Chapter 6

BIOCHEMICAL COMPOSITION

6.1. INTRODUCTION

Molluscs in general, and bivalves in particular, have conquered a wide variable environment for their successful inhabitance and a varied environment presupposes important adaptations at the morphological, physiological and biochemical levels, which may be of ecological singificance. An understanding of the biochemical composition of marine and estuarine organisms is highly essential, since an understanding of the metabolism of different populations provide an estimate of their energy content and an insight into the understanding of the biogeochemical circulation of elements.

India has a rich and varied bivalve resource and has been the subject of extensive biochemical investigations not only because of their importance as food for man but also because of their significant role in the economy of many littoral states. Although a considerable body of information has been accumulated on the biochemical composition of bivalves in relation to sex, size and season, data on the biochemical composition of the same species inhabiting different areas so as to estimate the relative nutritive value of the species with reference to geographical distribution, are rather scarce. Studies by earlier workers have shown that seasonal metabolic cycles in bivalves are a reflection of complex interactions between food availability, environmental parameters, growth and reproductive activities (Bayne, 1976; Gabbot, 1983). Similarly, owing to variation in hydrographical conditions of the environment, there is a distinct seasonal fluctuation in

the abundance of bivalves and the present estimations have therefore been restricted to months during which the specific groups are available in sufficient quantities.

Further, the present study envisages an evaluation of the biochemical composition from more of a commercial view point than a physiological or sex wise determination, since in commercial utilisation, generally, greater stress is given to better "quality" of the meat. Hence if estimation such as these are based on sex, will be little appreciated. Moreover, species enjoying a wide geographical distribution are often likely to give rise to locally adapted populations or ecotypes and a comparative account on the biochemical status of such individual populations would be worthwhile. Hence in the present study, pooled estimations were made of the various biochemical components without regard for the sex of individuals.

6.2. REVIEW OF LITERATURE

Much of the early work on the seasonal changes in biochemical composition of Mytilus edulis and M. galloprovincialis have been reviewed in detail by Giese (1969) and the more recent ones are of De Zwaan and Zande (1972), Gabbot and Bayne (1973) and Bayne et al. (1982) in Mytilus edulis and Ansell et al. (1980) in Donax trunculus L. These studies have demonstrated that the changes in body weight are mainly due to changes in carbohydrate or glycogen content.

Seasonal variations in quality and quantity of tissues in Mytilus have been investigated in different geographic areas by several authors

(De Zwaan and Zande, 1972; Telembici and Dimoftache, 1972; Gabbot and Bayne, 1973; Dare and Edwards, 1975; Pieters et al., 1980). These studies have demonstrated that seasonal variation in biochemical composition have different patterns depending on the different latitudes and are strongly influenced by temperature and phytoplankton availability. It is fairly well established that, in fishes, marked changes do occur in the chemical constitution of tissues from season to season (Love, 1970; Shreni, 1980).

Studies on the biochemistry of Indian bivalves have chiefly focused on the biochemical composition, seasonal changes in composition and calorific values. Analyses have mostly been made on pooled, homogenized animals with little or no distinction of sex, gonadal condition or environmental parameters (Venkataraman and Chari, 1951; Durve and Bal, 1961; George and Nair, 1975; Shafee, 1978). Studies on the biochemical constituents and food values of five commercially important edible bivalves of Kerala by Suryanarayan and Alexander (1972) revealed that the bivalve meat compared favourably with the common food fishes with regard to their calorific value and hence would be an excellent and economic source of nutrition for man. Ansell (1974a, b, c) has reported that spawning in Abra alba, Chlamys septemradiata and Nucula sulcata is accompanied by a rapid decline in the total carbon and calorific value and the highest values of energy content coincided with the maximum lipid content of the tissue. Supporting these findings, Nair and Shynamma (1975) and Ansari et al. (1981), working on the seasonal variations in calorific value and lipid content of Villorita cyprinoides var. cochinensis reported that the calorific values are directly proportional to the lipid content. Similar studies have been carried out by several others

(Krishnakumari, et al., 1977; Nagabhushanam and Mane, 1978; Shafee, 1978). As pointed out by Giese (1969), such data are only of limited use in the study of the relation of biochemistry of the animal to its nutritional stages.

Studies on the seasonal variations in biochemical composition have been carried out in a number of bivalve species from the Indian sub-continent. Durve and Bal (1961) investigated into the seasonal variation in all the biochemical components including inorganic constituents and the calorific values of Crassostrea gryphoides and inferred that this species is good for consumption from late October to June. Similar studies on other Crassostrea species have also been reported by several others (Krishnakumari et al., 1976; Joseph, 1979; Joseph and Madhyastha, 1986; Easterson and Kandasami, 1988). In Meretrix meretrix, Nagabhushanam and Deshmukh, (1974) found that the glycogen content was related to gonad development and depicted an increase during active gametogenesis. Studies by Venkataraman and Chari (1951) revealed an increase in fat content in Meretrix casta during periods of gonad development. In Meretrix meretrix, the glycogen and protein content showed a steady fall during premonsoon period with an equally steady rise during postmonsoon period; the fat content depicting a reverse trend (Salih, 1979). Lakshmanan and Nambisan (1980) observed a significant negative correlation between carbohydrate and protein in Meretrix casta and Villorita cyprinoides var. cochinensis. In Meretrix casta, Balasubramanyan and Natarajan (1988) recorded a high percentage of protein in the mantle while the gonad and digestive gland had higher percentage of carbohydrate and lipid respectively. Similar observations have also been made in other Meretrix species (Durve and George, 1973; Krishnakumari et al, 1976; Jayabal and Kalyani, 1986). Biochemical analyses were carried out

in Katelysia marmorata (Joshi and Bal, 1965) and K. opima (Mane, 1974) with a view to estimate their nutritive value. Two distinct periods of variation in biochemical composition were observed in Cellana radiata while estimating their nutritive value (Suryanarayan and Nair, 1976). Krishnakumari et al. (1976) estimated the variations in nutritive value and the suitable periods for harvesting Mytilus viridis and such estimations have also been made in the species of Paphia (Nagabhushanam and Dhamne, 1975; Mane and Nagabhushanam, 1979), Perna viridis (Ramachandran, 1980) and Sunetta scripta (Katticaran, 1988).

6.3. MATERIALS AND METHODS

6.3.1. SPECIMENS EMPLOYED

Details of the specimens employed, sampling stations, collection and laboratory conditioning etc. have been described earlier (vide pages, chapter 2 and 3). Sexually mature individuals were avoided and only immature specimens were utilised.

6.3.2. ESTIMATION OF BIOCHEMICAL CONSTITUENTS

a) Water content

To estimate the percentage water content in unit weight of adductor muscle of the three species, the specimens were first forced open with a scalpel, washed with distilled water and the excess water was blotted using filter paper. The adductor muscle was then removed asceptically,

transferred to previously weighed aluminium foil dishes and weighed immediately to determine the wet weight of the tissue. They were then dried at 70-80°C for 48 h and the dry weights taken to constancy. The difference between the fresh wet weight and dry weight yields the water content of the tissue and is expressed as percentage wet weight, or

$$\text{Water content} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100 = \% \text{ wet weight}$$

b) Protein

Protein content in the tissue was determined by the method of Lowry et al. (1951). A unit weight of the adductor muscle was dissolved in 0.1 N Sodium hydroxide with gentle warming in a water bath. The following procedure was adopted for analysis of the extract.

1. Add 0.2 ml of the sample to 1 ml of 10% trichloro acetic acid.
2. Centrifuge at 3000 RPM for 15 minutes and decant the supernatant.
3. Dissolve the precipitate in 1 ml of 0.1 N Sodium hydroxide.
4. Pipette out 0.5 ml of this into another test tube and add 0.5 ml of distilled water to make up to 1 ml.
5. Add 5 ml of alkaline copper reagent to this and mix well.
6. After 10 minutes, add 0.5 ml of Folin's reagent and mix well. Leave the resulting solution for 30 minutes at room temperature.
7. Using bovine serum, prepare standards similarly. Read the optical density (OD) of the blank, standard and samples at 500 nm in a Spectrophotometer (Hitachi, Model 200 - 20).

c) Glycogen

To estimate the total glycogen content in the adductor muscle, Montgomory's method (1957) was employed. The extract was prepared as described for protein estimation and later analysed according to the following procedure.

1. Add 0.2 ml of the extract to 1 ml of 10% trichloro acetic acid.
2. Centrifuge at 2500 RPM for 10 minutes. Take the supernatant.
3. To 1 ml of the supernatant, add 3 ml of 95% ethyl alcohol and shake well.
4. Keep that undisturbed in a refrigerator for about 12-24 h.
5. Centrifuge at 2500 RPM for 15 minutes. Remove the supernatant gently and take the pellet.
6. Add 0.1 ml of 80% phenol to the pellet.
7. Then add, 2 ml of distilled water followed by 5 ml of Con. Suphuric acid and shake well.
8. Standards are prepared similarly using glucose.
9. Prepare a 'blank' that contains 2 ml of distilled water, 0.1 ml of 80% phenol and 5 ml Con. Sulphuric acid.
10. Allow the preparations to stand for 30 minutes at room temperature.
11. Read the OD of the blank, standards and samples, at 490 nm in a Spectrophotometer.

d) Lipid

The method of Barnes and Blackstock (1973) was used to estimate

the lipid level in the adductor muscle. Weighed tissue samples were extracted with chloroform : methanol (2:1) mixture and subjected to further analysis as follows:

1. To the extract, add 1 ml of methanol, 2 ml of chloroform and 2 ml of chloroform : methanol mixture again.
2. Add 0.2 volume of 0.9% Sodium chloride solution. Pour this into a separating funnel and mix thoroughly. Allow it to stand for few hours.
3. Separate the lower phase into a clean test tube. Make up the volume of the lower phase to the original quantity of chloroform added before.
4. Measure out 0.5 ml of extract into a clean test tube and allow it to dry in a vacuum desiccator over silica gel. Dissolve in 0.5 ml of Con. Sulphuric acid and mix well. Plug the tubes with non-absorbent cotton wool, place in boiling water bath for 10 minutes and cool.
5. Pipette out 0.2 ml of this acid digest into a dry test tube and add 5 ml of sulphophospho-vanillin reagent. Mix well and allow to stand for 30 minutes.
6. Prepare standards using cholesterol (8 mg/4 ml of chloroform : methanol mixture).
7. Read the blank, standard and samples at 520 nm using a spectrophotometer.

6.4. RESULTS

The approximate biochemical composition of the adductor muscle

of the three bivalve species under investigation is presented in Tables 21-23 and Fig. 19-21. 'One way ANOVA' was carried out to assess whether there were any significant variation in the biochemical composition analysed between the different stations from where each species were sampled. The results thus obtained has been treated under species heads to make the presentation more meaningful. The different biochemical constituents such as protein, glycogen and lipid are expressed in µg per mg (µg/mg) wet weight of the tissue, while water content in the tissue is expressed as percentage wet weight.

6.4.1. VILLORITA CYPRINOIDES

The adductor muscle of V. cyprinoides sampled from the five stations (vide pages, chapter 2) were found to have protein as the major biochemical constituent, despite the minor variations between sampling sites. The highest protein concentration was observed at station 3 (53.32 µg/mg) while individuals from station 2 had a low value of 42.50 µg/mg. Specimens from stations 1, 4 and 5 had protein levels of 43.40 µg/mg, 52.10 µg/mg and 50.12 ug/mg respecively as shown in Table 21 and Fig 19. Analysis of variance (ANOVA) revealed that the protein concentration was significantly different only between stations 1 and 3, 1 and 4, 2 and 3, and 2 and 4 ($P \leqslant 0.01$) (Table 24a).

Glycogen represents the most variable biochemical component in an organism. Clearcut variation in the glycogen content in the adductor muscle was evident among the different populations tested. Significant

muscle of individuals from stations 1 to 5.

	PROTEIN (µg/mg)	GLYCOGEN (µg/mg)	LIPID (µg/mg)	WATER CONTENT (%)
Stn. 1	43.40	31.21	14.05	82.00
Stn. 2	42.50	36.94	14.86	82.21
Stn. 3	53.32	38.03	17.70	80.50
Stn. 4	52.10	31.48	18.75	79.38
Stn. 5	50.12	29.49	17.78	82.62

Table 22. Crassostrea madrasensis. Biochemical composition of the adductor muscle of individuals from stations 1 to 3.

	PROTEIN (µg/mg)	GLYCOGEN (µg/mg)	LIPID (µg/mg)	WATER CONTENT (%)
Stn. 1	56.82	28.92	9.83	80.58
Stn. 2	56.98	28.73	10.42	80.38
Stn. 3	60.69	31.39	11.19	81.83

Table 23. Perna viridis. Biochemical composition of the adductor muscle of individuals from stations 1 and 2.

	PROTEIN (µg/mg)	GLYCOGEN (µg/mg)	LIPID (µg/mg)	WATER CONTENT (%)
Stn. 1	62.68	29.03	17.24	77.80
Stn. 2	66.27	34.13	20.61	79.10

difference was observed between stations 1 and 2, 1 and 3, 2 and 5, 3 and 4, and 3 and 5 (Table 24b). Specimens from station 3 depicted the highest glycogen content (38.03 µg/mg) while the lowest concentration was observed among individuals from station 5 (29.49 µg/mg). Among the other three populations, those from station 2 had 36.94 µg/mg glycogen in their muscle tissue while individuals from station 2 and 4 recorded an almost identical concentration of 31.21 µg/mg and 31.48 µg/mg (Table 21).

It is evident from Table 21 that the lipid level in the adductor muscle of V. cyprinoides from the five stations were more or less identical and that the variation was not statistically significant ($P \leq 0.01$) (Table 24c). Individuals from station 4 recorded the highest lipid level (18.75 µg/mg) although their counterparts from stations 1 and 2 had only 14.05 µg/mg and 14.86 µg/mg respectively. A moderate concentration of 17.70 µg/mg and 17.78 µg/mg were reported in respect of individuals sampled from stations 3 and 5.

As with the other biochemical constituents, the water content of the muscle tissue was also found to vary in a similar manner. The water content varied from 79.38 to 82.62% in the different populations of the species. Stations 1 and 4, 2 and 3, 2 and 4, and 4 and 5 showed statistically significant variation in the amount of water in the adductor muscle of individuals (Table 24d). A water content of 82.62% was obtained for individuals from station 5; the corresponding value being 82.00%, 82.21%, 80.50% and 79.38% for individuals from stations 1, 2, 3 and 4 respectively (Table 21).

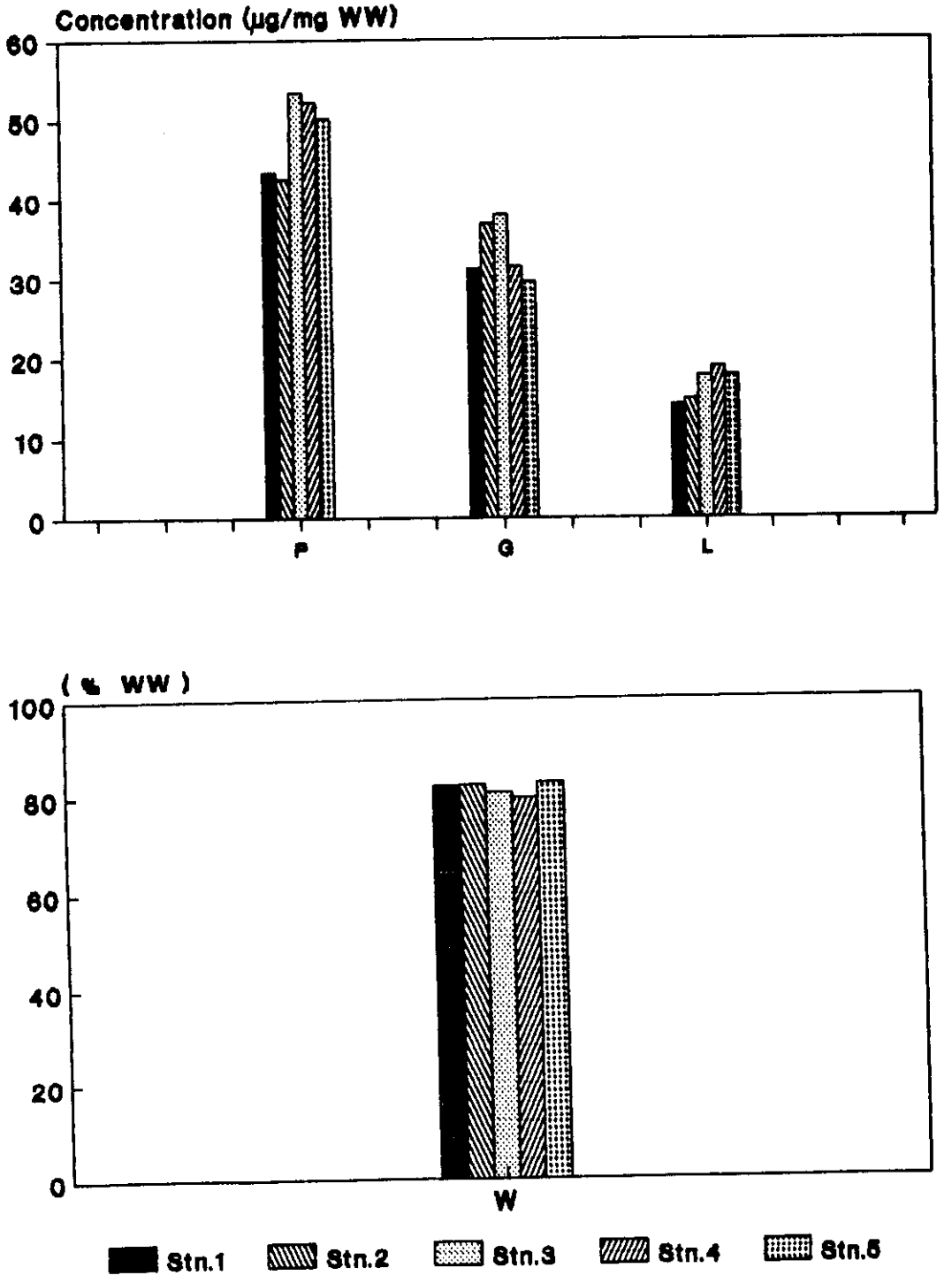

Fig. 19 Villorita cyprinoides. Various biochemical constituents ((P) Protein (G) Glycogen (L) Lipid (W) Water content) in the adductor muscle of individuals from stations 1 to 5.

Table 24 a-d. Villorita cyprinoides. Analysis of variance (ANOVA) of the various biochemical components in the adductor muscle of individuals from stations 1 to 5, along with their level of significance.

24a. Protein

Source	SS	Df	Mean Sqr	F
Total	5846.38	149	--	
Station	3017.25	4	754.313	3.89*
Error	28129.13	145	193.994	

* $P \leq 0.01$

24b. Glycogen

Source	SS	Df	Mean Sqr	F
Total	19645.39	149	--	
Station	1734.125	4	433.531	3.51*
Error	17911.27	145	123.526	

* $P \leq 0.01$

24c. Lipid

Source	SS	Df	Mean Sqr	F
Total	7711.183	149	--	
Station	419.191	4	104.798	2.08
Error	7291.992	145	50.29	

24d. Water content

Source	SS	Df	Mean Sqr	F
Total	977.064	149	--	
Station	151.25	4	37.813	5.49*
Error	825.813	145	6.882	

* $P \leq 0.01$

6.4.2. CRASSOSTREA MADRASENSIS

No significant variation was observed in the biochemical composition of the adductor muscle of C. madrasensis collected from the three stations. Individuals from station 3 were characterised by a relatively high concentration of the different components : the values being, protein 60.69 µg/mg, glycogen 31.39 µg/mg, lipid 10.19 µg/mg and water content 81.83%. On the other hand, individuals from the most saline area (station 1) had the following biochemical composition : protein 56.82 µg/mg, glycogen 28.92 µg/mg, lipid 9.83 µg/mg and water content of 80.58%, the same being 56.98 µg/mg, 28.73 µg/mg, 10.42 µg/mg and 80.38% for the moderately saline station 2 (Table 22 and Fig 20). The results of the analysis of variance (ANOVA) of the various components are detailed in Tables 25 a-d.

6.4.3. PERNA VIRIDIS

Despite the statistically significant variation ($P \leq 0.01$) in lipid and water content, individuals of P. viridis collected from the two stations had an almost identical glycogen and protein levels. The protein content of the adductor muscle of specimens from station 1 was 62.68 µg/mg while that for station 2 was 66.27 µg/mg; glycogen content being 29.03 µg/mg and 34.13 µg/mg respectively for individuals from stations 1 and 2. On the other hand, the lipid levels in the tissue were 17.24 µg/mg and 20.61 µg/mg and water content was 77.80% and 79.10% respectively (Table 23 and Fig 21). No significant variation was observed in the protein and glycogen levels (Table 26 a-d).

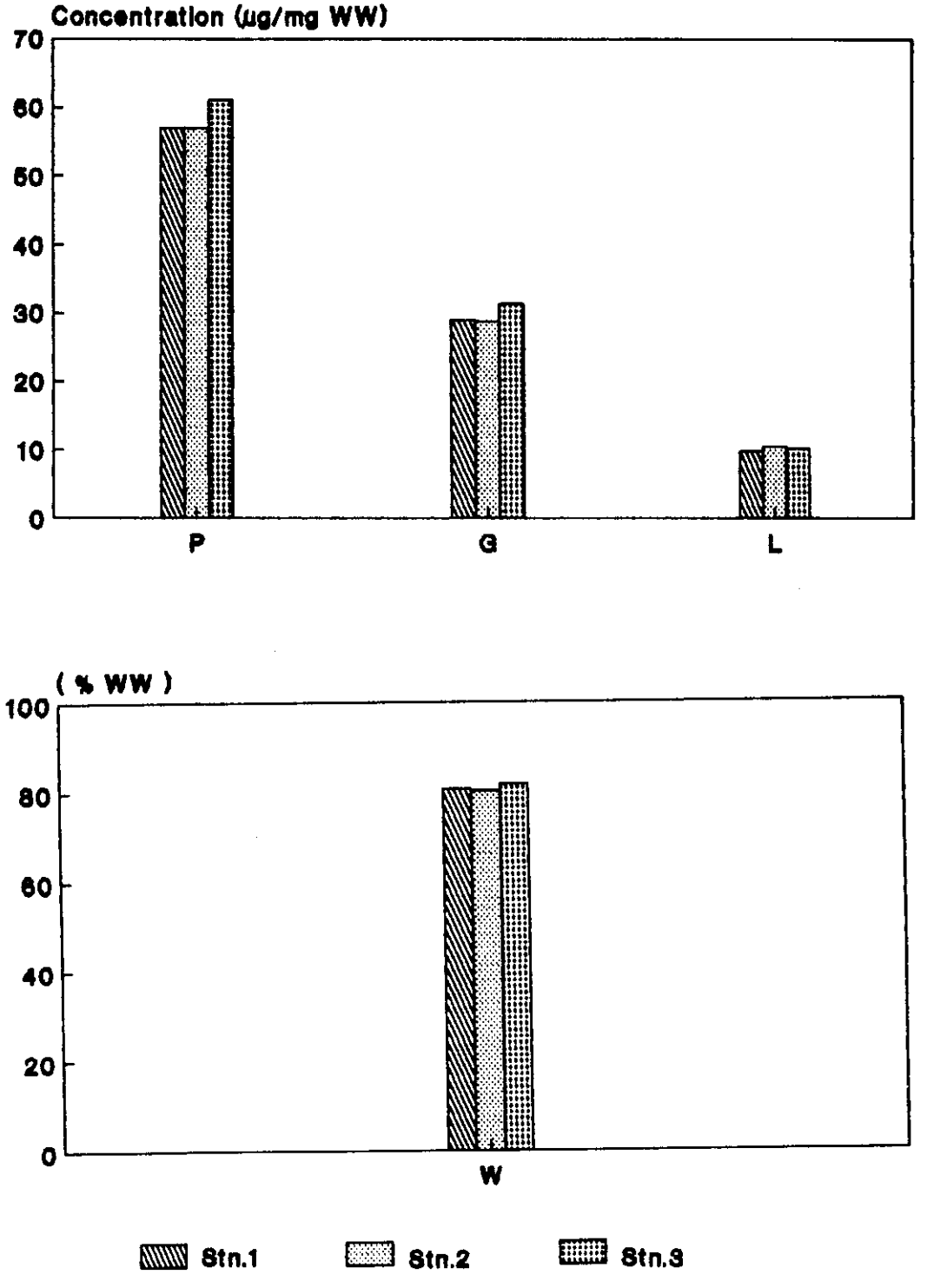

Fig.20 Crassostrea madrasensis. Various biochemical constituents ((P) Protein (G) Glycogen (L) Lipid (W) Water content) in the adductor muscle of individuals from stations 1 to 3.

Table 25 a-d. Crassostrea madrasensis. Analysis of variance (ANOVA) of the biochemical components in the adductor muscle of individuals from stations 1 to 3, along with their level of significance.

25a. Protein

Source	SS	Df	Mean Sqr	F
Total	23346.93	89	--	
Station	287.219	2	143.609	0.54
Error	23159.72	87	266.204	

25b. Glycogen

Source	SS	Df	Mean Sqr	F
Total	12687.24	89	--	
Station	132.422	2	66.211	0.46
Error	12554.82	87	144.308	

25c. Lipid

Source	SS	Df	Mean Sqr	F
Total	1945.081	89	--	
Station	27.785	2	13.893	0.63
Error	1917.296	87	22.038	

25d. Water content

Source	SS	Df	Mean Sqr	F
Total	903.313	89	--	
Station	29.313	2	14.656	1.21
Error	874	87	12.139	

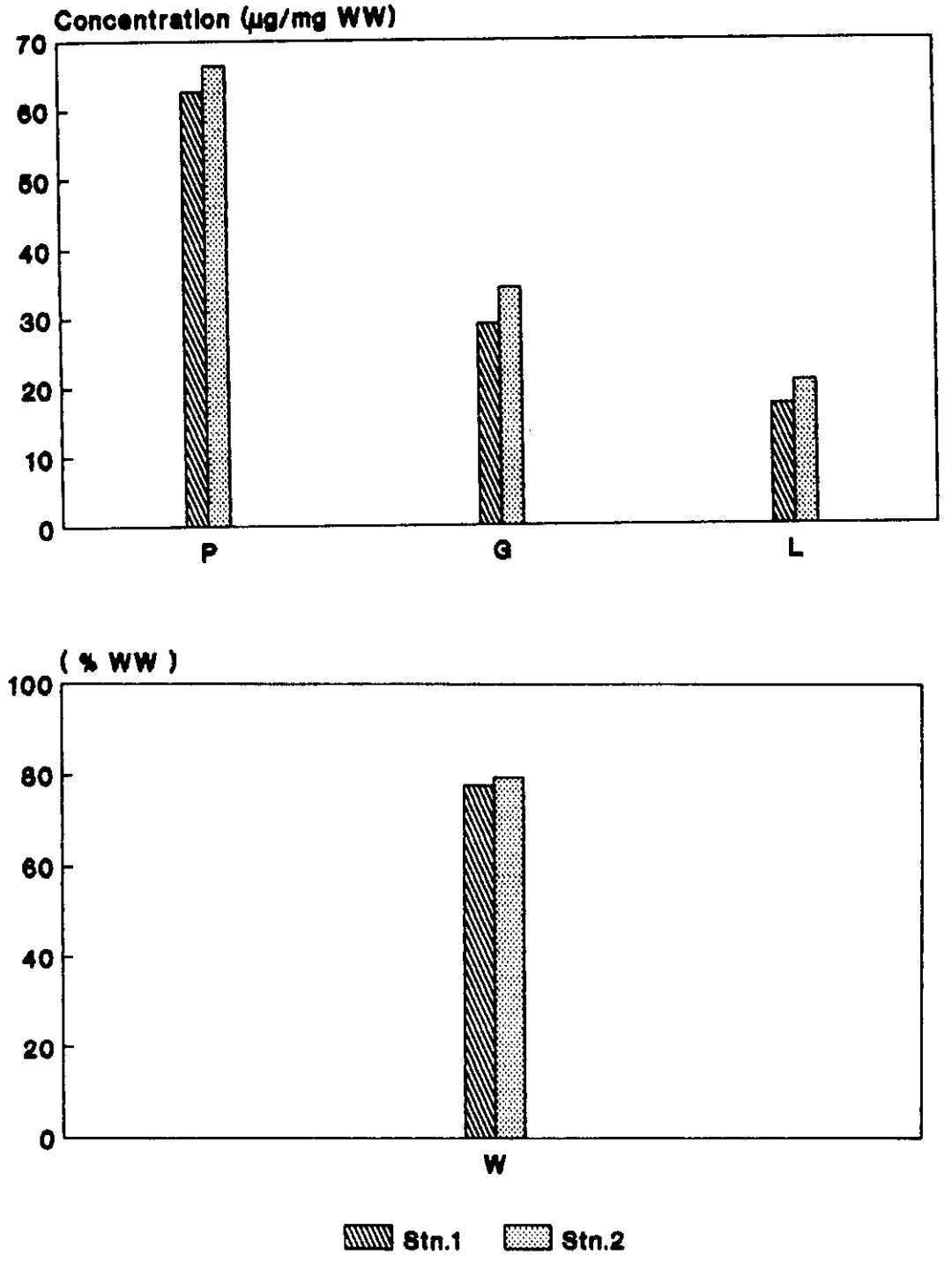

Fig. 21 Perna viridis. Various biochemical constituents ((P) Protein, (G) Glycogen, (L) Lipid (W) Water content) in the adductor muscle of individuals from stations 1 and 2.

Table 26 a-d. Perna viridis. Analysis of variance (ANOVA) of the various biochemical components in the adductor muscle of individuals from stations 1 and 2, along with their level of significance.

26a. Protein

Source	SS	Df	Mean Sqr	F
Total	15634.95	59	--	
Station	193.594	1	193.594	0.73
Error	15441.34	58	266.23	

26b. Glycogen

Source	SS	Df	Mean Sqr	F
Total	7084.004	59	--	
Station	393.465	1	393.465	3.41
Error	6690.539	58	115.354	

26c. Lipid

Source	SS	Df	Mean Sqr	F
Total	1328.549	59	--	
Station	170.684	1	170.684	8.55
Error	1157.965	58	19.965	

26d. Water content

Source	SS	Df	Mean Sqr	F
Total	287.001	59	--	
Station	30.188	1	30.188	5.64
Error	256.813	58	5.35	

6.5. DISCUSSION

Organisms store reserve energy in the form of lipid, glycogen or protein. Regarding fat and glycogen levels, some molluscs especially bivalve species have a glycogen economy with a corresponding low lipid level (Giese, 1966). Synthesis and accumulation of protein have been regarded as the main denominator of true growth by several investigators (Giese, 1969). Further, the nutrient substances accumulated in the form of protein may serve as an energy source during periods of starvation (Giese, 1966; Love, 1970). Detailed comparisons and broad generalizations are difficult in such biochemical studies. This is mainly due to variations in the habitat, season and breeding periodicity of the species concerned. Morevoer, aspects like age and physiological state of such experimental animals are also found to have significant influence.

It is evident from the results obtained that the biochemical constituents of the adductor muscle exhibited some degree of variation among the different populations of the three species sampled from areas of varying salinity regimes. Further, in all the three species, the protein content was relatively high when compared to lipid and glycogen. Thus, of the five populations of V. cyprinoides compared, the highest levels of protein and glycogen in the muscle tissue (53.32 µg/mg and 38.03 µg/mg respectively) were reported from station 3;the lipid content was highest at station 4 (18.75 µg/mg) while the highest water content (82.62%) was reported from station 5 (Table 21). In the case of C. madrasensis, the highest values of protein, glycogen, lipid and water content were reported in individuals from station

3 (Table 22); in P. viridis, the highest value being recorded from station 2 (Table 23).

The storage and utilisation of glycogen reserves reflect the complex interactions between food supply and temperature, and between growth and annual reproductive cycle. The form of the reproductive cycle varies considerably between species and with geographical locality : some species having definite annual cycles while others may breed more or less continuously (Nair and Shynamma, 1975; Ramachandran, 1980; Reddy, 1983; Easterson and Kandasami, 1988). Biochemical studies in bivalves have revealed that glycogen accumulates mainly during the non-reproductive period in the summer (Easterson and Kandasami, 1988) and some bivalves are known to store large amounts of glycogen (11-37%) in their tissues. While in Martesia fragilis values as high as 52% has been reported for glycogen (Srinivasan and Krishnaswamy, 1963), values ranging from 10-35% of glycogen has also been observed in the soft parts of the mussel Mytilus edulis (De Zwaan and Zande, 1972) with extremes of about 60% in Ostrea edulis (Walne, 1970). On the other hand, in most lamellibranchs, the protein content remains at a relatively high level throughout the year and decreases during the period of gametogenic activity and breeding season. The maximum glycogen content in tissues has been observed during the period of gametogenic cycle and decreases with an increase in the fat content. Both glycogen and fat are found to decrease during the spawning period (Naghabhushanam and Thalikhedkar, 1977). Despite all these facts, the results obtained during the present study involving immature individuals of uniform size revealed that the variation found among populations of the same species can be attributed to factors other than those

associated with maturation.

Several workers have reported on the effect of salinity on the water content of marine bivalves (Joshi and Bal, 1965; Deshmukh, 1972; Nagabhushanam and Mane, 1978). In oysters, the water content in the adductor muscle was found to increase up to 92% during periods of low salinity and this increase was ascribed to the loss of salts and gain of water (Nagabhushanam and Mane, 1978). Similar observations were made in the case of Meretrix meretrix (Deshmukh, 1972). Katelysia opima (Nagabhushanam and Mane, 1978), Donax cuneatus (Nagabhushanam and Talikedkar, 1977) and several other species. The increase in water content observed among populations of V. cyprinoides, C. madrasensis and P. viridis sampled from the comparatively less saline areas, in the present study may be explained as due to the effects of salinity on the salt concentration of tissues. It has been observed that in some bivalves, the seasonal fluctuations in the chemical constituents of tissues are reciprocal with the variation in water content (Venkataraman and Chari, 1951; Joshi and Bal, 1965; Deshmukh, 1972). The decrease in glycogen content reported during the present study could be attributed to the utilisation of carbohydrate reserves which are beleived to occur during periods of stress, such as low salinity, rather than to a mere increase in water content. Similar observations were made in respect of C. madrasensis and P. viridis (Table 22 and 23). The marginal variations in moisture content observed between the different populations of the test species may however be explained as due to variations in the habitat salinity.

Besides an inverse relationship between the water content and lipid

levels were evident in almost all populations of the three species. Thus, populations of V. cyprinoides which were characterised by high water content, had a reduced lipid level in their tissues (Table 21). Similar observations were made in the case of C. madrasensis also (Table 22). However, no such relationship was evident among populations of P. viridis (Table 23).

The present series of analyses were carried out employing only immature specimens and avoiding sexually mature ones with a view to minimize the possible fluctuation in the organic constituents associated with reproductive cycle. It is therefore reasonable to assume that the variations in biochemical constituents observed during the present study may perhaps be due to the resultant effect of fluctuations in salinity of the ambient water, nutritional conditions available at different areas and the physiological status of the animal (Suryanarayan and Nair, 1976). A perusal of the data further reveals that mussels are more nutritious than clams and oysters (Fig. 19, 20 and 21) with a relatively high protein content than other biochemical constituents.