Global Warming's Impact on Living Organisms: A Quantitative Analysis of the Effect of Temperature on Bacteria

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Personal Section

As global warming becomes more apparent, the effects of temperature on living organisms are causing great concern. However, most information regarding the impact of rising temperature on life is descriptive and inexact. Along with AP Calculus, I took AP Biology my sophomore year and was fascinated by the idea of emergent properties, the idea that life is not a simple sum of its respective parts; interactions between the different levels of biology create emergent properties that cause, for example, a cell to behave differently from a mixture of its component molecules. I wondered how increasing temperature affects organisms, particularly bacteria, and how its effect on organisms differs from its effect on the chemical reactions that the organism is composed of.

During my junior year, through AP Statistics and AP Chemistry, I learned the mathematical skills to effectively analyze the these relationships. From my chemistry course, I learned that the Arrhenius equation describes simple chemical reaction rates as a function of temperature; I now had a way to quantify temperature's effect on chemical reactions. From my

statistics course, I learned the quantitative tools needed to compare temperature's effects on bacteria and simple chemical reaction rates.

I then conducted my experiment at Exelixis, Inc., but the majority of the project took shape at home, during the background study, the design of the experiment, and the analysis of the data. I am grateful to both my AP Biology teacher Ms. Katherine Ward and my AP Statistics teacher Mrs. Andrea Gould for reviewing my work and supporting me. When I learned of the Intel International Science and Engineering Fair (ISEF), I wanted to seize the opportunity to participate. Unlike most participants, however, I had the obstacles of being new to science fairs and having few school resources to help me pursue my interest. On my own, I coordinated paperwork and meetings with Mr. Nakagiri, the local fair director, and enrolled for participation at the San Mateo County Science and Technology Fair. While other veteran participants, who had entered their projects with the help of their respective schools, were confidently assembling their poster boards, I was rereading the county fair instructions, struggling to find the best ways to sturdily stack one poster board on the other, and memorizing the times of scheduled events. I had no idea what to expect. Although I was confident that my scientific reasoning was sound, I didn't know if my lack of experience in science fairs would hinder me from advancing to the regional competition.

Luckily, it did not and in March 2010 my project won the Grand Prize at the San Francisco Bay Area Science Fair and advanced to the Intel International Science and Engineering Fair (ISEF). But even after ISEF, I felt I could incorporate more analysis into my project. After adding my expanded analysis, I entered my project into the Intel Science Talent Search, in which I was selected as a semifinalist.

Through this experience, I have learned how rewarding it is to apply mathematics to other sciences, particularly the biological sciences, which are often very descriptive. With quantitative analyses, many intricate aspects of biology can still be further explored.

Introduction

The impacts of global warming have significant implications for the fate of our globe. Increased temperature will harm plants and animals in the sea and force animals and plants on land to search for new habitats. Climate change will cause flooding, drought and an increase in the number of damaging storms. Furthermore, it will lead to crop yield decline and an increase in human disease (1). Rising average temperatures are affecting the global environment; these changes include disappearing glaciers, lengthening growing seasons, shifting plant and animal ranges and earlier flowering of trees (2).

There is increasing research being done to understand rising temperature's effect on biological functions. For example, Sakata *et al* discovered that temperature can affect the tissue specific auxin signaling in plants. Auxin production and regulation is essential for the modulation of cell proliferation, differentiation and development in plants. Higher temperatures resulted in male tissue specific auxin reduction, leading to abortive pollen development (3). Research conducted by Cingi *et al* demonstrated significant negative impacts of elevated water temperature on the fertilization and embryonic development of whitefish Coregononus lavaretus. Rising temperature increases the proportion of unfertilized and abnormally dividing eggs, deformed embryos and consequently mortality (4). Moreover, global warming causes hypoxia in water, damaging ecosystems. Climate change's impact is becoming increasingly significant (5).

While there has been quantitative research of global warming's effect on the physical world, most of the information of its effect on biological systems is generally descriptive and inexact. This project focuses on the effects of temperature on living systems through mathematical analysis using bacteria as example. Temperature's effects on living systems were compared to those on non-living systems, through simulations based on chemical principles.

In relation to temperature, enzymatic reactions seen in biological systems behave similarly to simple chemical reactions. The Arrhenius equation predicts that increases in temperature result in increases in both the rates of enzyme-catalyzed reactions and the rates of simple chemical reactions (6). However, living organisms are not a simple sum of their respective reactions; living systems are highly integrated and regulated, resulting in emergent properties. Consequently, observed growth rates of *E. coli* bacteria as a function of temperature may deviate from the projected growth rates described by the Arrhenius equation.

The Arrhenius equation describes the dependence of the rate constant (k) on temperature for most chemical and enzymatic reactions (7). Rate constants for most reactions follow the equation of the form

where A is the frequency factor (a constant), E_a is the activation energy, R is the gas constant, and T is the absolute temperature. The Arrhenius equation describes the relationship between the rate constant and temperature as exponential.

The Q_{10} is a measure of the change in rate of a chemical reaction as a consequence of a temperature increase of 10°C. The Q_{10} is calculated as:

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where R is reaction rate and T is temperature in °C or K. In general, for every increase of 10°C, the reaction rate constant *k* will be multiplied by a factor of 1.2 to 3.

The growth of *E. coli* is a direct reflection of its metabolic output. Metabolism is the totality of an organism's chemical reactions; therefore, we can compare the growth rates of *E. coli* to the predicted chemical reaction rates in response to temperature changes. The comparison between the observed bacterial growth rates and the predicted chemical reaction rates should reveal fundamental differences between life and simple chemical reactions.

Materials and Methods

The Tetracycline (Tc) resistant *E. coli* strain XL-1 blue was used. The culture medium was LB with Tc (15 µg/mL). A single bacterial colony was inoculated into 50 mL LB+Tc in a 250 mL baffled flask; this culture was incubated overnight at 37°C at 225 rpm. 1 mL of this culture was then added to each of 18 250 mL flasks, each containing 50 mL LB+Tc. Triplicates were used for each of the six temperature settings. Our six temperature settings were: 11°C, 18°C, 28°C, 37°C, 41°C, and 43°C. All cultures were tested in several incubators of the same model at 225 rpm. Trials with temperatures above 43°C and below 11°C were attempted but not obtainable due to limitations of incubator capability (Multitron, Infors). The flasks were then placed into the appropriate incubators at the appropriate temperature. A period of time was allowed for the bacteria to adapt to the new environment. 1 mL was drawn from each flask and its optical density (OD) was measured at a wavelength of 600 nm with LB+Tc as a blank control.

These were used as the t_0 data points. After various time periods, more 1 mL samples were drawn from each flask and analyzed for its OD; the optical density versus time was used to determine the growth rate constant, *k*. The OD instrument used was Genesys 10 uv Scanning, Thermo Scientific. Bacterial growth in batch conditions can be described with 4 phases: the lag phase, exponential growth phase, stationary growth phase and death phase. All data used for this study were taken from the exponential growth phase, when there are no limiting factors other than temperature, such as nutrient depletion; thus, the only explanatory variable in our experiment was temperature.

Microsoft Excel 2007 and GraphPad Prism v.4.0 were used for curve fitting and graphic analysis. Basic statistical methods can be found by Yates *et al* (8).

Data analysis and equations used in this study were as follow:

Standard deviations were calculated using:

(1)

The growth constants were obtained:

(2)

The bacterial doubling time was deduced as follows

— (3)

Pearson Correlation Coefficients between x and y were calculated based on:

— — — (4)

Spearman's rank correlation coefficient was calculated using:

——— (5)

is given by the equation only when there are no tied ranks in the data.

Where: n: sample size; x_i: individual x values; : sample mean; s_x: x variable standard deviation; s_y: y variable standard deviation; d_i: $d_i=x_i-y_i$, where n raw scores (X_i, Y_i) are converted to ranks (x_i, y_i); k: growth rate constant; N: number of bacteria; N_0: initial bacteria population.

Results

Bacterial growth can be categorized into three phases: the adaptation phase, the exponential phase or log phase, and the stationary phase. The adaptation phase is the period in which the bacteria are inoculated (or introduced) into a new medium or environment. Bacterial growth then enters into exponential phase, in which the doubling time becomes constant. The bacteria will then eventually enter into the stationary phase, in which the nutrients become limited. In these experiments, data from the exponential growth phase were used for analysis, in which only temperature is the explanatory variable (Figure 1). The OD data were collected at various time points and temperatures were plotted and analyzed. The standard deviations were obtained based on equation 1. The growth rate constants (k) at various temperatures were experimentally measured and their rates were obtained by non-linear regressions using equation 2. Bacterial doubling time was calculated with equation 3. The greater standard deviations at low temperatures (e.g.11ºC) were reflected in the extremely slow growth rates of the bacteria. This was also the reason that the coefficient of determination (r^2) was poor at lower temperature.

The obtained growth rate constants at various temperatures can be summarized in Table 1. The doubling times (time it takes for bacteria to complete one generation) are wildly different: 45 hours at 11 ºC, 9.4 hours at 18 ºC and 1.9 hours at 28 ºC. Bacterial growth reaches a maximum at 37 ºC, with a doubling time of 1.6 hours. Above the optimal temperature, the doubling time dropped to 1.7 hours at 41 ºC and 2.7 hours at 43 ºC.

The graphic presentation of growth rate constants (k) as function of temperature indicated a non-linear relationship (Figure 2). Before reaching the k_{max}, the k value increases as

temperature increases. Above k_{max}, the k value decayed rapidly. Due to incubator temperature control limitations in this experiment, the maximum temperature was 43 ºC.

To investigate temperature's effect on living organisms and non-living systems, a predictable fundamental chemical principle was used. The Q_{10} is the value that reflects the rate change of a chemical reaction as temperature increases 10 ºC. Normal Q_{10} values for most chemical reactions are from 1.2 to 3.0. These two Q_{10} values, 1.2 and 3.0, were applied to simulate the growth rate constants as function of temperatures (Figure 3). Using the growth rate constant k at 11 ºC as the initial value, the k values were simulated using the equation:

—

where R is reaction rate and T is temperature in °C. Comparison of measured bacteria growth rate constants with the simulated growth rate constants based on chemical principles revealed fundamental differences (see *conclusions and future work*).

The relatively poor correlation based on Pearson's correlation coefficient (equation 4) is due to uncorrelated k above the optimal temperature for *E. coli* (Figure 4). At temperatures above the critical point, biological systems dramatically diverge from simple chemical systems. In principle, simple chemical rates exponentially increase indefinitely as the temperature increases; however, biological growth rates reach maximums at critical points and then sharply decrease to 0 (point of death).

The data taken from temperatures below the critical point were reanalyzed with Pearson's correlation coefficient and Spearman's rank correlation coefficient (calculated by equation 5) (Figure 5). The results show that below the critical point, there is a moderately strong correlation

between the observed biological growth rate and predicted rate. In addition, there is a perfect monotonic, rank order correlation; as the predicted rate increases, so does our observed *k*.

Figure 1. Bacterial growth rates at various temperatures. ODs at 600 nm were measured at various time points during the exponential growth phase.

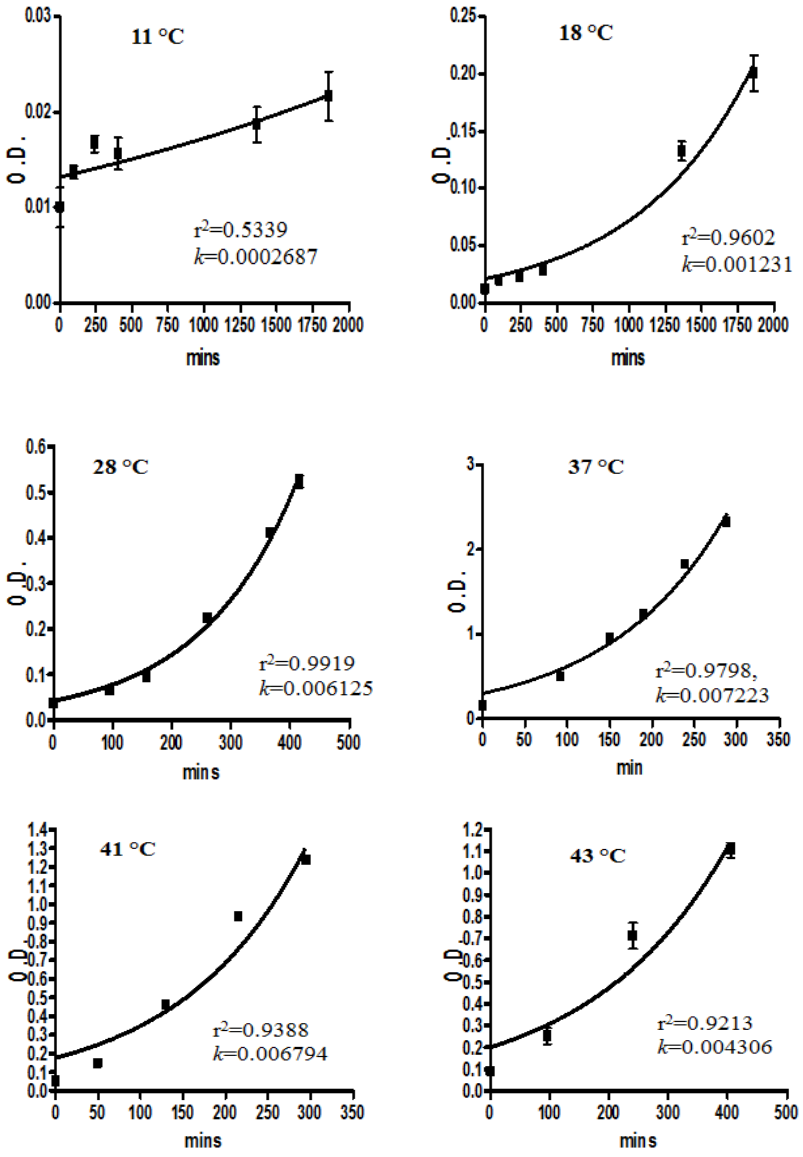


Table 1. The bacterial growth rate constants and bacterial doubling times at various temperatures were recorded.

	11°C	18°C	28°C	37°C	41°C	43°C
k **(min^{-1})**	**0.0002687**	**0.001231**	**0.006125**	**0.007223**	**0.006794**	**0.004306**
k **(hr^{-1})**	**0.016122**	**0.07386**	**0.3675**	**0.43338**	**0.40764**	**0.25836**
Doubling time **(min)**	**2679**	**562.8**	**113.2**	**95.96**	**102.0**	**161.0**

Figure 2. Bacterial growth rate constant (*k*) at various temperatures (ºC).

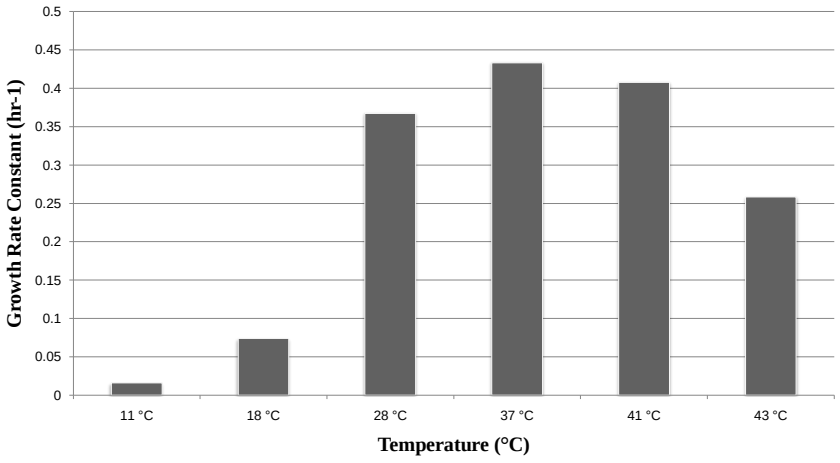


Figure 3. Comparison of observed and simulated growth rates as functions of temperature. The initial value used for the simulations was the observed initial value, *k*=0.016122 at 11 ºC .

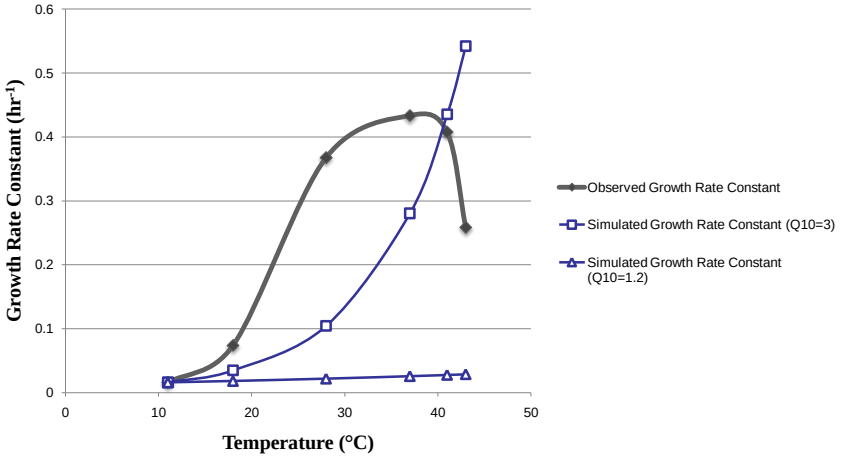
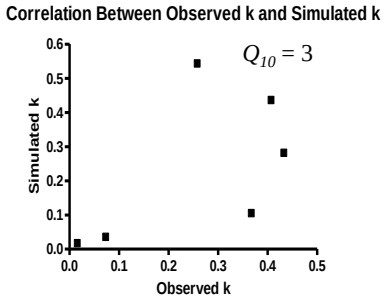
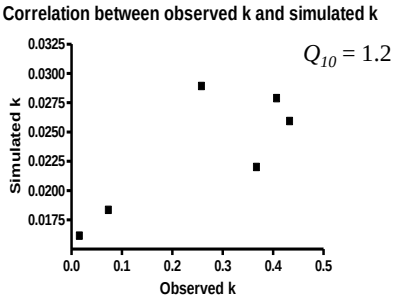


Figure 4. Pearson correlations between the observed growth rate constant (*k*) and simulated growth rate constant (*k*) were calculated and graphed.

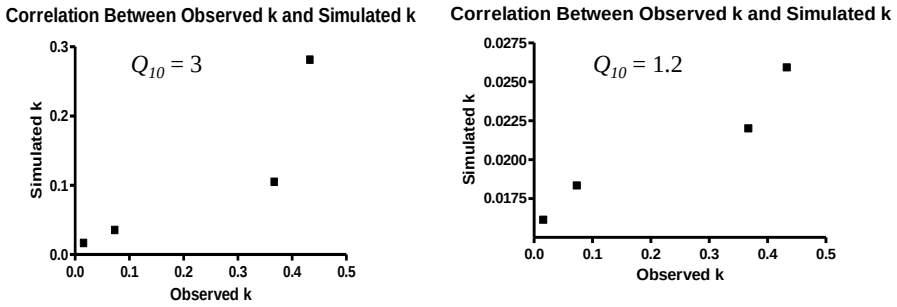


Pearson r=0.5820



Pearson r=0.7862

Figure 5. Pearson and Spearman correlations between the observed growth rate constant (*k*) and simulated growth rate constant (*k*). These two graphs exclude data at 41ºC and 43 ºC, which were above the optimal temperature for *E.coli*.



Pearson r=0.8723
Spearman ρ=1.00

Pearson r=0.9624
Spearman ρ=1.00

Conclusions and Future Work

Unlike simple chemical reactions, life can only exist within narrow temperature ranges. Living organisms possess critical transition temperatures, above which metabolic outputs rapidly diminish, leading to death. At low temperatures, bacteria significantly upregulate their metabolic rate; their growth rate constant *k* is much larger than the predicted rate based on simple chemical principles. Below optimal temperatures, both the growth rate constant and the simple chemical reaction rate constant increase as temperature increases, albeit at different rates.

Thorough quantitative analysis revealed that life is significantly more sensitive and susceptible to temperature changes than are simple chemical reactions; minor alterations in temperature have the potential to substantially alter metabolic rates in living organisms. The enormous effects of temperature on life are particularly alarming as we face global warming, as even relatively small increases in temperature in any temperature range pose risks to the delicate nature of life's regulatory abilities.

Future work will be to further confirm this observation in various bacteria and to investigate how the genes are regulated at low and high temperatures. I am also fascinated by the speed at which *E. coli* may adapt to higher temperatures and the mechanisms behind it.

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