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Response of a freshwater air-breathing fish, *Clarias batrachus* to salinity stress: an experimental case for their farming in brackishwater areas in Andaman, India

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Abstract The study was conducted to assess the effects of salinity on growth and biochemical composition of freshwater catfish, *Clarias batrachus*. A static nonrenewable acute toxicity bioassay test was conducted and LC_{50} of salinity for 96-h exposure to the fingerling (14.5 cm) was 12.52 ‰. Based on these results, two sublethal salinity levels, viz. 4 and 8 ‰ were selected to study the long-term effects of salinity on *C. batrachus* for a period of 90 days. From the study, it was found that growth and survival rate were less in saline water (4 and 8 ‰). Maximum growth and survival were recorded in freshwater (0 ‰ salinity) and subsequently at 4 and 8 ‰. To assess the biochemical alteration, few important biomarkers were estimated. At the end of 90 days rearing period, glucose level in the brain and blood of *C. batrachus* was found to decrease with salinity. The level of liver and muscle glycogen in the fish reared at 4 ‰ was lower than that of control. Ascorbic acid in all organs under study was found to decrease with increasing salinity, which was attributed to stress mitigation effect of vitamin C. Acetylcholine esterase (AchE) activity recorded a gradual decrease with increasing salinity. Metabolic enzymes, alkaline phosphatase (ALP) activity and adenosine triphosphosphtase (ATPase) activity also reduced both in liver and muscle tissues with increasing salinity. From the present investigation, it can be concluded that exposure to higher salinity significantly ($P < 0.01$) affects the growth and physiological response of *Clarias batrachus*.

Keywords Ascorbic acid · Glucose · Stress · Enzyme · Catfish

Introduction

Salinity tolerance limits of freshwater fishes are of ecological significance in assessing the distribution of fish and their impact on ecosystems (Kilambi and Zdinak 1980). It is an important environmental factor, which influences growth performance of many fish

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(Kang'ombe and Brown 2008). Salinity effects have been studied in several species of fish in ponds, tanks, raceways and cages (Cruz et al. 1990; Watanabe et al. 1990). Fluctuation of salinity is very pronounced in tropics where the climate is characterized by wet and dry seasons (Suresh and Lin 1992; Boyd and Tucker 1998). In Western Australia, salinity in farm ponds fluctuates from less than 0.5 ‰ in the wet season to over 3 ‰ in dry season (Boyd and Tucker 1998). Especially in island conditions like Andaman, where annual average rainfall is over 3,000 mm, pond water salinity changes due to high precipitation during monsoon and high evaporation during the dry season. Therefore, it is important to understand the salinity tolerance of freshwater fish species in areas where sea water intruded.

Clarias batrachus is a freshwater Indian catfish popularly known as magur. It is well distributed in Indian subcontinent (Jayaram 1981) fetching very high price in the domestic market (Das 2002). They are obligatory air breathers and normally inhabit in low lying water bodies, swamps, marshy and derelict waters, rivers, etc. (Chondor 1999). They are very hardy in nature (Kumar et al. 2012, Verma et al. 2011) and can thrive in adverse ecological conditions like vey low dissolved oxygen, high turbidity, etc. *Clarias batrachus* is an omnivore fish having broad feeding habits at different life stages (Hora and Pillay 1962). However, in recent times, the magur population has decreased at an alarming rate due to high fishing pressure as well as habitat destruction (Sahoo et al. 2010; Khedkar et al. 2010) and has become a vulnerable species (CAMP 1998).

During the *tsunami* in 2004, a large portion of coastal areas of Andaman and Nicobar islands (ANI) was completely submerged by seawater (Raja et al. 2009). Total cultivated land available before the tsunami was 50,000 ha that came down to 43,339 ha (Sarma et al. 2010a), and area under paddy has drastically come down from 12,000 ha to less than 8,000 ha due to submergence of low lying areas or seawater intrusion (Deshmukh and Din 2008). Due to salination of freshwater, many freshwater species might be under severe stress and threat due to their inability to cope up with extreme physiological and osmotic stress. Biochemical constituents and enzymes have been explored as potential biomarkers for variety of organisms as these parameters are highly sensitive and conserved between species and less variable (Agrahari and Gopal 2009). Hence, there is an urgent need to know the actual physiological consequences of salinity on an organism and also to ascertain whether some of the freshwater species can be farmed in the low saline brackishwater areas. Hence, the present study was undertaken to study the sublethal effect of salinity on growth and biochemical parameters of *Clarias batrachus* reared under different salinity exposure for a period of 90 days.

Materials and methods

Experimental animals

Healthy fingerlings of *Clarias batrachus* were collected from Magur Hatchery unit of Central Agricultural Research Institute (CARI), Andaman. The collected fingerlings were transported in oxygenated bags to the laboratory, located 12 km away from the hatchery. The fish were acclimatized to the laboratory conditions for a period of 1 week prior to the experiment.

Treatments

A static nonrenewable acute toxicity bioassay was conducted according to standard method (APHA, 1998 and Reish and Oshida, 1987) to determine LC_{50} concentration of salinity for fingerlings (14.5 cm) of *Clarias batrachus* following exposure of 96 h. Initially, range finding test was conducted and recorded mortality percentage of 0 and 100 %. Subsequently, definitive test was conducted for four different test media (11 to 14 ‰) with 3 replications for each treatment (10 fishes per tank), and the data obtained from the experiment were processed by probit analysis using a personal computer. The median lethal concentration of salinity at the end of 96-h exposure for fingerling (14.5 cm) was 12.52 ‰. Based on LC_{50} for 96 h of *C. batrachus* fingerlings (12.52 ‰), three different sublethal salinities, viz. 0, 4 and 8 ‰ were identified to assess the effect of salinity on the fishes. The water quality parameters that were maintained in rearing tanks for the experiment are depicted in Table 1. Filtered seawater (33 ‰ salinity) was used in preparing desired salinity in the experiment. Freshwater ($\sim$0 ‰) was collected from municipal supply and stored in separate tanks (1,000 l) at least 5 days prior to using them. Before 24 h of utilization, both freshwater and seawater were collected separately in FRP (fiber reinforced plastic also called fiber reinforced polymer) tanks and vigorously aerated. Required salinity (4 and 8 ‰) was made by mixing fresh water with the seawater appropriately.

Experimental design

Completely randomized design was followed in the present experiment. 90 fish (14.10 ± 0.1 cm; 18.96 ± 0.37 g) were distributed in three different groups with 3 replicates per treatment. Stocking density maintained for the experiment was 10 fish/tank. The experiment was conducted in 200 l identical FRP tanks in which 150 l of water was maintained. Three different salinity treatments, viz. 0, 4 and 8 ‰ were selected based on the acute toxicity results, and the rearing experiment was conducted for a period of 90 days. Round-the-clock aeration was provided in all the tanks from a centralized air blower. The uneaten food and fecal matters were removed on daily basis by siphoning. Complete water exchange was done on weekly basis.

Table 1 Average water quality parameters in the rearing tanks of *Clarias batrachus* for a period of 90 days of culture in different salinities

Parameters	Salinity		
	0 ‰	4 ‰	8 ‰
Water temperature	28.23 ± 0.31 with a range of 26.4–30.20 °C		
Turbidity	30.768 ± 1.57	28.764 ± 0.078	29.588 ± 0.254
DO	5.118 ± 0.267	5.206 ± 0.245	4.966 ± 0.428
DO%	68.627 ± 3.32	70.641 ± 2.41	65.288 ± 8.07
pH	7.954 ± 0.235	7.836 ± 0.12	7.912 ± 0.099
Salinity	0.262 ± 0.064	4.262 ± 0.09	8.197 ± 0.034

Units: temperature = °C, turbidity = NTU, DO = mg/l, pH = hydrogen ion concentration, salinity = g/l

Growth and survival study

Monthly sampling was carried out to ascertain weight gain and survival of the fish using the following formulae:

$$\text{Weight gain}\,(\%) = \frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \times 100$$

$$\text{Survival}\,(\%) = \frac{\text{Number of fish survived after 90 days}}{\text{Initial number of fish stocked}} \times 100.$$

Feed preparation and feeding

The basal diet was formulated using wheat flour, rice bran, groundnut oil cake, vegetable oil, fish meal, minced meat and vitamin. Minced meat was prepared by finely chopping the flesh portion of dressed mackerel fish. All ingredients, except mineral–vitamin mixture and minced meat, were mixed in a plastic bowl and dough was prepared using water. The dough was then steamed for about 15 min in a pressure cooker. The vitamin–mineral mixture and minced meat were mixed after cooling. This moist feed was used for feeding fishes at the rate of 5 % body weight per day. Total feed quantity was divided into two parts and distributed in the morning and afternoon hours. The proximate composition of the feed was estimated following Malik and Sirohi (2004) and moisture, crude protein, fat and ash content were found to be 58.18 ± 2.40 %, 35.48 ± 4.84 %, 4.76 ± 0.33 % and 4.65 ± 0.86 % respectively.

Sample preparation

At the end of 90 days, 6 fish per treatment (2 per tank) were taken out, anaesthetized with clove oil (50 µl/l) and different organs taken out for biochemical and enzymatic analysis. For the determination of enzyme activity, liver, muscle and brain were dissected out, and 5 % homogenate was prepared in chilled sucrose solutions (0.25 M) in a mechanical tissue homogenizer. The homogenate was centrifuged at 5,000*g* at 4 °C for 10 min, supernatant collected and stored at −20 °C for further analysis. Blood was collected by puncturing the caudal vein using a medical syringe (No. 23), which was previously rinsed with 2.7 % EDTA solution (as an anticoagulant) and shaken gently in order to prevent hemolysis of blood.

Biochemical studies

Glucose was estimated from the blood, brain and muscle of the fish using arsenomolybdate reagent by the method of Nelson and Somogyi (1945). For glucose, the blood was de-proteinized (v/v) with zinc sulfate and barium hydroxide, centrifuged at 5,000*g* for 5 min, and the supernatant was used for the estimation of glucose. Tissue homogenates were used for the estimation of glucose from brain and muscle. Glycogen content was estimated from the liver and muscle of the fish using anthrone reagent as described by Hassid and Abraham (1957). Ascorbic acid content in the kidney and brain tissues was determined using 2,4-dinitro-phenylhydrazine at 540 nm according to the method described by Roe and Keuther (1943). UV–VIS spectrophotometer (E-Merck, Germany) was used for determination of optical density.

Enzyme assay

Acetylcholine esterase (AchE; EC 3.1.1.7) was assayed by the method described by Hestrin (1949), using a mixture of 0.07 M phosphate buffer (pH 7.2), 4 mM acetylcholine (pH 4.0), substrate–buffer mixture (1/10 dilution) and 0.2 ml of tissue homogenate. The mixture was incubated at 37 °C for 30 min, and the OD (optical density) was recorded at 540 nm. Alkaline phosphatase activity was determined by the method of Garen and Levinthal (1960). The assay mixture consisted of 0.2 ml bicarbonate buffer (0.2 M), 0.1 ml of 0.1 M $MgCl_2$, 0.05 ml tissue homogenate, 0.5 ml of distilled water and 0.1 ml of freshly prepared 0.1 M para-nitrophenyl phosphate. The reaction mixture was incubated in water bath at 37 °C for 15 min, and the reaction was stopped by 1.0 ml of 0.1 N NaOH. OD was taken at 410 nm. Total adenosine triphosphatase (ATPase; EC 3.6.1.3) was assayed in a reaction mixture of 0.1 M Tris–HCl buffer (pH 7.8), 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 5 mM ATP and 0.1 ml tissue homogenate. The mixture was incubated for 15 min, and the reaction was terminated by means of 10 % TCA (Post and Sen 1967). Phosphate liberated was estimated at 660 nm (Fiske and Subbarow 1925). Lactate dehydrogenase (LDH; EC_1.1.1.27) was assayed using 0.2 mM NADH solution in 0.1 M phosphate buffer (pH 7.5) and 0.1 ml tissue homogenate (after 10 times dilution of the homogenate). The reaction was initiated by adding 0.2 mM sodium pyruvate as the substrate, and OD recorded at 340 nm for 3 min at an interval of 30 s (Wroblewski and Ladue 1955).

Data analysis

The data obtained in the present experiment were subjected to one-way ANOVA using Statistical Package, SPSS version 11. Duncan's multiple range tests were used to determine the differences among treatment mean, which were significant at $P < 0.05$. For growth analysis, two-way ANOVA was conducted taking into consideration the monthly weight gain in different salinities.

Results

Data pertaining to average water quality parameters of the rearing media of *C. batrachus* are presented in Table 1. All parameters like temperature, dissolved oxygen (DO), turbidity, pH and salinity is within the acceptable limit for culturing fishes. In the rearing media, though we have mentioned as 0 ‰ but actual concentration of the salinity was 0.26 ‰ only.

Growth and survival

Data pertaining to growth and mortality of *C. batrachus* are presented in Figs. 1 and 2. There was no significant difference ($P > 0.05$) in weight gain among the fishes exposed to different salinities. However, weight gain in each month was statistically significant ($P < 0.05$). The sublethal salinity exposure of *C. batrachus* for 90 days caused a reduction in the weight gain ($P > 0.05$) compared to control. At the end of rearing period, the weight gain observed at 0, 4 and 8 ‰ salinity were 62.7, 57.8 and 54.5 %, respectively. The survival rate also decreased at higher salinity ($P > 0.05$). At the end of 90 days, the survival rate in the corresponding experiments was 96.7, 93.3 and 83.3 %, respectively.

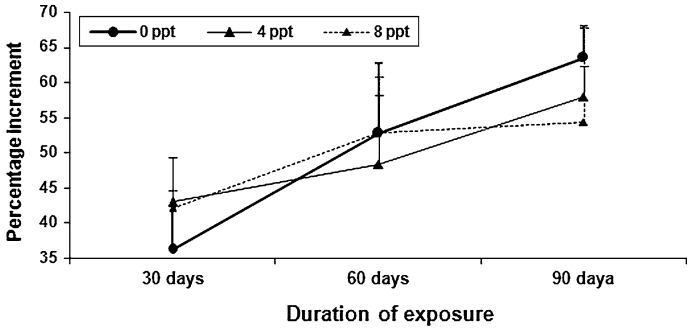

Fig. 1 Percentage weight gain *C. batrachus* exposed to different salinities for a period of 90 days

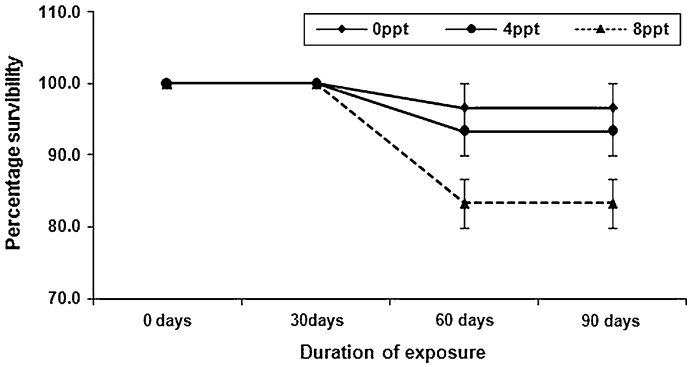

Fig. 2 Percentage survival of *C. batrachus* exposed to different salinities for a period of 90 days of rearing

Glucose

Glucose level was estimated in blood, brain and muscle of *C. batrachus* at the end of 90-day rearing period (Fig. 3). There was a significant ($P < 0.05$) difference in blood glucose level *in C. batrachus* exposed to sublethal dose of salinity compared with 0 ‰ salinity level. Blood glucose level was increased by 29.11 % at 4 ‰ salinity and decreased by 28.8 % at 8 ‰ salinity compared to control at the end of 90 days. However, there was no difference in blood glucose level between control and 4 ‰ and between 4 and 8 ‰. Brain glucose levels were also significantly reduced ($P < 0.05$) in fishes exposed to medium levels of salinity. At the end of 90-day rearing period, 37.0 and 19.83 % reduction in glucose level was observed in comparison with control at 4 and 8 ‰ salinity; although no significant ($P > 0.05$) difference was found between 4 and 8 ‰ (Fig. 3). In fish muscle, the glucose levels were not significantly ($P > 0.05$) affected by the treatments when exposed to salinity stress.

Glycogen

Glycogen level was estimated in liver and muscle tissues of *C. batrachus* after 90 days of exposure to salinity (Fig. 4). There was a significant decrease ($P < 0.05$) in the glycogen level in liver tissues after 90 days. Reduction of glycogen in liver was 48.2 and 45.8 % at 4 and 8 ‰ salinity, respectively, compared with the control. However, in muscle tissue, the glycogen level was not significantly ($P > 0.05$) affected by the salinity.

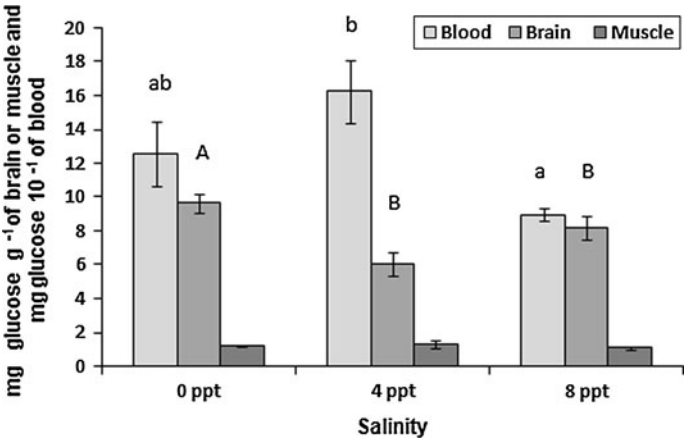

Fig. 3 Glucose level in different organs of *Clarias batrachus* exposed to different salinities at the end of 90 days of rearing. Data are expressed as mean ± SE, $n = 5$. *Bar* with *different alphabet letters* (*a* and *b* for blood, *A* and *B* for brain) differ significantly ($P < 0.05$)

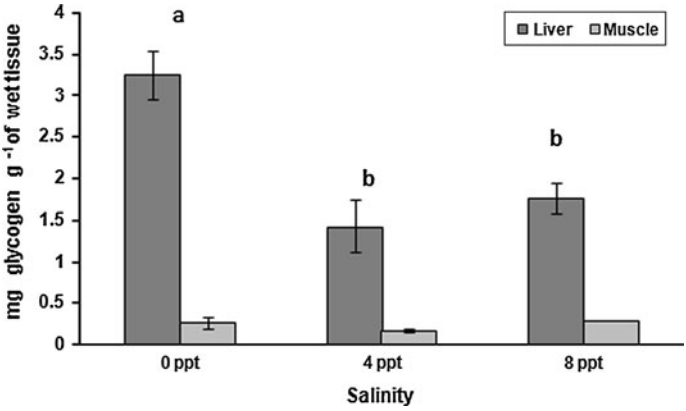

Fig. 4 Glycogen level in liver and muscle of *Clarias batrachus* exposed to different salinities at the end of 90 days of rearing. Data are expressed as mean ± SE, $n = 5$. *Bar* with *different alphabet letters* (*a* and *b* for liver) differ significantly ($P < 0.05$)

Ascorbic acid

Ascorbic acid level was found to be significantly ($P < 0.05$) reduced after 90 days of exposure in liver, kidney, brain and muscle (Fig. 5). It reduced by 15.70 and 31.07 % in liver; by 17.64 and 34.38 % in kidney; by 35.67 and 46.22 % in brain and by 13.79 and 22.70 % in muscle at 4 and 8 ‰ salnity, respectively, after 90 days of exposure.

Enzyme activity

Data pertaining to different enzymatic activities in *C. batrachus* are presented in Table 2. AchE activity in brain tissues of *C. batrachus* exposed to different salinities indicated that activity decreased progressively ($P < 0.05$) when salinity was increased from 0 to 8 ‰

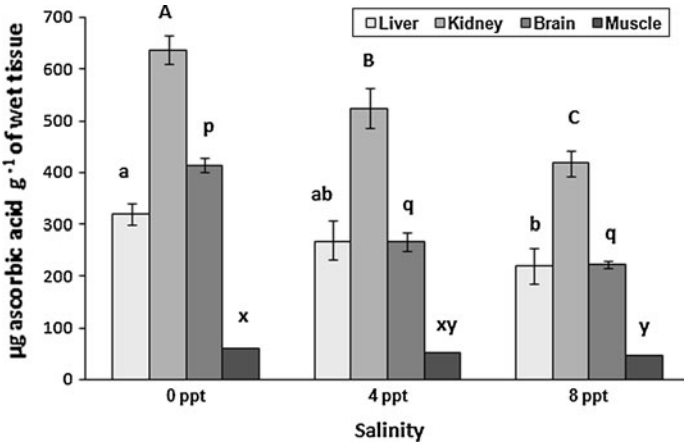

Fig. 5 Ascorbic acid level in different tissues of *Clarias batrachus* exposed to different salinities at the end of 90 days of rearing. Data are expressed as mean ± SE, $n = 5$. *Bar* with *different alphabet letters* (*a* and *b* for brain, *A*, *B* and *C* for kidney, *p* and *q* for brain, x and y for muscle) differ significantly ($P < 0.05$)

salinity. Inhibition of AchE to the extent of 39.3 and 54.45 % was recorded at 4 and 8 ‰ salinity ALP in liver ($P < 0.05$) and muscle ($P > 0.05$) of *C. batrachus* also showed a decreasing trend with increasing salinity. In muscle, ALP activity was reduced by 30.8 and 49.3 % at 4 and 8 ‰, respectively, compared with the control value, while in liver, it was 35.15 and 67.19 % lower at the same salinities. The ATPase activity in liver and muscle of *C. batrachus* also showed a decreasing ($P < 0.05$) trend with increasing salinity. A 55.6 and 60 % reduction at liver tissues, 43.5, and 51 % reduction in the muscles corresponding to 4 and 8 ‰ salinity were recorded at the end of 90-day rearing period. LDH activity was also significantly ($P < 0.05$) affected in the muscle tissues at the end of 90 days of exposure, and at the end of 90 days, 33.4 and 8.82 % reduction corresponding to 4 and 8 ‰ salinity were recorded. The muscle LDH activity was higher at 8 ‰ compared to 4 salinity. Though LDH activity in liver tissues was not significantly ($P > 0.05$) affected but its activity was reduced at both the salinity levels (7 and 9.7 % *a*t 4 and 8 ‰, respectively).

Discussion

The present study revealed that the growth of *Clarias batrachus* is affected by salinity. This finding is similar to the earlier reports in *Tilapia rendalli* (Kang'ombe and Brown 2008), *Mystus vittatus* (Arunachalam and Ravichandra Reddy 1978), *Oreochromis niloticus* (Schofield et al. 2010) and *Cyprinus carpio* fingerlings (Wang et al. 1996). Survival rate of *C. batrachus* was also reduced at higher salinities. Low survival rates of *C. batrachus* at higher salinity were comparable to those reported in Rainbow trout by McKay and Gjerde (1985). De-Boeck et al. (2000) reported that salt exposure reduced food intake by 70 % in *C. carpio* and had adverse effect on growth and survival in fishes.

In the present study, blood glucose level was higher at 4 ‰ salinity compared with control (0 ‰), indicating hyperglycemic condition in *C. batrachus*. Stress in fish has been shown to cause a primary response, involving neuro-hormonal stimulation resulting in an increase in corticosteroid and catecholamine secretions (Karşi and Yildiz 2005). These primary stress responses cause a number of physiological changes known as 'secondary

Table 2 Enzymatic activities in different organs of *Clarias batrachus* exposed to salinities at the end of 90 days of culture period

Parameters	Organs	Salinity		
		0 ‰	4 ‰	8 ‰
AChE activity	Brain	0.168 ± 0.03^{a}	$0.102 \pm 0.02^{a,b}$	0.076 ± 0.01^{b}
ALP activity	Muscle	2.900 ± 0.37	2.005 ± 0.68	1.467 ± 0.54
	Liver	16.773 ± 1.32^{a}	10.876 ± 0.75^{b}	5.737 ± 0.96^{c}
ATP activity	Muscle	1.358 ± 0.18^{a}	0.766 ± 0.07^{b}	0.665 ± 0.17^{b}
	Liver	1.958 ± 0.41^{a}	0.868 ± 0.07^{b}	0.783 ± 0.13^{b}
LDH activity	Muscle	160.724 ± 14.73^{a}	106.948 ± 21.69^{b}	146.448 ± 7.36^{b}
	Liver	42.762 ± 6.13	39.728 ± 6.72	38.580 ± 1.37

Data are expressed as mean ± SE ($n = 5$). Different superscript, lower case letters (a, b, c) in the same row indicate significant difference (Duncan's multiple range test, $P < 0.05$). Enzyme activities are expressed as follows: ACHE, μ moles of acetyl choline hydrolyzed/mg protein/minute at 37 °C; ALP, n moles of paranitrophenol released/mg protein/minute at 37 °C; ATPase: enzyme activity expressed in µg of phosphorus released/mg protein/minute at 37 °C; LDH, Δ 0.01 OD/mg protein/minute at 25 °C

effects' (Foo and Lam 1993). Increased plasma glucose level is used as an indicator of the secondary stress response (Mommsen et al. 1999). Jeanette et al. (2007) recorded significant increase in plasma glucose levels in *Oreochromis mossambicus* with the increase in salinity, which they attributed to the stress response and/or increased energy demand for maintenance of hydro-mineral balance at higher salinity. However, in the present study, blood glucose level was lower at 8 ‰ salinity, which could be attributed to the higher utilization of glucose (Martínez-Álvarez et al. 2002) and might be due to reduced appetite of the fishes at higher salinity (Usher et al. 1991; Plaut 1998). In brain, there was significant decrease ($P < 0.05$) in glucose level both at 4 and 8 ‰ salinity. Brain generally requires only glucose for energy purpose and reduced glucose flow into the brain at higher salinity might be due to the compensatory mechanism developed by the species to combat stress. During stress, demand of glucose in different tissues is higher in order to supply the energy for osmoregulatory mechanisms (Krumschnabel and Lackner 1993; Plaut 1998), whereupon glyconeogenesis even increases (Jürss and Bittorf 1990). However, reduced glucose supply to the brain might have detrimental effect on brain tissue.

In the present study, glycogen level in liver tissues significantly reduced ($P < 0.05$), indicating high rate of catabolism. Similar to the present study, depletion of liver glycogen was recorded in *C. carpio* when exposed to salt stress (De-Boeck et al. 2000) and in liver tissue of *C. batrachus* exposed to pesticides stress (Begum 2004). The results reveal that at higher salinity fish might have performed high rate of glycogenolysis activity to meet high-energy demand and that resulted in reduced glycogen levels in liver. Stress-associated hyperglycemia is an expected result of stress or exhaustive exercise in fishes (Barton and Iwama 1991; Hrubec et al. 1997). Chan and Woo (1978) reported that cortisol has shown to promote catabolism of peripheral tissues through increased gluconeogenesis leading to hyperglycemia.

Antioxidant vitamin C is well known for its major antistress activity (Azad et al. 2007, Misra et al. 2007; Norouzitallab et al. 2009). The antioxidant activity of ascorbic acid is based on its ability to react with free radicals. Most fish species cannot synthesize ascorbic acid and have to depend on external source to meet their needs (Chatterjee et al. 1975). In fish, liver and head kidney are the most significant storage organs for vitamin C (Gabaudan

and Verlhac 1992). Heath (1995) reported a reduction in the level of vitamin C in fish subjected to sublethal levels of organic and inorganic substances. In the present study, vitamin C level reduced significantly ($P < 0.05$) in all the organs under study with an increase in salinity (Fig. 5). The reduction in the ascorbic acid level might be due to high rate of utilization of vitamin C at higher salinity. Reduction of vitamin C in fish due to different pesticides has been reported in many fish (Madhuban and Kaviraj 2003, 2009; Sarma et al. 2010b; Sarma et al. 2009). Madhuban and Kaviraj (2009) opined that depletion of ascorbic acid is probably a defensive reaction of fish to combat stress, which thus enhances the requirement of ascorbic acid during stress. In the present study, vitamin C might have been used up for detoxification process (Mauck et al. 1978) or might have been used up for preventing peroxidation of cells (Winston and Di-Guilo 1991), which in turn might have caused a functional reduction in vitamin C content in different tissues thereby exposing the tissue to possible cell injury.

Acetylcholine esterase (AchE) is one of the most widely used enzymes as a biomarker for environmental pollution (Sarma et al. 2010b). In the present study, AchE activity in the brain of *C. batrachus* was inhibited significantly (39.3 at 4 ‰ and 54.45 at 8 ‰) with increasing salinity. Reduction in AchE enzyme activity indicates an accumulation of acetylcholine in the brain tissue, interfering with energy metabolism of the nervous system, preventing transmission of nervous impulses, and thereby causing behavioral alteration (Sarma et al. 2010b). It has been reported that fish can tolerate the loss of AchE activity up to 70–80 % before death (Coppage and Mathew 1974). Pavlov (1996) reported 75 and 70.6 % inhibition of AchE activity in tilapia and perch respectively when they were exposed to acute salinity stress. As high as 79.03 % inhibition of AchE activity was observed in *C. batrachus* exposed to pesticides (Yadav et al. 1998). In another study, 55–62 % inhibition of brain AchE activity was recorded in *Girardinichthys viviparous*, exposed to polluted water (López-López et al. 2006).

The ALP activities in liver and muscle were also inhibited at higher salinities. Similar to the present study, 27 % inhibition of ALP activity in liver tissues of *Oreochromis mossambicus* was reported by Rao (2006) when exposed to insecticides. Reduction of ALP was also reported in *Heteropneustes fossilis* (Johal et al. 2002) and *Clarias gariepinus* (Madhuban and Kaviraj 2009) upon different stressors. Madhuban and Kaviraj (2009) opined that the decrease of alkaline phosphatase activity might result in altered cell membrane transport, decrease in glycogen level and inhibitory effect on cell growth and proliferation. In the present study, the inhibition of ALP in *C. batrachus* might be due to continuous exposure to higher salinity, which causes depression in the overall impact of the enzyme.

Adenosine triphosphatase (ATPases) are membrane-bound enzymes involved in the immediate release of energy which are responsible for a large part of basic metabolic and physiological activities (Smitha and Philip 2011). Measurement of ATPases could also be used as a surrogate biomarker to assess the exposure to chemical pollutants (Parveza et al. 2006). In the present study, it was found that the activity of ATPase was reduced in liver and muscle of *C. batrachus* when exposed to salinity for a period of 90 days. The reduction in ATPase activity could indicate the alterations in the structure and functions of the membrane or may be due to direct inhibition of long-term exposure to high salinity on the enzymes. ATPase is related to immediate release of energy, and reduction in its activity might have significantly affected the fishes in terms of the energy balance and ion transport (Sarma et al. 2010b). Reduction in ATPase activity after the exposure to various stressors has been reported earlier in *C. batrachus* (Begum 2009), *O. mossambicus* (Smitha and Philip 2011) and *Channa punctata* (Parveza et al. 2006). Rao and Rao (1983) reported

reduced ATPase activity in *Tilapia mossambica* exposed to methyl parathion suggesting the inhibition of active transport system and oxidative phosphorylation.

The LDH is the terminal enzyme of glycolytic pathway and used extensively as a biomarker for various environment-monitoring programmes. In the present study, LDH activity in liver tissue was also reduced with increasing salinity as observed by Agrahari and Gopal (2009) in *Channa punctatus* when exposed to monocrotophos. It was attributed to the high rate of glycolysis, which is the only energy-producing pathway for the animals under stress. Rao (2006) reported 25 % inhibition in muscle LDH activity in *O. mossambicus* exposed to insecticides. In the present study, the reduction in LDH activity was higher at 4 ‰ than at 8 ‰ salinity ($P > 0.05$). Lower muscle LDH activity in fish at 4 ‰ might be due to faster rate of glycolysis, LDH is a marker enzyme of tissue damage and elevated LDH activity is reported to cause liver necrosis (Ramesh et al. 1993).

From the present investigation, it can be summarized that long-term exposure to salinity affects the growth and biochemical composition of tissues in *C. batrachus*. It may be presumed that the impact of salinity on weaker and younger fishes may be many folds higher than the subadult individuals of *C. batrachus,* as used in this study. Hence, a comprehensive study to assess the impact of higher salinity on different freshwater species across the life stages would throw more light on the extent of vulnerability of different fishes to the situations linked to climate change and provide necessary guidelines in the selection of suitable fishes for aquaculture in low saline brackishwater areas.

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