Chapter II

Carbohydrate Metabolism

RESULTS

Significant variations in the haemolymph and tissue metabolic variables were observed when shrimps subjected to salinity stress. In the case of metabolic variables, an increase could be observed in shrimps at 30 ppt except TG, which were maximum in shrimps at 0 ppt salinity. Following *V.harveyi* and WSSV challenge, there was a significant enhancement in haemolymph metabolic variables in general on pathogenic infected shrimps. Infected shrimps subjected to salinity stress, a pronounced increased in haemolymph and decreased in tissue metabolic variables was observed in shrimps maintained at 0 ppt, pathogenicity of *V.harveyi* was higher from 30 ppt to 10 ppt and lower in 0 ppt salinity. Yoganandhan *et al.* (2003) reported similar increase in haemolymph metabolites in WSSV-infected *F.indicus*. A similar enhancement of metabolic variables in haemolymph of *P.monodon* was also observed in the previous study after *V.harveyi* challenge. Decrease in tissue metabolites at the later stages of infection may be attributed to the mobilization of energy reserves from the reserve tissues- gill and muscle to meet the energy requirements toward off infection.

Free amino acids: Figure (2.1 & 2.2) present results on the concentration of FAA decreased from 254.6±0.11 to 119.9±0.18 mg/g in muscle and 3.9±0.12 to 0.6±0.12 mg/ml in haemolymph were recorded respectively in under salinity stress PLs. Following *V.harveyi* challenged shrimps shows significantly decreased FAA concentration in muscle (291.6 ± 0.03 to 120.2 ± 0.05 mg/g) and 4.6 ± 0.18 to 0.8 ± 0.22 mg/ml in haemolymph at low salinity adaptation were observed. Similarity same decreasing trend was followed by WSSV infected shrimps at 0 ppt salinity. The mean ± SD values decreased from 335.6 ± 0.04 to 121.5 ± 0.24 mg/g in muscle and 5.1 ± 0.11 to 0.7 ± 0.04 mg/ml in haemolymph of viral infected shrimps were recorded. Higher TFAA concentration was recorded in infected shrimps held at 30 ppt compared to under salinity stress respectively.

Lipids: Shrimps subjected to 0 ppt stress showed a significantly decreased in total lipid level were observed in muscle (43.5 ± 0.02 to 10.2 ± 0.02 mg/g) and gill (28.6 ± 0.12 to 5.6 ± 0.05 mg/g) compared to controls. Similar decreasing levels were observed in *V.harveyi* challenged shrimps subjected to hypo osmotic stress respectively. Muscle lipid levels were decreased from 30.6 ± 0.21 to 8.5 ± 0.05 mg/g and 36.6 ± 0.04 to 9.5 ± 0.12 mg/g in gill were recorded. Viral infected shrimps shows gradual decrease of lipids in

muscle (36.6 ± 0.04 to 9.5 ± 0.12 mg/g) and gill (22.4 ± 0.01 to 5.4 ± 0.11 mg/g) were registered at 0 ppt salinity compared to high salinity (Figure-2.3 & 2.4).

Free Fatty Acids: Figure- (2.5 & 2.6) present results represents a gradual decrease register from 34.6±0.15 to 9.2 ± 0.12 in muscle and 15.5±0.13 to 1.1±0.13 mg/g in gill free fatty acids concentration was noted in shrimps at hypo osmotic stress ($P<0.05$) compared to control group. When challenged with Pathogen decreasing was observed in the FFA concentration in shrimps at all reducing salinities compared to under salinity stress shrimps. *V.harveyi* challenged Shrimps maintained at 0 ppt registered comparatively lower FFA levels in muscle 22.7 ± 0.24 to 7.9 ± 0.25 and gill 8.5 ± 0.06 to 0.8 ± 0.05 mg/g were recorded respectively. Similarly viral infected shrimps showed same decreasing trend was followed. The FFA levels were progressively reduced from 27.8 ± 0.02 to 8.5 ± 0.15 in muscle and 11.5 ± 0.15 to 0.9 ± 0.12 mg/g in gill respectively in viral infected shrimps maintained at 0 ppt.

Triglycerides: The results clearly demonstrated that there was a reversible enhanced from 10.7 ± 0.11 to 41.6 ± 0.13 in gill and 20.5 ± 0.12 to 58.6 ± 0.23 mg/g in muscle were observed in triglycerides level at 0 ppt after transfer from 30 ppt salinity ($P< 0.05$) respectively. Following *V.harveyi* challenge, a steady increase was noticed in the triglycerides concentration in shrimps that was more pronounced at low salinity. Triglycerides levels were recorded (Mean ± S.D) in muscle 28.9 ± 0.21 to 58.6 ± 0.35 and 16.2 ± 0.01 to 44.3 ± 0.15 mg/g in gill tissues at low salinity were recorded. Similar results were observed in viral infected shrimps at low salinity stress. Comparatively high in triglycerides concentrations were obtained in infected shrimps held at 0ppt compared to under salinity stress shrimps respectively (Figure- 2.7 & 2.8).

Proteins: Figure (2.9 & 2.10) present results on the concentration of proteins decreased from 187.9 ± 0.02 to 87.6 ± 0.11 mg/g in muscle and 32.3 ± 0.12 to 7.6 ± 0.25 mg/g in gill were recorded respectively under salinity stress PLs. Following *V.harveyi* challenged shrimps shows significantly decreased protein concentration in muscle (150.8 ± 0.21 to 78.6 ± 0.05 mg/g) and 26.4 ± 0.01 to 7.1 ± 0.13 mg/g in gill at low salinity adaptation were observed. Similarity same decreasing trend was followed by WSSV infected shrimps at 0 ppt salinity. The mean ± SD values from 167.9 ± 0.23 to 82.6 ± 0.23 in muscle and 26.4 ± 0.05 to 7.1 ± 0.13 mg/g in gill of viral infected shrimps were

recorded. Higher protein concentration was recorded in infected shrimps held at 30 ppt compared to under salinity stress respectively.

Carbohydrates: Figure (2.11 & 2.12) present results represents a gradual decrease register from 20.5 ± 0.11 to 2.9 ± 0.11 in gill and 56.4 ± 0.10 to 25.6 ± 0.01 mg/g in muscle free fatty acids concentration was noted in shrimps at hypo osmotic stress ($P < 0.05$) compared to control group. When challenged with Pathogen decreasing was observed in the FFA concentration in shrimps at all reducing salinities compared to under salinity stress shrimps. *V.harveyi* challenged shrimps maintained at 0 ppt registered comparatively lower FFA levels in muscle 22.7 ± 0.24 to 23.4 ± 0.02 and gill 10.5 ± 0.15 to 2.4 ± 0.32 mg/g were recorded respectively. Similarly viral infected shrimps shows same decreasing trend was followed. The FFA levels were progressively reduced from 48.6 ± 0.14 to 4.6 ± 0.13 in muscle and 15.6 ± 0.02 to 2.6 ± 0.03 mg/g in gill respectively in viral infected shrimps maintained at 0 ppt.

Glycogen: The results evidently show that the concentration of glycogen decreased significantly in shrimps subjected to hypo osmotic stress ($P < 0.05$) and increased at control group in muscle and gills. The values 68.4 ± 0.12 to 34.6 ± 0.11 mg/g in muscle and 27.6 ± 0.11 to 5.2 ± 0.02 mg/g in gill were recorded at low salinity levels. *V.harveyi* challenged groups shows decreased glycogen concentration in muscle from 50.2 ± 0.32 to 29.1± 0.02 and 16.5 ± 0.22 to 4.2 ± 0.01 mg/g in gills at 0 ppt salinity adaptation. Glycogen levels were significantly lower at reducing salinities in under salinity stress compared pathogenic challenge shrimps. Viral infected PLs showed a reducing from 57.9 ± 0.15 to 32.2 ± 0.04 mg/g glycogen levels in muscle and 20.5 ± 0.02 to 4.7 ± 0.21 mg/g in gill were recorded respectively (Fig- 2.13 & 2. 14).

Figure - 2.1: Total Free Amino acid content in the haemolymph of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05).

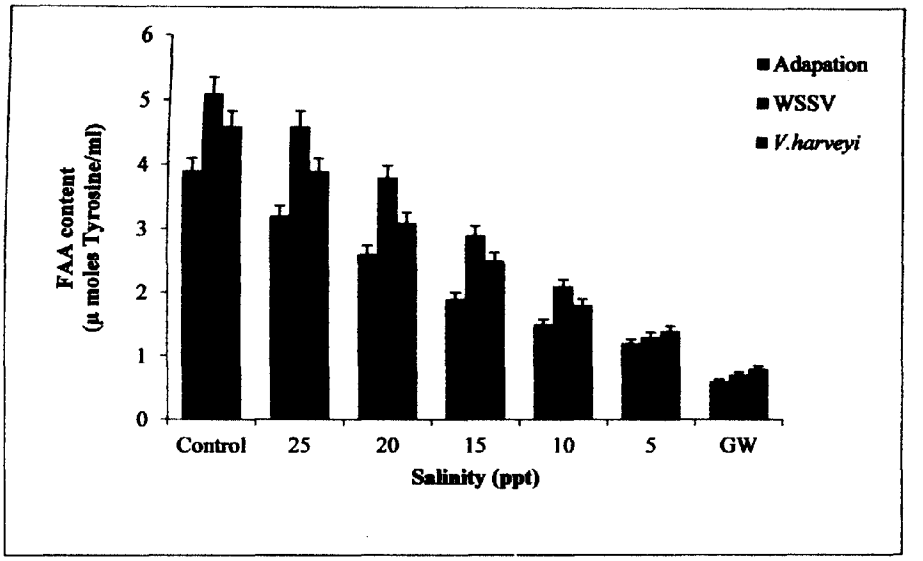

Figure 2.2: Total Free amino acid content in muscle of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05)

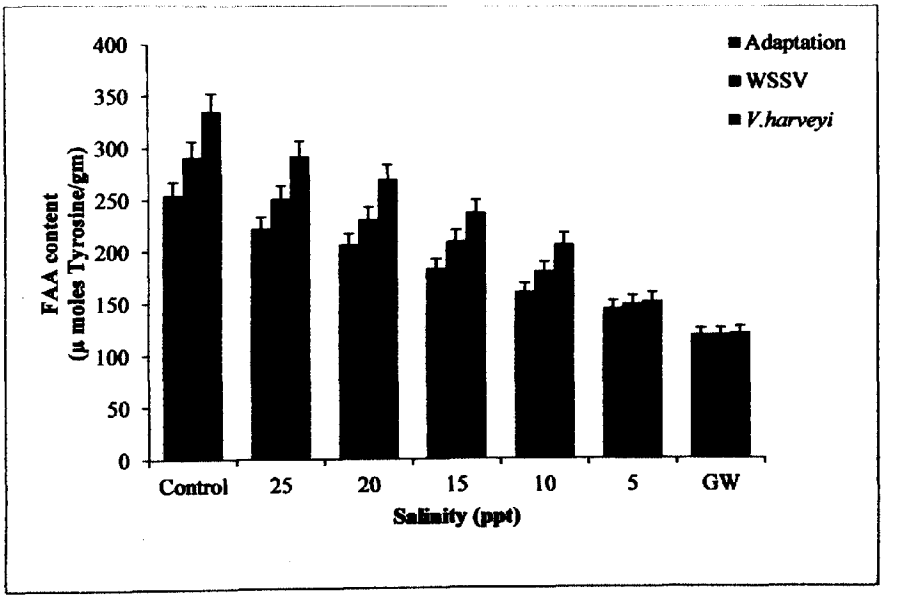

Figure-2.3: Lipid content in the gill of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05).

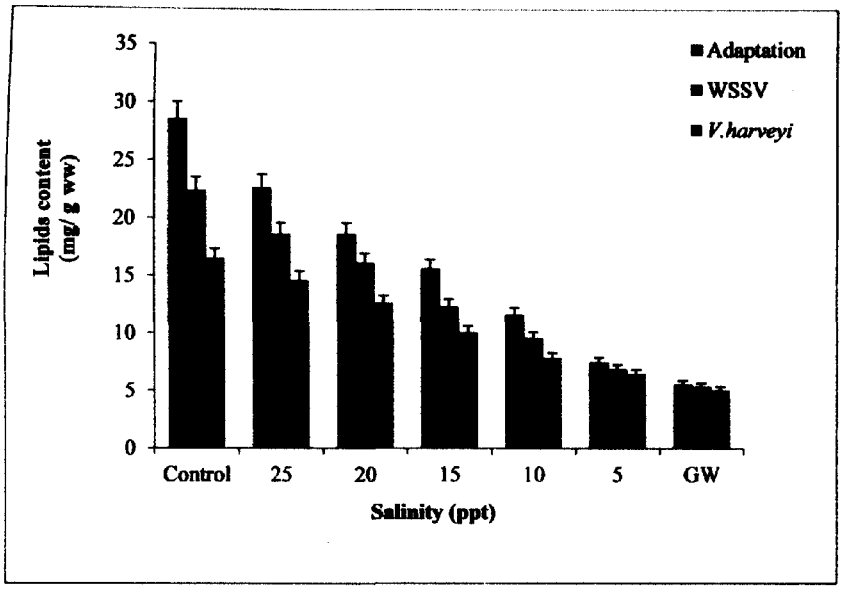

Figure-2.4: Lipid content in the muscle of PLs of *P.monodon* subjected to salinity Stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05).

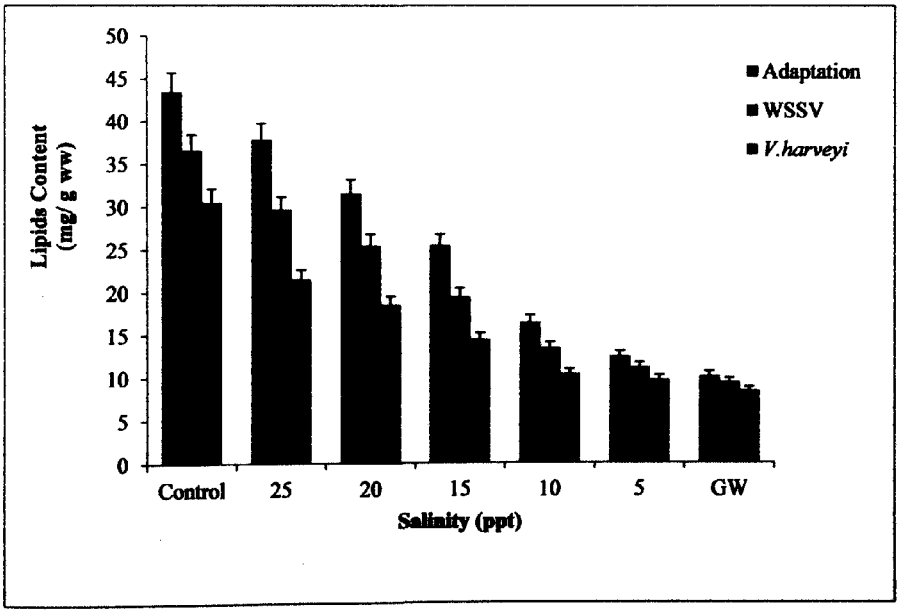

Figure-2.5: Free fatty acid content in gill of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05).

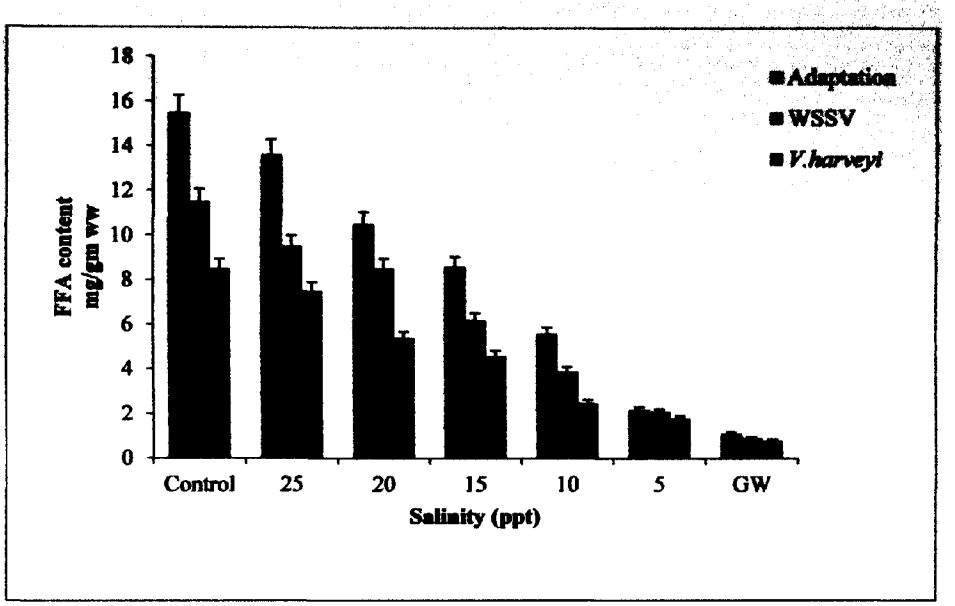

Figure-2.6: Free fatty acid content in muscle of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05)

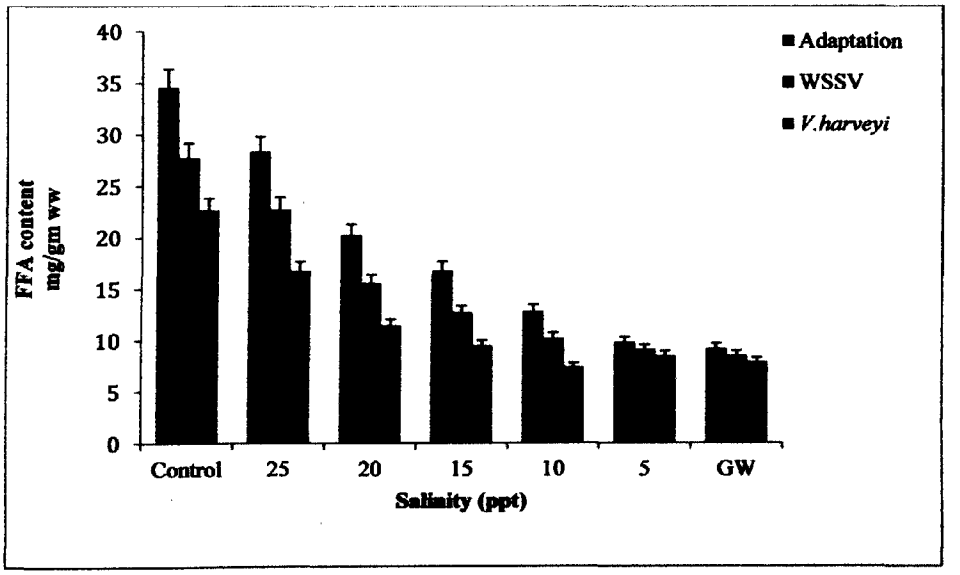

Figure-2.7: Triglyceride content in gill of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05).

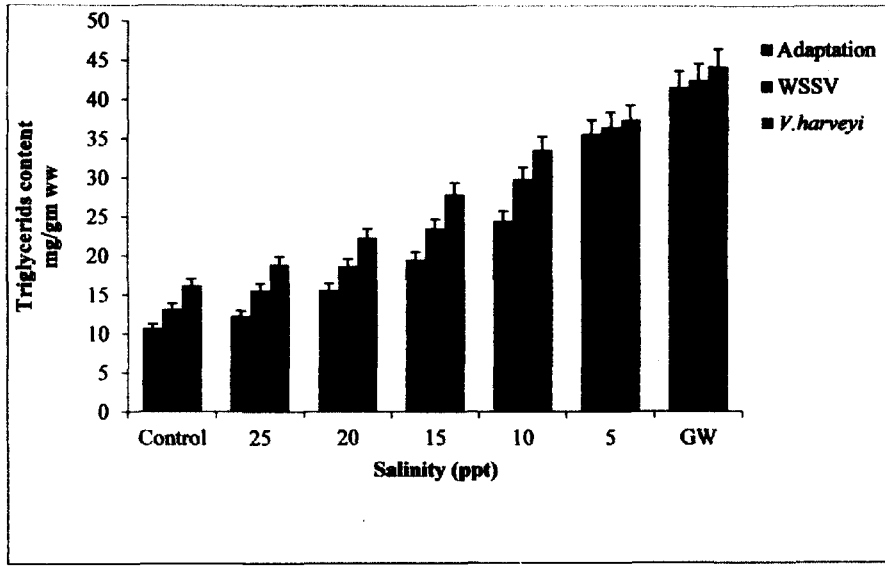

Figure- 2.8: Triglyceride content in muscle of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (value are mean ± SD of 6 observations; P< 0.05).

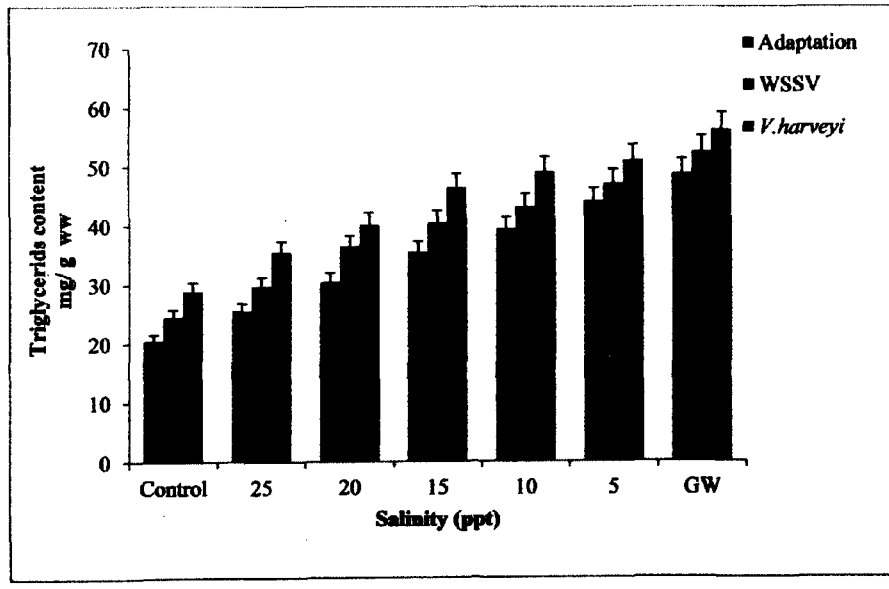

Figure- 2.9: Protein content in gill of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (Value are mean ± SD of 6 observations; P< 0.05).

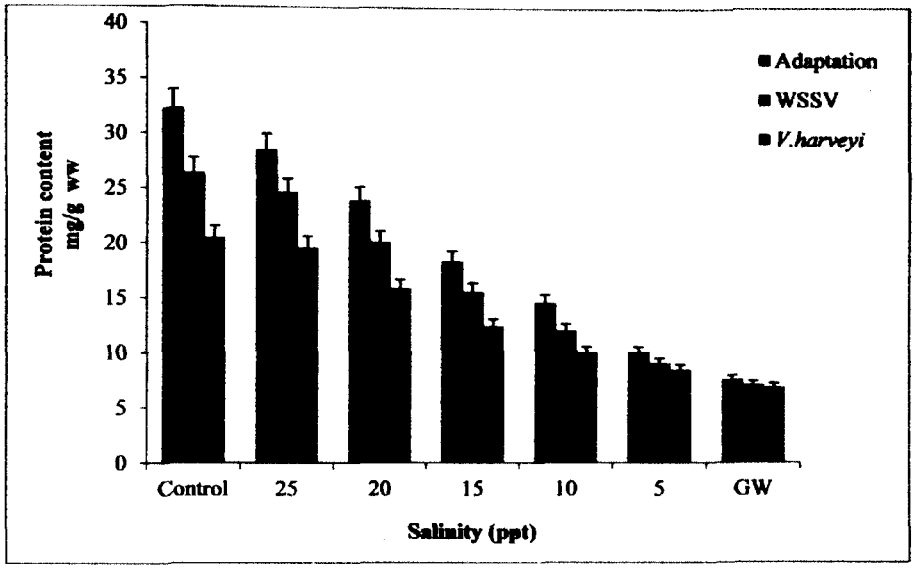

Figure-2.10: Protein content in muscle of PLs of *P.monodon* subjected to salinity Stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (Value are mean ± SD of 6 observations; P< 0.05).

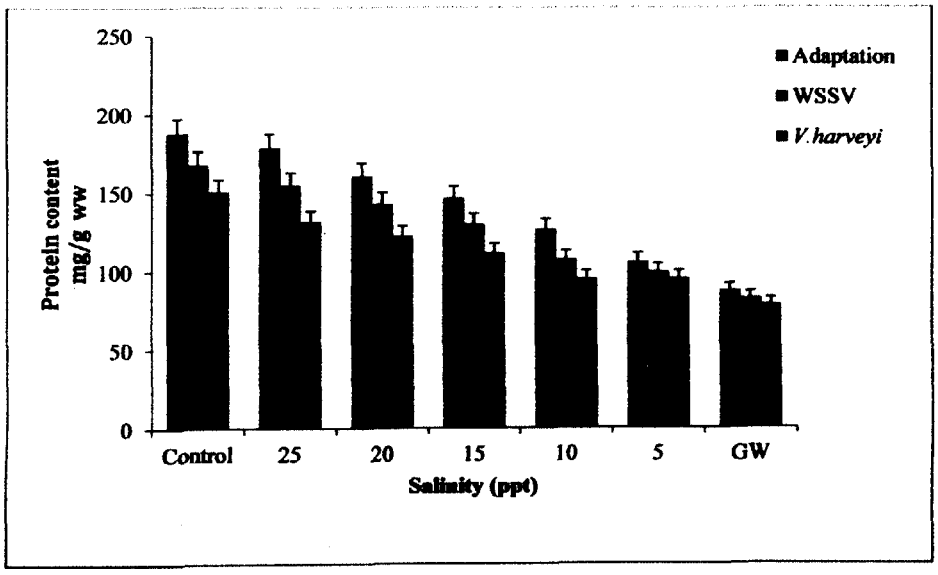

Figure- 2.11: Carbohydrate content in gill of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (Value are mean ± SD of 6 observations; P< 0.05).

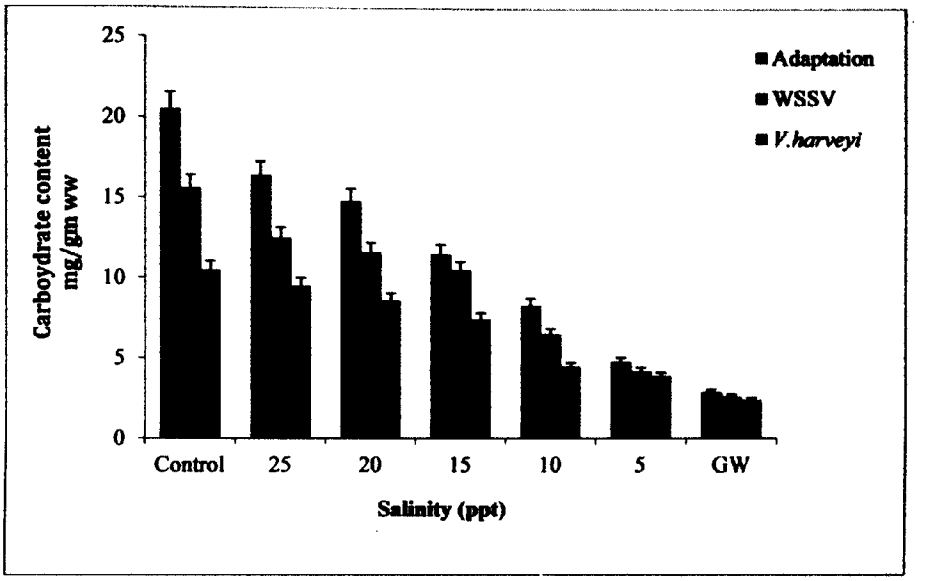

Figure-2.12: Carbohydrate content in muscle of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (Value are mean ± SD of 6 observations; P< 0.05).

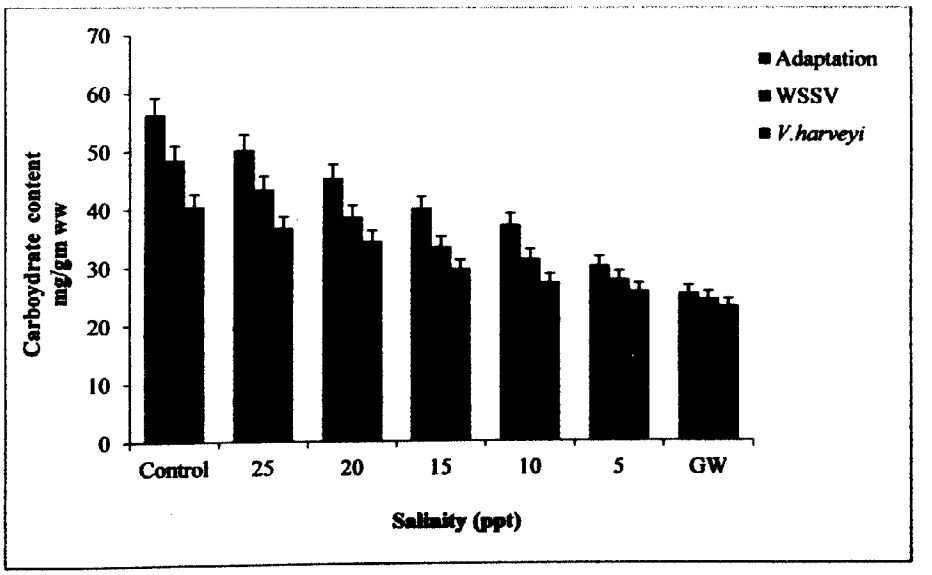

Figure-2.13: Glycogen content in gill of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (Value are mean ± SD of 6 observations; P< 0.05).

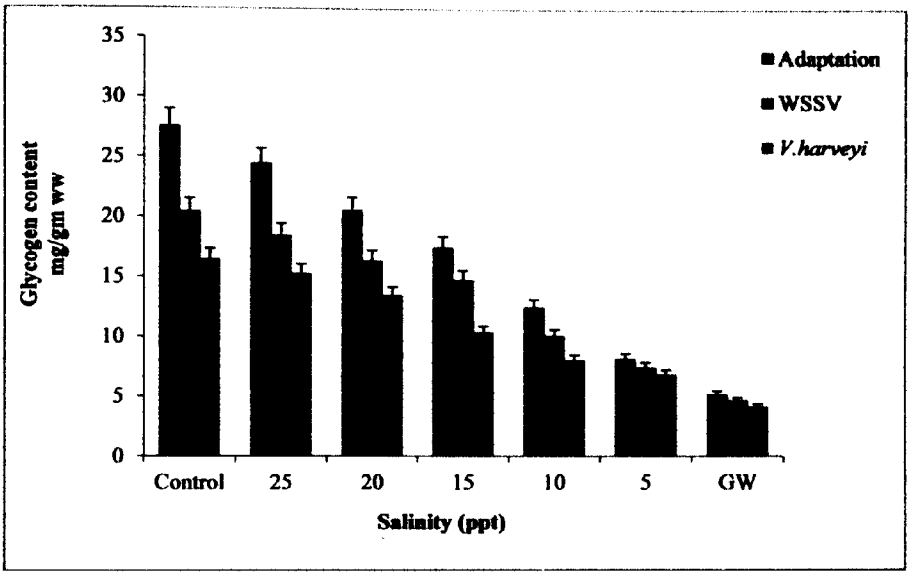

Figure-2.14: Glycogen content in muscle of PLs of *P.monodon* subjected to salinity stress and challenged with *V.harveyi*, White Spot Syndrome Virus. (Value are mean ± SD of 6 observations; P< 0.05).

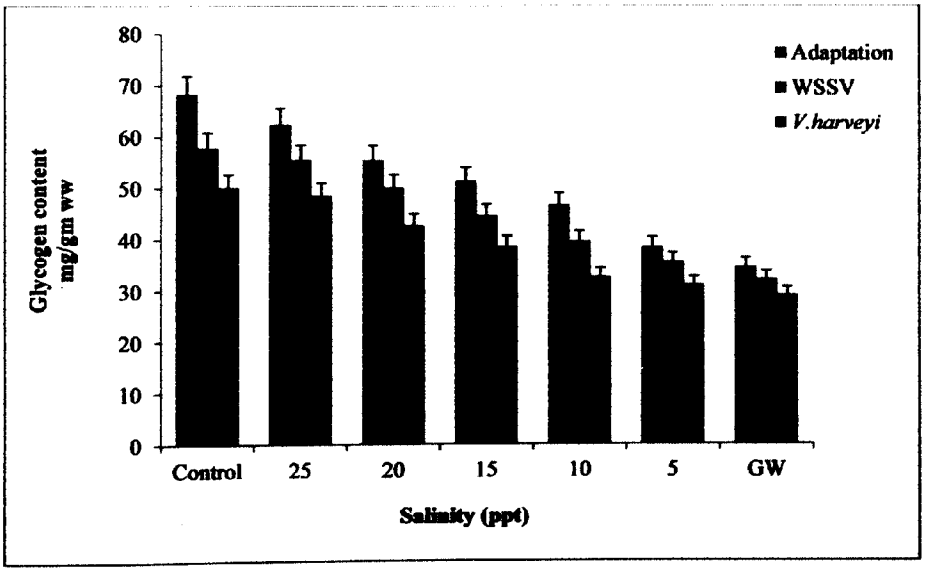

Table-2.1 Influence of salinity changes on biochemical variables in gills of PLs of *Penaeus monodon* during *V.harveyi* and White Spot Syndrome Virus challenge.(values are mean ± SD of six individual observations).

S.No	Name of the Experiment	Under Stress Condition	Salinity (ppt)						
			30	25	20	15	10	5	GW
1.	Free amino acids	Adaptation	3.9 ± 0.14	3.2 ± 0.11	2.6 ± 0.03	1.9 ± 0.01	1.5 ± 0.05	1.2 ± 0.04	0.6 ± 0.07
		WSSV	5.1 ± 0.11	4.6 ± 0.05	3.8 ± 0.02	2.9 ± 0.15	2.1 ± 0.18	1.3 ± 0.09	0.7 ± 0.04
		V.harveyi	4.6 ± 0.18	3.9 ± 0.22	3.1 ± 0.11	2.5 ± 0.24	1.8 ± 0.12	1.4 ± 0.23	0.8 ± 0.22
2.	Lipid	Adaptation	28.6 ± 0.12	22.6 ± 0.05	18.6 ± 0.15	15.6 ± 0.11	11.6 ± 0.12	7.5 ± 0.04	5.6 ± 0.05
		WSSV	22.4 ± 0.01	18.5 ± 0.04	16.1 ± 0.08	12.3 ± 0.15	9.6 ± 0.16	6.9 ± 0.11	5.4 ± 0.11
		V.harveyi	16.5 ± 0.11	14.6 ± 0.05	12.6 ± 0.15	10.1 ± 0.14	7.9 ± 0.24	6.5 ± 0.21	5.1 ± 0.08
3.	Free fatty acids	Adaptation	15.5 ± 0.11	13.6 ± 0.32	10.5 ± 0.15	8.6 ± 0.08	5.6 ± 0.04	2.2 ± 0.02	1.1 ± 0.21
		WSSV	11.5 ± 0.15	9.5 ± 0.02	8.5 ± 0.11	6.2 ± 0.05	3.9 ± 0.01	2.1 ± 0.09	0.9 ± 0.12
		V.harveyi	8.5 ± 0.06	7.5 ± 0.09	5.4 ± 0.05	4.6 ± 0.11	2.5 ± 0.24	1.8 ± 0.22	0.8 ± 0.05
4.	Triglycerides	Adaptation	10.7 ± 0.11	12.3 ± 0.07	15.6 ± 0.01	19.5 ± 0.15	24.5 ± 0.19	35.6 ± 0.09	41.6 ± 0.13
		WSSV	13.2 ± 0.05	15.6 ± 0.23	18.6 ± 0.12	23.5 ± 0.06	29.8 ± 0.07	36.5 ± 0.06	42.5 ± 0.11
		V.harveyi	16.2 ± 0.01	18.9 ± 0.22	22.3 ± 0.32	27.9 ± 0.05	33.6 ± 0.12	37.4 ± 0.03	44.3 ± 0.15

5	Protein	Adaptation	32.3 ± 0.12	28.5 ± 0.01	23.9 ± 0.06	18.4 ± 0.14	14.6 ± 0.03	10.1 ± 0.08	7.6 ± 0.25
		WSSV	26.4 ± 0.05	24.6 ± 0.21	20.1 ± 0.06	15.6 ± 0.07	12.1 ± 0.12	9.1 ± 0.25	7.1 ± 0.13
		V.harveyi	20.5 ± 0.11	19.6 ± 0.01	15.9 ± 0.25	12.5 ± 0.12	10.1 ± 0.16	8.5 ± 0.04	6.9 ± 0.06
6	Carbohydrate	Adaptation	20.5 ± 0.11	16.4 ± 0.01	14.8 ± 0.04	11.5 ± 0.01	8.3 ± 0.14	4.8 ± 0.21	2.9 ± 0.11
		WSSV	15.6 ± 0.02	12.5 ± 0.24	11.6 ± 0.11	10.5 ± 0.01	6.5 ± 0.04	4.2 ± 0.22	2.6 ± 0.03
		V.harveyi	10.5 ± 0.15	9.5 ± 0.01	8.6 ± 0.22	7.4 ± 0.11	4.5 ± 0.11	3.9 ± 0.24	2.4 ± 0.32
7	Glycogen	Adaptation	27.6 ± 0.11	24.5 ± 0.04	20.6 ± 0.21	17.5 ± 0.32	12.5 ± 0.03	8.2 ± 0.23	5.2 ± 0.02
		WSSV	20.5 ± 0.02	18.5 ± 0.35	16.4 ± 0.22	14.8 ± 0.15	10.1 ± 0.01	7.5 ± 0.32	4.7 ± 0.21
		V.harveyi	16.5 ± 0.22	15.3 ± 0.03	13.5 ± 0.51	10.4 ± 0.22	8.1 ± 0.15	6.9 ± 0.04	4.2 ± 0.01

Values similarly marked are significantly different ($P<0.05$) from each other up to 30 ppt to 0 ppt, after that not significantly different values were observed between under salinity shock and challenged shrimps.

Table-2.2 Influence of salinity changes on biochemical variables in muscle of PLs of *Penaeus monodon* during *V.harveyi* and White spot syndrome virus challenge.(values are mean ± SD of six individual observations).

S.NO	Name of the Experiment	Under Stress Condition	Salinity (ppt)						
			30	25	20	15	10	5	GW
1.	Free amino acids	Adaptation	254.6 ± 0.01	222.6 ± 0.12	207.1 ± 0.14	183.6 ± 0.13	161.5 ± 0.14	145.5 ± 0.12	119.9 ± 0.11
		WSSV	291.6 ± 0.03	251.4 ± 0.17	231.6 ± 0.02	210.4 ± 0.02	181.4 ± 0.15	150.1 ± 0.18	120.2 ± 0.05
		V.harveyi	335.6 ± 0.04	292.5 ± 0.02	270.6 ± 0.01	238.5 ± 0.04	207.6 ± 0.02	152.8 ± 0.03	121.5 ± 0.24
2.	Lipid	Adaptation	43.5 ± 0.02	37.9 ± 0.15	31.6 ± 0.16	25.5 ± 0.25	16.5 ± 0.15	12.5 ± 0.16	10.2 ± 0.02
		WSSV	36.6 ± 0.04	29.7 ± 0.02	25.5 ± 0.02	19.3 ± 0.25	13.6 ± 0.18	11.2 ± 0.21	9.5 ± 0.12
		V.harveyi	30.6 ± 0.21	21.6 ± 0.03	18.6 ± 0.23	14.5 ± 0.21	0.9 ± 0.04	9.8 ± 0.16	8.5 ± 0.05
3.	Free fatty acids	Adaptation	34.6 ± 0.15	28.4 ± 10.5	20.3 ± 0.02	16.8 ± 0.15	12.8 ± 0.25	9.8 ± 0.14	9.2 ± 0.21
		WSSV	27.8 ± 0.02	22.8 ± 0.21	15.6± 0.32	12.7 ± 0.14	10.2 ± 0.23	9.1 ± 0.01	8.5 ± 0.15
		V.harveyi	22.7 ± 0.24	16.8 ± 0.21	11.5± 0.02	9.5 ± 0.21	7.4 ± 0.45	8.5 ± 0.12	7.9 ± 0.25

4.	Triglycerides	Adaptation	20.5 ± 0.12	25.6 ± 0.05	30.5 ± 0.02	35.6 ± 0.13	40.3 ± 0.21	54.3 ± 0.02	58.6 ± 0.23
		WSSV	24.5 ± 0.03	29.8 ± 0.03	36.5 ± 0.32	40.5 ± 0.03	46.5 ± 0.03	56.2 ± 0.03	59.6 ± 0.21
		V.harveyi	28.9 ± 0.21	35.6 ± 0.02	42.2 ± 0.22	46.5 ± 0.15	54.2 ± 0.02	58.6 ± 0.23	58.6 ± 0.35
5	Protein	Adaptation	187.9 ± 0.02	178.6 ± 0.05	160.7 ± 0.12	146.7 ± 0.21	126.8 ± 0.17	106.2 ± 0.01	87.6 ± 0.11
		WSSV	167.9 ± 0.23	154.7 ± 0.12	142.7 ± 0.21	130.1 ± 0.32	107.6 ± 0.32	99.6 ± 0.15	82.6 ± 0.23
		V.harveyi	150.8 ± 0.21	131.7 ± 0.11	122.5 ± 0.15	111.9 ± 0.02	95.4 ± 0.36	93.6 ± 0.14	78.6 ± 0.05
6	Carbohydrate	Adaptation	56.4 ± 0.10	50.4 ± 0.24	45.5± 0.20	40.2± 0.11	37.4 ± 0.04	30.4 ± 0.31	25.6 ± 0.01
		WSSV	48.6 ± 0.14	43.6 ± 0.02	38.8± 0.13	33.6± 0.21	31.5 ± 0.14	28.2 ± 0.02	24.6 ± 0.13
		V.harveyi	40.6 ± 0.21	37.2 ± 0.11	34.6 ± 0.02	29.8 ± 0.31	27.5± 0.21	26.2 ± 0.04	23.4 ± 0.02
7	Glycogen	Adaptation	68.4 ± 0.12	62.4 ± 0.01	55.5 ± 0.06	51.4 ± 0.14	46.7 ± 0.03	38.5 ± 0.31	34.6 ± 0.11
		WSSV	57.9 ± 0.15	55.6 ± 0.05	50.1 ± 0.32	44.6 ± 0.04	39.6 ± 0.22	35.6 ± 0.24	32.2 ± 0.04
		V.harveyi	50.2 ± 0.32	48.6 ± 0.21	42.8 ± 0.15	38.7 ± 0.01	32.7 ± 0.14	31.6 ± 0.23	29.1± 0.02

Values similarly marked are significantly different ($P<0.05$) from each other up to 30 ppt to 0 ppt, after that not significantly different values were observed between under salinity shock and challenged shrimps.

DISCUSSION

The levels of various bio molecules such as proteins, free amino acids, total carbohydrates, lipids, glycogen, free fatty acids and triglycerides in different tissues of organism are expressions of adaptive mechanism and strategies for adaptation in a particular environment. Many biotic factors (breeding cycle of the animal, availability of the food) and abiotic factors (salinity and tidal cycles) strongly affect the biochemistry and physiology of crustaceans (Rosa & Nunes, 2003 and Vinagre *et al.*, 2006).

Concentration of tissue metabolites in shrimps subjected to salinity stress was less compared to that in the infected shrimps at 30 ppt following challenge. However, there was difference in the performance of gill and muscle metabolic variables between the shrimps at 0 ppt and 30 ppt stress except for the Triglyceride level (TG) and FAA. A significant reduction of tissue metabolites in shrimps under salinity stress could be explained as a deviation in the energy flow to support osmotic work as they were under a dual stress (salinity stress and pathogenic stress). A metabolic stress probably resulted as they were spending less energy for osmoregulation and they can able to function effectively against infection. According to previous workers salinity itself has very little effect on the metabolic rate of euryhaline shrimp (Bishop *et al.*, 1980 and Gaudy & Sloane, 1981). Since *P.monodon* were subjected to salinity acclimatisation in the present investigation, a rapid change in the osmotic concentration of the haemolymph caused osmotic stress and consequent metabolic adjustments. An increase in tissue metabolites was seen at 30 ppt with the exception of TG. Metabolic rate also might have altered significantly in low salinity compared to those maintained at high salinity. The disturbed animal naturally required time to reach a steady state of equilibrium. *P.monodon* required 3-4 days to stabilize the haemolymph as reported by Castille and Lawrence (1981). The entry of virus placed an additional burden on the metabolic requirements of the animal contributing to a relative reduction in the levels of metabolic constituents in the tissue. Following *V.harveyi* challenge, there was an overall decrease in the levels of tissue metabolic variables in *P.monodon* maintained at 30 ppt and gradually increased with decreasing salinities. Most probably, metabolites were transported to the haemolymph from gill and muscle to meet the energy requirements to ward off infection. Yoganandhan *et al.* (2003) could observe a reduction in the total carbohydrate and glucose levels in muscle and hepatopancreas of

WSSV-infected *F. indicus* and a corresponding increase in the haemolymph. Compared to those at 30 ppt, a general reduction in the metabolic variables could be observed in shrimps under salinity stress and following pathogenic challenged shrimps.

Free amino acids (FAA) are known to play a major role in osmoregulation of marine invertebrates (Deaton *et al.*, 1984 and DallaVia, 1986). In cray fish and crab, more than 40 to 60% of the intracellular osmolarity has been contributed by FAA (Shaw, 1958 and Robertson, 1961). In the present study, decreased concentration of amino acids was noted in the shrimps at lower salinity and highest concentration of FAA being at 30%. Which may be a physiological adaptation for osmoregulation. As far as osmotic stress is concerned, it has been demonstrated that euryhaline crabs, such as *C.maenas* and *Callinectes sapidus*, showed increased blood protein content associated with decreased blood osmolality. This process might be related to the stored of amino acids leaking out of the cells during the volume control process (Pequeux *et al.*, 1979). In rotifer, *Brachionus plicatilis* free amino acid content increased due to an increase in salinity from 0.5% to 28% (Frolov *et al.*, 1991).

Gills (1998) also found that the amount of total protein in the haemolymph of euryhaline crustaceans decreased significantly after transfer to a more concentrated salinity. The concentrated FAA found in the haemolymph lower than that in their muscle . The effect of environmental salinity on the FAA composition and concentration in penaeid shrimp has also been examined by Dalla (1986). The shrimp employ a mechanism common among invertebrates for adjusting intracellular volume: decreasing the intracellular pool of organic osmolytes as measured by changes in muscle and haemolymph TNPS. As extracellular fluid becomes less concentrated, the osmotic equilibrium with the intracellular fluid is disrupted, water is gain from the intracellular compartment and cell swelling results. Large changes in cell volume can disrupt normal cell function and lead to cell death (Deaton & Pierce, 1994). Many invertebrates adjust intracellular osmotic concentration (therefore cell volume) by regulating the size of an intracellular pool of amino acids. This pool is decreased in response to an decrease in salinity in order to retain osmotically obligated water and thus restore cell volume. In *P.monodon* the decrease in the muscle TNPS pool coincided with the decrease in haemolymph osmolality.

Total free amino acids level increased in haemolymph of *V.harveyi* challenged shrimps maintained at 30 ppt. Lo *et al.* (1997) reported the high concentrations of amino acids in the haemolymph of crustaceans due to WSSV heavy load, confirmed by PCR detection. Another possibility for the increase of amino acids content in haemolymph and decrease in muscle as well as gill of infected shrimps is that baculoviruses (Beckage, 1996).

The results presented in Fig- (2.1 & 2.2) suggest that FAA seemed to play an important role in osmoregulation. But there is also a general energy expenditure that is required to maintain ionic pumps for ion exchange and other energy costs associated with osmoregulation. Decrease in total FAA concentration at low salinity is correlated with the appearance of the capacity of hyperosmoregulate in post larvae. Same results were observed in Lobster *Homarus gammarus* (Haond *et al.*, 1999). The amount of FAA in the haemolymph of *P.monodon* was less than 1/50 of that in the muscle. Most of the haemolymph osmolarity is regulated by inorganic ions. Under normal condition, the flux direction of FAA is from haemolymph to cell, not from cell to haemolymph. Results revealed high salinities show higher concentration of FAA, it represents they were abundant and probably more available to tissue cells at this salinity. On the other hand, low concentration of FAA implied more organic molecule could be used or building blocks of cells instead of just an osmolarity regulation reservoir in the haemolymph. Similarly bacterial and viral infected shrimps shows higher FAA concentration levels compared to under salinity stress group. Shrimps maintained at 0 ppt registered comparatively lower FFA levels on challenged shrimps, but no significant differences between under salinity stress shrimps and pathogenic infected shrimps.

The quantity and quality of lipids play significant role throughout life cycle of crustaceans (Zhukova *et al.*, 1998). The lipid reserves and its mobilization are also very important during the osmoregulation of crustaceans. A decrease in lipid concentration was observed after the hypo-osmotic stress, while an increase was observed in control group respectively. Although the effect of salinity on the concentration of lipids and their variation in fatty acid composition are well reported in the literature (Chapelle *et al.*, 1982 and Zwingelstein *et al.*, 1998), studies looking at the effect of salinity on total lipid concentration in crustacean tissues are still scarce. Frolov *et al.* (1991) described a decrease in lipid content in the rotifer *B.plicatilis* at salinities lower than 17 ppt. Lemos

et al. (2001) also recently demonstrated salinity change-induced alteration in lipid content in early postlarvae *Farfantepenaeus paulensis*. He reported that lipid concentration in postlarvae VI–VII was lower at 5 ppt salinity.

Gills lipid concentration seems to be related to hypo-osmotic stress in PLs. At this point, it is interesting to note that lipids not only play a metabolic role in providing energy for almost all Endergonic processes, but they are also extremely important in maintaining the structural and physiological integrity of cellular and sub cellular membranes. During salinity adaptation, two types of mechanisms can be employed to maintain ionic haemolymph homeostasis: (1) 'limiting processes' acting on permeability properties of epithelial structures; and (2) 'compensatory processes' driving the active movement of water and ions. Both mechanisms are essentially linked to lipid metabolism. It is well known that phospholipids play an important role in membrane structure, which ultimately affects ion permeability. They also play a dynamic role in the function of membrane-bound proteins and can modulate enzymatic activity (Fourcans & Jain, 1974; Sandermann, 1978 and Smith & Miller, 1980). This is especially true for the Na^+ active transport, since Na^+K^+-ATPase needs to be closely associated to phospholipids to accomplish its hydrolytic activity and, presumably, its ion transport function (Kimelberg & Papahadjopoulos, 1974 and Chapelle, 1986). The reduction of gill total lipid content during hypo-osmotic stress could indicate that these compounds are serving as energy substrates.

The results presented in Table (2.1 & 2.2) suggest that lipids seem to be the major energy store in crustaceans. Moreover, they are extremely important in maintaining structural and physiological integrity of cellular and sub cellular membranes. During salinity adaptation, energy-demanding mechanisms for haemolymph osmotic and ionic regulation are activated. Thus, the main goal of this work was to verify the possible involvement of lipids as an energy source in the osmotic adaptation process. Gill and muscle lipids were significantly lower in PLs subjected to hypo-osmotic stress than maintained at the control salinity. Our results represents that lipid mobilization and involvement of these compounds in the hypo osmotic acclimation process in *P.monodon*. Like wise, bacterial and viral infected shrimps showed less lipid concentration than under salinity stress PLs.

The proportions of fatty acids in gills were also affected by the salinity challenge, although it only lasted 3 h. In the neutral fraction, the decrease in several saturated and monounsaturated fatty acids, together with an increase in polyunsaturated fatty acids following the exposure to dilute media probably indicate a selective fatty acid oxidation to obtain energy. *C.hanges* in the membrane fatty acid composition in relation to salinity have been widely studied. Chapelle *et al.* (1977) found no differences in the saturation state of fatty acids in the gills of the crab *Eriocheir sinensis* acclimated to freshwater for 14 days. Thuet *et al.* (1988) observed decreased permeability in lobsters exposed to dilute seawater within 3 h of transfer. Thus, postlarvae exposed to a low-salinity challenge could modify the proportion of certain fatty acids present in the membranes as a first step to decrease permeability. The present work was based on the hypothesis that the fatty acid composition in gills could be modified by the salinity, and that changes in the fatty acid composition would affect the osmoregulatory mechanisms. The decrease of fatty acid level in tissue is a usual phenomenon in the infected shrimp (Hameed, 1989), the reason of which is yet to be defined. However, an decrease in lipid concentration occurred after the gradual osmotic shock to 0 ppt, supposedly related to the osmotic acclimation process.

The present results represents fatty acids levels decreased after adapted to hypo salinity stress. However, an increase was noted on 30 ppt. Fatty acids may be used to repair membranes in case of severe osmotic stress that may result in the alteration of membrane permeability. The low Fatty acids concentrations seen at all salinities were considered a clear indication that stress had affected lipid transport. Significant reduction of tissue metabolites in infected shrimp under salinity stress may be attributable to deviation of energy flow towards supporting osmotic adjustment because they are under dual stress (both salinity and infection-related stress). These fatty acids are important for proper growth and maturation (Fig- 2.5 & 2.6).

Increased concentration of triglycerides in all the tissues of *S.serrata* of hypo saline group indicates sparing of fat for the supply of energy in new habitat. Chapelle (1977) reported an increase in triglyceride content in the Chinese crab *Eriocheir sinensis* upon acclimation to fresh water. In increments of tissue triglycerides observed in hypo saline acclimation group may be due to initiation of *de novo* synthesis of fatty acid. *De novo* synthesis of fatty acid upon acclimation in hypo saline water was

confirmed with the observed increased level of fatty acid in hepatopancreas of the crab. The results presented in suggest that Triglycerides seem to play a important role in osmoregulation. They supply energy for PLs when adapted to low salinity levels.

The results presented in Fig- (2.7 & 2.8) suggest that triglycerides concentration in gill and muscle of *P.monodon* acclimated to different salinities. The tissue triglycerides may be provide energy for stress conditions. Likewise, pathogenic group shrimp shows higher triglyceride levels in hypo osmotic shock were observed respectively.

Torres *et al.* (2002) reported that decrease in the protein content of zoea 1 decapods crustacean larvae at the lowest salinity. Proteins are essential in all living organisms, performing various roles ranging from structural to catalytic. The protein pool of any organism is in a continual state of flux, with new proteins entering the pool through protein synthesis and being removed through protein degradation. The rhythm between continual synthesis and degradation of protein is not only vital for tissue maintenance and animal growth but is also important in allowing animals to adapt to the changing environmental conditions (Fraser *et al.*, 2002). Decreased levels of total protein, observed in hypo saline group of present study clearlysignifies the mobilization of protein as a source of energy in the tissue. In low salinity, shrimp need to use protein as source of amino acids to maintain the osmotic pressure and for growth (Claybrook, 1983). During salinity acclimation, a very rapid change in free amino-acid content occurs (Gerard & Gilles, 1972) suggested that the regulation of cell volume after a hypoosmotic change is a rapid process in crustacean. The potential use of protein as a metabolic reserve has also been suggested by Bhat & Wagh (1992) for marine zooplankton. The decline in protein reserves in study after a 4-day exposure low salinity supports the hypothesis that *N. integer* can actively use protein as an energy source under stress conditions. The regulation of intracellular osmotic effectors affects the amino-acid metabolism and hence the protein composition under osmotic stress. Extracellular osmoregulation, is associated with energy expenditure for active ion transport, involving the breakdown of energy-rich compounds such as lipids. These mechanisms produce biochemical changes in terms of lipids and proteins in response to salinity variation (Torres *et al.*, 2002).

In haemolmph pronounced increase in total protein levels at 30 ppt on bacterial challenge groups compared to those under salinity stress groups may be possibly due to decrease in muscle and gills proteins mobilized in to haemolymph for specific immune proteins production against infection. In shrimps under salinity stress proteins might have also been used as an energy source for the production of ATP necessary for osmoregulation as suggested by Noga (2000). Harwood *et al.* (1994) reported that the presence of an abundant viral protein in the haemolymph of *Manduca sexta* larvae infected with *polydnavirus*. Sahul Hameed *et al.* (1998) studied the distribution of WSSV in different tissues including haemolymph by pathogenicity experiments as well as Western blot and their results showed the high concentration of WSSV in the haemolymph.

The results presented in Fig- (2.9 & 2.10) suggest that Protein concentration in the tissue of gill and muscle of *P.monodon* adapted to different salinities. Decreased in protein concentration in gill and muscle of PLs acclimated to low salinities. This indicates protein could impact the availability of energy and the ability of the shrimp to maintain itself at new environmental adaptation. Protein content were gradually reduced with decreases in environmental salinity. Muscle showed higher content of proteins than gills at low salinity adaptation. However, bacterial and viral infected shrimps shows less Protein concentration levels compared to under salinity stress group. Shrimps maintained at 0 ppt registered lower protein levels in tissues of challenged shrimps, but no significant differences between under salinity stress shrimps and infected shrimps were observed respectively.

Carbohydrates are the immediate source of energy in the cells. They play a major role in the cellular metabolism by serving as fuel and providing energy to the cells. Fluctuations in salinity reflect fluctuations in energy demands of the animal, changes in carbohydrate metabolism that would meet the changing energy demands may be expected to stress (Lacerda & Sawaya, 1986 and Santos & Nay, 1987). When crustaceans placed in a medium of lower osmotic pressure, energy-consuming processes are required to maintain the internal osmotic pressure constant. Glucose delivers fast energy in the form of ATP via the process of glycolysis and oxidative phosphorylation and is the major circulating carbohydrate in crustaceans (Morris, 1999). Although lipid and protein utilisation is variable in crustaceans, generally,

carbohydrate is used before lipid and protein as the preferred fuel for metabolic processes (Garret & Grisham, 1995 and Morris, 1999). Depletion in the levels of total carbohydrates by 50 to 70% in hypo saline water acclimated PLs also indicates oxidation of carbohydrates to meet the energy demands. This is essential to maintain internal osmotic pressure of the organism to enable efficient osmoregulation in order to prevent water accumulation and salt loss from their body. Besides, the mobilization of carbohydrates may lead to biosynthesis of fat. Adaptation to hypo-saline environment involves the mobilization of more protein and carbohydrate in order to meet the extra energy demand or which the respiratory rate of mud crab, *Scylla serrata* was increased by 55 to 80% when they were acclimated to low saline water.

The present study indicates change in tissue (carbohydrate) after transfer to dilute sea water. The exposed to low salinity implies the mobilization and use of energy substrate for the function of $Na^{+}K^{+}$-ATPase (or) a general condition of stress. At least in the first case, this energy may be obtained directly from reserve stored in gills in the form of carbohydrate. The strong osmoregulating shrimp *P.monodon* transfer from high to low salinity resulted in marked drop in tissues carbohydrate in end of the experiment. Viral and bacterial infected group results represent decline carbohydrate content in lower salinity compared with high salinity and under hypo osmotic stress (Fig- 2.11 & 2.12).

Glycogen plays a central role in providing energy for metabolism. It is primarily stored in animal tissue as a glycogen (Roach *et al.*, 1998). Glycogen is the principal energy source in both vertebrates and invertebrate, especially during environmental fluctuations (Karlsson 1979; Oliera *et al.*, 2004 and Bacca *et al.*, 2005). Glycogen content in both gill and muscle was significantly depleted after transfer to fresh water. But the depletion occurred earlier in gill than in the muscle in *Oreochromis mossambicus* (Chia-His Chang *et al.*, 2007). The glycogen levels increased in haemolymph and decreased in muscle and gill of WSSV infected shrimp in comparison with healthy shrimp Yoganandhan & Thirupathi *et al.* (2003). Generally, the glucose level increases in infected or stressed animals to ward off the infection or stress. The possibility of high levels of glucose and carbohydrate in haemolymph might be due to the transport of glycogen and carbohydrate from gill and muscle to haemolymph. During stress, shrimp use glycogen as a source of energy (Paterson, 1993).

The results presented in Fig- (2.13 & 2.14) suggest that glycogen concentration in gill and muscle of *P.monodon* acclimated to different salinities. The mobilization of muscle glycogen may provide carbohydrate reserves to fuel glycolysis in gill for operation of ion regulation mechanism during acclimated to salinity change. Carbohydrate plays in energy metabolism for osmoregulation. Energy may be mainly supplied by the oxidation of glucose obtained from the circulation as aresults of carbohydrate metabolism. While glycogen as a local carbohydrate reserve in gills. This results represents decreased in glycogen in gill and muscle of PLs acclimated to low salinities. The glycogen levels were found to be lower in the tissues of pathogenic infected shrimp compared to under salinity stress.

Our results demonstrated that PLs of *P.monodon* can survive in low salinity adaptation. We observed significant depletion in the levels of total carbohydrates as well as glycogen, total proteins and fatty acids along with increased level of free amino acid and triglycerides during low salinity acclimated period, clearly indicates the utilization of these bio molecules provide energy for new environmental adaption via influence of osmoregulation. Significant reduction of tissue metabolites in infected shrimp under salinity stress may be attributable to deviation of energy flow toward supporting osmotic adjustment because they are under dual stress.