Effect of Ultraviolet Light Exposure on *Tetrahymena Thermophila* Growth Rates

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Abstract

As climate change and ozone depletion contribute to increasing ground-level ultraviolet (UV) radiation, organisms must be able to grow in conditions of increasing UV light to have a chance at survival. Our experiment investigated the relationship between the exposure to UV light and the growth rate of *Tetrahymena thermophila*, a unicellular eukaryote which increases expression of DNA repair enzymes when exposed to UV. We tested the hypothesis that brief UV exposures would have little effect on the growth of *T. thermophila*, but longer exposures would decrease growth rates by exposing *T. thermophila* cultures to 254 nm UV light for either 1 minute or 5 minutes. The treatments were then incubated at 30°C and samples were taken from each of the treatments every hour for four hours; additional samples were taken 21 hours and 46 hours after the initial UV exposure. The cells were counted using a hemocytometer and growth rates were calculated for each replicate and treatment. The growth rate of *T. thermophila* did not differ significantly among the two treatments and the control ($p>0.05$). The results of this study could be used to inform further research on the growth and survival of microbes under UV exposure.

Introduction

As the temperature of the earth continues to rise, climate change and ozone depletion are becoming serious threats to life on our planet (Barnes et al., 2019). One consequence of ozone layer depletion is increased penetration of ultraviolet (UV) light (Barnes et al., 2019), a known mutagen. To survive in this changing environment, organisms must be able to grow and adapt to these conditions.

Tetrahymena thermophila is a unicellular eukaryote that can be found in temperate, freshwater environments; it reproduces via sexual reproduction, in which pairs of cells mate via conjugation to produce progeny with unique combinations of macronucleus chromosomes (Collins and Gorovsky, 2005). *T. thermophila*'s size and ease of growth and manipulation have made it a popular organism for educational purposes and biological research (Cassidy-Hanley, 2012). *T. thermophila* is sensitive to UV light; high levels cause DNA and cell envelope damage (Campbell and Romero, 1998; Peng et al., 2009) and increased rates of cell death (Martindale & Pearlman, 1979). However, studies have also demonstrated that *T. thermophila* is capable of DNA repair after UV exposure (Brunk & Hanawalt, 1967), due to UV-induced upregulation of proteins involved in DNA repair and meiosis (Campbell & Romero, 1998; Howard-Till et al., 2011; Chi et al., 2013). These studies, however, did not examine the effect of UV exposure on the growth rates of *T. thermophila.*

Our study therefore aims to determine the effect of UV light on the growth rate of *Tetrahymena thermophila*. The growth of *T. thermophila* cultures exposed to UV light for either 1 or 5 minutes were calculated and compared to controls not exposed to UV. The growth rates were analyzed to test the null hypothesis that UV exposure does not affect *T. thermophila* growth, and the alternate hypothesis that UV exposure will change *T. thermophila* growth rates. If UV light causes damage to the structural and genetic integrity of *T. thermophila* but also increases expression of proteins that can repair the damage, then we predict that short periods of UV exposure will have little effect on growth rates but longer exposures will decrease the growth rate of *T. thermophila*.

Methods and Materials

UV Radiation Materials

Mineralight UVS-11 ultraviolet lamps with a wavelength of 254 nm were used as UV sources. The lamps were placed into cutouts in cardboard boxes, which suspended them 25 cm above the Petri dishes containing the cell cultures. To prevent other light sources from affecting the cultures during the treatment, aluminum foil was placed over the lamps and cutouts, and the boxes were closed during the treatment. The control cultures were placed in a closed cardboard box without a UV lamp during the treatment.

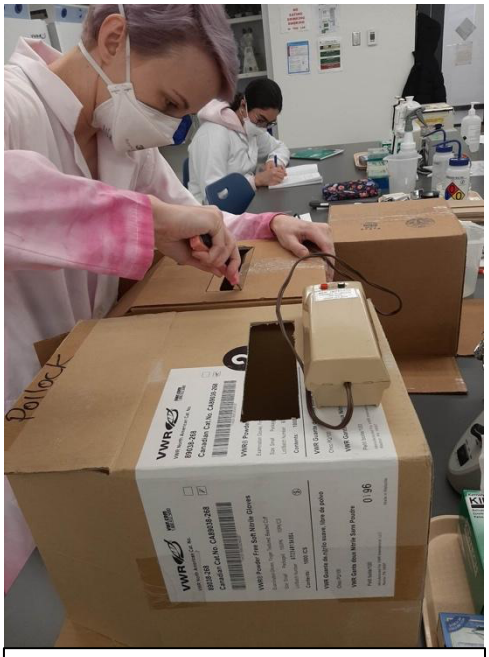

Figure 1. Design of the UV radiation treatment

Cell Counting Materials

Leitz Biomed and Zeiss Axiostar/Axios microscopes set on the 10x objective lens were used to obtain cell growth information. The Hausser Scientific Fuchs-Rosenthal Ultra Plane counter chambers were used to count the cell concentration of the cultures.

Incubation

VWR incubators set at 30° C were used to incubate the *Tetrahymena* cultures.

Biological Materials

The *T. thermophila* cultures and media were obtained from the lab technician of the BIOL 342 course at the University of British Columbia (UBC). The concentration of the initial *T. thermophila* culture was calculated as 9.2×10^4 cells/mL. It was therefore not diluted to reach the ideal initial concentration for growth curves of approximately 1.0×10^5 to 3.0×10^5

cells/mL. The Iodine-Potassium Iodine solution (IKI) fixative was also provided by the university.

Procedures

We first placed 7 mL of the initial *T. thermophila* culture into each of the nine 60 mm Petri dishes, to create 3 samples each for the control, the short UV treatment, and the long UV treatment. Using the UV exposure set-up described above (see Figure 1), we exposed the short treatment samples to UV for 1 minute and the long treatment samples for 5 minutes; the control samples were not exposed. All samples were placed inside closed cardboard boxes until all treatments were complete.

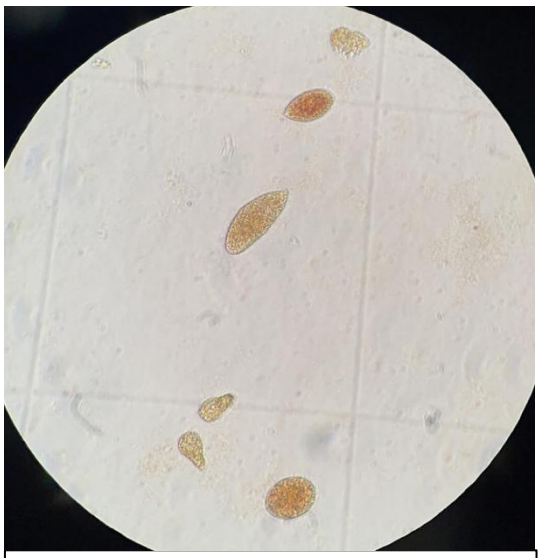

Figure 2. Tetrahymena observed using 40x objective lens. Organism on the top right showing significant cell envelope damage not counted.

After the exposure treatment, we transferred 5 mL of culture from each Petri dish into labeled 10 mL test tubes, one for each replicate. The test tubes were placed in an incubator set at 30° C. During each sampling period, we transferred a 100 µL sample from each test tube into a labeled counting tube also containing 10 µL of fixative. The first four growth samples were taken in approximately 1-hour increments, starting immediately after the treatment. Additional samples were taken approximately 21 hours and 46 hours after treatment. Cell counts for each sample were performed immediately, using a haemocytometer and microscopes. We counted approximately 100-200 cells for each sample, recording the total number and the size of haemocytometer divisions the cells were in. Only Tetrahymena cells with visibly intact cellular envelopes were counted; cells with visible damage, including broken envelopes and shriveled appearance, were excluded.

Data and Statistical Analysis

Experimental data were organized using Google Sheets, and analyzed using R Studio. After plotting cell concentrations as a function of time, we used linear regression analysis for each replicate and treatment to find slopes and intercepts of the growth rates. We used one-way ANOVA to compare the slopes of the growth rates for the replicates in each treatment.

Results

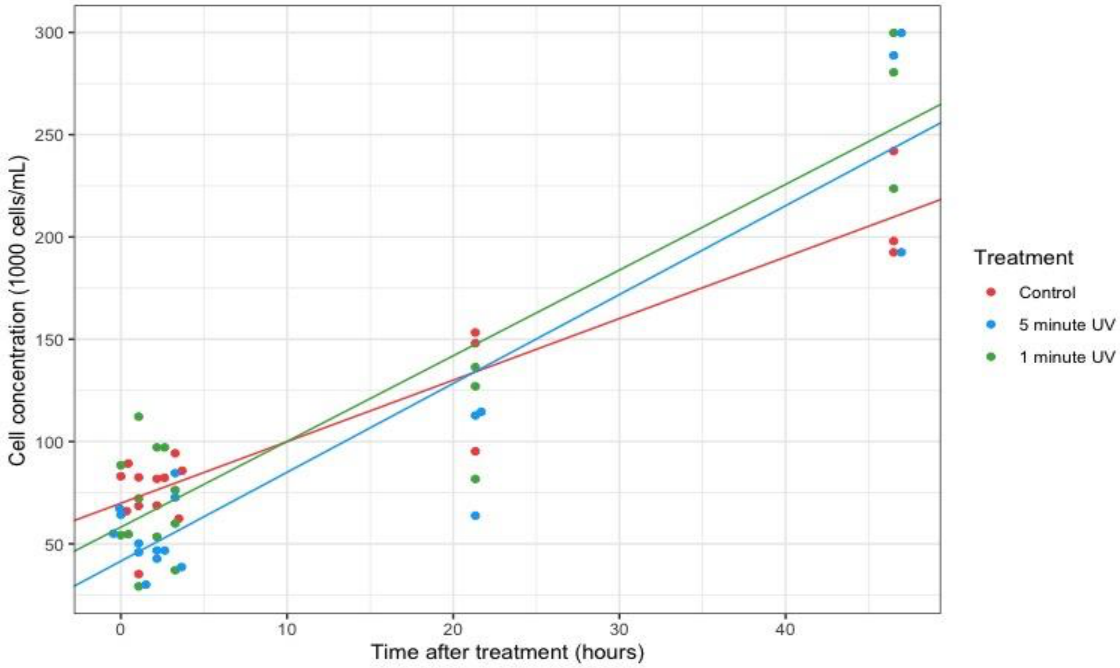

Figure 3. Cell culture concentrations (in thousands of cells per mL) across sampling times for all replicates and treatment conditions. Dots represent calculated cell concentrations for individual samples at different sampling times. Lines are the mean linear regression lines, representing the mean growth rate of each treatment.

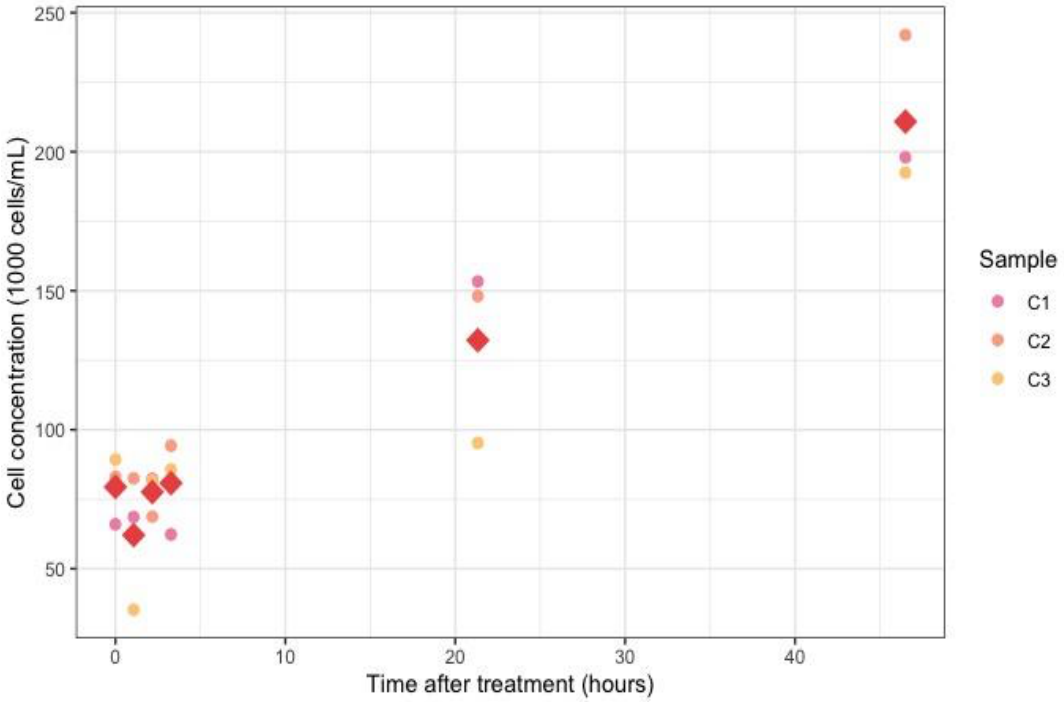

Figure 4. *Tetrahymena* cell concentrations (in thousands of cells per mL) across sampling times for the control treatment (no UV exposure). Dots represent the cell concentration for different replicates. Diamonds represent the mean cell concentration for each sampling time.

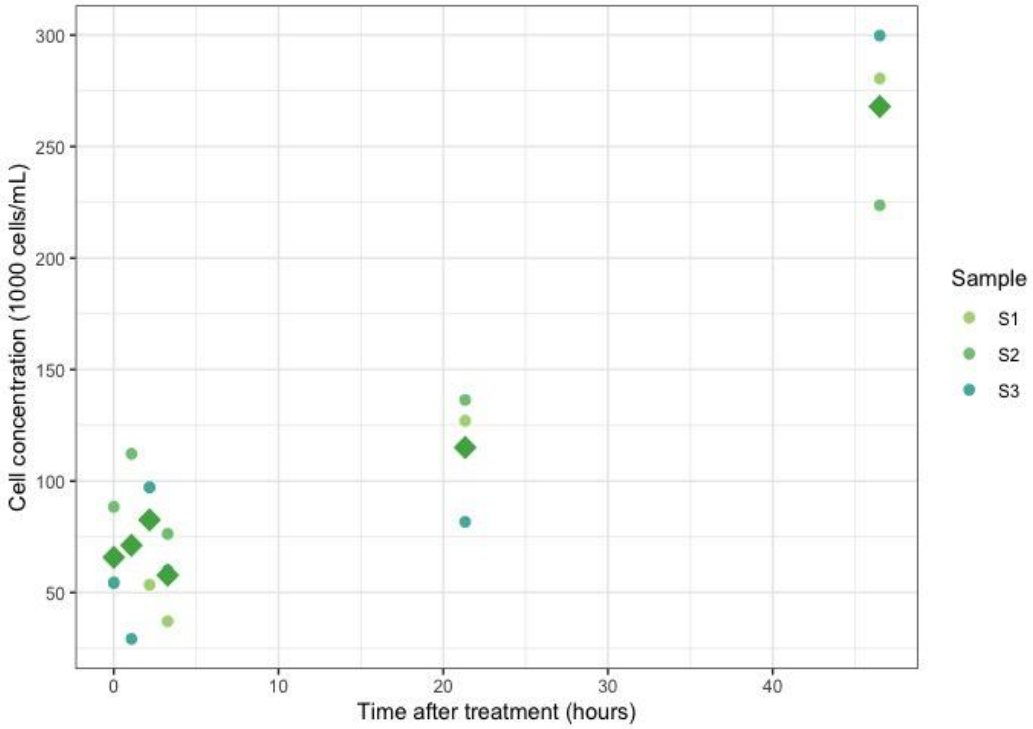

Figure 5. *Tetrahymena* cell concentrations (in thousands of cells per mL) across sampling times for the short UV treatment (1 minute exposure). Dots represent the cell concentrations for different replicates. Diamonds represent the mean cell concentration for each sampling time.

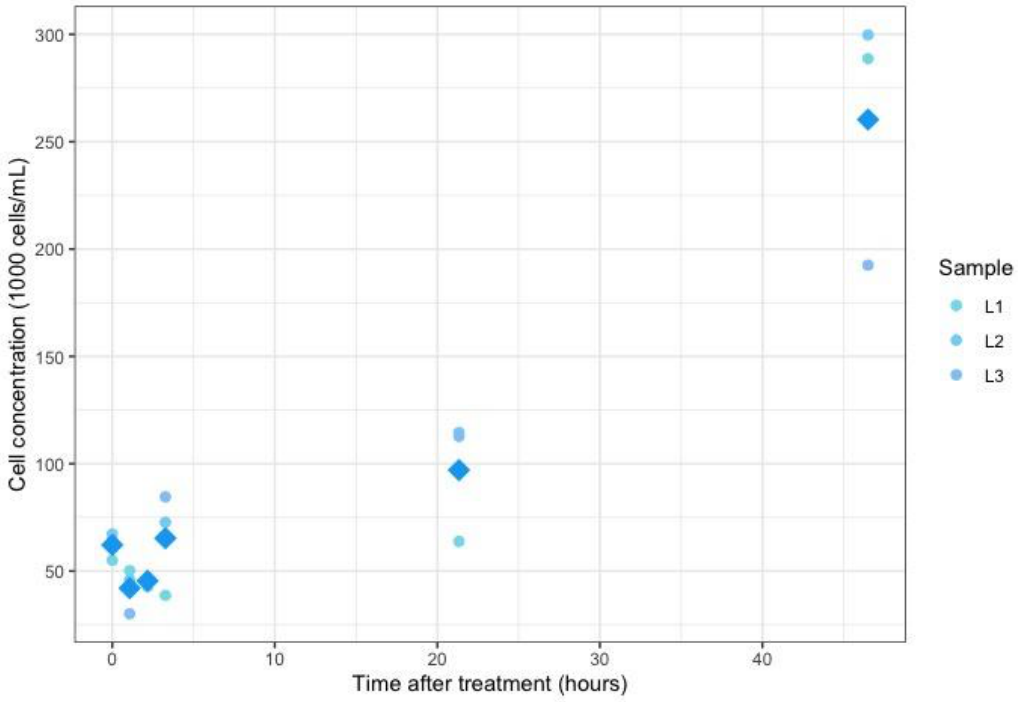

Figure 6. *Tetrahymena* cell concentrations (in thousands of cells per mL) across sampling times for the long UV treatment (5 minute exposure). Dots represent the cell concentrations for different replicates. Diamonds represent the mean cell concentration for each sampling time.

The growth rates were approximately linear for all replicates, with all nine linear regression analyses returning *p*-values below 0.05. Regression was also performed on log-transformed concentration data, but the fit to the data was less significant. The mean linear growth rates, as shown in Figure 3, were 3010 cells/hour for the control, 4190 cells/hour for the 1 minute UV treatment, and 4345 cells/hour for the 5 minute UV treatment; however the differences in growth rates among the different treatments were not significant (one-way ANOVA, p=0.2801). As seen in Figures 4 through 6, the controls had the highest mean cell concentration immediately after treatment, while the long treatment had the lowest. After 3 hours 15 minutes, both the control and long treatments had mean concentrations slightly higher than their respective t_0 concentrations, despite an initial drop. In contrast, the mean concentration of the short treatment initially increased, then dropped below the starting concentration at t_3. All groups had substantial increases in mean concentration between t_3 and t_4, approximately 18 hours apart, and an even larger increase at t_5, approximately 25 hours later. The long and short UV treatments had much larger relative increases (168% and 133%, respectively) in this time than the control treatment (59% increase). Accordingly, the final mean concentrations of the short and long treatments (approximately 268 000 and 260 000 cells / mL, respectively) were greater than the final mean concentration of the control (211 000 cells / mL).

Discussion

Since our results were not statistically significant, we cannot reject the null hypothesis that UV exposure does not affect the growth rate of *T. thermophila*. While there was insufficient support for our alternate hypothesis - that high UV exposure would decrease growth rates and low UV exposure would have little effect on growth rates - we did note some interesting trends and qualitative observations. Compared to the controls, the cultures exposed to UV showed more signs of cell wall damage and had lower live cell concentrations in the first 24 hours after treatment.More intriguingly, they had, on average, higher growth rates and higher final cell concentrations, approximately 47 hours after treatment. Studies have established that UV exposure causes increased expression of certain genes in *Tetrahymena*; the products of these genes repair DNA damage and are required for propagation through the cell cycle (Campbell & Romero, 1998; Marsh et al, 2000; Howard-Till, et al., 2011: Chi et al., 2013). Though not definitive, our results may suggest that the UV exposure did induce both cell damage and increased repair-protein expression; the higher growth rates in the exposed cultures may be due to the action of the proteins repairing the DNA damage and even encouraging advancement through the cell cycle for the surviving cells.

There are several possible reasons that our results were not significant. The primary cause is likely the small sample size, as there were only 3 replicates for each of the

treatments. We noted that though the growth rates for both UV treatments somewhat resembled an exponential curve, linear regression was significant for all replicates. Due to time constraints imposed by lab opening times and the schedules of the authors, we only had six irregularly-spaced sampling times, with substantial time between t_4 and t_5; trends and the true nature of growth curves may have been clearer with more sampling times at regularly-spaced intervals. A baseline concentration obtained from each sample immediately before treatment could also improve data interpretation.

We also suspect that the UV lamps we used contributed to the insignificance of our results. They appeared to be several decades old, with minimal information on the packaging. The radiation dose delivered could not be determined with any certainty. The same models were used for a study in 1979 by Beckwith and Malberger, who determined that the lamps delivered 3.5 ergs/mm^2/second when placed 25 cm above a surface. We chose our treatment lengths based on this measurement, and the finding that a total of 250 ergs/mm^2 causes sub-lethal DNA damage in *T. pyriformis* (Brunk & Hanawalt, 1967). However, it seems likely that the lamps lost efficiency over time, and the actual radiation delivered to our cultures was lower than initially estimated - perhaps too low to cause substantial DNA damage.

Another source of error was the counting procedure for the samples. Some samples were counted multiple times by different people, and then averaged, while other samples were counted only once. Further, all the samples for t_5 were counted by the same author. Differences in counting technique or precision likely introduced bias into the samples for t_5 and into the samples counted fewer times.

A comparison of our experiment to previous studies reveals differences in methodology, particularly UV dose and exposure time, and the use of chemical agents. While we used similar exposure times as some other researchers (Campbell and Romero, 1998), others used much longer exposures, from 20 minutes to 6 hours (Calkins et al., 1986; Peng et al., 2009; Gao et al., 2013). Direct comparisons, however, are difficult because the energy delivered can vary depending on the UV source and the distance between the source and cell culture. Some studies also combined other factors with UV exposure, including caffeine (Calkins et al., 1986), UV filters (Gao et al., 2013), and titanium dioxide (Peng et al., 2009), to investigate the combined effects of UV irradiation and chemicals. While these studies found significant cell damage, death or decreased growth rates in *Tetrahymena,* they used additional environmental stressors, while we investigated the effect of UV alone.

Conclusion

We aimed to determine whether UV light has an effect on *T. Thermophila* growth rates. Over a period of approximately 47 hours after exposing tetrahymena cultures to 254 nm UV light for either one or five minutes, we did not find significant differences in growth rates among the treatments and controls. Therefore, we cannot reject the null hypothesis that UV exposure has no effect on *T. thermophila* growth rates. While our results did not support our predictions, the finding that *T. thermophila* can survive short exposures to UV light is supported by past studies about *Tetrahymena's* DNA repair mechanisms. Further research could examine the survivability of microbes exposed to UV, the impact of UV-induced gene expression on growth rates, and the microbe coping mechanisms for environmental stressors on a genetic level.

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