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IMPACT OF TEMPERATURE CHANGE AND DISSOLVED ORGANIC CARBON ON PHOTOTOXICITY OF PHOTOSENSITIZER ON *CYCLOPS* IN DOON VALLEY

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Abstract

Ultraviolet (UV-B) radiation wave length (280-315 nm) has increased because of stratospheric ozone depletion. Among the harmful effects of UV-B radiation, photoinduced toxicity of environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs), has been documented. Phototoxicity resulting either in the production of reactive oxygen or modification of the PAH to new chemical species. Active oxygen is biologically damaging, and the photomodification products are often more toxic than parent compound. In natural waters, retene (7-isopropyl-1-methyl-phenanthrene) is mainly formed anaerobically from resin acids, oleoresinous constituents in coniferous trees, water contaminated by treated pulp and paper mill effluents. UV radiation penetration in aquatic habitats is modulated by factors as dissolved organic carbon, suspended particles, phytoplankton and reflection. Certain chemicals as retene become phototoxic in presence of ultraviolet radiation. Natural photosensitizers are present in many organisms as bacteria, protozoa, plants and animals.

Thus a study has been performed to investigate the adverse effect of UV-B on crustacean species due to climatic condition. *Cyclops* were collected and cultured in laboratory. Solar and artificial UV radiation was given at two different temperatures to *Cyclops* in presence of retene photosensitizer. Mortality, morphological, behavioral and physiological changes were recorded in presence of dissolved organic carbon also. Zooplanktons are negatively affected when exposed to high intensities of UV radiation, DOC reduces the oxidative stress caused by photosensitizers and decrease the mortality in *Cyclops* thus protect the aquatic organisms from solar UV induced harmful radiation. It is observed that with the increase in temperature the body size of *Cyclops* reduces. *Cyclops* may avoid exposure to high levels of UV-B by staying in deep water layers or by undergoing diel vertical migration, behavioral avoidance, and by producing photoprotective compounds. Results indicate that climate change alter response to UV through temperature mediated effect on aquatic ecosystem and biodiversity.

INTRODUCTION:

Ultraviolet radiation is the most photochemically reactive wavelength of solar energy reaching the surface of the earth, and has a broad range of effects on aquatic biogeochemistry, biota, and ecosystems. As a result of anthropogenic impacts on the atmosphere of the earth, UV radiation exposure in arctic environments is changing substantially. Climate change is very likely to be accompanied by shifts in biological UV radiation exposure in Arctic river, lake, and wetland environments via three mechanisms (Vincent et al., 2003): changes in stratospheric ozone levels, changes in snow and ice-cover duration, and changes in the colored materials dissolved in natural waters that act as sunscreens against UV radiation. Anthropogenic emissions of ozone depleting substances have declined since the ratification of the Montreal Protocol and its amendments, future levels of ozone and UV radiation in the Arctic are uncertain, depending not only on continued compliance with the Protocol and changes in legislation, but also on climate change effects on temperatures and trace gases (Callaghan et al., 2004; Wrona et al., 2006). To understand the overall impact of changes in UV radiation levels, the synergistic and antagonistic processes resulting from climate change have to be considered since they have the potential to modify the underwater UV radiation regime and consequently the stress on aquatic organism.

Many factors are known to control zooplankton and *Cyclops* community structure in lakes including temperature, food limitation, predation and as well as seasonal variation in these factors. One mechanism that may contribute to the relationship between DOC and zooplankton community structure is the ability of DOC to regulate the level of potentially damaging ultraviolet radiation (UVR). DOC is the primary regulator of variation in ultraviolet radiation (UVR) among freshwater lakes of glacial origin (Laurion et al., 1997). Zooplankton have adapted to these environmental conditions by evolving several defense strategies against UVR. Example- late Copepodid and adult life stages of copepod avoid the upper meters of the water column during the day (Tartarotti et al., 1999). During the last decade, the focus has shifted from individual species to ecosystem responses and changes in food webs and trophic cascades (Vinebrooke and Leavitt, 1998). Organisms are adapted to their habitat and cope with UVR differently which results in great species-specific variability in UVR protection (Leech and Williamson, 2001). UV radiation is likely to cause a variety of direct and indirect negative effects on zooplankton populations. The majority of studies concerning direct UV induced damage on zooplankton have reported increased mortality rates (Zagarese et al., 2003). Adaptations and abilities of species to respond to UVR have been challenged with increase in UVR that are beyond the natural range in the animal’s habitat (ACIA, 2005). These increases have occurred mainly at high altitudes where the depletion of ozone in the stratosphere has caused great intensities of UVR to reach the ground level. The darkest lakes can be considered well-protected from UVR, however UV-irradiated DOC may promote the formation of reactive oxygen species (ROS) that in turn are harmful and toxic to organisms including zooplankton (Souza et al., 2007). Many lakes, especially northern lakes that have had a stable low DOC concentration and hence have been exposed to UVR in the same manner for decades and even centuries, are now changing in their optical properties due to climate change- induced melting of permafrost soils and the subsequent release of DOC into the downstream receiving waters. Structural drivers such as temperature and food availability show little or no change on a daily basis but serve to create vertical habitat gradients in the water column. Dynamic drivers namely ultraviolet radiation and visual predation pressure show strong changes over a 24 hr period and provide the proximate cues that drive diel changes in zooplankton vertical position but may also have ultimate adaptive significance (Hylander et al., 2010). Studies have demonstrated that *Daphnia* are negatively phototoxic to UV and show downward swimming behavior upon exposure to UV radiation (Milla and Barbara, 2010). The genus *Daphnia* and *Cyclops* are ecologically important and ubiquitous freshwater zooplankton genus that provides important trophic linkagers exert control over water quality and serves as a model organism for ecosystem, genomic and eco-evolutionary processes (Tiberty et al., 2013, Hylander et al., 2014). Thus a study has been performed to investigate the effect of dissolved organic carbon and temperature in presence of UV B-radiation and retene on *Cyclops*.

STUDY AREA AND METHODOLOGY:

Doon valley situated between 29°-30' to 31°-28' North latitude and 78°-03' to 79°-32' East longitude was selected for the present study. Zooplanktons were collected from Doon valley and after identification of *Cyclops* species they were cultured in the laboratory (Songlake and Tisher 2001). *Cyclops bicaspidatus* species was selected for the present study due to high abundance. *Cyclops* were divided into 8 groups with three replicates and experimental protocols were designed. The first experimental setup was provided with normal temperature 25 ± 2°C. Group one was control, group two exposed to retene, group three was given natural solar radiation, , group four exposed to artificial UV-B, group five exposed to artificial high intensity of UV-B, group six exposed to solar radiation and retene, group seven exposed to artificial UV-B and retene and group eight was exposed to artificial high UV-B and retene. Second experimental setup having eight groups was prepared and was maintained at high temperature 40± 2° C and a third experiment was set up with normal temperature in the presence of dissolved organic carbon (DOC).

Solar radiation was given between 11:00 a.m. to 2:00 p.m., for two hours per day. Solar radiation of average intensity of 0.780 mw/cm^2 during experiment period in the month of October was used. Artificial ultraviolet-B with same intensity and high intensity of 1.20 mw/cm^2 was given by Philips UV-B lamps. UV-B radiation was measured using Coleparmer radiometer having Vilber Laurmet France calibrated sensor with spectral sensitivity 312 nm. Retene (7-isopropyl-1-methyl-phenanthrene) and Sigma Aldrich humic acid, a coal based commercial source of DOC was used in the experiment. Mortality, movement, morphological, behavioral and physiological changes were recorded. Statistical inferences were drawn by using Students 't' test (Fisher, 1963).

RESULT AND DISCUSSION:

Slight change in behavior, movement, morphology and pigmentation was observed which increased with increase in UV-B dose and intensity. Study shows that the size of *Cyclops* decreases with the increase in temperature. The responses of *Cyclops* depend on the various other factors like transparency of the reservoir, concentration of dissolved organic carbon (DOC). In the experimental setup with high and normal temperature, highest mortality was observed in group eight with artificial UV-B high intensity followed by low dose and intensity of artificial UV-B and retene and lowest in group one i.e. control. Mortality in *Cyclops* was low in presence of DOC. DOC possibly reduce the penetration of UV radiation in water and protect the *Cyclops*.

Artificial UV radiation is more toxic than natural solar radiation. Intensity and dose dependent change in mortality of *Cyclops* was observed (Table1-3, Fig.-1). UV may interact with other dynamic drivers such as predation pressure to produce pattern in migration that vary seasonally and independently of UV exposure level. Photoenhanced toxicity may occur through two processes, photomodification and photosensitization. Photomodification is the structural modification of chemicals in water to more toxic/ reactive compounds. In photosensitization, the bioaccumulated chemical transfer light energy to other molecules causing cells damage.

Cyclops occurs circumpolar and plays an important role in food web. Results are supported by studies on amphibian larva and *Pheretima* (Formicki et al., 2003, Kumar et al., 2010). Thus results revealed high mortality at low DOC concentration under condition of exposure to UV radiation and were similar to those obtained for cladocerans of the genus *Daphnia* in northern hemisphere (Williamson et al., 2001). On the basis of experimental and field works, zooplankton species of large and deep lakes with fish populations are vulnerable to exposure to UV radiation. In aquatic system the negative effect of UVR is minimized by dissolved organic carbon, which is known to attenuate UVR across water column.

Table 1 Mortality rate in *Cyclops* in the presence of retene photosensitizer at 25°C temperature with different intensities of UV-B radiation.

GROUP	TREATMENT	MORTALITY	
		4th DAY	6th DAY
1.	Control	4 ± 0.18 NS	5 ± 0.31 NS
2.	Retene	4 ± 0.4 NS	6 ± 0.33 NS
3.	Solar radiation	6 ± 0.5 NS	6.5± 1.4 NS
4.	Artificial UV-B	6 ± 0.8 NS	7± 1.06 NS
5.	Artificial UV-B High	9 ± 0.42 *	12 ± 0.72*
6.	Solar+ retene	18 ± 1.08 *	20 ± 1.05*
7.	UV-B L + retene	20 ± 1.4*	24 ± 0.9*
8.	UV-B H + retene	23 ± 1.2*	25 ± 1.6*

Results are mean ± S.E. of 5 observations in each group, P value *0.05, NS not significant.

Table 2 Mortality rate in *Cyclops* in presence of retene photosensitizer at high temperature 40°C with different intensities of UV-B radiation.

GROUP	TREATM ENT	MORTALITY	
		4th DAY	6thDAY
1.	Control	4 ± 0.18 NS	5 ± 0.31NS
2.	Retene	4 ± 0.41 NS	5 ± 0.03NS
3.	Solar radiation	6 ± 0.5 NS	7 ± 0.45 NS
4.	Artificial UV-B	7 ± 0.8 NS	8± 0.86 NS
5.	Artificial UV-B high	10 ± 0.92*	14± 0.71*
6.	Solar + retene	20 ± 1.08 *	24 ± 1.05*
7.	UV-B L + retene	22 ± 1.4*	25 ± 0.9*
8.	UV-B H + retene	24 ± 1.2*	28 ± 1.1*

Results are mean ± S.E. of 5 observations in each group, P value *0.05, NS not significant

DOC reduces the penetration of UV-B in water and decreases the mortality in *Cyclops bicuspidatus*, thus protect the aquatic organisms from solar UV induced harmful effect and indirectly affect biodiversity and productivity of ecosystems. The results of this study are also supported by the experiment that exposed *Cyclops bicuspidatus* individuals to UV radiation and visible light under controlled conditions; here the copepods were not affected significantly by exposition to UV radiation (De los Rios, 2005). Similar evidence for photorepair effect in the presence of light was obtained for high UV radiation tolerant species of shallow Patagonian water bodies. Vertical migration is a well known behavioural response induced by *Cyclops*, perhaps more often than UVR. Moreover many photoprotective strategies may also be complimentary and hence cannot be detected in all UVR situations. Lack of vertical migration in copepods in the absence of visually hunting predators may, for e.g. be explained by their high concentration of MMAs (Mycosporine like amino acids) that allows an efficient enough shield from UVR for the copepods to stay in surface water. Zooplankton may also use a broadband strategy to cope with

UVR. Species that accumulate multiple photoprotective pigments, may have a lower concentration of a certain pigment for a given irradiance than its co-specimen that uses a single protective strategy. The spectral sensitivity of phytoplankton communities to ultraviolet radiation- induced photoinhibition differs among clear and humid temperate lakes (Harrison et al., 2011).

Table 3 Mortality rate in *Cyclops* in the presence of retene photosensitizer and DOC with artificial UV-B radiation:

GROUP	TREATMENT	MORTALITY 4th Day	MORTALITY 6th Day
1	Control	2 ± 0.18 ^{NS}	6 ± 0.31 ^{NS}
2.	Retene	4 ± 0.41^{NS}	5± 0.03 ^{NS}
3.	Solar radiation	4 ± 0.5^{NS}	6.5 ± 1.4^{NS}
4.	Artificial UV-B	5 ± 0.8 ^{NS}	7 ± 1.06 ^{NS}
5.	Artificial UV-B high	8 ± 0.42 *	12 ± 0.72*
6.	Solar + retene	12 ± 1.08 *	18 ± 1.05*
7.	UV-B L + retene	16 ± 1.4*	20 ± 0.9*
8.	UV-B H + retene	18± 1.2*	23 ± 1.6*

Results are mean ± S.E. of 5 observations in each group. P value *0.05, NS not significant.

Studies suggest that ultraviolet radiation may also be important in zooplankton DVM (zooplankton diel vertical migration) in some systems proposed the transparency regulator hypothesis (Williamson et al., 2011). Crustacean zooplankton more generally show greater day time surface avoidance in high transparency system (Rose et al., 2012). It is observed that with the increase in temperature the body size of *Cyclops* reduces. *Cyclops* may avoid exposure to high levels of UV-B by staying in deep water layers or by undergoing diel vertical migration, behavioral avoidance, photoprotective compounds. Result indicates that climate change alter response to UV through temperature mediated effect on aquatic ecosystem and biodiversity. *Daphnia* abundance was significantly higher in –UV treatment than in +UV treatment in both the presence and absence of fish in high UV lakes indicating UV avoidance (Fischer et al., 2015).

This study is also helpful in studying the future consequences of enhanced UV-B radiation related to cataract and erythema. DOC can generate a potential screen effect / photobleaching against UV radiation. It is also possible that the interactions between UV radiation and DOC generate oxidants and free radicals. This possibility was investigated in field studies in the northern hemisphere by Hessen who revealed the dual role of substances such as melamine, mycosporine like aminoacids or carotenoids: their protective role against UV radiation and antioxidant role to avoid the damage generated by the free radicals originating from the interaction between UV radiation and DOC. DOC reduces the oxidative stress caused by photosensitizers and decrease the mortality in *Cyclops* thus protect the aquatic organisms from solar UV induced harmful radiation. It is observed that with the increase in temperature the body size of *Cyclops* reduces. *Cyclops* may avoid exposure to high levels of UV-B by staying in deep water layers or by undergoing diel vertical migration, behavioral avoidance, photoprotective compounds. Zooplankton are negatively affected when exposed to high intensities of UV radiation, DOC reduces the oxidative stress caused by photosensitizers and decrease the mortality in *Cyclops* thus protect the aquatic organisms from solar UV induced harmful radiation. Result indicates that climate change alter response to UV through temperature mediated effect on aquatic ecosystem and biodiversity.

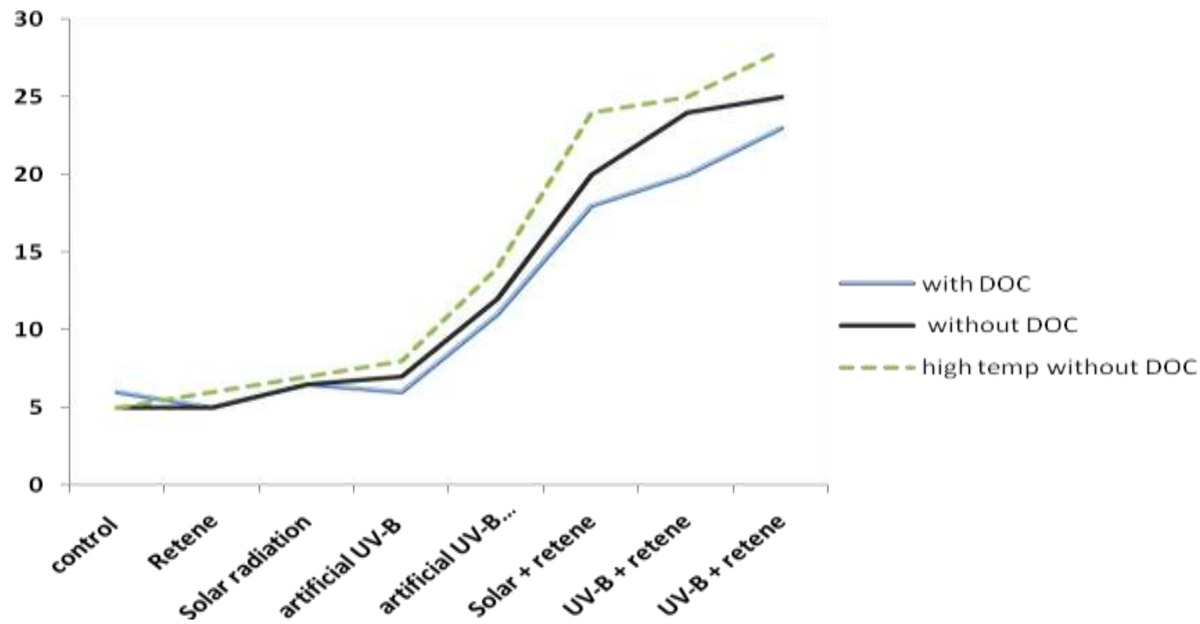

Fig. 1 Mortality rate in *Cyclops* in presence of retene and artificial UV-B at different temperature and DOC.

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