Effects of temperature on oxygen consumption and biochemical contents in fresh water crab, *Barytelphusa guerini*

M.S. KHAGOKPAM, R.S. OINAM, G.A. KADAM AND R.P. MALI

**ABSTRACT**

The influence of temperature on oxygen consumption in whole animal, glycogen and protein content in tissues (leg muscle and hepatopancreas) of *Barytelphusa guerini* was determined at cold (18 ± 0.5°C), warm (35 ± 0.5°C) and normal temperatures. Oxygen consumption increased significantly (p < 0.05) under warm condition and decreased insignificantly under cold condition. Leg muscle and hepatopancreas glycogen were found to be decreased in both the conditioned temperatures (cold and warm) except for hepatopancreas in warm condition where the value was increased significantly (p < 0.001). Whereas the protein content in both the tissues were found to be increased on cold and warm acclimation. It was concluded that the high rate of intermediary metabolism during thermal stress was supported by utilizing oxygen and involvement of various biochemical and physiological adjustments.

**Key words :** *Barytelphusa guerini*, Temperature, Oxygen consumption, Glycogen, Protein

Temperature is an environmental parameter that plays a key role in determining animal distribution in any environment. Relationships between animal occurrence and survival have been clearly recognized for a long time as being related to maximum and minimum temperatures. Precht *et al.* (1973) stated that the temperature influences 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. Temperature is known to affect the chemical composition of aquatic organisms (Landau, 1992 and Brown *et al.*, 1994).

Rate of respiration and metabolic rate is normally assessed in terms of oxygen consumption which is a highly complex physiological process and is influenced by various extrinsic and intrinsic factors such as – temperature (Pörtner *et al.*, 2004; Valeria *et al.*, 2008 and Mandic *et al.*, 2009); salinity (Hagerman, 1970 and Jones, 1974); oxygen tension (McMahon *et al.*, 1974); body size (Kapoor, 1974) and starvation (Kotaiah and Rajabai, 1975). With increasing concern about global warming, habitat fragmentation and introductions of exotic species, it is imperative to understand how changes in species composition scale up to affect large-scale ecosystem processes (Vitousek 1990; Lawton and Jones, 1995; Hector *et al.*, 2001).

Diwan and Nagabhushanam (1976) described that the variations in chemical constitution of tissues have been associated with differences in environmental temperatures. The effects of temperature acclimation on some aspects of carbohydrate metabolism in decapod Crustacea have also been reported (Dean and Vernberg, 1965). A much higher concentration of glycogen and significantly lower lipid reserves in the tissues of fish acclimated to 5°C than fish acclimated to either 15 or 25°C have also been reported (Johnston and Maitland, 1980; Stone and Sidell, 1981 and Johnston and Dunn, 1987).

The effect of temperature on protein synthesis and degradation rate under cold condition in fresh water male crab, *Barytelphusa guerini* have been reported (Ambore, 1974 and Kadam, 1980). Berger and Emlet (2007) have also shown that acclimation of *B. glandula* to relatively higher temperatures resulted in higher levels of protein synthesis. A mechanism facilitating the release of oxygen at cell level linked with O₂ demand at the cost of increased in protein synthesis have also been reported.

A comprehensive study of the aquatic poikilotherm in relation to thermal acclimation has been proposed to investigate the effect of cold and warm temperatures on the oxygen consumption in order to observe the changes in the metabolic profiles which enable us to arrive at a clear understanding of the total oxygen consumption of fresh water male crab, *Barytelphusa guerini*. 

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Simultaneously changes in total protein content and glycogen content were also studied after acclimation to different temperatures.

**MATERIALS AND METHODS**

Suggesting a high degree of temperature adaptability to laboratory conditions and its wide range of natural habitat (Kadam, 1980), the fresh water male crab, *Barytelphusa guerini* has been chosen as the experimental animal. The male crabs with body weight (35 – 45 g) were collected from the paddy fields of the adjoining districts of Nanded (Maharashtra) and Nizamabad (Andhra Pradesh) for the present investigation. The crabs were brought to the laboratory and kept submerged in glass troughs containing sufficient quantity of tap water (pH: 7 – 7.5; Total hardness: 100 – 112 mg/L and Dissolved oxygen: 4.5 – 5.7 mg/L). During their sojourn in the laboratory, the animals were given ad libitum quantities of minced meat daily in the evening so that it coincides with the time of feeding in their natural habitat. The water in the troughs was replaced daily with fresh dechlorinated tap water.

The water temperature was around 24 ± 1⁰C during winter months and 33 ± 1⁰C during summer months. After one week of their adaptive sojourn in the laboratory in order to obviate the effect of environmental changes, the animals were divided into three groups and maintained at their respective temperatures consecutively for 20 days. The first set of the animals was maintained at the laboratory temperature only to serve as control or laboratory adapted normal animals. The second set of animals was subjected to cold temperature (18 ± 0.5⁰C); while the third set of animals was subjected to warm temperature (35 ± 0.5⁰C) and served as experimental animals. Uninjured intermoult stage (C₄) crabs were selected for the present investigation and the animals were not fed 24 hours prior to the commencement of the experiments to eliminate the influence of differential diet (Gilbert, 1959).

The total oxygen consumption of the crab was measured by using the apparatus as described by Saroja (1959). The amount of dissolved oxygen content of the water samples was estimated by the standard Winkler’s iodometric method (Welsh and Smith, 1960). All duplications were done at the same period of the day to maintain constant temperature. The oxygen consumed by the animal in one hour was calculated and represented as ml of O₂/hr of the animal. The glycogen content of the tissue in normal and experimental animals was estimated by Anthrone Method (Seifter *et al.*, 1950); since, the anthrone reaction forms the basis of a rapid and conventional method for the determination of glycogen. The values were read from the standard graph of glucose and the amount was expressed in mg of glycogen/gm wet weight of tissue. For the estimation of total protein content, the method recommended by Lowry *et al.* (1951) was employed and the amount of total protein content was expressed in mg of protein/gm wet weight of tissue.

Each experiment was repeated at least 8 times and the mean of the eight experiments with standard deviations were used to make comparisons with other experiments. Data were statistically analyzed using means with standard deviations and Student ‘T’ Test. The values of p = 0.05 were taken as significant.

**RESULTS AND DISCUSSION**

The influence of the acclimation to different temperatures on total oxygen consumption of whole animal and on the tissues (leg muscle and hepatopancreas) of fresh water male crab, *Barytelphusa guerini* was determined and the results are presented in Table 1 and in Fig. 1.

From the data given in Table 1, it is clear that total oxygen consumption decreased in cold acclimation temperature and increased in warm acclimation temperature. The glycogen content in leg muscle decreased in both cold and warm conditions whereas in hepatopancreas, the values were found to be decreasing in cold temperature and increasing in warm temperature. The values of total protein content in leg muscle and hepatopancreas were found to be increased in both the conditions.

The physiological mechanisms limiting and adjusting cold and heat tolerance have regained interest in the light of global warming and associated shifts in the geographical distribution of ectothermic animals. In
accordance with Shelford’s law of tolerance decreasing whole animal aerobic scope characterizes the onset of thermal limitation at low and high pejus thresholds (Shelford, 1931). This led to the hypothesis of a unifying concept, proposing that a mismatch between the O$_2$ demand and O$_2$ transport from gills to cells is the primary mechanism restricting animal tolerance to thermal extremes (Pörtner and Knust, 2007). And the aerobic scope of an animal is indicated by falling oxygen levels in the body fluids and the progressively limited capacity of circulatory and ventilatory mechanisms during thermal stress (Frederich and Pörtner, 2000; Satoris et al., 2003 and Metzger et al., 2007).

A decrease in oxygen consumption rate in a wide range of experimental temperatures which happen to be statistically insignificant was reported in White and North Sea snails (Sokolova and Pörtner, 2003). Whereas a statistically insignificant was reported in White and North range of experimental temperatures which happen to be statistically insignificant was reported in White and North Sea snails (Sokolova and Pörtner, 2003). Whereas a statistically 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); which was expected as both studies used a closed bottle system. As temperature directly affects the rate of all biological processes the increase in respiration rate with an increase in temperature is not surprising (Schmidt – Nielsen, 1983).

It is conceivable that visceral blood oxygenation levels increased during warming to meet rising metabolic oxygen demands. This is possibly being a trade-off at the expense of reduced blood supply to the less active muscular tissue (Johnston and Dunn, 1987). The oxygenation levels can be increased or remain unchanged in more vital organs such as liver when oxygen supply to muscle is reduced (Mark et al., 2002). While Pörtner et al. (2005) and Wittmann et al. (2008) described that the increase in oxygen consumption rates with rising temperature is typically exponential within the passive thermal tolerance window set by upper and lower critical temperatures. And in contrast, non-exponential temperature-dependent oxygen consumption has been previously detected in larvae of Cancer irroratus (Sastry, 1979).

Finally it can be inferred that thermal acclimation caused a negligible significant variation in total oxygen consumption of fresh water male crab, Barytelphusa guerini. The adjustment to increase and decrease in total oxygen consumption was facilitated by activities involving biochemical and physiological adjustments where glucose and fatty acids are broken down to provide energy by producing more ATPs and of ATPases to support the increased rate of metabolism, locomotory, respiratory, and circulatory activities. Andrejew (1948) reported that glycogen serves as a reserve of carbon and energy. However, the demonstration of glycogen as a cell constituent suggests that the reserves may not be exclusively lipid (German et al., 1961). Antoine and Tepper (1969) also signify that, in Mycobacterium phlei, glycogen is a more labile storage material than lipid and is utilized before lipid.

A significant depletion in the glycogen content in the tissues (leg muscle and hepatopancreas) was observed in freshwater crab, Barytelphusa guerini exposed to cold and warm temperatures in the present investigation (Table 1) and it is supported by the findings of Kulkarni and Nagabhushanam (1978) and Krishnamoorthy (1979) who had briefed that the alteration in biochemical parameters to be eco-physiologically significance in counteracting the ambient thermal fluxes. Glucose being the predominant substrate in the energy yielding process, carbohydrate metabolism has been shown to play an important role in the energetic of thermal stress or adaptation (Bonthu, 1995). However, there has been an increase in the glycogen content in hepatopancreas upon exposure to warm temperatures (Table 1). Generally in poikilotherms maintenance of energy requirements are considerably lowered upon acclimation to cold temperature (Hochachka and Somero, 1973).

Considering the biochemical mechanisms in

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<th>Table 1 : Total oxygen consumption, glycogen content and protein content of fresh water male crab</th>
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Acclimation to be similar in different organisms, three possible modes of triggering and control are suggested by Rao (1966) namely, direct effects of temperature, effects of the nervous system and, most important of all, regulation through the release of hormones or hormone-like substances. From the large number of studies carried out to date, it seems that animal cells utilise a multitude of volume regulatory mechanisms, including transport of inorganic and organic osmolytes across the cell membrane and alterations in metabolism to modify levels of organic metabolites (Lang et al., 1998). Several metabolic pathways are sensitive to cell volume changes, including glycogen synthesis and glycolysis, leading to changes in the amount of carbohydrate metabolites that contribute to cellular osmolarity (Al-Habori et al., 1992).

It is known that the protein metabolism, just like carbohydrate or lipid metabolism, shows some adaptive changes in poikilotherms when exposed to thermal stress. Enhanced rate of general protein synthesis measured by the incorporation of labelled amino acids into proteins has been reported after cold acclimation in the liver of gold fish (Das and Prosser, 1967). A significant increase in protein content in Poecilobdella viridis during warm and cold acclimation have been also reported by Kulkarni and Nagabhushanam (1978), and the same pattern was noticed in leg muscle and hepatopancreas of fresh water male crab, Barytelphusa guerini in the present investigation (Table 1).

Acclimation relatively to higher temperatures resulting in higher levels of protein synthesis has been reported in B. glandula (Berger and Emlet, 2007). Also of interest is the possibility that disassembly of the protein synthetic system at high temperatures is associated with a shift to production of heat shock proteins, as in other organisms (Lemaux et al., 1978 and Moran et al., 1978). Protein synthesis rates may also be influenced by membrane transitions in different temperature range and another factor that may play a role is the requirement for adjustment of blood pH with temperature change (Rahn and Baumgardner, 1972).

A remarkable increase of the total protein content in liver and gill and less in muscle of the goldfish during cold adaptation have been reported Das and Prosser (1967). Robertson et al. (2001) also reported an increase in whole body protein synthesis rates accompanied by increased in both RNA activity and RNA/protein ratio in the baltic isopod crustacean, Saduria entomon. Significant recovery from drop in serum protein concentrations following metabolic compensation has also been reported (Ennis, 1973) and depletion of available organic reserves by starvation resulting in a loss of the ability to respond to cold exposure by a compensatory increase in metabolic rate was also reported (Vernberg, 1959). These observations suggest that there exists an integrative mechanism capable of preventing temperature acclimation when available organic reserves are required for what appear to be more vital metabolic needs and a prolonged encounter to low temperatures necessitate a compensatory metabolic reorganization (Passano, 1960).

In conclusion, the present study suggests that the capacity of oxygen delivery is set to a level just sufficient to meet maximum oxygen demand between the average highs and lows of laboratory temperatures. The increase in activity during thermal stress and the need for transporting and utilizing oxygen to support high level of activity involves various biochemical and physiological adjustments, indicating high rate of intermediary metabolism, the substrates for which may come from the stored glycogen and fat in addition to ingested food and the variation in tissues constituents at different temperatures which reflect the differences in their energy requirements for maintenance of body physiology during thermal stress. Therefore, the recorded data indicate that temperature has a role in maintaining normal physiological functions of the fresh water male crab, Barytelphusa guerini which are of vital statistics for sustaining life. Further investigation emphasizing the importance of taking into account of other relevant factors (e.g. seasonal amplitude of temperatures, food availability and activity levels of animals) are required when studying metabolic adaptations to temperature.

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LITERATURE CITED


