Thermal stress on oxygen consumption and haemolymph biochemical constituents in fresh water crab Barytelphusa guerini

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Abstract:

The effect of temperature on whole animal body oxygen consumption and protein, carbohydrate content in tissue (Haemolymph) of *Barytelphusa guerini* was determined at cold, warm and normal temperatures. Oxygen consumption increased under warm condition and decreased under cold condition. In haemolymph, carbohydrates were found to be decreased in cold condition and increased in warm condition when compared to normal temperatures. Whereas, the haemolymph, protein levels were found to be decreased in cold condition and increased in warm condition. From the results, it indicated that protein and carbohydrate levels were most important biochemical constituents that came into the action to overcome stressful conditions that generally prevail in *Barytelphusa guerini*. This study will help to model temperature dependent growth in the field and assist in designing the best possible temperature and diets for crabs.

Key Words: Crab, Temperature, Oxygen Consumption, Haemolymph, Carbohydrate, Protein.

Introduction:

Temperature is a measure of hotness and coldness in an animal's body. It is usually a function of the rate of molecular agitation which is controlled to a large extent by the rate of physic-chemical reactions in the body of the animal (Hardy, 1979).

Temperature stress provokes energy-demanding responses at the cellular level, which eventually may reduce the organisms' competition and reproduction abilities (Tomanek, L. and M.J.Zuzow, 2010, Feder, M.E., 1999). Hence, temperature stress is a significant physiological and ecological factor. In evolution, those species apparently are more successful that better cope with the physiological effects of stress, i.e. respond with less expense of energy. (Henkel, S.K and G.E.Hofman, 2008).

Since crabs are poikilotherms, it is expected that temperature will grossly affect their metabolic rates. Though metabolic rate of an animal tends to increase with increasing temperature, Aldrich (1975) noted that because of the complex interactions of environmental, demographic and physiological factors it may not be surprising to notice individual variability of oxygen consumption rates in some crustaceans.

Temperature is generally known to influence the oxygen consumption of animals. Factors affecting the metabolic rate of invertebrates can be either endogenous (body size, respiratory surfaces, activity, nutritional status and state of reproductive cycle or exogenous (temperature, salinity, pH, photoperiod and oxygen concentration among others (Newell et al., 1979).

Relationships between animal occurrence and survival have been clearly recognized for a long period as being related to maximum and minimum temperatures. Precht et al. (1973) stated that the temperature influence on the unchanging or steady systems (normal physiological conditions) which are more important than those on the changing systems (during growth, reproduction, development etc.) for better assessment of the thermal stress. The present study is to investigate the effect of temperature and pH variations on the oxygen consumption rate of *Barytelphusa guerini* in the laboratory as a contribution to the biology of the species.

Material and Methods:

Fresh water crabs, *Barytelphusa guerini* have been caught from paddy field area of Hyderabad. The crabs have been transported to the laboratory in aerated plastic troughs. They have been acclimated for seven days in an aquarium under laboratory condition. The water in the aquarium was changed every day. The crabs were fed daily on small and immature grasshoppers before the water changed.

The selected temperatures of the experiment were 22^0C, 26^0C, and 30^0C. At the required temperature and constant pH of 7.0, water sample was siphoned into an oxygen bottle and fixed for oxygen determination. The mean value of the oxygen so obtained was taken as the initial oxygen concentration for all the respiratory chambers. The crabs were weighed, and introduced into air-tight and bubble-free chambers. After 60 minutes (1hour), water samples were siphoned from each of the chambers and fixed for oxygen determination. The difference between the value obtained after 60minutes and the initial value of oxygen in control chamber was regarded as the amount of oxygen consumed by the crabs per hour, while the weight-specific oxygen consumption was calculated as the amount of oxygen consumed by a unit body weight of the crab per hour. The amount of dissolved oxygen content of the water samples was estimated by the Standard Winkler's Iodometric method (welsh and smith, 1960). The oxygen consumed by the animal in one hour was calculated and expressed as ml of O2/g. body weight/hour.

The protein content of the haemolymph in normal and experimental animals was estimated by Lowry et al., (1951). Estimation of carbohydrate was done according to the methodology of Roe, (1955).

The data were statistically analysed using means with standard deviations and student's t-test.

Results and Discussion:

The fresh water crab, *Barytelphusa guerini*, on exposure to various temperatures revealed that a significant alterations in the whole body oxygen consumption and haemolymph biochemical constituents (figure-1 and 2).

Figure-1: Effect of temperature on whole body oxygen consumption and haemolymph biochemical constituents.

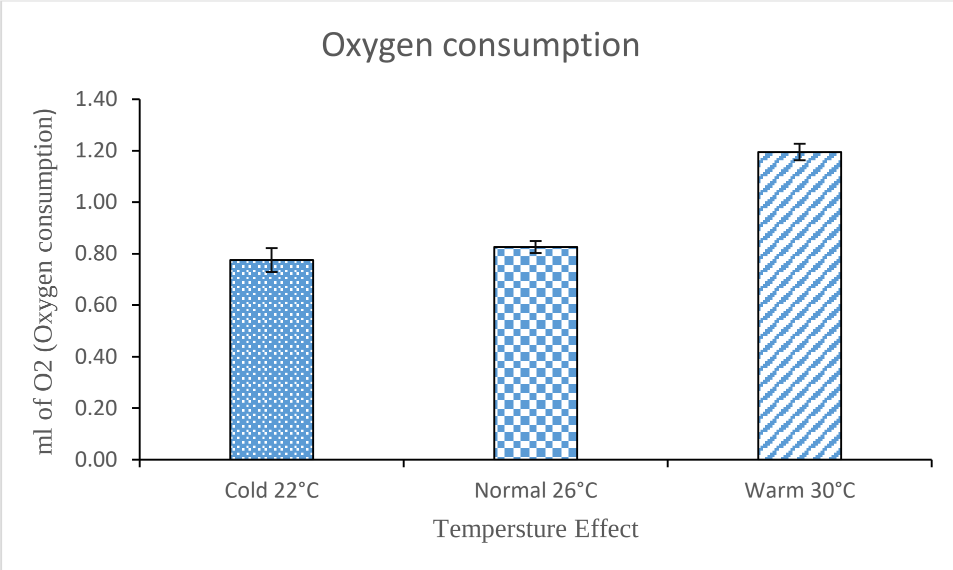
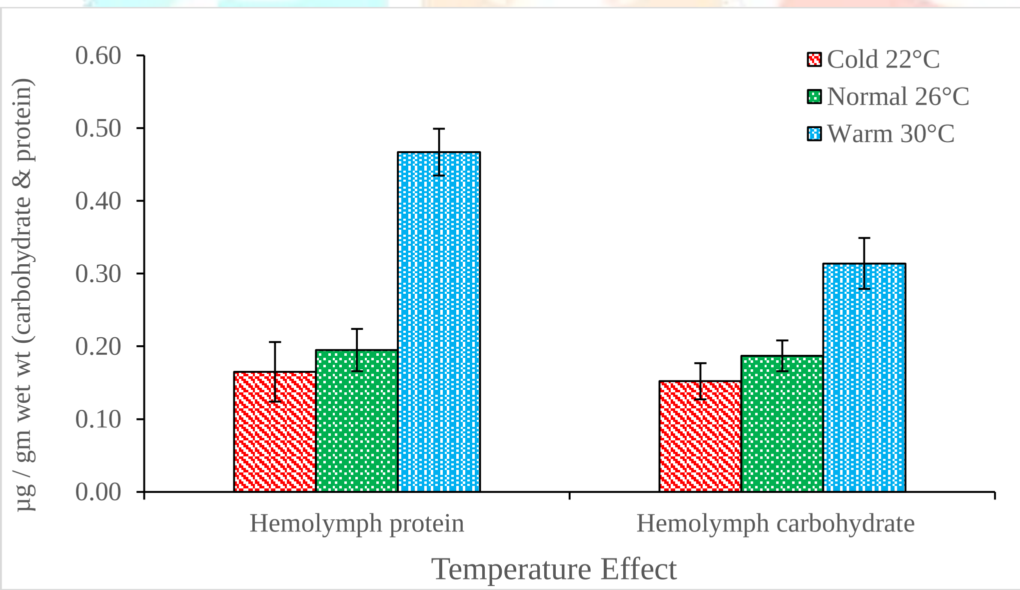


Figure-2: Effect of temperature on crab tissue haemolymph biochemical constituents.



The figure-1 shows the rate of oxygen consumption and biochemical parameters protein and carbohydrate of the crab's haemolymph. The rate of oxygen consumption decreased (0.78±0.05) under cold stress and increased (1.20±0.03) under heat stress when compared to normal temperature (0.83±0.02). The figure-2 shows the protein level in the haemolymph tend to increase under warm condition (0.47±0.03) and decreased under cold condition (0.17±0.04), when compared to the normal condition (0.20±0.03). The levels of haemolymph carbohydrates increased at 30^0C (0.31±0.04) and a decrease at 22^0C (0.15±0.03), when compared to the control at 26^0C (0.19±0.02). All the values are significant at $P<0.05$.

A decrease in oxygen consumption rate in a wide range of experimental temperature which happen to be statistically insignificant was reported in White and North Sea snails (Sokolova and Portner, 2003).Whereas a significant increased in oxygen consumption in H.*Brachysoma* with increasing acclimation temperature between 15 to 31˚C and 33 to 36˚C have been reported by Dalvi (2009) indicating better capability for adapting to higher temperatures. The increased in the respiration rate of *Barytelphusa guerini* with the increased in temperature observed in the present study corresponds to results obtained in previous studies (Achituv and Cook,1984 and Emmerson, 1985).

The metabolic rate of crustaceans depends on a number of internal and external variables. Temperature is one of the external factors that influences the life pattern of poikilothermous animals. It influences the animals directly and indirectly. Johnson et al. (1954) interprets the direct effect of temperature on organisms in terms of activation energies of key biochemical reactions.

Temperature affects the organisms indirectly through its effect on the metabolic rate of the organism. A change of external temperature results in a change of oxygen consumption (metabolic rate). In S.*flower*i oxygen consumption was positively correlated with temperatures between 16 ˚C and 31 ˚C. At 31 ˚C the oxygen consumption was 2.4 times greater than at 16 ˚C. The relationship between oxygen consumption and temperature has been attributed to the physiological processes and reactions taking place in the animal's body. Mc Mohon *et al*. (1978) also explained the pattern of increase of metabolic rate with temperature as a result of bronchial water flow to supply extra oxygen demand in the crab, *Cancer magister*.

In crustacean, an increase in haemolymph glucose levels is considered a response to stress (Hall and van Ham, 1998; Lorenzon, 2005). The release of glucose into the haemolymph is mediated by the crustacean hyperglycaemic hormone (CHH) through the mobilisation of intracellular glycogen stores (Stentiford et al., 2001). Hyperglycaemia has been documented in crustacean following exposure to a variety of stressors, such as emersion (Durand et al., 2000), cold shock (Kuo and Yang, 1999), anoxia and carbon dioxide (Hall and van Ham, 1998).nitrite (Yildiz and Benli, 2004), and pollutants (Lorenzon et al., 2000). Increases in water temperature resulted in increased blood CHH in Cancer pagurus (Wilcockson et al., 2002). In Uca minax, a temperature value of 10 ˚C caused a significant decrease in haemolymph glucose levels (Dean and Vernberg, 1965), Whereas in P.interruptus,increases in temperature from 20 ˚C to 27 ˚C induced an increase in haemolymph glucose levels (Ocampo et al., 2003). Powell and Rowley (2008) suggested that increases in haemolymph glucose levels may be due to the mobilisation of glucose from muscles in stressed crabs.

Many intertidal animals live under conditions where temperatures regularly approach the upper lethal limit and in such cases, it is likely that mechanisms will be required both to repair thermal damage and to protect the organism from further thermal damage as the temperature increases. It is expected that the ability to exist at an environmental temperature is expressed in the physiological and biochemical responses of the animal. In H.americanus, significant differences in total protein concentrations were observed between animals kept at 6 ˚C and those maintained at 15 ˚C (Lorenzon et al., 2000). It can be hypothesised that crabs maintained at 30 ˚C used haemolymph proteins to cope with high temperature,considering that proteins are the primary source of energy in crustacean (Helland et al., 2003;Sanchez-Paz et al., 2007). In the present study, the crabs were exposed to varying water temperatures of 30^0C, 26^0C, and 22 ^{0}C for 2hrs, which resulted in changes in the biochemical constituents. The protein and carbohydrates levels were increased with increase in temperature (30^0C) and decreased with decrease in temperature (22^0C), when compared to the normal (26^0C).

In conclusion, the present study demonstrated that both the lowest and the highest temperature tested influenced immune parameters in crabs. In any case, the present study also indicated that *Barytelphusa guerini* modulated its cellular and biochemical parameters in order to cope with temperature. This suggested that when crabs suffer temperature stress in the environment, they can respond promptly by modulating immune parameters. However, both results of the present study and those from the literature suggest differing immunomodulation patterns in crustacean species depending on their thermal optimum.

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References:

1. Tomanek, L. and M.J.Zuzow, 2010.The proteomic response of the muscle congeners mytilus galloprovincialis and M.trossulus to acute heat stress: implications for thermal tolerance limits and metabolic costs of thermal stress. Journal Experimental Biology, 213:3559-3574.

2. Feder, M. E., 1999. Organisamal,ecological and evolutionary aspects of heat-shock proteins and the stress response: established conclusions and unresolved issues. American Zoologist, 39: 857-864.

3. Henkel,S.K andG.E.Hofmann,2008.Differing patterns of hsp70gene expression in invasive and native kelp species:evidence for acclimation-induced variation.Journal of applied phycology.20(5);465-474.

4. Diwan,A.D and R.Nagabhushanam,1976.Stidies on heat tolerance in the freshwater crab,Bartelphusa cunicularis.Hydrobiologio,50:65-70.

5. Prech et al.,(1973)Christopherson,J.,Honsel,H and Larcher,W.(1973).Temperature and Life,Springer-Verlag,Berlin,Heidelberg.

6. Portner, H.O., Mark,F.C and Bock,C(2004).Oxygen limited thermal tolerance in fish?Answers obtained by nuclear magnetic resonance techniques.Respir.Physiol&Neurobiol.,141(3):243-260.

7. Mandic,M. Todgham,A.E and Richards,J.G.(2009).Mechanisms and evolution of hypoxia tolerance in fish.Proc.R.Soc.B,276:735-744.

8. Hagerman,L.(1970).Oxygen consumption of Crangon Vulgaris (Fao)in relation to salinity.Ophelia,7(2).283-292.

9. Jones,M.B(1974)Survival and oxygen consumption in various salinities of three species of Idotea (crustacean,isopoda)from different labitats.Comp.Biochem.Physiol.,48A:501-506.

10.McMahon,B.R.,Burgren,w.w.and Wilkens, J.L.(1974).Respiratory responses to long-term hypoxic stress in the crayfish,Orconectes virilise.J.Exp.Biol.,60:195-206.

11. Kapoor,N.N.(1974).Some studies on the respiration of stoneflynymph, paragnetina media (walker).hydrobiologia,44:37-41.

12.Kotaiah,Kand Rajabai, B.S.(1975).Starvation stress on metabolism of the tropical freshwater crab, Paratelphusa hydrodromus(herbst)with reference to size, sex and sudden changes of temperature.Indian.J.Exp.Biol.,13:180-184.

13.Newell,R.C.Roy,A.and Armitage, K.B.(1979).An analysis of factors affecting the oxygen consumption of the isopoda Lagia Oceania.Physiological zoology,49(1):109-137.

14. Bell, G.H., Davidson,J.N and Scarborough,H.(1970).Textbook of physiology and biochemistry,Neil and co.ltd.Edinburgh,1268pp.

15.Wolvekamp,H.P. and Waterman,T.H.(1969).Respiration.pages35-100.In:Waterman,T.H.(ed).Physiology of Crustacea I. Academic Press,NewYork.

16. Bullock, T.H., 1955.Compensation for temperature in the metabolism and activity of poikilotherms. Biological Reviews, 30:311-342.

17. Schlieker, C.,B.Bukan and A.Mogk,2002.Prevention and reversion of protein aggregation by molecular chaperones in E.Coli:implications for their applicability in biotechnology. Journal of Biotechnology, 96: 13-21.

18. Amsler, M. and R.V.George, 1984.seasonal variation in the biochemical composition of the embryos of callinectes sapidus. Journal of crustacean biology4 (4); 546-553.