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Biochemical and stress responses of rohu *Labeo rohita* and mrigal *Cirrhinus mrigala* in relation to acclimation temperatures

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The biochemical and stress responses of two Indian major carps, rohu *Labeo rohita* and mrigal *Cirrhinus mrigala* were studied after acclimating them to four preset temperatures (26, 31, 33 and 36° C) for 30 days. The blood glucose and liver glycogen levels showed an inverse trend in both the species and were significantly different in *L. rohita* at higher temperatures. The decrease in the liver glycogen level of *C. mrigala*, however, was not significant. Plasma cortisol levels increased significantly whereas the ascorbic acid content in the brain and kidney of both the species decreased significantly with increasing temperatures. Total lipid content in the liver of both the species decreased significantly with increasing acclimation temperatures. The phospholipid concentration decreased in *L. rohita* with increasing acclimation temperatures, and in *C. mrigala* the values decreased up to 33° C and increased at 36° C. In *C. mrigala*, the cholesterol level decreased up to 33° C and then increased at 36° C, but the absolute value was lower in comparison to *L. rohita*. The cholesterol levels, however, were not significantly different in *L. rohita*. Triglycerides and free fatty acids concentrations decreased significantly with increasing acclimation temperatures in both the species. The present study indicates species-specific metabolic responses of *L. rohita* and *C. mrigala* to thermal acclimation.

Key words: ascorbic acid; carps; cortisol; glucose; lipid; thermal.

INTRODUCTION

Temperature of aquatic environment is one of the most pervasive environmental factors, which influence the survival, distribution and metabolism of poikilotherms. Failure to adapt to temperature fluctuations, however, results in altered metabolic pathways and subsequent mortality (Forghaly *et al.*, 1973). Fishes live in intimate contact with the aquatic environment and being

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ectothermic, their physiology depends on the environment, which may be reflected in their tissue biochemistry (Wilson & Taylor, 1993). Therefore, biological functions are critically dependent on environmental temperature.

Drastic diurnal variations in the water temperature of shallow-water ponds or the thermocline of deep-water bodies or that caused by human activities (by means of thermal discharges) pose common threats to fishes. These thermal effects may be additive or synergistic with those of other stimuli (*e.g.* low water pH, algae and oxygen storage) (Wagner *et al.*, 1997), which are relevant to fishes in natural water bodies as well as under laboratory conditions (Barton & Schreck, 1987; Donaldson, 1990; Pearson *et al.*, 1999). Different approaches for measuring compensatory or adaptive changes in animals either involves studies pertaining to natural fluctuations of environmental variable (acclimatization) or with a single laboratory controlled variable, *i.e.* acclimation (Cossins & Bowler, 1987). Acclimation is the sum total of the adjustments, which makes fishes able to adapt themselves to long-term changes in their environment. Other environmental factors, *e.g.* oxygen level and salinity, are also considered pertinent (Adeyemo *et al.*, 2003), which may results in the alteration of hormones, metabolic pathways, enzymes and behaviour of fishes to regain homeostasis by means of acclimation. These metabolic changes may be considered as an important measure for assessing the effect of environmental perturbation, especially temperature.

It has been established that, at the organism level, compensatory responses are evident in response to thermal acclimation (Gladwell *et al.*, 1976; Chatterjee *et al.*, 2004; Das *et al.*, 2004; Manush *et al.*, 2004), thermal preferenda (Brett, 1971), growth (Das *et al.*, 2005) and metabolism (Hazel & Prosser, 1974). In order to maintain physiological homeostasis during unfavourable temperatures, teleosts have developed their own specific adaptive mechanism both behavioural and physiological (Hazel & Prosser, 1974; Prosser & Heath, 1991).

Indian major carps (IMC) represents the most important freshwater fishes in Asia in terms of high commercial value and consumer preferences. Cultured fishes are often classified according to their trophic niche that they occupy in a water body. Among the three species of IMCs, catla *Catla catla* (Hamilton) occupies the surface layer, rohu *Labeo rohita* (Hamilton) the midwater layer and mrigal *Cirrhinus mrigala* (Hamilton) the lowest niche in a pond, making them an ideal combination for polyculture (Jhingran, 1991). Field observations of culture systems have reported that IMC of size range 65–70 mm can thrive well between 18·3 and 37·8° C, while temperatures $<16{\cdot}7$ and $>39{\cdot}5$° C are fatal (Jhingran, 1991). The optimum temperature for the growth of IMC is suggested to be between 27 and 32° C (ICAR, 2005). The optimum temperature for the growth of *L. rohita* fry in laboratory acclimation studies, however, was observed to be between 31 and 33° C (Das *et al.*, 2005). Investigations on laboratory-acclimated IMC have shown that their metabolism (evaluated *via* oxygen consumption rate) increases with increasing acclimation temperatures from 26 to 36° C (Das *et al.*, 2004). Further, an increase in the gluconeogenic and glycogenolytic pathways of *L. rohita* with increasing temperatures (from 26 to 36° C) for maintaining homeostasis was also observed by Das *et al.* (2006). Very little is known, however, about the species-specific responses to thermal acclimation, especially pertaining to utilization and mobilization of

the energy reserves and stress responses, in tropical freshwater fishes. In this perspective, the present study was undertaken to determine the effects of different acclimation temperatures (26, 31, 33 and 36° C) on biochemical variables namely, blood glucose, liver glycogen, brain and kidney ascorbic acid and total lipid, phospholipids, cholesterol, free fatty acids and triglycerides in the liver tissue of *L. rohita* and *C. mrigala*.

MATERIALS AND METHODS

EXPERIMENTAL FISHES

Juveniles of *L. rohita* (mean ± S.E. 29·16 ± 1·16 g) and *C. mrigala* (23·01 ± 1·04 g) were brought from the Pancham fish farm, Saphale, Maharashtra, India to the Central Institute of Fisheries Education, Mumbai, India and were acclimated to laboratory conditions (26° C, range ± 1° C) for 30 days. Photoperiod of 12L:12D was maintained during the entire experimental period. Fishes were fed with pelleted feed (35% crude protein) as recommended for *L. rohita* (Renukardhyay & Varghese, 1986), supplemented with 0·10% vitamin C, during the study. Feeding was twice daily (0800 and 2000 hours) at 3% of body mass, and waste feed and faeces were siphoned off each day before dispensing the feed. Every day, water (40–50%) was removed from the tanks and replaced with the same volume of fresh chlorine-free water. Fishes were deprived of food for a day prior to sampling.

THERMAL ACCLIMATION PROCEDURE

A total of 24 fishes each of *L. rohita* and *C. mrigala* were equally distributed and acclimated to four different temperatures (26, 31, 33 and 36° C). Fishes were maintained in two different groups, for each species with six fishes per acclimation temperature. Acclimation was carried out in thermostatic aquaria (175 l water capacity and sensitivity ±0·2° C) at the rate of 1° C day^{-1} from laboratory temperature (26° C) to reach experimental temperatures (26, 31, 33 and 36° C). To avoid the aquarium effects, the fishes were maintained for a period of 1 week in their respective aquarium prior to acclimation procedures. A lag period, of 5 days between 31 and 36° C and 3 days between 33 and 36° C, was maintained to increase the temperatures, so that the prescribed temperatures were reached on the same day. Subsequently, the fishes were maintained for a period of another 30 days prior to sampling. An acclimation temperature of 30 days has been used by many researchers for studying different physiological variables in fishes (Bennett & Beitinger, 1997; Panepucci *et al.*, 1999; Beitinger *et al.*, 2000). In a previous investigations on the effect of acclimation temperatures on thermal tolerance and oxygen consumption in IMC, it was observed that fishes were completely acclimated after 30 days (Das *et al.*, 2004, 2005). Therefore, in the present study it was assumed also that the experimental fishes were adequately acclimated to perform metabolic studies.

SAMPLING AND ANALYSIS

At the end of acclimation period, fishes were anaesthetized using CIFECALM (200 $\mu l\ l^{-1}$), an herbal anaesthetic formulation containing natural alcoholic extracts of *Eugenia caryophyllata* and *Mentha arvensis* (developed by Central Institute of Fisheries Education, Mumbai, India). Blood samples were drawn from the caudal vein using syringes, which were previously rinsed with 2·7% ethylene diamine tetraacetic acid (EDTA, as anti-coagulant) and transferred immediately into a test tube containing EDTA. An aliquot of 0·25 ml blood was used for estimation of blood glucose and remaining blood was centrifuged at 5000 *g* for 10 min in a cooling centrifuge to separate

plasma. Plasma was collected in glass vials and immediately stored at −20° C for subsequent plasma cortisol assay. Fishes were immediately dissected and samples of liver, kidney and brain were removed and weighed for further analysis. Glycogen and lipid content were determined in the liver tissue, whereas ascorbic acid was determined in the kidney and brain tissues. Liver tissues (0·3 g) were placed in pre-weighed test tubes containing 3 ml of 30% potassium hydroxide for glycogen estimation.

PLASMA CORTISOL

Plasma cortisol was determined by solid-phase radio immuno-assay (RIA) kit method using ^{125}iodine as the radioisotope. The kit was purchased from Diagnostic Product Corporation (www.dpcweb.com). Gamma counter (Electronic Corporation Ltd; http://www.ecil.co.in/index.aspx/) was used for RIA study.

BLOOD GLUCOSE, LIVER GLYCOGEN, KIDNEY AND BRAIN ASCORBIC ACID

Blood glucose was estimated following the method of Nelson (1944) using arsenomolybdate reagent. Quantitative estimation of liver glycogen was done using anthrone reagent as described by Hassid & Abraham (1957). Ascorbic acid in the kidney and brain tissues was determined according to the method of Roe & Keuther (1943). All the analyses were carried out using UV-VIS spectrophotometer (E-Merck; www.germany.merck.de).

ISOLATION OF LIPIDS

Lipid was extracted from the liver tissue of fishes using chloroform: methanol (2:1) as solvent (Folch *et al.*, 1957) and filtered using Whatman filter paper no. 41. Filtrates were then washed in 1/5 volume of 0·85% of NaCl solution. The lipid extracts were finally transferred into a pre-weighed conical flask, and the solvent was evaporated by passing nitrogen gas. The residue was dissolved in chloroform at 2 mg ml^{-1} and was then used for quantitative determination of total lipid, phospholipid, cholesterol and triglycerides.

TOTAL LIPID, PHOSPHOLIPID, CHOLESTEROL AND TRIGLYCERIDES

Total lipid, phospholipids and cholesterol were determined spectrophotometrically following the procedure described by Marsh & Weinstein (1966), Wanger *et al.* (1962) and Zlatkis *et al.* (1953), respectively. Triglyceride content was determined by subtracting the value of cholesterol and phospholipid from its corresponding total lipid value (Sinku *et al.*, 2003).

STATISTICAL ANALYSIS

Statistical significance of blood glucose, liver glycogen, plasma cortisol and ascorbic acid levels in the brain and kidney and lipid composition (total lipid, phospholipid, cholesterol and free fatty acids) were analysed by one-way ANOVA using SPSS version 11.0 (www.spss.com). Tukey's multiple range tests was used for *post hoc* comparison of means ($P < 0{\cdot}05$) amongst different acclimation temperatures. Species-specific variation was tested between *L. rohita* and *C. mrigala* by using a *t*-test.

RESULTS

Data on blood glucose, plasma cortisol, liver glycogen and ascorbic acid levels in the brain and kidney of *L. rohita* and *C. mrigala* acclimated to four

different temperatures (26, 31, 33 and 36° C) are given in Tables I and II. Blood glucose and plasma cortisol levels increased significantly ($P < 0{\cdot}05$) with increasing acclimation temperature in both the species, however, the values did not change significantly ($P > 0{\cdot}05$) between the species at their respective acclimation temperature (Table I). The liver glycogen contents of both the species decreased with increasing acclimation temperatures. The values, however, significantly ($P < 0{\cdot}05$) decreased in *L. rohita* (Table I). The brain and kidney ascorbic acid concentration in both species decreased significantly ($P < 0{\cdot}05$) with increasing acclimation temperatures (Table II). The values significantly ($P > 0{\cdot}05$) decreased at 33° C in the kidney and brain tissues of *L. rohita* (Table II). It was observed that, at ambient temperature (26° C), the level of ascorbic acid in the kidney of *L. rohita* was less than that of *C. mrigala* (Table II).

Data on the effect of acclimation temperatures on total lipid, phospholipid, cholesterol, triglycerides and free fatty acid in the liver tissue are presented in Table III. The values for total lipid, phospholipid, cholesterol, triglycerides and free fatty acids remained significantly ($P < 0{\cdot}05$) less in *C. mrigala* irrespective of the acclimation temperatures. Total lipid, phospholipid, triglyceride and free fatty acid concentrations decreased significantly ($P < 0{\cdot}05$) with increasing acclimation temperatures in both the species. Cholesterol level was not affected by increasing acclimation temperatures in *L. rohita* ($P > 0{\cdot}05$). In *C. mrigala*, cholesterol level showed an irregular trend but was statistically significant ($P < 0{\cdot}05$).

TABLE I. Effect of acclimation temperatures (26, 31, 33 and 36° C) and species-specific variation on the blood glucose, plasma cortisol and liver glycogen levels of *Labeo rohita* and *Cirrhinus mrigala*. Values are expressed as mean ± S.E. ($n = 6$)

Variable	Tissue	Acclimation temperature (° C)	Species	
			L. rohita	*C. mrigala*
Glucose (mg 100 ml^{-1})	Blood	26	$17{\cdot}51 \pm 1{\cdot}07^{a}$	$8{\cdot}76 \pm 0{\cdot}29^{a}$
		31	$21{\cdot}34 \pm 2{\cdot}46^{ab}$	$13{\cdot}64 \pm 1{\cdot}17^{a}$
		33	$28{\cdot}62 \pm 0{\cdot}77^{bc}$	$21{\cdot}20 \pm 2{\cdot}88^{b}$
		36	$35{\cdot}61 \pm 2{\cdot}56^{c}$	$33{\cdot}15 \pm 1{\cdot}64^{c}$
Cortisol (ng ml^{-1})	Plasma	26	$21{\cdot}83 \pm 1{\cdot}35^{a}$	$13{\cdot}00 \pm 0{\cdot}76^{a}$
		31	$32{\cdot}50 \pm 1{\cdot}59^{b}$	$22{\cdot}16 \pm 1{\cdot}58^{b}$
		33	$42{\cdot}83 \pm 1{\cdot}30^{c}$	$29{\cdot}83 \pm 1{\cdot}41^{c}$
		36	$44{\cdot}91 \pm 1{\cdot}67^{c}$	$36{\cdot}33 \pm 1{\cdot}80^{d}$
Glycogen (mg g^{-1} wet tissue)	Liver	26	$10{\cdot}81 \pm 0{\cdot}86^{a}$	$17{\cdot}75 \pm 1{\cdot}56$
		31	$9{\cdot}72 \pm 0{\cdot}16^{aA}$	$17{\cdot}17 \pm 0{\cdot}81^{B}$
		33	$6{\cdot}90 \pm 0{\cdot}59^{b}$	$16{\cdot}38 \pm 0{\cdot}93$
		36	$4{\cdot}39 \pm 0{\cdot}42^{c}$	$14{\cdot}58 \pm 0{\cdot}63$

Different superscript lower-case letters in the same column indicate significant difference amongst different acclimation temperatures in each species (Tukey's multiple range test). Different superscript upper-case letters in the same row indicate significant difference ($P < 0{\cdot}05$) (overall mean values) amongst different species (*t*-test).

TABLE II. Effect of acclimation temperatures (26, 31, 33 and 36° C) and species-specific variations on the kidney and brain ascorbic acid levels of *Labeo rohita* and *Cirrhinus mrigala*. Values are mean ± S.E. ($n = 6$)

Variable	Tissue	Acclimation temperature (° C)	Species	
			L. rohita	*C. mrigala*
Ascorbic acid (μg g^{-1} wet tissue)	Kidney	26	$328{\cdot}71 \pm 7{\cdot}55^{aA}$	$535{\cdot}85 \pm 27{\cdot}14^{aB}$
		31	$282{\cdot}19 \pm 24{\cdot}92^{a}$	$450{\cdot}06 \pm 14{\cdot}30^{a}$
		33	$149{\cdot}36 \pm 2{\cdot}20^{bA}$	$371{\cdot}30 \pm 22{\cdot}52^{bB}$
		36	$143{\cdot}25 \pm 8{\cdot}58^{b}$	$297{\cdot}13 \pm 6{\cdot}75^{c}$
	Brain	26	$241{\cdot}51 \pm 9{\cdot}55^{a}$	$308{\cdot}15 \pm 10{\cdot}35^{a}$
		31	$230{\cdot}43 \pm 4{\cdot}72^{a}$	$297{\cdot}46 \pm 12{\cdot}28^{a}$
		33	$151{\cdot}59 \pm 11{\cdot}37^{bA}$	$289{\cdot}40 \pm 24{\cdot}08^{aB}$
		36	$111{\cdot}19 \pm 6{\cdot}34^{b}$	$217{\cdot}49 \pm 5{\cdot}56^{b}$

Different superscript lower-case letters in the same column indicate significant difference amongst different acclimation temperatures in each species (Tukey's multiple range test). Different superscript upper-case letters in the same row indicate significant difference ($P < 0{\cdot}05$) (overall mean values) amongst different species (*t*-test).

TABLE III. Effect of acclimation temperatures (26, 31, 33 and 36° C) and species-specific variations on the lipid profile of the liver tissue in *Labeo rohita* and *Cirrhinus mrigala*. Values are means ± S.E. ($n = 6$)

Variable	Acclimation temperature (° C)	Species	
		L. rohita	*C. mrigala*
Total lipid (mg g^{-1} wet tissue)	26	$72{\cdot}80 \pm 3{\cdot}40^{aB}$	$20{\cdot}42 \pm 1{\cdot}64^{aA}$
	31	$65{\cdot}86 \pm 2{\cdot}60^{aB}$	$18{\cdot}49 \pm 2{\cdot}94^{aA}$
	33	$58{\cdot}20 \pm 1{\cdot}11^{abB}$	$12{\cdot}73 \pm 0{\cdot}41^{bA}$
	36	$40{\cdot}63 \pm 4{\cdot}89^{bB}$	$10{\cdot}95 \pm 0{\cdot}13^{bA}$
Phospholipid (mg lecithin g^{-1} wet tissue)	26	$16{\cdot}84 \pm 0{\cdot}43^{aB}$	$5{\cdot}41 \pm 0{\cdot}52^{aA}$
	31	$13{\cdot}62 \pm 1{\cdot}36^{aB}$	$3{\cdot}26 \pm 0{\cdot}19^{bdA}$
	33	$9{\cdot}22 \pm 0{\cdot}79^{bB}$	$1{\cdot}53 \pm 0{\cdot}12^{cA}$
	36	$8{\cdot}91 \pm 0{\cdot}16^{bB}$	$1{\cdot}64 \pm 0{\cdot}15^{dA}$
Cholesterol (mg g^{-1} wet tissue)	26	$7{\cdot}94 \pm 0{\cdot}54^{B}$	$5{\cdot}54 \pm 0{\cdot}13^{aA}$
	31	$9{\cdot}82 \pm 0{\cdot}78^{B}$	$3{\cdot}40 \pm 0{\cdot}12^{bA}$
	33	$10{\cdot}36 \pm 0{\cdot}42^{B}$	$2{\cdot}31 \pm 0{\cdot}23^{cA}$
	36	$12{\cdot}25 \pm 1{\cdot}69^{B}$	$3{\cdot}32 \pm 0{\cdot}32^{bcA}$
Triglycerides and free fatty acids (mg g^{-1} wet tissue)	26	$48{\cdot}06 \pm 2{\cdot}77^{aB}$	$9{\cdot}45 \pm 1{\cdot}37^{a}$
	31	$43{\cdot}50 \pm 1{\cdot}73^{aB}$	$6{\cdot}40 \pm 0{\cdot}10^{abA}$
	33	$37{\cdot}40 \pm 2{\cdot}35^{abB}$	$8{\cdot}88 \pm 0{\cdot}23^{abA}$
	36	$19{\cdot}46 \pm 3{\cdot}71^{bB}$	$5{\cdot}98 \pm 0{\cdot}21^{bcA}$

Different superscript lower-case letters in the same column indicate significant difference amongst different acclimation temperatures in each species (Tukey's multiple range test). Different superscript upper-case letters in the same row indicate significant difference ($P < 0{\cdot}05$) (overall mean values) amongst different species (*t*-test).

DISCUSSION

In the present study, elevation in the plasma cortisol and glucose levels with increasing acclimation temperature indicates an increase in the level of stress in both *L. rohita* and *C. mrigala*. The primary stress response in fishes is characterized by rapid increase in the level of stress hormones particularly cortisol (Wendelaar Bonga, 1997; Barton, 2002). Increase in the blood cortisol and glucose in response to different stressors such as, temperature, confinement and handling is reported in various fish species (Kindle & Whitmore, 1986; Davis & Parker, 1990; Pérez-Casanova *et al.*, 2008). Hyperglycaemia is the most evident indicator of secondary stress response in fishes (Chatterjee *et al.*, 2006). Many workers have reported rise in the glucose level of fishes and shellfish exposed to various kinds of stressors such as handling, transportation, temperature and claw ablation (Wedemeyer, 1976; Mazeaud *et al.*, 1977; Strange, 1980; Barton & Schreck, 1987; Iverson *et al.*, 1998; Manush *et al.*, 2005). The increase in the plasma cortisol levels due to stress results in the production and mobilization of energy reserves, the release of glucose by the liver in fishes (Wendelaar Bonga, 1997), which ensures the availability of adequate energy required for maintaining homeostasis (Ackerman *et al.*, 2000). Therefore, it can be suggested that the increase in the blood glucose levels with increasing acclimation temperatures as observed in the present study could be a compensatory mechanism to satisfy the energy demand at higher temperatures (Barton & Schreck, 1987; Vijayan & Moon, 1994).

Cortisol, either directly or indirectly, increases glycogen mobilization (Reid *et al.*, 1992; Vijayan & Leatherland, 1992; Vijayan *et al.*, 1993) or directly affects the gluconeogenesis in fishes (Vijayan *et al.*, 1994). The rise in the glucose level is due to glycogenolysis in the earlier stages and gluconeogenesis in the later stages of stress in fishes (Barton & Iwama, 1991; Reubush & Heath, 1996). Therefore, in the present study, the increased blood glucose and reduced hepatic glycogen concentration in *L. rohita* and *C. mrigala*, with the increase in the acclimation temperatures, could be due to increased glycogenolysis and gluconeogenesis mediated by cortisol. In an earlier study on the metabolic changes in *L. rohita* acclimated to 31, 33 and 33° C, an increase in the glycogenolytic and gluconeogenic pathways with increasing acclimation temperatures was observed (Das *et al.*, 2006), which supports the present study findings. In the present study, the liver glycogen contents in *L. rohita* decreased significantly ($P < 0{\cdot}05$) with increasing acclimation temperatures. This indicates that hyperglycaemia in *L. rohita* could predominantly be due to glycogenolysis. The study also suggests that, at higher temperatures, the magnitude of reduction in the glycogen level, and energy demand and utilization in *L. rohita* is profound as compared to *C. mrigala.*

Vitamin C is well known for its anti-oxidative property and has also been used as an immuno-modulator in aquaculture (Cruz de Menezes *et al.*, 2006; Norouzitallab *et al.*, 2008). In the present study, the ascorbic acid contents in the brain and kidney tissues of both the species was reduced with increasing acclimation temperatures. Teleosts do not have an active L-gulonolactone oxidase, the terminal enzyme in the ascorbic acid synthesis pathway (Dabrowski, 1990*a*). Thus, fishes are sensitive to a threshold level of vitamin C as they are

unable to synthesize it *de novo* (Dabrowski, 1990*b*). Temperature stress is reported to modulate the antioxidants and induce production of reactive oxygen species leading to lipid peroxidation (LPO) in fishes (Flanagan *et al.*, 1998; Guderley, 2004). Parihar & Dubey (1995) have reported decline in the ascorbic acid contents and an increase in the LPO level in the gills and air sac membrane of stinging catfish *Heteropneustes fossilis* (Bloch) due to temperature stress. Although, in the present study, the fishes were fed with uniform amount of feed supplemented with vitamin C, the reduction in the ascorbic acid levels with increasing acclimation temperatures indicates increased oxidative stress. Similar results were found in thornfish *Therapon jarbua* (Forskål) when exposed to thermal stress (Chien *et al.*, 1999). Comparative study on the vitamin C levels in the present study also indicated that the magnitude of reduction at higher acclimation temperatures is greater in *L. rohita* than that in *C. mrigala.*

Generally, lipids are stored as triacylglycerols and the constituent fatty acids tend to be longer and polyunsaturated (Sheridan, 1994). Free fatty acids are lipid substrates that rapidly get circulated amongst the tissues, whereas triacylglycerols and phospholipids are slowly delivered (Weber & Zwingelstein, 1995). As poikilotherms, fishes show perturbation in their membrane structure in response to temperature fluctuation. Consequently, membrane lipid structure may become altered (Hazel, 1995). A decreasing trend of total lipid in the present study indicated the mobilization of lipid for energy utilization. Lipids undergo rapid breakdown, resynthesis and interconversion in response to different stimuli (Chetty & Indira, 1994). Similar results were reported in sunshine bass *Morone chrysops* (Rafinesque) that liver lipid catabolism increases in respect to the increasing temperatures (26·7–32·2° C) (Keembiyehetty & Wilson, 1998).

Phospholipids constitute *c.* 5–65% of the membrane lipids, which assist in the formation of lipoproteins, enhance fat mobilization and assist in the absorption of fat-soluble vitamins in the intestine (Das, 2002). In the present study, phospholipid concentration in *L. rohita* decreased with increasing acclimation temperatures, whereas in *C. mrigala* the values decreased up to 33° C and then increased at 36° C. This may be due to the adaptation of various components of cellular membrane in *C. mrigala* to preserve the physiological integrity of cells.

The role of cholesterol in membrane structure and as a precursor for the synthesis of steroid hormone and bile acids is well documented. The adenocorticotropic hormone (ACTH) stimulates the hydrolysis of cholesteryl esters, thereby providing cholesterol for hormone production (Montgomery *et al.*, 1980). Increase in the cholesterol levels with increasing acclimation temperatures could be due to the hydrolysis of cholesterol esters for cortisol synthesis under the influence of ACTH. In *C. mrigala*, the cholesterol level decreased up to 33° C and then increased at 36° C, but the absolute value was lower in comparison to *L. rohita*. The cholesterol levels, however, were not significantly different in *L. rohita*. This result could be due to the feedback regulation of cortisol production, resulting in lower stress response in *C. mrigala*. The elevation of membrane cholesterol content may be an effective mechanism for stabilizing plasma membranes against the perturbing effects of high temperatures

in poikilotherms (Robertson & Hazel, 1997). Triglycerides are usually mobilized for energy production. Vijayan *et al.* (1990) reported similar findings when brook trout *Salvenilus fontinalis* (Mitchill) were subjected to crowding and confinement stress. It was proved that modification of lipid components must have assisted in the maintenance of proper membrane fluidity and permeability for efficient functioning of the nervous system (Roots, 1965). Though the test temperatures were not uniformly selected (26, 31, 33 and 36° C), significant changes in the lipid components at higher acclimation temperature (31, 33 and 36° C) may be due to the utilization of stored lipid to cope with the increasing energy demands apart from maintenance of cell membrane fluidity.

This investigation presents the species-specific responses of *L. rohita* and *C. mrigala* to increasing acclimation temperatures. Species-specific variation in the observed biochemical variables may be due to the difference in the ecological niches occupied by the two species. The present investigation indicates that thermal acclimation is an essential physiological phenomenon in the life of ectotherms and is strongly dependent on their ambient temperature. Subsequently, acclimation elicits compensatory responses that allow the fishes to adapt themselves to the new environment.

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