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EFFECT OF TEMPERATURE ON BIOMOLECULES IN PENAEUS INDICUS

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ABSTRACT

Temperature affects nearly all biological processes including the structure of proteins and biological membranes and rates of biochemical and physiological reactions in all aquatic organisms. In the present study, the effect of temperature on biochemical constituents in *Penaeus indicus* showed much variation. The protein content showed fluctuations in muscle and hepatopancreas and in gill tissue it decreased at 20 ^{o}C for shorter time of exposure. An increase in protein content was noted in all the tested tissues at 37^{o}C for short duration. Fluctuations in carbohydrate content were noted in the muscle, gill and hepatopancreas at 20 ^{o}C during short duration of exposure. Lipid content was fluctuated in all the tissues at 20 ^{o}C for shorter duration and it decreased in all the analyzed tissues for longer time of exposure. Elevated lipid content was observed in all the tested tissues at 37^{o}C for shorter exposure time. But fluctuations were noted in all tissues at longer duration of exposure. The presence of these alterations may serve as "biomarkers," signaling exposure to stressors or adverse effects, which can be set right.

KEYWORDS: Carbohydrate, Lipid, Protein, White Prawn

INTRODUCTION

Of all the environmental factors that affect aquatic organisms, temperature is the most all pervasive. Depending upon the magnitude and rates of the temperature changes, there may be minor readjustments of the rates of metabolism and growth or major changes in the distribution of species, affecting the aquatic ecosystem. Temperature has been recognized as a dominant environmental factor and has been shown to influence biological systems at numerous levels of organization (Cossins and Bowler, 1987; Johnston and Bennett, 1996). Water temperature is probably the most important environmental factor because it directly affects oxygen consumption, metabolism, growth, moulting and survival (Staples and Heales 1991; Chen *et al.*, 1995). In addition, temperature has a direct effect on other environmental factors such as increasing salinity and decreasing dissolved oxygen. Both conditions also affect the physiological stability of the animal.

Temperature affects nearly all biological processes including the structure of proteins and biological membranes and rates of biochemical and physiological reactions (Hazel 1995; Somero 1995). Effects of temperature on the physiology of ectothermic animals have been implicated as a pervasive cause (Clarke 2003; Bradshaw *et al.*, 2004). Vargas Albores *et al.*, (1998) reported that under the influence of temperature, Yellow leg shrimp *Penaeus californiensis* showed a significant increase in total protein.

The organism *Penaeus indicus* is a representative of an ecological and commercial important group. They are abundant and widely available in Kanyakumari district, Tamil Nadu, South India. They are amenable to laboratory testing and easily maintained. Hence for the purpose of the present study, *Penaeus indicus,* the white prawn is chosen as the experimental organism and the study was carried out in Centre for Marine Science and Technology, Kanyakumari district.

MATERIALS AND METHODS

The white shrimp, *Penaeus indicus* weighing approximately 5g were maintained with adequate aeration for 5 days at 28^{o}C (control) with 5 shrimps per trough. The experimental shrimps were exposed to hypothermic and hyperthermic condition. For hypothermic treatment, the temperature was reduced from 28^{o}C to 20^{o}C and for hyperthermic treatment; the temperature was raised from 28^{o}C to 37^{o}C in BOD incubator. Shrimps were exposed at 20 ^{o}C and 37^{o}C for about 60 minutes and during this experiment the shrimps were withdrawn at time intervals of 15, 30, 45 and 60 minutes and at time intervals of 2, 4, 6 and 8 hours exposure and sacrificed. Gills, hepatopancreas and muscles were dissected and used for biochemical analysis. Protein content was extracted and estimated following the method of Lowry *et al.*, (1951). Carbohydrate content of the sample was estimated by the method of Carrol *et al.,* (1956). Extraction of total lipid was done following the method of Folch *et al.,* (1957) and estimation was done using the method of Barnes and Black Stock (1973).

RESULTS

The biomolecules protein, carbohydrate and lipid in muscle, gills and hepatopancreas were analysed in *P. indicus* stressed at 20^{o}C and 37^{o}C and also at different exposure periods are provided in the following Tables 1 to 4. The *P. indicus* stressed at 20^{o}C for short duration from 15 to 60 minutes showed fluctuation in protein content in muscle and hepatopancreas. In gill tissue, the protein content decreased as the time of exposure increased and the values ranged from 6.840±0.02 mg to 5.850±0.03 mg (Table 1).

The protein content in all the tested tissues showed a declining trend and the values were ranged from 8.24±0.06 mg to 7.09±0.01 mg, from 5.82±0.04 mg to 4.41±0.03 mg and from 3.35±0.03 mg to 2.86±0.06 mg in muscle, gills and hepatopancreas respectively when the exposure time increased from 2 to 8 hours (Table 2). In muscle, gills and hepatopancreas of *P. indicus* stressed at 37^{o}C for 15 to 60 minutes of exposure showed an increasing trend in the protein content and the values ranged from 9.03±0.09 mg to 9.24±0.06 mg, from 6.91±0.09 mg to 7.52±0.07 mg and from 4.72±0.08 mg to 5.08 ±0.06 mg respectively (Table 3). In *P. indicus* stressed at 37^{o}C for 2 to 8 hours showed variations in protein content in all the analyzed tissues. In muscle tissue, maximum value of 9.47±0.08 mg was registered at 2 hours and minimum of 7.62±0.06 mg at 8 hours of exposure. A maximum value of 7.56±0.06 mg was noted at 2 hours and minimum of 6.62±0.09 mg at 8 hours of exposure in gill tissue. But in hepatopancreas, the protein content increased at all the exposure time compared to the control shrimp (Table 4).

The carbohydrate content of muscle, gills and hepatopancreas of *P. indicus* exposed to 20^{o}C showed much fluctuation as the exposure time increased from 15 minutes to 60 minutes (Table 1). Likewise in *P. indicus* stressed at 20^{o}C for longer exposure duration altered the carbohydrate content in all the tested tissues. There noticed a linear reduction and the values were ranged from 2.04±0.03 mg to 1.42±0.06 mg, from 1.35±0.08 mg to 0.72±0.07 mg and from 1.03±0.05 mg to 0.72±0.06 mg in muscle, gills and hepatopancreas respectively (Table 2). There were changes observed in carbohydrate content of *P. indicus* exposed to 37^{o}C for short period of 15 to 60 minutes. Carbohydrate content of muscle, gills and hepatopancreas showed an increasing trend when compared to the control value (Table 3). In case of *P. indicus* stressed at 37^{o}C for longer duration (2 to 8 hours), the carbohydrate content fluctuated much in all the tested tissue (Table 4).

P. indicus exposed at 20^{o}C for short period of 15 to 60 minutes registered variations in lipid content in all the tested tissues (Table 2.1). When *P. indicus* stressed at 20^{o}C for longer duration, a linear decline in lipid was noted in all the analyzed tissues and it ranged from 0.48±0.005 mg to 0.44±0.002 mg, from 0.406±0.004 mg to 0.378±0.005 mg and from 0.69±0.002 mg to 0.59±0.003 mg in muscle, gills and hepatopancreas respectively as the time of exposure increased from 2 to 8 hours (Table 2). Exposure of *P. indicus* at 37^{o}C for short duration revealed an elevation of lipid content in all tested tissues. Compared to the control values, lipid content increased in muscle at all the tested time intervals and its values ranged from 0.668±0.002 mg to 0.601±0.006 mg as time of exposure increased. Fluctuations in lipid content were observed in *P. indicus* stressed at 37^{o}C for 2 to 8 hours. (Table 4)

DISCUSSIONS

In the present investigation, the temperature has induced alterations in the biochemical constituents such as protein, carbohydrate and lipid. The protein content in muscle, gills and hepatopancreas of *P. indicus* showed a slight decreasing trend at 20°C at short duration (minutes) temperature exposure. The decrease in the protein content was much pronounced in all the tissues at 20°C at long duration (hours) temperature exposure. The protein level showed an increasing trend in all the tissues at shorter exposure period (minutes) at 37°C and also at acute stress. Total protein values were increased at 37°C at longer duration till 6 hours and then decreased at 8 hours. The continual synthesis and degradation of proteins is not only vital for tissue maintenance and animal growth but is also important in allowing animals to adapt to changing environmental conditions, to replace denatured or damaged proteins, to mobilize amino acids and to allow metabolic regulation (Hawkins, 1991).

In the hermit crab, *Uca pugilator*, temperature modified the concentration of protein (Dean and Vernberg, 1966). Similar effect was reported in crab, Carcinus *maenas* by Truscott and White (1990). Total serum protein showed an increase in the yellowleg shrimp, *Penaeus californiensis* at 28°C and 32°C but no changes were observed at 18°C, 22°C and 25°C (Vargas *et al*., 1998). Lucía Ocampo (2003) reported that proteins level increased with increased temperature in *Panulirus interruptus*. At elevated temperature, oysters *Crassostrea gigas* had significantly higher levels of protein (Muki *et al.*, 1992**).** The protein content at 20 ± 2 °C and 20 ± 4 °C were slightly higher than those at constant temperature of 20°C in *Ulva pertusa* (Qiaohan Wang et al., 2007). Body proteins of juvenile *Farfantepenaeus californiensis* were 15.1% wt/wt at the higher temperature and 12.5% wt/wt at the lower temperature **(**Ocampo et *al.*, 2001). Protein was unaffected in *Chaetoceros* cf. *wighamii* at lower temperatures, 20 and 25 °C (Sirlei *et al,* 2005).

Hyperglycemia as a secondary stress response has been documented in most species of fish in response to a wide range of stresses. Elevated blood glucose can result from a reduced utilization of glucose or stimulation of gluconeogenesis and/or glycogenolysis. In the present study, the carbohydrate content in muscle, gills and hepatopancreas of *P. indicus* increased at both 20 °C and 37 °C at short exposure time. Whereas the carbohydrate level in all the tissues were decreased at 20 °C at longer exposure period (hours) and at 37 °C it increased at 2 and 4 hours in muscle and gills and till 6hours in hepatopancreas and then decreased at 8 hours. Santos and Keller (1993) reported that in crustaceans, elevation of hemolymph glucose is also observed in vivo when they are submitted to stressful condition, elevated temperature in *Orconectes limosus*. Sirlei *et al.*, (2005) have observed that the carbohydrate content was higher at lower temperatures (20 and 25 °C) in *Chaetoceros* cf. *wighamii*. Total reducing sugar levels of crab, *Uca minax* acclimated to different temperatures indicated that the concentration of glucose in blood is depressed at the lower temperatures, and short periods of acclimation to higher temperatures resulted in a higher blood glucose level **(**Dean and Vernberg, 1965).

Changes in hemolymph glucose concentration reflect the process of physiological compensation to temperature shock. At elevated temperature, oysters *Crassostrea gigas* had significantly higher levels of carbohydrate (Muki *et al*., 1992**).** Qiaohan Wang *et al*., (2007) have indicated that total carbohydrate content has significantly increased as the temperature increased in *Ulva pertusa.*

Any type of stress reduces the energy resources of an organism. To compensate for these stresses induced events, an animal will be forced to spend more energy. The higher lipid depletion rate at higher temperature occurs because lipids are the main source of energy during ontogeny of aquatic organisms (Holland, 1978).

In the present work, the lipid level was increased at 37 °C in muscle, gills and hepatopancreas of *P. indicus* at shorter exposure period (minutes) and at longer exposure time of 2, 4 and 6 hours and also at acute stress. At 20°C lipid content decreased in all tissues for longer exposure duration and for shorter period the level increased at 15 and 30 minutes and then decreased at 45 and 60 minutes. James *et al*., (2001) has reported that Bass *Micropterus salmoides* raised at 32 °C had higher lipid levels in the liver. Higher levels of triglycerides were observed in lipids of shrimp *Litopenaeus vannamei* hepatopancreas kept at 32°C (Martin Perez-Velazquez *et al*., 2003). Lipid content was higher at lower temperatures (20 and 25 °C) in *Chaetoceros* cf. *wighamii* (Sirlei *et al.,* 2005). The variation in lipid content in the tested tissues of *P. indicus* may be attributed to the fluctuation in energy requirement due to temperature induced stress.

CONCLUSIONS

This study revealed that temperature alters the biomolecules in the organisms to cope up with the temperature stress. The elevation in protein concentration is believed to adapt to environmental changes. Similarly, increase in carbohydrate is an indication of stress response. The variations in lipid level depends on the energy needed to combat the thermal stress.

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APPENDICES

Table 1: Effect of Temperature on Change in Biochemical Constituents in Different Tissues of Penaeus Indicus, Exposed to 20ºc at 15, 30, 45, and 60 Minutes of Exposure Time. The Results Are Expressed in Mg per 100 Mg Wet. Tissue: Each Value (X ± SD) is the Average of Three Estimates

Tissues Tested	Biochemical Constituents	Control (28^0C)	Duration of Exposure at 20^0C (Min.)			
			15	30	45	60
Muscle	Protein	8.786 ± 0.08	8.862±0.04	8.880 ±0.06	8.482±0.02	8.312±0.04
	Carbohydrate	2.342 ± 0.06	2.620 ± 0.02	2.681 ±0.05	2.420±0.04	2.036±0.06
	Lipid	0.536±0.005	0.561 ± 0.02	0.565±0.002	0.521±0.007	0.502±0.005
Gills	Protein	6.862 ± 0.01	6.840±0.004	6.818 ±0.02	5.986 ±0.06	5.850 ± 0.03
	Carbohydrate	1.487 ± 0.05	1.524 ± 0.05	1.556 ±0.04	1.464 ±0.06	1.362 ± 0.04
	Lipid	0.421±0.002	0.464±0.002	0.430±0.005	0.412±0.004	0.410±0.005
Hepatopancreas	Protein	4.210 ± 0.02	4.250 ± 0.01	4.230 ±0.02	4.066 ±0.03	3.852 ±0.01
	Carbohydrate	1.620 ± 0.01	1.640 ± 0.02	1.516 ±0.03	1.216 ±0.04	1.120 ± 0.04
	Lipid	0.742±0.002	0.746±0.004	0.746±0.003	0.738±0.001	0.704±0.002

Table 2: Effect of Temperature on Change in Biochemical Constituents in Different Tissues of Penaeus Indicus, Exposed to 20ºc at 2, 4, 6 and 8 Hours of Exposure Time. The Results Are Expressed in Mg per 100 Mg Wet. Tissue: Each Value (X ± SD) is the Average of Three Estimates

Tissues Tested	Biochemical Constituents	Control (28^0C)	Duration Of Exposure At 20^0C (H)			
			2	4	6	8
Muscle	Protein	8.786 ± 0.08	8.24 ±0.06	7.97±0.08	7.26±0.02	7.09±0.01
	Carbohydrate	2.342 ± 0.06	2.04 ±0.03	1.82±0.03	1.81±0.02	1.42±0.06
	Lipid	0.536±0.005	0.48±0.005	0.48±0.006	0.46±0.006	0.44±0.002
Gills	Protein	6.862 ± 0.01	5.82 ± 0.04	5.28 ±0.02	4.79 ±0.06	4.41 ± 0.03
	Carbohydrate	1.487 ± 0.05	1.35 ± 0.08	1.10 ±0.06	0.80 ± 0.05	0.72 ± 0.07
	Lipid	0.421±0.002	0.406±0.004	0.402±0.06	0.390±0.006	0.37±0.005
Hepatopancreas	Protein	4.210 ± 0.02	3.35 ± 0.03	3.03 ±0.02	3.00 ± 0.01	2.86 ± 0.06
	Carbohydrate	1.620 ± 0.01	1.03 ± 0.05	0.86 ±0.04	0.80 ± 0.03	0.72 ± 0.06
	Lipid	0.742±0.002	0.69 ± 0.002	0.64±0.003	0.61 ± 0.002	0.59±0.003

Table 3: Effect of Temperature on Change in Biochemical Constituents in Different Tissues of Penaeus Indicus, Exposed to 37ºc at 15, 30, 45, and 60 Minutes of Exposure Time. The Results Are Expressed in Mg per 100 Mg Wet. Tissue: Each Value (X ± SD) is the Average of Three Estimates

Tissues Tested	Biochemical Constituents	Control (28^0C)	Duration Of Exposure At 37^0C (Min.)			
			15	30	45	60
Muscle	Protein	8.786 ± 0.08	9.03±0.09	9.28±0.07	9.13±0.07	9.24 ± 0.06
	Carbohydrate	2.342 ± 0.06	2.72 ± 0.03	2.86 ± 0.02	2.98 ± 0.03	3.00 ± 0.05
	Lipid	0.536±0.005	0.668±0.002	0.601±0.005	0.622±0.003	0.601±0.006
Gills	Protein	6.862 ± 0.01	6.91 ± 0.09	6.96 ± 0.01	7.03 ± 0.11	7.52 ±0.07
	Carbohydrate	1.487 ± 0.05	1.58 ± 0.03	1.68 ± 0.03	1.75 ± 0.01	1.79 ±0.04
	Lipid	0.421±0.002	0.47 ± 0.003	0.50 ± 0.001	0.54 ± 0.001	0.54 ±0.003
Hepatopancreas	Protein	4.210 ±0.02	4.72 ± 0.08	4.83 ± 0.07	5.02 ± 0.06	5.08 ±0.06
	Carbohydrate	1.620 ±0.01	1.78 ± 0.04	1.92 ± 0.02	1.96±0.03	2.00 ±0.04
	Lipid	0.742±0.002	0.83±0.002	0.95±0.003	0.98±0.002	1.110±0.007

Table 4: Effect of Temperature on Change in Biochemical Constituents in Different Tissues of Penaeus Indicus, Exposed to 37ºc at 2, 4, 6 and 8 Hours of Exposure Time. The Results Are Expressed in Mg per 100 Mg Wet. Tissue: Each Value (X ± SD) is the Average of Three Estimates

Tissues Tested	Biochemical Constituents	Control (28^0C)	Duration Of Exposure At 20^0C (H)			
			2	4	6	8
Muscle	Protein	8.7860.08	9.47±0.08	9.14±0.06	8.46±0.07	7.62 ±0.06
	Carbohydrate	2.342±0.06	3.23±0.05	2.79±0.06	2.14±0.04	2.02 ±0.03
	Lipid	0.536±0.005	0.594±0.001	0.587±0.002	0.566±0.002	0.502±0.003
Gills	Protein	6.862 ± 0.01	7.56 ± 0.06	7.26 ± 0.07	7.05 ± 0.08	6.62 ± 0.09
	Carbohydrate	1.487 ± 0.05	1.80 ± 0.03	1.74 ± 0.03	1.47 ± 0.04	1.41 ± 0.02
	Lipid	0.421±0.002	0.54±0.002	0.52±0.001	0.44±0.001	0.40 ± 0.003
Hepatopancreas	Protein	4.210 ± 0.02	5.17±0.04	5.05±0.05	4.71 ± 0.06	4.26 ±0.03
	Carbohydrate	1.620 ± 0.01	2.25±0.03	2.06±0.02	1.82 ± 0.02	1.54 ±0.03
	Lipid	0.742±0.002	1.06±0.005	0.92±0.005	0.81±0.004	0.72±0.005