The Effect of Temperature on the Motility of *Euglena gracilis*

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ABSTRACT

This study investigated the effect of temperature on the motility of *Euglena gracilis*. A culture of *E. gracilis* was divided into four groups of test tubes and incubated at different temperatures (11°C, 17°C, 27°C, 34°C) for 60 mins. After incubation, the swimming speed of *E. gracilis* was measured under a compound microscope using a Dino-lite eyepiece camera. The results obtained, demonstrated a trend where the rate of forward swimming for *E. gracilis* increased as temperature increased until 27°C. The rate of forward swimming started to decline when the temperature surpassed 27°C. A one-way analysis of variance (ANOVA) resulted in a p-value of 1.64×10^{-8} indicating statistically significant differences in the results. The increase in speed at higher temperatures up to 27°C may be attributed to increased metabolic activity, since 27°C falls in the optimal temperature for *E. gracilis* growth (Buetow, 1962). The decrease in speed above optimal temperature may have been due to denaturation of the metabolic enzymes, hindering structures like the flagella (Humphries, 2013). Our prediction was met as the speed increased as temperature increased until *E. gracilis'* optimal temperature, at which point the speed began to decline beyond the optimal temperature. We reject the null hypothesis that there would be no change in motility at varied temperatures and support the alternative hypothesis that there would be a change in motility.

II. INTRODUCTION

Euglena gracilis is a single-celled mixotrophic protist used widely throughout scientific research to address fundamental questions in biochemistry, ecology, and cellular and molecular biology (Schwartzbach & Shigeoka, 2017). It is a particularly important organism due to its role in freshwater ecosystems. Previous research has shown that protists like *E. gracilis* occupy a key role in the ecological food web, as they form the base of many freshwater ecosystems (Chittenden et al., 2010). In particular Chittenden et al. (2010) found that *E. gracilis* are an important food source for salmon fry in the Pacific Northwest. As such, *E. gracilis* make up a significant portion of the foundation of the freshwater food web and are responsible for feeding and sustaining organisms at all higher trophic levels.

An organism's rate of swimming determines the frequency at which it encounters food and predators within its environment, thus, an organism's ability to move is a vital component of its fitness (Beveridge, Petchey, & Humphries, 2010). Therefore, environmental factors that affect an organism's motility can have a significant impact on its fitness and its interactions within the ecological food web (Beveridge et al., 2010). Temperature in freshwater ecosystems can vary greatly due to seasonal conditions, depth levels, and climate change (Beveridge et al., 2010; Woodward, Perkins, & Brown, 2010). These variations in temperature can impact an array of biological processes including metabolic rate, reproductive behaviours and notably, motility (Woodward et al., 2010). Temperature dependency of swimming speed has been extensively studied in some protists (e.g. Shortess, 1942; Glaser, 1924), but the effects of temperature changes on the rate of swimming of *E. gracilis* are not fully understood.

The objective of this study is to determine the effect of temperature on the motility of *E. gracilis*. Previous research has shown that the motility of protists increases with temperature, until it reaches a maximum speed, and decreases rapidly as temperatures go beyond this point. We hypothesize that the average rate of swimming of *E. gracilis* will change as temperature changes. The optimal temperature range for growth of *E. gracilis* has been determined to be 25°C - 30°C (Buetow, 1962). We predict that the rate of swimming of *E. gracilis* will increase up until it reaches the upper limit of this temperature range. Subsequently, as the temperature conditions surpass this maximum value, the rate of swimming of *E. gracilis* is expected to decline. We tested our hypothesis by measuring and comparing the rate of swimming of *E. gracilis* at four different temperature conditions: 11°C, 17°C, 27°C, and 34°C.

III. METHODS & MATERIALS

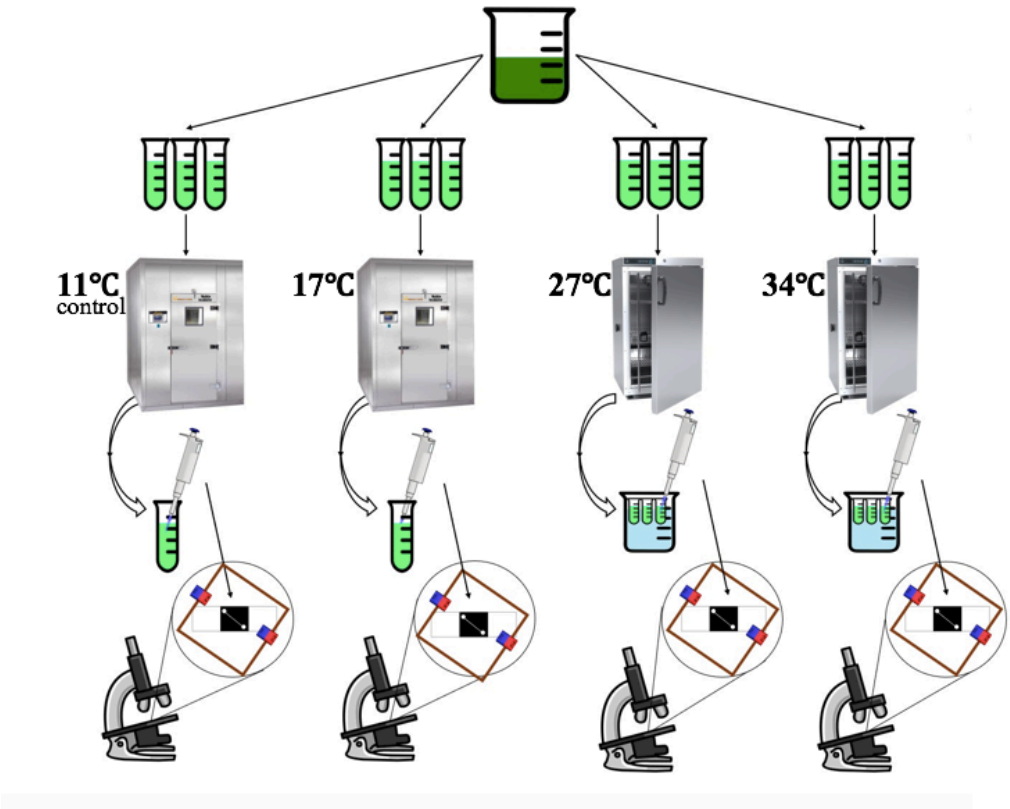

Figure 1. Flow chart outlining general procedure. Initial culture was divided into three groups of four and incubated at four temperature treatments for 1 hour. Subsequently, we transferred 5µL of the culture on to track slides and imaged the movement using Dino-Lite software under a compound microscope.

Culture and Set up:

We obtained 75mL of *E. gracilis* grown in UTEX culture medium from the University of Texas (Appendix 1) and 20mL of *E. gracilis* free UTEX culture medium. Subsequently, we prepared four groups of three test tubes, where each group corresponded to one of our temperature treatments (Figure 1). We then dispensed 5mL aliquots of the *E. gracilis* culture into every test tube. We incubated the four groups at each of our temperature conditions: 11°C, 17°C,

27°C and 34°C. The 11°C treatment served as our control, since it falls within the range of average water temperatures of streams in the Pacific Northwest when salmon fry are feeding (Moore, 2006; Wild Fish Conservancy, 2015). We incubated the test tubes at the given temperatures for one hour before observing motility. The 11°C and 17°C incubators were walk-in incubators and allowed us to observe motility within the incubator, thereby ensuring the temperature was kept consistent. However, the 27°C and 34°C incubators were smaller and had no room for walk-in, as such, we removed the samples from the incubators to observe *E. gracilis*. To ensure that the temperature was kept consistent for these treatments, we placed the samples in beakers of water — incubated alongside the cultures at the respective temperature treatments — while we observed the motility of *E. gracilis* from each test tube (Figure 1). We incubated track slides (Figure 2) alongside the cultures to ensure the difference in temperature between the samples and the slides did not impact *E. gracilis* motion.

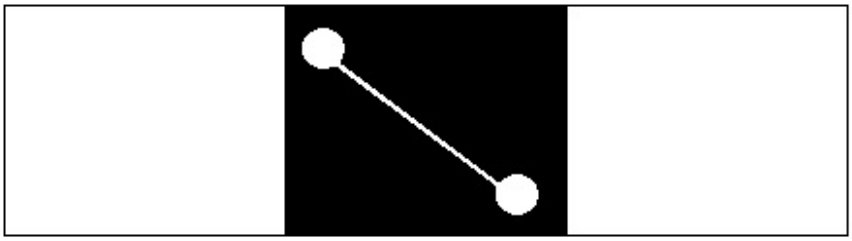

Figure 2. Track slides consisting of two chambers connected by a miniscule track, designed by Gill, G., Park, J. J., & Rathbone, B. (2012).

Track slides:

Under normal conditions, we observed that *E. gracilis* displayed erratic motion that would be difficult to track and measure. Thus, to simplify the observation of *E. gracilis*, and ensure it moved along a predictable path, we used microscope track slides designed by Gill,

Park, & Rathbone (2012). The slides consisted of two chambers carved out of black wax, connected by a miniscule track.

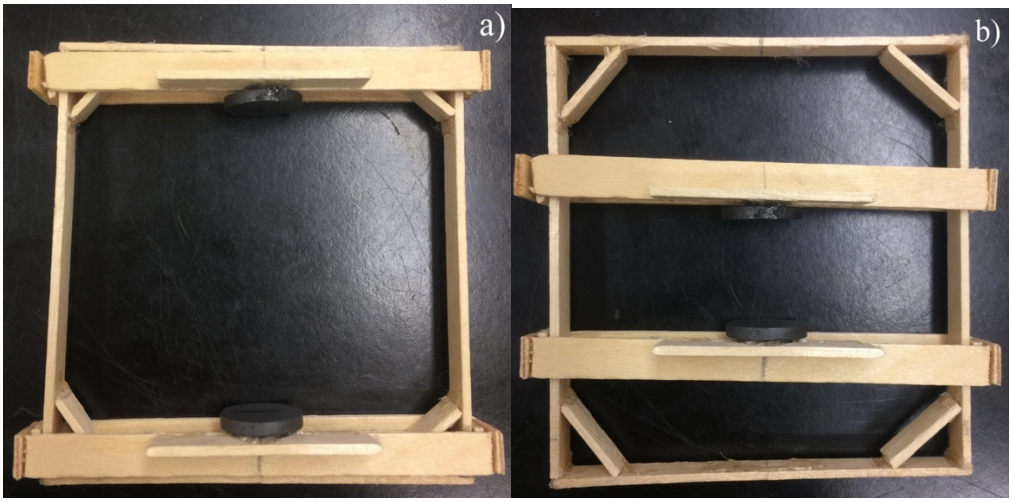

Figure 3. Magnetic apparatus with adjustable magnet sliders. a) Magnet sliders pushed to opposite ends, displaying maximum permissible distance between magnets. b) Magnet sliders pushed to intermediate position, with decreased distance between magnets.

Construction of Magnetic Apparatus:

Ascoli et al (1978), determined that *E. gracilis* orient themselves parallel to the direction of a magnetic field. Thus, magnets can be used to control the erratic swimming motions of *E. gracilis*, and allow for easier measurement of motility. We designed a magnetic apparatus to orient *E. gracilis* along the magnetic field (Figure 3). We adhered together four wooden sticks, each 10cm in length, using a Stanley Bostitch Heavy Duty Glue Gun. Subsequently, we attached two circular magnets (3mm in thickness and 2cm in diameter) to adjustable rails on either side of a 10cm square. To ensure the formation of a magnetic field, we placed the magnets in an orientation where the North and South magnetic poles faced one another. We used this apparatus to control the orientation of *E. gracilis* thereby simplifying our observation and analysis of their swimming.

Observing motility:

We set up a Zeiss Axiostar compound microscope using Köhler illumination following the steps outlined in the Biol 342 lab manual (Department of Botany, 2018). We inserted a Dino-

lite eyepiece camera (model am4023X-R4) into the microscope eyepiece slot and used DinoXcope software to calibrate and focus the camera to the field of view. Once the one hour incubation period was complete, we removed the cultures from their respective incubators and prepared them for viewing under the microscope. Cultures incubated at 27°C and 34°C were kept in water baths to ensure their temperature was kept consistent. We pipetted 65 µL UTEX medium into one chamber of the track slide and 5 µL of *E. gracilis* culture into the other chamber. Subsequently, the track slide was placed onto the microscope, and the magnetic apparatus was placed atop it. We used the Dino-lite eyepiece to record videos observing the motility of *E. gracilis* for each replicate at our four temperature treatments.

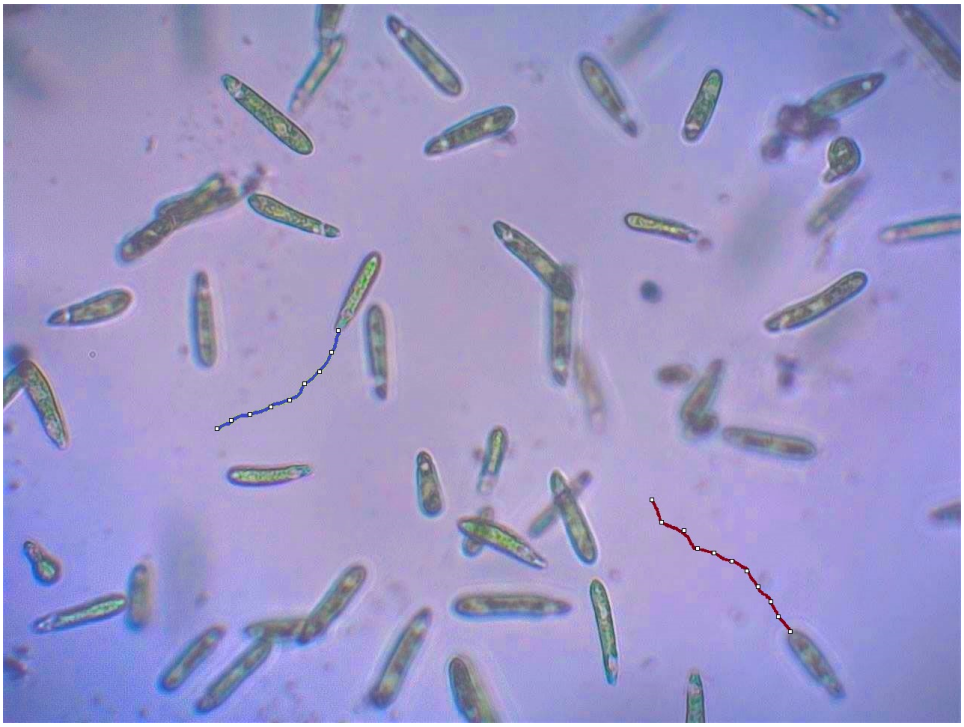

Figure 4. Tracked movement of *E. gracilis*, represented by blue and red lines, viewed using Dino-Lite camera and traced using Manual Tracking Plugin in ImageJ.

Video Analysis:

We used Adobe Photoshop to break down the videos of each replicate into a frame-by-frame image sequence, consisting of 500 frames. From these, we randomly selected twenty-five frames and exported them onto ImageJ. We then selected ten *E. gracilis* cells based on whether they remained within the field of view in all 25 frames. We tracked these 10 pseudo-replicates in ImageJ using the Manual Tracking Plugin (Figure 4). We then averaged the rates of swimming of the ten organisms to determine the rate of swimming for each replicate. Subsequently, we averaged the rates of swimming of the three replicates to determine the average rate of swimming at each temperature treatment (Figure 5).

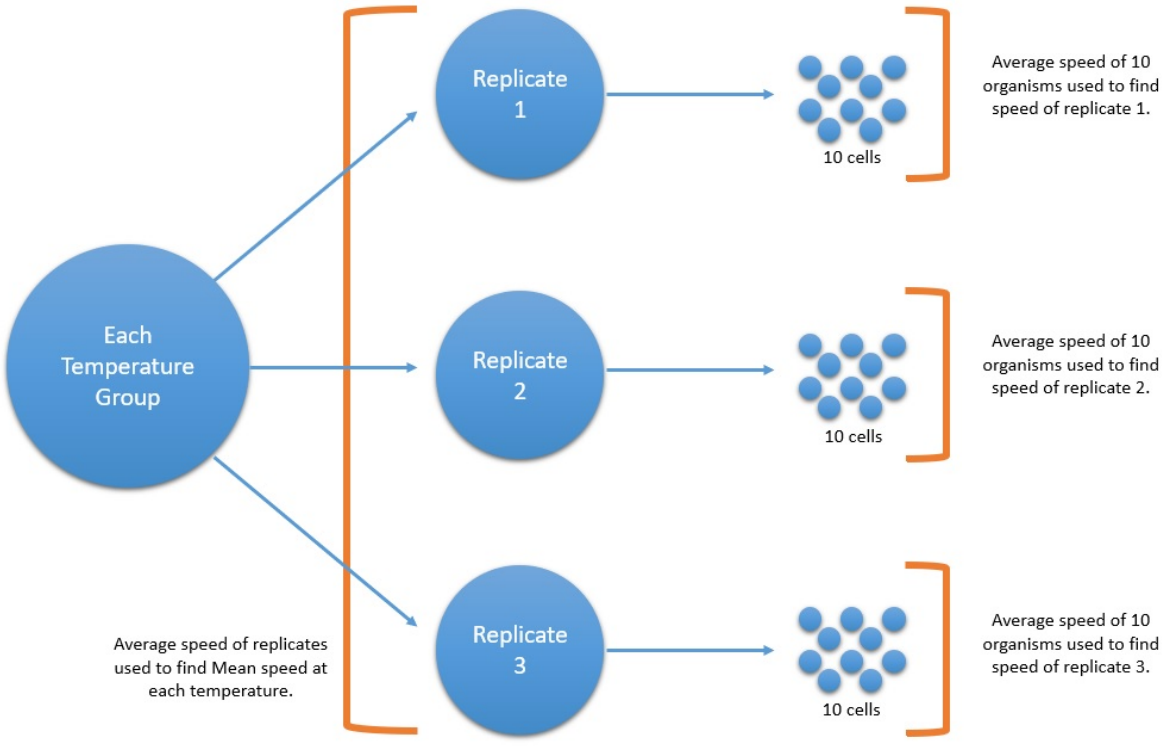

Figure 5. Chart detailing the process of determining the rate of swimming of each replicate at each temperature treatment.

Data Analysis

After calculating the average rate of swimming at each temperature treatment, we performed a one-way ANOVA to determine if there was a difference between the average rates at each temperature. We determined that there was a significant difference. Subsequently, we used a Dunnett's test to compare the differences in motility between our treatments (17°C, 27°C and 34°C) and our control (11°C). We used GraphPad Prism 8 software to analyze our data.

IV. RESULTS

Differences in *E. gracilis* motility were observed at the different temperature treatments. At the lowest temperature treatment (11°C), very few of the *E. gracilis* cells in the field of view were motile; the majority were either stationary or moving back and forth in one place. As we moved to higher temperature treatments, we observed that a larger proportion of organisms within the field of view were active and motile. A trend of increasing swimming speed with increasing temperature was observed. An average swimming rate of 19.22 μm/sec was observed at 11°C. The swimming rate increased to 80.44μm/sec at 17°C and reached a maximum of 95.63μm/sec at 27°C. When the temperature reached 34°C and surpassed the optimal temperature range for growth, swimming decreased to a rate of 80.73μm/sec. The average rate of swimming and 95% confidence intervals at 11°C, 17°C, 27°C and 34°C are: 19.22±6.9 μm/sec , 80.44±7.8 μm/sec , 95.63±13.1 μm/sec and 80.73±3.7 μm/sec respectively, as shown in Figure 6.

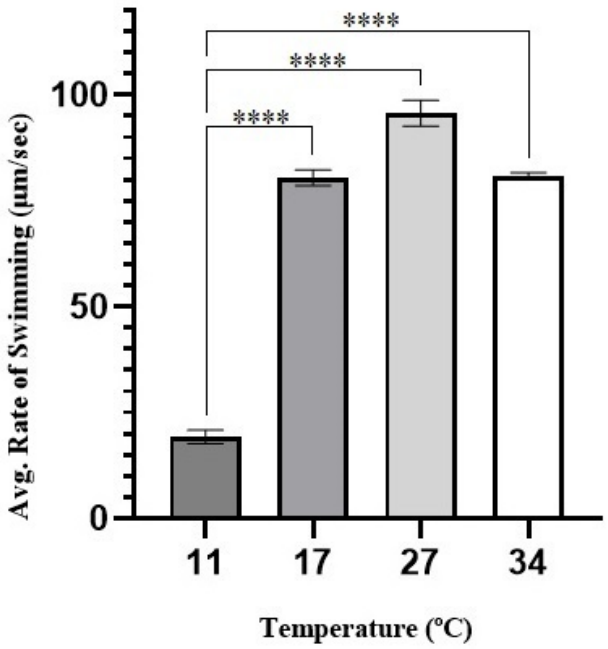

Figure 6. Bar graph representing the rate of swimming at each temperatures, as exhibited by *E. gracilis*. The mean swimming rate, measured from 30 *E. gracilis* cells at each temperature, is 19.22µm/sec, 80.44µm/sec, 95.63µm/sec, 80.73µm/sec, respectively from left to right. Error bars represent standard error. One-way ANOVA analysis gave an f-value of 292.157 which is greater than the F-critical value 4.06618, and p-value of 1.64×10^{-8} which is smaller than the alpha value of 0.05. Degrees of freedom between groups is 3, while degrees of freedom within groups is 8. Swimming speeds at all temperature treatments are statistically and significantly different from the control (11°C) as denoted by the asterisks. **** $p \leq 0.0001$.

A p-value of 1.64×10^{-8} and an F-value of 292.157 were obtained from the one-way ANOVA, these are less than the α value (0.05) and greater than the F-critical value (4.06618) respectively, indicating a significant difference between the means observed. The results of the Dunnett's multiple comparisons test indicate that the difference between the mean swimming rate of the control group (11°C), and the mean swimming rate of the experimental treatments (17°C, 27°C and 34°C) are statistically significant.

V. DISCUSSION

The results indicated that there was a significant difference in the rate of swimming of *E. gracilis* at 17°C, 27°C and 34°C when compared to the control (11°C). Based on our results, we reject the null hypothesis that *E. gracilis* would experience no change in motility at varied temperatures and support the alternative hypothesis that the average rate of swimming of *E. gracilis* would change as the temperature changed. We predicted that the rate of swimming of *E. gracilis* would increase up until it reached the upper limit of its optimal temperature range (25°C - 30°C). Subsequently, we predicted that as the temperature conditions surpassed this maximum value, the rate of swimming of *E. gracilis* would decline. Our predictions were met since *E. gracilis* exhibited the highest rate of swimming at the centre of its optimal temperature range; 27°C. As the temperature condition increased beyond this point (34°C) the rate of swimming of *E. gracilis* decreased (Figure 6). This noticeable bell curve like trend is similar to temperature dependent locomotion observed in *Peranema* which are structurally similar to *E. gracilis* (Shortess, 1942). Notably, we observed that *E. gracilis* exhibits low motility at cold temperature conditions (11°C).

The initial rise in motility for *E. gracilis* could be attributed to the viscosity of the UTEX culture medium. It is known that *E. gracilis* uses its flagella for locomotion, when the flagella moves within a fluid, it experiences viscous drag (Humphries, 2013). In systems operating at low Reynolds numbers, this drag force dominates motility dynamics and swimming speeds are heavily dependent on the viscosity of the solution (Beveridge et al., 2010; Humphries, 2013). Additionally, the viscosity of a liquid is known to decrease as temperature increases (Humphries, 2013). Thus, *E. gracilis'* increased motility at higher temperatures could be due to a lower

viscous drag on the flagella which would increase the speed of the organism (Humphries, 2013). Moreover, the initial increase in motility of *E. gracilis* could also be attributed to the effect of increasing temperatures leading to an increase in the reaction rates of the biological processes that underlie flagellar locomotion (Beveridge et al., 2010). Furthermore, the decrease in motility observed at 34°C could be due to the denaturation of important metabolic enzymes that impact the flagellar motion of *E. gracilis* (Humphries, 2013). Additionally, the decreased motility at higher temperatures could also be attributed to temperature-dependent changes in enzyme efficiencies that impact the molecular mechanisms of flagellar motion. In his study, Humphries (2013) states that the rate of flagellar beating is proportional to the amount of energy available to an organism. This energy is characterized by the hydrolysis of energy transfer molecules such as ATP, which in protists is regulated by a temperature-dependent diffusion process (Humphries, 2013). The delivery of this energy supply could be impacted at high temperatures, resulting in reduced energy available for locomotion.

Global temperatures continue to rise at unprecedented rates as climate change takes effect (Woodward et al., 2010). Freshwater streams in particular are highly vulnerable to these temperature effects due to their fragmentation and relative isolation within a terrestrial landscape (Woodward et al., 2010). We can extrapolate that organisms residing within this streams will be significantly impacted by these temperature effects. In particular, *E. gracilis*' will likely experience an increase in swimming speed as temperatures continue to increase. This increase in motility, would likely benefit *E. gracilis* by allowing it to move to nutrient rich areas, and obtain prey before other phytoplankton (Ross and Sharples, 2007). This increase in motility could also increase *E. gracilis'* ability to evade predators, thereby increasing its fitness (Visser, 2007).

However, the increased fitness of *E. gracilis* could have negative impacts on the ecosystem as a whole. Previous studies have determined that protists like *E. gracilis* form the base of the ecological food web. They are responsible for feeding organisms at higher trophic levels, in particular, they are a key source of nutrition for salmon fry in the Pacific Northwest (Chittenden et al., 2010). Thus, increased predator evasion by *E. gracilis* could decrease the amount of prey available to salmon fry and negatively impact the survivability of salmon in freshwater ecosystems.

Potential sources of uncertainty could be the usage of different incubators for certain temperature treatments. The incubators used for 11°C and 17°C were walk-in incubators, while those used for 27°C and 34°C were smaller chambers, that we could not walk in to. Thus, the set-up and video recording of *E. gracilis* was done at room temperature for the 27°C and 34°C treatments, since the culture had to be taken out of the incubators and placed onto the microscope to be observed. Although we tried to mitigate the temperature transition by way of placing the culture test tubes in incubated water baths and incubating the track slides to decrease potential heat loss, the temperature of the culture could have varied after it was removed from the incubators. Another potential source of error could be that due to time constraints, the experiment was conducted on two different days. In addition, a different culture of *E. gracilis* was used on the second day which could also potentially contribute to uncertainty.

Future studies should be conducted to examine trends in *E. gracilis* motility at temperature points that lie between our selected treatments. Additionally, further research should be done to examine why magnetic fields influence *E. gracilis* and to what degree the magnetic field governs their orientation. Moreover, further examination must be conducted to determine

the exact mechanisms that underlie flagellar motility in *E. gracilis* and how they may be influenced by variations in temperature.

VI. CONCLUSION

The swimming speed of *E. gracilis* was studied at various temperatures to examine the effect on their motility. We observed an increasing trend from the *E. gracilis* observed at 11°C to a more motile group at 27°C. At 34°C, the speed decreased compared to 27°C. The results found reject the null hypothesis of no change in movement speed due to varied temperatures. Our results also support our prediction that the motility increased from 10-30°C and dropped off after their maximum growth temperature range. The reason why there is a bell curve like trend could be due to the increased temperature assisting reaction rates and warming up the medium in which they swim in that reduces friction on the flagella. After the optimal temperature range was surpassed, the increased temperature could be high enough to denature enzymes associated with the flagellar movement. The denaturing of these enzymes could be responsible for the decrease in motility after exceeding their optimal temperature range.

VII. ACKNOWLEDGMENTS

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IX. APPENDIX

#	Component	Amount Stock Solution Concentration
1	Sodium acetate (Fisher BP 333)	1 g/L
2	Beef extract (Sigma B-4888)	1 g/L
3	Tryptone (Sigma T 9410)	2 g/L
4	Yeast extract (Bacto, Difco)	2 g/L
5	CaCl2·2H2O (Sigma C-3881)	0.01 g/L

Table 1. Recipe from UTEX Culture Collection of Algae from the University of Texas: 950mL of deionized water to make 1 litre of medium.

Temperature: 11°C

Sample	PR 1	PR 2	PR 3	PR 4	PR 5	PR 6	PR 7	PR 8	PR 9	PR 10	Average	Average Speed @ 11°C
R1	27.509	17.639	33.932	27.512	20.39	20.969	21.336	17.444	18.138	19.27	22.41	
R2	14.532	21.672	15.326	23.629	12.28	17.018	13.785	32.88	12.414	11.999	17.55	**19.21666667**
R3	24.911	13.582	14.41	25.902	13.651	26.967	16.112	18.326	10.536	12.484	17.69	

Temperature: 17°C

Sample	PR 1	PR 2	PR 3	PR 4	PR 5	PR 6	PR 7	PR 8	PR 9	PR 10	Average	Average Speed @ 17°C
R1	70.853	77.421	57.831	76.184	83.671	97.779	75.135	112.479	77.06	70.87	79.87	
R2	59.848	77.539	49.618	103.17	51.646	62.579	80.355	146.662	66.676	78.166	77.64	**80.44**
R3	113.067	94.609	132.442	14.759	20.166	91.364	118.584	139.494	86.773	26.818	83.81	

Temperature: 27°C

Sample	PR 1	PR 2	PR 3	PR 4	PR 5	PR 6	PR 7	PR 8	PR 9	PR 10	Average	Average Speed @ 27°C
R1	92.231	82.603	72.982	119.377	87.375	95.219	67.406	47.897	121.123	113.895	90.0108	
R2	153.382	46.308	179.465	86.111	79.34	111.278	65.885	67.936	46.891	127.895	96.45	**95.63026667**
R3	74.475	144.218	60.775	126.178	95.081	76.666	130.463	120.021	134.239	42.14	100.43	

Temperature: 34°C

Sample	PR 1	PR 2	PR 3	PR 4	PR 5	PR 6	PR 7	PR 8	PR 9	PR 10	Average	Average Speed @ 34°C
R1	84.916	104.533	69.971	58.047	70.998	52.182	96.468	96.743	79.43	108.133	82.04	
R2	105.085	56.819	75.217	62.242	96.702	81.796	66.37	77.446	81.733	107.244	81.07	**80.73333333**
R3	59.686	81.63	76.742	80.047	61.288	66.938	107.342	97.756	78.647	80.844	79.09	

Table 2. Raw Data collected for the *E. gracilis* swimming speed at each temperature treatment using the ImageJ software. "R#" represents replicate number and "PR#" refers to pseudo-replicate number.