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Energy Budget in Terms of ATP, ADP, AMP, and Energy Charge in The Osmoregulatory Tissues of Indian Major Carp Catla catla (Hamilton) Under Thermal-Stress and Thermal-Adaptation

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Abstract

The heat-adapted (32°C) Catla catla registered as increased level of ATP and energy charge with concomitant lower levels of ADP, and AMP when compared to cold-adapted (22°C) fishes. All the osmoregulatory tissues like the gill, kidney, and intestine indicate greater ATP turnover adaptation to higher temperatures. In order to differentiate thermal stress from thermal adaptation, the 22°C adapted were readapted to a temperature change of 22°C □32°C at the rate of l°C/hour as in the case of stress and at the rate of l°C/60 hrs as in the case of adaptation During the time course of experiments there is a gradual stepping-up in the levels of ATP and energy charge and a gradual stepping-down in ADP and AMP in all tissues. Further, there is a complete filling-up process in ATP and values reached gradually the original level of these parameters of the control of 32°C adapted fishes. The heat-adapted fishes exhibited a fairly good amount of recovery ranging from 83% to 88% in these adenylate nucleotides. On the other hand in the temperature-stressed fishes the adenylate nucleotides did not reach the control values and the filling processes with ATP could not be completed. Further, the % recovery in these parameters is far less 47 % to 57%, when compared to temperature adapted fishes. The continuous thermal-stress (l°C/hour) action upon this fish Catla catla resulted in stress-adaptation (adaptation resulted due to stress)

Keywords: *ATP, ADP, AMP, Energy charge, Thermal-stress, and Thermal-adaptation*

1. Introduction

Temperature is one of the most important environmental factors with tremendous influence on the metabolism, activity and distribution of animals. Many studies on energy metabolism and growth of fish have been primarily concerned with aspects related to fisheries ecology and management. Thermal adaptation involves a number of active processes with energy expenditure for metabolic re-organizations. (Hochachka, 1967, 1969; Hochachka and Somero1973; Basha Mohideen and Kunnemann, 1979). The variations in adenylate energy nucleotides concentration are correlated to the energy budget and hence energy demands of the animal (Caldwell, 1967; 1969). Further, it is known that the primary energy source, the adenylate nucleotides like ATP, ADP and AMP serves as effective steric modulators in many important metabolic reactions. (Atkinson, 1970) refers to the ATP-ADP-AMP status of a cell as its energy charge, recently some extensive studies have been published on sockeye salmon, Oncorhynchus nerka (Brett, 1979), rainbow trout, Salmo gairdneri (Staples and Nomura, 1976; and Rasmussen,1984; Cho et al. 1982), a great deal of work investigated since several decades in the thermal adaptation of poikilotherms. Hence it appears imperative that studies of this nature should be extended at different levels of organization of the animal namely, the whole animal level, cellular level, and sub-cellular level involving various aspects of the energetics of the animal during thermal stress. Thanks to the pioneering works of Precht and his collaborators on this new approach of differentiation of environmental stress from environmental adaptation. The effects of environmental factors on energy metabolism and growth in fish have recently been reviewed by Webb (1978) and Brett (1979), following the classification of Fry (1971).In recent times it was found necessary and possible to distinguish and differentiate the adaptations, may be thermal or osmotic or any other, from other phenomenon like “stress effects” or “stress-adaptation” which could be easily mistaken for the adaptation in general (Kunnemann and Precht, 1975; Gngo, 1975; Basha Mohideen, 1984; Abdul Rahim, 1989; Dhanunjaya, 1990; Shankar Naik, 1993; Basha Mohideen et al, 2001). The adenylate energy nucleotides like ATP, ADP, and AMP and the Energy charge of the fish adapted to 22°C and 32°C were measured separately and it was continued till the attainment of a constant level in adenylate energy nucleotides

1. The 22°C adapted fishes were re-adapted to a slow temperature change at the rate of 1°C/60hrs from a temperature range of 22°C to 32°C for a period of 35 days (heat-adaptation)
2. The 22°C adapted fishes were re-adapted to an abrupt temperature change at the rate of 1°C/hr from a temperature range of 22°C to 32°C for a period of 35 days (heat-stress)
3. The 32°C adapted fishes were re-adapted to a slow temperature change at the rate of 1°C/60hrs from a temperature range of 32°C to 22°C for a period of 35 days (cold-adaptation)
4. The 32°C adapted fishes were re-adapted to an abrupt temperature change at the rate of 1°C/hr from a temperature range of 32°C to 22°C for a period of 35 days (cold-stress)

2. Method

The levels of concentration of adenylate energy nucleotides like ATP, ADP, and AMP were estimated separately in the gill, kidney, and intestine; in Catla catla of 22°C and 32°C adapted control fishes as well as the experimental fishes which were subjected to heat-stress, cold-stress, heat adaptation and cold-adaptation. The estimation in 22°C and 32°C temperature-adapted fishes served as controls. Prior to estimation, the fish were anesthetized with methane sulfonate (MS 222) in order to make the movements of the fish stop instantaneously, thereby keeping the stationery concentration of the nucleotides unchanged. The estimation of adenylate energy nucleotides like ATP, ADP, and AMP was carried out by adopting the method of Bergmeyer (1974). The measurements were carried out in an ELICO spectrophotometer at the wavelength of 360 nm. The ATP, ADP, and AMP concentrations were expressed in p mole/gm wet weight of the tissue. The levels of adenylate energy charge (AEC) were calculated separately in the gill, kidney, and intestine, of Catla catla subjected to temperature-adaptation (heat-adaptation and cold-adaptation) and the temperature-stress (heat-stress and cold-stress) besides in the 22°C adapted and 32°C adapted control fishes. The adenylate energy charge was calculated by the following formula as given by Atkinson (1977).

$$\mathrm{AEC} = \frac{\mathrm{ATP} + 1/2\ \mathrm{ADP}}{\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP}}$$

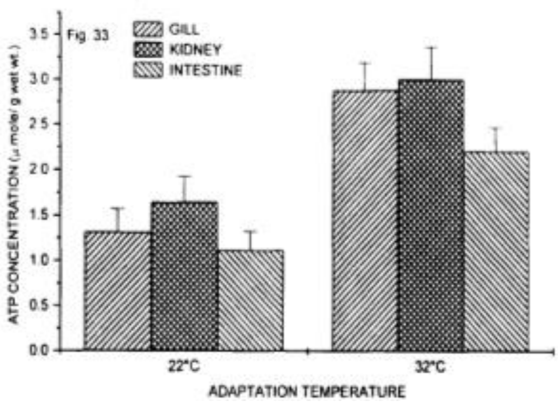

Figure 1 ATP Concentration in Osmoregulatory Tissues of Catla-catla at Different Temperatures

Figure 1 Histogram showing the ATP concentration (u moles/gm wet weight) in osmoregulatory tissues like gill, kidney, and intestine in Catla catla adapted to 22°C and 32°C temperatures. Each histogram is a mean of six individual measurements.

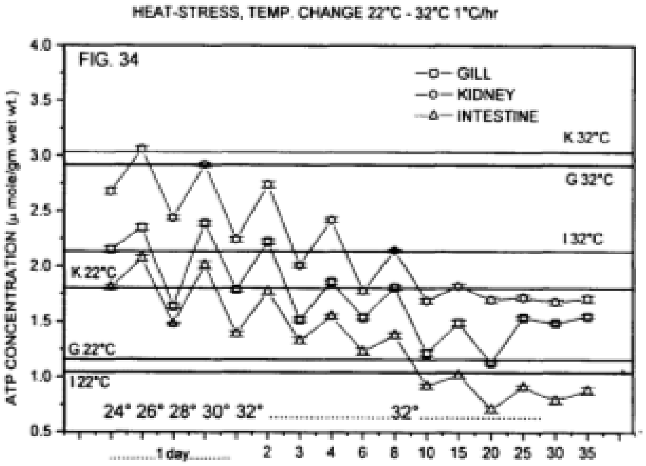

Figure 2 **ATP Concentration in Osmoregulatory Tissues Under Heat Stress in Catla catla**

Figure 2 ATP concentration (u mole/gm. wet weight) in osmoregulatory tissues like gill(), kidney (O), and intestine (A) in Catla catla subjected to an abrupt temperature change from 22°C to 32°C (heat-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

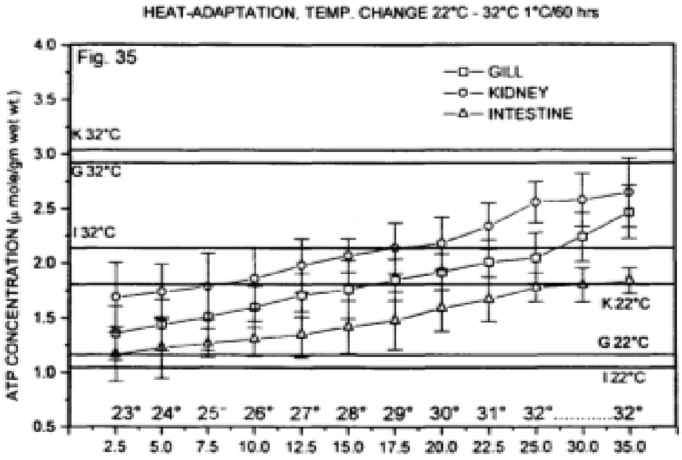

Figure 3 **ATP Concentration in Osmoregulatory Tissues During Heat Adaptation in Catla catla**

Figure 3 ATP concentration (µ moles/gm wet. weight) in osmoregulatory tissues like gill(), kidney (O), and intestine (A) in Catla catla subjected to a slow temperature change from 32°C to 22°C (heat-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

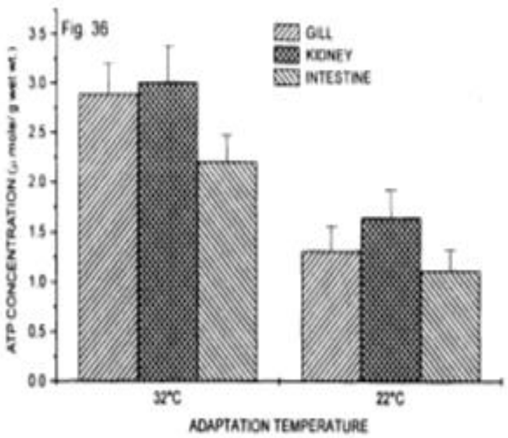

Figure 4 **ATP Concentration in Osmoregulatory Tissues Adapted to Different Temperatures in Catla catla**

Figure 4 Histogram showing the ATP concentration (µ mole/gm. wet. weight) in osmoregulatory tissues like gill, kidney, and intestine adapted to 32°C and 22°C temperatures. Each histogram is a mean of six individual measurements.

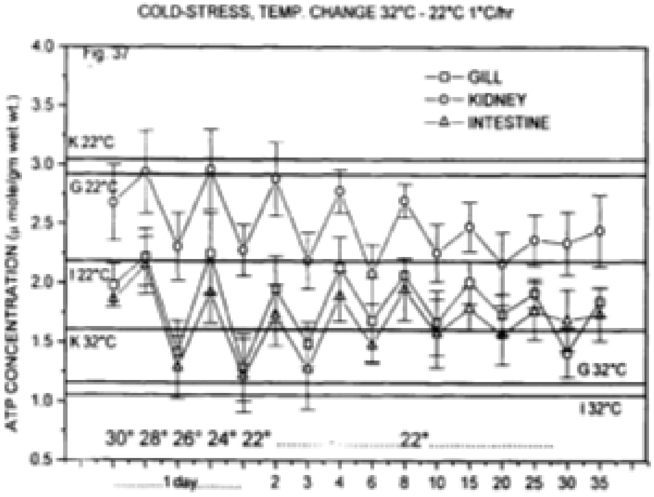

Figure 5 **ATP Concentration in Osmoregulatory Tissues Under Cold Stress in Catla catla**

Figure 5 ATP concentration (µ mole/gm. wet. weight) in osmoregulatory tissues like gill(), kidney (O) and intestine (A) in Catla catla subjected to an abrupt temperature change. from 32°C to 22°C (cold-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

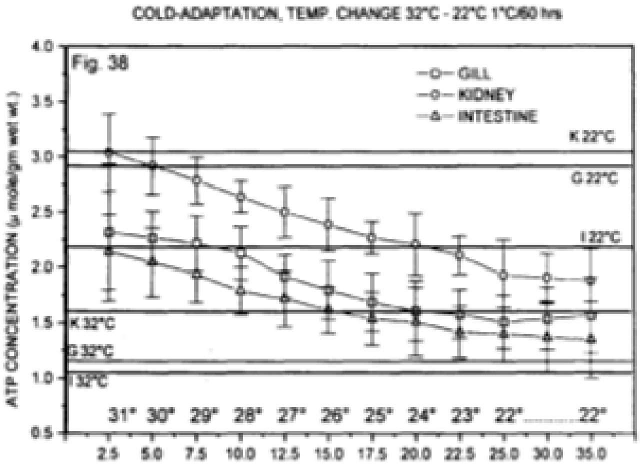

Figure 6 **ATP Concentration in Osmoregulatory Tissues During Cold Adaptation in Catla catla**

Figure 6 ATP concentration (µ mole/gm. wet weight) in osmoregulatory tissues like gill (), kidney (O), and intestine (A) in Catla catla subjected to a slow temperature change from 32°C to 22°C (cold-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

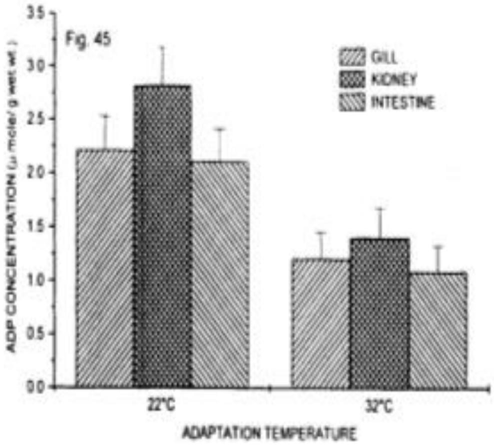

Figure 7 **ADP Concentration in Osmoregulatory Tissues Adapted to Different Temperatures in Catla catla**

Figure 7 Histograms showing the levels of ADP concentration (µ mole/gm. wet weight) in osmoregulatory tissues like gill, kidney and intestine in Catla catla adapted to 22°C and 32°C temperatures. Each histogram is a mean of six individual measurements.

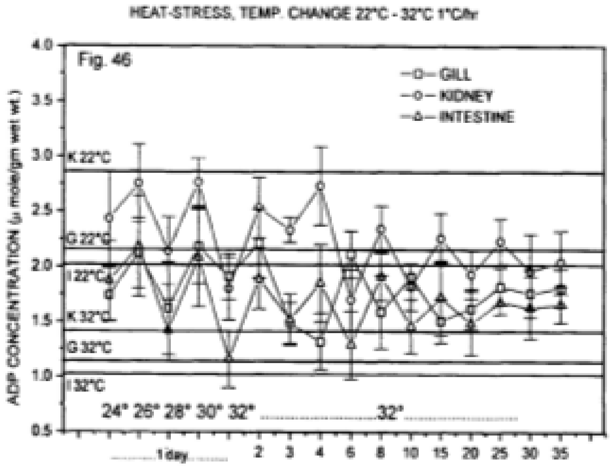

Figure 8 **ADP Concentration in Osmoregulatory Tissues Under Heat Stress in Catla catla**

Figure 8 ADP concentration (u mole/gm. wet weight) in osmoregulatory tissues like gill(), kidney (O) and intestine (A) in Catla catla subjected to an abrupt temperature change from 22°C to 32°C (heat-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

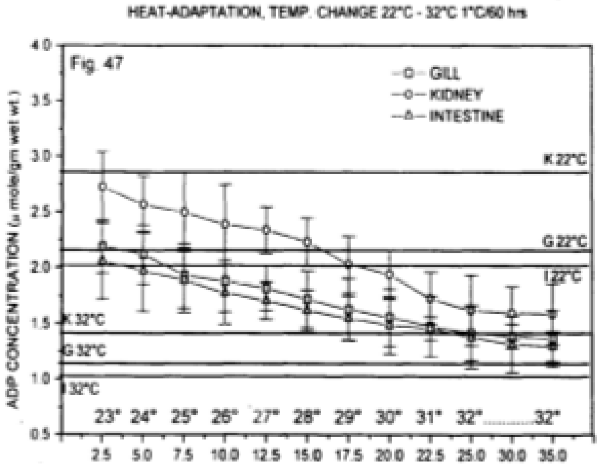

Figure 9 **ADP Concentration in Osmoregulatory Tissues During Heat Adaptation in Catla catla**

Figure 9 ADP concentration (u mole/gm. wet weight) in osmoregulatory tissues like gill(), kidney (O) and intestine (A) in Catla catla subjected to a slow temperature change from 22°C to 32°C (heat-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

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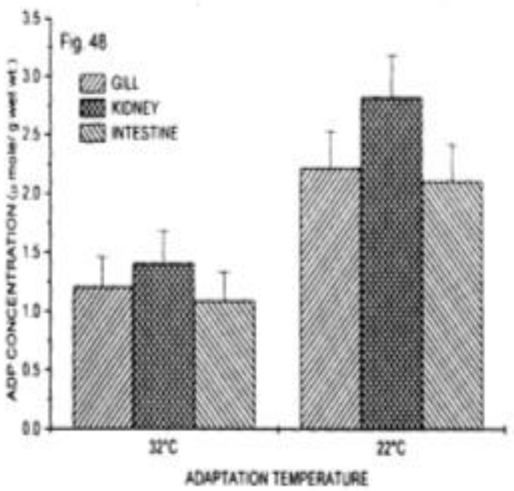

Figure 10 ADP Concentration in Osmoregulatory Tissues at 32°C and 22°C in Catla catla

Figure 10 Histograms showing the level of ADP concentration in osmoregulatory tissues like gill, kidney, and intestine adapted to 32°C and 22°C temperatures. Each histogram is a mean of six individual measurements.

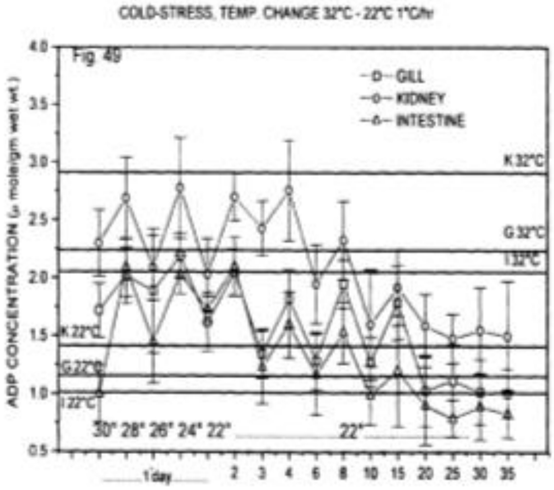

Figure 11 ADP Concentration in Osmoregulatory Tissues During Cold Stress in Catla catla

Figure 11 ADP concentration (u mole/gm. wet weight) in osmoregulatory tissues like gill(), kidney (O), and intestine (A) in Catla catla subjected to an abrupt temperature change from 32°C to 22°C (cold-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

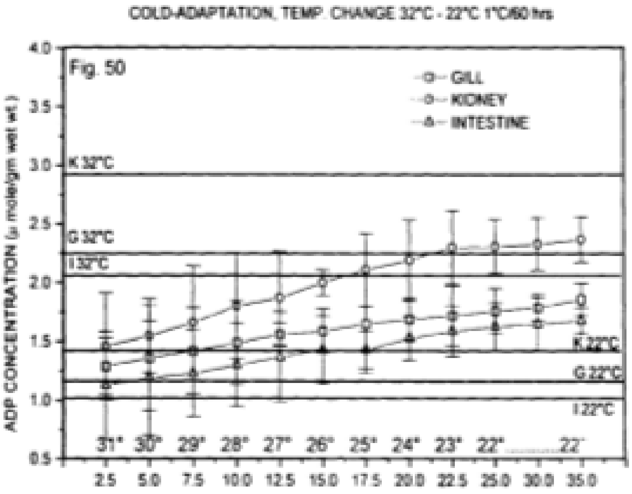

Figure 12 ADP Concentration in Osmoregulatory Tissues During Cold Adaptation in Catla catla

Figure 12 ADP concentration (u moles/gm. wet weight) in osmoregulatory tissues like gill(), kidney (O), and intestine (A) in Catla catla subjected to a slow temperature change from 32°C to 22°C (cold-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

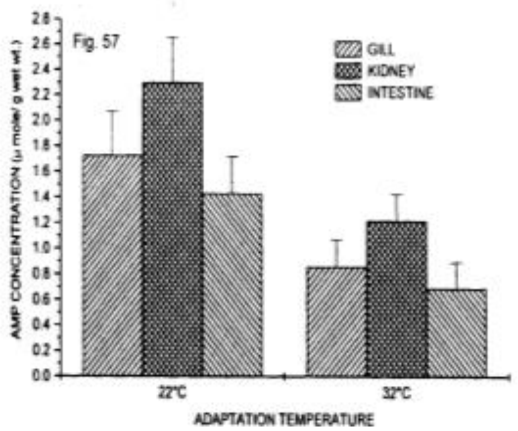

Figure 13 AMP Concentration in Osmoregulatory Tissues Adapted to Different Temperatures in Catla catla

Figure 13 Histograms showing the level of AMP concentration (u mole/gm. wet weight) in osmoregulatory tissues like gill, kidney and intestine in Catla catla adapted to 22°C and 32°C temperatures. Each histogram is a mean of six individual measurements.

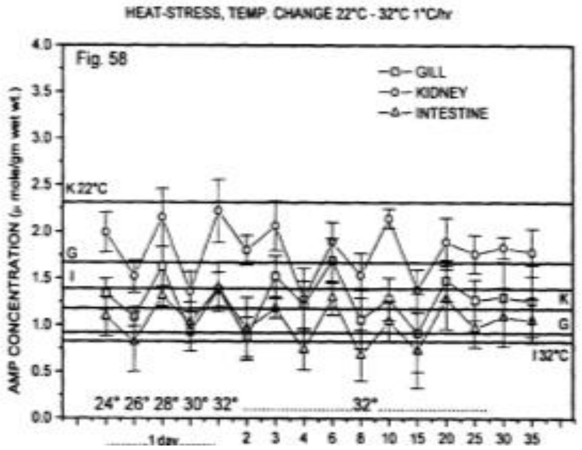

Figure 14 AMP Concentration in Osmoregulatory Tissues Under Heat Stress in Catla catla

Figure 14 AMP concentration (u mole/gm wet weight) in osmoregulatory tissues like gill (0), kidney (O) and intestine (A) in Catla catla subjected to an abrupt temperature change from 22°C to 32°C (heat-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

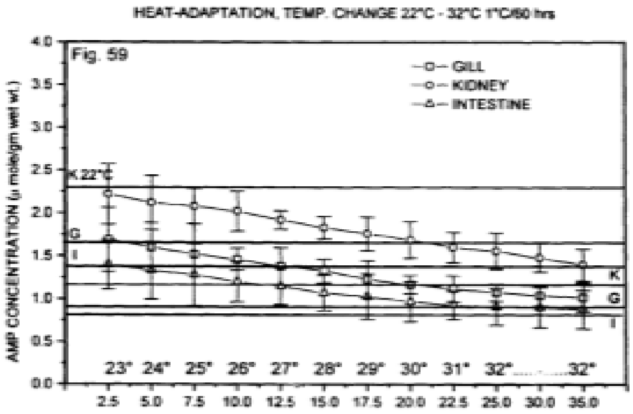

Figure 15 AMP Concentration in Non-Osmoregulatory Tissues During Heat Adaptation in Catla catla

Figure 15 AMP concentration (µ mole/gm. wet weight) in non-osmoregulatory tissues like gill (0), kidney (O), and intestine (A) in Catla catla subjected to a slow temperature change from 22°C to 32°C (heat-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

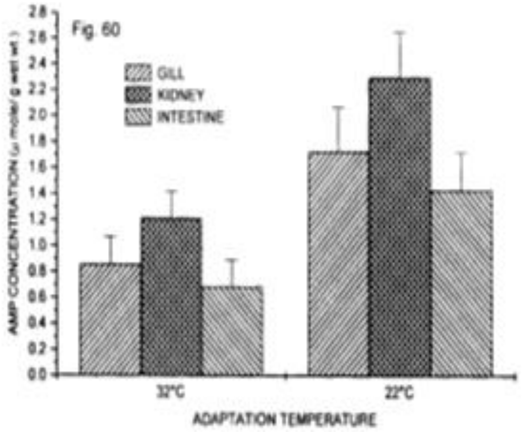

Figure 16 AMP Concentration in Osmoregulatory Tissues Adapted to Different Temperatures in Catla catla

Figure 16 Histograms showing the level of AMP concentration (µ mole/gm wet weight) in osmoregulatory tissues like gill, kidney and intestine in Catla catla adapted to 32°C and 22°C temperatures. Each histogram is a mean of six individual measurements.

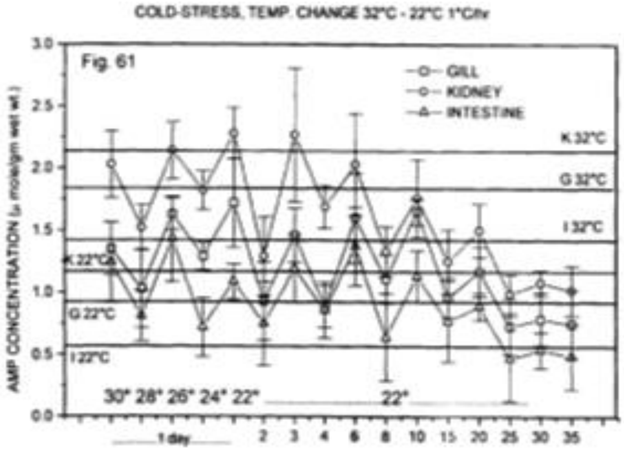

Figure 17 AMP Concentration in Osmoregulatory Tissues Under Cold Stress in Catla catla

Figure 17 AMP concentration (µ mole/gm. wet weight) in osmoregulatory tissues like gill(), kidney (O) and intestine (A) in Catla catla subjected to an abrupt temperature change from 32°C to 22°C (cold-stress) at the rate of 1°C/hr. Each point is a mean of six îndividual measurements. Vertical bars represent standard deviation.

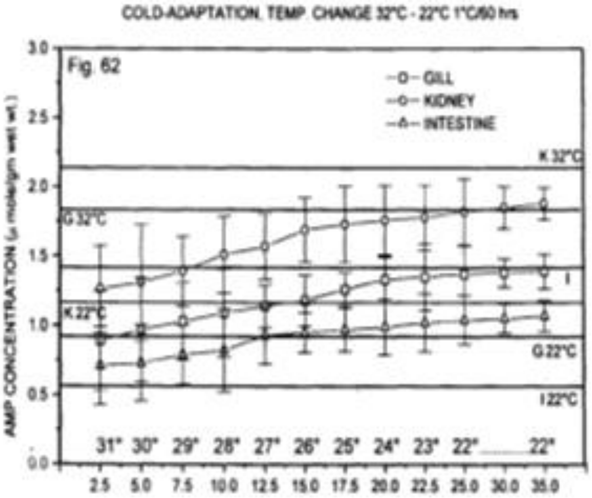

Figure 18 **AMP Concentration in Non-Osmoregulatory Tissues During Cold Adaptation in Catla catla**

Figure 18 AMP concentration (u mole/gm. wet weight) in non-osmoregulatory tissues like gill(), kidney (O), and intestine (A) in Catla catla subjected to a slow temperature change from 32°C to 22°C (cold-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

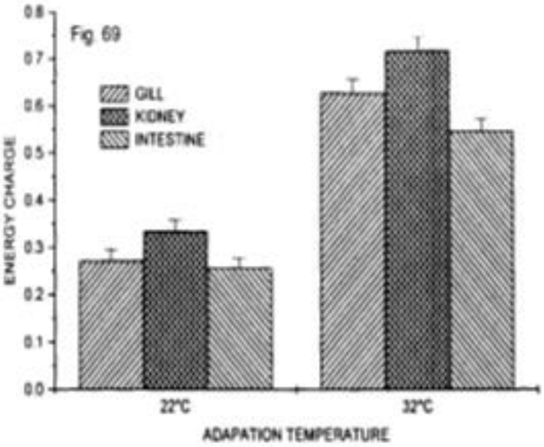

Figure 19 **Energy Charge in Osmoregulatory Tissues Adapted to Different Temperatures in Catla catla**

Figure 19 Histograms showing the energy charge in osmoregulatory tissues like gill, kidney, and intestine in Catla catla adapted to 22°C and 32°C temperatures. Each histogram is an individual measurement.

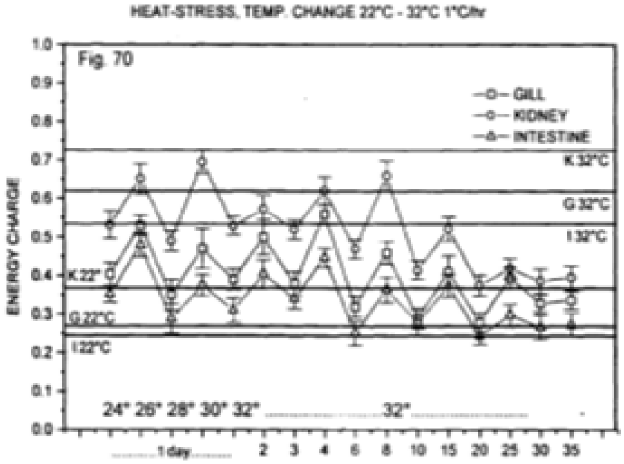

Figure 20 **Energy Charge in Osmoregulatory Tissues Under Heat Stress in Catla catla**

Figure 20 Energy charge in osmoregulatory tissues like gill (), kidney (O) and intestine (A) in Catla catla subjected to an abrupt temperature change from 22°C to 32°C (heat-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

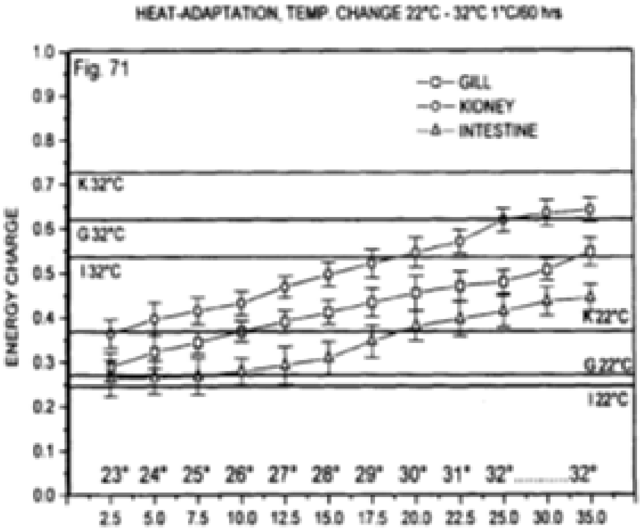

Figure 21 **Energy Charge in Osmoregulatory Tissues During Heat Adaptation in Catla catla**

Figure 21 Energy charge in osmoregulatory tissues like gill(), kidney (O) and intestine (A)in Catla catla subjected to a slow temperature change from 22°C to 32°C (heat-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

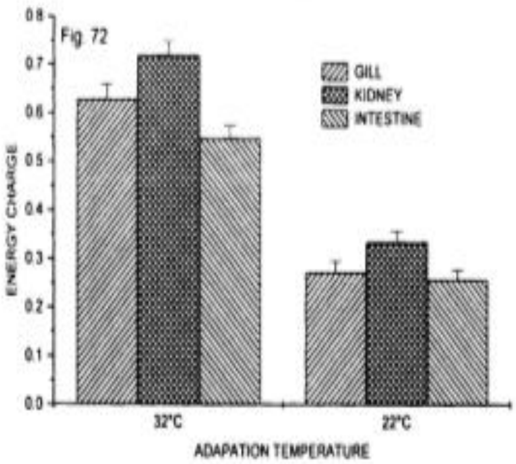

Figure 22 Energy Charge in Osmoregulatory Tissues Adapted to Different Temperatures in Catla catla

Figure 22 Histograms showing the energy charge in osmoregulatory tissues like gill, kidney and intestine in Catla catla adapted to 32°C and 22°C temperatures. Each histogram is a mean of six individual measurements.

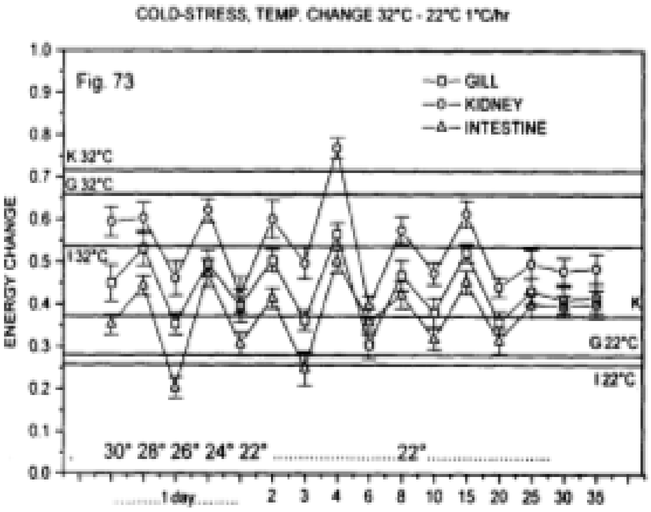

Figure 23 Energy Charge in Osmoregulatory Tissues Under Cold Stress in Catla catla

Figure 23 Energy charge osmoregulatory tissues like gill(), kidney (O) and intestine (A) in Catla catla subjected to an abrupt temperature change from 32°C to 22°C (cold-stress) at the rate of 1°C/hr. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

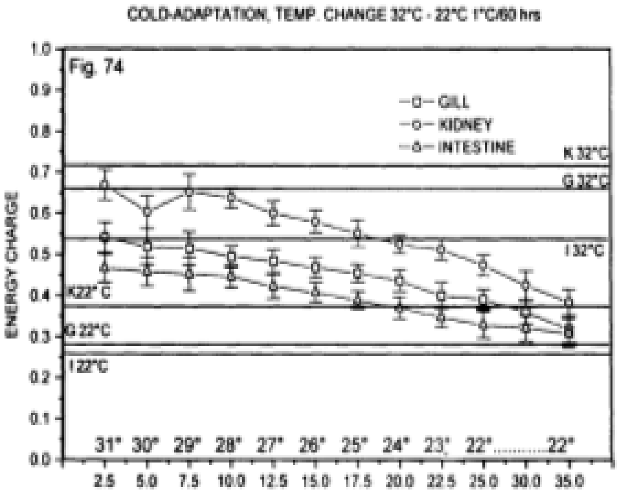

Figure 24 Energy Charge in Osmoregulatory Tissues During Cold Adaptation in Catla catla

Figure 24 Energy charge in osmoregulatory tissues like gill(), kidney (O) and intestine (A) in Catla catla subjected to a slow temperature change from 32°C to 22°C (cold-adaptation) at the rate of 1°C/60 hrs. Each point is a mean of six individual measurements. Vertical bars represent standard deviation.

3. Results

The ATP content and energy charge are significantly higher in 32°C adapted fish tissues like gill, kidney, and intestine (osmoregulatory tissues), than in 22°C adapted fish tissues Conversely the ADP, AMP levels were found to be lower in 32°C adapted fish tissues This indicates a greater turnover of ATP at higher temperature (32°C).\The levels of ATP, ADP, AMP and energy charge in all these tissues in 22°C and 32°C temperature adapted fishes are represented in the form of histograms (Figs. 33,36,45,48,57,60,69 and 78). For differentiation of thermal-adaptation process from the phenomenon of the thermal-stress; the 22°C temperature adapted fishes were re-adapted from 22 to 32°C to a slow rise in the ambient temperature (heat-adaptation) at the rate of 1/60 hrs (Figs : 35,47,59, and 71) and to an abrupt raise of temperature (heat-stress) at the rate of l°C/hr (Figs. 34,46,58 and70). In the case of cold-adaptation the 32°C temperature adapted fishes were re-adapted from 32°C to 22°C to a slow change in temperature (cold-adaptation) at the rate of l°C/60 hrs (Figs.38,50, 62 and 74) and to an abrupt change of temperature (cold-stress) at the rate of l°C/hr (Fig. 37,49 ,61 and

73) for a period of 35 days. In these figures the straight horizontal lines represent the original levels of adenylate nucleotides ATP, ADP, AMP and energy charge in different tissues of 22°C and 32°C temperature-adapted fishes which served as controls. In the case of heat-adapted fishes which were subjected to a very slow change in ambient temperature a gradual stepping-up in the level of ATP and energy charge but a gradual stepping-down in the levels of ADP and AMP in all the tissues was observed. These heat-adapted fishes exhibited a fairly good amount of recovery ranging from 55% to 96% in the above parameters, in all the tissues of the fish, *Catla catla* (There is an inverse relationship between the levels of ATP and energy charge to that of ADP and AMP. In that, there is an elevation in the levels of ATP and energy charge with a corresponding decline in the levels of ADP and AMP. The percent recovery in these parameters during heat adaptation is found to be greater in the order of kidney > gill > intestine

Discussion

In this fish, *Catla catla* increased ATP and energy charge levels and corresponding decreased AMP and an intermediate level of ADP between ATP and AMP were noticed in different organs like gill, kidney, intestine, of cold 22°C adapted fishes. In all these organs an increase in energy charge an adaptation to warm reflects that the tissues are fully charged with ATP to meet the heavy energy demands of this fish during higher temperature, which comes under loading stress involving extra energy when compared to adaptation to cold. it may be said that in *Catla catla* the overall energy efficiency during thermal adaptation especially heat-adaptation is higher than cold-adaptation The osmoregulatory tissues are found to be more sensitive and at the same time more efficient in the process of recovery during cold and heat-adaptation. the phenomenon of temperature stress is different from temperature adaptation And it may be concluded that environmental stress is different from environmental adaptation. Such differentiation was very well established with regard to environmental-temperature in the case of fishes, and to some extent in mice (Gronow, 1974a,b; Grigo, 1975a; Dunnendhal, 1975; Kunnemann and Precht, 1975; Kunnemann and Basha Mohideen, 1976; Basha Mohideen, 1985; Basha Mohideen *et ai*, 1987). Studies of this nature which could differentiate environmental stress from environmental-adaptation with reference to ambient temperature are highly useful in the evaluation of rates of temperature that act as stressors and induce stress situations and on the other in the evaluation of “safe” and “ideal” rates of temperatures which do not act as stressor but, result in the slow and easy compensation of adaptation without physiological load on the part of the animal. Thus, studies of this nature are highly appreciable in monitoring thermal pollution and in evaluating methods and techniques concerned with the safe rearing and conservation of useful fauna of the aquatic habitat.

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