0.26
	June	15.1—19.9	44.1—72.2	57.6	0.31
	July	19.8—20.9	56.1—77.6	69.2	0.45
	August	16.0—20.0	50.0—65.3	55.4	0.30
	September	16.5—20.5	52.6—80.5	62.5	0.33
	October	18.0—19.1	44.4—75.0	57.5	0.27

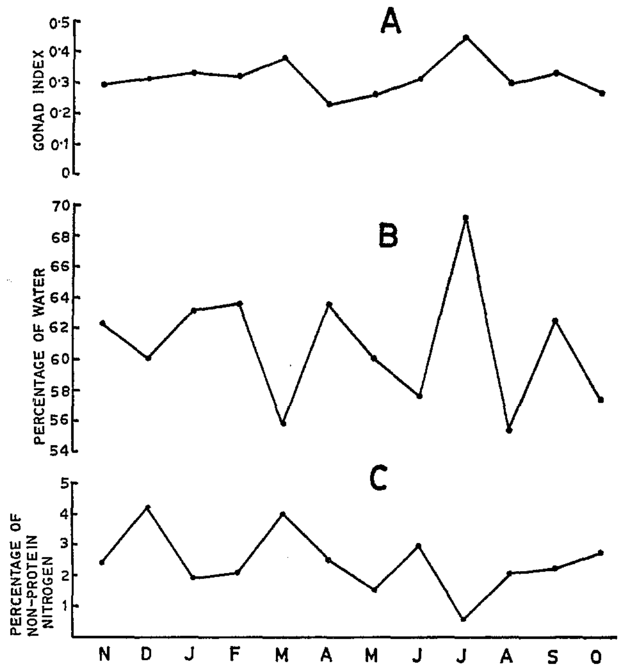

Fig. 4. *Uca annulipes.* Changes in gonad index and various biochemical constituents of testis from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

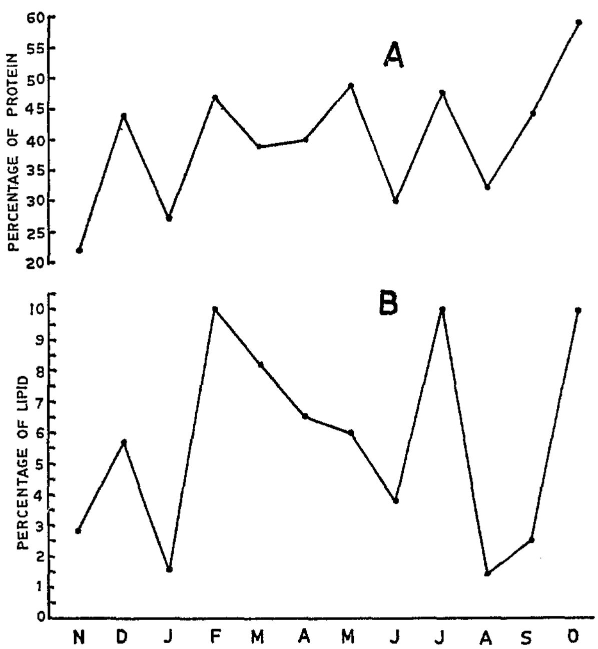

Fig. 5. *Uca annulipes.* Changes in various biochemical constituents of testis (percentage of dry weight) from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content

activity, protein, lipid and glycogen tend to show an increase with the increase in the size of the gonad.

The results of the determination of water content, non-protein nitrogen, protein, lipid and glycogen content of the testis of *Uca annulipes* are presented in Tables 5 and 6 and Figs. 4 and 5.

These results suggest that there is no significant correlation between the fluctuations of various chemical constituents of the testis and the reproductive cycle of the males. This may probably be due to the protracted and almost continuous activity of the male gonad in this crab.

The overall averages of the various biochemical constituents of the gonads of *Uca annulipes* for the entire year are also given in Tables 3 and 5. It is evident that, while the ovary is distinctly richer than the testis in lipid content, the testis appears to be richer than the ovary in water content, non-protein nitrogen and protein.

Fluctuations in the Biochemical Constituents of the Liver in Relation to the Reproductive Cycle

The data for the various biochemical components such as water, non-protein nitrogen, protein, lipid and glycogen of the liver of female *Uca annulipes* are presented in Table 7 and Figs. 6 and 7. An inverse relationship is evident between the water and lipid contents of the liver, which stores lipid in considerable quantities.

The protein content of the liver is less than that of the ovary, and this feature has been noticed in the other two species also examined during the present study. The lipid content of the liver is higher than that

Table 7. *Uca annulipes. Changes in biochemical composition of liver of females (1963/1964); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1963	November	1.88	58.4	7.93	2.22	5.71	35.7	29.58	6.56
	December	8.60	55.2	5.37	1.31	4.06	25.4	35.79	7.37
1964	January	6.44	61.7	6.62	2.26	4.36	27.2	29.36	3.63
	February	3.80	62.3	4.47	1.53	2.94	18.4	23.62	4.69
	March	3.29	64.0	3.73	2.62	1.11	6.9	18.75	3.34
	April	2.1	67.6	3.32	1.21	2.11	13.2	15.63	3.82
	May	1.64	65.1	4.21	2.31	1.9	11.9	19.29	4.56
	June	1.91	66.2	3.42	1.97	1.44	9.0	17.67	3.38
	July	2.03	67.3	5.63	2.93	2.7	16.8	21.53	6.26
	August	3.31	60.4	6.31	1.28	5.03	31.4	27.78	5.36
	September	5.36	59.4	7.22	1.72	5.51	34.4	32.43	6.67
	October	4.27	55.6	6.63	1.94	4.69	29.3	36.36	7.59

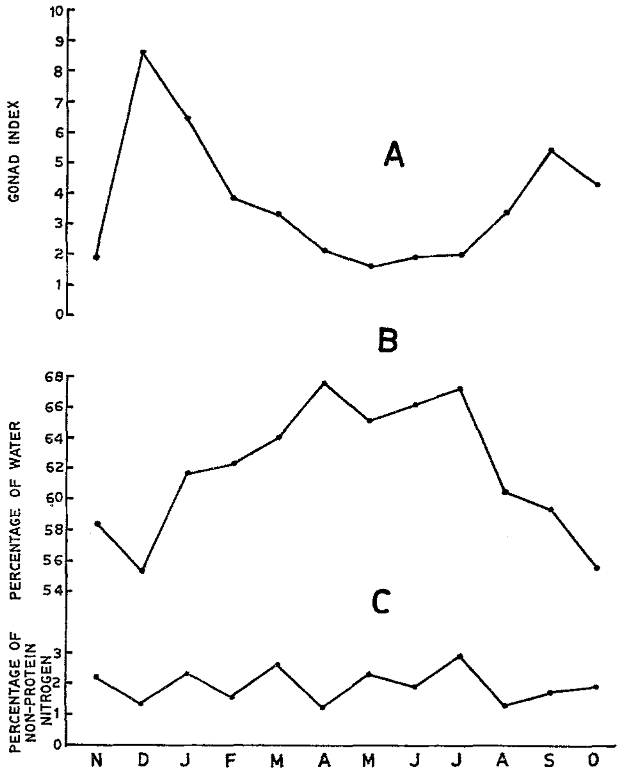

Fig. 6. *Uca annulipes.* Changes in gonad index and various biochemical constituents of liver of female from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

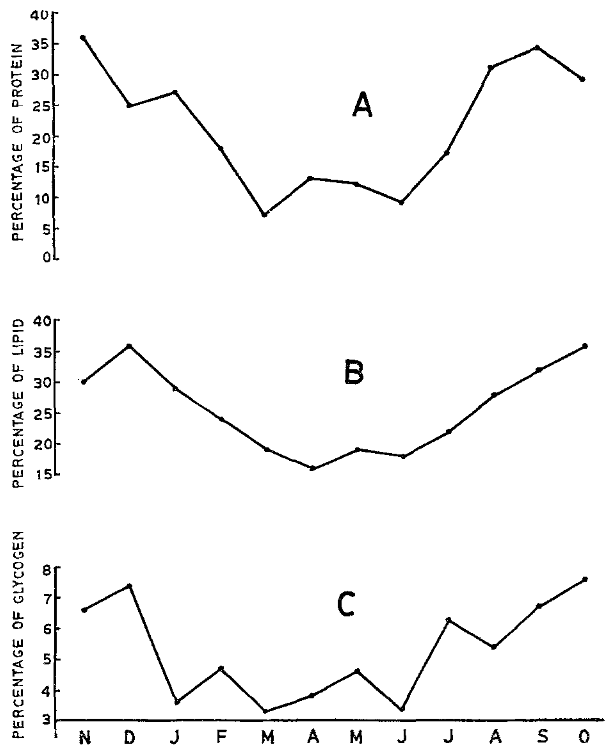

Fig. 7. *Uca annulipes.* Changes in various biochemical constituents of liver (percentage of dry weight) of female from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

of the ovary. In the liver of female *Uca annulipes*, a comparatively higher value of lipid content is noticeable during the breeding months. It is possible that the lipid requirements during development or recuperation of the ovary could be met from the liver. However, the available data do not clearly give any indication of a definite storage and subsequent transfer of this material to the gonad for gamete production as in temperate forms. Since much lipid is needed for the formation of oocytes, the possibility of a transfer of this substrate from the liver to the gonad cannot be ruled out.

The apparently steady values for lipid content in the liver during the breeding season suggest that the amount likely to be transferred to the gonad for breeding purposes is rapidly reinforced in the liver at almost the same rate, so that the depletion of this constituent in the liver is not clearly noticeable. The glycogen content of the liver is more than that of the gonad. When compared to the liver of the other crustaceans in the present study, that of *Uca annulipes* has greater quantities of glycogen. This is probably indicative of storage of this substance. From the liver, there in the possibility of a transfer of this constituent to the ovary, since the ovary of this species contains fair quantities of glycogen.

The data on the water, non-protein nitrogen, protein, lipid and glycogen content of the liver of males are presented in Table 8 and Figs. 8 and 9. The liver shows a comparatively higher value for water content than the gonad per unit weight of tissue. There is apparently no clear indication of any mobilization of stored protein from the liver to the testis. The protein values of the liver are comparatively less than those of the gonads, and values for the liver of males are higher than those of females. There is no evidence of any clear correlation between the lipid content of the liver and the activity of the testis. The data show that the lipid content of the liver is greater than that of the testis. A correlation between the glycogen content of the liver and breeding activity in males is not clearly discernible.

In these tropical habitats, even when food is available fairly uniformly all the year round, the production of successive broods may entail large amounts of organic substances. In *Uca annulipes*, seasonal storage of large quantities of substances does not seem to take place. However, the liver seems to function as a storage

Table 8. *Uca annulipes. Changes in biochemical composition of liver of males (1963/1964); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1963	November	0.29	59.8	7.36	1.02	6.34	39.65	27.18	7.72
	December	0.31	55.1	5.93	2.31	3.64	22.75	34.72	3.83
1964	January	0.33	53.8	6.57	2.36	4.21	26.31	37.43	4.66
	February	0.32	58.4	3.91	1.03	2.87	17.94	25.07	2.73
	March	0.38	62.7	2.35	1.03	1.33	8.28	19.88	3.41
	April	0.23	61.6	4.04	1.08	2.96	18.53	22.94	4.39
	May	0.26	65.7	3.89	1.13	2.76	17.25	16.07	7.43
	June	0.31	62.6	5.44	1.78	3.65	22.83	22.57	8.52
	July	0.45	61.6	6.39	2.56	3.83	23.94	21.36	8.37
	August	0.30	59.3	7.08	1.36	5.72	35.3	26.67	7.67
	September	0.33	55.3	6.95	1.68	5.27	32.93	35.52	3.74
	October	0.27	52.2	7.20	2.35	4.85	30.31	38.38	5.08

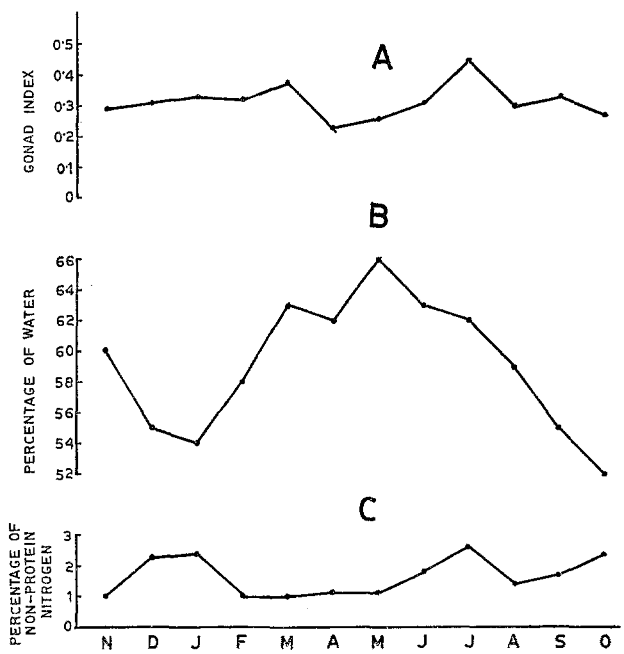

Fig. 8. *Uca annulipes*. Changes in gonad index and various biochemical constituents of liver of male from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

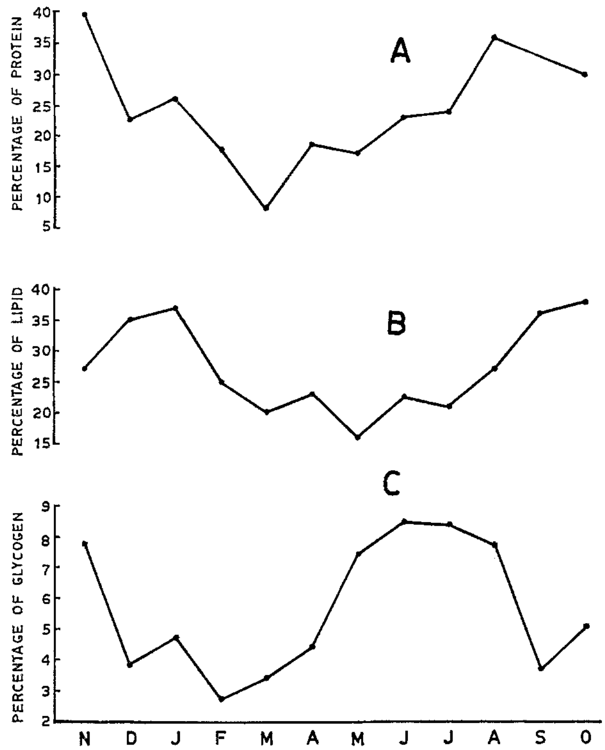

Fig. 9. *Uca annulipes*. Changes in various biochemical constituents of liver (percentage of dry weight) of male from November, 1963 to October, 1964. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

organ. Owing to the availability of a fairly steady supply of food and a protracted breeding season, with the production of several successive broods during the year, a steady flow of organic substances seems to be maintained without any large-scale storage in any organ. This fact is clear from the analysis of the constituents of the liver of *U. annulipes* during the yearly cycle. However, the noticeable waxing and waning of the constituents in both the gonad and the liver, although without distinct correlation, are indications of the physiological state of the animal, related to feeding and, to some extent, to brood formation, besides other factors. Thus, even though the liver has less amounts of nitrogen than the gonad, glycogen and lipid contents are more than those in the gonad, and these materials are, therefore, probably stored in the liver depending upon the availability of food. In the case of the ovary, when the developing oocytes need more protein, lipid and glycogen, these can be readily mobilized to the ovary from their sites of storage in the liver. In the males, clear evidence of such a transfer of materials is lacking.

Portunus pelagicus

Changes in the Biochemical Composition of the Gonads During the Reproductive Cycle

Data on the determination of various biochemical constituents such as water, protein, non-protein nitrogen, lipid and glycogen of the ovaries of *Portunus pelagicus* are presented in Tables 9, 10 and 11 and Figs. 10 and 11. It will be seen that the ripe and ripening ovaries in all the months contain less water than the spent or immature ones. This fact is clearer in *P. pelagicus* than in *Uca annulipes*. The fluctuation in non-protein nitrogen is not substantial. However, it is noteworthy that the low values coincide with the months (December to March) of high gonad indices. Thus, the non-protein nitrogen values apparently show an inverse relationship with the growth of the ovary. The high values in protein occurring during the other months (breeding period) indicate the presence of large quantities of this constituent in the ripe and ripening ovaries.

Table 9. *Portunus pelagicus. Biochemical composition of ovary (1964/1965); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1964	February	1.76	64.4	9.76	2.16	7.61	47.44	8.93	2.44
	March	2.3	67.6	10.04	2.50	7.5	46.89	8.3	3.34
	April	0.29	69.5	9.8	3.26	6.54	40.84	9.38	2.31
	May	0.33	60.3	9.4	2.6	6.79	42.41	6.27	2.47
	June	0.31	69.4	9.67	2.82	6.25	39.05	4.95	1.63
	July	0.71	69.8	9.35	2.76	6.59	40.98	6.47	1.94
	August	1.39	68.9	10.11	2.86	7.22	45.18	7.35	2.71
	September	0.95	72.1	10.1	3.25	6.83	42.71	10.39	2.76
	October	0.62	72.1	10.07	3.1	6.88	43.02	6.59	2.24
	November	0.92	74.8	9.76	2.7	7.06	44.12	8.40	2.6
	December	3.90	53.9	10.34	2.5	7.84	49.1	8.2	2.30
1965	January	5.44	55.1	10.39	2.39	7.9	49.36	12.46	2.61
Average			64.8		2.73		44.2	8.14	2.61

Table 10. *Portunus pelagicus. Carapace-width range, percentage range and mean of water content of ovary and gonad index of females (1964/1965)*

Year	Month	Carapace-width range (mm)	Range of water content (%)	Mean (%)	Gonad index
1964	February	100.0—154.0	45.9—70.4	64.43	1.76
	March	98.0—134.0	43.8—76.3	67.6	2.3
	April	106.0—130.0	54.7—76.5	69.5	0.29
	May	116.5—140.0	47.1—70.8	60.3	0.33
	June	115.5—157.0	60.5—80.0	69.4	0.31
	July	118.5—161.0	62.3—85.3	69.8	0.71
	August	126.0—161.0	50.9—78.8	68.9	1.39
	September	122.0—151.0	58.3—85.1	72.13	0.95
	October	113.5—145.0	49.9—80.1	72.1	0.62
	November	122.0—150.0	65.9—79.5	74.77	0.92
	December	139.0—154.0	41.0—75.7	54.0	3.90
1965	January	95.0—143.0	40.9—84.4	55.1	5.44

Table 11. *Portunus pelagicus. Water content of ovary (1964/1965)*

Year	Month	Gonad index	Water content of ovary (overall average %)	Water content of ripe ovary (average %)	Water content of spent ovary (average %)
1964	February	1.76	64.4	60.5	68.8
	March	2.3	67.6	54.2	71.1
	April	0.3	69.5	61.7	72.9
	May	0.33	60.3	68.9	70.8
	June	0.31	69.4	66.3	67.8
	July	0.71	69.8	63.6	69.4
	August	1.39	68.9	51.8	73.2
	September	0.96	72.1	63.0	76.2
	October	0.63	72.1	64.7	74.5
	November	0.92	74.8	69.9	76.2
	December	3.9	54.0	48.5	64.9
1965	January	5.4	55.1	49.4	77.8

High values in lipid occur during the breeding season, from September to April, with the exception of October. This indicates that ripe and ripening ovaries during these months contain considerable quantities of lipid in the developing oocytes. It appears that the spent ovary is poor in this constituent. Unlike protein and lipid, the glycogen values do not show any significant correlation with the ovarian cycle. This suggests that glycogen may not form a significant substrate in the ovary, and does not substantially contribute towards oocyte formation.

Thus, generally, in the ovary of *Portunus pelagicus*, water and non-protein nitrogen show low values during the months of high gonad activity (December and January to March), whereas protein and lipid increase during the months when ripe and ripening individuals dominate in the population. The glycogen content in the ovary of this species is low, and this constituent does not indicate noticeable fluctuations during the annual cycle.

The data on the determination of water content, protein, non-protein nitrogen, lipid and glycogen of the testis of *Portunus pelagicus* are presented in Table 12 and Figs. 12 and 13. The highest gonad index coincides with the highest value for water content, and also the lowest value of water content with the lowest gonad index value. Thus, there apparently exists a correlation between the water content and gonad growth in males, contrary to our observations in females. The data showing the range of water content obtained from individual determination of the testis are given in Table 13. This shows a greater degree of fluctuation of the water content in the testis than in the ovary. It is noteworthy that the highest and lowest values of water content of the ovary do not coincide with those of the testis, and this indicates the difference in the physiological state of the two sexes. The water content of both ovary and testis shows some noticeable trends. The fluctuations are more pronounced in the testis than in the ovary. It is also clear that the water content of the testis is maximum when the gonad index is highest, and minimum when the gonad index is lowest. On the other hand, in the ovary, the trend has been the higher the gonad index, the lower the

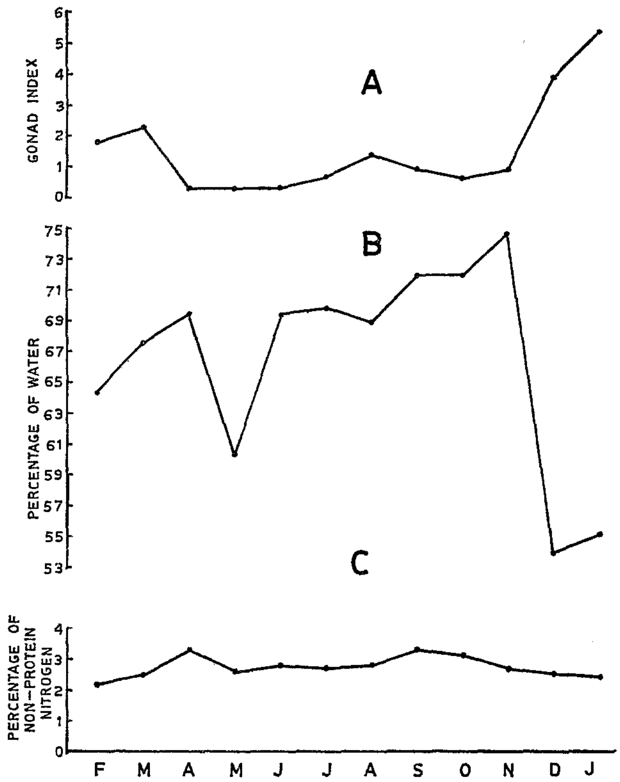

Fig. 10. *Portunus pelagicus*. Changes in gonad index and various biochemical constituents of ovary from February, 1964 to January, 1965. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

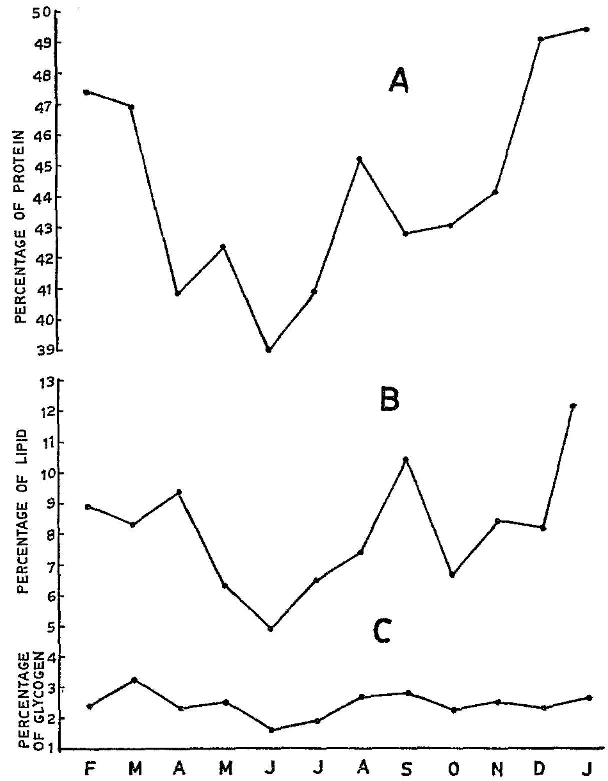

Fig. 11. *Portunus pelagicus*. Changes in various biochemical constituents of ovary (percentage of dry weight) from February 1964 to January, 1965. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

Table 12. *Portunus pelagicus*. *Biochemical composition of testis (1964/1965); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1964	February	0.30	61.4	10.6	2.07	8.52	53.26	6.42	3.45
	March	0.18	55.2	11.47	1.59	9.37	58.58	4.19	3.28
	April	0.24	60.3	10.82	2.30	8.51	53.21	9.0	1.93
	May	0.23	58.8	9.98	1.94	8.03	50.2	4.53	2.26
	June	0.47	65.8	9.66	1.91	7.75	48.37	3.94	1.80
	July	0.41	64.3	9.51	1.87	7.63	47.70	4.89	1.96
	August	0.29	63.1	9.85	1.91	7.93	52.96	3.62	1.51
	September	0.32	64.0	9.87	1.93	8.05	50.30	3.85	1.72
	October	0.43	69.7	11.23	1.81	9.42	58.89	4.57	1.52
	November	0.98	75.7	11.87	2.09	9.78	61.13	7.7	0.94
	December	—	—	—	—	—	—	—	—
1965	January	0.47	67.8	10.28	2.05	8.24	51.46	8.14	2.25
Average			64.1		1.95		53.2	5.52	1.96

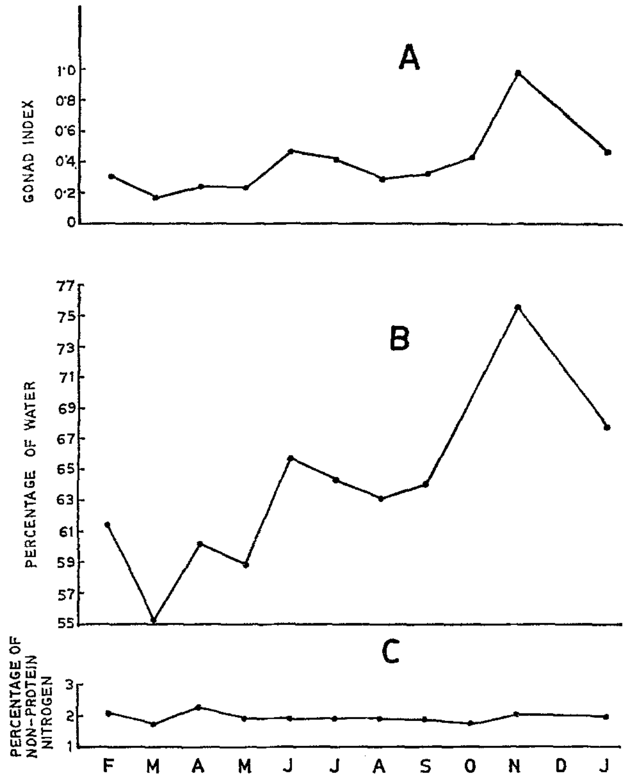

Fig. 12. *Portunus pelagicus*. Changes in gonad index and various biochemical constituents of testis from February, 1964 to January, 1965. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

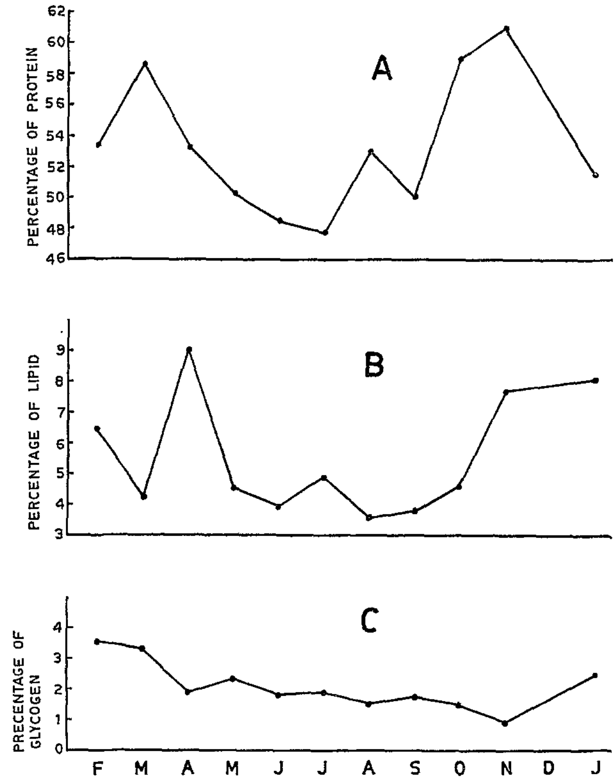

Fig. 13. *Portunus pelagicus*. Changes in various biochemical constituents of testis (percentage of dry weight) from February, 1964 to January, 1965. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

water content. In *P. pelagicus*, the water and protein contents increase with the growth of the testis. Whereas lipid and non-protein nitrogen do not show any indication of a relationship with the development of the testis, glycogen seems to show an inverse relationship.

The overall averages of various chemical constituents of the gonads of *Portunus pelagicus* for the entire year are given in Tables 9 and 12. It may be noted that the ovary is distinctly richer than the testis in non-protein nitrogen, fat and glycogen,

Table 13. *Portunus pelagicus. Carapace-width range, range and mean water content of testis and gonad index of males (1964/1965)*

Year	Month	Carapace-width range (mm)	Range of water content (%)	Mean (%)	Gonad index
1964	February	68.0—132.0	48.9—88.0	61.4	0.30
	March	90.0—145.0	35.1—70.1	55.2	0.18
	April	73.5—123.5	40.9—68.9	60.3	0.24
	May	109.0—134.0	45.7—70.2	58.8	0.23
	June	115.5—155.0	54.1—77.0	65.8	0.47
	July	99.0—138.0	50.0—83.0	64.3	0.41
	August	107.0—157.0	58.9—68.2	63.1	0.29
	September	111.0—135.0	50.6—71.6	64.0	0.32
	October	125.0—144.0	61.2—82.0	69.7	0.43
	November	129.0—130.0	74.5—76.4	75.7	0.98
	December	—	—	—	—
1965	January	103.0—132.0	64.0—72.4	67.8	0.47

Table 14. *Portunus pelagicus. Biochemical composition of gonads of females and males during different stages of gonadal maturity; see Table 3*

Sex and condition	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
Females (spent or unripe)	0.08	76.0	10.3	2.12	8.17	51.08	4.72	2.25
	0.19	72.8	9.68	2.60	7.08	44.23	5.04	1.9
	0.32	72.5	10.64	2.65	7.98	49.9	5.19	2.52
	0.77	75.3	10.58	2.98	7.6	47.5	4.47	3.69
Average	0.34	74.2	10.3	2.59	7.71	48.18	4.86	2.59
Females (ripe)	4.72	53.8	10.4	1.93	8.46	52.89	7.73	3.88
	7.24	50.8	9.18	2.21	6.97	43.57	11.26	1.99
	8.05	48.5	12.01	2.89	9.12	56.98	13.57	2.82
	9.65	43.0	11.07	2.10	8.97	55.06	10.64	4.6
Average	7.42	49.0	10.67	2.28	8.38	52.13	10.8	3.32
Males (spent)	0.04	73.6	10.43	2.24	8.19	51.19	4.45	2.88
	0.25	72.9	9.00	1.57	7.44	46.51	6.28	2.22
	0.31	73.2	10.91	2.70	8.21	51.28	5.64	3.6
	0.40	70.2	7.8	1.59	6.21	38.78	7.71	1.28
Average	0.25	72.0	9.54	2.02	7.51	46.94	6.02	2.5
Males (ripe)	0.51	71.8	10.84	1.35	9.49	59.31	5.96	1.37
	0.70	63.9	10.92	2.16	8.76	54.74	4.49	2.78
	0.83	67.9	10.61	1.28	9.34	58.34	6.59	4.69
	1.03	74.9	11.62	2.19	9.42	58.9	5.13	1.99
Average	0.77	70.9	10.9	1.75	9.25	57.82	5.54	2.71

whereas the testis is richer than the ovary in protein content.

The data on the biochemical composition of the gonads of low and high gonad indices are compared in Table 14 and Fig. 14. Thus, the differences in the chemical constitution of gonads during the course of the reproductive cycle are most clearly illustrated. From the data, it is evident that the water content of the ovary is inversely proportional to the gonad index values; the higher the gonad index, the lower the water content. The range in average water content between ripe and spent ovaries is fairly wide, about 25%. Although the non-protein nitrogen values do not fluctuate significantly during growth of the ovary, the values are comparatively lower in the ripe ovary than either in the spent or in the unripe ovary. On an average, protein, lipid and glycogen show a greater increase in the ripe ovary than in the unripe or spent ovary.

In the testis, the fluctuation of water content is not so marked as that in the ovary. The non-protein nitrogen values also show a decline in per cent weight as the gonad index of the testis increases. While the protein values show a distinct increase, glycogen values show only a slight increase in the ripe testis. The lipid content of the testis tends to show a decline as it ripens.

It is, therefore, evident from the data that some constituents seem to be synthesised more rapidly in particular phases, probably depending on the physiological state of the individual and the rate at which biochemical synthesis takes places.

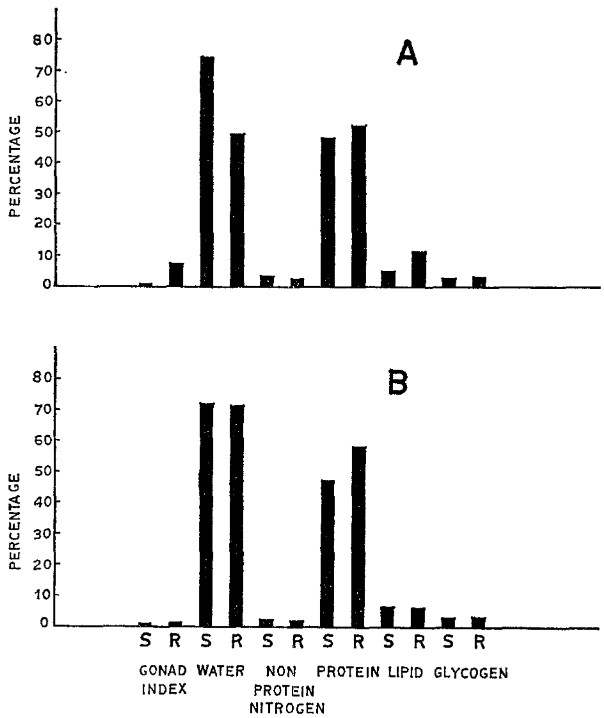

Fig. 14. *Portunus pelagicus.* Histograms illustrating biochemical composition of ovary and testis during different stages of gonadal maturity. (Values are averages of 3 to 4 determinations in each stage. Water content: percentage wet weight; other constituents: percentage dry weight). (A) Ovary; (B) testis. R: ripe or ripening; S: spent or resting

Table 15. *Portunus pelagicus. Biochemical composition of ovary, muscle, and liver during different months of year 1964/1965; see Table 3*

Year	Month	Gonad index	Tissue	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1964	February	3.93	Ovary	9.72	2.10	7.62	47.64	11.58	3.12
			Muscle	11.68	3.98	7.7	48.11	3.62	0.53
			Liver	7.44	4.43	3.1	19.37	19.06	1.87
		0.55	Ovary	8.21	2.89	5.32	33.26	3.82	3.18
			Muscle	12.98	4.22	8.76	54.78	2.08	0.34
			Liver	6.31	3.26	3.05	19.04	39.22	2.89
	March	7.41	Ovary	9.18	2.21	6.97	43.57	15.0	2.53
			Muscle	11.08	3.52	7.56	47.25	2.80	0.43
			Liver	7.53	4.0	3.53	22.03	16.66	2.15
		0.51	Ovary	8.27	3.64	4.63	28.93	4.78	2.58
			Muscle	12.82	4.31	8.51	53.19	1.62	0.32
			Liver	7.31	4.34	2.97	18.58	27.92	5.08
	April	0.73	Ovary	9.91	2.49	7.43	46.41	8.75	3.03
			Muscle	10.51	3.81	6.70	41.88	2.07	0.22
			Liver	6.18	3.28	2.9	18.59	15.59	3.85
		0.19	Ovary	9.68	2.60	7.08	44.23	5.31	1.9
			Muscle	11.07	3.08	7.99	49.93	1.93	0.62
			Liver	7.09	3.73	3.36	20.99	16.60	5.28
	May	1.01	Ovary	10.44	1.76	8.67	54.21	9.45	3.01
			Muscle	11.07	4.16	6.91	43.16	2.53	0.43
			Liver	7.21	4.21	3.00	18.77	20.82	3.27
		0.48	Ovary	9.42	3.02	6.4	40.06	6.21	1.82
			Muscle	12.33	4.04	8.29	51.83	2.27	0.43
			Liver	6.26	2.72	3.54	22.13	21.38	3.89
	June	2.15	Ovary	9.43	2.24	7.19	44.93	12.78	1.05
			Muscle	10.39	3.62	6.77	42.32	1.01	0.65
			Liver	7.21	3.53	3.77	23.56	25.36	4.43
		0.22	Ovary	9.42	4.38	5.12	31.98	5.80	1.15
			Muscle	11.07	4.32	6.75	42.21	2.08	0.56
			Liver	6.62	3.28	3.34	20.86	22.43	3.61
	July	1.89	Ovary	9.34	1.80	7.54	47.1	13.81	1.46
			Muscle	11.79	3.63	8.17	51.03	2.08	0.63
			Liver	5.08	3.30	2.78	17.36	21.14	5.34
		0.32	Ovary	10.64	2.65	7.98	49.9	5.19	2.52
			Muscle	12.91	4.59	8.32	51.99	1.39	0.65
			Liver	6.63	4.01	2.62	16.39	20.36	5.89
	August	5.34	Ovary	10.64	1.15	9.33	58.29	13.49	2.62
			Muscle	12.30	4.63	7.68	47.98	3.83	0.24
			Liver	6.22	3.08	3.14	19.62	25.17	3.39
		0.77	Ovary	10.58	2.98	7.6	47.5	4.47	3.69
			Muscle	11.51	4.72	6.79	42.45	2.33	0.18
			Liver	5.61	2.36	3.25	20.28	26.92	4.62
	September	2.66	Ovary	11.06	1.55	9.51	59.43	12.64	2.52
			Muscle	12.36	4.75	7.61	47.56	1.73	0.32
			Liver	6.07	3.08	2.99	18.71	20.90	3.28
		0.45	Ovary	9.41	3.99	5.42	33.84	7.67	1.23
			Muscle	11.19	4.40	6.79	42.41	2.07	0.26
			Liver	6.73	3.72	3.01	18.84	22.11	5.07
	October	1.97	Ovary	9.11	2.16	6.95	43.44	12.72	2.57
			Muscle	12.79	3.07	9.72	60.73	1.39	0.58
			Liver	5.37	2.28	3.09	19.32	25.89	3.21
		0.33	Ovary	8.20	4.13	4.07	25.43	8.19	3.27
			Muscle	11.63	3.53	8.1	50.61	3.16	0.45
			Liver	5.76	2.7	3.06	19.1	30.21	3.81
	November	1.73	Ovary	9.91	2.72	7.18	44.9	9.94	2.24
			Muscle	11.31	4.89	6.42	40.12	2.61	0.43
			Liver	6.26	3.07	3.19	19.2	15.98	5.83
		0.59	Ovary	11.38	3.40	7.98	49.86	5.16	1.84
			Muscle	12.09	4.31	7.78	48.6	5.00	0.30
			Liver	5.27	2.68	2.59	16.18	23.29	1.11

Table 15 (Continued)

Year	Month	Gonad index	Tissue	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
	December	7.24	Ovary	9.18	2.21	6.97	43.57	14.0	1.99
			Muscle	12.46	3.21	9.25	57.79	6.15	0.44
			Liver	9.44	3.69	5.75	35.95	18.33	3.13
		0.65	Ovary	9.62	4.41	5.21	32.56	4.55	1.43
			Muscle	11.89	4.07	7.82	48.9	2.82	0.22
			Liver	7.44	3.95	3.49	21.8	38.0	4.15
1965	January	9.69	Ovary	10.45	1.76	8.69	54.3	11.89	2.02
			Muscle	12.88	4.01	8.87	55.45	1.36	0.30
			Liver	5.79	2.63	3.17	19.8	20.84	4.28
		0.67	Ovary	9.61	3.22	6.39	39.91	5.06	1.98
			Muscle	12.14	3.47	8.68	54.23	5.11	0.69
			Liver	6.58	3.48	3.10	19.38	31.75	3.6

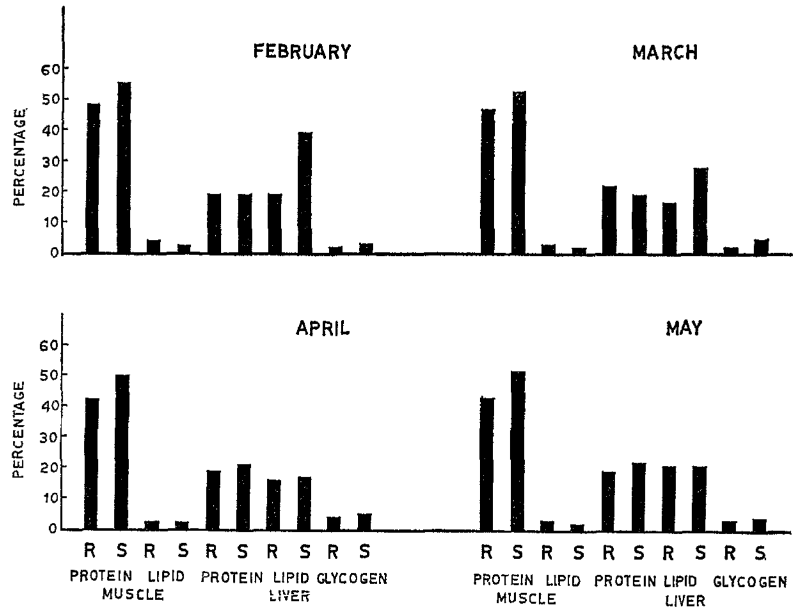

Fig. 15. *Portunus pelagicus.* Histograms illustrating biochemical composition of muscle and liver during different months. (Values expressed as percentage dry weight). R: ripe or ripening; S: spent or resting

Changes in the Biochemical Composition of Muscle and Liver During the Reproductive Cycle

While the testes do not apparently show any wide fluctuations in biochemical constituents relative to the reproductive cycle, the ovaries give evidence of fluctuations with regard to their total content of lipid and protein. However, glycogen shows no noticeable pattern of fluctuation. In certain instances, protein is found to occur in greater quantities in the unripe ovary, or almost the same quantities are found in both ripe and unripe ovaries in terms of unit dry weight of the gonad, and this may be due to individual differences in the rate of biochemical synthesis in the ovary as indicated above. With a view to examining whether there is any prior storage of organic materials in some organs of the body and a subsequent transfer to gonadal synthetic centres at the time of gonad maturation, the liver (hepatopancreas) and the muscles from the posterior region of the cephalothorax of *Portunus pelagicus* were examined with reference to their biochemical constituents during the different months of the year.

Such an investigation in *Portunus pelagicus* indicates that the muscle contains large quantities of protein. In the hepatopancreas, while the protein content is low, the lipid content is considerably higher than that of the muscle or the gonad. From Table 15 and Figs. 15—17 it is evident that there is no direct correlation between the fluctuation of the protein content of the ovary and that of muscles in the

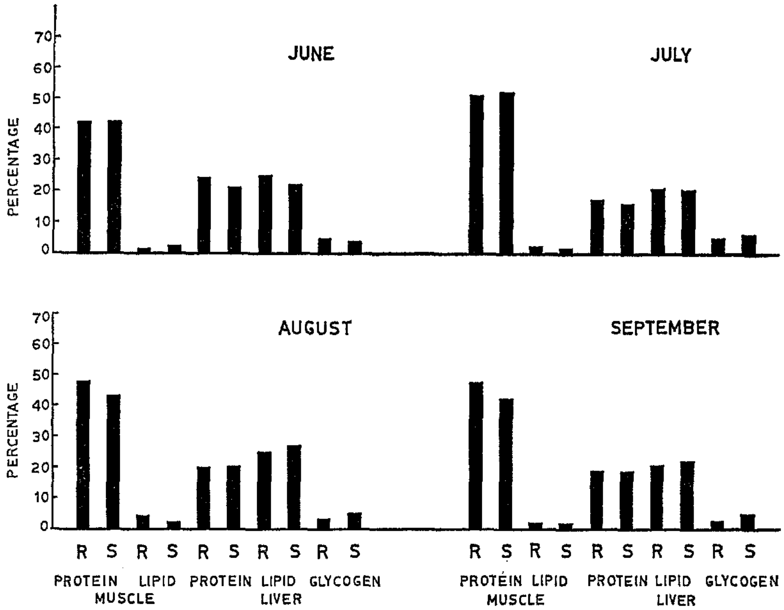

Fig. 16. *Portunus pelagicus*. Histograms illustrating biochemical composition of muscle and liver during different months. (Values expressed as percentage dry weight). R: ripe or ripening; S: spent or resting

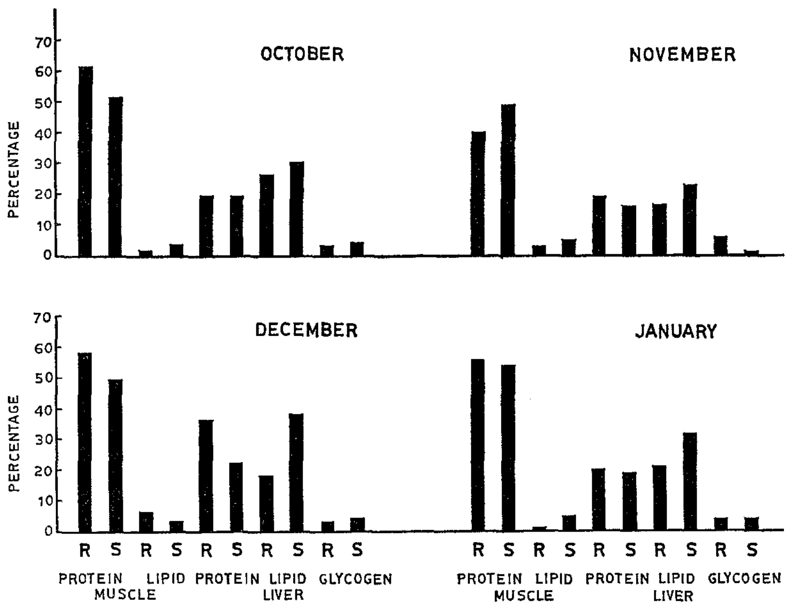

Fig. 17. *Portunus pelagicus*. Histograms illustrating biochemical composition of muscle and liver during different months. (Values expressed as percentage dry weight). R: ripe or ripening; S: spent or resting

different months. Nevertheless, during the period February to May and in November, the muscles of females with ripe ovaries show significantly less protein than that of unripe or spent females. During the months of June and July, there is no difference in the protein values of the muscles of ripening and unripe individuals, whereas during the period August to January, with the exception of November, the muscles of ripe females contain more protein. Hence, when the food supply is inadequate or when successive broods are produced, the depletion in the protein content of the muscle of crabs with ripening ovaries may be due to a transfer of this material from the muscle to the ovary. This may be the condition during the period February to May and in November. As stated above, lipid is present in the muscles only in small quantities, and storage of this material in the muscle for subsequent utilisation by the gonad seems to be only a remote possibility.

The hepatopancreas is known to be a storage organ in decapod crustaceans, and it is evident that considerable quantities of lipid are stored in this organ. The lipid content shows wide variations in individuals with ripe and unripe ovaries in certain months. From Table 15 and Figs. 15—17 it can be seen that, during the period December to March, there is a marked decrease in the values of lipid in the hepatopancreas of females with ripening ovaries. This period obviously includes the peak breeding season and, therefore, the females may require more organic materials than are available directly from the food supply for reproductive activity. At this time, the much needed lipid for developing ova might be mobilised from the hepatopancreas, which stores considerable quantities of this material. In other months, the reproductive activities are not intense, and the available food may probably be sufficient to meet the requirements of gonadal activity. This is evident from the fact that, during the months of June and July, the ripening individuals have more lipid in the liver than unripe specimens. During May, and in the period August to November, the ripening females have comparatively less lipid content in the liver than the unripe females, but this difference is not very significant.

It seems that some glycogen is also stored in the hepatopancreas. Glycogen values are higher in the liver when compared to those in either the muscles or the gonads. In certain months, such as January, June and November, there is more hepatopancreas-glycogen in ripening individuals, whereas in all other months glycogen values are low in the hepatopancreas of ripening individuals. Since glycogen is only a minor constituent of the ovary, as seen from the present study, there is apparently no need for any large-scale mobilisation of this constituent into the ovary from reserves during the reproductive cycle.

In conclusion, there is an indication of possible transfer of organic substances such as protein from the muscles, and lipid from the hepatopancreas, at least during the peak periods of breeding activity. This is due to the fact that the production of successive broods in the peak breeding season may require large amounts of organic substances. Since glycogen is a minor constituent of the gonad, this substance does not apparently provide an indication of any mobilisation from reserves at a significant rate, even during the height of the breeding season.

From the data presented in Table 14 and Fig. 14, it may be seen that, in *Portunus pelagicus*, there is little variation in the productivity of organic materials in the gonads on the basis of per cent content, whereas the actual state of affairs seems to be different. This may be due to the fact that some materials are synthesised more rapidly than others at a particular stage. It has also been observed that the water content of the ovary declines to some extent along with a general increase in the total mass of organic substances in the ovary. With a view to ascertaining the organic productivity of the gonad, the increase in the total mass of these organs as well as the total quantity of organic substances produced therein from a spent to a fully gravid gonad has been examined. The increase of materials during the growth of the gonad is assessed from 3 different angles as performed by Giese *et al.* (1958) in the purple sea urchin *Strongylocentrotus purpuratus*: (1) by dividing the gonad index of a gravid individual by that of a spent specimen of the same size; (2)by dividing the wet weight of the gonad of a ripe individual by the wet weight of the spent gonad; (3) by dividing the dry weight of the gravid gonad by that of the spent gonad, thereby calculating the increase in dry mass. Similarly, the amount of

Table 16. *Portunus pelagicus. Increase in organic constituents of gonads during growth from shrunken state to maximal size (in mg; total weight in g)*

Status of gonad	Female		Relative increase	Male		Relative increase
	Spent	Ripe		Spent	Ripe	
Gonad index	0.081	8.049	99.4	0.041	1.027	25
Wet weight (g)	0.061	6.7	109.8	0.0464	1.0176	21.9
Dry weight (g)	0.029	3.698	127.5	0.0254	0.3486	13.7
Total nitrogen (mg)	2.9	444.0	153.1	2.65	40.5	15.2
Non-protein nitrogen (mg)	0.6	107.0	178.3	0.57	7.64	13.4
Protein nitrogen (mg)	2.3	337.0	146.5	2.08	32.84	15.8
Protein (mg)	14.8	2100.0	141.8	13.0	205.3	15.8
Lipid (mg)	1.3	501.0	385.3	1.13	17.87	15.7
Glycogen (mg)	0.6	100.0	166.6	0.73	6.92	9.4

biochemical constituents in the spent and gravid individuals could also be calculated by multiplying the weight of the gonad in grams by the per cent content of each of the constituents. The content of each constituent in the gravid gonad is divided by the content of that constituent in the spent gonad of the animal, and the value thus obtained has been taken as the relative increase in mass of the constituent in question during the growth of the gonad from the spent to the gravid state. Such data for *P. pelagicus* are given in Table 16.

From Table 16 it will be seen that while the gonad index of female *Portunus pelagicus* increases 99.4 times, the wet weight of the ovary increases by about 109.8 times, the dry weight 127.5 times, total nitrogen in the ovary 153.1 times, non-protein nitrogen 178.3 times, protein 141.8 times, lipid 385.3 times and glycogen 166.6 times. It may also be noted that the lipid content of the ovary increases much more than all other constituents.

In male *Portunus pelagicus*, the gonad index of the testis increases about 25 times, whereas the wet weight of testis increases 21.9 times, dry weight 13.7 times, total nitrogen 15.2 times, non-protein nitrogen 13.4 times, protein 15.8 times, lipid 15.7 times and glycogen 9.4 times. In the testis, the relative increase of non-protein nitrogen, protein and lipid is more evident than that of glycogen.

Metapenaeus affinis

Changes in the Water Content of the Body

The average values of water content of the body of female and male *Metapenaeus affinis* for different months are presented in Table 17 and Fig. 18. In the females, in all the months except April, the values are above 70%, and comparatively lower values occur during the breeding months, i.e., from November to April; in the pre-breeding period, however, there is apparently considerable accumulation of water. This is in contrast to the condition noticed in female *Uca annulipes*, where the maximum water content occurs in the months with breeding activities. The exact reason for this apparent disparity in the water content of the body of the females of these two species during the breeding season is not known. It is probable that the habitat of these species may be of some significance in this context. In contrast to the condition in male *U. annulipes*, which has a uniform value of water content, male *M. affinis* show fluctuation in the water content. This may be due to the presence of a thick

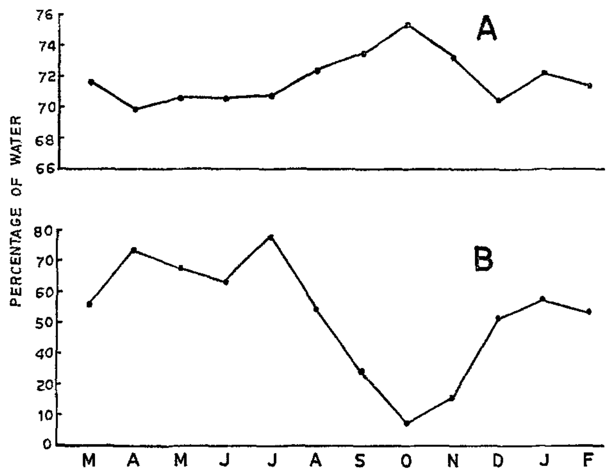

Fig. 18. *Metapenaeus affinis*. Fluctuations in water content of entire body from March, 1964 to February, 1965. Fluctuations in (A) water content of females (each point represents average for month); (B) water content of males (each point represents average for month)

Table 17. *Metapenaeus affinis. Length range and mean water content of entire animals, both males and females (1964/1965)*

Year	Month	Length range (mm)		Water content (%)	
		Males	Females	Males	Females
1964	March	126—129	125—170	71.1	71.6
	April	128—135	119—158	74.7	69.8
	May	115—135	121—162	73.6	70.6
	June	105—117	130—159	72.7	70.6
	July	119—162	120—171	75.6	70.7
	August	115—135	132—156	70.9	72.4
	September	142—126	118—145	66.9	73.6
	October	116—130	119—170	63.5	75.4
	November	117—131	125—171	65.1	73.3
	December	116—129	128—156	70.4	70.5
1965	January	120—145	118—171	71.6	72.3
	February	130—144	116—164	70.7	71.4

exoskeleton in the former species or due to the purely aquatic habitat of the latter species.

Changes in the Biochemical Composition of the Gonads During the Reproductive Cycle

Data on the determination of biochemical constituents such as water, non-protein nitrogen, protein, lipid and glycogen in the ovary of *Metapenaeus affinis* for the different months of the year are presented in Tables 18, 19 and 20 and Figs. 19 and 20. It is evident that, in the females of this species, as in *Portunus pelagicus*, an inverse relationship apparently exists between the gonad index and water content — the higher the gonad index, the lower the water content. This fact is clear from the data in Table 19, wherein the water content for ripe and for spent ovaries are treated separately. It will be seen that, as in *P. pelagicus*, the ripe ovaries of *M. affinis* contain less water than the spent ovary.

In the ovaries of *Metapenaeus affinis*, the protein and lipid contents are comparatively higher than those in the other two species studied, and these two materials show well defined fluctuations in relation to the gonadal cycle. Lipid and protein contents of the ovaries are maximum during the peak period of gonadal activity, and minimum during the non-reproductive period. Glycogen does not show significant fluctuations in relation to the breeding cycle; however, a slight increase of this constituent is seen during the non-breeding period. The non-protein nitrogen shows a general trend towards a decrease as the ovary matures. An inverse relationship apparently exists between the lipid content and the water content of the ovary.

The data for the determination of biochemical components such as water content, non-protein nitro-

Table 18. *Metapenaeus affinis. Changes in biochemical composition of ovary (1964/1965); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1964	March	3.07	67.0	10.79	4.03	6.76	43.21	13.89	1.49
	April	4.62	59.3	10.65	5.08	5.57	34.84	14.06	1.89
	May	2.89	63.2	10.38	3.73	6.63	41.42	10.92	1.63
	June	1.84	69.8	9.85	3.57	6.28	39.26	8.19	1.71
	July	1.3	70.8	10.66	4.23	6.42	40.14	13.58	1.09
	August	2.84	68.0	10.6	3.79	6.76	41.17	10.80	1.44
	September	2.86	63.5	10.89	3.84	7.04	44.32	11.47	1.57
	October	4.49	69.8	10.47	3.34	7.15	44.95	12.71	1.37
	November	4.15	66.9	10.51	3.84	6.56	41.88	13.43	1.41
	December	11.73	60.2	11.08	3.66	7.43	46.28	17.91	1.51
1965	January	5.67	63.5	10.35	4.05	6.20	38.76	14.83	1.33
	February	10.19	57.9	10.65	3.68	7.12	44.89	15.97	1.82
Average			59.4		3.83		41.7	13.14	1.52

Table 19. *Metapenaeus affinis. Water content of ovary (1964/1965)*

Year	Month	Gonad index	Average water content of ovary (%)		
			All stages	Ripe and ripening	Unripe and spent
1964	March	3.07	67.0	64.3	71.2
	April	4.62	59.3	56.7	61.4
	May	2.89	63.2	59.6	64.8
	June	1.84	69.8	67.6	70.7
	July	1.3	70.8	66.9	70.1
	August	2.84	68.0	65.1	68.8
	September	2.86	63.5	56.5	71.4
	October	4.49	69.8	65.9	70.6
	November	4.15	66.9	61.8	70.2
	December	11.73	60.2	58.4	61.3
1965	January	5.67	63.5	60.9	66.1
	February	10.19	57.9	55.1	59.8

Table 20. *Metapenaeus affinis. Length-range of prawn, range and mean of water content of ovary and gonad index of females (1964/1965)*

Year	Month	Length-range (mm)	Range of water content (%)	Mean (%)	Gonad index
1964	March	120.0—150.0	63.3—74.0	67.0	3.07
	April	107.0—149.0	47.6—65.7	59.3	4.62
	May	108.0—131.5	54.1—73.1	63.2	2.89
	June	118.0—131.5	63.4—74.0	69.8	1.84
	July	112.5—171.0	61.5—83.4	70.8	1.3
	August	130.5—170.0	65.1—71.9	68.0	2.84
	September	128.0—142.0	45.2—75.5	63.5	2.86
	October	119.0—149.0	64.7—74.2	69.8	4.49
	November	133.0—157.0	54.4—73.8	66.9	4.15
	December	135.0—163.0	58.6—65.3	60.2	11.73
1965	January	140.0—169.0	60.0—68.9	63.5	5.67
	February	130.0—171.0	53.2—65.5	57.9	1.190

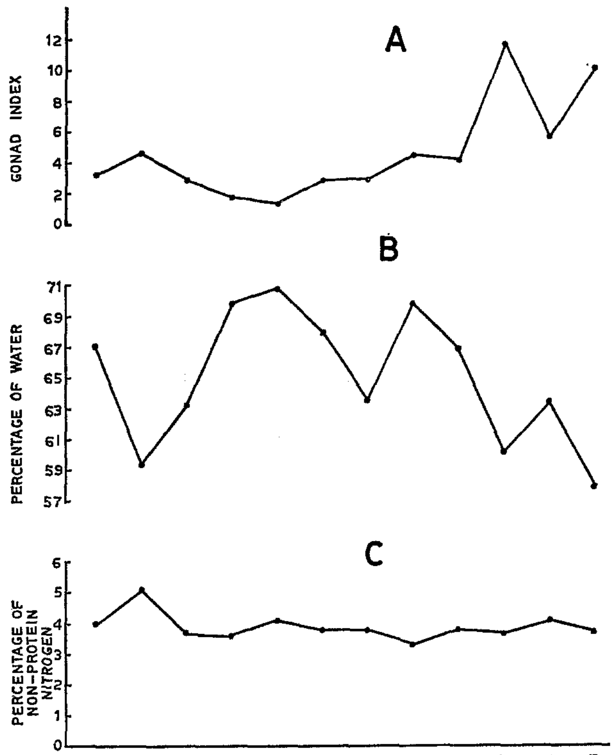

Fig. 19. *Metapenaeus affinis.* Changes in gonad index and various biochemical constituents of ovary from March, 1964 to February, 1965. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

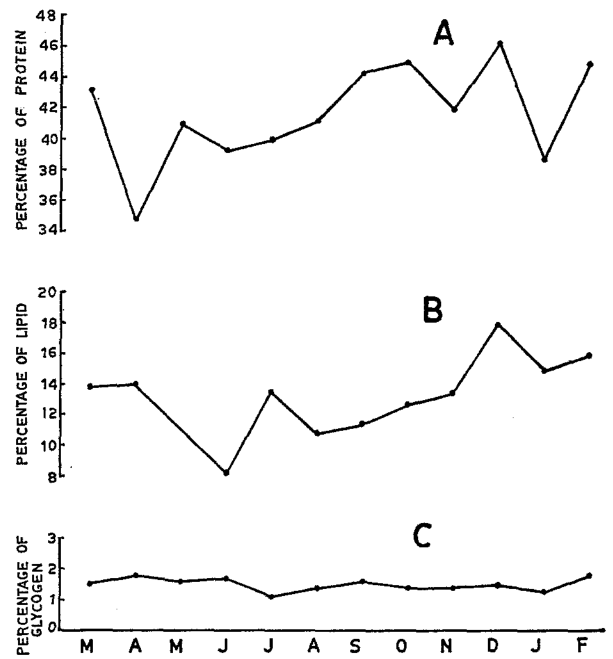

Fig. 20. *Metapenaeus affinis.* Changes in various biochemical constituents of ovary (percentage of dry weight) from March, 1964 to February, 1965. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

gen, protein, lipid and glycogen of the testis of *Metapenaeus affinis* are presented in Tables 21 and 22 and Figs. 21 and 22. In the testis, the water content does not fluctuate monthly with the growth of the testis. In the testis, it is seen that, during certain months, the values of non-protein nitrogen show an increase with the increase of the weight of the testis. The protein values also show definite increase as the gonad matures. The lipid content of the testis does not seem to give an indication of a similar fluctuation. Similarly, correlation between the water and lipid contents of the testis is lacking. In contrast to the condition

Table 21. *Metapenaeus affinis. Changes in biochemical composition of testis (1964/1965); see Table 3*

Year	Month	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1964	March	0.94	63.7	10.76	4.95	5.82	35.87	8.96	2.65
	April	0.96	63.9	11.46	3.39	8.07	50.44	6.63	3.43
	May	0.93	62.7	9.31	1.94	7.41	46.28	2.60	2.25
	June	0.56	71.0	9.35	3.03	6.32	39.53	5.05	2.42
	July	0.96	66.5	9.81	1.87	7.94	49.27	2.78	1.74
	August	0.76	66.5	12.34	3.02	9.32	58.26	7.42	1.83
	September	0.88	68.8	10.56	3.02	7.54	47.13	5.92	2.36
	October	1.11	66.7	11.12	2.86	8.26	51.25	8.72	2.71
	November	1.05	65.8	11.64	4.15	7.49	46.82	2.86	3.99
	December	1.09	66.6	12.15	4.18	7.97	49.84	3.01	3.81
1965	January	0.99	65.0	12.21	3.97	8.24	38.16	7.01	4.52
	February	0.86	62.7	12.20	4.42	7.78	47.12	9.46	3.49
Average			64.1		3.4		46.6	5.86	2.93

Table 22. *Metapenaeus affinis. Length range of prawn, range and mean of water content of testis and gonad index of males (1964/1965)*

Year	Month	Length range (mm)	Range of water content (%)	Mean (%)	Gonad index
1964	March	122.5—135.0	50.9—70.4	63.7	0.94
	April	115.0—140.0	59.8—69.6	63.9	0.96
	May	100.0—133.0	56.3—67.7	62.7	0.93
	June	105.0—147.0	60.0—77.4	71.0	0.56
	July	118.0—165.0	63.6—69.7	66.5	0.96
	August	110.0—130.5	63.4—71.4	66.5	0.76
	September	114.5—129.5	64.5—73.5	68.8	0.88
	October	115.0—127.5	57.9—73.6	66.7	1.11
	November	124.0—140.0	63.1—74.7	65.8	1.05
	December	131.0—138.0	60.6—64.4	66.6	1.09
1965	January	119.0—141.0	53.2—85.7	65.0	0.99
	February	131.0—142.0	52.4—68.9	62.7	0.86

noticed in the ovary, the glycogen content of the testis shows increase in months with high gonad activity. The overall averages of various biochemical constituents of the gonads of *M. affinis* for the entire year are given in Tables 18 and 21. It can be seen that, while the ovary is distinctly richer than the testis in non-protein nitrogen and lipid, the testis is richer in water content, protein and glycogen content.

Data on the biochemical composition of the gonads of individuals of low and high gonad indices are compared in Table 23 and Fig. 23. Thus, the variations in the chemical constituents of gonads during the course of the reproductive cycle are most clearly demonstrated. From the data, it is clear that, as in *Portunus pelagicus*, the water content of the ovary in *Metapenaeus affinis* has an inverse relationship with the gonad index — the higher the gonad index, the lower the water content. The range in water content from the spent to the ripe condition is about 10%. Non-protein nitrogen values, on an average, tend to be slightly less in the ripe ovary than at other times, and this constituent does not give indications of wide fluctuations during the different maturity stages of the ovary. The fluctuation of the lipid content is considerably more than that in the protein and glycogen content of the ovary.

The water content of the testis does not show any relationship with an increase in gonad index, and the variations are not significant. The spent and unripe testes contain more non-protein nitrogen. On an average, the glycogen and non-protein nitrogen values in the ripe testes are lower than those in the unripe testes, while the protein and lipid values in the ripe testes apparently show an increase over the unripe testes. As in *Portunus pelagicus*, in *Metapenaeus affinis* also, these organic substances are not laid systematically in the various stages of gonadal development, some being synthesised more rapidly at a particular stage, depending upon the physiological state of the individual and the rate at which biochemical synthesis takes place in the gonads.

From an analysis of the biochemical composition in the gonads of individuals of high and low gonad indices of *Portunus pelagicus* and *Metapenaeus affinis*, it is clear that the water content of the ovary shows, in general, an inverse relationship with the gonad index, but the testes do not indicate a similar relationship. The non-protein nitrogen in the ovary and in the testis in both species shows a decrease in value as the gonad matures, but this is not always the case. On the other hand, the protein content increases as the ovary and testis mature in both species. The lipid increases with maturity of the ovary in both species, and also in the testis of *M. affinis*, while its level declines with maturity of the testis in *P. pelagicus*. Glycogen shows at least some increase with maturity of the ovary in both species, and the testis of *P. pelagicus*; it decreases in the testis of *M. affinis*. These data suggest that there is apparently no similarity in the accumulation of organic materials in the gonads of different species and even in the males and females of the same species. Thus, some difference between the annual cycle and the individual reproductive cycle may be seen in respect to fluctuation of the biochemical composition. The annual cycle alone has been considered to be important, and the individual cycles merely emphasise the fact that the biochemical composition of the gonads is individualistic.

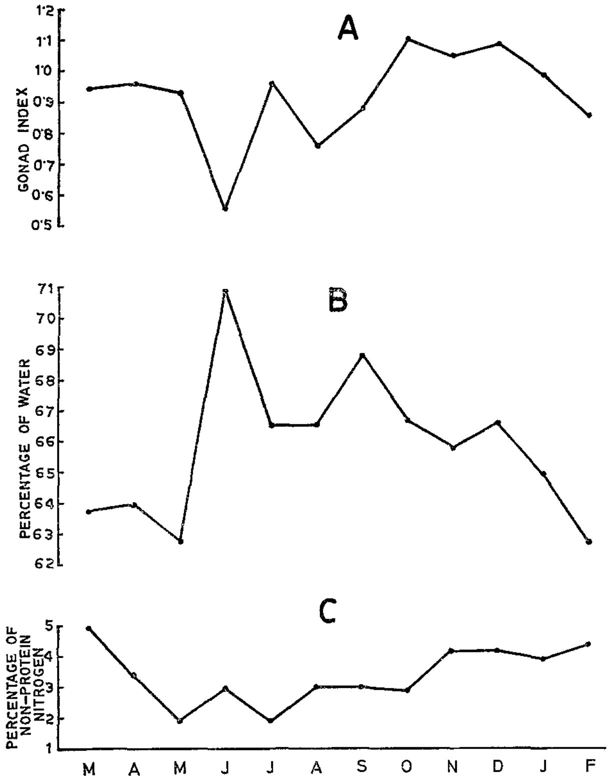

Fig. 21. *Metapenaeus affinis*. Changes in gonad index and various biochemical constituents of testis from March, 1964 to February, 1965. Each point represents average for month. Fluctuations in (A) gonad index; (B) water content (percentage wet weight); (C) non-protein nitrogen content (percentage dry weight)

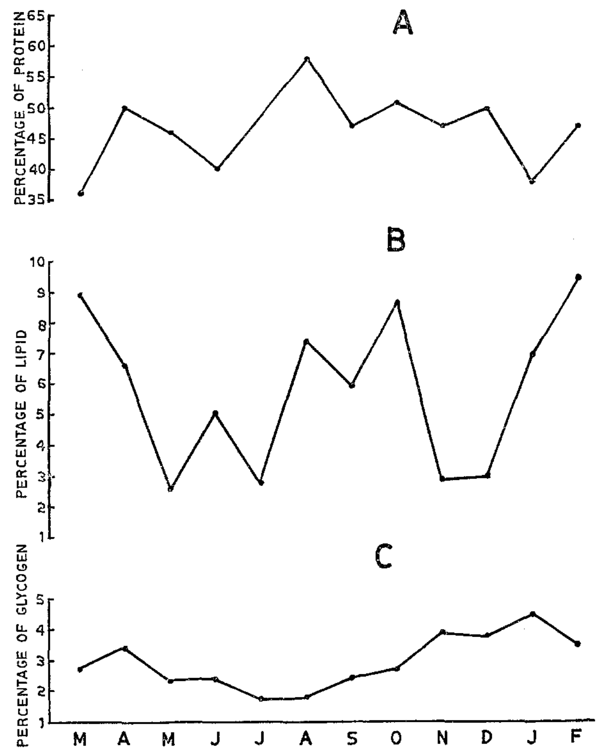

Fig. 22. *Metapenaeus affinis*. Changes in various biochemical constituents of testis (percentage dry weight) from March, 1964 to February, 1965. Each point represents average for month. Fluctuations in (A) protein content; (B) lipid content; (C) glycogen content

Changes in the Biochemical Composition of Muscle and Liver During the Reproductive Cycle

As in *Portunus pelagicus*, the testis of *Metapenaeus affinis* also shows no marked fluctuations in biochemical components during the reproductive cycle. However, females do display variations in the total lipid and protein content of the ovary. It has been observed that the chemical composition of the internal organs of *M. affinis* exhibiting identical gonad indices shows differences. With a view to examining whether there is any indication of storage of organic

Table 23. *Metapenaeus affinis. Biochemical composition of gonad of females and males during different stages of gonadal maturity; see Table 3*

Sex and condition	Gonad index	Water content	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
Females (spent or unripe)	0.8	69.6	10.08	4.49	5.59	34.91	9.71	1.44
	1.21	75.5	11.5	4.81	6.68	41.72	10.21	1.42
	1.38	73.7	9.65	3.66	5.99	37.43	11.20	0.39
	1.87	69.3	10.11	4.14	5.96	37.25	10.69	1.34
Average	1.31	71.9	10.33	4.28	6.01	37.83	10.46	1.14
Females (ripe)	7.01	65.1	10.38	3.62	6.77	42.26	14.85	0.95
	9.57	65.2	10.16	3.57	6.6	41.23	17.49	1.2
	11.44	54.9	10.51	4.83	5.67	35.46	21.03	1.45
	13.21	61.6	10.17	4.28	5.9	36.84	16.10	1.57
Average	10.3	61.9	10.3	4.07	6.25	38.95	17.39	1.29
Males (spent)	0.4	73.5	8.34	2.36	6.08	37.97	2.33	2.64
	0.70	65.7	12.59	5.00	7.59	47.41	2.62	3.77
	0.78	69.7	11.25	5.52	5.73	35.79	9.03	3.25
Average	0.63	69.6	10.73	4.4	6.46	40.39	4.66	3.22
Males (ripe)	1.03	62.6	9.85	2.33	7.52	46.98	4.35	1.48
	1.14	70.4	11.15	5.41	5.74	35.85	9.95	4.16
	1.37	69.6	10.58	3.04	7.54	47.13	7.92	2.36
	1.79	73.6	11.56	1.87	9.69	59.47	8.14	3.06
Average	1.08	69.2	10.79	3.18	7.62	47.35	7.59	2.77

materials prior to gonad maturation, and a subsequent transfer of these materials to the sites of gametogenesis, the hepatopancreas, which is known to be a storage organ, and the muscle from a given part of the body, i.e., from the first abdominal segment, have been analysed for their various biochemical constituents throughout all the months of the year.

In *Metapenaeus affinis*, the muscles are rich in protein and poor in fat and glycogen. The hepatopancreas is rich in fat, whereas its protein content is comparatively low, and the glycogen content is higher than that of the muscles or gonad (Table 24 and Figs. 24—26). The lipid content of the liver shows marked fluctuation in individuals at different stages of gonad maturity during certain months. From Table 24 and Figs. 24—26, it is evident that, during the period September to January, with the exception of December (which includes breeding months as well), there is less fat in the liver of ripe individuals than in unripe specimens. It may also be noted that in April, which represents a minor peak in the breeding activity, a low value of lipid in the hepatopancreas of ripe individuals also occurs. The increased value of hepatopancreas-lipid in ripe individuals in May and August is suggestive of an accumulation of this substance during these months. This indicates that fat is an important organic material in *M. affinis*, and it is inferred that there is a certain degree of relationship between the hepatopancreas lipid and lipid content of the ovary. This is more evident during the breeding season. When gonad activation is rapid and succes-

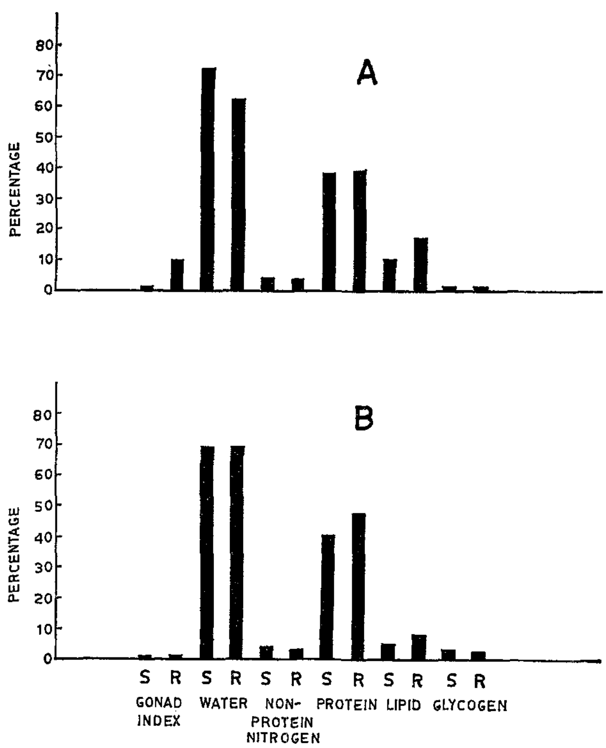

Fig. 23. *Metapenaeus affinis*. Histograms illustrating biochemical composition of ovary and testis during different stages of gonadal maturity. (Values are averages of 3 to 4 determinations in each stage. Water content: percentage wet weight; other constituents: percentage dry weight). (A) ovary; (B) testis. R: ripe or ripening; S: spent or resting

Table 24. *Metapenaeus affinis. Biochemical composition of ovary, muscle, and liver during different months of year 1964/1965; see Table 3*

Year	Month	Gonad index	Tissue	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
1964	March	6.49	Ovary	10.38	4.81	5.57	38.83	11.63	1.93
			Muscle	13.7	3.00	10.7	66.84	3.36	0.23
			Liver	8.24	4.87	3.37	21.04	13.3	4.31
		1.32	Ovary	10.71	4.54	6.17	32.28	10.34	1.61
			Muscle	12.26	3.71	8.55	53.43	1.32	0.62
			Liver	7.31	3.66	2.65	16.57	15.08	3.16
	April	9.77	Ovary	9.23	3.62	6.61	41.3	17.46	1.63
			Muscle	13.31	4.67	8.64	53.99	4.31	0.59
			Liver	8.05	5.79	2.26	13.13	31.85	3.41
		1.87	Ovary	10.11	4.14	5.96	37.25	10.69	1.34
			Muscle	13.33	4.61	8.72	54.51	2.52	0.49
			Liver	8.18	4.73	3.45	19.03	35.80	4.32
	May	8.27	Ovary	11.56	3.86	7.71	48.16	10.64	1.27
			Muscle	11.67	3.13	8.55	53.41	3.11	0.50
			Liver	9.08	5.30	3.78	23.63	28.72	3.64
		1.42	Ovary	10.71	3.25	7.46	46.63	8.36	1.16
			Muscle	12.22	3.85	8.36	52.28	2.01	0.42
			Liver	7.72	4.52	3.20	20.19	27.97	4.79
	June	3.49	Ovary	10.39	2.78	7.61	47.54	10.83	2.22
			Muscle	13.64	3.07	10.57	65.97	2.08	0.52
			Liver	6.37	3.18	3.19	19.94	15.62	4.66
		1.14	Ovary	10.57	3.72	6.85	43.8	5.62	1.84
			Muscle	12.36	4.70	8.66	54.11	4.07	0.47
			Liver	6.27	3.88	2.38	14.89	17.77	3.78
	July	4.45	Ovary	11.53	3.9	7.63	47.71	13.83	0.89
			Muscle	13.44	4.46	8.98	56.15	3.32	0.71
			Liver	7.26	4.30	2.96	17.51	20.17	5.09
		1.24	Ovary	9.65	3.66	5.99	37.43	6.20	0.39
			Muscle	13.12	4.37	8.75	54.68	4.39	0.65
			Liver	5.20	2.16	3.05	19.03	22.58	4.07
	August	7.62	Ovary	10.01	2.53	7.46	46.64	15.63	0.91
			Muscle	10.39	2.83	7.56	47.25	4.37	0.54
			Liver	7.72	4.59	3.13	18.57	18.88	3.09
		1.44	Ovary	10.79	4.49	6.3	39.34	9.71	1.44
			Muscle	12.18	1.36	11.81	63.83	3.97	0.64
			Liver	6.21	3.01	3.20	20.01	16.54	1.22
	September	3.98	Ovary	9.87	2.81	7.06	44.11	19.32	1.22
			Muscle	12.35	4.61	7.74	48.39	2.62	0.62
			Liver	5.91	2.56	3.35	21.75	25.37	3.08
		1.20	Ovary	10.27	3.76	6.51	40.69	9.96	1.58
			Muscle	13.54	4.30	9.24	57.73	3.01	0.56
			Liver	6.62	3.2	3.42	21.38	27.36	5.93
	October	9.57	Ovary	10.16	3.57	6.6	41.23	20.49	1.2
			Muscle	11.91	3.86	8.05	50.32	2.52	0.42
			Liver	8.16	4.59	3.57	22.27	18.92	4.87
		1.31	Ovary	10.08	3.98	6.2	41.22	11.72	1.22
			Muscle	12.12	4.4	7.72	48.26	4.84	0.48
			Liver	7.47	4.46	3.01	18.84	32.66	5.59
	November	7.55	Ovary	11.64	3.34	8.3	51.76	16.59	1.1
			Muscle	13.36	3.28	10.08	63.01	3.73	0.56
			Liver	8.66	5.54	3.12	19.47	21.79	4.01
		1.78	Ovary	10.12	4.19	5.93	37.06	10.85	2.12
			Muscle	10.97	2.47	8.5	53.12	2.85	0.72
			Liver	7.54	4.63	2.91	18.21	31.73	3.82
	December	12.7	Ovary	10.11	3.36	6.85	42.83	19.01	1.12
			Muscle	11.21	3.33	7.88	49.24	3.57	0.4
			Liver	7.8	5.58	2.22	13.85	20.0	4.09

Table 24 (Continued)

Year	Month	Gonad index	Tissue	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Protein	Lipid	Glycogen
	December	1.71	Ovary	9.91	5.54	4.37	27.29	12.5	1.91
			Muscle	10.39	3.50	6.89	43.04	4.68	0.96
			Liver	9.52	6.50	3.02	18.87	10.55	5.01
1965	January	10.13	Ovary	11.45	3.14	8.31	51.92	14.55	3.25
			Muscle	12.04	4.21	7.83	48.93	33.37	0.46
			Liver	7.72	4.34	3.38	21.13	16.51	3.65
		1.42	Ovary	9.6	4.34	5.26	32.88	10.63	1.32
			Muscle	13.17	3.35	9.82	61.4	1.35	0.43
			Liver	7.17	4.16	2.01	12.56	28.36	3.27
	February	11.81	Ovary	10.51	3.73	6.78	42.36	16.9	1.21
			Muscle	13.14	4.23	8.91	55.68	2.09	0.51
			Liver	6.27	2.37	3.90	24.39	18.80	4.82
		1.23	Ovary	9.62	3.53	6.18	38.64	10.6	1.63
			Muscle	12.16	3.30	8.85	55.32	3.71	0.68
			Liver	6.28	3.35	2.92	18.28	19.22	3.73

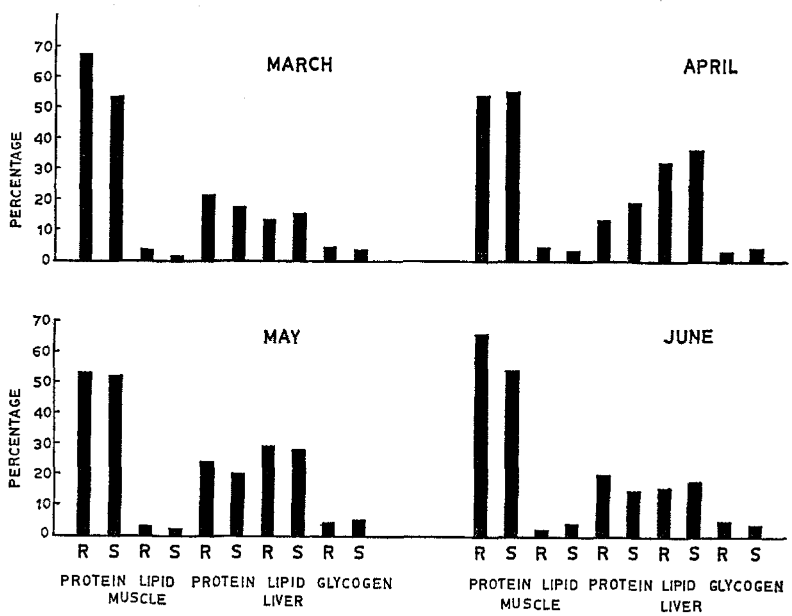

Fig. 24. *Metapenaeus affinis.* Histograms illustrating composition of muscle and liver during different months. (Values expressed as percentage dry weight). R: ripe or ripening; S: spent or resting

sive, involving the utilisation of substantial quantities of lipid for storage in the ova, and when the fat supply from the food is insufficient to meet the demands from the ovary, the lipid that is stored in the hepatopancreas is likely to be transferred to the ovary.

The glycogen values of the liver are comparatively higher than those of the gonad or the muscle. This may be due to the storage of glycogen in the hepatopancreas. A significant correlation between the glycogen content of the liver and the reproductive cycle of this species is, however, not evident. The ripening individuals have less glycogen in their liver during the months of April and May, September, October and December, whereas in all the other months, with the exception of November, the ripening individuals have more glycogen per unit weight of liver. In November, the glycogen content of the liver of females at different stages of gonad maturity shows no significant variation. Since no clear fluctuation in the glycogen content of the liver is evident in relation to the reproduc-

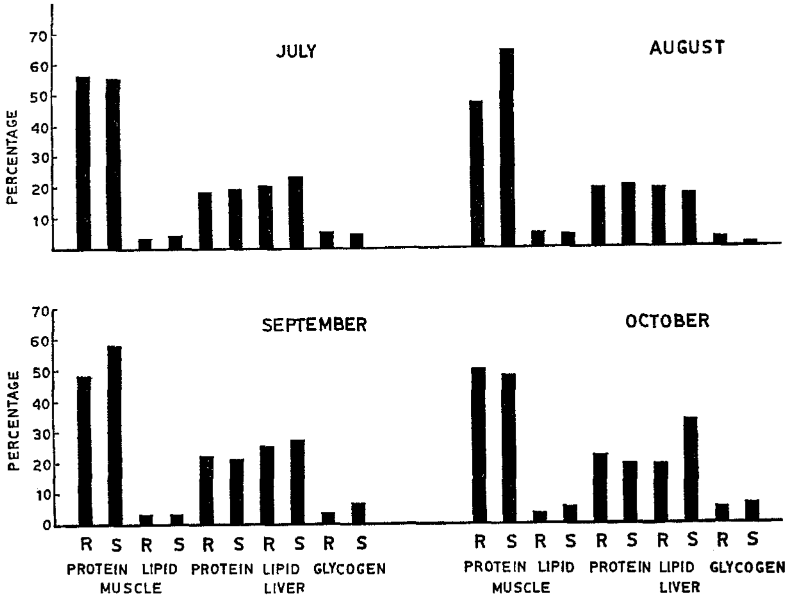

Fig. 25. *Metapenaeus affinis*. Histograms illustrating biochemical composition of muscle and liver during different months. (Values expressed as percentage dry weight). R: ripe or ripening; S: spent or resting

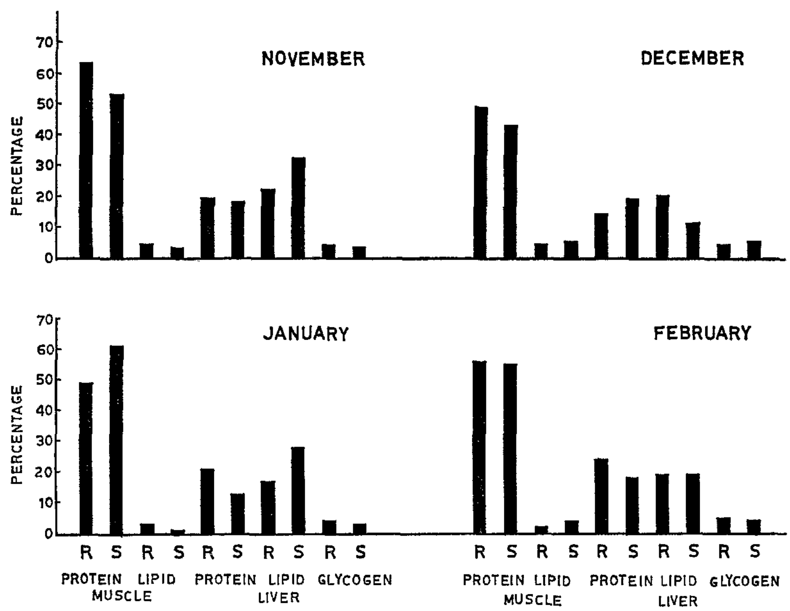

Fig. 26. *Metapenaeus affinis*. Histograms illustrating biochemical composition of muscle and liver during different months. (Values expressed as percentage dry weight). R: ripe or ripening; S: Spent or resting

tive cycle, it is inferred that glycogen is not mobilised into the gonad from storage organs during the time of gonad activation and growth. Thus, it may be stated that, in this species, none of the organic substrates other than the lipid of the hepatopancreas seems to be mobilised in sufficient quantities into the ovary during the breeding period.

The relative increase of the various biochemical constituents of the gonads has been calculated as for *Portunus pelagicus* (*vide supra*), and the results are presented in Table 25. It will be seen that, while the gonad index of female *Metapenaeus affinis* increases by about 16.6 times, the wet weight of the gonad increases 28 times, the dry weight 35.3 times, total nitrogen 36.2 times, non-protein nitrogen 33.7 times, protein 37.3 times, lipid 58.7 times and glycogen 41.6 times. In *M. affinis*, also, lipid is the constituent which increases relatively more than any other substrate. It is interesting to note that the relative increase of the organic substances in the ovary of *M. affinis* is not so conspicuous as in *P. pelagicus*.

In male *Metapenaeus affinis*, the gonad index of the testis increases only about 4.5 times, the wet weight of the testis 6.3 times, the dry weight 4.9 times, total nitrogen 6.8 times, non-protein nitrogen 3.9 times protein 7.7 times, lipid 17.2 times and glycogen 5.7 times.

Thus, the relative increase of the substrates in the prawn *Metapenaeus affinis*, is less than that noticed in the crab *Portunus pelagicus*. In both species, lipid is the constituent which increases in substantial quantities during the growth of the gonad.

Table 25. *Metapenaeus affinis. Increase in organic constituents of gonads during growth from shrunken to maximum size (in mg; total weight in g)*

Status of gonad	Female		Relative increase	Male		Relative increase
	Spent	Ripe		Spent	Ripe	
Gonad index	0.796	13.206	16.6	0.396	1.789	4.5
Wet weight (g)	0.148	4.1476	28.0	0.038	0.2424	6.3
Dry weight (g)	0.045	1.592	35.3	0.013	0.0644	4.9
Total nitrogen (mg)	4.5	162.9	36.2	1.08	7.44	6.8
Non-protein nitrogen (mg)	2.02	68.1	33.7	0.307	1.202	3.9
Protein nitrogen (mg)	2.51	93.8	37.4	0.789	6.24	7.8
Protein (mg)	15.7	586.5	37.3	4.9	38.29	7.7
Lipid (mg)	4.36	256.3	58.7	0.303	5.24	17.2
Glycogen (mg)	0.6	25.0	41.6	0.343	1.97	5.7

Discussion

The main purpose of the present study was to gather as much information as possible on the general biochemical make-up and the systematic variations of the different constituents in the gonad, the liver and the muscle in relation to the annual reproductive cycle in three representative crustaceans *Uca annulipes*, *Portunus pelagicus*, and *Metapenaeus affinis*, selected from different ecological habitats. Systematic data on the biochemical changes of the gonads and other organs associated with the reproductive cycle of marine invertebrates are limited, being confined to the observations of Giese and his associates (Giese, 1959; Giese and Araki, 1962; Giese *et al.*, 1964; Pearse and Giese, 1966). These authors have shown that gonad production involves intense biochemical synthesis; and have indicated that organic reserves may or may not be stored prior to gonad development, and that when storage is evident, transfer of material to the reproductive organ must take place subsequent to storage. While such information is available in the case of echinoderms and molluscs, our knowledge regarding these in the crustaceans is based on the works of George and Patel (1956) and Barnes *et al.* (1963).

It is clear from the data collected for the three species of crustaceans of the present study, that there are seasonal as well as individual variations in the biochemical composition of the gonads. The variability of chemical constitution of the gonads noticeable during the year may be just another index of the lack of synchronised spawning. In similar observations in the purple sea-urchin *Strongylocentrotus purpuratus*, Giese *et al.* (1958) observed that, at almost all times, the population is rather inhomogeneous with respect to the gonad cycle, some animals having fairly well developed gonads, others having poorly developed or spent gonads. Biochemical inhomogeneity of different individuals may, therefore, reflect population inhomogeneity in gonad development. Even when animals of similar gonad index are compared, biochemical differences are clearly evident in these crustaceans also. Yet another factor which plays a role in the variability of chemical composition of the gonad is the availability of nutrients to each individual in the population. However, the food may have unequal nutritive quality at different times. The availability of nutrients may, therefore, vary even though the bulk of food taken in may be the same.

In this context, it is worthwhile to examine here the nature of fluctuations of the various biochemical components of the gonads, the liver and the muscle in *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* in relation to the reproductive cycle of these species. While the water content of the entire body of male *U. annulipes* shows no fluctuation, a higher percentage of water has been noticed in the body of females during the breeding season. As in the case of male *U. annulipes*, in male *M. affinis* also, a correlation between the water content of the body of the adult and breeding activity is not noticeable. In contrast to female *U. annulipes*, those of *M. affinis* gave evidence of an accumulation of water in the pre-breeding months, but the peak breeding period shows only a low water content. Similar data for purposes of comparison regarding other crustaceans are few. In barnacles of the boreo-arctic region, an increase of water content of the body after copulation, and a slight rise of water content along with a loss of body weight owing to transfer of materials to the female gonad have been noticed (Barnes *et al.*, 1963). In a bivalve mollusc, *Crassostrea virginica*, Durve and Bal (1961) noted that, during the development of gonads as well as in the spawning season, there is a rise in the water content of the body, and a fall in water content during the resting period.

The ash content of the body of male *Uca annulipes* does not indicate any correlation with the breeding season; in females, an increase of ash content during the breeding season is evident — this may be due to the accumulation of mineral salts in the developing gonad. Greenfield *et al.* (1958) observed in echinoderms that ripening of the gametes is also correlated, in some cases, with an increase in ash concentration. There is no correlation between the water and ash content of the body in *U. annulipes*. Durve and Bal (1961) noted, in *Crassostrea virginica*, that, during the rapid development of gonads, all values (water, ash, fat, glycogen and protein) were high except the mineral content.

The water content of the gonad and body of *Uca annulipes* and *Metapenaeus affinis* does not indicate a distinct relationship. A comparison of water content of the gonads of all three species studied here shows that, in the testis, an increase in water content is apparently absent with the ripening of this organ; but in the ovary, particularly in the case of *M. affinis* and *Portunus pelagicus*, the water content and the gonad development present an inverse relationship. In the case of *U. annulipes*, this relationship is not well pronounced. Giese *et al.* (1958) noted a decrease in water content in the gonads of *Strongylocentrotus purpuratus* during maturity of the gonad, and observed that the relative water content in the ovary tends to decline to some extent along with a general increase in the total mass of other substances in the ovary. Greenfield *et al.* (1958), in echinoderms, showed that the water content decreases with the ripening of the gametes, especially in the females and this, according to these authors, is possibly due to the accumulation of fat. George and Patel (1956) observed in the case of the Bombay lobster and the prawn an inverse relationship between water and fat content of the gonad, and that this increase in fat content is evidently associated with gonad development. This seems to be the case in the present species as well.

The non-protein nitrogen showed an increase in the ovary of *Uca annulipes* during the breeding season, whereas in the testis no such correlation was evident. The non-protein nitrogen in the ovary of *Portunus pelagicus* and *Metapenaeus affinis* decreased with the growth of the gonad, whereas in the testis, while an increase was seen in some individuals, a decrease was also noticed in others during the gonad growth, leading to a condition wherein correlations were difficult. Giese *et al.* (1958), Greenfield *et al.* (1958) and Giese and Araki (1962) have investigated the non-protein nitrogen content in some species of echinoderms and chitons. It is interesting to note certain points of similarity with regard to the fluctuations of this constituent in the gonads of these species. In *Strongylocentrotus purpuratus*, a decrease in non-protein nitrogen content of the ovary was noticed as in the case of the ovary of *P. pelagicus* and *M. affinis* in the present study. However, in the testis of the 3 species of the present study, a distinct relationship was not evident. In female chitons also, a similar decrease had been noticed in ripe females by Giese and Araki (1962). The observations of Greenfield *et al.* (1958) suggest an increase of non-protein nitrogen during the growth of both the testis and the ovary, and the result apparently agrees with the results obtained for female *U. annulipes* in the present study. These authors attribute the increase noted in the male to the accumulation of nucleic acids in the testis.

An increase in the protein values of gonads during the breeding season was discernible in both sexes of all species studied here. These results are in conformity with the conditions noticed in barnacles (Barnes *et al.*, 1963), echinoderms (Giese *et al.*, 1958; Greenfield *et al.*, 1958) and molluscs (Giese and Araki, 1962; Tucker and Giese, 1962).

A scrutiny of the trends in lipid fluctuations reveals that there is a general increase of this substance during the breeding season in all three species. This condition was especially evident in the females. Barnes *et al.* (1963) noticed an increase in fat content of the ovary during the breeding season in *Balanus balanoides* and *B. balanus*. It is noteworthy that similar increase in this substrate with gonad growth (especially in females) has been reported for echinoderms (Giese *et al.*, 1958; Greenfield *et al.*, 1958) and chitons (Giese and Araki, 1962; Tucker and Giese, 1962). Even though conspicuous variations have not been noticed in the lipid content of the males of these crustaceans, a decrease has been reported with development of the testis in *Stronylocentrotus purpuratus* and *Mopalia hindsii*, giving evidence of an inverse relationship between testis growth and lipid content (Giese *et al.*, 1958; Giese and Araki, 1962). However, in *Katherina tunicata* (Giese and Araki, 1962), a decrease in lipid content has been indicated in the spent testis. In this connection, it is interesting to note that, in *Panulirus polyphagus*, George and Patel (1956) noticed an increase in the fat content of the ovary during the reproductive period, whereas that of the testis was slightly reduced; the fat content of the testis was found to undergo little change whatsoever during the different months. Thus, the fat store was evidently utilised in the female for the development of eggs, but not conspicuously so for the development of sperms. As Giese and Araki (1962) suggested in the case of *M. hindsii*, it is possible that, in the case of these crustaceans also, a large amount of lipid goes into the eggs as a reserve for larval nutrition; consequently, after spawning, ovaries have a smaller content of lipid.

The glycogen content increased per unit weight of the ovary in *Uca annulipes* during gonad growth, a condition similar to that noticed in echinoderms (Giese *et al.*, 1958; Greenfield *et al.*, 1958). In the ovary of *Portunus pelagicus*, no such correlation was found to exist. In *Metapenaeus affinis*, there was no marked increase in glycogen in the testis and in the ovary with gonadal growth. The glycogen content of the testis of *P. pelagicus* decreased with growth of the gonad, a condition noticed in the gonad of *Katherina tunicata* and *Mopalia hindsii* (Giese and Araki, 1962). Contrary to the tendency towards accumulation of glycogen in the developing gonads of echinoderms (Giese *et al.*, 1958; Greenfield *et al.*, 1958) in *Cryptochiton stelleri* (Tucker and Giese, 1962), and in chitons, *K. tunicata* and *M. hindsii* (Giese and Araki, 1962), the results suggest an increase of this material in the spent gonads. The crustaceans seem to agree with the chitons in this respect, even though glycogen is not a major constituent in their gonads. The observations of Barnes *et al.* (1963) on barnacles indicate a similar trend. Barnes *et al.* state that the young ovary is richer in soluble glycogen, and the fall in glycogen may reflect transformation of this substance into others, but probably it represents utilisation of glycogen in the respiration of the ovary.

It has been found that, in sea stars (Greenfield *et al.*, 1958) which have pyloric caecae for storage, organic materials may be stored for subsequent use in gonad production. Barnes *et al.* (1963) have found evidence of a storage of organic materials in the boreo-arctic barnacles and state that "it is not inferred that stored material does not play a part in gonad development in the decapods: no information is available on this point". During the present investigation, the major organs of three decapod crustaceans have been analysed with a view to obtaining information on the possible storage and transfer of materials to the gonads at the time of gamete production. In the case of male *Uca annulipes*, no evidence of storage or subsequent transfer of materials to the testis could be found, owing probably to the almost uninterrupted activity of the testis in this species. On the other hand, in the case of females, it has been noticed that, at the height of the breeding season, there are indications of a possible transfer of the hepatopancreatic lipid and glycogen to the ovary. In the case of *Portunus pelagicus* also, such transfer of hepatopancreatic lipid apparently takes place during the height of the breeding season. In certain months, such as January and November, the hepatopancreatic glycogen shows depletion in ripening females. However, in the ovary of *P. pelagicus*, glycogen has been shown to be a minor constituent and, therefore, transfer does not seem to be very necessary. A similar case has been reported in *Pisaster ochraceus* by Greenfield *et al.* (1958). In the case of *Metapenaeus affinis*, there is no evidence of a transfer of any other organic substances than the hepatopancreatic lipid at the peak period of gonadal activity in females. Among other crustaceans also, there is an indication of a possible transfer of organic reserves in other tissues to the developing ovary at the height of the breeding season. (George and Patel, 1956; Barnes *et al.*, 1963). However, such transfer of organic materials from other tissues to the gonad was not evident in the case of the common barnacle *Balanus amphitrite communis*, whose reproductive biology has been recently studied (Pillay and Nair, 1972).

Storage of materials depends upon the possession of specialised tissues or storage organs. In the sea star *Pisaster ochraceus*, a considerable increase of the gonad is followed by storage in hepatic caecae, and the hepatic caecae shrink as the gonad increases in size (Farmanfarmaian *et al.*, 1958). The relationship is, thus, almost quantitatively reciprocal. Although a similar analogy may be drawn between the hepatopancreas and gonads of decapod crustaceans, the quantity involved as well as the nature of the change seems to be less conspicuous in the tropical crustaceans of the present study. In *Haliotis cracherodii*, Boolootian *et al.* (1962) observed that the size of the hepatic gland exhibits a distinct inverse relation to gonad activity and that the maximum growth of the hepatic gland corresponds to gonadal quiescence. The precipitous diminution in the size of the hepatic gland corresponds to the growth period of the gonad. This suggests that materials are mobilised from the hepatic gland for gonad growth. It appears that the hepatic gland stockpiles nutrients essential to gamete development. Stockpiling may occur at a season when the food supply (quantitatively, qualitatively, or both) is most effective. In an allied species, *H. rufescens*, no such cycle has been observed, both the hepatic caecae and the gonad being well developed almost throughout the year. It would thus, appear that nutrients required for both hepatic and gonadal growth are always available. This lends support to the fact that, in tropical forms which possess protracted breeding periods, storage and subsequent transfer of materials may not be universal, and in the crustaceans of the present study, the absence of a noticeable and distinct transfer of stored materials may be attributed to the same principle, or it may be the result of the physiological state of the individuals of the species. This fact is confirmed by Rahaman (1967) who has recently reported from the east coast of India that when the gonad index showed high values hepatic index also had high values. This simultaneous increase cannot occur when there is a direct transfer of organic materials from the hepato-pancreas to the gonads.

In terms of organic production of the animal, the noticeable increase in organic constituents represents the major changes of the gonads. The data on the biochemical composition of the gonads calculated on the basis of unit dry weight do not emphasise the biochemical change in the gonads quite as well as do the data showing the total amount of each organic constituent in the entire gonad. This is due to the fact that the gonad increases in size several fold during the course of a reproductive cycle. The relative increase of each substance has, therefore, been obtained by multiplying the amount per unit weight by the weight of the gonad. When it is realised that, in the course of one reproductive cycle, the gonad weight may increase several times, it becomes clear that a small increase in the amount of a constituent per unit weight may mean a very large total increase per animal. From the results of the relative increase of various substances in the gonads, it is evident that lipid and protein are the important constituents in these crustaceans, and particularly lipid of the ovary, which increase several fold during the reproductive cycle (Tables 16 and 25). Lipid appears to be the most significant substance in the metabolism of crustaceans and, in this respect, crustaceans resemble chitons and echinoderms.

The overall averages of the various constituents of the gonads for the entire year for all three species provide interesting information (Table 26). In all three species studied, the ovary is richer than the testis in lipid, whereas the testis is richer in both protein and water content, as noticed by Giese *et al.* (1958) in sea urchins. However, non-protein nitrogen content varies in different species, the ovary being richer than the testis in this constituent in *Metapenaeus affinis* and *Portunus pelagicus*, whereas the testis of *Uca annulipes* has more non-protein nitrogen than the ovary. In the case of glycogen, a definite pattern of variation is not evident, as observed by Giese *et al.* (1958), since the ovary of *P. pelagicus* contains more glycogen than the testis and, in *M. affinis*, the testis is richer in glycogen than the ovary.

The differences discernible in the constituents of the gonads of the crustaceans may be due to the difference in the feeding habits imposed by the habitats of the species, besides other factors. *Uca annulipes* is a semiterrestrial crab living in the intertidal belt along the backwaters, where a rich supply of food is available throughout the year in the form of a detrital mixture consisting of photosynthetic organisms such as littoral diatoms and materials formed by the disintegration of other marine organisms. In this semiterrestrial crab, the body has a lower water content than that of the typical marine form *Metapenaeus affinis*. In the case of *M. affinis*, the chief source of nourishment seems to be the bottom organic detritus, which must be richer in organic constituents than the food available for *U. annulipes*. *Portunus pelagicus*, the swimming crab, is a carnivore, and occurs in a fairly wide range of localities in the sea, and sometimes even in the backwaters.

When viewed against the background of the ecological situations in which the three species occur, the differences in the biochemical constituents seem to be reasonable. Among the organic substances, lipid has been found to be the most important constituent of the ovary of all species, especially the detritus feeders *Uca annulipes* and *Metapenaeus affinis*. In the case of the carnivorous crab *Portunus pelagicus*, the ovary is richer in protein than the ovary of the other two detritus feeders.

Table 26. *Uca annulipes, Metapenaeus affinis and Portunus pelagicus. Overall averages of various constituents of testis and ovary showing differences in values; see Table 3*

Species	Gonad	Water content	Non-protein nitrogen	Protein	Lipid	Glycogen
Uca annulipes	Ovary	52.1	1.8	34.8	15.72	2.83
	Testis	61.1	2.45	40.1	5.71	—
Portunus pelagicus	Ovary	64.8	2.73	44.2	8.14	2.61
	Testis	64.1	1.95	53.2	5.52	1.96
Metapenaeus affiinis	Ovary	59.4	3.83	41.7	13.14	1.52
	Testis	64.1	3.4	46.6	5.86	2.93

Summary

1. The changes in the water content of the body of *Uca annulipes* (Latreille) and *Metapenaeus affinis* (Milne-Edwards), the ash content of the body of *U. annulipes*, and the biochemical constituents (water, nitrogen, non-protein nitrogen, protein, lipid and glycogen) of the gonad, liver and muscle of *U. annulipes, Portunus pelagicus* (Linnaeus) and *M. affinis*, were studied monthly to determine their variability in the course of the reproductive cycle.

2. In female *Uca annulipes*, body water content fluctuates during the breeding season, while a similar change is not noticed in the males. During the breeding season, the body of the females generally contains more ash than during other seasons; no such relationship is observed in males. Generally, in the ovary of *U. annulipes*, while water and non-protein nitrogen show no distinct correlation with gonad activity, protein, lipid and glycogen tend to exhibit an increase during the breeding season. In the testis of *U. annulipes*, a significant correlation between the fluctuations of various chemical constituents and breeding activity is not evident. The ovary of *U. annulipes* is distinctly richer in lipid than the testis, but the testis appears richer in water, non-protein nitrogen and protein than the ovary.

3. Generally, in the ovary of *Portunus pelagicus*, water and non-protein nitrogen show low values during the months of high gonad activity, whereas protein and lipid increase during the months when ripe and ripening individuals dominate. Glycogen does not seem to fluctuate during the annual cycle. In males, the water and protein content increases with the growth of the testis. Lipid and non-protein nitrogen do not fluctuate during the reproductive cycle; glycogen seems to have an inverse relationship with gonad activity. In *P. pelagicus*, the ovary is distinctly richer than the testis in non-protein nitrogen, fat and glycogen, whereas the testis is richer than the ovary in protein content.

4. In the adult body of females and males of *Metapenaeus affinis*, water content shows no fluctuations in relation to reproductive cycle, but there is an accumulation of water in the body of females during the pre-breeding season. In the ovary, protein and lipid fluctuate during the annual reproductive cycle, but glycogen does not show similar trends. An inverse relationship between the water and lipid content of the ovary has been observed. In the testis of *M. affinis*, water content does not fluctuate in the yearly cycle; neither does the lipid content of the testis. The non-protein nitrogen, protein and glycogen show an increase during the months with high gonad development.

5. Simultaneous analysis of the biochemical constituents of liver and muscle from a definite region of the body of these crustaceans does not give evidence of any significant seasonal storage of organic substances prior to the breeding season for subsequent transfer to the gonads. However, a general depletion of the hepatopancreatic lipid in reproducing forms at the height of the breeding season has been noticed.

Acknowledgements. One of us (K.K.P.) is grateful to the Ministry of Education for the award of a scholarship, during the tenure of which the work was carried out. The authors record their thanks to the officers of the Chemistry Section of the C.M.F.R.I., Cochin, India, for facilities, help and criticism during the course of the work.

Literature Cited

Barnes, H., M. Barnes and D. M. Finlayson: The seasonal changes in body weight, biochemical composition and oxygen uptake of two common boreo-arctic cirripedes, *Balanus balanoides* and *B. balanus*. J. mar. biol. Ass. U.K. **43**, 185—211 (1963).

Boolootian, R. A., A. Farmanfarmaian and A. C. Giese: On the reproductive cycle and breeding habits of two western species of *Haliotis*. Biol. Bull. mar. biol. Lab., Woods Hole **122**, 183—193 (1962).

Daniel, R. J.: Liverpool University Lancashire. Sea Fisheries. Lab. Rep. No. 31, 27—50 (1923). (As quoted by Giese, 1959).

Durve, V. S. and D. V. Bal: Studies on the chemical composition of the oyster. *Crassostrea gryphoides* (Schlotheim). J. zool. Soc. India **13**, 70—77 (1961).

Farmanfarmain, A., A. C. Giese, R. A. Boolootian and J. Bennett: Annual reproductive cycles in four species of west coast star fishes. J. exp. Zool. **138**, 355—367 (1958).

Fraga, I. F.: Consejo Super. investigaciones cientificas, Patronato Juan de la cierva. Investigación pesq. **4**, 109—125 (1956). (As quoted by Giese, 1959).

George, J. C. and B. S. Patel: The seasonal variation in the fat content of the liver and gonads in a marine and a fresh water decapod. J. Anim. Morph. Physiol. **3**, 49—55 (1956).

Giese, A. C.: Comparative physiology: annual reproductive cycles of marine invertebrates. A. Rev. Physiol. **21**, 547—576 (1959).

— and G. Araki: Chemical changes with reproductive activity of the chitons *Katherina tunicata* and *Mopalia hindsii*. J. exp. Zool. **151**, 259—267 (1962).

—, L. Greenfield, H. Huang, A. Farmanfarmaian, R. A. Boolootian and R. Lasker: Organic productivity in the reproductive cycle of the purple sea urchin. Biol. Bull. mar. biol. Lab., Woods Hole **116**, 49—58 (1958).

—, S. Krishnaswamy, B. S. Vasu and J. Lawrence: Reproductive and biochemical studies on a sea urchin, *Stomopneustes variolaris*, from Madras Harbour. Comp. Biochem. Physiol. **13**, 367—380 (1964).

Greenfield. L., A. C. Giese, A. Farmanfarmaian and R. A. Boolootian: Cyclic biochemical changes in several echinoderms. J. exp. Zool. **139**, 506—524 (1958).

Korringa, P.: Recent advances in oyster biology. Q. Rev. Biol. **27**, 266—308 (1952).

Mendel, B. and P. L. Hoogland: A rapid method for the determination of blood sugar. Lancet **11**, p. 6 (1950).

Pearse, J. S. and A. C. Giese: Food, reproduction and organic constitution of the common Antarctic echinoid *Sterechinus neumayeri* (Meissner). Biol. Bull. mar. biol. Lab., Woods Hole **130**, 387—401 (1966).

Pillay, K. K. and N. B. Nair: The annual reproductive cycles of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) from the South-west coast of India. Mar. Biol. **11**, 152—166 (1971).

— — The reproductive biology of the sessile barnacle, *Balanus amphitrite communis* (Darwin) of the South-West coast of India. Indian J. mar. Sci. **1**, (1972). (In Press).

Rahaman, A. A.: Reproductive and nutritional cycles of the crab, *Portunus pelagicus* (Linnaeus) (Decapoda: Brachyura) of the Madras Coast. Proc. Indian Acad. Sci. (Sect. B) **65**, 76—82 (1967).

Rao, K. S.: Reproductive and nutritional cycles of *Oreaster* (*Pentaceros*) *hedemanni* Lutken. J. mar. biol. Ass. India 8, 254—272 (1966).

— DNA content of gonads of the starfish, *Oreaster* (*Pentaceros*) *hedemanni* Lutken during reproductive cycle. Indian J. exp. Biol. **6**, 180—181 (1968).

Raymont, J. E. G. and S. Krishnaswamy: Carbohydrates in some marine plankton animals. J. mar. biol. Ass. U.K. **39**, 239—248 (1960).

Renaud, L.: Le cycle des reserves organiques chez les crustacés decapodes. Annls Inst. océanogr., Monaco **24**, 259—357 (1949).

Russo, G.: Boll. accad. Gioenia **51**, 39—43 (1923). (As quoted by Giese, 1959).

— Arch. Sci. biol. (Bologna) 8, 161—181 (1926). (As quoted by Giese, 1959).

Saraswathy, M. and N. B. Nair: Biochemical changes in relation to breeding cycles of *Nausitora hedleyi* Schepman. Curr. Sci. **38**, 158—160 (1969).

Steyermark, A. L.: Quantitative organic microanalysis, Philadelphia: Blakiston Company 1961.

Stott, F. C.: The spawning of *Echinus esculentus* and some changes in gonad composition. J. exp. Biol. 8, 133—150 (1931).

Tanaka, S. and H. Hatana: Studies on the seasonal changes in the chemical constituents of the pearl oyster. Publs Seto mar. biol. Lab. **2**, 341—355 (1952).

Tucker, J. S. and A. C. Giese: Reproductive cycle of *Cryptochiton stelleri* (Middendorff). J. exp. Zool. **150**, 33—43 (1962).

Vinogradov, A. B.: Elementary composition of marine organisms. Elementary composition of Arthropoda. Mem. Sears Fdn mar. Res. **11**, 375—429 (1953).

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Date of final manuscript acceptance: September 18, 1972. Communicated by N. K. Panikkar, Panaji