The Effect of Paraformaldehyde on the Phenotypic Appearance of *Paramecium tetraurelia*

Course Name and Lab Section

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Introduction

Background Information

Paramecium tetraurelia, a unicellular, ciliated protist that thrives in fresh-water, relies on the defensive function of its trichocysts and its cilia to protect itself from predators and other external threats. By changing the environment, or solution, to which it is exposed to, the overall defense mechanism of *P. tetraurelia* can be more clearly understood through close observation of resulting phenotypic changes. Moreover in doing so, the impacts of the solution itself on the organism can also be explored and thus we can gain deeper understanding of the compound in question.

P. tetraurelia is one of the twenty-six species in the genus of *Paramecium*. As a eukaryotic organism, it has membrane-bound organelles. Moreover, the size of a *P. tetraurelia* can range in size from 100-350 μm in length (Elwess, 2017). Any change in cell length (or width) to beyond the normal range is an abnormal phenotypic change and can thus serve as one of the few indicators of the *P. tetraurelia* undergoing a self-defense mechanism.

The cilia that cover the *P. tetraurelia* cell body surface serve two main purposes, in addition to general cell-to-cell communication. Firstly they serve the purpose of wafting small bacteria and algae into the gullet of the cell membrane. Bacteria, such as *Klebsiella pneunomiae* and algae typically serve as a primary food source for the *P. tetraurelia* and undergo endocytosis into the cell for further nutrient breakdown (Berger, 1981). Cellular waste is then excreted through the cell's anal pore. Secondly, the cilia also serve the purpose of aiding the movement of the *P. tetraurelia* – particularly by propelling the cell away from an unfavorable environmental condition (Elwess, 2017). Upon exposure to the unfavorable environment the Ca^{2+} channels open, which allows the rush of calcium ions into the cell (Nauli, 2016). This triggers the cell to reverse direction by propelling cilia in the opposite direction – an effective avoidance response. The ability of the *P. tetraurelia* to steer its way away from danger works hand-in-hand with the cell's trichocysts, which are located in an alternating pattern with the cilia on the cell's surface, in further protecting the cell and ensuring survival.

Trichocysts function as defensive organelles in *P. tetraurelia* (Miyake, 1996). Trichocysts in protozoans are ejected in response to external stimuli – particularly threatening ones. Much like with changing direction of movement using the cilia, with a rapid increase in calcium ion concentration within the cell (due to the opening of the Ca^{2+} channels), the rate of the discharge of trichocysts also increases (Elwess, 2017). While there are several types of trichocysts, *P. tetraurelia* has filamentous trichocysts. Filamentous trichocysts are spear-like structures that protrude from the cell. The discharge of the trichocysts thus causes a physical phenotypic change in the *P. tetraurelia* – another one of the few indicators that the *P. tetraurelia* is undergoing a self-defense mechanism.

Another structural change in *Paramecia* that result in visible phenotypic changes in the cell is the contractile vacuole activity of the *P. tetraurelia* (Bilinski, 1981). *P. tetraurelia* has contractile vacuoles located on the cell surface near the end of the cell. These vacuoles serve the purpose of water regulation within the cell and as excretory structures since metabolic waste is also expelled with the water. Vacuoles consist of canals that contain vacuole fluid with an osmolarity.

This osmolarity is controlled by C^- and K^+ ions, which is higher than that of the cytoplasm. The difference if osmopolarity allows water to enter the canals via osmosis. With the entrance of water into the vacuole through the canal, the pore opens. The vacuole can then contract, expelling the contained water through the pore. *P. tetraurelia* cells are capable of regulated exocytosis when exposed to certain external stimuli as a form of self-defense (Wu, 2013). Thus, presence of regulated exocytosis is also another viable phenotype change to consider as an indicator that the *P. tetraurelia* is undergoing a self-defense mechanism.

P. tetraurelia can reproduce both by asexual fission as well as by sexual reproduction. Moreover, they divide by binary fission, which can also further be observed as a supporting factor of the impacts of a changing environment on *P. tetraurelia* cells. For example, during asexual reproduction the *P. tetraurelia*'s macronucleus and then cell split up (Elwess, 2017). Any changes to this process (or the rate of the process) can serve as a direct indication of the effects of the unfavorable environment on the *P. tetraurelia* cells. Furthermore, changes in the processes of any sexual reproduction in the *Paramecia*, such as cell alignment, cell fusion, micronuclei disintegration, etc., can further support any conclusions drawn from observations of any phenotypic changes in regards to the impact of a change in the surrounding environment on *P. tetraurelia*.

In order to better understand the impacts of exposing *P. tetraurelia* to an unfavorable environmental condition, the exposure of *Paramecia* to a Paraformaldehyde solution is explored in this experiment. Paraformaldehyde, a white crystalline solid formed by the polymerization of formaldehyde, is a soluble alkali. Common uses of Paraformaldehyde include fumigation, disinfection and as a fungicide. The uses of the chemical alone give insight on the potential fatal impacts it could have on a cell – particularly the cell of a *P. tetraurelia.* The impact of Paraformaldehyde, or specifically the preservation-chemical formaldehyde, has many resulting phenotypic changes in a cell (Oettlé, 1948). Cell shrinkage is a common incidence, resulting in alteration in the structure and organelle delocalization. Deformation of the cytoskeleton is another phenotypic change that could occur as a result of exposing the *P. tetraurelia* to a Paraformaldehyde solution. Finally, the vesiculation of cell membranes is also a consequence of exposing *Paramecia* to Paraformaldehyde as it breaks down the membrane-cytoskeleton.

Due to the many potential phenotypic impacts Paraformaldehyde can cause to the *Paramecia*, it makes it an ideal solution to explore. Not only does it give us insight and better understanding on the chemical and how it works, but also on the defense mechanisms of *P. tetraurelia* and the resulting phenotypic changes. C. N. C. Crawford carried out a similar study where he studied the action of Formaldehyde (depolarized paraformaldehyde) on living cells using phase-contrast microscopy (Oettlé, 1948). Using *Salamander spermatognia* and *Ssnail amoebocyte* cells and various strengths of formaldehyde solution, he was able to observe morphological changes in the cells. Observations in his study include cell swelling/shrinkage, formation "bubbles" from the cells, nuclear changes, and changes in overall cytoplasmic structure.

Purpose

The purpose of this experiment was to expose *Paramecium tetraurelia* to various concentrations of Valproate and observe any phenotypic changes that occurred to the *P. tetraurelia* cells.

In doing so, we were able to gain further understanding of the defense mechanisms of *P. tetraurelia* upon exposure to an unfavorable environmental condition, as well as the impacts of Valproate on a unicellular, ciliated organism..

Hypothesis

With an increase in the concentration (mg/ml) of the Valproate solution, an increase in GABA

size and width (µm) of *P. tetraurelia* will occur.

This will occur because the expected impacts of the Paraformaldehyde on a cell include phenotypic changes such as cell swelling/shrinkage, cellular vesiculation, nuclear changes, and changes in overall cytoplasmic structure. Moreover, the discharge of the trichocysts as a response to the changing environment as a self-defense mechanism will cause an increase in overall cell length and width (µm).

Materials and Methods

Culturing *Paramecium*

Stock cultures of *Paramecium tetraurelia* were maintained at SUNY Plattsburgh, over a period of three weeks. Each week, sterile wheat medium was inoculated with a type of bacteria called *Klebsiella pneunomiae*, which served as a food source for the *P. tetraurelia* to live and grow. The cultured bacteria were grown overnight while shaking in an incubator at 37°C. The shaking incubator served the purpose of ensuring constant agitation, constant access of oxygen and nutrient supply for the bacteria, optimum bacterial reproduction, and the prevention of bacterial clump formation.

The following day, after allowing the flask to cool to room temperature for five minutes, 1ml of *P. tetraurelia* was added to the inoculated wheat flask. This was repeated for the next two weeks; all experiments were done using three-week old *Paramecia*.

Stock solutions(s)

4% of paraformaldehyde was used in all experiments, obtained from the Science Department at SUNY Plattsburgh. Different volumes of water were added, using micropipettes, to different volumes of the 4% paraformaldehyde to obtain the final three different solutions of diluted paraformaldehyde. The final three different dilutions of the paraformaldehyde used were: 0.0045%, 0.005%, and 0.007%.

In order to find the ideal final three dilution settings, several different dilutions of the 4% paraformaldehyde with water were tested on samples of the cultured *P. tetraurelia* over a period of three weeks, prior to the experiment. After several observations using the EVOS inverted microscope on the impact of each respective dilution of paraformaldehyde on the *Paramecium tetraurelia*, the final three dilution ratios were chosen.

These three dilutions were chosen because they were the most tolerable dilutions that the *P. tetraurelia* could be exposed to without immediate cell death. Moreover the exposure of these specific dilutions of paraformaldehyde allowed slow enough phenotypic changes with the *P. tetraurelia*, making observation of the respective changes easier to spot. Stronger solutions of the paraformaldehyde proved to be ineffective in exploring the effects of paraformaldehyde on the phenotypic changes of *P. tetraurelia*, and ultimately the overall purpose of this experiment. This is because with stronger solutions of paraformaldehyde, the *P. tetraurelia* mutated and blistered at too fast a rate for efficient observation, and also resulted in immediate cell death.

The control solution for this experiment was water without any presence of paraformaldehyde, serving the purpose of to ensure that any phenotypic changes that were occurring to the *P. tetraurelia* were a direct result of the exposure to paraformaldehyde and not as a result of another external factor.

Experimental Set-Up

For each of the three solutions of diluted paraformaldehyde, three separate trials were done for increased accuracy. In addition, three trials of the exposure of the *Paramecium tetraurelia* to a water solution were done as the control setting. Thus, eight reaction-plate mixing wells were used, each

containing 3ml of the previously cultured *Paramecium* and 150µm of the respective diluted paraformaldehyde solution. The final three contained 3ml of *Paramecium* without any paraformaldehyde. The wells were then instantly placed under an EVOS inverted microscope for immediate observation of any potential phenotypic changes in the *Paramecia* upon exposure to the paraformaldehyde solution. Any visual observations were noted, and images of the *Paramecia* were captured and saved for further analysis using the EVOS imaging system.

Once data had been collected for the initial observation(s), the reaction-plate mixing well tray was covered, sealed and then left undisturbed for a week at constant temperature and exposure to light. These two variables were controlled to maintain accuracy and to ensure consistency of the variables, the tray was left in a controlled environment in the lab at Hudson Hall, SUNY Plattsburgh. Observations were then taken a week later under the EVOS inverted microscope. This was done to more clearly understand both the long-term and short-term effects of paraformaldehyde on the phenotypic appearance of *P. tetraurelia*.

Safety Precautions

When carrying out the different sections of the experiment, from culturing the *Paramecia*, to making the stock solution of Paraformaldehyde, to the experimental set-up – safety precautions must be taken. When culturing the *Paramecia* it is important to be careful when dealing with the *Paramecia* itself however most importantly, when handling the bacteria *K. pneunomiae*. To ensure maximum safety, gloves must be worn at all times when handling the bacteria and as soon as the cell have be cultured, ensure that the conical flasks are covered in aluminum. Exposure to the bacteria could result in increased risk of pneumonia.

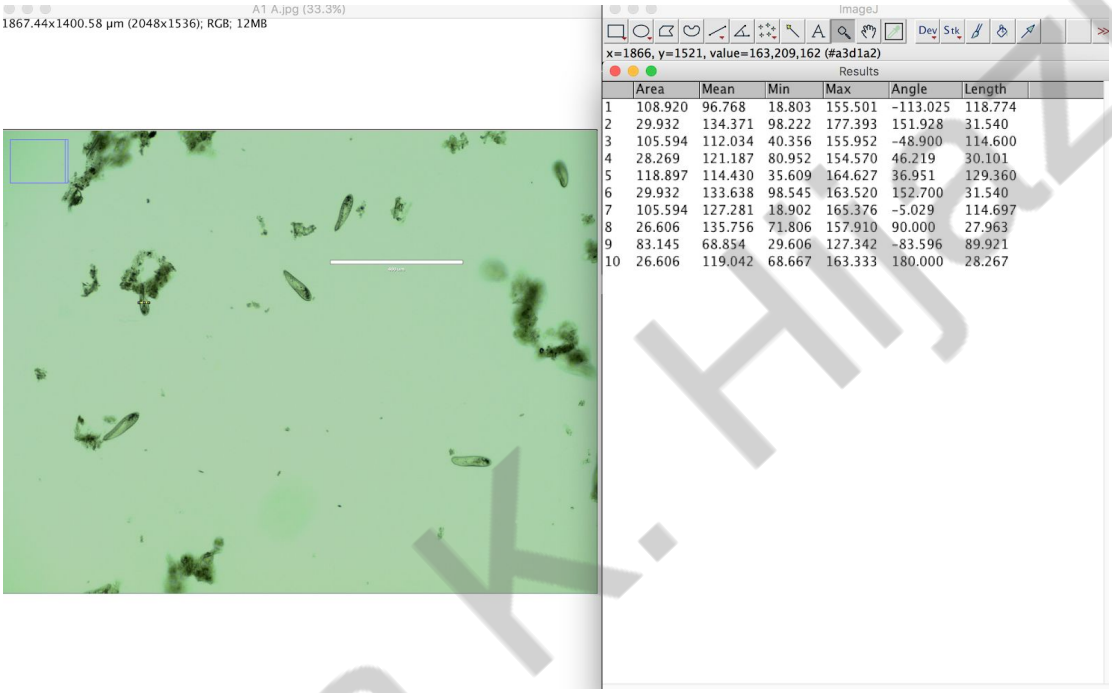
When making the stock solution of the Paraformaldehyde, it is also important to wear gloves at all times and avoid any skin or eye contact, inhalation and/or ingestion. According to the Material Safety Data Sheets (MSDS) of Paraformaldehyde, wash out thoroughly any area that comes in contact with the Paraformaldehyde and seek immediate medical attention (Paraformaldehyde, n.d.).

Results

Raw Data

In order to measure the width and length of the *Paramecium* in each respective sample, the software ImageJ was used (as shown in Figure 1). As seen in Figure 1.1, the length and width was measured alternatively for ten *Paramecium* and the resulting data was inputted into Microsoft Excel for further processing.

Figure 1 - Using the software ImageJ in order to measure the length and width of the *Paramecium* in each sample (um)



The averages were calculated using Microsoft Excel (as shown in Tables 1-4) for each setting.

Table 1 – Average length and width (µm) of the *Paramecia* in the control setting (no Paraformaldehyde)

	Length (µm)	Width (µm)
Paramecium 1	118.77	31.54
Paramecium 2	114.60	30.10
Paramecium 3	129.36	31.54
Paramecium 4	114.70	27.96
Paramecium 5	89.92	28.27
Paramecium 6	112.31	29.37
Paramecium 7	121.60	32.16
Paramecium 8	114.18	25.59
Paramecium 9	101.41	26.29
Paramecium 10	110.47	29.37
Average	**112.73**	**29.22**

Table 2 - Average length and width (µm) of the *Paramecia* with 0.0045% Paraformaldehyde

	Length (µm)	Width (µm)
Paramecium 1	112.8	28.92
Paramecium 2	115.32	25.93
Paramecium 3	118.17	28.71
Paramecium 4	90.16	28.47
Paramecium 5	128.38	26.12
Paramecium 6	105.67	27.88
Paramecium 7	122.34	39.01
Paramecium 8	117.39	29.4
Paramecium 9	107.47	25.86
Paramecium 10	115.23	35.74
Average	**113.293**	**29.604**

Table 3 - Average length and width (µm) of the *Paramecia* with 0.005% Paraformaldehyde

	Length (µm)	Width (µm)
Paramecium 1	130.49	31.95
Paramecium 2	124.63	32.65
Paramecium 3	103.96	20.36
Paramecium 4	116.99	26.39
Paramecium 5	117.62	31.27
Paramecium 6	113.1	28.01
Paramecium 7	128.03	30.77
Paramecium 8	111.47	30.55
Paramecium 9	118.16	28.53
Paramecium 10	116.09	31.43
Average	**118.054**	**29.191**

Table 4 - Average length and width (µm) of the *Paramecia* with 0.007% Paraformaldehyde

	Length (µm)	Width (µm)
Paramecium 1	106.17	24.74
Paramecium 2	126.93	31.73
Paramecium 3	122.67	30.14
Paramecium 4	113.28	34.07
Paramecium 5	117.75	25.08
Paramecium 6	121.57	37.05
Paramecium 7	118.32	27.19
Paramecium 8	105.62	28.9
Paramecium 9	267.33	64.2
Paramecium 10	259.36	60.75
Average	**145.9**	**36.385**

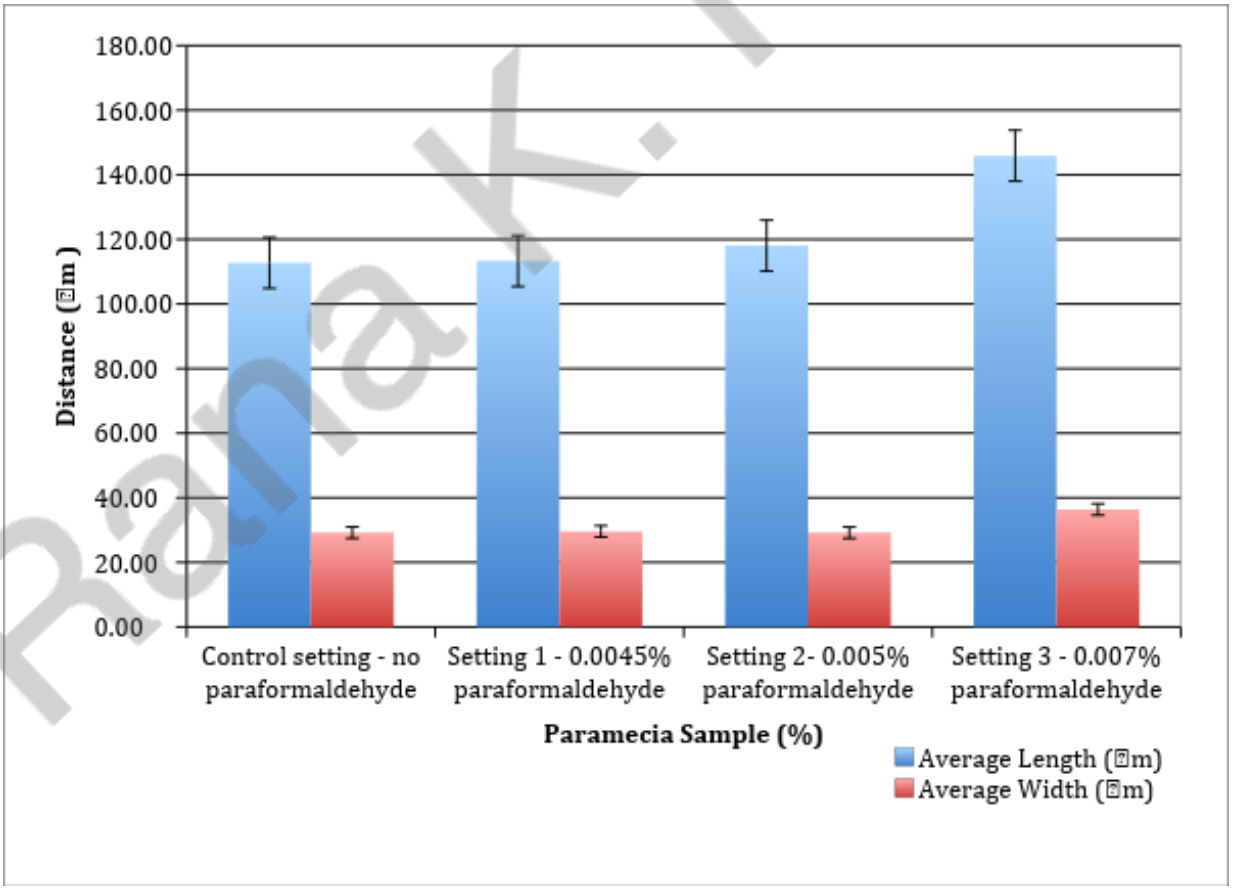
Processed Data

The average length and width of the *P. tetraurelia* in each *Paramecia* sample is summarized in Table 5. This was then plotted on a bar graph using Microsoft Excel, as seen in Figure 2.

Table 5 – Summary of the average length and width of the P. tetraurelia in each Paramecia sample

	Average Length (µm)	Average Width (µm)
Control setting - no paraformaldehyde	112.73	29.22
Setting 1 - 0.0045% paraformaldehyde	113.293	29.604
Setting 2- 0.005% paraformaldehyde	118.054	29.191
Setting 3 - 0.007% paraformaldehyde	145.9	36.385

Figure 2 - Graph of average length and width (µm) of the *P. tetraurelia* in each *Paramecia* Sample (error bars using Standard Error)



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