The effect of temperature on the population size of *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila is a ciliated protozoan that inhabits freshwater ponds and is commonly used as a model organism (Cassidy-Hanley, 2012). Like all model organisms, the growth medium and temperature are two important factors. Taking that into account, the purpose of our experiment was to determine the optimal temperature for the growth of *T. thermophila* by observing the relationship between an increase in temperature and population size. To perform this study, we incubated *T. thermophila* cultures at 11^{o}C, 17^{o}C, 30^{o}C and 40^{o}C with three replicates per temperature. We collected mean population sizes every 2 hours in a 10-hour period as well as at 24 hours of incubation for further data analysis and observation. After 24 hours of incubation, we found that the mean population size of *T. thermophila* incubated at 40^{o}C was significantly larger than the mean population sizes at 11^{o}C, 17^{o}C and 30^{o}C. This result allows us to reject our null hypothesis and provide support for our alternate hypothesis: stating that an increase in temperature increases the population size of *T. thermophila* and that the optimal temperature resides close to 40^{o}C.

Introduction

Tetrahymena thermophila is a ciliate protozoan commonly used as a model organism in fields such as biology and biomedicine (Cassidy-Hanley, 2012). The growth rate of *T. thermophila* is based on a number of factors, but the two most significant factors are the growth medium and temperature (Cassidy-Hanley, 2012).

Temperature is an important factor on the growth rate of *T. thermophila* because it is correlated with the speed of various key metabolic components. For example, an increased temperature results in a more efficient transport of RNA during protein synthesis, leading to faster transcription of cilia (Nägel and Wunderlich, 1976). Also, an increased temperature is correlated with increased efficiency of phagocytosis due to the use of cilia in transport and

uptake of foods (Luan *et al.* 2012). Ultimately, this increased speed in metabolic function leads to a faster transition through the growth cycle of *T. thermophila*, which in turn results in a faster rate of reproduction.

However, *T. thermophila* can only tolerate temperatures up to a certain limit before growth rate starts declining (Frankel and Nelsen, 2001). Proteins, which are essential to life, start to denature when exposed to temperatures which are too high (Daniel *et al.* 2013). For example, Frankel and Nelson (2001) state that the oral apparatus, which is used in food vacuole formation in *T. thermophila*, can have abnormalities when exposed to temperatures past its optimal point.

Nonetheless, *T. thermophila* are known to grow in a wide range of temperatures. Cassidy-Hanley (2012) states that the optimum doubling time of 2 hours for *T. thermophila* is best achieved at temperatures of 32°C with an upper limit near 40°C. However, Frankel and Nelson (2001) found that *T. thermophila* grew more efficiently at temperatures closer to 40°C rather than 30°C.

In our experiment, we measured the effects on the population size of *T. thermophila* when exposed to four different temperatures, 11°C, 17°C, 30°C and 40°C, over a period of 24 hours. The objective of our study is to see the responses of *T. thermophila* as temperature is increased towards 40°C, since there is variation within the literature. We came up with the following hypotheses for our study:

H_a: Increasing the temperature will increase the population size of *Tetrahymena thermophila.*

H_o: Increasing the temperature decreases or has no effect on the population size of *Tetrahymena thermophila.*

This experiment is important because it attempts to clear up the misconceptions and confusion about the optimal temperature for the growth of *T. thermophila* that are brought up by the disagreements within the literature. Our findings may be helpful for future experiments or studies concerning the growth rate of *T. thermophila*.

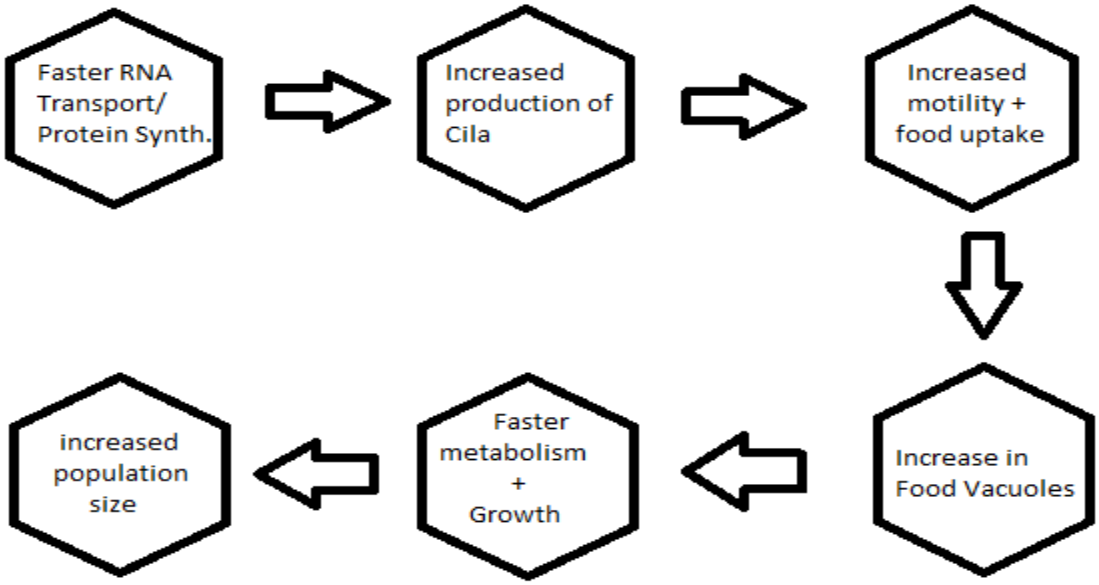

Figure 1. Biological processes of *T. thermophila* at optimal temperature

Methods

In preparation of our starting cell culture, we first determined the initial concentration of cells in an undiluted stock solution of *T. thermophila* grown in an SSP medium *(*see results section for calculations). From this concentration, we determined the volume required to prepare a large sample of 5000 cells/mL dilution (Figure 2). We pipetted 6 mL of

this diluted sample into 12 separate test tubes. From the 12 tubes, we prepared three replicates for each of the following four incubation temperatures: 11 ^{o}C, 17 ^{o}C, 30 ^{o}C and 40 ^{o}C. The replicates at 11 ^{o}C, 17 ^{o}C, 30 ^{o}C were kept in incubators whereas the replicates cultured at 40 ^{o}C were kept in a hot water bath due to temperature restrictions of the incubators. We covered the test tubes racks in each incubator with a cardboard box to maintain constant light intensity for all treatment levels (Figure 3). We counted cells every 2 hours for a total period of 10 hours, using the same clock as our timer for all replicates. We also counted cells for each replicate after 24 hours of incubation to further analyze and compare the mean population sizes.

Figure 2. Preparing the diluted stock solution of *Tetrahymena thermophila.*

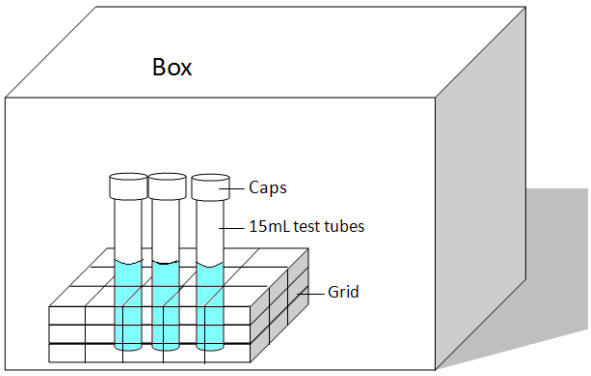

Figure 3. Experimental set-up in incubator. Three 15mL test tubes containing *T. thermophila* sample kept under a box to ensure constant light intensity.

We calculated the population size by counting cells on a Max Levy Fuchs haemocytometer grid (Figure 4) under an Axiostar compound microscope set to 10X magnification (100X total magnification). Before counting the cells, we mixed 50 µL of the sample with 5 µL of glutaraldehyde in a microcentrifuge tube. We mixed the sample by pipetting up and down to ensure the cells were evenly distributed and pipetted 15 µL of the sample onto the haemocytometer to fully cover the grid. This was done for all 3 replicates of each specific incubation temperature at our pre-determined data collection times. When counting the cells on the grid of the haemocytometer, we only counted a total of 5 cells that were touching the top and left boundaries of each square. To ensure consistent data, we counted the same squares for each replicate sample. Finally, we determined the population density by finding the mean cell counts per replicate and multiplying it by the respective dilution and fixative correction factor.

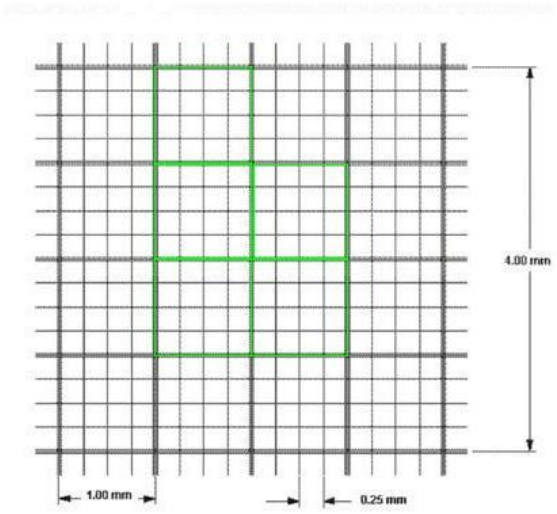

Figure 4. A diagram of Max Levy Fuchs Rosenthal haemocytometer was used to count *T. thermophila* (Cobey *et al.* 2013)

Statistical methods of analysis used were 95% confidence intervals for each mean population size at each incubation temperature. The overlap or non-overlap of the confidence intervals determined if we rejected or failed to reject the null hypothesis. To further strengthen our results, a t-test was performed to determine if mean population sizes between two temperatures significantly differ from each other.

Results

In Table 1, the mean population sizes for all temperatures with 95% confidence intervals at all time intervals are presented. The population size for all samples grew over time, and the general trend is that samples at higher temperatures tended to have higher population sizes. The trend was more obvious at later time intervals.

Table 1. Average population size of *T. thermophila* grown at 11 ^{o}C, 17 ^{o}C, 30 ^{o}C and 40^{o}C counted every two hours, with 95 confidence intervals.

Population size (# of cells/mL)		Temperature							
		11℃	(95 C.I.)	17℃	(95 C.I.)	30℃	(95 C.I.)	40℃	(95 C.I.)
Time for growing (hr)	0	5000	± 1132	5000	± 1132	5000	± 1132	5000	± 1132
	2	7333	± 2875	9167	± 4713	9533	± 1901	7700	± 1245
	4	6233	± 719	12833	± 5613	13200	± 1245	8433	± 719
	6	13200	± 4488	10633	± 5749	20533	± 4371	15033	± 719
	8	7333	± 719	12467	± 4001	17233	± 8473	21267	± 4371
	10	7700	± 3734	23283	± 3428	13017	± 5883	24200	± 2156
	24	5500	± 2244	12833	± 5783	48217	± 3593	58667	± 3752

We focused on the mean population sizes at 11°C, 17°C, 30°C and 40°C after 24 hours of incubation (Figure 5). At this time interval, population size increased with an increase in temperature. The 95% confidence intervals for the highest incubation temperatures of 40°C and 30°C do not overlap with any other means at this time period.

The mean population size at 11°C and 17°C are the only confidence intervals that overlap after 24 hours of incubation. The replicates grown at 40°C have the highest population size and the replicates incubated at 11°C have the smallest population size.

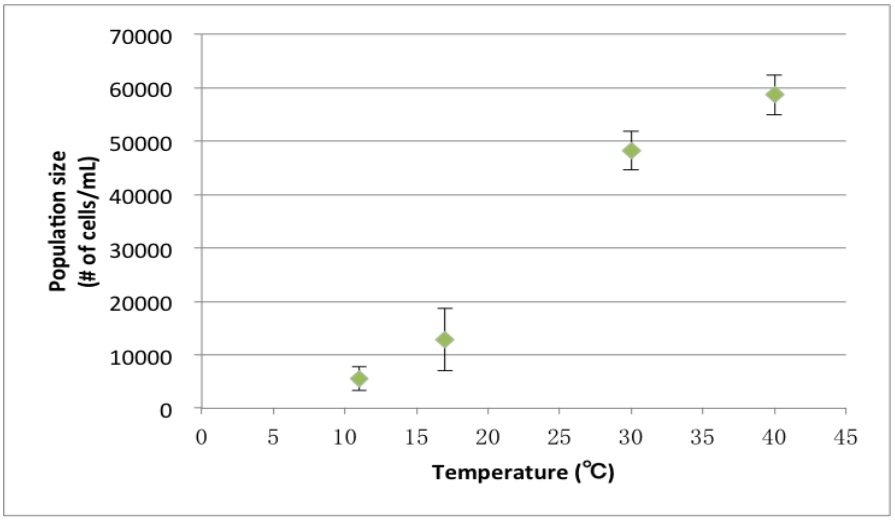

Figure 5. Average population sizes (# of cells/mL) of *Tetrahymena thermophila* incubated at 11 ^{o}C, 17 ^{o}C, 30 ^{o}C and 40 ^{o}C for a duration of 24 hours. Error bars show 95% C.I. n=3.

Furthermore, in order to confirm whether two mean population sizes are significantly different, we performed the t-test for any two experimental groups without 95% confidence intervals bars overlapping at durations of 10 hours and 24 hours. T-test results for samples incubated for 10 hours (Table 2) confirmed that populations growing at 40 ^{o}C had significantly larger number of cells than those at 11 ^{o}C and 30 ^{o}C. From results shown in Table 3, experimental groups at 40 ^{o}C had significantly greater population sizes than all the rest after 24 hours of incubation.

Table 2. T-test: after 10 hours of growing, any two populations without error bar overlapping were significantly different. Degree of freedom = 4. Sample size = 3. Two sided 95% P value = 2.776. Negative values of t represent temperature 2 had greater population size than temperature 1; vice versa.

Temperature 1, X_1	30℃	11℃	17℃	11℃
Temperature 2, X_2	40℃	40℃	30℃	17℃
t	-4.948	-10.607	4.180	-8.521
Significant or not	Yes	Yes	Yes	Yes

Table 3. T-test: after 24 hours of growing, any two temperatures without error bar overlapping were significantly different. Degree of freedom = 4. Sample size = 3. Two sided 95% P value = 2.776. Negative values of t represent temperature 2 had greater population size than temperature 1; vice versa.

Temperature 1, X_1	30℃	17℃	11℃	17℃
Temperature 2, X_2	40℃	40℃	40℃	30℃
t	-5.576	-18.430	-33.712	-14.405
Significant or not	Yes	Yes	Yes	Yes

We observed that after 24 hours of incubations, the *T. thermophila* sample in the test tubes kept at 40°C and 30°C were cloudy in appearance (Figure 6). The test tubes at treatment levels 11°C and 17°C were not. The 40°C test tube was slightly more cloudy than the test tube incubated at 30°C.

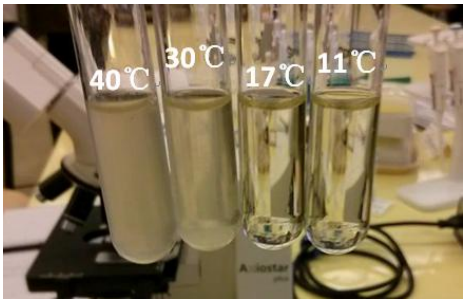

Figure 6. *T. thermophila* samples from left to right: 40 ^{o}C, 30 ^{o}C, 17 ^{o}C and 11 ^{o}C after 24 hours of incubation.

Sample Calculations:

1. To calculate corrected number of cells per mL
Correction factor = (volume of cell solution + volume of fixative) / volume of cell solution

Cell concentration = average number of cells in 5 squares x (5 x 10^3) x correction factor

e.g., Mixing 50μL cell solution and 5μL fixative. The correction factor would be:

$$\frac{50\mu L+5\mu L}{50\mu L} = 1.1$$

Numbers of cells in 5 squares respectively are: 16, 12, 8, 9, 11.
The cell concentration would be:

$$\alpha=\frac{16+12+8+9+11}{5} \text{x } (5 \text{ x } 10^3) \text{ x1.1 } = 61.6 \text{ x } 10^3 \text{ cells/mL}$$

2. To determine how much cultural solution is needed to add to the initial culture in order to make a 5000 cells/mL *T. thermophila* culture.

Initial cell concentration = α (calculated as 1. above)
Total volume of initial culture needed to make a 10,000 cells/mL culture per tube = V (unknown)
Volume for each tube to start growing = 6mL

$$V = \frac{5000 \text{ cells/mL x } 6 \text{ mL}}{\alpha}$$

Total required volume for 12 replicates = 6mL x 12 = 72mL
Volume of cultural medium needed, β = 72mL- 12 x V

e.g.,. α = 61.6 x 10^3 cells/mL

$$V = \frac{5000 \text{ cells/mL x } 6 \text{ mL}}{61.6 \text{ x } 10^3 \text{ cells/mL}} = 0.487 \text{ mL}$$

β = 72mL - 12 x 0.487mL = 66.156mL

3. To calculate the 95% C.I., we used the following equation:

$$s^2 = \frac{\sum(x-\bar{x})^2}{n-1} \qquad \text{C.I.} = \bar{x} \pm 1.96\frac{s}{\sqrt{n}}$$

x: population size
x□: mean population size
s: standard deviation
n: number of replicates

e.g., Replicates at 40°C after a duration of 24 hours had population sizes (cells/mL): x =

58300, 62150, 55550.

Therefore, x□ = $\frac{58300 + 62150 + 55550}{3} \approx 58667$

$$s^2 = \frac{(58300-58667)^2 + (62150-58667)^2 + (55550-58667)^2}{(3-1)} \approx 10989225$$

95 C.I. = 1.96 x $\sqrt{\frac{10989225}{3}}$= 3752

4. T-test calculations to determine whether two temperatures had significantly different population sizes, by using the following equations.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \qquad s^2 = \frac{\sum(x-\bar{x}_1)^2 + \sum(x-\bar{x}_2)^2}{n_1+n_2-2}$$

x = population size

x□ = mean population size

n = number of replicates

When the absolute value of t is greater than 2.776 (from two sided t-distribution), the two temperatures had significantly different population sizes; vice versa.

e.g., Replicates at 30°C after 24 hours had population sizes (cells/mL): X1 = 50050, 50050, 44550. Replicates at 40°C after 24 hours had population sizes (cells/mL): X2 = 58300, 62150, 55550.

Therefore, x□1 =$\frac{50050 + 50050 + 44550}{3}$ ≈ 48217

x□2 = $\frac{58300 + 62150 + 55550}{3}$ ≈ 58667

s^2

$$= \frac{[(50050-48217)^2+(50050-48217)^2+(44550-48217)^2]+[(58300-58667)^2 \ +(62150-58667)^2+(55550-58667)^2]}{(3+3-2)}$$

= 10537083

t = $\frac{(48217 - 58667)}{\sqrt{10537083 \text{ x } (⅓+⅓)}}$ = -5.576

| t | = 5.576 > 2.776.

Discussion

Samples of *T. thermophila* grown at 40^{o}C had a significantly higher population size at the end of the experiment (24 hours of incubation) than the treatment levels at 11^{o}C, 17^{o}C and 30^{o}C. The replicates incubated at 11^{o}C and 17^{o}C showed significantly lower population sizes compared to 30^{o}C after 24 hours. Based on this data, we reject our null hypothesis and find support for our alternate hypothesis, which states that increasing the temperature will increase the population size of *T. thermophila.*

Our results are consistent with the findings of Frankel and Nelsen (2001) who found that *T. thermophila* optimum growth is best achieved when grown at 35-39^{o}C. We observed optimal growth at 40^{o}C based on the larger mean population size we calculated. Our results do not agree with that of Cassidy-Hanley (2001), which states that the *T. thermophila* grew best at temperatures close to 30^{o}C rather than 40^{o}C.

Nagel and Wunderlich (1976) observed RNA-transport occurred significantly slower at lower temperatures due to the clustering of lipids in the nuclear membrane. This clustering of lipids in the nuclear membrane may have affected protein synthesis, resulting in a lowered formation of important structures such as cilia. This would have decreased the speed of processes which require the use of cilia, such as motility and uptake of foods needed for growth. In turn, this would have resulted in a decreased efficiency of phagocytosis. This is to be expected based on the biological model outlined in figure 1.

Luan *et al.* (2012) noted that the average number of food vacuoles in each cell increased as *T. thermophila* was exposed to higher temperatures. Luan *et al.* (2012) also found that as the temperature increased from 12°C to 30°C, a significant difference in food

vacuoles was observed. Since we found the population size to be greatest at our highest temperature of 40°C, it is probable that this could be due to an increased amount of food vacuoles the cells had at this temperature. The greater number of food vacuoles could have resulted in a faster transition through the cell cycle, resulting in a faster doubling time (Alfred *et al.* 2001). *T. thermophila* exposed to the lower treatment levels of 11°C and 17°C may not have had enough food vacuoles to effectively meet its growth requirements to complete its cell cycle resulting in less replication and a smaller population size.

It also could have been expected that the growth rate of *T. thermophila* would have decreased as temperatures increased closer to the upper limit of 40°C due to the possibility of the denaturation of proteins (Daniel *et al.* 2013). However, the population size was still the greatest at 40°C rather than at 30°C. Frankel and Nelson (2001) found that even though there were signs of protein denaturation at 40.5°C, such as abnormalities in the oral apparatus, that food vacuole formation was not affected. This could mean that proteins could start to denature at around 40°C, but these denaturations do not alter *T. thermophila* enough to significantly affect its growth rate. One reason that the oral apparatus of *T. thermophila* may be resistant to denaturation could be due to the presence of proteins called chaperonin 60's, or cpn60's (Maguire *et al.* 2002). Cpn60's are a class of chaperone proteins which are important in the folding of proteins under conditions of stress, such as at high temperatures. Maguire *et al.* (2012) states that Cpn60's have been discovered in *T. thermophila*, and that it is thought that these chaperone proteins play a role in the formation of the oral apparatus. This may explain the continued increase of population growth of *T. thermophila*, even at high temperatures up to 40°C.

As seen in Figure 4, one of the replicates grown at 30°C and one at 40°C were cloudy in appearance after 24 hours of incubation. The replicates grown at 11°C and 17°C were not. This could be due to a high density of *T. thermophila* in the test tube. Due to the highest population size being at 40°C, the increased density of cells at this time may explain why we see cloudiness at 40°C and slightly less cloudiness at 30°C due to a smaller population size. The second possible explanation for the cloudiness is bacterial contamination (Cassidy-Hanley 2012). We failed to flame the test tubes before collecting samples for cell counting for the first four hours of our experiment. Since *T. thermophila* are extremely sensitive to impurities, the lack of sterilization could have affected the sample of *T. thermophila* in this test tube. However, because we did not observe any bacterial organisms in the haemocytometer when we were counting the replicates, we believe that the clouding was due to the higher density of *T. thermophila.*

Several factors may have influenced our results leading to possible errors and variation in our data. Firstly, the incubators set at 11°C and 17°C were not stable throughout the entire experiment. We discovered that the incubator set to 11°C sometimes fluctuated to 13°C when we collected samples for cell counting. This also occurred at the incubator set to 17°C in which it changed to 15°C. This could have contributed to possible errors in our results.

Also, a source of variation was that the collection of samples from each of the tubes for counting was done by three different members of the group. Because of this, there may have been variation in the mixing of the test tubes (done by pipetting up and down within the tubes) before samples were taken for counting. This may have lead to the extraction of

samples which were not representative of the true cell densities of the replicates due to inconsistent mixing.

Another possible source of variation was from the handling of the test tubes. Hellunglarson *et al.* (1992) states that when cultures of *T. thermophila* are shaken, their growth rates are significantly stunted, sometimes becoming twice as slow. Due to human error, there may have been shaking of some test tubes *T. thermophila* when taking samples for the replicates or when moving the samples into the incubators. This may have affected the cell densities in our replicates.

Finally, a source of variation due to human error may be from our cell counting procedures. In order to reduce variation, it would have been ideal to have one person count all of the replicates. However, due to time constraints, three different people from our group were involved in counting the cell densities. Also, there may have been mistakes made by the people in our group when counting the *T. thermophila* in the haemocytometer, either by counting the wrong number of cells or when writing down the number in our lab notebooks.

Conclusion

After analyzing the mean population sizes of *T. thermophila* we can reject our null hypothesis and provide support for our alternate hypothesis that increasing temperature increases the population size. Through our studies we found that the optimal temperature is closer to 40°C. Although this experiment presents the effect of temperature on the population density of *T. thermophila,* we hope that this study will provide useful information

for further studies on *T. thermophila* and its relationship between population size and other abiotic factors.

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