Volume 7 Issue 2 2021

ISSN 2454-3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 7, No. 2, 308-323 (2021)

International Journal of Zoological Investigations
ISSN: 2454-3055
Contents available at Journals Home Page: www.ijzi.net

Evaluation of Oxidative Stress Enzymes of Spawn and Fry of *Clarias batrachus* (Linn. 1758) Exposed to Artificial UV-B Radiation: A Biomarker Analysis

Mandal Arghya[1], Das Subhas[1,2], Patra Atanu[1], Mondal Niladri Sekhar[1,3] and Ghosh Apurba Ratan[1*]

[1]Ecotoxicology Lab, Department of Environmental Science, The University of Burdwan, Purba Bardhaman, West Bengal, India
[2]Panacea the Aqua Clinic, Galsi-II Block, Purba Bardhaman, Department of Fisheries, Govt. of West Bengal, India
[3]Department of Environmental Science, Netaji Subhas Open University, DD-26, Sector-I, Salt Lake City, Kolkata - 700 064

**Corresponding Author*

Received: 20th May, 2021; **Accepted:** 15th June, 2021; **Published online:** 2nd July, 2021

https://doi.org/10.33745/ijzi.2021.v07i02.001

Abstract: The present study investigated the impact of the artificial exposure of UV-B radiation, based on different time span and intervals, on spawn and fry of *Clarias batrachus* at the level of oxidative stress enzymes. The spawn and fry of *C. batrachus* were exposed for time span of 5 and 10 days at a duration of 5, 10 and 15 min per day. The specific growth rate (SGR%), weight gain (%WG) and oxidative stress enzymes, *i.e.,* superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), protein carbonyl (CP), nitric oxide synthase (NOS) and trypsin were analysed. The specific growth rate and the per cent weight gain of spawn and fry of *C. batrachus* were decreased as compared to control. The results of the oxidative stress enzymes, *viz*., SOD, CAT, GSH and NOS were decreased in both spawn and fry of *C. batrachus* in comparison to control. CP values were increased for both the spawn and fry as compared to control. Trypsin in spawn and fry decreased gradually. This study has been able to explore the impact of UV-B exposure on spawn and fry of *C. batrachus* in regard to these oxidative stress enzymes, which can be established as biomarkers in monitoring the UV-B radiation pollution. Finally, it infers that the harmful radiations may cause deterioration in fish health as well as yield, so, UV-B radiation becomes a major concern to the aquaculture industry, especially, with regard to sustainable fish production.

Keywords: UV-B radiation, Oxidative stress enzymes, Biomarkers, SGR, WG, *Clarias batrachus*

Citation: Mandal Arghya, Das Subhas, Patra Atanu, Mondal Niladri Sekhar and Ghosh Apurba Ratan: Evaluation of oxidative stress enzymes of spawn and fry of *Clarias batrachus* (Linn. 1758) exposed to artificial UV-B radiation: A biomarker analysis. Intern. J. Zool. Invest. 7 (2): 308-323, 2021.
https://doi.org/10.33745/ijzi.2021.v07i02.001

Introduction

Stratospheric ozone depletion is inflicted the incidence of increase of the most energetic and potentially damaging daylight component of UV-B radiation (280-315 nm) (Madronich, 1993), which is now a great concern towards global climate change. Enhancement of solar UV-B radiation and its penetration depth are also responsible for the serious ecosystem damage including the aquatic organisms (Blaustein *et al*., 2003). Many studies were carried out during the last decades which

focused on determining the solar ultraviolet fluxes (UVR- 280-320 nm) reaching the Earth's surface (Frederick *et al*., 1994), and their effects on different ecosystems (Björn *et al*., 1996; Whitehead *et al*., 2000). It has now been considered that normal solar UVR can also cause ecological stresses to aquatic organisms because of their sensitivity on ambient levels of UVR. Fish act as one of the important bio-indicators in monitoring the health of aquatic environment due to its early responsiveness to sequential environmental changes. UV-B radiation is directly affecting the primary and secondary aquatic community like zooplankton, crustaceans, amphibians, fish and corals (Jokinen *et al*., 2011; Sucré *et al*., 2012). Exposure of UV-B radiation to planktonic community, eggs and larvae of freshwater fish resulted into an increased mortality and morbidity and may lead to poor propagation of adult populations (Häder *et al*., 2007; Jokinen *et al*., 2011; Singh *et al*., 2013). The ultraviolet radiation is associated with the production of free active oxygen species (ROS) including free radicals from oxygen and other oxygen-derived compounds like superoxide (O_2^-), hydroxyl (OH^-), nitric oxide (NO) *etc*., that cause oxidative damage resulting into damage to DNA strand and finally cell death (Aebi, 1984; Browman *et al*., 2000; Lamare *et al*., 2004; Obermüller *et al*., 2005; Häder *et al*., 2007; Sucré *et al*., 2012; Ibrahim, 2015). Pro-oxidants are capable of generating free radicals during its metabolism and producing prolonged biological effects extending from minutes to years (Chen, 2009). Proteins, nucleic acids and lipids are the primary target components of UV-B radiation. Damage induced by UV radiation can be occurred at the levels of cellular, tissue, organ and molecular level leading to increase of the incidence of mortality of gametes and fish spawn (Mitchell *et al*., 2004; Häder *et al*., 2010; Dahms and Lee, 2010; Alves and Agustí, 2020). Finally, they also oxidize cellular thiols (-SH) and extract hydrogen atoms (H^+) from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Halliwell and Gutteridge, 2015). Under normal physiological condition, cellular ROS generation is counter balanced by the action of antioxidant enzymes and other redox molecules; the antioxidant defence systems like catalase (CAT), super oxide dismutase (SOD), glutathione (GSH) are meant to decrease ROS from body. UV-B disrupts the balance between the production and removal of H_2O_2 and subsequently accumulation of H_2O_2 initiates the signalling responses leading to the induction of enzymatic antioxidant defence system (Pieri *et al*., 1995). Induction of UV-B radiation may interrupt the enzymatic antioxidant defence systems to overcome ROS production, which are mainly attributed by ascorbate peroxidase, total peroxidases and catalase enzymes derived from SOD (Hamilos *et al*., 1989). Exposure of UV-B radiation to *Spodoptera litura* caused DNA damage and increased production of ROS and simultaneously reduction of POX, CAT and GST activities (Ibrahim, 2015).

Thus, evaluation of these enzymes may give an important indication of pathological discourse. But the study on the effects of UV-B radiation on fish spawn and fry, which are the key components in pisciculture, is scanty, therefore, the present study is intended to evaluate the growth related parameters, *viz*., SGR and WG, and simultaneously to determine these oxidative stress enzymes as biomarkers and to establish their mechanism of actions.

Materials and Methods

Fish sample procurement and dose indication:

Healthy and disease-free spawn and fry of *Clarias batrachus* (Linnaeus, 1758) were procured from a registered fish farm (Chandimata SHG, Purba Bardhaman, W.B.) and transferred to the aquarium and acclimatized for 48 h and then distributed in the aquarium with the pre-filled decloronized water. Four (4) sets of aquaria [control (one set) and treated (three sets) comprising 3 replica in each, altogether total twelve (12) aquaria] were used for spawn and

same for fry subsequently and separately under dose- and exposure-specific study. A preburnt UV-B lamp [Philips Tube Light; NAAVA-LT8W T5] was used for incidence of the radiation doses. The doses were given for the duration of 5, 10, 15 min/day in the registered aquarium for the periods of 5 and 10 days to spawn and same for fry also. During experimentation, mortality record was maintained in control as well as in treated sets for both the spawn and fry samples. During acclimatization and exposure periods they were fed with *Tubifex* sp. (Commercial name, Royal Feast Tubifex Worm). Desired samples were collected from respective control and treated aquaria after 5 and 10 days of exposures with scheduled doses and were marked as: for 5 days: 5D5M, 5D10M, 5D15M and for 10 days: 10D5M, 10D10M, 10D15M for both the spawn and fry.

Specific growth rate (SGR%):

Specific growth rate was calculated using the formula (Mandal *et al*., 2020):

$$(\text{SGR }\%) = \frac{\text{FW-IW}}{\text{N}} \times 100$$

Where, FW is final weight of the sp.; IW is initial weight of the sp. and N is number of days experiment continued.

Weight gain (%WG):

Weight gain (%) was calculated by using the formula (Das *et al*., 2021):

$$\text{WG }(\%) = \frac{\text{FMW-IMW}}{\text{IMW}} \times 100$$

Where, FMW is final mean live body weight of the sp. and IMW is initial mean live weight of the sp.

Biochemical analysis:

Superoxide dismutase (SOD):

The reaction mixture containing 1.2 ml of solution A (50 mM sodium carbonate in 0.1 mM EDTA buffer, pH 10.8), 0.5 ml solution B [96 µM NBT (Nitroblue Tetrazolium)] and 0.1 ml of solution C (0.6% Triton X-100) were incubated at 37 C for 10 min. Reaction was initiated by adding 0.1 ml of 20 mM hydroxylamine HCl (pH 6.0). The rate of reduction of NBT dye by O_2^- anion generated due to photoactivation of hydroxylamine HCl and was recorded at 560 nm for 3 min for blank. Then 0.1 ml PMS [phenazinemethosulfate (5-methyl-phenazinium methyl sulfate)] was immediately added after addition of hydroxylamine HCl to the reaction mixture. After mixing thoroughly, 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was recorded at 560 nm for 3 min (Kono, 1978).

Catalase (CAT):

For catalase activity the whole body was homogenized in phosphate buffer (50 mM, pH 7.0). Then the contents were centrifuged and 2.0 ml of the supernatant was used along with 1.0 ml of phosphate buffer containing 10 mM of hydrogen peroxide as substrate for catalase. The decrease in absorbance of the sample was measured at 240 nm in UV–visible spectrophotometer (Hitachi, Model No. U-3310) against blank. The blank contains 3.0 ml of phosphate buffer solution without hydrogen peroxide. The values were expressed as millimolar (mM) of hydrogen peroxide decomposed/min/mg of protein. Homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution for estimation of proteins with folin–phenol reagent using bovine serum albumin as standard.

Glutathione (GSH):

For estimation of cellular GSH level (Ebrahim and Dakshinamurti, 1986) the cell pellets were re-suspended in 200 µl of ice cold $_{dd}H_2O$ and sonicated for 30 sec. Afterwards, 50 µl of 25% metaphosphoric acid was added and sonicated for 2 min and then the mixture was centrifuged at 13,000 rpm for 20 minutes. Then 20 µl of the supernatant was transferred into a cuvette containing 2 ml of H_2O followed by addition and mixing of 500 µl of 0.1 M sodium phosphate (pH 8.0) and 100 µl of 0.1% o-phthaladehyde (dissolved in methanol). Now, the mixture was

kept in the dark at room temperature for 20 min and measured at 340 nm.

Protein carbonyl (CP):

Protein carbonyl content was assayed (Bayer *et al*., 1979) as modified (Floor and Wetzel, 1998). Soluble protein (0.5 ml) was reacted with 10 mM DNPH in 2M hydrochloric acid for 1 h at room temperature and precipitated with 6% of trichloroacetic acid (TCA). The pelleted protein was washed thrice by re-suspension in ethanol/ethyl acetate (1:1). Proteins were then solubilized in 6 M of guanidine hydrochloride, 50% of formic acid and centrifuged at 16,000 rpm for 5 min to remove remnants or insoluble material if any. The carbonyl content was measured spectrophotometrically (Shimadzu UV–vis spectrophotometer, Japan, Model no- UV-1800) at 366 nm. Assay was performed in triplicate and a tissue blank incubated with 2M of HCl without DNPH for each sample. The results were expressed in nanomoles of DNPH incorporated/mg protein based on the molar extinction coefficient of 21,000 per M/cm.

Nitric oxide synthase (NOS):

Nitric oxide synthase (NOS) of the sample was measured as per the method (Singh *et al*., 2015). The tissue sample (100 mg) was homogenized in 1 ml of phosphate buffer saline (pH 7.4) and centrifuged at 10,000 rpm for 10 min at 4 C. 100 µl of supernatant was mixed with equal volume of Griess reagent (1% of sulfanilamide and 0.1% of naphthylethylenediamine in 5% of phosphoric acid) and incubated at room temperature for 10 min. The absorbance was recorded at 540 nm. The nitrite concentration was determined from the nitrite standard curve and was expressed as m mol/mg tissue.

Trypsin:

Trypsin activity was measured by using synthetic substrates, N-α-benzoyl-DL-Arginine-p-nitroanilidine (BAPNA, 1.0 mM) (Erlanger *et al*., 1961). The pre-incubated experimental or control mixtures were added with 0.05 M of Tris-HCl buffer containing 0.02 M of $CaCl_2.2H_2O$ (pH 7.4) and respective synthetic substrates. The reaction mixture was incubated at 30 ^{0}C and absorbance (410 nm) was determined at an interval of 1 min for 5 min. The trypsin amidase activity ((Enzyme activity unit mixture of mg/protein/min) was calculated using the formula (Ágeirsson *et al*., 1989):

$$\text{Specific activity units} = \frac{(\text{Abs410/min}) \times 1000 \times \text{ml of reaction mixture}}{\text{Extinction coefficient of chromogen} \times \text{mg protein in reaction}}$$

The extinction coefficients of p-nitroanilidine liberated from chromogen BAPNA is 8800 (Charu and Ragini, 2020).

Statistical analysis:

Analysis of variance (One-way ANOVA) followed by Tukey's test at the significance level of 0.05 using Origin pro-ver. 8.5 was adopted for statistical analysis of the enzyme activity, SGR% and %WG. PCA (Principal Component Analysis: A multivariate technique, involved in analysing a set of data in tabular form in which observations are reflected by several inter-correlated quantitative dependent variables) was also done using PAST software (Ver. 4.04) after standardization of the variables to mean of zero and unit variance.

Results

Specific growth rate (SGR%):

In the present study, the average value of SGR in control spawn of *C. batrachus* was 24.39±0.1% (on 6th day) and 15.42±0.1% (on 11th day) (Table 1), i.e., after completion of the experimental periods. In case of 5 d experiment (on 6th day), after 5, 10, 15 min of exposures the values were 17.12±0.3, 12.52±0.2, 11.46±0.3%, respectively; during 10 d experiment (on 11th day), after 5, 10, 15 min of exposures the values were 9.09±0.2, 6.43±0.2, 6.09±0.1%, respectively (Table 1).

SGR value in control fry of *C. batrachus* on 6th day and 11th day was 13.77±0.1% and 17.11±0.2% respectively (Table 1); during 5 d (on 6th day), after the exposures of 5, 10, 15 min, the values were 9.95±0.1, 5.57±0.2, 2.90±0.1%, respectively; and on 10 d period (on 11th day), after the same

exposures of 5, 10, 15 min the average values were 9.01±0.1, 5.88±0.2, 4.07±0.1%, respectively (Table 1). In the present study, the specific growth rate for both the spawn and fry of *C. batrachus* was decreased in comparison to control, and the lowest (SGR%) was found under the dose of 10D15M for both the spawn and fry (Table 1).

Weight gain (%WG):

The average value of WG in control spawn of *C. batrachus* was 238.24±3.4% (on 6th day) and 347.05±4.1% (on 11th day) (Table 2). In 5 d experiment (on 6th day), after 5, 10, 15 min of exposures the values were 117.65±2.5, 73.53±1.9, 63.24±1.4%, respectively, whereas in 10 d experiment (on 11th day), after 5, 10, 15 min of exposures the values were 130.88±2.7, 76.47±2.1, 69.11±1.6%, respectively (Table 2).

In fry of *C. batrachus*, the %WG in control (on 6th day) was 99.16±1.1% and on 11th day it was 453.63±4.2% (Table 2); during treatment of 5d (on 6th day), after the exposures of 5, 10, 15 min the values were 64.53±1.4, 33.12±1.1, 15.64±1.1%, respectively; and on 10 d (11th day), after the exposures of 5, 10, 15 min the average values were 146.37±2.1, 80.17±1.7, 50.28±1.5%, respectively (Table 2). In the present experiment, the per cent weight gain for both the spawn and fry of *C. batrachus* was decreased as compared to control, and the lowest WG was found in the dose of 5D15M for both the spawn and fry (Table 2).

Superoxide dismutase (SOD):

Superoxide dismutase (SOD), one of the antioxidant proteins catalyzes the dismutation of superoxide anion to hydrogen peroxide, which is subsequently detoxified to oxygen and water by catalase or glutathione peroxidase. SOD does not bind to cellular membranes and is rapidly excreted from the kidney. In the present experiment, the average value of SOD in control spawn of *C. batrachus* was 0.95±0.02 unit/mg of protein/min (Table 3a). In 5 d experiment, after 5, 10, 15 min of exposures the values were 0.85±0.02, 0.75±0.02, 0.72±0.01 unit/mg of protein/min, respectively; in 10 d experiment, after 5, 10, 15 min of exposures the values were 0.60±0.03, 0.55±0.02, 0.48±0.01 unit/mg of protein/min, respectively (Table 3a).

The value of superoxide dismutase in control fry of *C. batrachus* was 1.76±0.01 unit/mg of protein/min (Table 3b); during 5 d after exposures of 5, 10, 15 min the values were 1.32±0.01, 1.01±0.01, 0.88±0.02 unit/mg of protein/min, respectively; and 10 d, after the exposures of 5, 10, 15 min the average values were 1.09±0.08, 0.73±0.01, 0.62±0.01 unit/mg of protein/min, respectively (Table 3b). In the present experiment, the SOD for both the spawn and fry of *C. batrachus* was decreased compared to control, and lowest SOD was found in the dose of 10D15M for both the spawn and fry (Tables 3a, b).

Catalase (CAT):

CAT catalyzes the degradation or reduction of hydrogen peroxide (H_2O_2) to water and molecular oxygen, consequently, completing the detoxification process that is imitated by SOD, here, the enzyme uses either iron or manganese as a cofactor and undergoes catalytic degradation. In this study, the average value of CAT in the control spawn of *C. batrachus* was 70.85±0.25 m mol/min/mg of protein (Table 3a). In case of 5 d, after the exposures of 5, 10, 15 min the average values were 61.47±0.62, 55.26±0.78, 49.55±0.40 m mol/min/mg of protein, respectively; and in 10 d experiment, the average values after 5, 10, 15 min exposures were 55.87±0.20, 51.34±0.52, 45.70±0.38 m mol/min/mg of protein, respectively (Table 3a).

The value of CAT for control fry of *C. batrachus* was 78.45±1.08 m mol/min/mg of protein (Table 3b). In 5 d experiment, after 5, 10, 15 min of exposures the values were 73.26±0.48, 67.76±0.51, 61.38±0.57 m mol/min/mg of protein, respectively, during 10 d exposure, after 5, 10, 15 min the average values were 62.28±0.32, 47.66±9.76, 50.58±0.34 m mol/min/mg of protein, respectively (Table 3a). In the present experiment,

Table 1: SGR % values of spawn and fry of *C. batrachus*

Sample	Condition	Control (%)	5 min/d (%)	10 min/d (%)	5 min/d (%)
Spawn	On 6th day	238.24±3.4^{d}	117.65±2.5^{c}	73.53±1.9^{b}	63.24±1.4^{a}
	On 11th day	347.05±4.1^{d}	130.88±2.7^{c}	76.47±2.1^{b}	69.11±1.6^{a}
Fry	On 6th day	99.16±1.1^{d}	64.53±1.4^{c}	33.12±1.1^{b}	15.64±1.1^{a}
	On 11th day	453.63±4.2^{d}	146.37±2.1^{c}	80.17±1.7^{b}	50.28±1.5^{a}

Values with same superscripts in the same row are not significantly different ($p<0.05$). $^{a, b, c}$ and ^{d} are the different groups for performing Tukey's test.

Table 2: Per cent WG values of spawn and fry of *C. batrachus*

Sample	Condition	Control (%)	5 min/d (%)	10 min/d (%)	15 min/d (%)
Spawn	On 6th day	24.39±0.1^{d}	17.12±0.3^{c}	12.52±0.2^{b}	11.46±0.3^{a,b}
	On 11th day	15.42±0.1^{d}	9.09±0.2^{c}	6.43±0.2^{b}	6.09±0.1^{a,b}
Fry	On 6th day	13.77±0.1 ^{d}	9.95±0.1 ^{c}	5.57±0.2 ^{b}	2.90±0.1 ^{a}
	On 11th day	17.11±0.2 ^{d}	9.01±0.1 ^{c}	5.88±0.2 ^{b}	4.07±0.1 ^{a}

Data are reported as Mean ± SEM (n=5). One way ANOVA followed by Tukey's test conducted. Values with same superscripts in the same row are not significantly different ($p<0.05$). $^{a, b, c}$ and ^{d} are the different groups for performing Tukey's test.

the CAT for both the spawn and fry of *C. batrachus* was decreased as compared to control, and lowest CAT was found in the dose of 10D15M and 10D10M for the spawn and fry, respectively (Tables 3a, b).

Glutathione (GSH):

Glutathione (GSH) is a key determinant of redox signalling, vital in detoxification of xenobiotics, and regulates cell proliferation, apoptosis, immune function, and fibrogenesis. In this study, the average value of the control spawn of *C. batrachus* was 5.24±0.01 m mol/mg of protein/min (Table3a). In case of 5 d experiment, after 5, 10, 15 min of exposures the average values were 5.10±0.00, 4.90±0.04, 4.55±0.04 m mol/mg of protein/min, respectively; and in 10 d experiment, after 5, 10, 15 min of exposures the values were 5.11±0.01, 4.18±0.01, 3.98±0.01 m mol/mg of protein/min, respectively (Table 3a).

In control fry of *C. batrachus* the average value of GSH was 6.13±0.01 m mol/mg of protein/min (Table 3b). In 5 d experiment, after 5, 10, 15 min of exposures the average values were 6.02±0.01, 5.84±0.01, 5.37±0.01m mol/mg of protein/min respectively, and in 10 d experiment, the values after 5, 10, 15 min exposures were 5.87±0.02, 5.17±0.02, 4.35±0.02 m mol/mg of protein/min,

Table 3a: Oxidative stress enzyme of the spawn of *C. batrachus*

Enzyme	Control	5D5M	5D10M	5D15M	10D5M	10D10M	10D15M
SOD (unit/mg of protein/min)	0.95±0.02 ^{g}	0.85±0.02 ^{f}	0.75±0.02 ^{e}	0.72±0.01 ^{d,e}	0.60±0.03 ^{c}	0.55±0.02 ^{b,c}	0.48±0.01 ^{a,b}
CAT (m mol/min/mg of protein)	70.85±0.25 ^{g}	61.47±0.62 ^{f}	55.26±0.78 ^{d,e}	49.55±0.40 ^{b}	55.87±0.20 ^{e}	51.34±0.52 ^{c}	45.70±0.38 ^{a}
GSH (m mol/mg of protein/ min)	5.24±0.01 ^{g}	5.10±0.00 ^{e,f}	4.90±0.04 ^{d}	4.55±0.04 ^{c}	5.11±0.01 ^{f}	4.18±0.01 ^{b}	3.98±0.01 ^{a}
CP (µ mol/mg of protein/min)	1.91±0.003 ^{a}	2.31±0.003 ^{b}	2.71±0.003 ^{d}	2.91±0.002 ^{e,f}	2.53±0.015 ^{c}	2.92±0.002 ^{f}	3.12±0.003 ^{g}
NOS (m mol/mg tissue)	24.33±0.33 ^{g}	23.73±0.15 ^{f,g}	19.37±0.32 ^{e}	15.53±0.23 ^{c}	18.20±0.20 ^{d,e}	11.23±0.19 ^{b}	7.27±0.22 ^{a}
TRYPSIN (u mg/protein/min)	0.13±0.01 ^{g}	0.11±0.01 ^{f,g}	0.09±0.00 ^{e,f}	0.05±0.00 ^{d,c,b}	0.06±0.00 ^{c}	0.06±0.01 ^{b,c}	0.04±0.00 ^{a,d,c,b}

Data are reported as Mean ± SEM (n=5). One way ANOVA followed by Tukey's test conducted. Values with same superscripts in the same row are not significantly different ($p<0.05$). ^{a,b,c,d,e,f,} and ^{g} are the different groups for performing Tukey's test.

Table 3b: Oxidative stress enzyme of the fry of *C. batrachus*

Enzyme	Control	5D5M	5D10M	5D15M	10D5M	10D10M	10D15M
SOD (unit/mg of protein/min)	1.76±0.01 ^{g}	1.32±0.01 ^{f}	1.01±0.01 ^{d,e}	0.88±0.02 ^{c,d}	1.09±0.08 ^{e}	0.73±0.01 ^{b,c}	0.62±0.01 ^{a,b}
CAT (m mol/min/mg of protein)	78.45±1.08 ^{g}	73.26±0.48 ^{f,g}	67.76±0.51 ^{e,g,f}	61.38±0.57 ^{c,g,f,e,d}	62.28±0.32 ^{d,g,f,e}	47.66±9.76 ^{a,b}	50.58±0.34 ^{b,e,c,d}
GSH (m mol/mg of protein/ min)	6.13±0.01 ^{g}	6.02±0.01 ^{f}	5.84±0.01 ^{d,e}	5.37±0.03 ^{c}	5.87±0.02 ^{e}	5.17±0.02 ^{b}	4.35±0.02 ^{a}
CP (µ mol/mg of protein/min)	2.01±0.003 ^{a}	2.54±0.002 ^{b}	2.81±0.003 ^{e}	3.17±0.002 ^{f}	2.76±0.013 ^{c}	3.22±0.002 ^{g}	2.71±0.004 ^{d}
NOS (m mol/mg tissue)	27.40±0.30 ^{g}	25.3±0.21 ^{f}	21.5±0.21 ^{e}	18.47±0.17 ^{c}	20.33±0.33 ^{d}	17.27±0.22 ^{b}	13.03±0.03 ^{a}
TRYPSIN (u mg/protein/min)	0.43±0.07 ^{g}	0.32±0.01 ^{f,g}	0.22±0.01 ^{d,f,e}	0.12±0.01 ^{a,d,c,e,b}	0.21±0.01 ^{c,f,d,e}	0.23±0.00 ^{e}	0.18±0.00 ^{b,d,c,e}

Data are reported as Mean ± SEM (n=5). One way ANOVA followed by Tukey's test conducted. Values with same superscripts in the same row are not significantly different ($p<0.05$). ^{a,b,c,d,e,f,} and ^{g} are the different groups for performing Tukey's test.

respectively (Table 3b). In the present experiment, GSH for both the spawn and fry of *C. batrachus* was decreased as compared to control, and lowest GSH was found in the dose of 10D15M for the spawn and fry (Tables 3a, b).

Protein carbonyl (CP):

Protein carbonylation is promoted by reactive oxygen species and is considered as irreversible and aims to induce protein degradation. Actually, it refers to the process that forms reactive ketones or aldehydes which can be reacted by 2,4-dinitrophenylhydrazine (DNPH) to form hydrazones. In the present experiment, an average value of CP in the control spawn of *C. batrachus* was 1.91± 0.003 µmol/mg of protein (Table 3a). In 5 d experiment, after 5, 10, 15 min of exposures the average values were 2.31±0.003, 2.71±0.003, 2.91±0.002 µmole/mg of protein, respectively; whereas in 10 d experiment, after 5, 10, 15 min of exposures the average values were 2.53±0.015, 2.92±0.002, 3.12±0.003 µmol/mg of protein, respectively (Table 3a).

In control fry of *C. batrachus*, the average value of CP was 2.01±0.003 µmol/mg of protein (Table 3b). In 5d experiment, after 5, 10, 15 min of exposures the average values were 2.54±0.002, 2.81±0.003, 3.17±0.002 µmol/mg of protein, respectively. In 10d experiment, after exposures of 5, 10, 15 min, the average values were 2.76±0.013, 3.22±0.002, 2.71±0.004 µmol/mg of protein, respectively (Table 3b). In the present experiment, the CP for both the spawn and fry of *C. batrachus* was increased compared to control, and highest CP was found in the dose of 10D15M and 10D10M for the spawn and fry, respectively (Tables 3a, b).

Nitric oxide synthase (NOS):

Nitric oxide (NO) controls servoregulatory functions such as neurotransmission (by stimulating NO-sensitive guanylyl cyclase) and regulates gene transcription and mRNA translation and produces post-translational modifications of proteins. It has numerous molecular targets and forms the potent oxidant peroxynitrite ($ONOO^-$), which can cause oxidative damage, nitration, and S-nitrosylation of biomolecules including proteins, lipids, and DNA. In this experiment, the average value of NOS in the control spawn of *C. batrachus* was 24.33±0.33 m mole/mg tissue (Table 3a). In 5 d experiment, after exposures of 5, 10, 15 min the average values were 23.73±0.15, 19.37±0.32, 15.53±0.23 m mol/mg of tissue, respectively. In 10 d experiment, after exposures of 5, 10, 15 min the average values were 18.20±0.20, 11.23±0.19, 7.27±0.22 m mol/mg tissue, respectively (Table 3a).

In control fry the value of NOS was 27.40±0.30 m mol/mg tissue (Table 3b). In 5 d experiment, after the exposures of 5, 10, 15 min the average values were 25.30±0.21, 21.50±0.21, 18.47±0.17 m mol/mg tissue, respectively, whereas in 10 d experiment after 5, 10, 15 min of exposures the values were 20.33±0.33, 17.27±0.22, 13.03±0.03 m mol/mg of tissue, respectively (Table 3b). In the present experiment, the NOS for both the spawn and fry of *C. batrachus* was decreased as compared to control, and lowest NOS was found in the dose of 10D15M for both the spawn and fry (Tables 3a, b).

Trypsin:

Trypsin is formed in the small intestine when its proenzyme is formed, i.e., the trypsinogen produced by the activated pancreas. In this study, the average value of trypsin in the control spawn of *C. batrachus* was 0.13±0.01 u mg/protein/min (Table 3a). In case of 5 d experiment, after 5, 10, 15 min of exposures the average values were 0.11±0.01, 0.09±0.00, 0.05±0.00 u mg/pro/min, respectively, and for 10 d experiment, after 5, 10, 15 min of exposures the average values were 0.06±0.00, 0.06±0.01, 0.04±0.00 u mg/protein/ min, respectively (Table 3a).

In control fry of *C. batrachus* the average values of trypsin was 0.43±0.07 u mg/protein/min (Table 3b). In 5 d, after 5, 10, 15 min of exposures the average values were 0.32±0.01, 0.22±0.01, 0.12±0.01 u mg/protein/min, respectively. In 10 d

experiment, after 5, 10, 15 min of exposures the average values were 0.21±0.01, 0.23±0.00, 0.18±0.01 u mg/protein/min, respectively (Table 3b). In the present experiment, the trypsin activity of spawn of *C. batrachus* decreased gradually and revealed maximum reduction in 5D15M and in 10D15M. In case of fry, the trend of reduction was same and became maximum at 5D15M and 10D15M (Tables 3a, b).

Principal component analysis (PCA):

SOD:

PC1-PC2 reflected that SOD activity in spawn of *C. batrachus* responded positively in the doses of 5D5M, 5D10M, 10D10M and 10D15M and negatively responded in the doses of 5D15M and 10D5M (Fig. 1 A). In fry of *C. batrachus* it responded positively in the doses of 5D5M, 5D10M and 5D15M and negatively in the doses of 10D5M, 10D10M and 10D15M (Fig. 1 B).

CAT:

PC1-PC2 reflected that CAT activity in spawn of *C. batrachus* was positively responded in the doses of 10D10M and 5D10M and negatively responded in the doses of 5D5M, 5D15M, 10D5M and 10D15M (Fig. 1 C). In fry of *C. batrachus* it responded positively in the doses of 5D5M and 5D15M and negatively in the doses of 5D10M, 10D5M, 10D10M and 10D15M (Fig. 1 D).

GSH:

PC1-PC2 reflected that GSH activity in spawn of *C. batrachus* responded positively in the doses of 10D10M and 5D15M and negatively responded in the doses of 5D5M, 5D10M, 10D5M and 10D15M (Fig. 1 E). In case of fry of *C. batrachus* it responded positively in the doses of 5D5M, 5D15M and 10D10M and negatively responded in the doses of 5D10M, 10D5Mand 10D15M (Fig. 1 F).

CP:

PC1-PC2 reflected that CP activity in spawn of *C. batrachus* responded positively in the doses of 5D5M, 10D5M and 10D10M and negatively in the doses of 5D10M, 5D15Mand 10D15M (Fig. 2 A). In fry of *C. batrachus*, PC1-PC2 of CP activity was also positively responded in the doses of 5D15M, 10D5M and 10D15M and negatively responded in the doses of 5D5M, 5D10M and 10D5M (Fig. 2 B).

NOS:

PC1-PC2 reflected that NOS activity in spawn of *C. batrachus* responded positively in the doses of 10D10M and 10D15M and negatively in the doses of 5D5M, 5D10M, 5D15M and 10D5M (Fig. 2 C). In fry of *C. batrachus* it responded positively in the doses of 5D5M and 10D10M and negatively in the doses of 5D10M, 5D15M, 10D5M and 10D15M (Fig. 2 D).

Trypsin:

PC1-PC2 reflected that Trypsin activity in spawn of *C. batrachus* positively responded in the doses of 5D5M, 5D15M and 10D10M and negatively in the doses of 5D10M, 5D5M, 10D5M and 10D15M (Fig. 2 E). In case of fry of *C. batrachus* it responded positively in the doses of 5D15M, 5D5M, 10D10M and 10D15M and negatively in the doses of 5D10M and 10D5M (Fig. 2 F).

Discussion

In the present study, SOD activity for spawn and fry of *C. batrachus* after different doses and two exposure periods was decreased in respect to control and the maximum reduction of SOD activity was found in the highest dose, i.e., 10D15M. Decreased SOD activity leads to less defence properties and it finally leads to the oxidative damage, damage to DNA and generation of ROS, because superoxide dismutases are the main antioxidants of defence pathways in response to oxidative stress (Zhang *et al*., 2020). The significant reduction of SOD activity under the exposures of UV-B and solar radiation in fish larvae that acted as sole biomarker for reactive oxygen species which caused damage to lipids, protein and DNA was also explained (Kumar *et al*., 2019). Being an important endogenous antioxidant enzyme it acts as a component of first line of defence system against the reactive oxygen

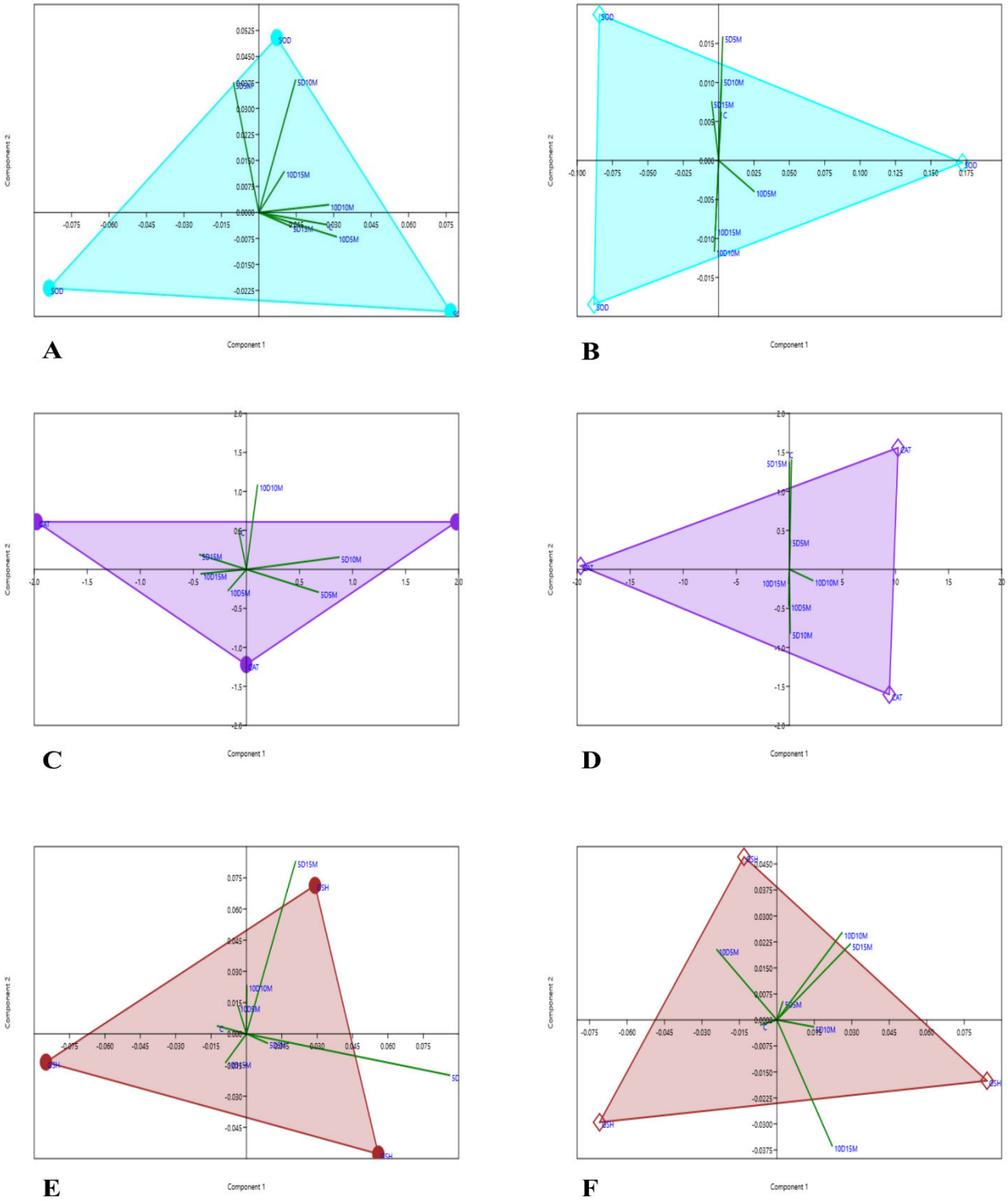

Fig. 1 (A-F): (A) Scatter plot showing relationship between PC 1 vs PC 2 in spawn of *C. batrachus* for the activity of SOD; (B) Scatter plot showing relationship between PC 1 vs PC 2 in fry of *C. batrachus* for the activity of SOD; (C) Scatter plot showing relationship between PC 1 vs PC 2 in spawn of *C. batrachus* for the activity of CAT; (D) Scatter plot showing relationship between PC 1 vs PC 2 in fry of *C. batrachus* for the activity of CAT; (E) Scatter plot showing relationship between PC 1 vs PC 2 in spawn of *C. batrachus* for the activity of GSH; (F) Scatter plot showing relationship between PC 1 vs PC 2 in fry of *C. batrachus* for the activity of GSH.

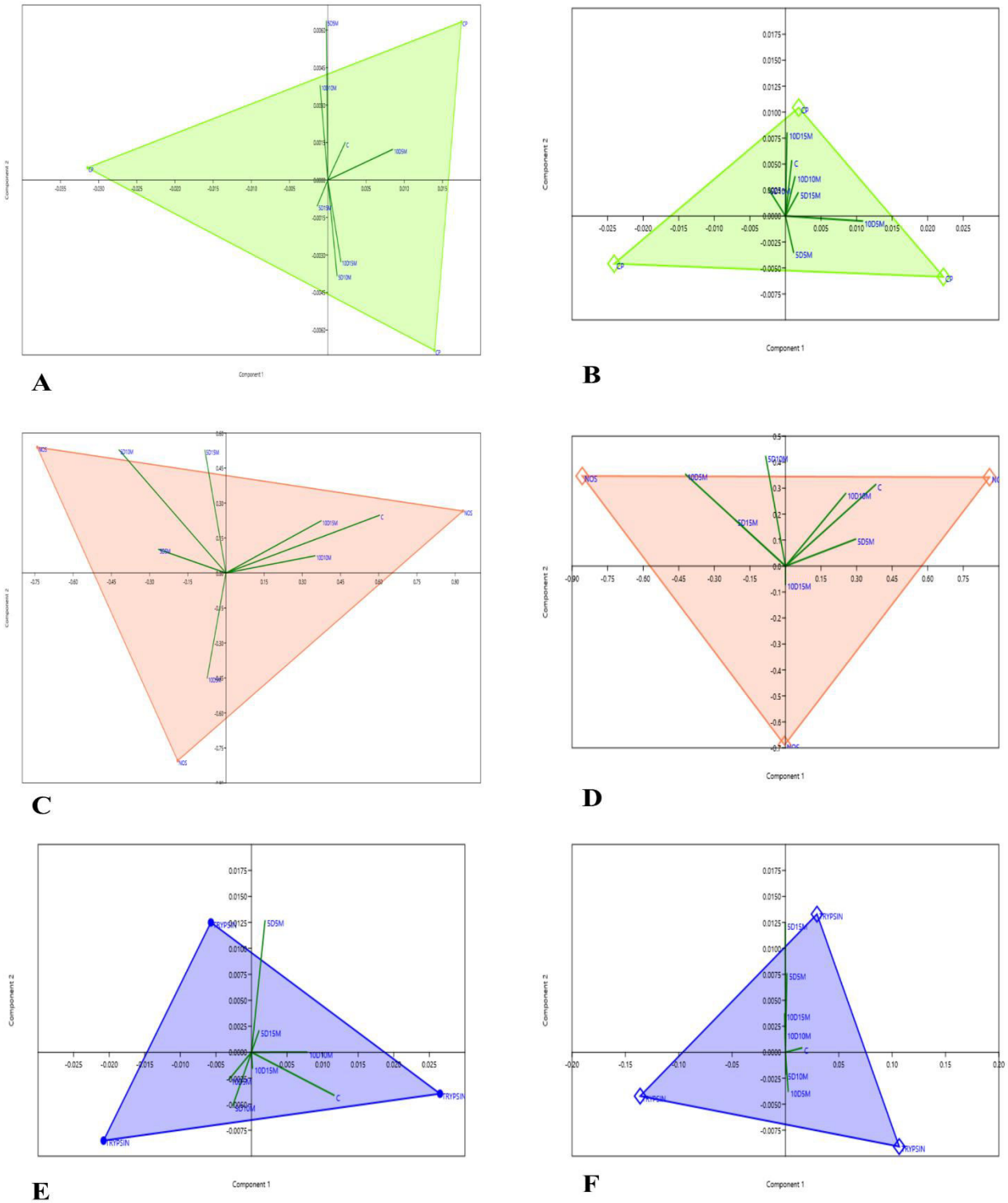

Fig. 2 (A-F): (A) Scatter plot showing relationship between PC 1 vs PC 2 in spawn of *C. batrachus* for the activity of CP; (B) Scatter plot showing relationship between PC 1 vs PC 2 in fry of *C. batrachus* for the activity of CP; (C) Scatter plot showing relationship between PC 1 vs PC 2 in spawn of *C. batrachus* for the activity of NOS; (D) Scatter plot showing relationship between PC 1 vs PC 2 in fry of *C. batrachus* for the activity of NOS; (E) Scatter plot showing relationship between PC 1 vs PC 2 in spawn of *C. batrachus* for the activity of Trypsin; (F) Scatter plot showing relationship between PC 1 vs PC 2 in fry of *C. batrachus* for the activity of Trypsin.

species (ROS) (Ighodaro and Akinloye, 2018). SOD activity was also significantly decreased under 254 nm UV radiation depending on the increasing time periods. It also disclosed a significant decrease on these antioxidant enzyme activities under 365 nm of UV radiation at 45 and 60 min of exposures time periods (Güven *et al*., 2015). The catalyse (CAT) activity of the spawn and fry of *C. batrachus* revealed decreased trend compared to the control, only at 10D5M the value increased slightly for both the spawn and fry, it may be possible that both of them intended to cope up with environmental condition. Similar results were found in another study, where catalase was decreased in case of fish fingerlings of *Tor tor* and *Schizothorax richardsonii* when treated with retene, solar UV radiation and artificial UV radiation (Kumar *et al*., 2019). Decreased CAT activity was also found in the different doses of 365 nm UV radiation in the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae treated with UV radiation (Güven *et al*., 2015). Similar results were also recorded under the exposure of sub-lethal concentration of sodium cyanide where CAT activity was decreased gradually suggesting the inhibitory effect of cyanides on the catalase system in the different tissues, namely, liver, gill, muscle and brain of the freshwater exotic carp, *Cyprinus carpio* (David *et al*., 2008). So, it can be explained that the decreased level of CAT activity is unable to utilize the oxygen to compete with the toxication process and catalytic degradation. GSH plays a major role in removal of many reactive species and it also modulates cell death (Forman *et al*., 2009). Due to ROS activity, GSH levels fall during apoptosis in many different cell types resulting into enhanced GSH efflux and decreased GCL activity (Lu, 2013). In the present study, GSH levels of spawn decreased and minimum value of GSH level was found in 10D15M dose. A study depicted the significantly decreased ($p< 0.05$) non-enzymatic antioxidant GSH in groups of African catfish, *Clarias gariepinus* exposed to ultraviolet-A radiation under the doses of 60 and 180 min per day (Ibrahim, 2015). In case of fry, the same pattern of reduction was estimated. Actually, this inhibition of the enzymatic activity may be due to the formation of an enzyme-inhibition complex, ion imbalance or the intracellular action of metal resulting into subsequent plasma membrane damage due to excess stress caused by UV radiation (Mekkawy *et al*., 2010). In this study, for both the spawn and fry, the GSH levels increased slightly in the dose of 10D5M, possibly they intended to convalesce from the UV-B radiation. It also indicated the vital role of GSH in detoxification of xenobiotics and regulation of cell proliferation. Several studies suggested that in many normal and malignant cell types increased the GSH level is associated with a proliferative response and is essential for cell cycle progression (Shaw and Chou, 1986; Hamilos *et al*., 1989; Pieri *et al*., 1995; Lu, 2013). Protein carbonylation is one type of protein oxidation that can be promoted by reactive oxygen species (Suzuki *et al*., 2010). In the foregoing study, the CP level increased in all the doses and periods of exposure than the control for both the spawn and fry of *C. batrachus.* Increased level of CP in this study was due to increase of ROS induced by ultraviolet radiation, because it is the oxidation product of protein caused by ROS and in this case reactive ketones and reactive aldehydes were formed and regulated by DNPH (2,4-dinitrophenylhydrazine) to form hydrazones (Stadtman and Levine, 2000; Stadtman, 2001; Levine, 2002; Moskovitz and Oien, 2010). Similar relationship was also observed between the dose of UV-B and the CP level, where CP level was significantly ($p< 0.05$) higher in the larvae of *Catla catla* exposed at the dose 1512 (15 min) mJ/cm^2 UV-B compared to the control group (Singh *et al*., 2015). A significant increase in protein carbonyls was also observed in the organs, *viz*., liver, kidney and gill in fish (*Channa punctata*) exposed to different toxic compounds (Parvez and Raisuddin, 2005). Nitric oxide synthase activity regulates the production of cell signalling molecule like nitric oxide (NO) that involves in the regulation of vital

functions. In the present study, NOS level was significantly reduced at different doses of UV-B radiation on spawn and fry of *C. batrachus* compared to control. Similar observation was reported in the larvae of *Catla catla* exposed to different doses of UV-B radiation, where nitric oxide synthase (NOS) level was reduced compared to control (Singh *et al*., 2015). NOS level of rohu larvae was also affected by UV-B radiation (Singh *et al*., 2013). Trypsin is the key enzyme for digestion of protein and it influences the protein and amino acid utilisation, and thus, it can be linked with the growth as studied in Atlantic salmon (*Salmo salar* L.) (Rungruangsak-Torrissen *et al*., 2006). In the present study, trypsin activity of fry of *C. batrachus* was more than spawn, but during UV-B exposure it revealed gradual reduction in both the cases showing maximum reduction at 5D15M and 10D15M conditions. The requirement of trypsin for protein hydrolysis for fry is more than spawn, therefore, secretion of trypsinogen from pancreas under the activation was less; but in the subsequent fry stage that requirement enhanced as evidenced from the results in control condition. During stress, under UVB radiation both the spawn and fry showed gradual decrease, with maximum in final exposure periods. Therefore, these analyses have drawn a correlation between lower SGR, WG value and higher reduced trypsin levels in comparatively older ages. It was also observed that an increment in irradiation time, caused a smaller formation of hippuric acid, and therefore, a reduction in the trypsin activity (Ibarz *et al*., 2009), which was in consonance with our present study.

Conclusion

From the present consequential discourse it can be inferred that UV-B radiation caused different oxidative damages, and the significant alterations in the activity of SOD, CAT, GSH, NOS and trypsin which became indicative to be established as biomarkers. Protein carbonylation prompted a very significant positive result in reference to dose and exposure. In both the spawn and fry, the SGR (%) and WG (%) indicated a significant reduced condition, which can be explained due to their lowering capacity of food intake and digestion which was also evidenced from reduced activity of trypsin. Moreover, under the specific doses like 10D5M and 10D10M, the enzymatic study exhibited a positive increment, because fish physiology induced to recover itself from the external stress, compared to the other doses. PCA study also reflects that how different enzymological parameters positively and negatively responded to the different doses. This harmful radiation causes deterioration in fish health, so, UV-B radiation now becomes a major challenge to the aquaculture industry, especially, with respect to climate change. Development of proper monitoring strategy can only help the fish farmers from the morbidity of fish spawn and fry, and finally their sustenance required for sustainable pisciculture.

Acknowledgements

The authors are thankful to Chandimata Fish Farm, Khano, Purba Bardhaman, WB, India for rendering constant support and help in our research work at field level. We are also obliged to DST-FIST sponsored Dept. of Environmental Science, The University of Burdwan, for providing laboratory facilities for performing the analytical part of the research work. 1st author is thankful to Ms. Samarpita Chakraborty for her constant support.

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