The Effects of Environmentally Realistic Concentrations of Caffeine on the Bioindicator Tetrahymena, and Testing of Caffeine in Local Waters

Zachary Booth

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OFFICIAL ABSTRACT and CERTIFICATION

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Zachary Booth
Columbia High School, East Greenbush New York, United States

Over the last few decades, contamination in different types of water sources has been a major concern. Specific contaminants, called PPCPs (pharmaceuticals and personal care products) have been shown to be alarming and concerning because of their potential to disrupt ecological processes. This study hopes to better understand the effects of caffeine on ecological growth, and the extent of caffeine in local waters. Tetrahymena were added to four different well plates, each containing different concentrations of caffeine- 0, .01, .05, and .1 micro-g/mL. At the immediate start of exposure, the number of cells were counted in each sample. Cells were counted again at 24 hours. Using these numbers, the number of generations and the generation time were calculated. The number of generations for each concentration- (0) 2.02, (.01) 0.15, (.05) 2.81, (1.0) 0. The generation time for each concentration- (0) 11.87, (.01) 157.89, (.05) 8.55, (1.0) 0. The number of generations in the amount of generation cycles completed in 24h. The generation time is the estimated amount of hours it would take for the Tetrahymena to complete a full generation cycle.

1. As a part of this research project, the student directly handled, manipulated, or interacted with (check ALL that apply):
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5. This abstract describes only procedures performed by me/us, reflects my/our own independent research, and represents one year's work only ■ Yes ☐ No
6. I/we hereby certify that the abstract and responses to the above statements are correct and properly reflect my/our own work. ■ Yes ☐ No

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Introduction

A PPCP (pharmaceuticals and personal care products) are classified as any product used for cosmetic or personal health reasons. This can include over-the-counter prescription drugs, deodorants and perfumes, or chemicals used in different beverages. PPCPs have been shown to be a rising public health concern among scientists, because of the unknown effects of contaminated water (Richmond et al).

Caffeine, a very prominent PPCP, has been found in considerable amounts in running water throughout the country. (Richmond et al). Different studies were performed testing the quantity of caffeine and other PPCPs in natural waters. In many, caffeine was extremely present.

Caffeine is found in more than just coffee and other caffeinated drinks, it is used in prescription and over-the-counter medications. It is considered the world's most popular drug. It is also found naturally in certain plants, but only one of which is native to North America, Yaupon (Ilex vomitoria). Yaupon is found in the southeastern United States. Because of its little natural presence in North America, it implies that the majority of caffeine concentrations found in natural waters is due to human activity (CAMH).

It has been shown that a large fraction of caffeine found in naturally occurring water is a result of wastewater treatment facilities, septic systems, and outdated sanitary sewage infrastructure. While these facilities remove up to 99% of contaminants before they enter the environment, other factors like large storms, runoff, and other waste have bound to contribute to water contamination. When this contaminated water enters natural streams and rivers, it has the potential to disrupt and harm ecosystems.

As stated, the presence of caffeine in water is a well documented statement. However, less is known about the particular impacts of caffeine on humans, and other organisms, particularly aquatic organisms.

Tetrahymena is a commonly used model organism. More importantly, it is shown to be a reliable model organism in toxicological studies (Maurya and Pandey). In my study, I used *Tetrahymena* as a model organism to pose as a bioindicator for the impacts of caffeine on ecosystems.

Methods and Materials

For my project, I created different water samples for different concentrations of caffeine, ranging from 0 to .1 micrograms per milliliter of caffeine powder. I then used these caffeine concentrations to test their effect on tetrahymena population growth.

I chose to observe the *Tetrahymena* population to quantify the effects of caffeine on overall *Tetrahymena* health, and potential ecosystem changes.

Creating Caffeine Concentrations

Four different samples of caffeine were created to use for the study. The five concentrations (with the addition of 0 mg/mL) were 0 mg/mL, .01 mg/mL, .05 mg/mL, and .1 mg/mL of caffeine. To create these samples, I crushed Nodoz caffeine tablets using a mortar and pestle into powder. Using a scale (to the nearest hundredth of a mg), I weighed out 0.1mg, 0.5mg, and 1mg of the caffeine powder, and added it to their own graduated cylinder. I then added 10mL of distilled water to each graduated cylinder and labeled them as my four caffeine concentration samples.

Preparing Tetrahymena

Tetrahymena and Tetrahymena medium were purchased from *Carolina.com*. Once unpacked, I let the *Tetrahymena* settle for 24 hours after being unpacked. The next day, 30 microliters of *Tetrhymena* were added to each medium, to create different populations of*Tetrahymena* for my experiment. The *Tetrahymena* were added to the medium to grow in population over 24 hours, then used for the behavior experiment.

Population Change

10 microliters of the *Tetrahymena/Tetrahymena* medium were added to a slide and viewed under a microscope with an AMScope camera and video recorder attached to it. 10 additional microliters of each caffeine solution were added to the slide. Immediately after exposure, 5 photos were taken of the *Tetrahymena* using the AMScope, and photos were uploaded to my computer. This was repeated for each caffeine concentration solution. Once the photos were taken, the amount of *Tetrahymena* in each photo was hand counted, and the average amount of *Tetrahymena* was recorded for each different caffeine concentration. 24 hours after exposure, the slide was put back under the microscope, and 5 new photos were taken for each caffeine concentration. The average amount of *Tetrahymena* in each photo was recorded for each caffeine concentration.

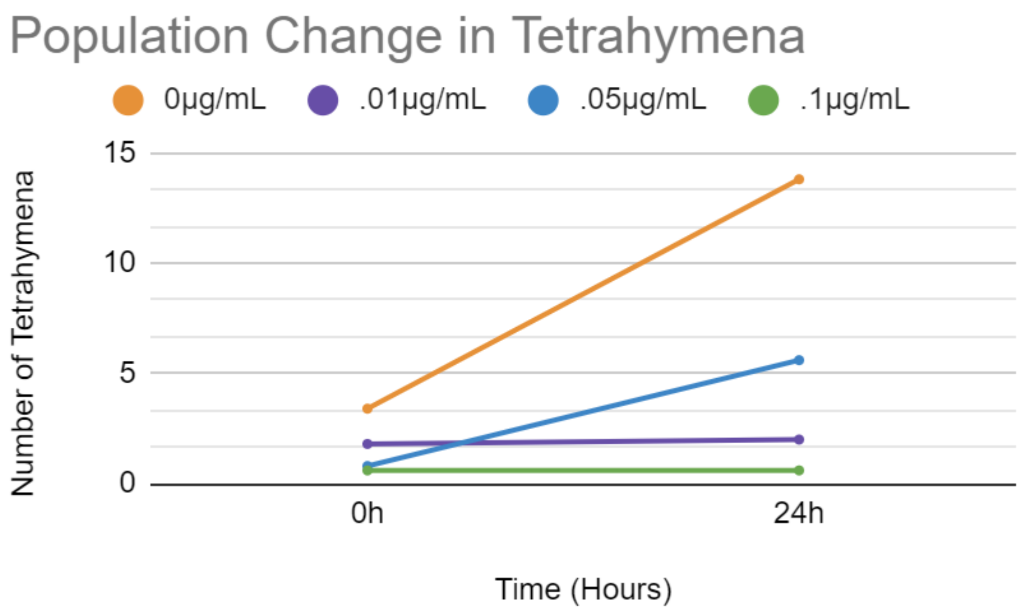
Using the population data from the photos at 0 h and 24 h after exposure to caffeine, the number of generations and generation time was calculated. The number of generations approximates the amount of reproductive cycles the *Tetrahymena* went through in the 24 hours since exposure. The generation time is an estimate of the amount of time it takes for the *Tetrahymena* to complete a full reproductive cycle.

Number of Generations Formula (n)- $\frac{\log N1 - \log N0}{\log 2}$

Generation TIme Formula (g)(hours)- time of growth(24h) / n

Results

Tetrahymena were shown to have a decreased number of generations over the 24 hour period, and a decreased generation time as the concentration of caffeine exposure increased. *Tetrahymena* showed a trend of decreasing population growth rates as the exposure to caffeine increased. At .1µg/mL, *Tetrahymena* had zero reproductive activity.



	0μg/mL	.01μg/mL	.05μg/mL	.1μg/mL
N (#)	2.02	.15	2.81	0
G (hours)	11.87	157.89	8.55	null

Conclusion and Discussion

This study aimed to observe the impacts of caffeine on the reproductive health of *Tetrahymena.* As shown, increased levels of caffeine decreased the growth rate of *Tetrahymena*, which poses a potential threat to ecosystems nationwide.

Because *Tetrahymena* is widely used as an ecosystem bioindicator, this study shows that caffeine can potentially harm ecosystem populations and overall health.

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