*Punjab Univ. J. Zool., Vol. 9, pp. 35-44, 1994*

# EFFECT OF ACCLIMATION TEMPERATURE ON PROXIMATE BIOCHEMICAL ANALYSIS OF SOME GASTROPOD SNAILS*

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**Abstract**: The present paper describes the relation of proximate biochemical changes as a function of acclimation temperature in four gastropod snails. The results showed that variation in the moisture, crude protein, fat, glycogen and ash contents of all the snail species studied were found associated with difference in acclimation temperature ($P < 0.001$).

**Key words**: Gastropod snails, acclimation temperature, proximate biochemical analysis.

## INRODUCTION

To ensure survival, all organisms show some degree of adjustment to the fluctuating environmental temperature, that influences the rate of almost all biological processes. Effect of temperature on various physiological and metabolic processes of pulmonate snails has been admirably reviewed by Aldridge (1983) and McMahon (1983). However, proximate biochemical changes in snails following acclimation to different temperatures seems to have received little attention. The only work of significance regarding effects of various acclimation temperatures on the survival and proximate biochemical anslysis of snail's tissues as well as some other related species have been reported by Nagabhushanum and Azmatunnisa (1976), Loomis and Deborah (1987), Wolmarans (1987), Barber *et al.* (1988) and Tanveer (1989b). Present study examines the proximate biochemical constituents of four gastropod snail species acclimated for 14 days at different temperatures ranging from 10-40 °C with an interval of 5 °C. The temperature acclimation relation of these snails is of considerable importance in facilitating further studies on their various physiological aspects.

## MATERIALS AND METHODS

Gastropod snails used in the present study were *Lymnaea acuminata* (*Lymnaeidae*), *Indoplanorbis exustus* (*Planorbidae*), *Physa acuta* (*Physidae*) and *Bellamaya bengalensis* (*Viviparidae*). Excluding *B. bengalensis* all these snail species are intermediate hosts for various helminth parasites of medical importance. The snails collected and maintained following the methods described by Tanveer (1989a) and Tanveer *et al.* (1989). In the present study unless otherwise stated laboratory bred snails were used. The required number of each snail species were removed from the

*Part of a thesis submitted to the University of the Punjab, Lahore, Pakistan for the award of Ph.D. degree in Zoology holding tank and acclimatized separately at different temperatures ranging from 10-40 °C with an interval of 5 °C for 14 days. *B. bengalensis* utilized in this study was 70 week old, while the rest three snail species were 24 weeks old. For each determination of proximate biochemical analysis, homogenized soft tissue material of 20 samples were treated individually for each snail species. Total water content of body was estimated by completely drying it at 100 °C untill a constant weight was reached. Glycogen was estimated from the dried samples by Kemp *et al.* (1954) method. The fat was extracted from the powdered tissue by using soxhlet apparatus. Protein level was measured by Kjeldahl micromethod (Hawk *et al.*, 1954) using the value of 6.25 as a conversion factor between nitrogen content and protein. Ash contents were determined by ashing the tissue in a muffle furnace.

The results correspond to the mean of percentage $\pm 5.0$ of all the determinations calculated both on wet and dry soft tissue basis. All the weighing were made to the nearest of 0.01 mg. Significance level was tested according to Student's `t' test (Sokal and Rohlf, 1969).

## RESULTS

The results presented as mean percentage on wet and dry weight basis were found statistically significant ($p < 0.001$) when analyzed by Student's `t' test (Figs. 1-4). The results clearly revealed the variation in the proximate chemical constituents of snails have been associated with difference in the ambient temperature. It was observed during present studies that increase in the temperature increased the body water content in all the snails under observation. This increase was more pronounced in *I. exustus* and *P. acuta* than *L. acuminata* and *B. bengalensis*. The results on dry weights were in the opposite direction. These changes in water content and dry weights due to temperature for the species under observation were highly significant ($p < 0.001$) when compared with the lowest temperature acclimated snails.

In general, there seemed to be a reciprocal relationship between the water content and the crude proteins of the species studied. As the water content increased, the protein content decreased. Snails kept at highest temperature had highest water contents but the lowest protein value.

Apart from the water, and crude protein content, crude fats, glycogen and ash content were also observed. Total fat and glycogen content of all the species showed an opposite response to water contents when described on wet weight basis. When observed on the dry weight basis, the fat content of *L. acuminata* remained constant when the temperature increased from 10 to 20 °C. After this, the fat content decreased. In the other three species, the fat content first increased with the inccrease in temperature (10-25 °C) and then decreased.

The glycogen contents showed a gradual decrease with increse in temperature. With the increase in temperature from 10 $^{0}$C to the optimum temperature of each snail, the ash contents when expressed on wet weight basis increased and further raising the temperature caused release of the minerals from the body. Similar trend was observed

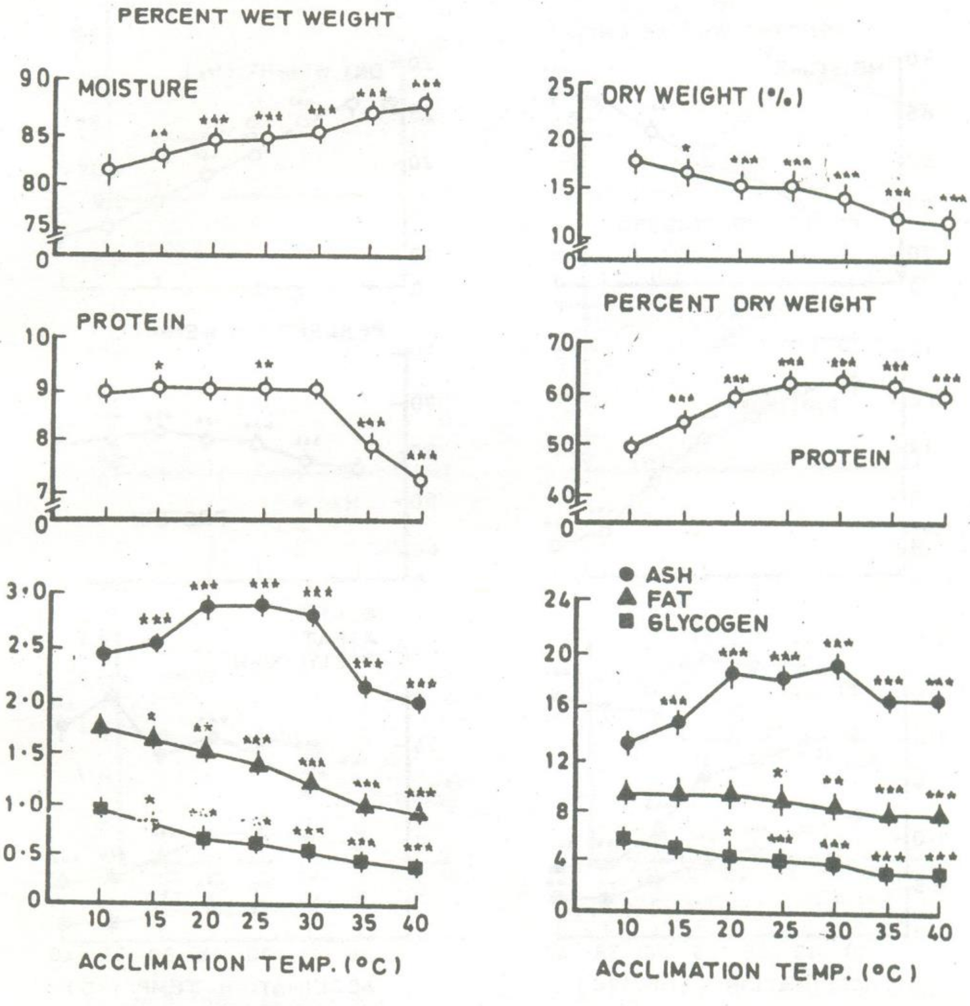


**Figure 1.** Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Lymnaea acuminata*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to `t' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

on the dry weight basis also. The response of *P. acuta* was different from rest of the three species, and therefore, warrants further comments. In this snail the ash, when described on the dry weight basis, showed an inverse relationship with the dry weight and a positive correlation with the moisture contents. This was not seen in other snail species.

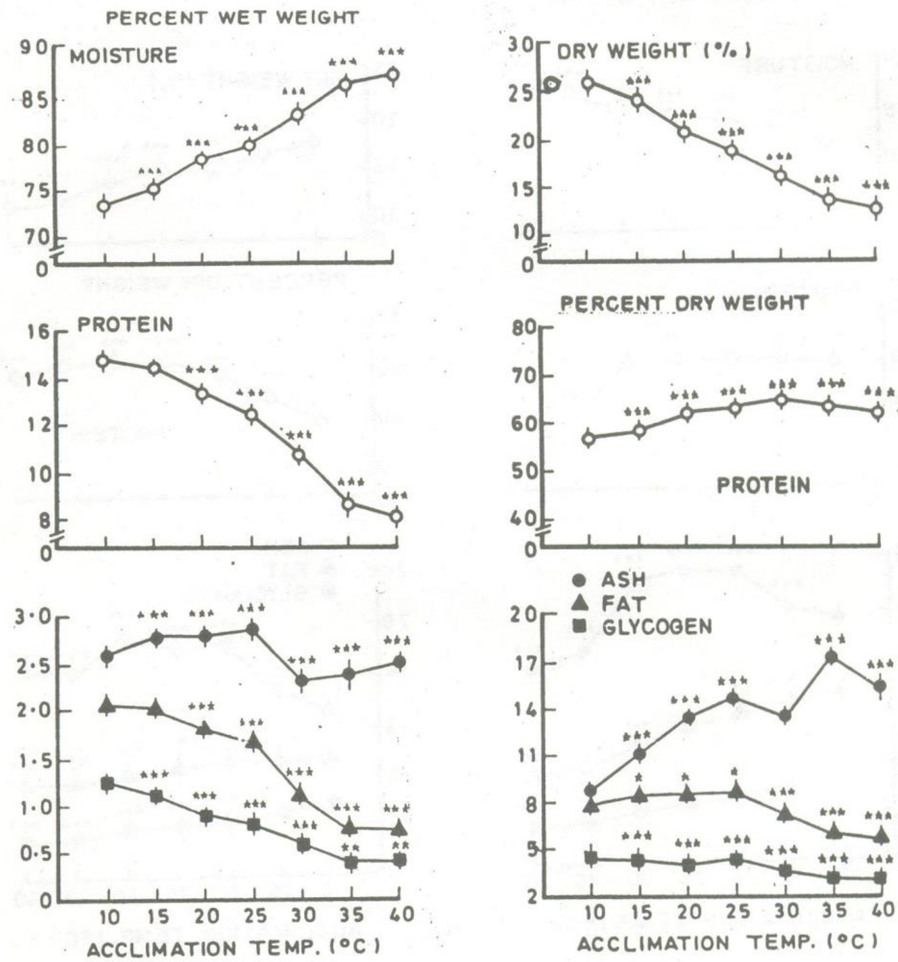


**Figure 2**. Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Indoplanorbis exustus*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to `t' test. * = P<0.05, ** = p<0.01, *** = p<0.001.

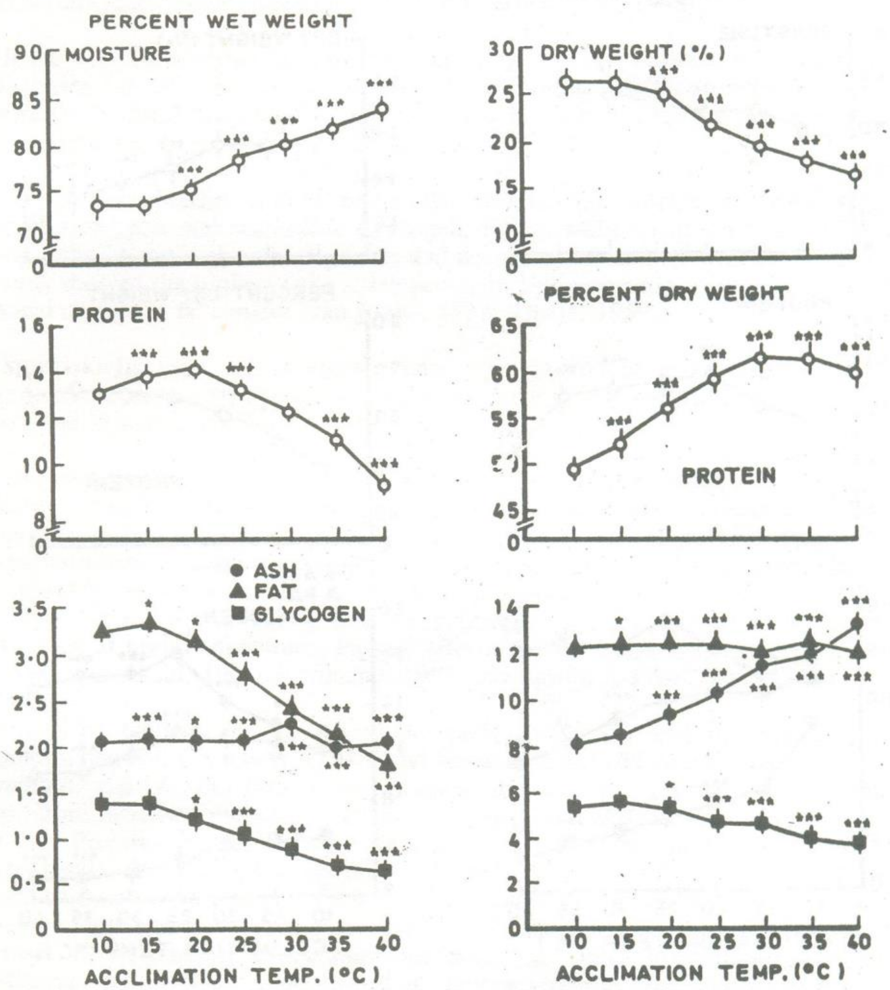


**Figure 3**. Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Physa acuta*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to 't' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

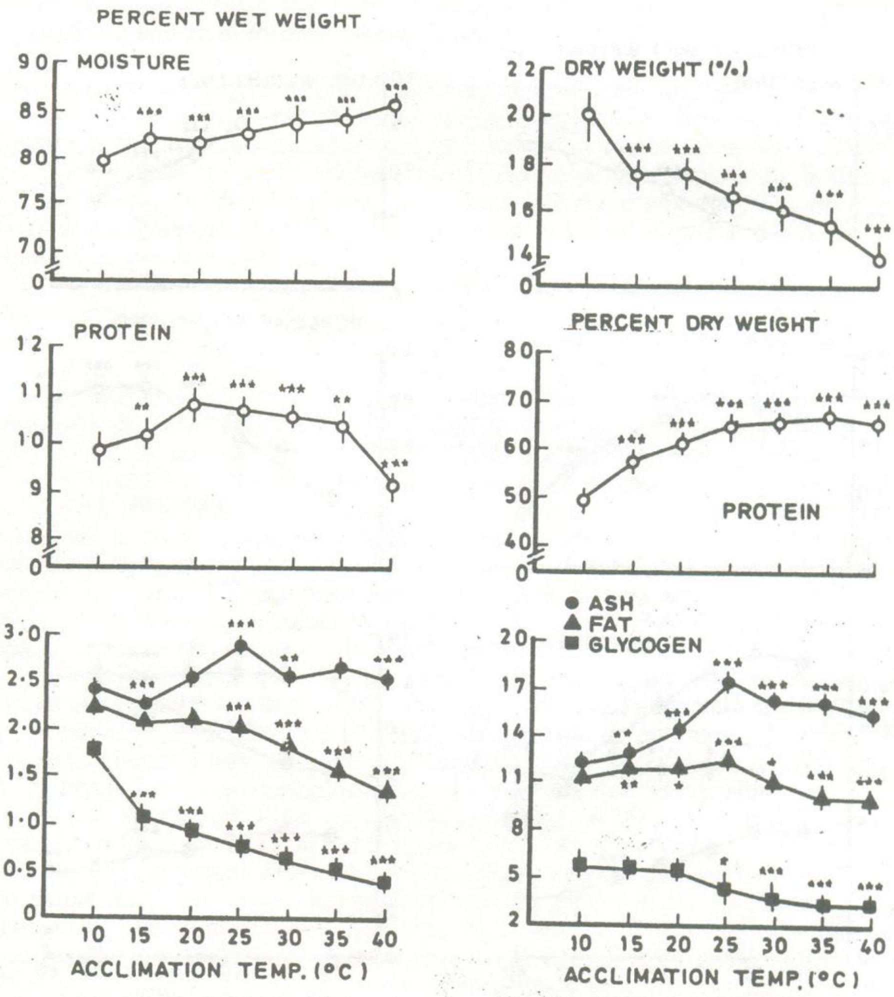


**Figure 4**. Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Bellamaya bengalensis*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to `t' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

## DISCUSSION

Glycogen has been found in the body of pulmonates and other molluscs (Goddard and Martin, 1966) and has been reported to the most readily catabolized substrate to meet the energetic burden posed by infection (Barber *et al.*, 1988).

It has also been shown that in molluscs, glycogen is mobilized more quickly than fats (Baker *et al.*, 1942). There is aslo indication, though indirect, that high temperataure (July) decreased the glycogen and fat contents but the proteins increased to a maximum and then decreased (Masumoto *et al.*, 1934).

Castro and Mattio (1987) relate the weight and energy reserves with the reproductive cycle and starvation. Glycogen, lipids, and protein contents increased in spring with the rapid growth of gonads and decreased in summer with spawning, while proteins showed the highest values. However, in Helix pomatia, there was no apparent seasonal change in fat content van Brand, 1931; Thiele, 1959).

Surprisingly, very little is known about other gastropods regarding this aspect and there is also controversy about the dominant source of energy whether glycogen or fat (Barry and Munday, 1959).

The decrease in the glycogen content of the body with increase in the temperature probably reflects an increase in metabolic rate at moderately increased temperature (around optimal temperature) and at extreme temperature, it is possible that the snails entered anaerobic metabolism thereby stripping their body reserves of glycogen. It will be interesting to study in these snails whether they exhibit the pasture effect or not. This type of study will also prove the contention made above regarding anaerobic metabolism at high temperature. Pasture effects in snails have been studied by various workers (Meenakshi, 1964; Hoffmann, 1983; Livingston and de Zwaan, 1983).

Effect of temperature on the whole body proximate analysis was studied by Nagabhushnan and Chintawar, (1976) and Nagabhushnan and Azmatunnisa (1976) in *L. acuminata* and *I. exustus*. These studies showed that increase in acclimation temperature increased the water content of the body but decreased the glycogen and fat contents. Proteins increased first but then showed a decreasing trend. Surprisingly, similar results have also been reported in the fish (Mearow and Houston, 1980). In the present study we have also estimated the water content, proteins, fats, glycogen and ash contents of the snails acclimated at 10-40 $^{O}$C. The results of the analysis showed that increase in acclimation temperature increases the water content while all other constituents are inversely proportional to the temperature. Although these results are similar to the results of Nagabhushnan and Chintawar (1976) and Nagabhushnan and Azmatunnisa (1976) but there are some points that warrant further comments.

Increasing the acclimation temperature increased the water contents of *L. acuminata* and *B. bengalensis* to the tune of 7.50% while *I. exustus* and *P. acuta* had their body water increased by 18.37 and 14.29 %. Increase in water contents of the body upon acclimation to high temperature has also been reported in other snails (van Brand, 1955 and Nagabhushnan and Chintawar (1976) and Nagabhushnan and Azmatunnisa (1976). Reasons for this increase are not known, but at high temperature it is possible that the snails switch to anaerobic metabolism (van Brand, 1955). This anaerobic metabolism will also explain the acute decrease in the glycogen and fat contents of the body. Another factor in increase in the water content of the body is increased breakdown of fats which are known to increase metabolic water.

Acclimation to high temperature decreased the proteins, glycogen and fat contents of the body of all the snails studied. Increase or decrease in acclimation temperature not only effects the ash, fat, carbohydrate, protein and water contents but various other excretory products as well. Wolmarans (1986) made a comparative study in the excretory products of five fresh water snail species (*Bulinus globosus*, *Bulinus tropicus*, *Biomphalaria glabrata*, *Lymnaea natalensis* and *Helisoma duryi*) at 4 $^{O}$C and 25 $^{O}$C and reported that concentration of their acids varied not only from species to species, but also in the same species at different experimental temperatures. It was found that concentration of most of the acids was higher at 4 $^{O}$C than at 25 $^{O}$C except *L. natalensis*.

In the present study increase in temperature from 10 to 40 $^{O}$C had the following changes on the three major energy yielding cellular constituents. Total body protein (nitrogen x 6.25) decreased 20.67, 46.09, 26.83 and 6.85%, total fat decreased 46.02, 63.20, 5.29 and 41.17% while glycogen decreased to the tune of 59.18, 64.62, 57.04 and 74.44% for *L. acuminata*, *I. exustus*, *P. acuta* and *B. bengalensis*, respectively. An analysis of these figures revealed that *L. acuminata* and *P. acuta* had more or less similar responses, but *I. exustus* and *B. bengalensis* differed from them. It is also clear from these values, that glycogen values decreased maximally, followed by fat and protein. This observation points to the fact that these snails had response similar to mammals, where aslo, glycogen is a preferred fuel for energy. When glycogen stores are nearly exhausted, fat provides the energy followed by the proteins. Another thing which is discernible from these data is that, glycogen also has protein sparing property. For example in *B. bengalensis*, decrease in protein is minimum, but comparable decrease in glycogen was maximum when compared with other snails. Similar examples can be given for *L. acuminata* and *P. acuta* which had nearly similar decrease in proteins and similar glycogen and fat values. Based on these biochemical studiees it appears that *B. bengalensis* is more resistant to temperature change, a response also seen in ecological results (Tanveer, 1989b). Further that *I. exustus* is least tolerant of high temperature. All these changes exhibited by these specific snails are mean, to countract the environmental changes and help survival of the species. The detail physiological and biochemical changes underlying these gross changes will have to be studied in order to put them in the right perspective to analyze their biology thoroughly.

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*Received: January 25, 1994*