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ABSTRACT

Students set in their ways are usually reluctant, as a general rule, to deal with open-ended investigative scenarios. In order to acquaint the student with the physical method and philosophical thought process of the discipline, the tone of the course must be set early on. The present study was conducted to develop scenarios and microbial model systems that would allow the student to apply biostatistical analyses to their work. Calibrated microscopes were used to measure the lengths and widths of replicated samples of various species of bacteria. These data were used to compute means, modes, medians, ranges, variances, standard deviations, and to establish frequency distributions and correlation coefficients. The use of biometric analyses introduced the student to the reality of scientific thought, allowing the novice student to appreciate scientific methodology on a whole new quantitative level. Findings show that students, once willing to accept the challenge, are stimulated and get excited when they settle into the practice of science. When the student is exposed to the way research unfolds in a laboratory or when they replicate a historic experiment, they seem to get a sense of belonging to a special group, "coming of age," which is the beginning of a true understanding of the discipline. (Contains 13 references.) (VWC)

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TEACHING THE METHODOLOGY OF SCIENCE:

THE UTILIZATION OF MICROBIAL MODEL SYSTEMS

FOR BIOMETRIC ANALYSES

Joseph A. Adamo Ph.D.

Ocean County College
Toms River, New Jersey 08753

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ABSTRACT

More often than not introductory science courses end up being courses about science and not a study of science and the way it works in the real world. The approach of "covering" the material for students required to take standardized tests usually requires the instructor to relinquish his or her academic freedom and creativity in order to teach to the test. Science is a process, a methodology, an intelligent way of solving problems. The Scientific Method is the beginning and the end, the α and the Ω of the discipline. Getting an undergraduate student to think quantitatively, analytically and from a scientific point of view is often a challenge and a major objective of the instructor of microbiology. The utilization of models and model systems in scientific research is often the genius of the work and a major way research unfolds in the laboratory. The use of models in research and science education is briefly discussed. We attempt to create an environment that will allow the student to experience science. Student response and reaction to the experience are included in the study.

The present study was conducted to develop scenarios and microbial model systems that would allow the student to apply bio-statistical analyses to their work. Calibrated microscopes were used to measure the lengths and widths of replicated samples of various species of bacteria. The lengths and widths were also measured of numerous samples of *Rhabditis*, a common, essentially microscopic, soil nematode and *Turbatrix aceti*, a nematode of historical significance. These data were used to compute means, modes, medians, ranges, variances, standard deviations and to establish frequency distributions and correlation coefficients. Frequency distributions were further employed to detect contaminants in pure cultures which were later verified by gram-staining. Microscopic data were also utilized to test hypotheses by way of t-tests. Various strains of *Escherichia coli*, were screened for antibiotic resistance using phenol red dextrose broth (PRDB). Selected bacteria and antibiotic combinations were used in a replicated dosage-growth study utilizing spectrophotometric measurements. These data were then subjected to analyses of variance (ANOVA) and multiple comparison tests. Students set in their ways are usually reluctant, as a general rule, to deal with open-ended investigative scenarios. In order to acquaint the student with the physical Method and philosophical thought process of the discipline the tone of the course must be set early on. The use of biometric analyses introduced the student to the reality of scientific thought, allowing the novice student to appreciate scientific methodology on a whole new quantitative level.

INTRODUCTION

Science education at the college introductory level has too often become one of traditional lecture with some laboratory work. That the laboratory hands-on aspect has been maintained is certainly fortunate, since the engine that drives the discipline of science is the Scientific Method. The methodology of science, the process, clearly requires experimental activity. Too often introductory courses teach about science not science itself and the instructor becomes primarily a disseminator of information, rather than a teacher of the thinking, creative process of science.

The Scientific Method:
Modern science is defined by the Scientific Method. The method is the beginning and the end, the limits of the discipline. The process is essentially an intelligent way of solving problems. Formally, the steps of the process include: making observations, posing problems, speculating hypotheses, designing experiments to test the speculations, collecting and analyzing data, and developing theories based upon well established validated hypotheses. The aim of science is to make and use theories. A theory is considered to be a temporary truth, a well established concept subject to modification as new data becomes available. Science is a work in progress. Science is a process, a methodology.

Modern Biology a Visual and Quantitative Study:
The study of modern biology is one of visualization and quantification. Biology like other sciences began with observations, primarily visual. The 14th-17th century Renaissance in Europe initiated a great deal of scientific experimentation, the development of the microscope and the birth of microbiology. Actually, the development of the field of microbiology had a great deal to do with experimental testing of Aristotle's concept of *Spontaneous Generation* which had a tremendous impact on the work of Louis Pasture, who is known as the "Father of Modern Experimental Microbiology". Max Dulbruck in 1941 established a quantitative phage course at Cold Spring Harbor Laboratory (Long Island, New York) for a group of researchers destined to establish the field we currently refer to as Molecular Biology and which led the young James Watson to collaborate with Francis Crick to divine the three dimensional structure of the DNA molecule. Max Dulbruck has been called the "Father of Molecular Biology". The "cutting edge", or as some would say the "bleeding edge" of modern biology is one of isolating and determining the way various molecules exist in space, i.e. visualization. The form of the molecule then is related to the way it works, the physiology (What is the molecular structure of the AIDs virus and how does it link up to the T cell?). All studies of this type seek visualization of one sort or another and require a tremendous amount of quantitative analyses.

Model Systems:

Various biological model systems are utilized to perform experimental work that allow the modern biologist to collect quantitative data and to visualize. Each model system is ideal for a particular type of study. For example *Drosophila melanogaster*, the fruit fly, having easily identifiable virgins, numerous detectable phenotype characteristics, generating large numbers of offspring in a short period of time, and its ease of breeding make these forms of life ideal for genetic studies. Some other major experimental biological model systems include: *Escherichia coli* (*E. coli*), probably the best known organism of all the 5×10^6 plus known creatures, its DNA also having been completely sequenced; *Saccharomyces cerevisiae* (brewer's yeast), the first eukaryote to have its DNA completely sequenced; *Neurospora crassa* (pink bread mold), involved in the development of new experimental approaches and the relationship between the protein molecule and the gene; *Caenorhabditis elegans*, a common nematode, the only multicellular organism to have its DNA completely sequenced and to have its entire embryology and development completely worked out from conception to death of the adult; and of course *Mus musculus*, the white mouse probably the most used model in the medical field. There are a number of significant models among the viral group including: Vaccinia virus, Tobacco Mosaic Virus (TMV), T_2, λ ,ΦX-174, and the influenza virus. Inanimate objects such as soap bubbles and lead shoot have also been used as model systems for the experimental study of cellular area, volume, structure and various other aspects of cellular biology.

In earlier work the author has created and utilized many model systems, both live and non-living, to visualize and/or to quantify biological concepts for research and teaching activities (Adamo et al., 1993; Adamo and Gealt, 1996a). The distributions generated by the rolling of a pair of dice were used to study the Normal Distribution (Adamo, 1963). Catsup, a very complex (non-Newtonian) fluid, was utilized to explore the chemistry and physiology of the movement of the *Amoeba* (Adamo, 1964). Salt crystals, beads and toothpicks allowed students to determine three dimensional measurements using two dimensional "micrographs", a common electron microscope technique (Adamo and Kalichstein, 1969). Three-ply string, glass slides, match sticks and rice petioles served to study the migration of pathogenic microscopic nematodes (Adamo et al., 1976a, 1976b). An actual model of an icosahedron was constructed to familiarize the student with the visual, quantitative and structural aspects of a basic virus particle (Adamo, 1993). The icosahedral ΦX–174 and the complex T_2 bacteriophages served as models for the development of a metabolic inhibition test (Adamo, 1996). We have used nematodes to model the natural transfer of DNA in the environment (Adamo and Gealt, 1998; 1996a) and as agents of disease transfer (Adamo and Gealt, 1996b). A simple eyedropper has even been utilized to

model serial sections and the derivation of complex three dimensional structure from a series of two dimensional views (Kalichstein et al., 1973).

The Current Study:

The present study was conducted to develop scenarios and microbial model systems that would allow the student to apply bio-statistical analyses to their visual and quantitative work. To shock, if need be, the student into experiencing the reality of the philosophy and methodology of the science of biology.

MATERIALS AND METHODS

Student Population:

The following studies were not all completed utilizing a single group of students. The report is a summary of experimental designs, data collected and analyses performed by four distinctly different groups in terms of space and time. Some of the work was completed by all four groups, while other portions were accomplished by one or two of the groups.

Calibration of the Microscope:

Each student calibrated an ocular micrometer in the usual manner, i.e. a particular eyepiece micrometer was compared to a stage micrometer, having known "grid" space values. The value of each ocular space was determined using a 4 X, 10 X, 40 X and 100 X objective. The student then created a table showing ocular space values for their particular scope, objectives and ocular for future use.

Measurements of Bacteria:

The students prepared bacterial smears of *Escherichia coli* using aseptic technique, allowing the smears to air dry and fixing using a Bunsen burner flame. The smears were then stained using methylene blue. Each student than created a data sheet and proceeded to measure the length and width of 25 *E. coli* cells using the micrometer. Other species of bacteria were measured using the same techniques.

Following data collection, each student: (a) calculated the average length and width of an *E. coli* cell; (b) determined the mode, median, range, variance and standard deviation for each set of data; (c) prepared a frequency distribution for the length and

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width data; (d) computed a correlation coefficient and (e) estimated the volume of one *E. coli* cell.

Using Frequency Distribution to Detect Contaminants:

Frequency distributions were utilized to detect a contaminant in a pure culture. Pure cultures, those containing only one species of organism, are required for experimental work. For this study cultures of *E. coli* and *Bacillus megatherium* were concentrated, mixed and supplied to the students. The students were not aware the culture was mixed, they assumed they had a pure culture. Bacteria were concentrated using 1 mL samples in microfuge tubes and centrifuged at 14,000 rpm for 3 mins. Students were instructed to prepare two bacterial smears the first of which was to be simple stained. The second slide was stored for future use, to be gram stained after the analysis of the length of 50 bacteria was completed. The students were instructed to use the stained slide to measure the length of 50 bacteria, to store the second slide and to construct a frequency distribution. *B. megatherium* is about 3-6 μm in length and gram positive while *E. coli* is about 1- 2.5 μm and gram negative. Random measurements result in a bimodal distribution indicating two species. After analyses the students were instructed to run a gram stain on the second slide. The results here clearly indicate a mixed culture, i.e. two species, should the bimodal curve elude student interpretation.

Measurements of Nematodes:

Two different sources of nematodes were utilized in the development of these techniques. The first was *Turbatrix aceti*, of historical significance (van Leeuwenhoek's "vinegar eels"). These were cultured in a simple apple and cider vinegar medium. The second were cultures of *Rhabditis*, isolated from campus soil and propagated on spread plates of pure cultures of *E. coli*. In the case of the spread plate cultures, students used sterile nutrient broth to flood a small area of a 6-7 day old petri plate freeing into fluid many active worms. In either case a drop of culture containing active nematodes was placed on a clean microscope slide. The slide was then placed on a hot plate (set very low) for a very short period of time. Enough heat was used to end active movement but not so much as to evaporate the fluid or cook the worms. Nematode activity can be viewed very conveniently using a dissecting low power scope. The heat treatment "heat-relaxes" the nematodes. This treatment relaxes the muscles of the worm causing them to straighten out for length measurement. Again each student created a data sheet and proceeded to collect measurements. Each student measured the length and width of 25 adult nematodes. A sample mean, mode, median, range, variance, standard deviation and frequency distribution were calculated for each set of data. A correlation coefficient was then computed (length vs. width).

Hypothesis Testing Comparing Two Means Utilizing t-Testing:

Using the nematode data collected earlier of *T. aceti* lengths paired students were asked to compare their means. The paired t-test was utilized testing the hypothesis that the means were equal.

Screening Bacteria for Antibiotic Sensitivity :

Students screened various strains of *E. coli* for antibiotic resistance using a number of different antibiotics and phenol red dextrose broth (PRDB). The PRDB medium was used for a metabolic inhibition test (Adamo, 1996; Adamo and Gealt, 1998). Those strains found to be resistant were used in a dosage-growth study. The results for *E. coli* χ1997 and nalidixic acid will be reported in this paper. This stain of *E. coli* was found to be resistant to this antibiotic (nalidixic acid). The experiment was replicated three times. Nine tubes of nutrient broth (6 mL each) were prepared and supplemented with antibiotic. Three tubes were supplemented with the recommended dose for this antibiotic (20μg/mL), three with half dose (10μg/mL) and the final three with a double dose (40μg/mL). The supplemented nutrient broth tubes were inoculated with a given amount of bacteria and incubated at 37 C for 18 hours. The cultures were then each mixed and 3 mL of each placed individually in a "Spectronic 20" cuvette. Each sample was then subjected to a spectrophotometric measurement of turbidity for optical density. Mean values were established and all the data was subjected to analysis of variance (ANOVA) and multiple comparison tests.

RESULTS AND DISCUSSION

As a general rule during the development of this work the data collected was subjected to computations of means, modes, medians, ranges, variance, standard deviation, correlation coefficients and frequency distributions. For the sake of brevity the results reported below are reduced and representative of that actually obtained.

Generally, even with science majors, the current population of undergraduate students in general, apparently prefers to attend a brief lecture, take a few (very few) notes, parrot back information on a straightforward no surprise or thought requiring test, meet once a week and have a simple "cookbook" laboratory experience. Will this be on the test? Do we have to know any of this material? Tell me exactly what I should know. Investigative, open ended, challenges are met with very little enthusiasm by most, at first. In general, there seems to be an inert laziness, or an overextended busyness of the student. The student really needs to experience

the process in order to determine whether this in fact is the area he or she wishes to pursue. There is a repetitiveness, a "donkey work" activity associated with scientific study. The student needs to experience the redundance of the work which is part of the scientific process.

Calibration:

Each student calibrated his/her own ocular micrometer and developed a table for the ocular space value data. Table 1 shows a typical student developed structure. Creating a table is an exercise in organization and presentation of data, an important part of any investigative work.

Many students find calibrating a microscope the first time to be a very frustrating experience. Measuring a large visual object is easy for the student but do exactly the same thing microscopically and it becomes extremely challenging.

Bacteria:

Each student prepared a number of bacterial smears and measured the length and width of 25 randomly selected samples for each species. The width measurements were done using oil and at the limits of the ocular micrometer utility where one space more or less was used to determine the width. This required significant student judgment and in part accounts for some of the data distribution. A table to organize the data collected was prepared. Table 2 shows a typical set of data collected by a student. Again creating a table is an exercise in organization and presentation, a valuable experience. The actual data collected was compared and peer evaluated an experience in accurate measurement technique and in variation in scientific work. Means, modes, medians and ranges were calculated (Table 3) and compared as were calculations of *E. coli* cell volume. The mean length was found to be 1.72 μm and the mean width to be 0.71μm (from student data shown in Table 2 and 3). Histograms were prepared for the length and width data and are shown in Figures 1 and 2. Using the equation for the volume of a cylinder($V = \pi r^2 h$) the volume of an *E. coli* cell was estimated to be 0.68 μm^3.

Nematodes :

Each student prepared a number of nematode slides and a data sheet to organize the data collected. Table 4 shows a typical set of data collected by a student. The data were compared and peer evaluated. Table 3 shows the results of the various student computed length and width central tendency statistics. Tables 4 and 5 show the *Rhabditis* length and width correlation computation data, the sums and the calculation of "r". The correlation was determined to be $r = 0.81$. Some students got values for "r" as high as 0.92, a very high, positive correlation.

Hypothesis Testing Comparing Two Means Utilizing t-Testing:

Using the nematode data collected earlier of *T. aceti* lengths paired students were asked to compare their means. The paired t-test was utilized testing the hypothesis that the means were equal.

Two student sets of data that had differing means (770.92 vs. 707) were selected for this report as the two students each felt they had the "right" answer and that the other was sloppy, criticizing each other. The data and computations are shown in Table 6. The results of the t-test demonstrated to the two students that their means were statistically the same, giving each some things to consider.

Screening Bacteria for Antibiotic Sensitivity :

The results for *E. coli* χ1997 and nalidixic acid will be reported in this paper. This strain of *E. coli* was found to be resistant to this antibiotic (nalidixic acid). A dosage-growth experiment was replicated three times. Nine tubes of 6 mL nutrient broth were supplemented with antibiotic. Three tubes with the recommended dose for this antibiotic (20µg/mL), three with half dose (10µg/mL) and the final three with a double dose (40µg/mL). The supplemented nutrient broth tubes were inoculated with a given amount of bacteria and incubated at 37 C for 18 hours. The cultures were then each mixed and 3 mL of each placed individually in a "Spectronic 20" cuvette. Each sample was then subjected to a spectrophotometric measurement of turbidity for optical density. Mean optical density values and the results of the ANOVA / multiple comparison test are given in Table 7. These data indicate inhibition of the bacterium as dosage of the antibiotic increased. Although not significant more growth was measured at the half dosage level than at the normal dose. However, there was a significant decrease in growth when the dosage was double that of the normal recommended dose.

Table 1. Shows one student's table of the space values using ocular #17 for the different objectives of microscope #3.

MAGNIFICATION	OBJECTIVE	SPACE VALUE (µm)
40 X	Scan	20.90
100 X	Low	7.14
400 X	High Dry	1.65
1,000 X	Oil	0.73

Table 2. Shows the length and width measurements of *Escherichia coli* cells in smears taken from 24h cultures grown at 37°C.

Sample #	Length (µm)	Width (µm)
1.	1.87	0.68
2.	1.53	0.68
3.	1.36	0.82
4.	1.97	0.75
5.	1.70	0.68
6.	1.56	0.61
7.	1.90	0.75
8.	1.70	0.85
9.	1.53	0.61
10.	1.36	0.75
11.	1.77	0.68
12.	2.04	0.68
13.	1.84	0.68
14.	2.11	0.82
15.	1.70	0.75
16.	1.36	0.68
17.	1.87	0.61
18.	1.63	0.68
19.	1.43	0.68
20.	1.90	0.68
21.	1.36	0.82
22.	1.53	0.75
23.	2.04	0.85
24.	1.90	0.68
25.	1.97	0.61

Table 3. Shows a compilation of *Eschericihia coli* and *Rhabditis* length and width central tendency statistical data.

	E.coli		*Rhabditis*	
Statistic	Length* (µm)	Width* (µm)	Length* (µm)	Width* (µm)
Mean	1.72	0.71	606	26.9
Mode	1.36	0.68	607	28.6
Median	1.70	0.68	607	28.6
Range	0.75	0.24	142	14.3

*n = 25

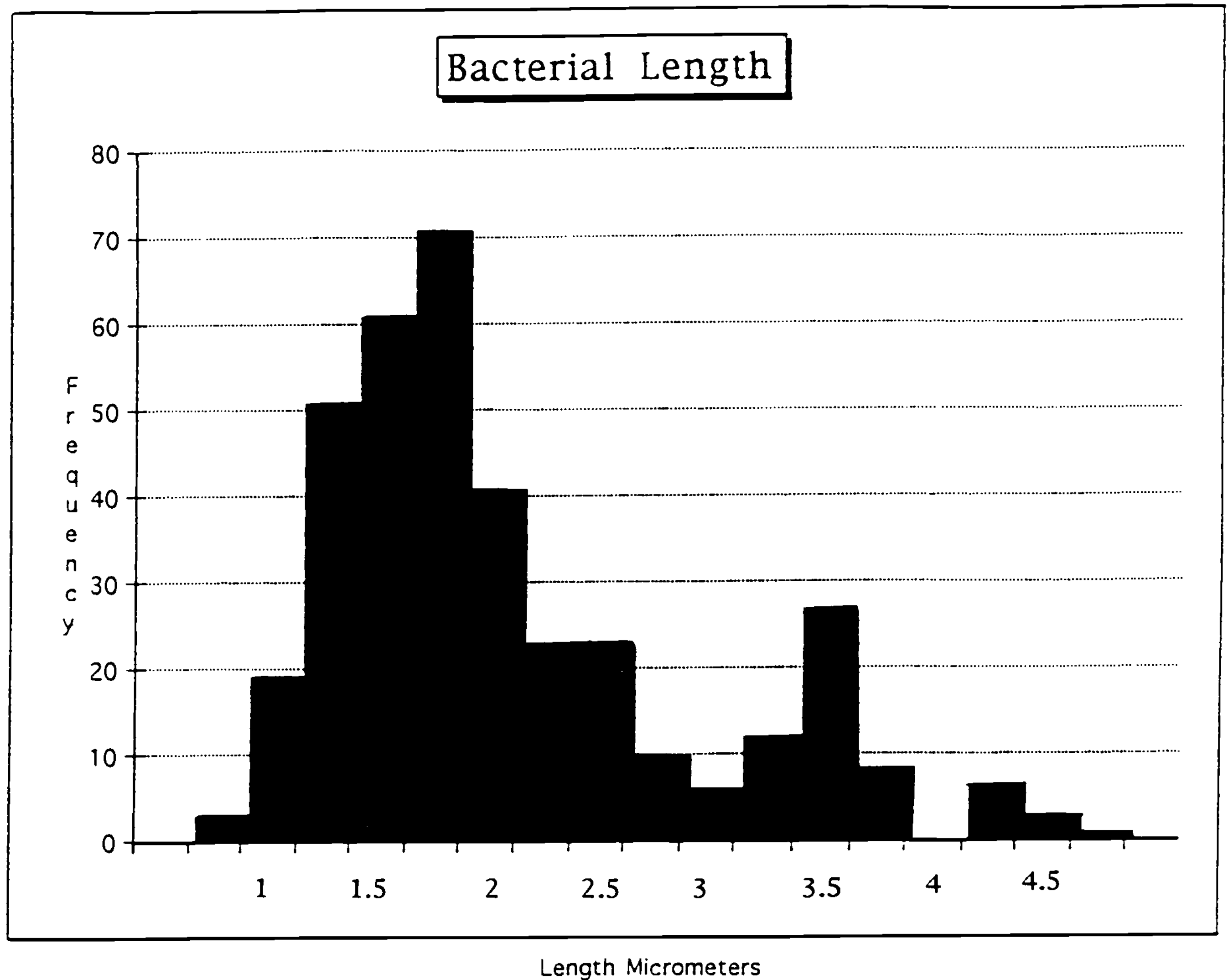

Figure 1. Shows the collective class distribution of *Escherichia coli* length measurements, for the *Frequency Distribution to Detect Contaminants Study.*

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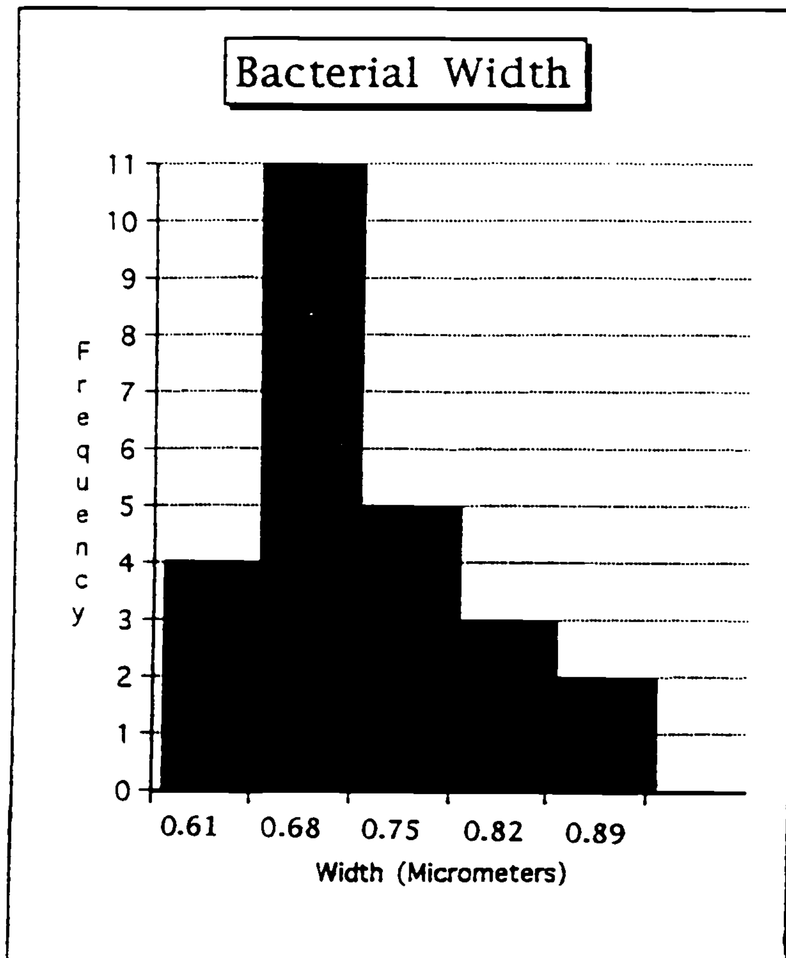

Figure 2. Shows the distribution of *Escherichia coli* width measurements from one student's data (found in Table 2).

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Table 4. Shows one student's *Rhabditis* length and width correlation computation data.

	(length) X	(width) Y	X^2	Y^2	XY
1.	650	28.6	422500	817.96	18590.0
2.	536	24.9	287296	620.01	13346.4
3.	564	24.9	318096	620.01	14043.6
4.	607	28.6	368449	817.96	17360.2
5.	607	28.6	368449	817.96	17360.2
6.	628	28.6	394384	817.96	17960.8
7.	571	21.4	326041	457.96	12219.4
8.	600	28.6	360000	817.96	17160.0
9.	557	21.4	310249	457.96	11919.8
10.	593	21.4	351649	457.96	12690.2
11.	607	28.6	368449	817.96	17360.2
12.	571	21.4	326041	457.96	12219.4
13.	643	28.6	413449	817.96	18389.8
14.	678	35.7	459684	1274.49	24204.6
15.	636	28.6	404496	817.96	18189.6
16.	600	28.6	360000	817.96	17160.0
17.	607	28.6	368449	817.96	17360.2
18.	628	28.6	394384	817.96	17960.8
19.	636	28.6	404496	817.96	18189.6
20.	614	28.6	376996	817.96	17560.4
21.	636	28.6	404496	817.96	18189.6
22.	557	21.4	310249	457.96	11919.8
23.	628	28.6	394384	817.96	17960.8
24.	586	21.4	343396	457.96	12540.4
25.	607	28.6	368449	817.96	17360.2

Table 5. Shows the sums of the *Rhabditis* length and width correlation computation data and the calculation of "r".

Totals	
$\Sigma X = 15,147$	$\Sigma Y = 671.5$
$\Sigma X^2 = 9,204,531$	$\Sigma Y^2 = 18,349.63$
$\Sigma XY = 409,216$	$n = 25$
Calculation of "r"	$r = 0.81$

Table 6. Shows two student's length measurements for *Turbatrix aceti* and computations to test the hypothesis that the means are equal.

Sample #	Length Student#1 (μm)	Length Student #2 (μm)
1.	936	711
2.	1289	587
3.	318	569
4.	583	587
5.	600	1280
6.	1112	533
7.	530	711
8.	512	533
9.	794	604
10.	1183	729
11.	812	836
12.	477	445
13.	565	302
14.	547	1067
15.	441	267
16.	530	1600
17.	600	924
18.	494	320
19.	1147	533
20.	1067	676
21.	1600	599
22.	899	899
23.	836	605
24.	836	922
25.	565	836

<u>Student #1</u>

$\Sigma X = 19273$

$n = 25$

$\bar{X} = 770.92$

$\Sigma X^2 = 17272183$

$S^2 = 100593.41$

$S = 317.16$

<u>Student #2</u>

$\Sigma X = 17675$

$n = 25$

$\bar{X} = 707$

$\Sigma X^2 = 14663927$

$S^2 = 90320.92$

$S = 300.53$

<u>t-Test Data</u>:

$H_O: \mu_1 = \mu_2$

$H_i: \mu_1 = \mu_2$

Assume: $(\sigma^2_1 = \sigma^2_2)$ or $(\sigma^2_1 \neq \sigma^2_2)$ DF=48

Then: t=-1.67185468 AR[-2.013 to +2.013]

Therefore: Accept Ho: $\mu_1 = \mu_2$

<u>Both means are the same</u>

Table 7. Shows the spectrophotometric growth measurements of *E. coli* χ1997 versus the antibiotic Nalidixic acid.

Nalidixic Acid/*E. coli* χ1997	
Dosage	Optical Density Means[1]
10 µg/mL	0.21a
20 µg/mL	0.19a
40 µg/mL	0.13b

[1] Means of 3 replicates each. Those means followed by the same letter are statistically the same, those by a different letter are significantly different P=0.05.

Future Work:

An experiment should be designed to utilize regression analyses. Using a standard replicated inoculum of *E. coli* in a given volume of a standard liquid medium, replicates could be incubated at several controlled temperatures i.e. 25, 30, 35, 37 and 42 C. After a given time of incubation the sample could be subjected to spectrophotometric analyses to determine growth at the various temperatures. Does the the temperature of incubation effect the growth of the bacteria? This would constitute a typical "cause and effect" regression analyses scenario.

Conclusion:

Here, as in much of my earlier work, I found that students, once willing to accept the challenge, are stimulated and get excited when they settle into the practice of science. When the student is exposed to the way research unfolds in a laboratory or when they replicate a historic experiment they seem to get a sense of belonging to a special group, "coming of age", the beginning of a true understanding of the discipline. Clearly early work of this nature sets the tone of the course and allows the novice student to appreciate the Scientific Method and Science on a whole new level.

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This paper will also be presented at the Annual Meeting of the American Society for Microbiology (A S M) to be held in Chicago, Illinois, May 30 - June 3, 1999.

The meeting this year celebrates the 100 th Anniversary of the society.

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Printed Name/Position/Title: Theodore K. Rabb, Director

Organization/Address: Mid-Career Fellowship Program at Princeton, 129 Dickinson Hall Princeton, NJ 08544-1017

Telephone: (609) 258-4994

FAX: (609) 258-5382

E-Mail Address: tkr@princeton.edu

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