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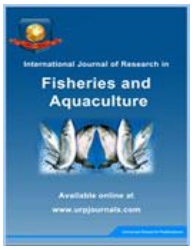

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Original Article

Adaptation to hypo-saline environment of mud crab, *Scylla serrata*: Metabolic changes

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Abstract

The present study envisaged demonstration of metabolism in the mud crab *Scylla serrata* during hyposaline adaptation. The crabs were collected from the estuarine regions of Goa, west coast of India in 6 pre-determined seasons (1 year) and were acclimated for 15 days (from new moon day to full moon day) every season at 1‰ (experimental group) and 12‰ (control group). Significant increase (P<0.001) in the rates of aquatic respiration (55-80%), ammonia (above 90%) and free amino acid (30-45%) excretion along with significant reduction (P<0.001) in the rates of urea (50-75%) and TMAO (15-45%) excretion were noticed in the crabs acclimated at 1‰ (experimental group) as compared to 12‰ (control group). In addition, analysis of crab tissue revealed significant increase in the concentration of tissue free amino acids (10-40%; P<0.001) and triglycerides (8-40%; P<0.005), whereas significant reduction was observed (P<0.001) in the concentration of total protein (10-35%), free sugars, total carbohydrates and free fatty acids (15-70%) in experimental group crabs as compared to control group. The above results demonstrated that 1‰ acclimation led to utilization/mobilization of greater amount of protein and carbohydrates in order to meet the energy demand and enabled the animal to overcome the osmoregulatory / metabolic challenge.

Keywords: Mud crab; salinity; adaptation; excretions; metabolism.

1. INTRODUCTION:

Aquatic biota comprises wide array of species those are directly and acutely exposed to several physical factors like temperature, salinity, tidal cycles, lunar cycles, etc. The capacity of euryhaline organisms to adapt themselves to salinity variation depends on several other environmental factors, which in turn depend on energy supply and demand. Exposure of Arctic char, *Salvelinus alpines* to seawater stimulated large changes in amino acid metabolism without affecting either lipid or carbohydrate metabolism [1] whereas in *Chasmagnathus granulata* changes in carbohydrate metabolism was noticed with the change in environmental salinity [2]. Distinct seasonal variations in the biochemical (total nitrogen, non protein nitrogen, protein, glucose, glycogen, lipid , etc.) tissue compositions in the flesh of *Portunus pelagicus*, in various tissues of *Aegla ligulata* and the whole body composition of cray fish, *Parasaticus brasiliensis* were observed by several workers [3-5]. All the above variations were co-related with the reproductive behaviour of the crustaceans. The observed variation in tissue biochemical composition of decapods larvae might be due to differential phylogenetic position as well as fluctuation in environmental factors like temperature, food availability and salinity [6].

Regulation of intracellular effectors affects the amino acid metabolism and thus, the protein metabolism under osmotic stress. However, few studies [7] have dealt with the participation of lipids in adaptive function during change in ambient salinity in the higher crustaceans. Chapelle [8] reported slight depletion in the triglycerides levels in muscles and hepatopancreas of the Chinese crab, *Eriocheir sinensis* when they were transferred from freshwater to seawater. Salinity variations are also known to cause modifications in muscle lipid composition in fishes [9]. Successive changes in salinity during catadromous migration in diadromic fishes have been shown to cause significant changes in their metabolism, which in turn affects their biochemical composition [10].

Successful salinity adaptation may require a metabolic reorganization to meet the increased energetic demands associated with the exposure to the newly induced salinity regime [11]. Bianchini *et al.* [12] reported that salinity influences the osmo- and ion- regulatory patterns of gills of *Neohelice granulata*, which are accompanied by morphological and structural changes in gills. All the above mentioned studies were restricted to temperate or polar crustaceans. However, there is lack of similar studies in tropical countries such as India where economically important species like *Scylla serrata*, *S. olivacea*, *S. tranquebarica* are abundant. Being euryhaline, they tolerate wide fluctuation in salinity; its adults breed in marine waters whereas, the larvae migrate to brackish water and develop into adults. Secondly, Goa, along the west coast of India, is characterised by large mangrove vegetation and large low lying wetland areas, locally termed "Khazan lands" with water holding capacity of 6-9 months and salinity range 1-10 ‰, which offers wide scope for mud crab aquaculture more particularly of *S. serrata* and *S. olivacea* which are more abundant in west coast. In view of the above, it is proposed that euryhaline species such as *Scylla sp.* could be cultured in very low salinity conditions aided by appropriate dietary supplementation. Once the metabolic adaptation of *Scylla sp.* in hypo-saline environment is fully understood, it could be easily cultured in Khazan lands and dietary supplements could be provided to boost its growth. The present study focuses to elucidate the metabolic changes in *Scylla serrata* owing to induced hyposaline environment.

2. MATERIALS AND METHODS:

2.1. Sample collection:

The Adult mud crabs *Scylla serrata* (approximately 5-7 cm minimum carapace width) were collected throughout the year from the Mandovi estuary Goa (Lat. 15^0 28'and Long. $73^0$52') where salinity ranged from 10 ± 15‰ throughout the year. Identification of the collected species was confirmed using the taxonomy keys of mud crab Keenan *et al.* [13].These organisms were maintained in outdoor aquarium tanks (90cm x 45cm x 45cm) in 30 lit water capacity with continuous aeration facility at the Goa University, Zoology Department. The water was changed after every alternate day with taking care of salinity and crabs were daily fed with bivalves. The entire study period was divided into six seasons namely summer (March and April), pre monsoon (May and June), monsoon (July and August), post monsoon (September and October), pre winter (November and December) and winter (January and February), and crabs were collected once every season.

2.2. Experimental design and setup:

Our previous study reported significant differences between the body metabolism of the mud crabs those collected on full moon day and new moon day, respectively [14]. In view of the above, the crabs were acclimatized for two days prior to new moon day under laboratory conditions (at 12 ‰), and subsequently segregated in two groups (each comprising 6 individuals with equal number of males and females). The first group exposed to 1 ‰ saline water and acclimated for 15 days (new moon to full moon day) served as the hyposaline group/experimental group. On the other hand, the second group maintained at 12 ‰ served as control group.

At the end of the experimental period (i.e. on full moon day), rates of aquatic respiration and excretion were estimated from the water samples. Estimations of aquatic respiratory rate by using sodium thiosulphate titrator and excretion (ammonia, urea, free amino acids and trimethylamine oxide) were carried out using reagents (phenol reagent, diacetyl monoxime reagent, Ninhydrin reagent and Fe-EDTA reagent, respectively) following [14]. Various tissues namely muscles, gills, hepatopancreas and heamolymph were collected to estimate various biochemical parameters. Heamolymph was collected with the help of syringe from the last pleopod of the crab. Tissues from individual crabs were homogenized separately in ice cold distilled water. A part of the homogenate was deproteinized and used for estimation of total carbohydrates and free sugars. From other part of homogenate free amino acid, triglycerides, free fatty acids and protein was extracted using different solvents, acid and alkali [15]. Total carbohydrates by using Anthrone reagent , free sugars by using Areseno-molybdate reagent , free amino acids by using ninhydrin reagent , total proteins by Folin's phenol reagent , triglycerides by using Chromatotropic acid and free fatty acids by using Sodium dithiocarbamate color reagent were quantified in different tissues as described by Pujari [16].

2.3. Data analysis:

Differences in observed biochemical parameters of mud crabs exposed to 1 ‰ and 12 ‰ were determined using Student's'*t*' test. In addition, seasonal variations in the above parameters were determined through one-way Analysis of variance (ANOVA). Criterion of significance level for Student '*t*' test as well as one-way ANOVA adopted was 5% (P <0.05).\

3. RESULTS:

3.1. Respiration and excretion:

In the present study, changes in metabolism of the mud crab *S. serrata* were determined for its hyposaline adaptation in six pre-determined seasons. Significant seasonal variations (F = 5 to 20, P <0.001) were observed in the rates of aquatic respiration as well as excretion (ammonia, urea and trimethylamine oxide) in mud crabs irrespective of the ambient salinity (Table 1). The maximum change in rates of ammonia and urea excretions were observed during pre winter (November and December) and post monsoon (September and October) respectively. However, the

Table 1: Effect of salinity adaptation on the rate of aquatic respiration (ml of oxygen consumed / g body wt / hr) and nitrogenous excretions (µg/g body wt/hr) of mud crab, *Scylla serrata* in different seasons. Mean values of six individuals and their standard error were tabulated

Parameters	Season	1 ‰	12 ‰
Oxygen consumption	Summer	0.084 ± 0.002	0.05 ± 0.002
	Pre-monsoon	0.093 ± 0.002	0.06 ± 0.002
	Monsoon	0.081 ± 0.002	0.05 ± 0.003
	Post-monsoon	0.065 ± 0.002	0.04 ± 0.002
	Pre-winter	0.079 ± 0.003	0.05 ± 0.001
	Winter	0.089 ± 0.003	0.05 ± 0.002
	ANOVA Level of significance	16.68 *P*< 0.05	6.74 *P*< 0.001
Ammonia excretion	Summer	115.5 ± 2.68	54.4 ± 1.37
	Pre-monsoon	128.7 ± 2.02	66.3 ± 2.22
	Monsoon	135.6 ± 2.81	71.5 ± 2.30
	Post-monsoon	121.6 ± 4.88	51.5 ± 4.22
	Pre-winter	104.5 ± 1.92	42.5 ± 1.93
	Winter	111.6 ± 3.66	55.3 ± 1.37
	ANOVA & Level of significance	13.07 *P*< 0.001	18.6 *P*< 0.001
Urea excretion	Summer	16.11 ± 0.83	36.3 ± 1.38
	Pre-monsoon	20.88 ± 1.34	43.4 ± 1.73
	Monsoon	10.98 ± 1.98	25.7 ± 1.45
	Post-monsoon	05.20 ± 0.31	20.7 ± 1.49
	Pre-winter	11.84 ± 1.14	28.2 ± 1.48
	Winter	14.50 ± 0.76	35.0 ± 1.59
	ANOVA Level of significance	20.04 *P*< 0.001	29.3 *P*< 0.001
Trimethyl amine oxide (TMAO) excretion	Summer	5.12 ± 0.31	7.32 ± 0.23
	Pre-monsoon	6.74 ± 0.15	7.98 ± 0.26
	Monsoon	3.34 ± 0.43	5.99 ± 0.23
	Post-monsoon	4.21 ± 0.24	6.74 ± 0.49
	Pre-winter	4.48 ± 0.25	6.85 ± 0.37
	Winter	6.14 ± 0.29	7.92 ± 0.19
	ANOVA Level of significance	18.89 *P*< 0.001	5.96 *P*< 0.001
Free amino acid (FAA) excretion	Summer	470.7 ± 11.57	332 ± 12.88
	Pre-monsoon	460.4 ± 09.66	315 ± 13.64
	Monsoon	475.0 ± 10.99	338 ± 17.56
	Post-monsoon	495.4 ± 13.64	377 ± 31.40
	Pre-winter	477.6 ± 09.22	345 ± 15.02
	Winter	475.9 ± 10.65	337 ± 15.05
	ANOVA Level of significance	1.065 *P*< 0.001	1.17 *P*< 0.001

maximum change in the rates of free amino acids (FAA) and trimethylamine oxide (TMAO) was observed during pre monsoon and monsoon period. Acclimation of the crabs to hypo-saline water revealed that there was 55-80% increase (P<0.001) in aquatic respiration (Table 1). In addition, there was ≥ 90% augmentation (P<0.001) in the rate of ammonia excretion, 30-45% increase (P<0.001) in free amino acid (FAA) excretion along with 50-75% decrease (P<0.001) in the urea excretion and 15-45% decrease (P<0.001) in TMAO excretion as compared to control group.

3.2. Tissue biochemical composition:

Analysis of the tissue of crab acclimated to hypo-saline water revealed about 10-40% (P<0.001) increase in the concentration of free amino acids along with 10-35% (P<0.001) decrease in the levels of protein in gills, hepatopancreas and heamolymph Figs. 1, 2 and Table 2. On the other hand, 25-30% decrease (P < 0.001) was observed in the concentration of muscle protein without any significant elevation of free amino acid. Overall, about 25-70% (P<0.001) decrease in the levels of free sugars and total carbohydrates were observed in all the tissues of the mud crabs due to acclimation in hyposaline water for a period of 15 days. These changes are shown in Figs. 3 & 4. Figure 5 reveals about 8-40% elevation (P<0.005) in the level of triglycerides in various tissue of the crab. The acclimation also led to 15-50% decrease (P<0.001) in the level of free fatty acid concentration in all the tissues except the hepatopancreas, where 15-30% increase (P<0.001) was noticed (Fig. 6).

Table 2: Effect of salinity adaptation on concentrations of various bio molecules in heamolymph (mg/dl of heamolymph) of mud crab, *Scylla serrata* in different seasons. Mean values of six individuals and their standard error were tabulated.

Parameters	Season	1 ‰	12 ‰
Protein	Summer	26.457 ± 0.041	34.282 ± 0.063
	Pre-monsoon	27.990 ± 0.107	35.865 ± 0.082
	Monsoon	26.567 ± 0.045	34.875 ± 0.076
	Post-monsoon	27.505 ± 0.095	33.000 ± 0.050
	Pre-winter	26.557 ± 0.060	32.852 ± 0.004
	Winter	25.337 ± 0.012	32.032 ± 0.012
	ANOVA	183.05	627.87
	Level of significance	*P*<0.001	*P*<0.001
Free amino acids	Summer	14.120 ± 0.021	11.370 ± 0.016
	Pre-monsoon	13.440 ± 0.047	10.900 ± 0.043
	Monsoon	13.560 ± 0.034	11.300 ± 0.041
	Post-monsoon	14.750 ± 0.043	13.540 ± 0.053
	Pre-winter	14.900 ± 0.025	12.250 ± 0.010
	Winter	15.120 ± 0.021	12.370 ± 0.016
	ANOVA	456.6	202.6
	Level of significance	*P*<0.001	*P*<0.001
Free sugars	Summer	0.216 ± 0.002	0.327 ± 0.002
	Pre-monsoon	0.167 ± 0.005	0.301 ± 0.002
	Monsoon	0.145 ± 0.005	0.265 ± 0.004
	Post-monsoon	0.124 ± 0.001	0.237 ± 0.008
	Pre-winter	0.132 ± 0.002	0.259 ± 0.002
	Winter	0.151 ± 0.003	0.268 ± 0.004
	ANOVA	96.47	58
	Level of significance	*P*<0.001	*P*<0.001
Carbohydrates	Summer	0.558 ± 0.003	0.740 ± 0.002
	Pre-monsoon	0.536 ± 0.002	0.726 ± 0.002
	Monsoon	0.512 ± 0.002	0.701 ± 0.005
	Post-monsoon	0.494 ± 0.014	0.697 ± 0.007
	Pre-winter	0.560 ± 0.001	0.776 ± 0.003
	Winter	0.599 ± 0.006	0.789 ± 0.003
	ANOVA	33.892	86.914
	Level of significance	*P*<0.001	*P*<0.001
Triglycerides	Summer	0.307 ± 0.001	0.224 ± 0.002
	Pre-monsoon	0.327 ± 0.002	0.246 ± 0.003
	Monsoon	0.302 ± 0.004	0.225 ± 0.004
	Post-monsoon	0.351 ± 0.004	0.258 ± 0.010
	Pre-winter	0.291 ± 0.002	0.208 ± 0.001
	Winter	0.320 ± 0.002	0.243 ± 0.003
	ANOVA	60.355	49.74
	Level of significance	*P*<0.005	*P*<0.005
Free fatty acids	Summer	0.311 ± 0.001	0.376 ± 0.002
	Pre-monsoon	0.298 ± 0.002	0.354 ± 0.003
	Monsoon	0.289 ± 0.004	0.342 ± 0.003
	Post-monsoon	0.283 ± 0.003	0.332 ± 0.006
	Pre-winter	0.316 ± 0.002	0.386 ± 0.006
	Winter	0.320 ± 0.004	0.393 ± 0.001
	ANOVA	27.428	38.865
	Level of significance	*P*<0.001	*P*<0.001

4. DISCUSSION:

The mud crab, *Scylla serrata* commonly inhabits sheltered estuaries, mud flats and mangrove forests. These crabs are exposed to large number of environmental variables including annual and daily tidal cycles, lunar cycles, etc. Adaptation of an organism to a particular environment involves an integrated response to changes in environmental parameters depending on its homeostatic control mechanism. Such integrated response may be exerted at different biochemical, physiological and behavioural levels. The effect of a single variable is largely overridden by the positive or negative synergism between various variables in given environment [17]. The levels of various bio molecules such as protein, free amino acids, total carbohydrates, free sugars, total fat and free fatty acids in different tissues of any 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, photoperiod, tidal cycles, etc.) strongly affect the biochemistry and physiology of crustaceans [18,19].

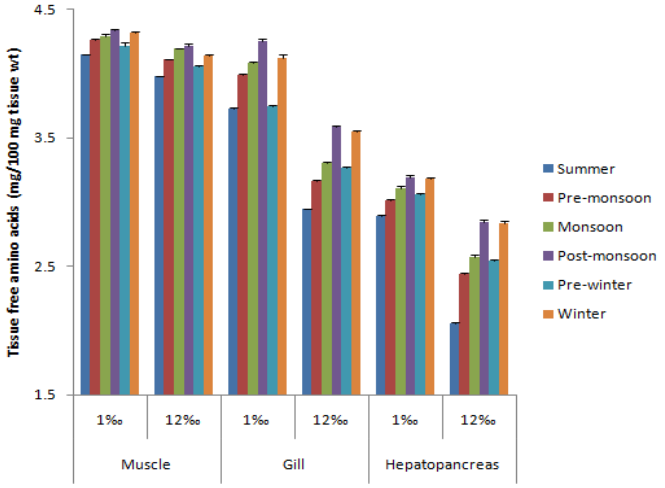

Figure 1. Effect of hyposaline adaptation on the tissue level concentrations of free amino acid in different tissues (mg/100mg) of mud crab *Scylla serrata*, during different seasons. Mean values of six individuals and their standard error were plotted.

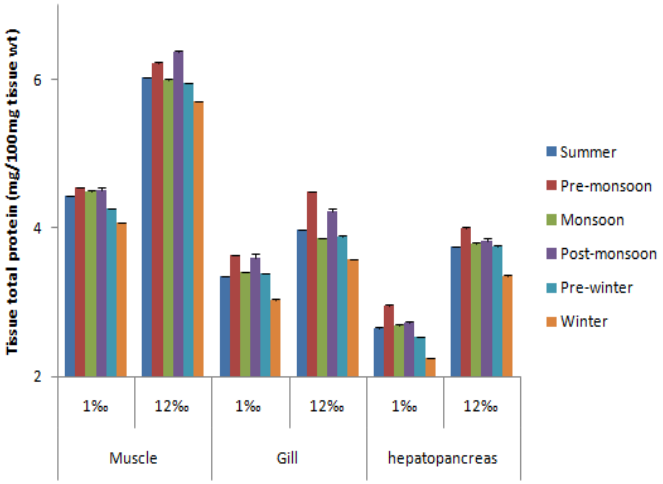

Figure 2. Effect of hyposaline adaptation on the tissue level concentrations of total protein in different tissues (mg/100mg) of mud crab *Scylla serrata*, during different seasons. Mean values of six individuals and their standard error were plotted.

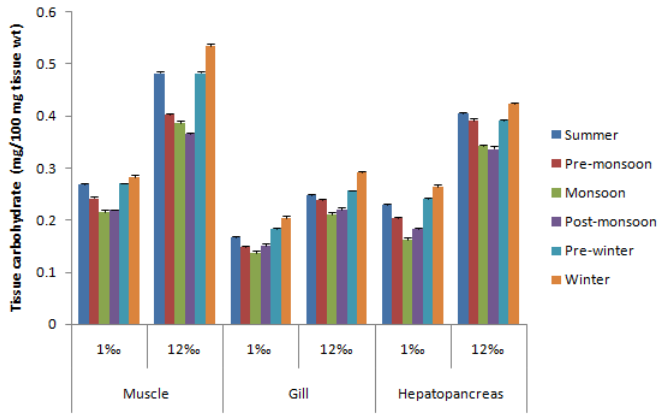

Figure 3. Effect of hyposaline adaptation on the tissue level concentrations of total carbohydrate in different tissues (mg/100mg) of mud crab *Scylla serrata*, during different seasons. Mean values of six individuals and their standard error were plotted.

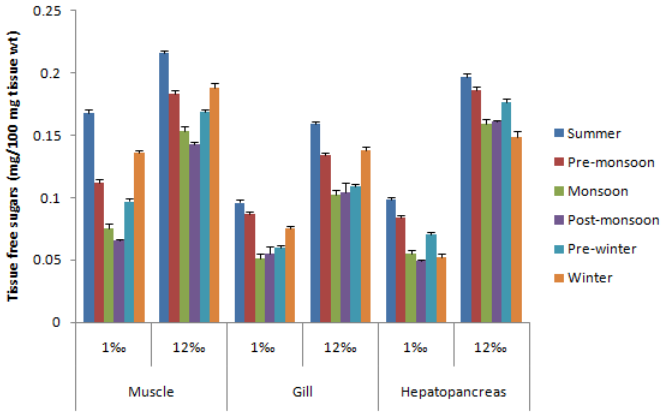

Figure 4. Effect of hyposaline adaptation on the tissue level concentrations of Free sugar in different tissues (mg/100mg) of mud crab *Scylla serrata*, during different seasons. Mean values of six individuals and their standard error were plotted.

Torres et al. [6] reported 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 synthesis and degradation of proteins is therefore a fundamental physiological process. 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 [20]. Significant increments of tissue free amino acids (Fig. 1), rates of free amino acids excretion, ammonia excretion (Table 1) and decreased levels of tissue total protein (Fig. 2), observed in hypo saline group of present study clearly signifies the mobilization of protein as a source of energy. Higher rates of ammonia and free amino acid excretion along with decrease in the rates of urea and TMAO excretion observed (Table 1) in hyposaline group, clearly indicates a shift towards ammoniotelism in mud crabs, *S. serrata*. This shift might be due to the increased

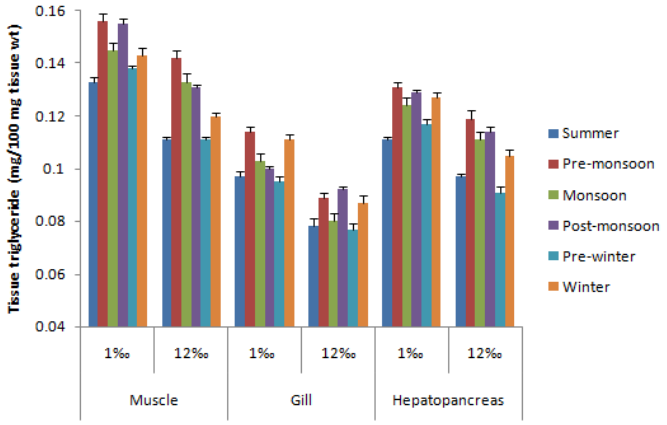

Figure 5. Effect of hyposaline adaptation on the tissue level concentrations of Triglycerides in different tissues (mg/100mg) of mud crab *Scylla serrata*, during different seasons. Mean values of six individuals and their standard error were plotted.

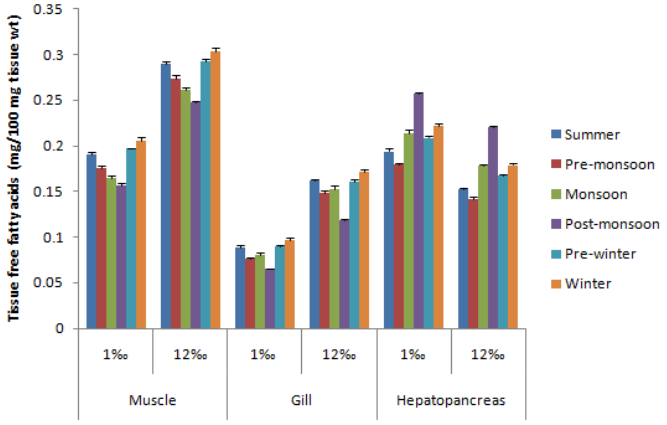

Figure 6. Effect of hyposaline adaptation on the tissue level concentrations of free fatty acid in different tissues (mg/100mg) of mud crab *Scylla serrata*, during different seasons. Mean values of six individuals and their standard error were plotted.

rate of protein catabolism or higher diffusiblity of ammonia in hypo-saline water. The active sodium uptake through the Na^+/NH_4^+ pump may help the organism to excrete ammonia directly rather than detoxifying it through the ornithine cycle. Activation of ion pump in the gill epithelial cell membrane upon acclimation in hypo saline water needs to be confirmed.

Depletion in the levels of total carbohydrates and free sugars by 50-70% (Figs. 3, 4) in hyposaline water acclimated crab 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. Increased concentration of triglycerides in all the tissues of *S. serrata* of hypo saline group (Fig. 6) indicates sparing of fat for the supply of energy in new habitat. Chapelle [8] reported an increase in triglyceride content in the Chinese crab *Eriocheir sinensis* upon acclimation to fresh water. In addition, there is no mobilization of lipids in hepatopancreas due to exposure in hypo-osmotic environment [21]. Thus, 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 (Fig. 6). Adaptation to hypo-saline environment involves the mobilization of more protein and carbohydrate in order to meet the extra energy demand for which the respiratory rate of mud crab was increased by 55-80% (Table. 1) when they were acclimated to 1 ‰ saline water. Chen and Chia [22] observed the decreased rate of respiration in *Scylla serrata* with increased salinity of the environment.

Higher accumulation of free amino acid and 5 - 18% reduction in the tissue total protein in gill, hepatopancreas and heamolymph of mud crab during winter as compared to summer month, irrespective of the ambient salinity of the water, indicate the utilization of more protein during winter in order to meet the energy demand of the body. A similar observation was made by Chen and Chia [22]. Higher rates of oxygen consumption along with nitrogen excretion are indicators of the endogenic conditions leading to gametogenesis of aquatic organisms [23]. We observed maximum rise in the rate of ammonia, urea excretion as well as TMAO excretion along with elevated rate of aquatic respiration (Table 1) during pre monsoon period, irrespective of their environment, clearly indicating the preparation of gametogenesis of mud crab. Although the mud crab *S. serrata* is continuous breeder throughout the year, it has two peak breeding cycle one in pre monsoon and another in post monsoon on the west coast of India [24]. The observed maximum depletion in the levels of total carbohydrates (Fig. 3) as well as free sugars (Fig. 4) and total protein (Fig. 2) along with increased level of free amino acid (Fig. 1) during monsoon or post monsoon period clearly indicates the utilization of carbohydrates and protein to gain the required energy for the breeding activity of crab.

The quantity and quality of lipid moieties play significant role throughout the life cycle of crustaceans [25]. The lipid reserves and its mobilization are also very important during the breeding activity of crustaceans [26]. A high energy yielding metabolism is very essential during the breeding activity that involves gametogenesis, vitellogenesis as well as spawning. The present study showed increase in the levels of triglyceride (Fig. 5) along with the decrease in free fatty acids

(Fig. 6) of hepatopancreas during pre monsoon period which serves as the preparatory phase for breeding period. Earlier reports [5, 27] also indicate utilization and mobilization of lipid reserves for the spawning activity of crabs. Therefore, in light of all these observations it can be inferred that the challenge to supply maximum energy for the breeding activity can be met through mobilization of fat rather than protein or carbohydrate.

Both (salinity and reproductive activity) appeared to be the main processes influencing the metabolism of the crab. However, the reproductive activity or the seasonal variations in the biochemical composition of the crab remained unaltered with the change in physical environment of the crab. The data presented here also indicates that lipids constitute an energy store, whereas carbohydrates, free sugars and proteins seems to be the main source of energy supply in mud crab *Scylla serrata* during acclimation to hypo saline environment.

Conclusion:

Mud crab *S. serrata* would be easily cultured in Khazan lands and feed rich in protein and carbohydrate would be ideal for boosting the growth of crab.

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References

[1] J. S. Bystriansky, N. T. Frick, J. S. Ballantyne, Intermediary metabolism of arctic char *Salvelinus alpines* during short term salinity exposure, J. Exp. Biol. 210 (2007) 1-15.

[2] E. A. Santos, L. E. M. Nery, Blood glucose regulation in an estuarine crab, *Chasmagnathus granulata* (Dana, 1851) exposed to different salinities. Comp. Biochem. Physiol. 87A (1987) 1033-1035.

[3] Z. Akbar, R. Qasim, P. J. A. Siddique, Seasonal variation in biochemical composition of edible crab *(Portunus pelagicus, Linneaus).* J Islam.Acad. Sci. 1 (1988) 127-133.

[4] G.T. Oliveira , F.A. Fernandes , G. Bond-Buckup , A. A. Bueno , R. S. M. Silva , 2003. Circadian and seasonal variations in the metabolism of carbohydrates in *Aegla lingulata* (Crustacea: Anomura: Aeglidae). Memoirs Museum Victoria, 60 (2003) 59-62.

[5] B. K. Durta, C. Zank, K. M. Da Silva, M. R. Conter, G. T. Oliveira, Seasonal variation in the intermediate metabolism of the crayfish *Parastacus brasiliensis* (Crustacea, Decapoda, Parastacidae) in the natural environment and experimental culture. Iheringia Serie Zoologia, 98 (2008) 355-361.

[6] G. Torres, L. Gimenez, K. Anger, Effects of reduced salinity on the biochemical composition (lipid, protein) of zoea 1 decapod crustacean larvae. J. Exp. Mar. Biol. Ecol. 227: (2002) 43-60.

[7] N. N. Fokina, Z. A. Nefedova, N. N. Nemova, V. V. Khalman, Modulating role of lipids and their fatty acids in adaptation of the White Sea mussels *Mytilus edulis* L. to environmental salinity change. J. Evol. Biochem. Physiol. 43 (2006) 379-387.

[8] S. Chapelle, Influence of salinity on the lipid composition and the fatty acid pattern of muscle and hepatopancreas of the Chinese crab *Eriocheir sinensis*. Arch. Int. Physiol. Biochim. 86 (1977) 393-401.

[9] M. E. L Cafsi, M. S. Romdhane, A. Chaouch, W. Masmoudi, S. Kheriji, F . Chanussot, A . Cherif, Quantitative needs of lipid by mullet, *Mugil cephalus*, fry during freshwater acclimation. Aquaculture, 225 (2003) 233-241.

[10] H. Dantagnan, A. S. Borquez, I. N. Valfebenito, I A. Salgado, E. A. Serrano, M. S. Izquierdo, Lipid and fatty acid composition during embryo and larval development of puye *Galaxias maculates* Jenyns, 1842, obtained from estuarine, freshwater and cultured populations. J. Fish Biol. 70 (2006) 779-781.

[11] S. S. Alvarellos, F. J. Arjona, M. P. Martin del Rio, J. M. Miguez, J. M Mancera, J. L. Soengas, Time course of osmoregulatory and metabolic changes during osmotic acclimation in *Sparus auratus.* J. Exp. Biol. 208 (2005) 4291-4304.

12] A. Bianchini, M. M. Lauer, L. E. M. Nery, E. L Colares, J. M. Monserrat, A. D. S. Filho, Biochemical and Physiological adaptations in the estuarine crab *Neohelice granulata* during salinity acclimation. Comp. Biochem. Physiol. 151 (2007) 423-436.

[13] C. P. Keenan, P. J. F. Davie, L. Mann, A revision of the genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). Raffles Bulletin of Zoology, 46 (1998) 217-245.

[14] S. Bhoite, R. Roy, Seasonal variation in the metabolism of mud crab *Scylla serrata* during new moon and full moon phases of lunar cycle. Indian J. Comp. Anim. Physiol. 28 (2010) 39-47.

[15] R. Roy, H. Dutta, A. B. Das, Role of environmental temperature in seasonal adaptation of an Indian air breathing fish, *Channa punctatus* (Bloch): Dynamics of tissue protein, free amino acids and RNA contents. Proc. Indian Nat. Sci. Acad. B57 (1991) 147-152.

[16] P. Pujari, P, PhD Thesis. Goa University, 2010.

[17] B. Salvato, V . Cuomo, P. De Muro, M. Beltramini, Effects of environmental parameters on the oxygen consumption of four marine invertebrates: a comparative factorial study. Mar. Biol. 138 (2001) 659-668.

[18] R. Rosa, M. L. Nunes, Biochemical composition of deep-sea decapods crustaceans with two different benthic life

strategies off the Portuguese south coast. Deep Sea. Res. Part I, 150 (2003) 119-130.
[19] A. S. Vinagre, A. P. N. Amaral, F. P. Ribarcki, E. F. Silveira, E. Perico, Seasonal variation of energy metabolism in ghost crab *Ocypode quadrata* at Siriu Beach (Brazil). Comp. Biochem. Physiol. 146 (2006) 514-519.
[20] K. P. P. Fraser, A. Clarke, L. S. Peck, Low temperature protein metabolism: seasonal changes in protein synthesis and RNA dynamics in the Antarctic limlet *Nacella concinna* strebel 1908. J. Exp. Biol. 205 (2002) 3077-3086.
[21] R. Luvizotto-Santos, J. T. Lee, Z. P. Branco, A. Bianchini, L. E. M. Nery, Lipids as energy source during salinity acclimation in the euryhaline crab *Chasmagnathus granulata* Dana, 1851 (Crustacea-Grapsidae). J. Exp. Zool. 295 (2003) 200-205.
[22] J. C. Chen, P. G. Chia, Oxygen uptake and nitrogen excretion of juvenile *Scylla serrata* at different salinity and temperature levels. J. Crust. Biol. 16 (1`996) 437-442.
[23] Y. Mao, Y. Zhou, H. Yang, R. Wang, Seasonal variation in metabolism of cultured pacific oyster, *Crassostrea gigas*, in Sanggou Bay, China. Aquaculture, 253 (2006) 322-333.
[24] P. N. Prasad, B. Neelakantan, Maturity and breeding of mud crab *Scylla serrata* (Forskal) (Decapoda: Brachyura: Portunidae), Proc. Indian Acad. Sci. (Anim. Sci.) 98 (1989) 341-349.
[25] N. V. Zhukova, A. B. Imbs, L. F. Yi, Diet induced changes in lipid and fatty acid composition of *Artemia salina*. Comp. Biochem. Physiol. 120 (1998) 499-506.
[26] B. E. Hasek, D. L. Felder, Biochemical contents of the ovary and hepatopancreas of *Uca longisignalis* and *Uca* nr. minax. Scientia Marina, 70 (2006) 505-517.
[27] D. Li, Y. Zhang, A. J. Sinclair, Seasonal variations of lipid content and composition in *perna viridis*. Lipids, 42(2007) 739-747.

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