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Effect of UV-B radiations on mortality and enzymes level in fish larvae of *Tor tor* and *Schizothorax richardsonii* on laboratory scale

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Abstract
Depletion of stratospheric ozone layer is resulting into increase in solar UV-B on earth surface. Ultra violet radiation is well known to cause many detrimental effects in aquatic organisms. The present study was performed to study the effect of solar ultraviolet radiation on fish fingerlings of *Tor tor* and *Schizothorax richardsonii* as a model system on laboratory scale. The effect of different intensities of natural solar and artificial UV-B radiation on fish larvae of *T. tor* and *S. richardsonii* in a presence of retene was investigated. Solar ultraviolet intensity showed seasonal and altitudinal variations in Garhwal region. Solar UV radiation level was lower (0.390 mw/cm^2) in the month of January- February at lower altitude and highest (1.192 mw/cm^2) in the month of July-August 2018 at higher altitude. Fish larvae exposed to artificial UV-B (average wavelength 312 nm and intensity of 750 mw/cm^2) with retene (50µg/l) showed increase in gills malandialdehyde level and caused larvae mortality as indicating that enhanced solar UV-B exposure could be lethal to fish fauna in aquatic ecosystem. Artificial UV-B had a stronger damaging effect on fish larvae than solar radiation exhibited highly toxic in presence of retene. The larvae of *S. richardsonii* was found more sensitive than *T. tor* as indicated by high mortality rate (30%) and high pigmented characteristics on dorsal side. The solar and ultraviolet radiation showed a positive effect on high pigmentation. These results suggest that on a short time scale, UV-B radiation causing developmental stress on fish larvae may contribute to assess the phototoxic behaviour of cold water fishes.

Keywords: Fish larvae, Fish mortality, UV-B radiation, *Tor tor, Schizothorax richardsonii*

INTRODUCTION

Stratospheric ozone depletion, e.g., the ozone “hole”, with the concomitant increase of the most energetic and potentially damaging daylight component UV-B, 280-315 nm (Madronich,1993), is a cause of great concern under global climate change. Intensity of solar UV-B radiation and the depth of UV penetration in to the water column are key factors in assessing the ecosystem potential for damage to aquatic organisms (Blautein *et al.,* 2002). Consequently, many studies carried out during the last decades have focused on determining solar ultraviolet (UVR- 280-400nm) fluxes reaching the Earth’s surface (Frederick *et al*., 1994), and effects upon various organisms were observed (Figueroa *et al*., 1996 and De Mora *et al*., 2000). In addition to the enhanced solar UV-B radiation caused by the stratospheric ozone depletion, it has now been recognized that normal solar UVR can also cause ecological stresses on aquatic organisms because they are very sensitive to ambient levels of UVR.

Living organisms of different communities vary in their responses towards ultraviolet radiation which largely depend on dose and duration of exposure of UVR and protective mechanism of the organism being exposed. Exposure of fish larvae towards UV radiation impacted negatively on their survival ratio and ultimately effect to ecosystem productivity in aquatic ecosystem. U-B radiation may impose considerable physiological stress and pathological effect resulting in a number of manifestations such as reduced growth, photoallergy, phototoxicity, photoaggravated skin diseases, increased rates of mortality, inhibition of growth rates and changes mortality (Sommaruga *et al*., 1996). Impaired reproduction, predisposition to diseases, reduced locomotory and predatory performances, or reduced capacity to tolerate subsequent stress (Scott *et al.,* 2004 and Arts *et al.,* 2010).Since many of the physiological conditions get affected by some very important factors(water temperature, diet, dissolved oxygen, pH, total hardness etc.) of aquatic ecosystem. UV-B radiation has severe effects on early life intervals of fishes such as lesions in the brain and retina, and reduced growth rate (Markkula *et al.,* 2009). On the other hand, fish have physiological, morphological and behavioural mechanisms of photoprotection, such as screening pigments (mycosporine -like amino acids and melanin), photorepairing (photolyase), excision repair, and avoidance behaviour (Speekmann *et al.,* 2000). In fish species, melanin pigmentation seems to provide an efficient photoprotection mechanism (Lin and Fisher, 2007). Exposure of UV- B radiation with retene and riboflavin cause increase in gill malandialdehyde and decrease in glutathione content. Artificial UV-B had a stronger damaging effect than solar radiation and became highly toxic in presence of retene (Kumar *et al*., 2011). Dargaei *et al.,* (2014) studied UV-B impacts on morphology and retina of *Oncorhynchus mykiss* larvae and concluded that the harmful effect of ultraviolet radiation on aquatic animals, due to ozone depletion a wide variety of body abnormalities and eye damages, none of the malformations were observed in control group. Several studies have reported that UV radiation reduces survival of fish larvae. UV-B radiation may impose considerable damaging of stem cell DNA, contribute to depletion of stem cells (ESCs and mesenchymal stem cells) and damage of stem cell niche, eventually leading to photoinduced skin aging (Uraiwan., *et al.,* 2015).

Because of lack in systematic and scanty scientific knowledge of phototoxicity on fish larvae and due to enhance of solar ultraviolet radiation in Himalayan region, an attempt has been made to assess the adverse effects of UV-B on metabolism and mortality rate of selected fish larvae of species, *T. tor* and *S. richardsonii* in laboratory condition.

MATERIALS AND METHODS

Uttarakhand Himalayan region situated between latitude from 28ºC 21’ to 30º 21’ North Latitude and 78º 30’ to 80º 30’.Different altitudinal sites were selected i.e. Haridwar (314 msl), Dehradun (450 msl), Tehri (1850 msl), Mussoorie (2100 msl) and Chamoli (3000 msl) for monitoring of natural solar radiation and measured every month by using Cole-parmer radiometer (U.S.A) having Vilber Laurmat France calibrated UV sensors with spectral sensitivity 312nm was performed in a year 2017-18 between 11:00am - 2:00pm for 3 hours on clear sunny days to compare the altitudinal differences in natural solar UV-B radiation.

Fish larvae (fingerlings) of *T. Tor* and *S. richardsonii* (weight range between 1.90 to1.99 gm and size range between 30 to 40 mm) were collected from Bhagirathi river, Uttarakhand (30º 22’ 54” N latitude and 78º 29’ 3” E longitude)) using the cast net (Figs. 3 A and B). The fish larvae were initially acclimatized in aquaria on supplementary feeds for two week and then shifted in six experimental aquariums, made up of glass having size dimensions (60cm X 45cm X 38cm). The experimental aquaria were fitted with vertical moving wooden sheet, which contained plastic mesh containing 120 fingerlings uplifted towards upper water surface on 10 cm to get maximum exposure of solar and UV radiation. The fish larvae of *Tor* and *Schizothorax* fishes were kept on wooden hatching trays at 10cm depth from water surface of aquaria (Fig. 1 and 2). The water temperature in the experimental aquaria was maintained between 16.2 to 17.5 ^{0}C. Fish fingerlings were well treated to avoid any signs of infection before the experiment. On the basis of experimental design, fish larvae were separated in to six groups 20 larva in each. Group I was kept as control. Group II was exposed to retene, Group III was given natural solar radiation between 11.00 am to 2.00 pm for three hours per day for 30 days during clear weather. Group IV exposed to artificial UV-B and group V exposed to solar radiation and retene. Group VI exposed to artificial UV-B and retene. Concentration of retene (50ug/l) was used as photo sensitizer in experimental aquarium. Artificial ultraviolet- B radiation was given by Philips UV-B lamp with average wavelength 312 nm for the same duration. Solar UV-B was measured by Cole -Parmer radiometer having Vilber Laurmat France calibrated UV-B sensor. Irradiation was carried out in the sunlight and for enhanced ultraviolet radiations; artificial UV-B lamp was used for scheduled duration (3hrs/day). Different intensity of solar and artificial ultraviolet-B radiation was used in the experiments. Horizontally placed 3ft long UV tubes were used for irradiation. Irradiation of the emitted light was measured by Cole Parmer Radiometer. After exposure of UV and solar radiation to fish larvae on 10 cm at water surface, the sieve reverted down to free movement of larvae in aquarium for rest of time. Artificial feed was given to fish larvae in all aquariums twice in a day as per 5% of body weight. Aerator was used for maintaining the quality parameters of water and related physico-chemical parameters were recorded at regular intervals using multi-parameter auto-analyser and colorimetric method. Mortality rate of *T. tor* and *S. richardsonii* larvae were recorded on daily basis. For mortality analysis, lipid peroxidation was assayed by measuring of malondialdehyde level in fish fingerlings by using thiobarbiturc acid (TBA) through the method (Smith and Anderson, 1987). The absorbance was recorded using

UV/ visible spectrophotometer at 532 nm. 1, 1, 3 tetramethoxy propane (Wako, Japan) was used as the standard. Catalase (E.C. 1.1.11.6) H_2O_2- H_2O_2 oxidoreductase enzyme activity in the fish tissue was determined by breakdown of hydrogen peroxide using titration method (Takahara *et al.,* 1960).Superoxide dismutase (SOD) activity was analysed as larval body analysis as described by (Hakkinen *et al.,*2004). The SOD activity was measured by the method of (Ukeda *et al.,* 1999).

RESULTS AND DISCUSSION

The amount of UV-B reaching the surface of earth depends on the thickness of the ozone layer and atmosphere. The variation of UV radiation reaching the earth surface has experimental evidence. It depends upon altitude, atmospheric condition and types of instrument used for measurement (Singh *et al*., 2012). Erythemal weighed UVR are reported to increase with altitude at an approximate rate of 5 to7% per kilometer and showed greatest increase occurring at solar zenith angle (SZA) ~ 60-70º (Zaratti *et al*., 2003 and Mckenzi *et* al., 2007). The present study observed that natural solar radiation was lowest in January-February and maximum in a month of July – August and there was about 4.5 to 6.8 **%** altitudinal increment per kilometer in Garhwal region of Uttarakhand. The results showed that intensity of solar ultraviolet radiation increases with increase in altitude based on seasonal variations (Fig. 4).

Fishes, unlike other surface dwelling organisms, avoid the harmful effect of Ultraviolet radiation by swimming to the deeper portion of the water body. However the effect of UV radiation may be more harmful on developmental and larval stages of fish species in natural aquatic ecosystem. Fish larvae are also affected by different environmental factors such as temperature, pH, light and environmental pollutants (Buzzi, 2002). The water quality

Table 1. Water quality parameters during experiments of fish larvae.

S.N.	Parameters	Range
1.	Temperature (ºC)	16.2-17.5
2.	pH	7.6-7.8
3.	TDS (mg/l)	120-140
4.	Conductivity (µS/cm)	125.8-152.5
5.	Total Hardness (mg/l)	89.2-102.3
6.	DO (mg/l)	9.1-10.14

Table 2. Chemical composition of supplementary fish feed.

Nutrients	Percentage
Protein	40.6%
Fat	6.6%
Starch	14.2%
Fiber	21.1%
Sugar	1%
Mineral/ash	12.4%
Moisture	4.7%

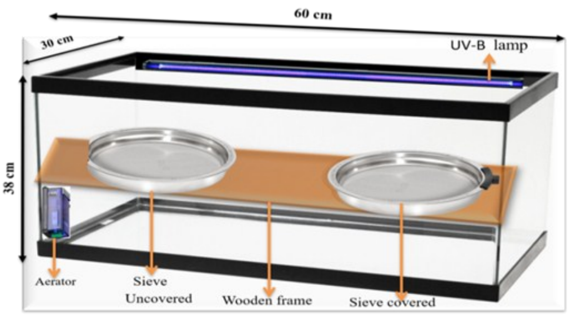

Fig. 1. *UV- B exposed and UV-B filtered experimental tank.*

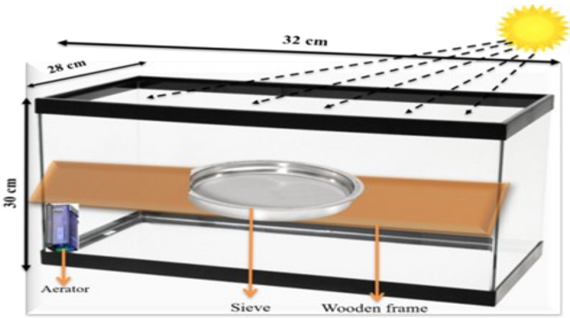

Fig. 2. *Natural solar UV exposed experimental tank.*

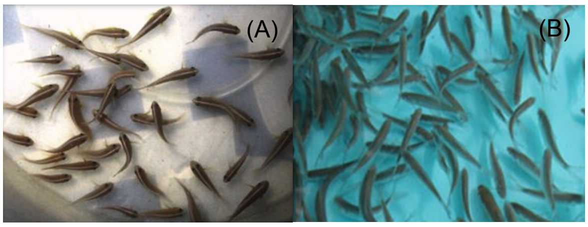

Fig. 3 (A-B). *Fingerlings : (A). T. tor (B). S. richardsonii.*

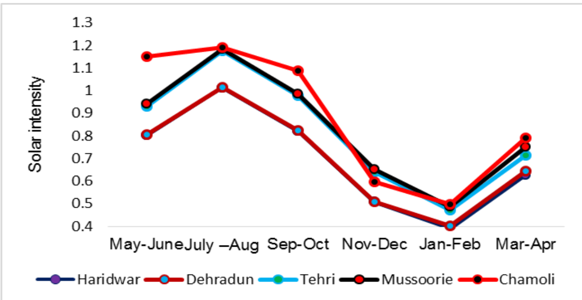

Fig. 4. *Seasonal and altitudinal variations of solar UV-B in Uttarakhand region.*

parameters in all fish experimental aquariums were maintained as per standard of cultural practices (Table 1). The present range of water temperature and dissolved oxygen contributed as optimum aquatic condition for the survival during the experiment. UV-B also participates in indirect damage to macromolecules, provokes free radical production and induces a significant decrease in antioxidents in biotic organisms (David and Davies, 2006). The hill stream fish species as *T. tor* and *S. richardsonii*, mostly preferred the high protein feed, hence composited supplementary feed of about 40%, 21% and 12.6 % of protein, crude fiber and

Table 3. Effect of solar and artificial UV radiation on mortality rate (%) in fish fingerlings of *T. tor* and *S. richardsonii.*

Group	Experiments	*T. tor*	*S. richardsonii*
Group I	Control	5±0.6	6±0.4
Group II	Retene	6.8±0.5	8±0.6
Group III	Solar radiation	8.0±0.4	10±0.7
Group IV	Artificial UV-B	12.0±0.6	16±0.6
Group V	Solar + Retene	18±0.8	23±0.5
Group VI	Artificial UV-B + Retene	24±0.2	30±0.6

Table 4. Effect of solar and artificial UV radiation on Lipid peroxidation (Malondialdehyde Nano Mole/Mg Protein) in fish fingerlings of *T. tor* and *S. richardsonii.*

Group	Experiments	*T. tor*	*S. richardsonii*
Group I	Control	7.18±0.18	7.56±0.28
Group II	Retene	8.35±0.50^{NS}	7.98±0.42^{NS}
Group III	Solar radiation	8.47±0.30	8.15±0.55^{NS}
Group IV	Artificial UV-B	8.60±0.58	8.22±0.40
Group V	Solar + Retene	9.10±0.65	8.50±0.62
Group VI	Artificial UV-B + Retene	9.37±0.82	8.90±0.40

Table 5. Effect of solar and artificial UV radiation on Superoxide dismutase (SOD) (unit/mg Protein) in fish fingerlings of *T. tor* and *S. richardsonii.*

Group	Experiments	*T. tor*	*S. richardsonii*
Group I	Control	0.65±0.05	0.58±0.04
Group II	Retene	0.60±0.04	0.55±0.02
Group III	Solar radiation	0.52±0.08	0.48±0.06
Group IV	Artificial UV-B	0.48±0.05	0.43±0.04
Group V	Solar + Retene	0.44±0.06	0.40±0.05
Group VI	Artificial UV-B + Retene	0.45±0.05	0.42±0.07

Table 6. Effect of solar and artificial UV radiation on Catalase in fish fingerlings of *T tor* and *S. richardsonii* with Retene.

Group	Experiments	*T. tor*	*S. richardsonii*
Group I	Control	82.6±1.52	78.5±1.64
Group II	Retene	74.5±1.80	74.3±1.76
Group III	Solar radiation	72.8±1.25	71.8±1.38
Group IV	Artificial UV-B	70.1±1.30	68.2±1.50
Group V	Solar + Retene	67.7±1.75	65.5±1.25
Group VI	Artificial UV-B + Retene	62.9±1.85	60.1±1.70

vitamin mineral respectively were used as feeding requirement during experiments (Table 2).

Result on mortality rate on fish within 30 days with 3 hour of exposure to solar(0.428mw/cm^2), artificial (750 mw/cm^2), UV-B and retene (50μg/l) indicated highest mortality in *S. richardsonii* (30%) as compared to *T. tor* (24%) with UV-B + retene (Table 3). This may be due to the fact that *Tor tor* being a bottom feeder species generally prefers to live in bottom which may avoid the direct exposure of harmful artificial UV radiation due to photoprotection changes in skin. While, *S. richardsonii* prefers to live in open water thus there are more chances to get UV exposure. Artificial UV-B + retene was found more toxic than natural solar + retene. Gills malondialdehyde level, a product of lipidperoxidation and cell injury indicated an increase in lipid peroxidation level in comparison to control. Non significant change was observed after exposure of individual retene and natural solar UV. An increase was observed after exposure to solar UV and artificial UV-B radiation in the presence of retene (Table 4). Antioxidative enzyme catalase indicated decrease in antioxidant potential, which decreased in all the groups in comparison to control. In the laboratory experiment UV-B induced reactive oxygen species, indicating as higher superoxide dismutase (SOD) activities in control as while UV – B and solar radiation contributed significantly to decrease the SOD units in fish larvae and acted as sole biomarker for reactive oxygen species that damaged to lipids, protein and DNA (Table 5). Significant decrease in catalase level (0.05) was found after artificial UV-B and retene. Glucose-6-Phosphatase activity decreased in all groups in comparison to control. Maximum reduction was observed after treatments with retene and artificial UV-B (Table 6). The larvae of *S. richardsonii* were found more sensitive than *T. tor* as indicated by high mortality rate and other pigmented characteristics observed on dorsal side. Hakkinen *et al*. (2002) and Bhandari and Sharma (2010) observed that due to less protection mechanism at early larval stage, sensitivity of *Coregonid* fish and *Phormidium corium* larvae to UV radiation was higher than other growing stages.

The studies on the effect of UV radiation have indicated that UVR may influence the respiratory systems in fishes mentioned by (Freitag *et al.,* 1998). It was also reported to induce pigmentation in white fish, *Coregonus albula*, larvae (Hakkinen *et al.,*2004). Alemanni *et al.,* (2003) also demonstrated an increase in oxygen consumption rate and restless behavior of juvenile rainbow trout (*Oncorhynchus mykiss)* due to the effect of UV radiations. Sharma *et al*., (2008) showed that fish survivorship model based on the exposure of UV-B radiation affects the survival rate of surface feeder fish, *Catla catla* larvae. UV radiations along with other ecological stressors are also harmful for fish species in aquatic ecosystem. Kumar *et al.,* (2011) observed that photo toxicity of naturally occurring retene affected the growth, swimming and behavioral pattern in hill stream fish species, *Barilius* and *Nemacheilus*.

The ultraviolet light had a positive effect on pigmentation, which also affects the coloration of arthropods and fish species (Kumar *et al.,* 2015). The UV light stimulates the chromate phores to produce more pigment on dorsal side of fish species in comparison of sun light. (Fukunish *et al.,* 2011) and (Laura, 2011) found significant reduction in growth, mortality, damages of corneal epithelium, skin lesion and other physiological functions in larval stages of sparid fishes and whale due to direct exposures of UV radiation. The present study also observed that solar and UV –B radiation on fish larvae of *T. tor* and *S. richardsonii* caused several detrimental effects as fin blistering, eyes bulging, body curving, loss of buoyancy and dark patches (pigmentation on dorsal side).

Conclusion

The present study on monitoring of solar UV radiation showed seasonal and altitudinal variations in Himalayan region of Uttarakhand. Solar ultraviolet radiation was found maximum in summer months of July and August (1.192 mw/cm^2) at higher altitude during the year 2017-18. Artificial UV-B had a stronger damaging effect than solar radiation and became highly toxic in a presence retene to fingerlings of *T. tor* and *S. richardsonii* fish species. Natural solar radiation contained combination of wavelength thus may have protective interaction than pure artificial UV radiation. The fingerlings of fish, *S. richardsonii* were found more sensitive than *T. tor* may be due to food habits, habitats and genetic variations. Hill stream fish fingerlings may be used as a model for aquatic phototoxicological studies as predicted ecosystem productivity modelling of aquatic ecosystem.

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