DU Journal of Undergraduate Research and Innovation

Volume 3, Issue 1, pp 60-73

Ultraviolet B Induced Protection Strategies in Unicellular Eukaryotic Microbes *Blepharisma* sp. and *Notohymena* sp. (Protista)

S. Sripoorna, Jeeva Susan Abraham, Swati Maurya, Hritik Kadian, Juhi Jaiswal, Seema Makhija, Ravi Toteja*

Ciliate Biology Laboratory, Department of Zoology, Acharya Narendra Dev College, University of Delhi, Delhi

Corresponding Authors: ravitoteja@andc.du.ac.in

ABSTRACT

The effect of ultraviolet radiation B (UVB) was studied on two freshwater ciliate species namely *Blepharisma* sp. (marked by presence of pink pigment, absence of cytoplasmic granules) and *Notohymena* sp. (marked by absence of pigment, presence of cytoplasmic granules). UVB radiations are considered as environmental stress factors for several aquatic organisms. However, not much information exists on the effect of UVB on the ciliates. Present study was carried out to understand the various modalities adopted by ciliates to withstand UVB exposure. It was found that UVB can lead to alteration of cell morphology, reduction in the cell movements, retardation of the cell growth, formation of cysts, increased absorption of UV by pigment/granules and UVB avoidance motile reactions. These were the few defense mechanisms observed in the present study which offer survival benefit to the organism.

Keywords: *Blepharisma* sp., *Notohymena* sp., UVB

INTRODUCTION

The UV radiations from the sun covers the wavelength ranging from 100 to 400 nm. They are divided into: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm) (1). Approximately 90% of UVB radiations and all of UVC get absorbed by the ozone, H_2O, O_2 and CO_2 present in the atmosphere. UVA radiation, along with 10% UVB reaches the Earth's surface and their levels vary which are influenced by a multitude of abiotic factors such as sun elevation, latitude, cloud cover, altitude, ozone and ground reflection (2). In addition, anthropogenic activities are responsible for the depletion of stratospheric ozone, leading to greater atmospheric transmission of UVB (3). This reduction of stratospheric ozone, especially in the Antarctic, has stimulated intensive efforts to understand and forecast the impact of increase in the UV radiations on the living systems. However, less amount of UVR is useful and essential for the production of vitamin D (4).

In the aquatic ecosystems, to what depth does the UVB radiation penetrates depends upon the turbidity in the water (5). It can affect both the phytoplankton and zooplankton in (6). With the reduction in the thickness of ozone layer, the extent of UV irradiance of our planet has increased over a period of time and the magnitude of its damage to the biological system has been a matter of grave concern (6, 7, 8).

Using different model systems, numerous reports have been documented that evaluate the negative effects of UVR, (5, 6, 7, 8, 9). In the present study, Ciliates, the single cell eukaryotic microorganism have been used because for their importance and abundance in aquatic ecosystems. Being the key consumers of planktons, diatoms, dinoflagellates, amoebae and bacteria, ciliates are the vital trophic links in the microbial food web. They in turn are eaten by higher zooplanktons (9). For cleaning water in the Sewage Treatment Plants (STPs), several ciliate species are known to have an important contribution. While *Frontonia leucas, Tetmemena pustulata, Coleps* are reported to feed on sewage, others like *Spirostomum minus, Urocentrum turbo, Vorticella* sp. feed on the bacterial population (10).

Ciliates are unicellular eukaryotes, with complex morphological features and physiology and are enormously perceptive to any changes in their environment. They sense a range of stimuli: mechanical, thermal, chemical, optical and gravitational (11) and because ciliates make a crucial part of the food web, it gets imperative to understand the effect of UVR radiations (UV-B in particular) on them (11, 12).

In the present study, two ciliate species, one with cytoplasmic pigment (*Blepharisma* sp.) and the other having cytoplasmic granules (*Notohymena* sp.) were selected to study the effect of UVB. The main aim of the study was to understand the morphological and behavioural response of the two species to the stress provided by UVB.

METHODOLOGY

Collection Site

The site for the collection of ciliates was Okhla Bird Sanctuary (28.5700° N, 77.3023° E) over the River Yamuna. In the sanctuary is present a large lake which was created by damming the River between Okhla village and Gautam Budhha Nagar (Figure I). Spreading over 4 square kilometres, the areas around the thorny scrub, grassland and a wetland that was formed as a result of creation of the Okhla Barrage. The sediment in the wetland consists of organic debris and fine sand. There is an extensive growth of water hyacinths on the banks and also inside the wetland.

Figure-I: Photographs of collection site, Okhla Bird Sanctuary, Delhi (A & B) and route map (C).

Water sampling

Fresh water samples were regularly collected throughout the year. The water samples were passed through a 120 μm nytex mesh to filter and collect ciliates as filtrate. Mixed planktonic cultures were initially grown at room temperature with addition of fresh boiled cabbage pieces to promote bacterial growth which served as food.

In vitro culture of ciliates

The freshwater ciliates isolated in this study were identified using Stereoscopic and Phase Contrast microscopy. Pringsheim's medium was used for maintaining the clonal cultures of each ciliate species and the temperature was kept at 22-23°C. The composition of the media was: $Ca(NO_3)_2$. $4H_2O$ (0.85 mM), KCl (0.35 mM), $MgSO_4.7H_2O$ (0.08 mM) and $Na_2HPO_4.2H_2O$ (0.11 mM) (13). To stimulate the growth of bacteria (which ciliates feed upon), boiled cabbage was also added to the medium.

UVB treatment of cells

Cell suspensions (about 50 cells/ml) were irradiated by fluorescent lamps (UVB, 302nm). Cells were exposed for time interval between 3 minutes to 25 minutes to the UVB intensity ranging from 29.6 X 10^{-10} J/M^2 to 187 X 10^{-10} J/M^2. Immediately after UV irradiation, the samples were examined and the cell suspension was divided in two parts: one part was examined by microscopy and was studied for behavioural analysis and the other part was fixed for studying nuclear changes. Control measurements were performed on the samples kept in the dark (controls). The photo recovery was studied by keeping the cells, which were irradiated for maximum time, under the light. The experiment was carried out in triplicates.

Morphological, behavioural and nuclear changes

UVB treated cells were evaluated for morphological, behavioural and nuclear changes. Morphological and behavioural changes were monitored by observing the cells under microscope at 40X. Cell size was determined with the help of

Scope Image Software. Nuclear changes were studied by Feulgen reaction. Cells were fixed in Carnoy's fixative (4:1 ratio of methanol and glacial acetic acid) for 20 minutes. After fixation, cells were treated with 1N HCl at 60ºC for 7 minutes and stained with Schiff's reagent for 30 min (14, 15). Stained cells were observed under microscope for detecting number, shape and size of macronuclei and micronuclei in control and UVB treated cells.

RESULTS

Morphological characteristics of Blepharisma sp.

Size (in life): 120-130 x 30-40 µm; Body flexible, anterior end bluntly pointed, posterior broadly rounded, AZM J-shaped, 2 macronuclear nodules joined by a thin thread-like segment and 8-12 micronuclei; Presence of subcellular pigment "Blepharismin", which renders it a pinkish appearance when observed *in vivo*.

Morphological characteristics of Notohymena sp.

Size (in life): 140-150 x 40-50 µm. Body flattened about 2:1 dorso-ventrally, flexible with yellowish-green cortical granules arranged in clusters. AZM covering 1/3rd of the body length and consists of 36 adoral membranelles, 18 FVT cirri, one RMC and one LMC which are almost confluent posteriorly, 6 dorsal rows, 2 macronuclei and undulating membranes in *Notohymena*-pattern.

UVB treatment of cells

Percentage survivability of *Bleharisma* sp. and *Notohymena* sp. decreases with the increase in exposure time as shown in Figure II.

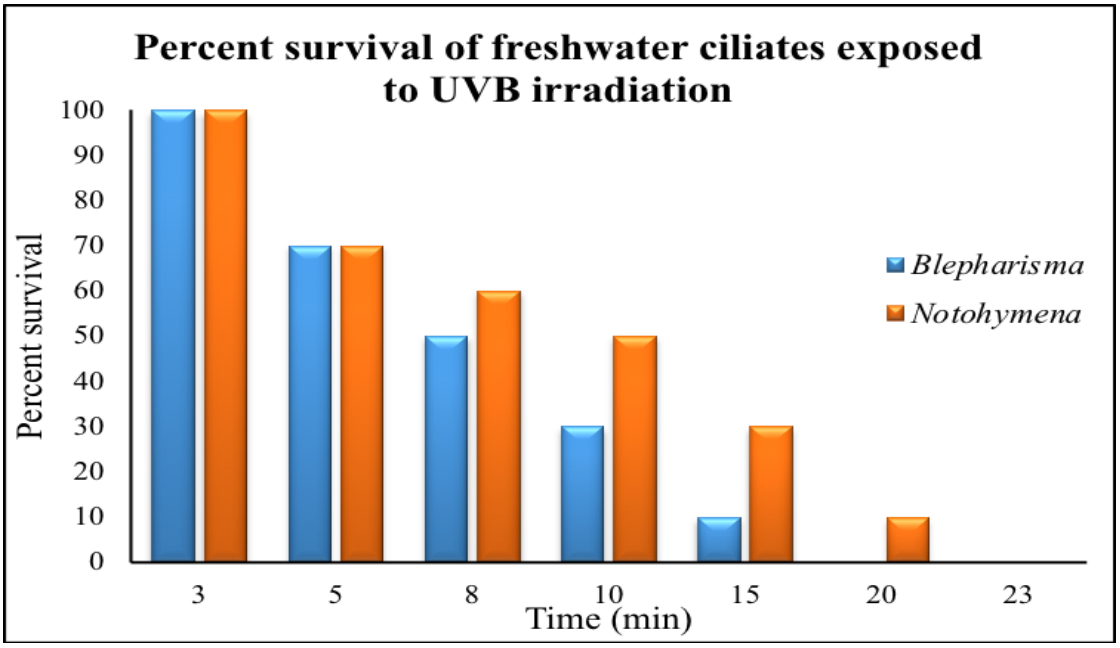

Figure II: Graph depicting the percent survival of *Bleharisma* sp. and *Notohymena* sp. when exposed to UVB irradiation for different time intervals.

Table I and Table II depict the morphological and behavioural changes appeared in *Blepharisma* sp. (Figure III) and *Notohymena* sp. (Figure III) after exposure to UVB at different time intervals.

Table-I: *Blepharisma* sp. exposed to UVB (302 nm)

Time of exposure (min)	**Morphological changes**
3	Aggregation and rounding of cells, swirling and slow movement
5	Cells settled down with very slow movement, 50% cells were encysted, showed slight loss in pigment, deformed shape, size was reduced
8	Most of the cells in cyst form, with some showing very slow movement and very light pigment
10	Very slow movement observed, most in cyst form, loss of pigment
15	Cells totally in cyst form, complete loss of pigment
20	Survival not observed

Table-II: *Notohymena* sp. exposed to UVB (302 nm)

Time of exposure (min)	**Morphological changes**
3	Slow movement, cells began to settle down
5	Aggregation of cells, reduction in cell size, rounding of cells and cyst formation, very slow movement, maximum number of cells are settled down
10	50% cells in cyst form, aggregates of cells observed, lack of movement or very slow movement, deformed shape
15	Most of the cells in cyst form
20	All the cells in cyst form
23	Survival not observed

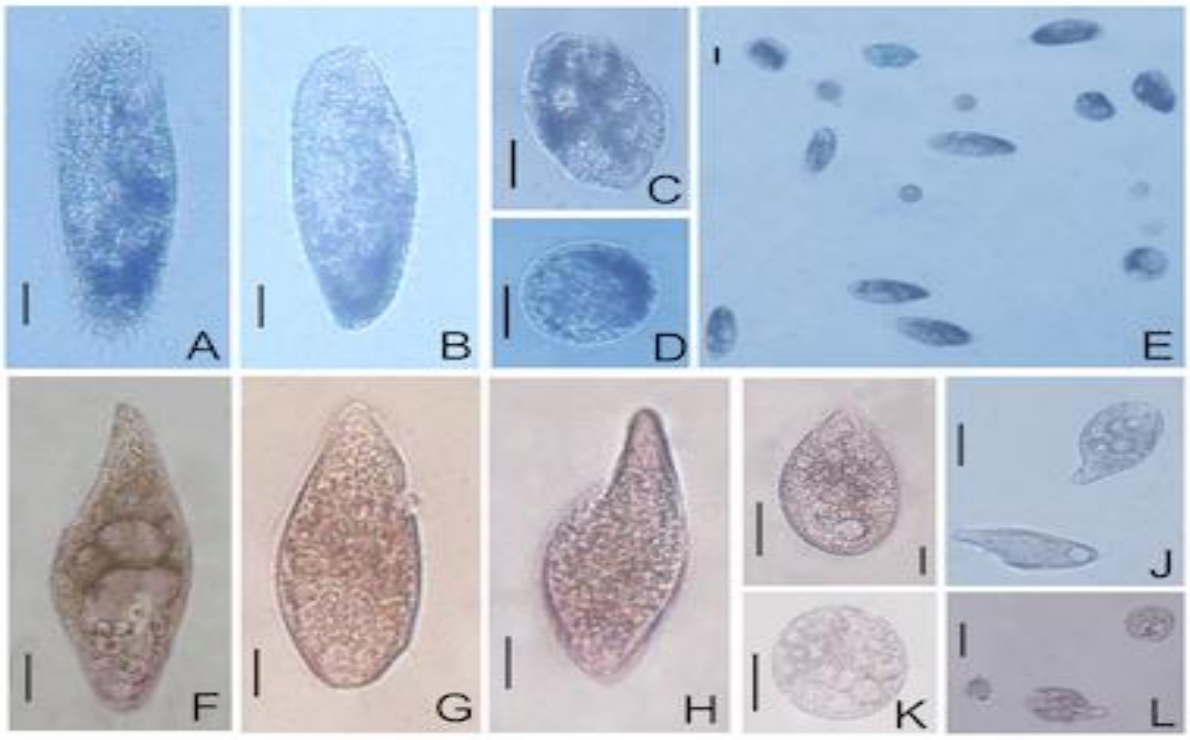

Figure-III: Cells of *Notohymena* sp. (A-E) and *Blepharisma* sp. (F-L) showing morphological deformities on exposure to UVB (302 nm). A & F: Normal cell, B, C, G-I: Deformed cells, D

& K: Cyst, E, J and L: Full microscopic view of cells in 10 X and 20 X magnification. Scale bar represents 20 µm in A-D and F-K, and 50 µm in E, J & L.

After treatment with UVB, the changes observed in morphology of micro and macronucleus are shown in Figure IV. Considerable degeneration of macronuclei of both *Blepherisma* sp. and *Notohymena* sp was seen. UVB exposure also led to the formation of amicronucleate mutants of both the species.

Photo recovery

Within 48 hours after the exposure to UVB, about 50% cells of *Blepharisma* sp. were found to revive from the cysts and the movement of the cells was very slow. In contrast, only 3-4 cells of *Notohymena* sp. were observed after 48 hours of UVB exposure and their movement was very slow too.

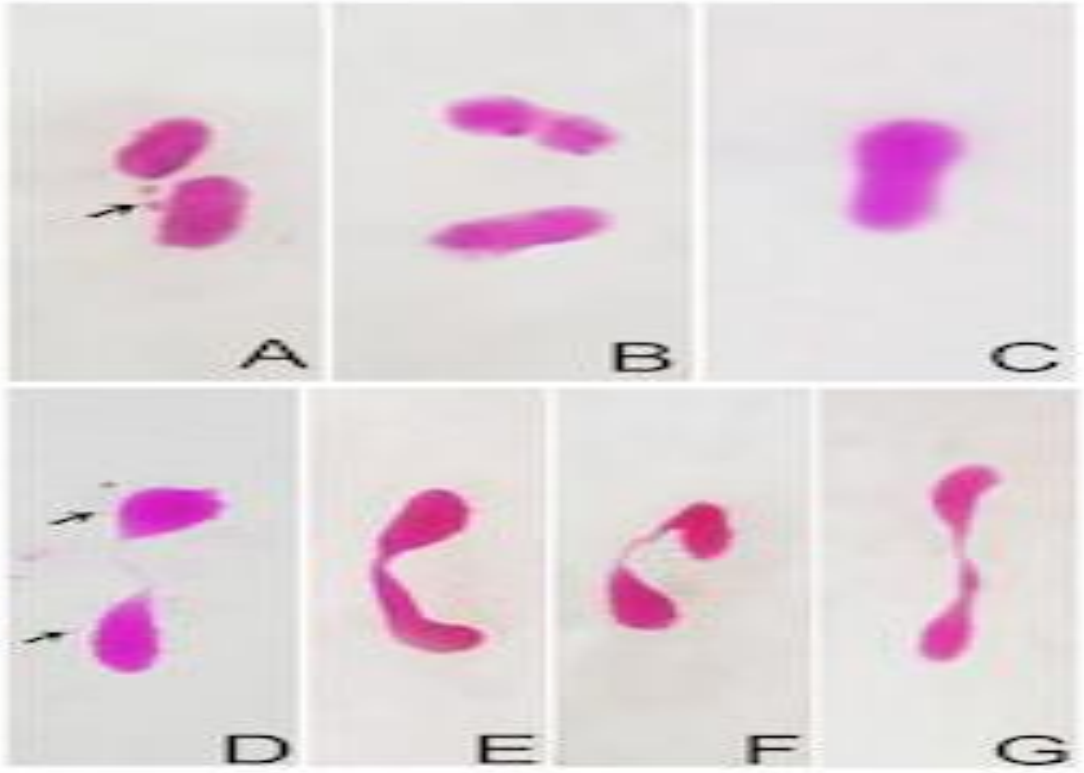

Figure-IV: Feulgen stained nuclei of *Notohymena* sp. (A-C) and *Blepharisma* sp. (D-G). A & D: Control, B, E & F: Macronuclei in UVB treated cells, micronucleus absent, C & G: Macronucleus of cells revived after UVB treatment.

DISCUSSION

The present study was aimed to study morphological and behavioural responses shown by two ciliate species, namely *Blepharisma* sp. (with pigment but devoid of granules) and *Notohymena* sp. (with cortical granules but devoid of pigment) to UVB by irradiating equivalent cells and measuring the cell viability at increasing intervals of exposure. In both the species, the cell death increased with the increase in the irradiation time. There have been several reports showing the direct relation between the cell viability and time of exposure to UVR (16, 17). The decrease in the cell viability observed on UVB exposure is because the cells undergo apoptosis (programmed cell death). In both the ciliate species, there has either been decrease in the number or complete loss of micronucleus. Micronucleus is the germ line nucleus and macronucleus is formed from micronucleus. Degeneration of macronucleus was also seen in both the species, which also suggests that under UV stress, cells undergo apoptosis. Similar stress-induced cell death has been shown in *Plasmodium* sp. *Trypanosoma* sp. (18, 19).

In this study, formation of cysts under UV stress was observed in both *Blepharisma* sp. and *Notohymena* sp. form. This is the most common strategy adopted by the ciliates when they are under stress (20, 21). Morphological changes like rounding of the cells, which is a step towards encystment was observed in both the ciliate species. During encystment, extreme cytoplasmic dehydration occurs which results in strong autophagic activity and decrease in the metabolic rate (20). As an effect of irradiation, significant changes in the cellular morphology have also been reported in several protozoan species showing variable sensitivity to the UV exposure. In *Blepharisma japonicum* also, severe impairment in its mobility (on exposure to UVB irradiation) has been shown (22). Similarly, changes in the mobility, morphological features, reproduction and infectivity of irradiated *Leishmania donovani* promastigotes (23) and inhibition of growth caused by UV radiation in *Crithidia fasciculata* (24) have been reported.

A strategy adopted by the ciliates to escape UV radiation is to form aggregates. It has been observed that under UVB, cells of both the species start forming aggregates and show change in their moving pattern. Experiments carried out both in the laboratory as well as in the field showed downward migration to be a common strategy in *Daphnia* sp. for UVR avoidance (25, 26, 27, 28, 29, 30, 31, 32)

Present study also indicates that *Notohymena* sp. is less prone to UVB exposure as compared to *Blepharisma* sp. This could be due to presence of cytoplasmic granules in *Notohymena* sp. which might be containing antioxidant enzymes that protect the cells from reactive oxygen species (ROS), produced on UV stress (33). UVB exposure has shown to increase the activity of antioxidant enzymes in *Daphnia longispina* (34) and in two species of genus *Anabaena* (35). Among ciliates, degree of resistance against UV-irradiation may significantly vary, as observed in *Fabrea salina,* which shows about tenfold greater resistance than that seen in *Blepharisma undulans* (9).

When the protective measures such as induction of stress genes like *hsp 70,* antioxidant enzymes and formation of cysts are not adequate, organisms are able to repair UV-induced damage only partially. Both the ciliate species in this study were found to recover the damage induced after 48 hours of UVB exposure. Exposure to UV is known to cause formation of thymidine dimers in DNA (36) which often lead to mutation and ultimately to cell death (37). The main mechanism for repairing thymidine dimers is the photo repair system (38) and evidence of enzymatic photo repair at the molecular level (in *Daphnia pulicaria*) has been given in a study by MacFadyen et al. (39).

The present study is focussed on the impact of UV rays on individual species of ciliates. While it helps us understand the effects of UV on the morphology and behavioural response of a ciliate species, comprehensive analysis is required to gauge the impact at the community level. Also, the molecular mechanisms like induction of stress gene (*hsp 70*) and antioxidant mechanism to combat UV stress in ciliates need to be studied further.

CONCLUSIONS

UVB exposure leads to substantial change in behavioral and morphological responses in ciliates. In response to it, organism adopts various defense mechanisms such as encystment, change in the motility, aggregation and these strategies provide a survival advantage to the organism.

ACKNOWLEDGEMENTS

This research work is supported by the ELITE scheme to support research by undergraduate students of Acharya Narendra Dev College. The authors appreciate the facilities provided by the Principal, Acharya Narendra Dev College, University of Delhi for carrying out the present study.

REFERENCES

1. Allen, J. (2001). Ultraviolet radiation: How it affects life on earth. *Earth Observatory*. Retrieved from https://earthobservatory.nasa.gov/Features/UVB/
2. Engelsen, O. (2010). The relationship between Ultraviolet Radiation Exposure and Vitamin D status. *Nutrients, 2*(5), 482-495.
3. Pettifor, J. M., Moodley, G. P., Hough F. S., Koch, H., Chen, T., Lu, Z. and Holick, M. F. (1996). The effect of season and latitude on *in vitro* vitamin D formation by sunlight in South Africa. *South African Medical Journal, 86*(10), 1270-1272.
4. Wacker, M. and Holick, M. F. (2013). Sunlight and Vitamin D. A global perspective for health. *Dermatoendocrinology, 5* (1), 51-108.
5. Davies-Colley, R. J. and Nagel, J. W. (2008). Predicting light penetration into river waters. *Journal of Geophysical Research, 113,* G03028.
6. Häder, D. P., Kumar, H. D., Smith, R. C. and Worrest, R. C. (2007). Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical and photobiology Sciences, 6,* 267-285.
7. Beardall, J., Stojkovic, S. and Larsen, S. (2009). Living in a high CO_2 world: impacts of global climate change on marine phytoplankton. *Plant Ecology and Diversity, 2* (2), 191-205.
8. Almeda, R., Harvey, T, R., Connelly, T. L., Baca, S., Buskey , E. J. (2016) Influence of UVB radiation on the lethal and sublethal toxicity of dispersed crude oil to planktonic copepod nauplii. *Chemosphere, 152,* 446-458.
9. Ghetti, F., Checcucci, G., and Bornman, J.F. (2001). *Environmental UV radiation: Impact on ecosystems and health and predictive models: Proceedings of the NATO advanced study institute on environmental UV radiation: Impact on ecosystems and human health and predictive models*. Pisa, Italy, Springer science and business media: Technology and engineering.

10. Dias, R.J.P., Wieloch, A.H. and D'Agosto, M. (2008). The influence of environmental characteristics on the distribution of ciliates (Protozoa, Ciliophora) in an urban stream of southeast Brazil. *Brazilian Journal of Biology, 68* (2), 287-295.
11. Kammerlander,B., Koinig, K. A., Rott, E., Sommaruga, R., Tartarotti, B., Trattnera, F. and Sonntag, B. (2016). Ciliate community structure and interactions within the planktonic food web in two alpine lakes of contrasting transparency. *Freshwater Biology, 61*, 1950-1965.
12. Sonntag, B., Summerer, M. and Sommaruga, R. (2011). Are freshwater mixotrophic ciliates less sensitive to solar ultraviolet radiation than heterotrophic ones? *Journal of Eukaryotic microbiology, 58*, 196-202.
13. Chapman-Andresen, C. (1958). Pinocytosis of inorganic salts by *Amoeba proteus* (Chaos diffluens). *Comptes rendus des travaux du laboratoire Carlsberg, 31,* 77-92.
14. Chieco, P. and Derenzini, M. (1999). The Feulgen reaction 75 years on. *Histochemistry and Cell Biology, 111*, 345-358.
15. Feulgen, R. and Rossenbeck, H. (1924). Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie, 135*, 203-248.
16. Imoto, K., Kobayashi, N., Katsumi, S., Nishiwaki, Y., Iwamoto, T.A., Yamamoto, A., Yamashina, Y., Shirai, T., Miyagawa, S., Dohi, Y., Sugiura, S., and Mori, T. (2002). The total amount of DNA damage determines ultraviolet-radiation-induced cytotoxicity after uniformor localized irradiation of human cells. *Journal of investigative dermatology, 119*(5), 1177-1182.
17. Wong, D.Y., Ranganath, T., and Kasko, A.M. (2015). Low-dose, long-wave UV light does not affect gene expression of human mesenchymal stem cells. *PLOS one*, 1-21.
18. Ameisen, J.C., Idziorek, T., Billaut-Mulot, O., Loyens, M., Tissier, J.P., Potentier, A. and Quaissi, A. (1995). Apoptosis in a unicellular eukaryote (Trypanosoma cruzi): implications for the evolutionary origin and role of programmed cell death in the control of cell proliferation, differentiation and survival. *Cell Death and Differentiation, 2*(4), 285-300.
19. Nguewa, P.A., Fuertes, M.A., Valladares, B., Alonso, C. and Pérez, J.M. (2004). Programmed cell death in trypanosomatids: a way to maximize their biological fitness? *Trends in Parasitology, 20*(8), 375-380.
20. Gutiérrez, J.C., Callejas, S., Borniquel, S., Benítez, L. and Martín-González, A. (2001). Ciliate cryptobiosis: a microbial strategy against environmental starvation. *International Microbiology, 4*, 151-157.

21. Slaveykova, V., Sonntag, B., and Gutiérrez, J.C. (2016). Stress and protists: No life without stress. *European journal of protistology, 55*, 39-49.
22. Lenci, F., Checcucci, G., Ghetti, F., Gioffrè, D. and Sgarbossa, A. (1997). Sensory perception and transduction of UV-B irradiation by the ciliate *Blepharisma japonicum*. *Biochimica et biophysica acta, 1336,* 23-27.
23. Molan, A.L. and Al-Harmni, K.I. (1989). Resistance produced in golden hamsters by inoculation with ultraviolet-irradiation *Leishmania donovani* promastigotes. *Japanese journal of parasitology, 38*(3), 113-119.
24. Barros, A.M.S. and Andrade, P.P. (1988). UV-induced growth inhibition in the trypanosomid *Crithidia fasciculata*. *Brazilian journal of medical and biological research, 21*, 493-497.
25. Storz, U.C., and Paul, R.J. (1998). Phototaxis in water fleas (*Daphnia magna*) is differently influenced by visible and UV light. *Journal of comparative physiology A, 183*, 709-717.
26. Leech, D.M. and Williamson, C.E. (2001). In situ exposure to ultraviolet radiation alters the depth distribution of *Daphnia*. *Limnology and oceanography, 46*(2), 416-420.
27. Rhode, S.C., Pawlowski, M. and Tollrian, R. (2001). The impact of ultraviolet radiation on the vertical distribution of zooplankton of the genus *Daphnia*. *Nature, 412*, 69-72.
28. Leech, D.M., Moeller, R.E. and Hargreaves, B.R. (2005a). Effects of ultraviolet radiation on the seasonal vertical distribution of zooplankton: a database analysis. *Archiv für Hydrobiologie, 162*, 445-464.
29. Leech, D.M., Padeletti, A. and Williamson, C.E. (2005b). Zooplankton behavioral responses to solar UV radiation vary within and among lakes. *Journal of Plankton Research, 27,* 461-471.
30. Fischer, J.M., Fields, P.A., Pryzbylkowski, P.G., Nicolai, J.L. and Neale, P.J. (2006). Sublethal exposure to UV radiation affects respiration rates of the freshwater cladoceran *Daphnia Catawba*. *Photochemistry and photobiology, 82*, 547-550.
31. Hansson, L. A., Hylander, S., and Sommaruga, R. (2007). Escape from UV threats in zooplankton: a cocktail of behavior and protective pigmentation. *Ecology, 88*, 1932-1939.
32. Hansson, L.A., and Hylander, S. (2009). Size-structured risk assessments govern *Daphnia* migration. *Proceedings of the Royal Society B, 276*, 331-336.
33. Kalyanaraman, B. (2013). Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. *Redox biology, 1*(1), 244-257.

34. Vega, M.P. and Pizarro, R.A. (2000). Oxidative stress and defence mechanisms of the freshwater cladoceran *Daphnia longispina* exposed to UV radiation. *Journal of photochemistry and photobiology B, 54*(2-3), 121-125.

35. Singh, G., Babele, P.K., Sinha, R.P., Tyagi, M.B. and Kumar, A. (2013). Enzymatic and non-enzymatic defence mechanisms against ultraviolet-B radiation in two *Anabaena* species. *Process biochemistry, 48*(5-6), 796-802.

36. Al-Adhami, B.H., Nichols, R.A.B., Kusel, J.R., O'Grady, J. and Smith, H.V. (2007). Detection of UV-induced thymine dimers in individual *Cryptosporidium parvum* and *Cryptosporidium hominis* oocysts by immunofluorescence microscopy. *Applied and environmental microbiology, 73*(3), 947-955.

37. Blaustein, A.R. and Belden, L.K. (2003). Amphibian defences against ultraviolet-B radiation. *Evolution and development, 5*(1), 89-97.

38. Rastogi, R.P., Richa, Kumar, A., Tyagi, M.B. and Sinha, R.P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of nucleic acids, 2010*, 1-32.

39. Macfadyen, E.J., Williamson, C.E., Grad, G., Lowery, M., Jeferey, W.H., and Mitchell, D.L. (2004). Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria. Global change biology, 10*, 408-416.