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**Detecting Plagiarism in Computer Programming Assignments  
Using Software Metrics**

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**Abstract**

Due to the highly flexible nature of computer programming, the process of modifying someone else's assignment for the purposes of cheating has become an almost trivial task to some students. On the other hand, the large numbers of students in programming classes makes it difficult for professors to catch these changes. Further complicating matters is that in lower-level classes, the assignments normally do not provide any variation. This paper examines the methods used in plagiarism detection systems and their effectiveness in lower level class settings.

## 1. Introduction

Merriam-Webster Online Dictionary [1] defines plagiarism as “an act or instance of plagiarizing”. It then defines plagiarizing as “to steal and pass off (the ideas or words of another) as one's own : use (another's production) without crediting the source“. When most people think of plagiarism, they think of writing papers, but it can be practically applied to any area of academics. It is out of the scope of this paper to speculate why students cheat, but it is safe to say that some students will cheat given the opportunity. For most of history, it has been the responsibility of the professor to set up rules to discourage plagiarism and to detect it when they are grading student work. However, given the large class sizes that institutions of learning face, it becomes quite a task to catch every instance of plagiarism.

With the rise of technology, the problem of detecting plagiarism has been partly turned over to the computer. Perhaps the most well known of these plagiarism detection tools is Turnitin [2]. Turnitin is designed to detect plagiarism in papers using a large database of stored papers and internet searches. When a teacher uses Turnitin for multiple years, it can easily catch reused papers and Turnitin can catch snippets of text stolen directly from books or websites. While Turnitin seems to be very good at detecting plagiarism in papers, it fails to catch plagiarism in computer programming assignments.

The author submitted two identical programming assignments which were then submitted to Turnitin to check for plagiarism. Turnitin's plagiarism detector returned a 0% similarity rating. While Turnitin does not detect plagiarism in programming assignments, systems have been developed to test for that kind of plagiarism. This paper looks at the problem of detecting plagiarism in those programming assignments and the detection systems that have been created.

## 2. The Problem

Programming assignments are similar to English papers in that the professor gives a topic or problem and it is up to the student to create a solution to that problem. Each student's implementation of the solution may be different while still solving the problem. The complication with programming assignments, especially at a lower level, is that the range of solutions is much smaller than the solutions of English papers. While every student in an English class may come up with a different idea or take on a

problem, programming students are often limited by the nature of the course. At the introductory level, assignments are often given to specifically introduce and enforce certain programming concepts. At this point, students do not have the experience needed to develop their own styles and methods of tackling their assignments. This results in many students turning in assignments that look very similar.

Even though this is the case, many professors have the ability to look at their classes' assignments and pick out which ones have been plagiarized. This becomes harder and harder for professors to do as class sizes grow. The problem that needs to be solved is how to catch these plagiarized assignments with an automated system.

### 3. Background and Related Work

This project used Joy and Luck's definitions of the two techniques for plagiarism. Instead of trying to paraphrase them, their definitions are presented below in it's entirety. [2]

“1) Lexical Changes: By lexical changes, we mean changes which could, in principle, be performed by a text editor. They do not require knowledge of the language sufficient to parse to a program.

1Comments can be reworded, added and omitted.

2Formatting can be changed.

3Identifier names can be modified.

4Line numbers can be change (in languages such as FORTRAN).

2) Structural Changes: A structural change requires the sort of knowledge of a program that would be necessary to parse it. It is highly language dependent.

- Loops can be replaced (e.g. a while loop in Pascal can be substituted for an until loop).
- Nested if statements can be replaced by case statements, and vice-versa.
- Statement order can be changed, provided this does not affect the meaning of the program.
- Procedure calls may be replaced by function calls, and vice-versa.
- Calls to a procedure may be replaced by a copy of the body of the procedure.
- Ordering of operands may be changed (e.g.  $x < y$  may become  $y \geq x$ .)”

Lexical changes are obviously the easiest method of attempting to plagiarize a programming assignment and most likely the plagiarism method of choice for introductory level programming classes. Changing variable names and comments will be the first thing a plagiarist will look at changing as they seem to be the most personal attribute of a piece of code. So, how can these changes be detected?

First, we can remove the comments and formatting because, broadly speaking, they have nothing to do with the function of the program. Even in the case of languages like COBOL, where spacing matters, spacing can be removed for the purposes of plagiarism testing. Likewise, using a parser, identifier names can be replaced by token representations. These tokens can then be compared by looking at the number of them that correspond and their function. In an introductory class, this method can cause problems if students have been shown that there is only one way to perform a certain action or they are following an example from a book. In this case, it is best for a professor to review the results of the token comparison with the non-tokenized code and make a decision based on that.

While this analysis may be good enough for some instructors, it is considered a weak test. Other systems have been designed to further this idea. [2][3] These systems count attributes of a program, like the tokens in the previous example. These systems are known as attribute counting systems. A popular method of attempting to detect plagiarism is to use what is known as the Halstead Metric. [11][12][13]

The Halstead Metric is based on four numbers that are taken from attribute counting. These numbers are:

$n_1$  = the number of distinct operators

$n_2$  = the number of distinct operands

$N_1$  = the total number of operators

$N_2$  = the total number of operands

These numbers are used to compute the following metrics to create a profile of a programming assignment. These metrics are:

Program Length =  $N_1 + N_2$

Program Vocabulary =  $n_1 + n_2$



$$\text{Volume} = N * (\text{LOG}_2 n)$$

$$\text{Difficulty} = (n1/2) * (N2/n2)$$

$$\text{Effort} = \text{Difficulty} * \text{Volume}$$

There is some debate about the effectiveness of the Halstead Metric. [7][8] One of the problems cited with it is that the Halstead metric ignores program structure and control flow which is an important thing to consider when determining plagiarism.

Leach[11] suggests that these problems can be solved by using the Halstead Metric in combination with the Cyclomatic Complexity, which is also known as the McCabe Complexity. [5][6] The Cyclomatic Complexity is calculated by constructing a graph of a program's control flow and then using the following formula:

$$\text{Cyclomatic Complexity (CC)} = E - N + P \quad \text{where}$$

E = the number of the edges of the graph

N = the number of nodes of the graph

p = the number of connected components.

Since the complexity is calculated by the program's control flow, it seemingly solves the weakness introduced by the Halstead metrics. Other researchers have added additional parameters to their detection programs along with the Halstead metric. Even with the addition of the Cyclomatic Complexity and the additional parameters, there is debate[2][3][8][9][11] about the effectiveness of these attribute counting systems. In an attempt to improve plagiarism detection systems, another type of system was developed: the structure-metric system.

Structure-metric systems focus on structural changes to a program more than lexical changes. While the Halstead Metric combined with the Cyclomatic Complexity may be considered a version of a structure-metric system, there are more advanced systems available. Here we will give a brief overview of three of those systems.

The Measure of Software Similarity [10] or MOSS for short, uses a method of document fingerprinting to determine similarity between two documents. This method is known as winnowing.

The basic idea behind winnowing is that fingerprint hashes are constructed for each section of a programming assignment. The size of a section is referred to as a window and the number of windows is determined by the MOSS system. These fingerprints are used to determine where the longest matching sections between programming assignments to detect plagiarism are.

MOSS is primarily a web-based system that requires a registration process to use. MOSS also requires the use of a submission script or e-mail to get files to the system and results from the system. MOSS returns data on the similarity percentage of a file against another file and the number of fingerprints and lines matched.

Another system, Jplag[9] takes an interesting take on the attribute counting system and turns it into a structure-metric system. Jplag first breaks the programming assignments down into tokens. Then the tokens are compared with tokens from other programming assignments. Jplag tries to match tokens from one program to another as much as possible and the similarity ratings between the token matches produces the similarity values which are returned for professor evaluation.

Like MOSS, Jplag is a primarily internet based system that also requires registration to use. Jplag offers an internet client with an HTML interface instead of having to use e-mail or a submission script to send assignments for testing. Results are also given in the HTML client. The results that Jplag returns include: A list of programs submitted, the language the programs were written in, the number of submissions, the number of invalid submissions, the number of matches displayed, the minimum length of a match, a distribution chart of the match percentages, a list of programs sorted by average similarity and a list of programs sorted by maximum similarity.

The last of these three systems is called Sherlock. [2] It is a part of an online submission system known as BOSS. Sherlock was created with the idea that the tokenizing process used in other detection systems would create too many false positives. It uses a five step approach to detecting plagiarism. Each pair of programs in a set of assignments is compared five times.

- “ In their original form
- with the maximum amount of whitespace removed

- with all comments removed
- with all comments and maximum amount of whitespace removed
- translated to a file of tokens”

During each of these five methods, program pairs are compared to look for “runs” which are defined as “a sequence of lines common to two files, where the sequence might not be contiguous.” Sherlock also allows for there to be interruptions inside of runs as long as they fall within a certain size limit.

Unlike MOSS and Jplag, Sherlock is not a web-based system. It can be ran on a personal computer or off of a local server and can be used separately from the BOSS submission system. Sherlock offers more initial options for detection preferences than the other systems included in this paper. Sherlock returns several different methods of viewing the detection results. One method presents the results as a graph with the thickness of the lines showing the level of similarity between individual programming assignments. Another method is similar to the results MOSS produces. The last method is a side by side comparison showing where each program is similar to another between the five different methods of detection.

#### **4. Materials**

For this project, the researcher had access to three plagiarism detection systems; MOSS, Sherlock and Jplag. Due to funding restrictions, the researcher could not gain access to a commercial metrics suite and had to simulate it. The researcher was also provided with a sample of programming assignments from a sophomore level programming class. Since the sample was provided in C++, all analysis was performed on C++ assignments.

#### **5. Method**

To perform the research, each individual's assignment was grouped together with the other assignments of the same number. Then these groups were each ran through the detection systems and their results were documented. Then the researcher constructed test cases using duplicate code and

modified code that could have resulted from plagiarism was introduced into the system to determine the strengths and weaknesses of the detection systems.

For this project, there was easy access to the MOSS, Jplag and Sherlock detection systems. But the researcher could not gain access to any system based on the Halstead Metric and Cyclomatic Complexity, this system had to be simulated.

The simulated detection system was based on the Halstead Metric with a few additions. Because it was not reasonable to construct Cyclomatic Complexity, the system counted items such as the number of loops of each type and the number and type of control flow statements such as if statements.

Joy and Luck make the assumption that the plagiarist will attempt to hide his work by making changes to a programming assignment. While this is probably true in most cases, we can not discount the fact that some plagiarists will not modify anything at all. Considering this, as a first line of detection, the simulated system removes, comments and standardizes white space the different files to make detection uniform. The Unix command diff was then performed on the files being compared to see if they were the same or not.

## **6. Results and Analysis**

In general, all of the detection systems returned high rates of similarity for all groups of assignments. The average similarity rating between all of the program was calculated to be 60%. Giving a ten percent leeway, everything under 50% was considered acceptable and everything above 70% was considered to be suspicious. Programs with similarity ratings over 90% were considered extremely suspicious and were most likely cases of plagiarism.

The simulated test system was the worst performing system out of the systems tested. While it did detect similarities between programs, the control flow metrics that were included did not match the standards of the Cyclomatic Complexity as they have been reported. While none of the student programs that were tested had a large amount of extraneous data, a constructed test program showed that extra data can easily throw off this system. Extraneous information that can skew the detection system in a plagiarist's favor includes: functions that are never called, extra variables that are never used and large

amounts of statements that do not change the function of the program. If the detection system can does not remove comments, then a large amount of comments can also skew the detection metrics.

While the simulated test system produced averages that were slightly lower than the other detection systems, the results were not so bad that one could say that it was ineffective at alerting an instructor of possible plagiarism. The large amount of extraneous data that can throw the system's metrics off would also be very visible to an instructor looking over the code themselves. It is worth noting that this metric system is possibly the most likely to be skewed due to its mainly attribute counting nature and weak structure-metric nature. It may also be the most likely to give a false-positive, but it is only speculation until further research can be performed.

The MOSS system was less forgiving than the simulated test system, but was not as strict as the other two systems. While no problems seemed to be found within the student programs, the special test-case programs indicated that MOSS is not as sensitive to code modifications with its default window size. With the winnowing algorithm that MOSS uses, it is possible to modify and position statements so that the fingerprinting window will account for it in different places in two different programs. This will make the fingerprints different for each program, making it harder to conclusively detect plagiarism. The fingerprinting system is also not very sensitive to other lexical modifications.

Jplag and Sherlock were very close in their similarity ratings, though there were some problems found with the Jplag system. By changing “while loops” to “for loops” and “case statements” to “if statements” and vice versa, something discussed in the definition of structural changes, Jplag can be confused. Another attack that was found to be effective against Jplag was also effective against the simulated system. That is the problem of introducing variables and functions that are not called or used. The results from this study of Jplag are consistent with the results from the creators of the system.[9] Their paper provides a more comprehensive look at the system than this paper does.

Sherlock's approach to detecting plagiarism effectively made all attacks that worked against the other detection systems invalid. If an attack was successful at the lexical level, it was caught on the token level and vice-versa. The results presentation from Sherlock was also superior to the other systems giving

an instructor more information about where the plagiarism occurred in a program instead of simply giving the program names as the other systems do.

## **7. Conclusions and Discussion**

Plagiarism detection systems are a good tool for helping an instructor look for instances of plagiarism, though they can return false positives. The results of the experiment conducted in this project suggest that these false positives will most likely occur more frequently in introductory level programming classes due to student inexperience and limited flexibility afforded in their assignments.

Research also suggests that metrics methods of detecting plagiarism, while easy to construct are not the best way of detecting plagiarism due to the number of ways they can be fooled. Structure-based systems, while not completely infallible are more robust and reliable when it comes to detecting plagiarism.

All three of the structure based plagiarism detection systems performed adequately but based on research results, Jplag and Sherlock outperform the MOSS detection system. The experiments suggest that Jplag and Sherlock are roughly even in capabilities, but Sherlock is resistant to more attacks than Jplag. The researcher would recommend Sherlock to any institution looking to institute a plagiarism detection program. The reasons for this are that while Jplag has a good interface and fast run times, it requires time to upload the files for processing. The Sherlock system, on the other hand can be ran locally and is easily modifiable to specific needs due to it's open source nature. Jplag's developers do offer assistance in developing one's own Jplag client, but it does not allow you to modify Jplag's internal workings as Sherlock does.

The detection systems provide a good starting point for instructors to look for plagiarism but they should only be used as a tool to begin further investigation of a student, not used to convict without first examining a program in more detail. An instructor's knowledge and intuition are invaluable in these matters. In order to help curb plagiarism, an instructor could attempt to allow more freedom and variation in their introductory programming assignments. Instructors could also put policies in their syllabus about sharing past programming assignments with other students.

Further research in this area could focus on fixing the problems that exist in the current detection systems and expanding the systems to include internet searches. Instructors could also study the effect of putting warnings on their syllabus concerning the use of these detection systems and the effect of allowing more freedom and variation in their introductory level programming assignments.

Another interesting research problem for the future would be to compare the similarity rates of more advanced programming classes compared to the lower level classes to see if programming skill level influences the similarity percentages across students in a class.

### 8. Acknowledgments

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**The Water Quality of Brush Creek:  
A Baseline Study**

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Read and accepted by:  
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***Abstract***

Brush Creek flows through Mercer County from its headwaters near Bluefield, WV to its confluence with the Bluestone River and is moderately threatened by commercial and residential development. The purpose of the project was to collect baseline data on the water chemistry of Brush Creek against which future changes in the health of the stream can be compared. Five sites were chosen from the length of brush creek and water samples were taken repetitively over a six-month period. The samples were analyzed for several standard parameters including pH, temperature, COD (chemical oxygen demand), and the presence of arsenic, copper, and phosphates.

The results show that the water quality of Brush Creek declines after passing through the city of Princeton, WV and the surrounding residential areas. However, some self-remediation of the stream occurs as it passes through less-developed areas prior to reaching the confluence with the Bluestone River. Suggestions for further studies on Brush Creek are given.

### ***Introduction***

Brush Creek originates near Bluefield, WV and flows indirectly northward. The creek travels through a portion of Bluefield before reaching the town of Princeton.<sup>i</sup> Over the past few decades, Princeton has developed around Brush Creek. Several plazas, two four-lane highways, and extensive residential areas all exist within a mile of Brush Creek.<sup>ii</sup> For the last few miles of its length, Brush Creek travels through a semi-protected zone partially owned by the Nature Conservancy. Eventually, it reaches its confluence with the Bluestone River, which is an important, local recreation area.<sup>iii</sup>

Over the past 100 years, the town of Princeton WV has developed from a rural, sparsely populated area into an active, business location. In 2000, the census bureau found there to be approximately 6,347 people in this town, which is consistent with historical trends for Princeton.<sup>iv</sup> Since 1930, Princeton has never claimed more than 8,393 residents, and never fewer than those in the 2000 census. On average, Princeton's population has been around 7,400 people for the past 70 years.<sup>v</sup> However, while the residential population has not changed much, the development in the area has massively increased over the past few decades. In the 1960's, building began on Interstate 77, which now passes directly through Princeton.<sup>vi</sup> Going north/south, this interstate is a heavily traveled road for vacationers, business people, and truck drivers alike.<sup>2</sup> On an average day, I-77 may see 50,000 vehicles traveling through the town of Wytheville, VA.<sup>16</sup> In the years that followed, Highway 460 was built and now travels almost alongside Brush Creek as it passes east/west through Princeton. This four-lane road carries a similar amount of drivers as I-77.<sup>6</sup>

Due to its convenient location between Beckley, WV (an already bustling city at the time of the development of I-77 and US 460) and Wytheville, VA, Princeton businesses quickly developed to accommodate the motorists traveling through the area. Hotels, gas stations, restaurants, and other businesses moved into the area in the early 1970's.<sup>6</sup> It is recorded that the town can hold an excess of 32,000 people per 2 square miles at a given time.<sup>6</sup> Princeton's development surrounded Brush Creek

during this time. Particularly, a large shopping plaza (commonly referred to as the Kroger Plaza) was built within yards of the stream.<sup>2</sup>

Brush Creek currently extends for 20.3 miles before it joins with Bluestone River.<sup>vii</sup> The Bluestone River was designated a National Scenic River in 1988 and remains a frequently visited scenic area. Also, the Bluestone flows through two state parks, Pipestem and Bluestone, which are frequented by hundreds of tourists every year.<sup>viii</sup> The quality of water in both Bluestone River and Brush Creek is extremely important to the recreation industry of southern West Virginia.

Water Quality Assessments, sometimes called Measurement Programs, can be conducted to evaluate the overall health of any stream, river, lake, or other body of water. Depending on the use of the water, different chemical and physical factors may be tested.<sup>ix</sup> The WV Department of Environmental Protection (DEP) does water assessments bi-annually on all the streams and rivers in the state. In doing this, they are assessing the water quality, habitat condition (geology of area), and benthic macroinvertebrate (visible bottom-dwelling aquatic life without backbones) community status. The main purposes of the DEP's study are to assess pollution trends, coordinate restoration projects, and determine the health of current and future drinking water sources (Brush Creek has never been a source for drinking water). Because of the time, the extent, and the nature of this study, the DEP limits factors of their assessment down to six critically important components: pH, iron, aluminum, and manganese concentrations, fecal coliform, and biological aspects.<sup>x</sup> While these factors give an indication of the health of a stream, additional study needs to be done to determine causes of pollution and locations of change in the stream.

According to the DEP's 2004 stream assessment report, Brush Creek is a Category 1 stream for its first 4.1 miles, and then almost immediately deteriorates into a Category 5 stream for the rest of its course. The DEP defines stream categories as follows.<sup>xi</sup>

- Category 1 - fully supporting all designated uses
- Category 2 - fully supporting some designated uses, but no or insufficient information exists to assess the other designated uses

- Category 3 - insufficient or no information exists to determine if any of the uses are being met
- Category 4 - waters that are impaired or threatened but do not need a Total Maximum Daily Load (TMDL)
  - Category 4a – waters that already have an approved TMDL but are still not meeting standards
  - Category 4b – waters that have other control mechanisms in place which are reasonably expected to return the water to meeting designated uses
  - Category 4c – waters that have been determined to be impaired by pollution or other natural factors
- Category 5 – waters that have been assessed as impaired and are expected to need a TMDL

In another DEP report, Brush Creek is shown to have received its Category 5 rating for biological factors. In 2004, these biological factors affected aquatic life in approximately 16.2 miles of Brush Creek.<sup>xiii</sup> The presence of chemical elements and metal compounds are known to change the environment in water and may affect the biology of the stream. Since chemical elements may exist in different forms, called species, in water, it can be difficult to tell the exact cause of these biological effects. Some common elements that can have a negative affect on aqueous life include excesses in copper, phosphorous, and the presence of various trace metals and metalloids such as aluminum, iron, and arsenic.<sup>9</sup>

The effect of man on water systems has not only been studied for a long time, it has been studied more extensively than almost any other area of environmental science.<sup>9</sup> Urbanization, like that of Princeton in recent years, can change the land, which in turn can change the rates of erosion into water, infiltration, overland flow, and evapo-transpiration. Construction of newly developed areas temporarily increases the amount of sediment in a stream, later the blacktopped areas cause less depression storage (water contained in natural depressions of land, like puddles) and thusly more runoff than before the development took place. At that point, the overall quality of the stream decreases somewhat exponentially as various factors affects others. In places where this occurs, assessment of the water is crucial.<sup>xiii</sup>

Because urbanization can affect a stream in a wide variety of ways, the assessments done by the DEP do not give a complete view of the actual health of Brush Creek. There are several unexamined factors that have the potential to be damaging to the environment. Some of these factors were examined in this study and, along with the information from the DEP study, give a better indication of the health of Brush Creek.

**Results**

Parameters examined were: temperature, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), and phosphate, arsenic, and copper concentrations. These were tested on a series of five sites that were chosen for their accessibility and location along Brush Creek.

**Temperature**

Not surprisingly, Figure 1 shows that the temperature goes through a marked decrease as the winter months approach. Also evident is an increase in temperature at both of the middle sites (Princeton and Brickyard Road). These sites were more open to sunlight than others, which is the likely cause of the higher temperatures.

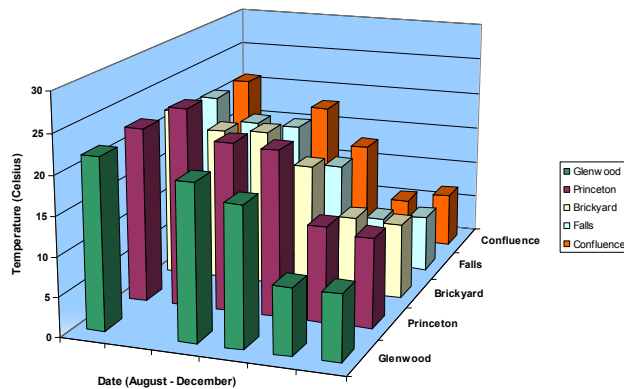


Figure 1: Temperature of Brush Creek (August – December 2005)

**pH**

Healthy streams have pH values ranging from 7.5 – 8.5 without much variation through the stream. Figure 2 shows that there is a significant change in pH between the Princeton and Brickyard Road

sites for Brush Creek. This is a strong indication that the quality of water is changing between these two points.

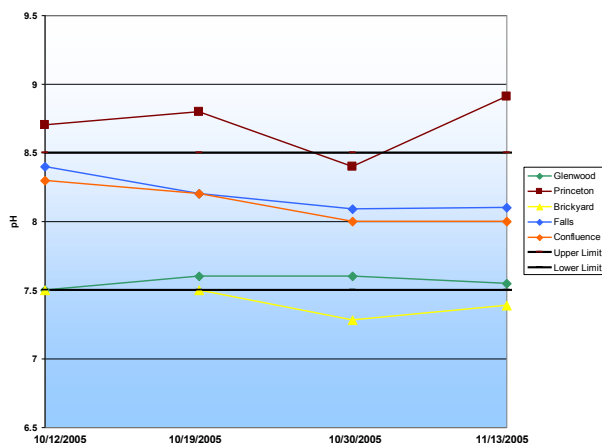


Figure 2: pH of various sites within Brush Creek (Autumn 2005)

### Total Suspended Solids (TSS)

The highest amounts for TSS were found to be in late October. It is hypothesized that this is due to seasonal factors and may be part of a larger seasonal trend. It is also found that the amount of suspended solids in the stream increases as it flows towards the confluence. The TSS data is shown in figure 3.

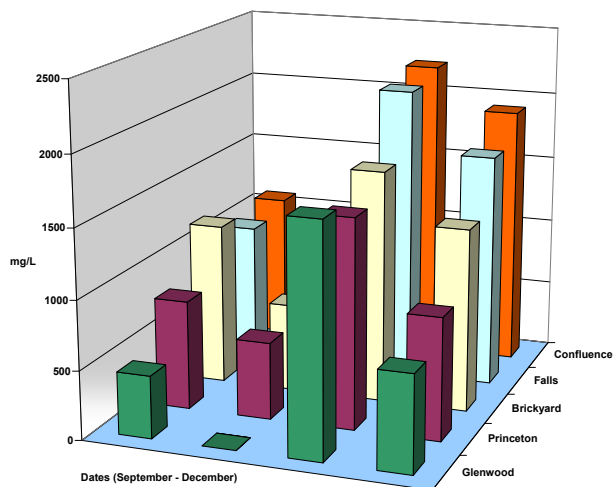


Figure 3: Total Suspended Solids (Autumn 2005)

### Total Dissolved Solids (TDS)

As with TSS, TDS was measured using the standard method from the Handbook of Water Analysis. As shown in Figure 4, the results show a large decrease after the first week of sampling. It is

not known at this time if this is an artifact from the testing procedure or if the end of a seasonal trend was observed. Reexamination of this parameter over a longer period of time is warranted. Despite this anomaly, the trend in TDS data seems to show an increase as the stream flows towards the confluence, similar to the trend in TSS data mentioned earlier.

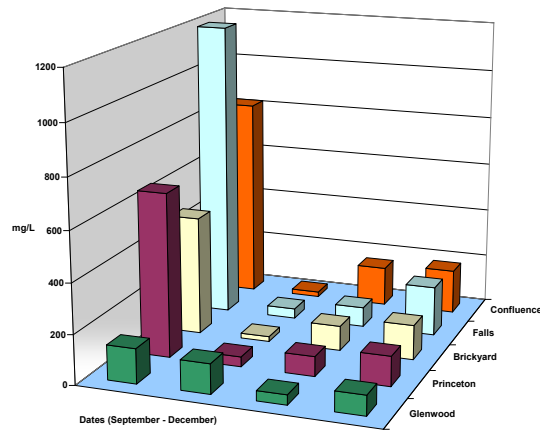


Figure 4: Total Dissolved Solids (Autumn 2005)

### Chemical Oxygen Demand (COD)

Chemical Oxygen Demand is an important measure that describes the amount of oxidizable organic material present in the stream. Large COD values are indicative of organic pollutants. COD values were determined more infrequently than other parameters and a complete calibration curve was not obtained. However, relative COD values are available and data from a representative week is shown in Figure 5. The data shows that COD values reach a maximum at the Brickyard Road site.

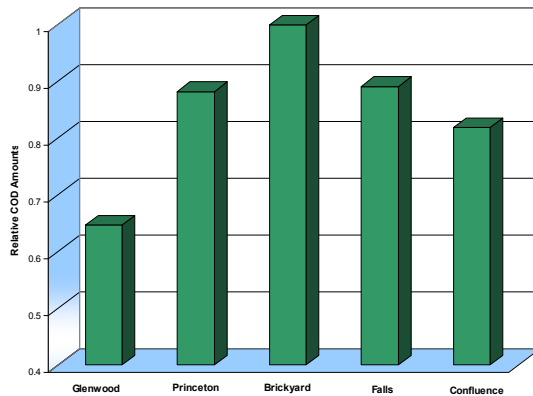


Figure 5: Relative COD values (week of November 13, 2005)

**Phosphates (Total Inorganic Phosphate)**

Phosphates come from detergents, pesticides, but primarily from fertilizers. Phosphate levels were consistently highest at the Brickyard Road site as can be seen in figure 6.

Not only does the amount of phosphates in Brush Creek increase dramatically between the Princeton and Brickyard sites, but also it decreases again as the water flows towards the confluence. This is an indication that there is self-remediation in the stream after the Brickyard Road site. This self-remediation may be due to dilutions that occur from smaller streams flowing into Brush Creek at various locations.

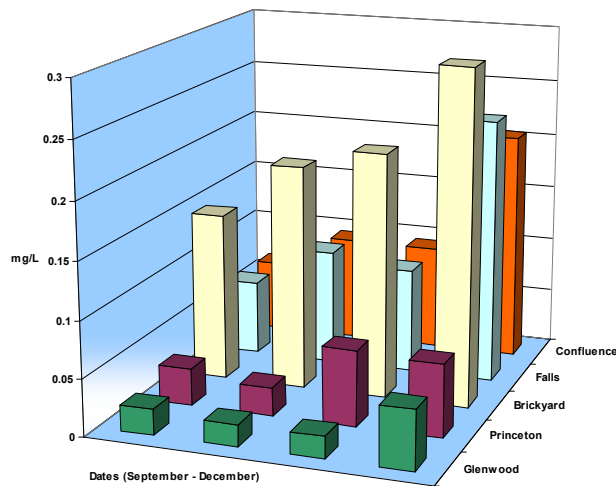


Figure 6: Inorganic Phosphate levels (Autumn 2005)

While containing the same data, the line graph in figure 7 may show the amount of self remediation more clearly than figure 6.

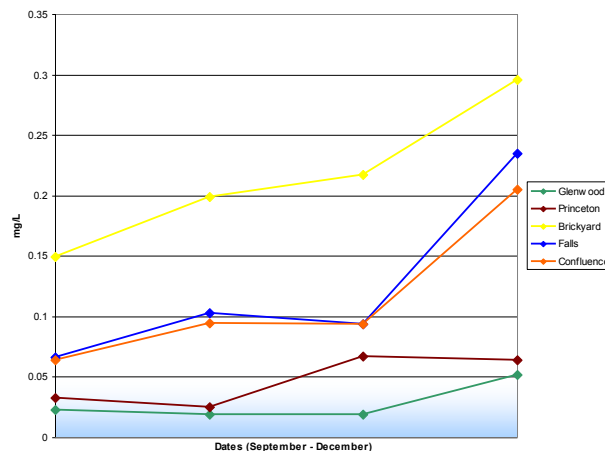


Figure 7: Inorganic Phosphate levels (Autumn 2005)

Note: same data as Figure 6



## Arsenic

Arsenic was tested semi-quantitatively using a kit manufactured by Hach. A large amount of scatter was present in the data with values ranging from no detectable arsenic to 30 ppb. Note that all measured values were below the current maximum allowable concentration in drinking water of 50 ppb.

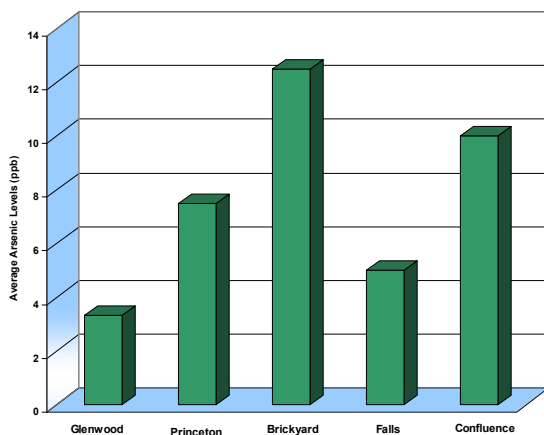
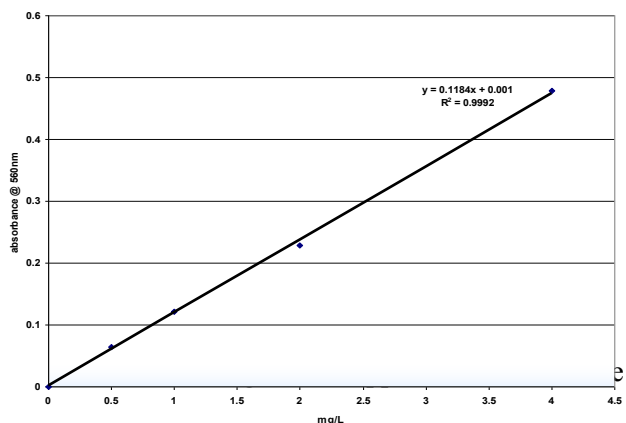


Figure 8: Average arsenic level (Autumn 2005)

## Copper

No copper was detected in any of the samples taken. Figure 9 shows the calibration curve for copper indicating a detection limit of less than 0.5 mg/L.



## Discussion and Conclusions

The data collected from Brush Creek shows that there is a significant change in water quality between the Princeton and Brickyard Road sites. While Princeton and Glenwood are generally similar in all examined parameters (except pH, *vide infra*), each parameter consistently changes prior to the Brickyard Road sampling site. There are significant changes in the pH of the stream before and after the

Princeton site, which is an indication that the over-all water quality is changing. pH drops dramatically between Princeton and Brickyard Road. Arsenic, COD, and phosphate levels reach a maximum at the Brickyard Road site. This effect is particularly noticeable for phosphate concentrations. With the exception of TSS and TDS, all parameters exhibit a trend toward more normal values as the stream approaches the confluence with the Bluestone River. While none of the parameters examined in this study have values that indicate that Brush Creek is seriously damaged or that recreational exposure poses a significant health risk, the data do clearly indicate that Brush Creek is negatively impacted by human activity.

Reasons for the change between Princeton and Brickyard Road may be linked to residential factors. The pollutants tested for in this study mainly come from non-industrial sources (homes and farmlands). Phosphates mainly come from fertilizers, but also from detergents and pesticides. Arsenic may leech into water from neighboring rock formations whenever the water levels change significantly.<sup>xiv</sup> COD gives an indication of the strength of sewage and other oxidizable organic materials in the water.<sup>xv</sup>

### **Recommendations for Long Term Monitoring**

Further testing should be done to determine if industrial pollutants from Princeton are having an equal or greater affect on the water of Brush Creek. Also, studies on Brush Creek should involve testing over a longer period of time to determine if seasonal trends affect the parameters that have been studied. It is possible that the large values in the first week's TDS data are due to the end of a seasonal trend. Also, further studies need to be conducted between the Princeton and Brickyard Road sites to determine how the change in water quality is occurring. Testing more sampling sites between these two would give an indication of this either being a gradual change or if there is a point source of pollution. Testing additional sampling sites between the Brickyard Road site and the Confluence could give an indication of the rate of self-remediation that is occurring in the stream. If most of the self-remediation is due to dilutions from smaller streams flowing into Brush Creek, the changes will be most apparent close to those streams.

While this study was limited by time constraints, the results clearly show changes in the water quality of Brush Creek that appear to be due to human activity. A year-long study of Brush Creek which would examine these same parameters, and possibly others, would be very beneficial to understanding the behavior of this stream. Testing once a week for a year would allow for analysis of seasonal trends and would help in determining if changes were significant or not.

The authors recommend that the following parameters be examined on a weekly basis for a one year period followed by monthly sampling on an on-going basis to assess the effects of continued development on the health of Brush Creek. Concentrations of chemical species should be measured both in aqueous solution and in stream sediment where appropriate.

- Inorganic species: trace metals (via ICP-MS), inorganic phosphate, inorganic nitrogen compounds (nitrates, nitrites, ammonia)
- Organic species: common pesticides, hydrocarbons typically found in urban run-off, volatile organics (EPA method 624), and COD
- Biological factors: Biochemical Oxygen Demand (BOD), fecal coliform, and benthic macroinvertebrates
- Other parameters; pH, temperature, TSS, TDS, and discharge data

### **Methods and Materials**

In order to determine the water quality of Brush Creek, two major steps had to be accomplished; sample collection and chemical analysis. The first step, sample collection, involved choosing sites that were representative of Brush Creek as a whole. Five were selected, beginning with the Glenwood site that is near the headwaters of the stream. Limited residential development is present at the headwaters of Brush Creek so pristine samples are not available. The Glenwood collection site is the site closest to the headwaters from which repetitive sampling can be obtained on a reliable basis. The Princeton site is second and is located directly past the Kroger Plaza on Stafford Drive next to the fire department training station. Changes in the water quality due to recent commercial development in the town of Princeton would be most noticeable at this location. The third site was along Brickyard Road, shortly past the town of Princeton. This spot is located in a relatively rural, residential section with farms and a limited amount

of commercial activity. The fourth site is known as Brush Creek Falls and is located directly past a small waterfall. A steadily decreasing amount of commercial and residential development is present between Brickyard Road and Brush Creek Falls. The last site is at the confluence with the Bluestone River. Due to its location, this site gives the clearest indication of whether or not self-remediation is taking place.

The precise location of each sampling site was recorded using the Global Positioning System (GPS). The GPS coordinates for each site are listed below and Figure 10 shows the approximate location of the sampling sites.

· Glenwood:	37° 20' 19'' W 81° 08' 67'' N	· Brickyard:	37° 24' 70'' W 81° 04' 28'' N
· Princeton:	37° 21' 71'' W 81° 05' 32'' N	· Falls:	37° 28' 00'' W 81° 03' 63'' N
	· Confluence:		37° 28' 65'' W 81° 03' 71'' N

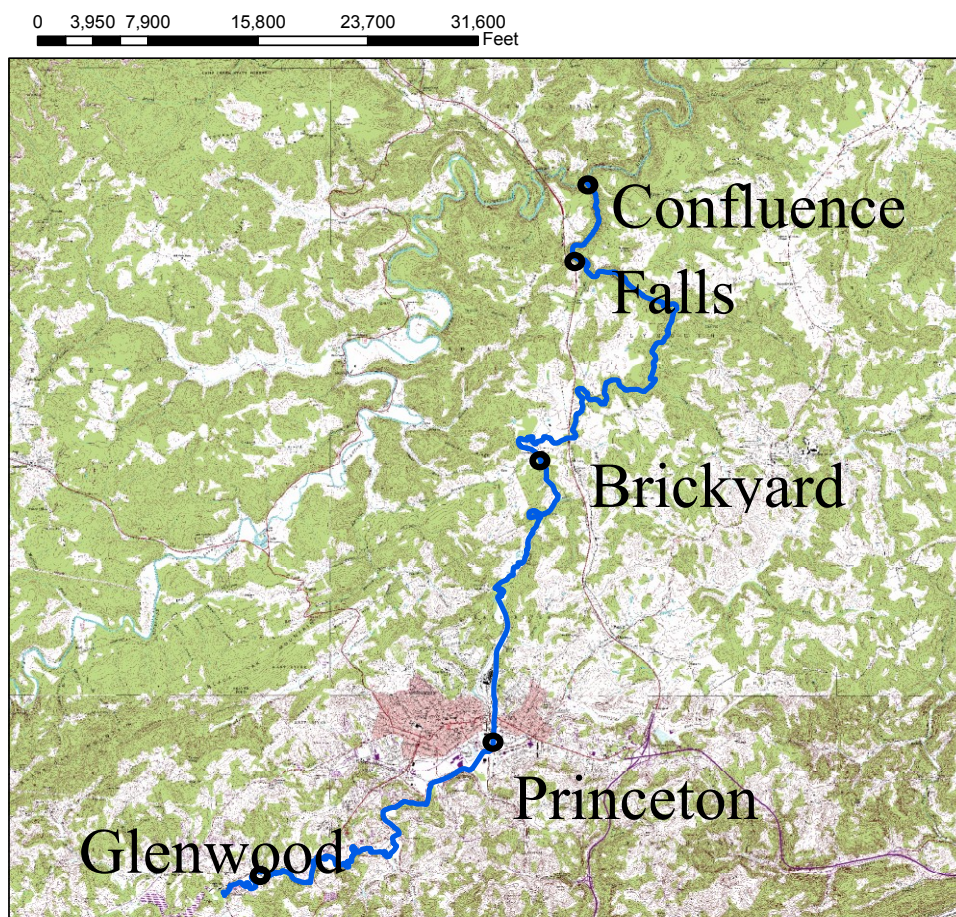


Figure 10: map of the sampling sites along Brush Creek

Once the sampling sites were chosen, testing could begin. Samples were taken in plastic bottles and the field conditions and relevant data were recorded in a field notebook.

### **Temperature**

Temperature was measured using a Checktemp™ temperature probe (Hanna Instruments) at each of the sampling sites. Probe was calibrated using ice water mixtures and boiling water.

### **pH**

pH was measured using either a pH probe connected to Turtle software installed on a PC after the samples were returned to the lab or in the field using a Checker™ pH probe (Hanna Instruments). The Checker probe was more precise and since it could be used in the field, provided a more representative measurement. pH probes were calibrated using commercially available pH 7, pH 10, and pH 4 buffers.

### **Total Suspended Solids**

The Total Suspended Solids (TSS) was measured using a standard method from the Handbook of Water Analysis.<sup>xvi</sup> This involved drying and weighing a sheet of filter paper and then passing 15mL of sample through the filter paper and drying it again. The increase in mass of the filter paper is taken as the amount of the TSS.

### **Total Dissolved Solids**

The Total Dissolved Solids (TDS) was also measured using a standard method from the Handbook of Water Analysis. This test used the filtrate from the TSS test. Five milliliters were collected from the filtrate and placed on a tared dish which was placed in a 105°C oven for at least five hours. The increase in mass the dish was taken to be the amount of Total Dissolved Solids.

### **Chemical Oxygen Demand**

The Chemical Oxygen Demand (COD) was measured using an open reflux method. Samples were mixed with a catalyst solution and a digestion solution prior to refluxing. The catalyst solution consisted of 100.97 g of concentrated sulfuric acid and 0.56 g of silver sulfate. The digestion solution was chromic acid produced from 16.7 mL of concentrated sulfuric acid, 1.7 g of mercuric sulfate, and 1.02 g

of potassium dichromate diluted to a 100 mL solution. One milliliter of sample mixed with 1.4 mL of catalyst solution and 0.6 mL of digestion solution was placed in a ten milliliter microscale reaction vial equipped with a water cooled condenser that was open to the atmosphere. This apparatus was placed in a sand bath on a hot plate and the mixture heated to 145 - 155°C for three to five hours. Orange chromium(VI) present in the dichromate ion is reduced to green chromium(III) as organic species in aqueous solution are oxidized. This color change can be followed quantitatively using visible spectroscopy. Following the digestion period, the samples were allowed to cool to room temperature prior to being diluted with 2 mL of deionized water. The concentration of remaining dichromate ion was determined by measuring the absorbance of the solution at 600 nm using a Varian DMS100S spectrometer. A control sample prepared with HPLC grade water was used as the reference point. Determination of absolute COD values requires preparation of a calibration curve using solutions containing known concentrations of potassium hydrogen phthalate. The calibration curve was not prepared in this study.

### **Phosphates**

The phosphate levels in Brush Creek were measured using a commercially available reagent from Hach. Pre-made reagent was obtained in packets. The contents of a packet were mixed with 10 mL of each sample and shaken vigorously for 10 minutes. Molybdate in the reagent reacted with phosphates in the sample to produce a phospho-molybdate complex. Ascorbic acid (also in the reagent) reduced this complex to give a characteristic blue color. Absorbance values were measured at 890 nm using a Varian DMS100S spectrometer. The concentrations were determined from a calibration curve prepared with known amounts of potassium dihydrogen phosphate.

### **Arsenic**

Arsenic was measured using a semi-quantitative, colorimetric test kit from Hach. All chemical transformations were done using the pre-made reagents supplied with the kit. The first step in this process was to oxidize hydrogen sulfide present in the sample into sulfates. This prevented interference from H<sub>2</sub>S gas. Second, the solution was neutralized, and a mixture of sulfamic acid and zinc added to create a

strongly reducing environment which would convert all arsenic in the solution to arsine gas. A test strip coated with mercuric bromide was placed at the top of the reaction chamber. Reaction of this strip with arsine gas produced characteristic colors which could be converted into arsenic concentrations colorimetrically using the chart provided with the reagent kit.

### **Copper**

The copper levels in Brush Creek were measured using a commercially available reagent from Hach. Pre-made reagent was obtained in packets. The contents of a packet were mixed with 10 mL of each sample and shaken vigorously for 10 minutes. A salt of bicinchoninic acid present in the reagent would produce a characteristic purple color in the presence of copper. Absorbance values were measured at 560 nm using a Varian DMS100S spectrometer. The concentrations were determined from a calibration curve prepared with known amounts of copper nitrate.

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**Momentum Improvement and Returns from investing in Fidelity Sector Funds**

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**Abstract**

Prior research in the area of momentum investing has shown that over the intermediate term time horizon, stocks that have outperformed in a previous time period tend to outperform in a subsequent time period. It has also been demonstrated that stocks returns over 6 to 12 month terms can largely be attributed to the returns in their respective industries. Thus, industry momentum creates the possibility of creating a trading strategy using industry sector funds. This study reviews a strategy in which portfolios are formed based on the change in quintile ranking of returns of an industry over two subsequent observation periods, which are then held for investment for a predetermined holding period. The results of the study show that -- for the strategy examined -- an investor would earn much higher absolute and risk adjusted returns as compared to those obtained from a simple momentum strategy of buying winners.

## **I. Introduction**

There is a growing body of research on the linkage between a stock's past and future returns. These studies have examined whether relative strength of a stock or industry group over the short, intermediate and long term carries over to future time periods. For the long term time horizon, De Bondt and Thaler (1985) show that stocks that have exhibited poor (superior) performance over the past 3-5 years time period tend to outperform (underperform) in the next. However, recent studies examining the short (three months) to intermediate term (six months and more) time horizons have reported that the correlation between past and future returns to be positive.

Jegadeesh and Titman (1993) document that a self financing strategy of creating an equally weighted portfolio of buying stocks whose performance was at the top decile and shorting those who's performance was in the bottom decile over a previous six month period, produced an excess return of around 1% per month.. Referred to as the (J, K) strategy it shows portfolios constructed on the basis of superior returns in the previous J months outperform their peers in a subsequent K month hold period. The study also shows that these abnormal returns tend to reverse themselves after the 13<sup>th</sup> month.

Moskowitz and Grinblatt (1999) attribute a large part of momentum returns in stocks to momentum of their respective industries. . Moskowitz and Grinblatt also argue that as a matter of strategy it is more practical to be long in an entire industry because of the greater diversification and reduced trading costs involved. This argument presumes that entire industries or their proxies are available as a single purchase.

Following the findings of Moskowitz and Grinblatt, O'Neal (2000) evaluated the performance of a momentum strategy that invested in Fidelity select sector mutual funds. In his research the funds were ranked based on their returns over the previous 6 or 12 months. Equally weighted portfolios consisting of the top 3 and top 6 funds were constructed and invested in for the next 6 or 12 months. Results of the study show that the momentum portfolio significantly outperformed a buy and hold strategy of investing in the S&P 500 for the sample period of May 1989 to April 1999. Because of the De Bondt and Thaler

(1985) result that superior performance by an industry group over a relatively long time period may be followed by periods of underperformance, Gomes & Islam (2005) conjectured that it may be possible to improve returns over a simple momentum strategy of buying and holding winners by excluding multi-period winners from the hold portfolio. Islam and Gomes accomplish this by forming portfolios based on change in quintile ranking of returns of S&P 500 industry groups. They demonstrate that portfolios chosen on the basis of large improvements of ranks provide annual returns significantly larger than those obtained from following a simple momentum strategy as well as the buy and hold strategy of the S&P 500 index. In Section II, we discuss our data source, methodological issues and results. In Section III, we examine the question optimal holding period once a portfolio has been formed. Section IV, concludes the discussion.

## **II. Data, Methodology ad Results**

The purpose of this paper is to apply the Islam and Gomes momentum change strategy to Fidelity sector Funds. As discussed in the literature review, prior research has shown that stocks or industry groups with high relative strength in one period continue to outperform others in a subsequent period or for several subsequent periods. While most of these studies considered stocks or industry groups, O'Neal (2000) extended the examination to sector mutual funds using data from Fidelity Sector Funds, and found that superior performance in one period leads to superior performance in subsequent periods. Gomes & Islam (2005) introduced momentum improvement as a basis of portfolio selection. Using the S+P 500 industry indexes for the period 1967 to 1997 they show that just as relative strength ranks and subsequent returns are positively correlated, so are the rank changes and subsequent returns correlated also. In this paper we will combine the Islam-Gomes study with the O'Neal study to examine if rank change in Fidelity sector mutual funds over two subsequent periods are positively correlated with returns in one or more ensuing periods.

### **Data**

The Dividend adjusted prices for the fidelity sector funds were obtained from the Yahoo Finance web site. The returns of the S+P 500 index will be used as a proxy for the market return, and were

obtained from the Yahoo Finance web site also. The Treasury bill rates will be used as a proxy for risk-free rates of interest. The Treasury bill rates were obtained from the various issues of Federal Reserve Statistical Releases on Selected Interest Rates.

### **Methodology**

The methodology for the study includes the following:

- (1) Compute evaluation period (1 to 24 months) returns for each Fidelity Sector Fund for the period January 1987 through May 2005
- (2) These returns are ranked into quintile rankings. The funds that performed in the top 20% will be designated quintile 1 group and the second best 20% as the quintile 2 groups etc... Thus there will be five groups with quintile ranks of 1 through 5.
- (3) For any two consecutive periods of equal length (such as a six month period followed by another six month period), the changes in quintile ranks will be computed for all funds and rank changes will be allocated. Accordingly if a fund's quintile rank increases from 5 to 1, its rank has changed by 4 and will be categorized accordingly for portfolio selection. Since a fund's quintile rank can increase or decrease, we will have nine portfolio groups with rank changes falling between 4 to -4.

Equally weighted portfolios are then constructed based on the rank change in the two previous periods of equal length. Thus we can form a portfolio based on the rank change of two consecutive six months periods (J,J) and hold the portfolio over K (1 to 24 months) subsequent periods. The strategy can be designated as a ((J,J),K) strategy.

Returns for the portfolios for different hold periods are then computed.

It is expected that for improvements, rank change and returns will be positively correlated. For negative rank change no such assertion is made. Both absolute return and risk adjusted returns will be computed for each rank change group. The risk adjusted returns are computed by using the Sharpe and the Treynor indexes. The beta coefficient and alpha value for each portfolio are computed regressing the market model.

$$(1) \quad R_i - R_f = \alpha_i + \beta_i (R_m - R_f) + u$$

where  $R_i$  is the portfolio return belonging to the  $i^{\text{th}}$  rank change group.  $\beta_i$  is the market risk of the  $i^{\text{th}}$  portfolio.

The risk adjusted returns will then be computed by applying the Sharpe as well as the Treynor indexes.

$$(2) \quad S_p = \frac{\overline{R_i} - \overline{R_f}}{\sigma_i}$$

$$(3) \quad T_p = \frac{\overline{R_i} - \overline{R_f}}{\beta_i}$$

where  $\overline{R_i}$  and  $\overline{R_f}$  are the average return in a portfolio and average risk free rate of interest for the holding period,  $\sigma_i$  is the portfolio standard deviation.

To examine whether rank change do make statistically significant contribution to portfolio return, we run the following dummy variable regression

$$(4) \quad R_i = b_0 + b_i (R_m - R_f) + a_4 D_4 + a_3 D_3 + \dots + A_3 D_3$$

where  $R_i$  is the return of the  $i^{\text{th}}$  rank change portfolio,  $i = 4$  to  $-3$ ,  $D_i$  are the dummy variables, and  $a_i$  are the shift parameters.

In Table 1 and 2, we present the average annual returns obtained from applying several simple momentum strategies and momentum change strategies. In Table 1, the numbers under the (6, 6) column show returns from buying sector funds with six month quintile rankings of 1 through 5, and holding them for the subsequent six months. Similar explanations apply to other columns. The results show that momentum strategy does give superior returns. For each of the observation and hold period examined, quintile 1 portfolio provides the best returns, and returns decline as quintile ranks decline.

**Table 1**

**Simple Momentum Strategy**  
**Average annual Returns: January 1987- May 2005**

<b><u>Quintile</u></b>	<b><u>( 6 , 6 )</u></b>	<b><u>( 6 , 12 )</u></b>	<b><u>( 12 , 6 )</u></b>	<b><u>( 12 , 12 )</u></b>
1 (best)	19.75%	19.08%	21.28%	18.34%
2	18.10%	17.41%	16.47%	16.60%
3	14.30%	14.44%	15.48%	15.15%
4	12.18%	13.85%	14.28%	15.29%
5	10.96%	12.04%	11.55%	14.61%

**Table 2**

**Rank Change Strategy Returns**  
**Average Annual Returns: January 1987- May 2005**

<u>Rank Change</u>	<u>((66), 6)</u>	<u>((66), 12)</u>	<u>((1212), 6)</u>	<u>((1212), 12)</u>
4	18.63%	22.38%	25.84%	22.51%
3	17.94%	19.91%	22.17%	21.95%
2	17.15%	18.97%	21.26%	22.16%
1	14.39%	15.31%	18.01%	17.09%
0	15.93%	15.80%	14.54%	13.54%
-1	16.26%	15.36%	13.98%	14.08%
-2	15.34%	14.41%	13.73%	13.42%
-3	15.10%	14.72%	10.53%	11.75%
-4	9.09%	9.02%	13.26%	15.91%

In Table 2, returns obtained from momentum change strategies are given. For example, the numbers under the ((66),6) columns show the returns pertaining to portfolios with a given rank changes under two consecutive six month observation periods, and held for a subsequent six month period. As is obvious, portfolios with the highest momentum change (rank change of 4) give the best returns followed closely by rank improvements of 3 and 2. Also, except the ((66), 6) strategy where returns are similar to the returns under the (6, 6) strategy, generally ((JJ), K) strategies with rank improvements of 2 or better give superior returns relative to the relevant simple momentum strategy by as much as 7% annually.

To see, how risk and returns are related we computed the Sharpe and Treynor indexes for each of the momentum change portfolios. Table 3 show these computations for the ((66), 6) and ((12 12), 12) portfolios. For the (66),6) strategies market risks for each of the portfolios are almost equal to one (risk of the market). However for the ((12,12)12) strategies the market risks are much lower for portfolios with large rank improvements. Both the Sharpe and Treynor indexes are generally larger as rank changes get larger, with the largest improvement registered by the portfolio with rank change of 4.

**Table 3**

**Risk Adjusted Returns: January 1987- May 2005**  
**Momentum Change Strategies: ((66), 6) and ((12, 12), 12) (6,6),(6) Strategy**

RC	Mean	Standard	Annualized	Sharp	BETA	Alpha	Treynor
	HP %		HP				
	Return	Deviation	Returns%	Ratio			

4	8.92%	16.81%	18.63%	0.3809	1.0548	10.08%	13.16
3	8.60%	15.65%	17.94%	0.3908	1.0299	11.31%	12.81
2	8.24%	14.47%	17.15%	0.3998	0.9900	9.98%	12.53
1	6.95%	11.18%	14.39%	0.4082	0.8091	8.21%	11.91
0	7.67%	11.53%	15.93%	0.4584	0.8657	9.39%	12.91
-1	7.82%	11.20%	16.26%	0.4865	0.8113	10.02%	14.19
-2	7.40%	14.80%	15.34%	0.3332	1.0746	7.78%	9.85
-3	7.29%	13.82%	15.10%	0.3504	0.9995	7.25%	10.36
-4	4.45%	17.64%	9.09%	0.1131	0.9890	2.67%	4.39

**(12,12),(12)**  
**Strategy**

4	22.51%	30.31%	22.51%	0.5855	0.1672	19.72%	106.13
3	22.35%	28.51%	22.35%	0.6169	0.5713	17.24%	30.79
2	21.68%	23.46%	21.68%	0.7211	0.6890	16.09%	24.55
1	16.95%	16.31%	16.95%	0.7470	0.7447	11.24%	16.36
0	13.64%	18.80%	13.64%	0.4724	0.8432	7.39%	10.53
-1	14.12%	17.32%	14.12%	0.5403	0.7612	8.31%	12.29
-2	13.72%	17.82%	13.72%	0.5029	0.7455	7.93%	12.02
-3	11.93%	22.20%	11.93%	0.3230	0.8372	5.05%	8.57
-4	17.39%	32.52%	17.39%	0.3883	1.0499	7.25%	12.03

To see, if the differences in the portfolio returns are statistically significant, the results from the dummy variable regression are given in Table 4.

**Table 4**

**Dummy Variable Regression**

Variable	(6,6) , (6)			(12,12) , (12)		
	Coefficients	t	Significance	Coefficients	t	Significance
Constant	0.0127	1.4626	0.1438	0.0906	5.1755	0.0000
4	0.0377	3.1027	0.0020	0.0678	2.7879	0.0054
3	0.0427	3.7630	0.0002	0.0671	2.9313	0.0034
2	0.0354	3.1620	0.0016	0.0629	2.8096	0.0050
1	0.0221	1.9789	0.0480	0.0177	0.7900	0.4296
0	0.0292	2.6236	0.0088	-0.0156	-0.6959	0.4866
-1	0.0308	2.7620	0.0058	-0.0107	-0.4795	0.6317
-2	0.0271	2.4201	0.0156	-0.0154	-0.6864	0.4926
-3	0.0225	1.9510	0.0512	-0.0392	-1.6978	0.0898

For the ((12, 12), 12) strategy positive and statistically significant shifts occur for rank changes of 2 or larger. Shifts associated with rank change of 1 or lower are negative and or statistically insignificant. For the ((66), 6) strategy although shifts associated with



all the rank change categories are positive, larger shifts are related to larger rank change;

also, the levels of significance are lower with larger rank change implying that more

robust results are related to groups where relative strength ranks have improved more.

To summarize, we have shown that generally momentum strategy based on momentum

improvement provide superior returns than a simple momentum strategy both on an

absolute and risk adjusted basis. One question that has not been addressed in the

previous literature is the optimal length of the holding period. We now address this issue.

**Optimal Holding Period**

As J and K are varied the returns of the portfolios formed vary as shown in Table 5. What is puzzling however is that in five out of the nine strategies annualized returns

**Table 5**

**Table of Best and Worst Returns for Each strategy.**

(JJ),K	RC	Mean HP % Return	Standard Deviation	Annualized HP Returns%	Sharp Ratio	BETA	Alpha	Treynor
11,1	4	2.48%	6.54%	34.21%	0.2620	0.9067	23.58%	32.9051
2,1	4	0.27%	6.80%	3.34%	-0.0098	0.9229	-0.39%	-1.2783
22,1	3	2.34%	5.91%	31.96%	0.2827	0.9921	1.51%	28.2672
23,24	3	28.61%	34.10%	13.41%	0.5480	0.7482	14.18%	11.5739
15,1	2	1.91%	5.05%	25.43%	0.2630	0.8851	1.15%	23.9725
18,1	2	0.96%	5.07%	12.15%	0.0994	0.9275	0.21%	8.6947
14,4	1	6.29%	10.37%	20.09%	0.4555	0.9349	4.20%	16.7814
7,2	1	1.85%	6.52%	11.65%	0.1549	0.9173	0.46%	7.7786
9,1	0	1.54%	4.92%	20.14%	0.2015	1.0114	0.73%	15.5294
3,3	0	2.91%	9.72%	12.15%	0.1675	1.0840	1.11%	6.9436
22,2	-1	3.15%	6.94%	20.43%	0.3321	0.9903	1.57%	16.6264
16,1	-1	0.66%	4.83%	8.25%	0.0536	0.9737	-0.04%	4.1913
5,2	-2	3.34%	7.15%	21.82%	0.3366	0.9711	1.72%	17.7893
11,2	-2	1.36%	7.43%	8.44%	0.0745	1.0112	-0.04%	3.9610
5,1	-3	1.61%	5.21%	21.10%	0.1979	1.0007	0.81%	16.6047
9,1	-3	0.37%	5.72%	4.54%	0.0011	1.0783	-0.45%	0.1012

12,23	-4	46.04%	71.90%	21.85%	0.5118	0.6339	31.62%	26.3734
10,3	-4	-0.22%	11.33%	-0.89%	-0.1008	0.9432	-2.10%	-5.7280

are maximized for a holding period of one month, and in three other cases they are between two to four months. At this stage of research, the reason for very short optimal holding period is not clear and needs further research.

Charts of how returns vary with formation as well as holding periods are given in Appendix 1.

It may be noted that for all the strategies the sharp ratio increased as the holding period was increased. For the positive rank change the peak sharp ratios are obtained with formation periods generally around 12 months. For the negative rank change portfolios the peak sharp ratio are found generally in the area of 24 months. As the holding period increases the beta of the portfolios on all strategies tend to decrease.

For the positive rank change portfolios the alpha tends to be greatest when both the formation and holding periods are in the intermediate term generally around twelve months. For the negative rank change portfolios the highest alpha was generally in the area of longer term holding and formation periods around 24 months. The Treynor indexes for the positive rank change portfolios tend to be the highest for the intermediate term formation and holding periods. Also, for the positive rank change portfolios returns tend to decrease with holding period whereas the reverse is true for negative rank change portfolios. The explanation for these results is not obvious and is subject to further research.

#### **IV. Conclusions**

The strategy of creating portfolios based on rank change over the two previous time periods holding them over subsequent periods show returns varying with changes in ranks, lengths of the formation and holding periods. In general, we found that larger absolute and risk adjusted returns are associated with larger rank changes. While this is our principal result, we also found that for the positive rank change portfolios annualized returns are maximized when holding periods are relatively short; Also, for positive rank change portfolios there is downward drift of annual returns as the hold period increases

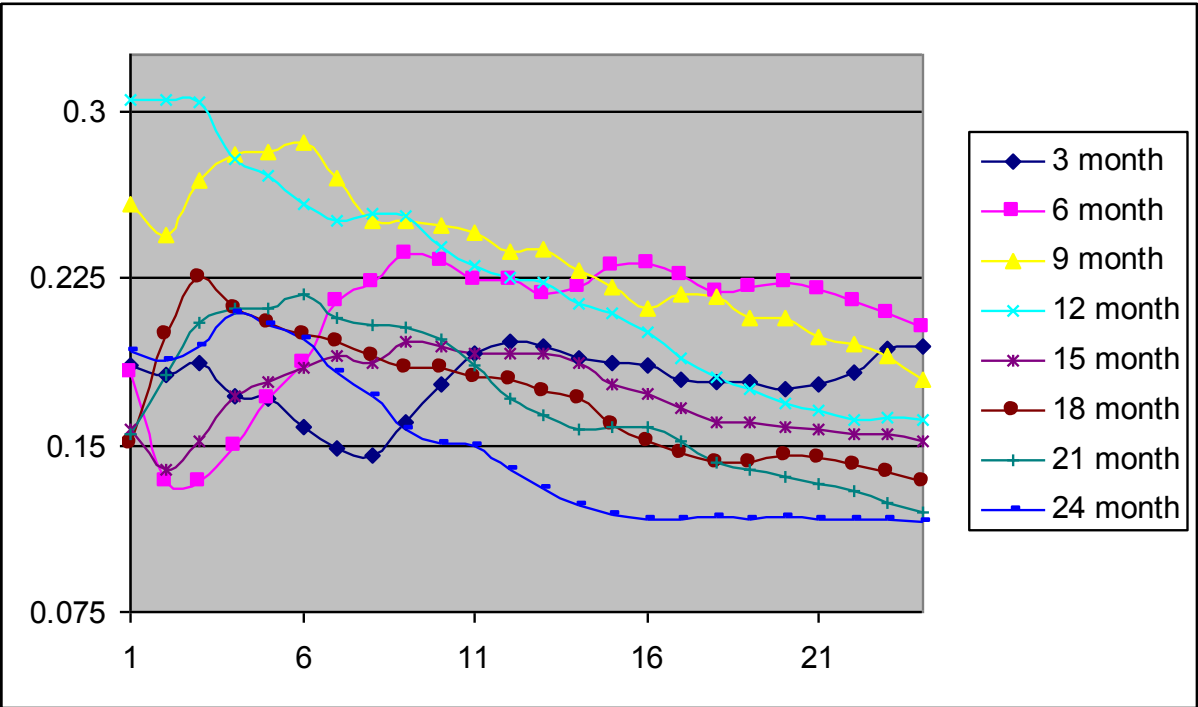
and the reverse is true for the negative rank change portfolios. These patterns seem to be similar to observations of long term return reversals noted by Debondt and Thaler, and need further investigation.

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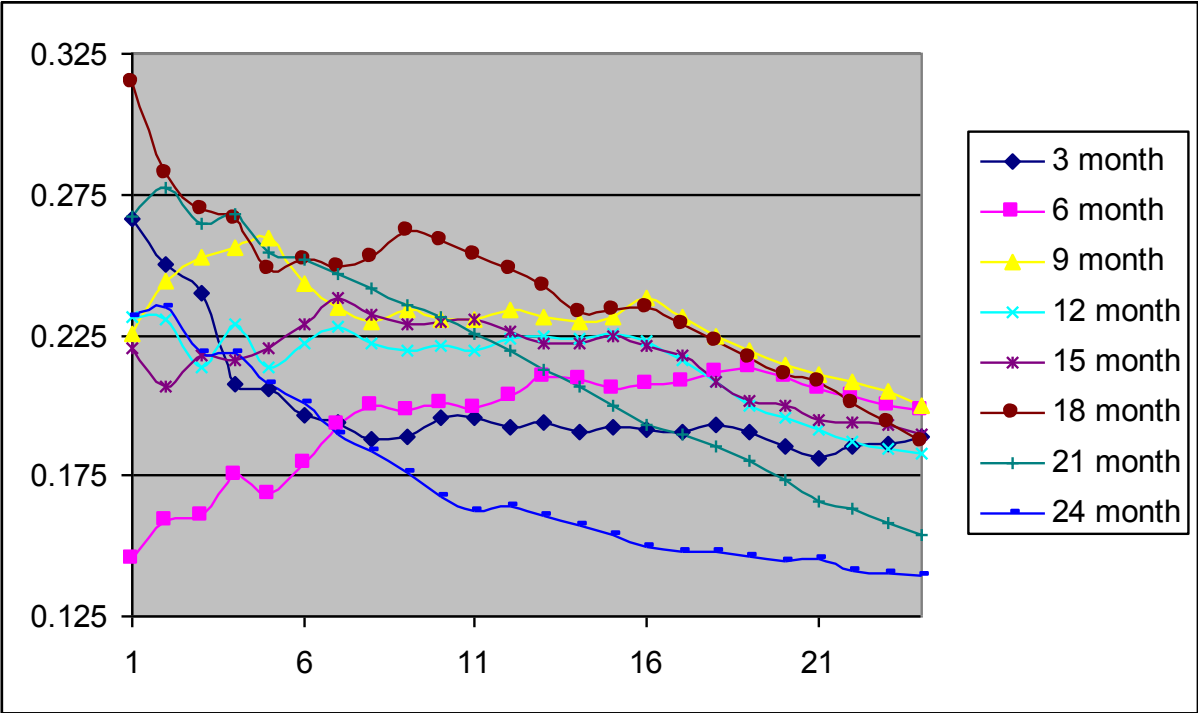
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Appendix 1

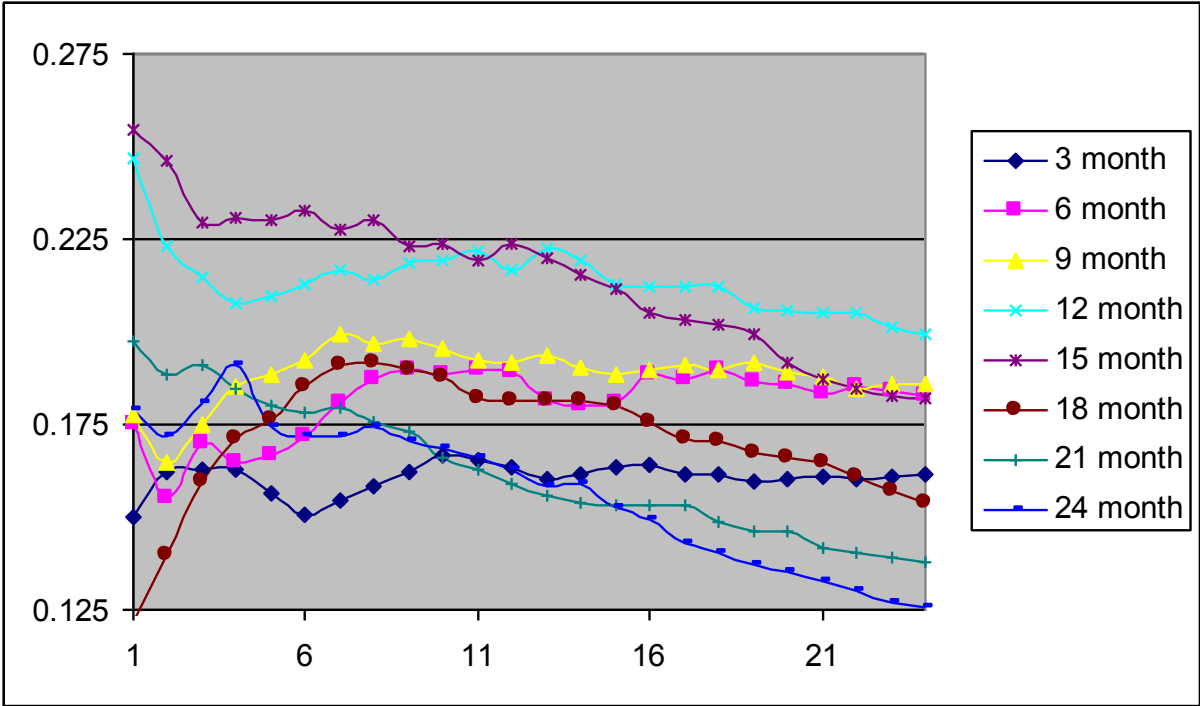
Charts of Returns as holding periods Increase for given Formation Periods.  
RC + 4



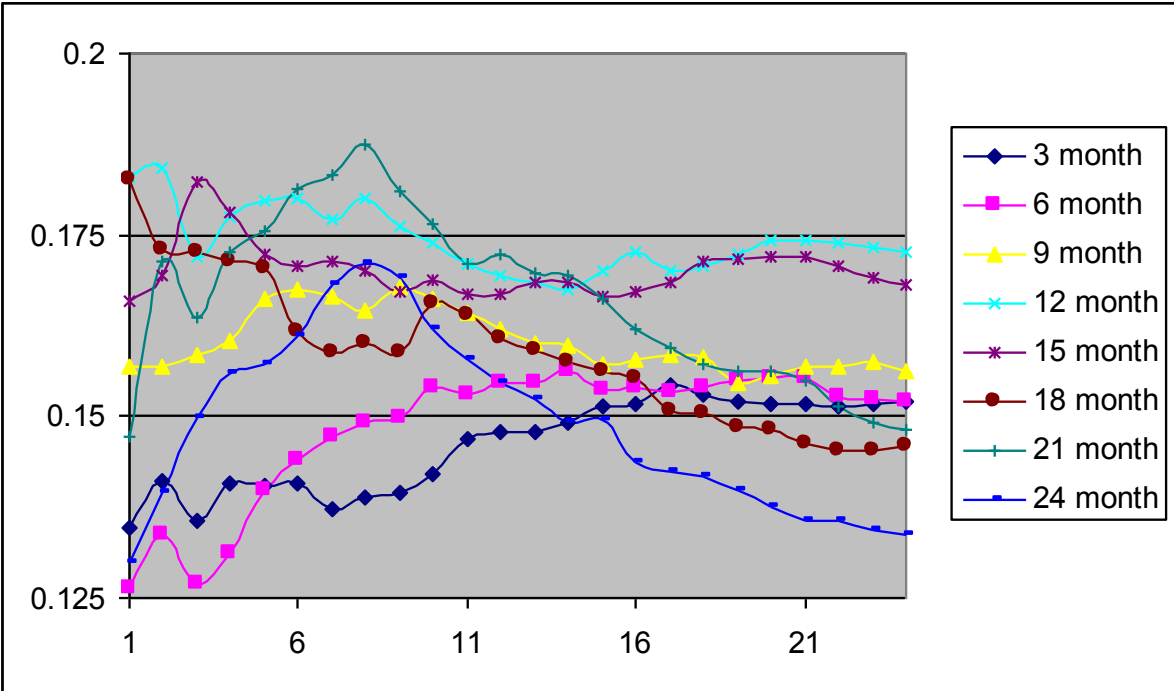
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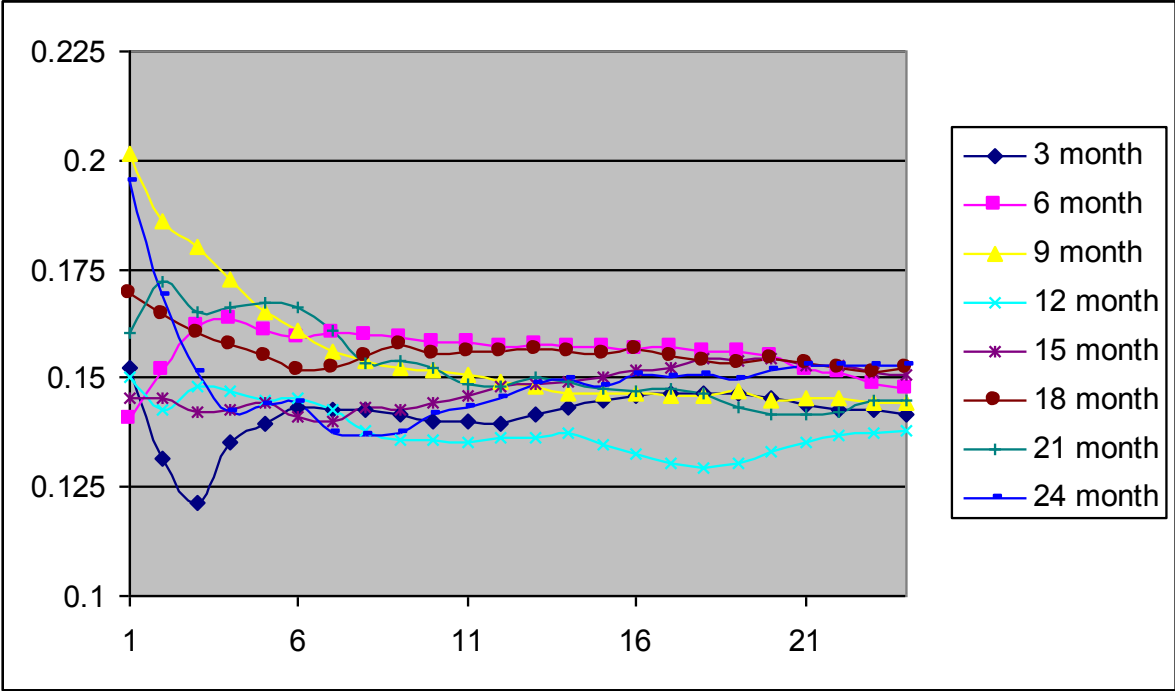
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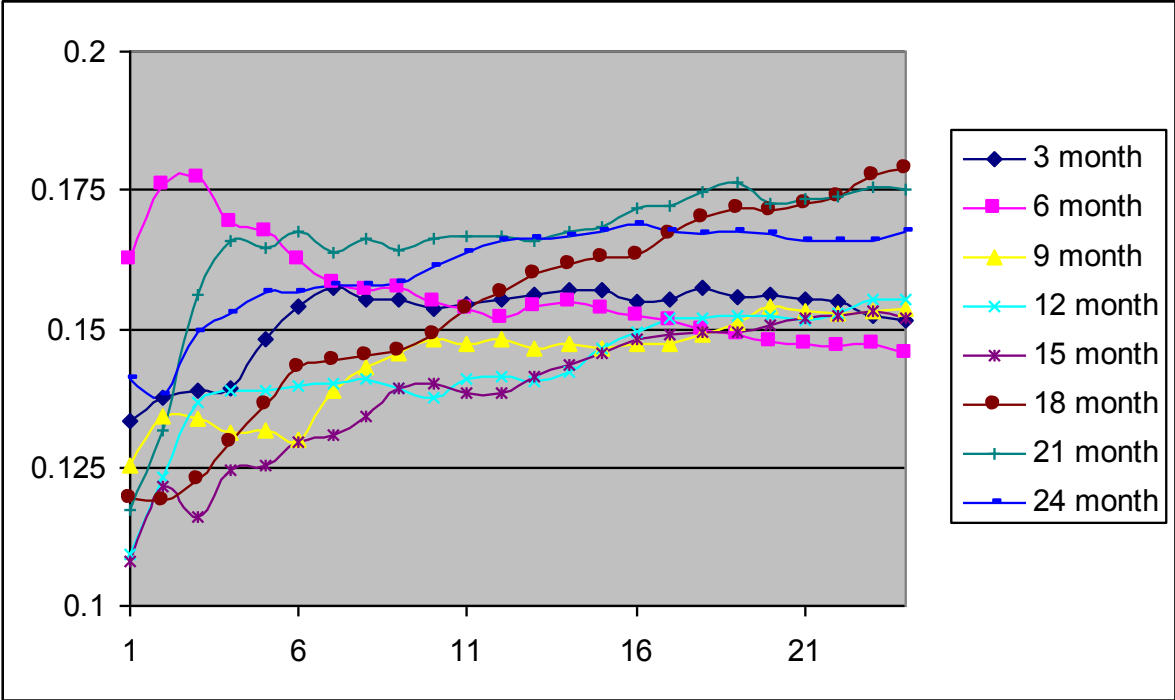
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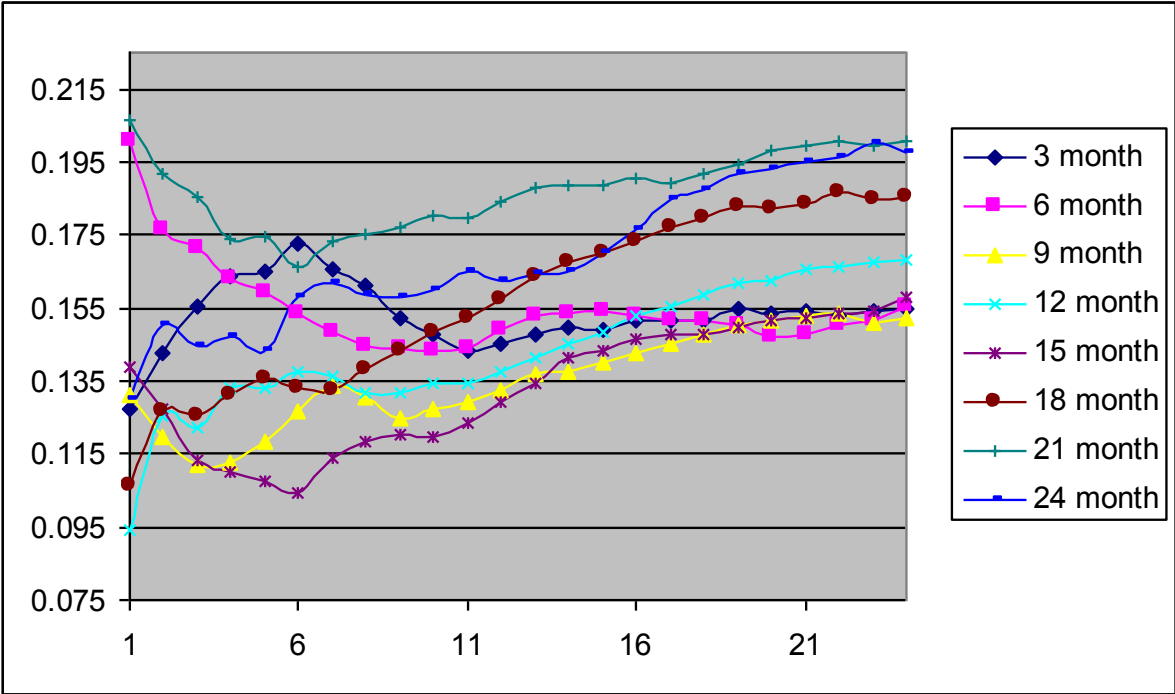
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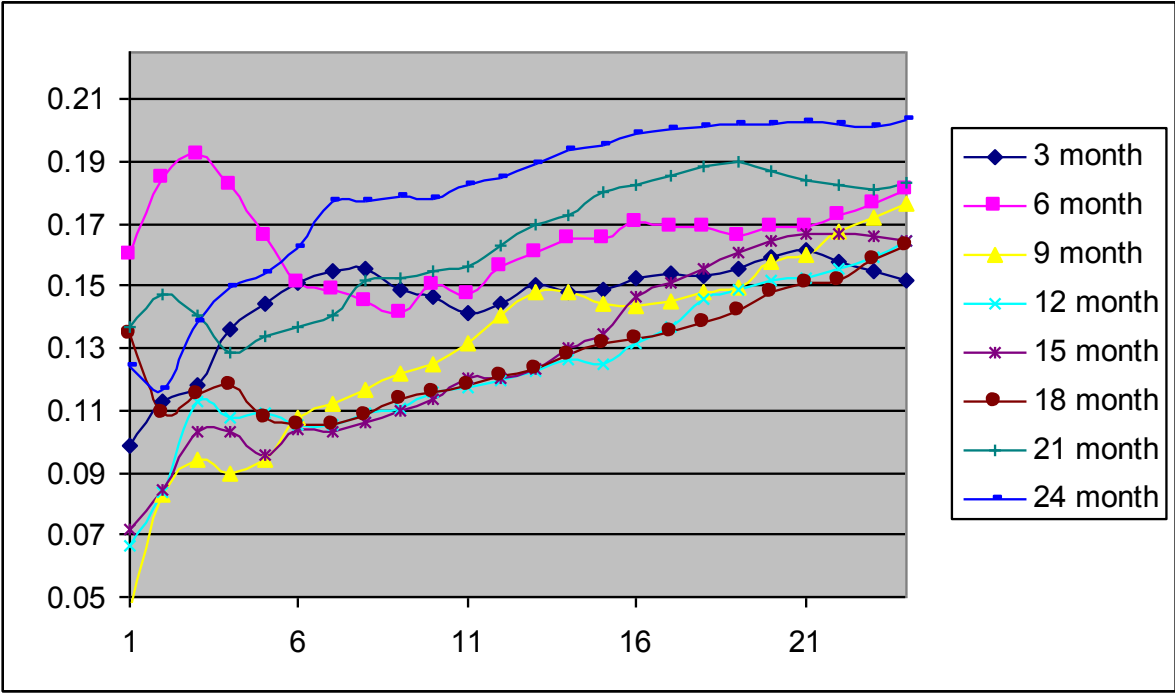
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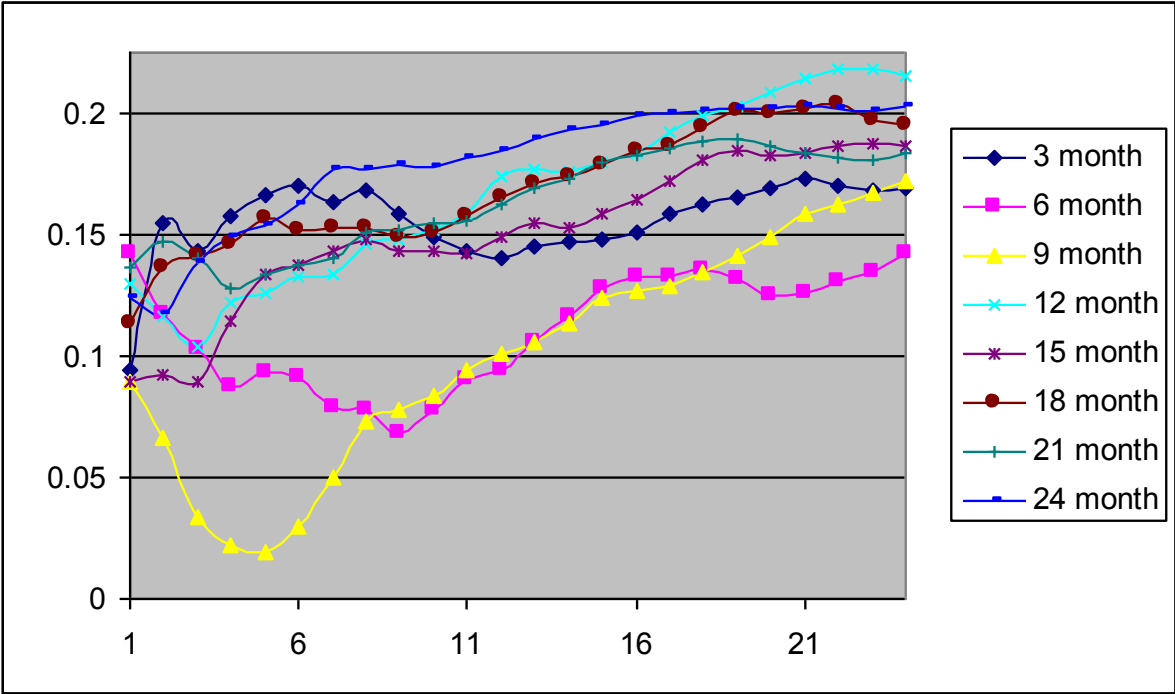
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RC -3

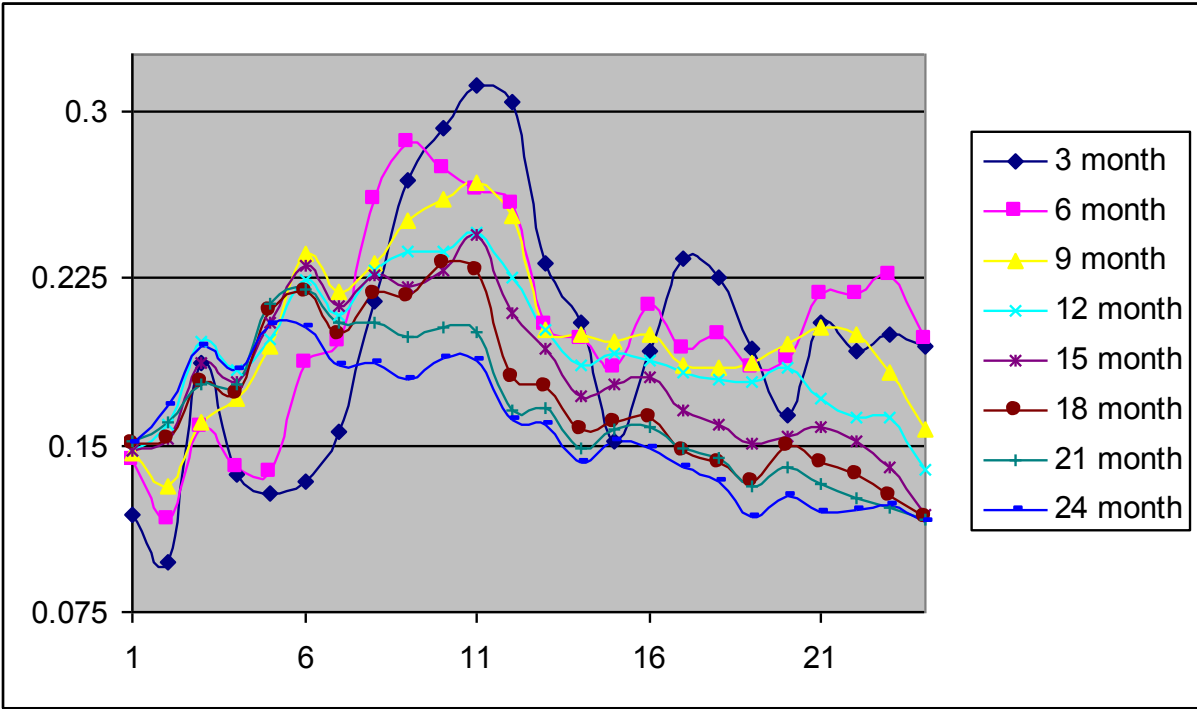


RC -4



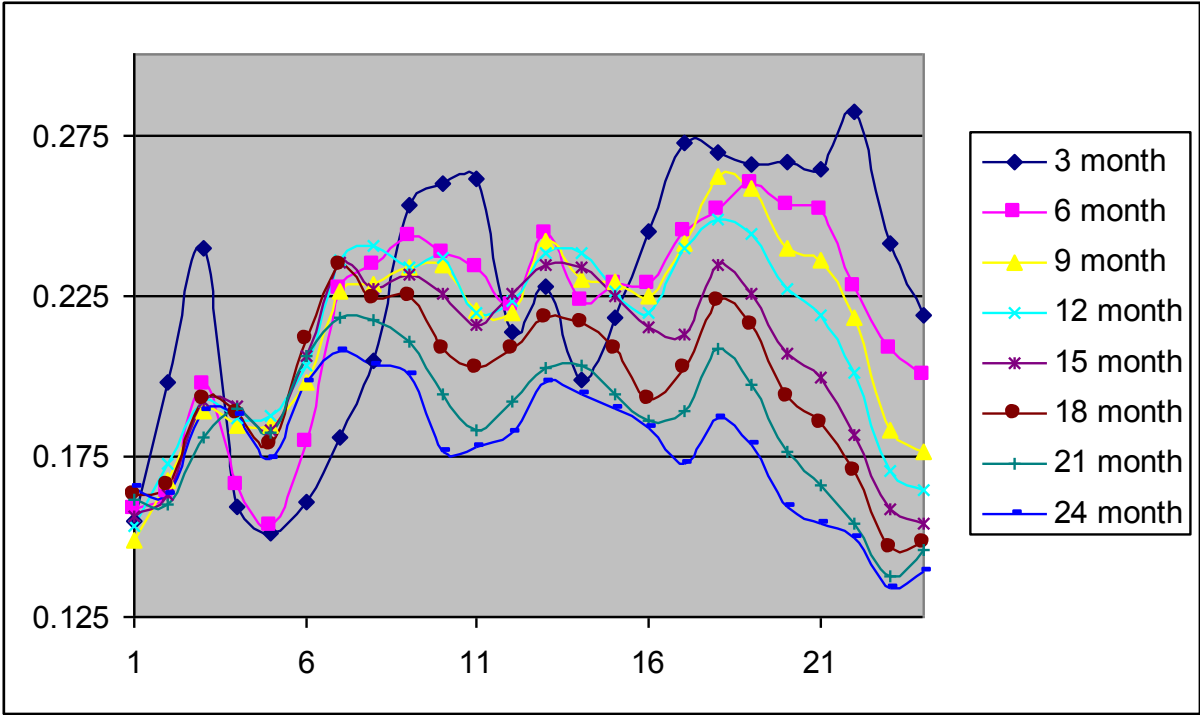
Charts of Returns as Formation periods Increase for given Holding Periods.

RC + 4

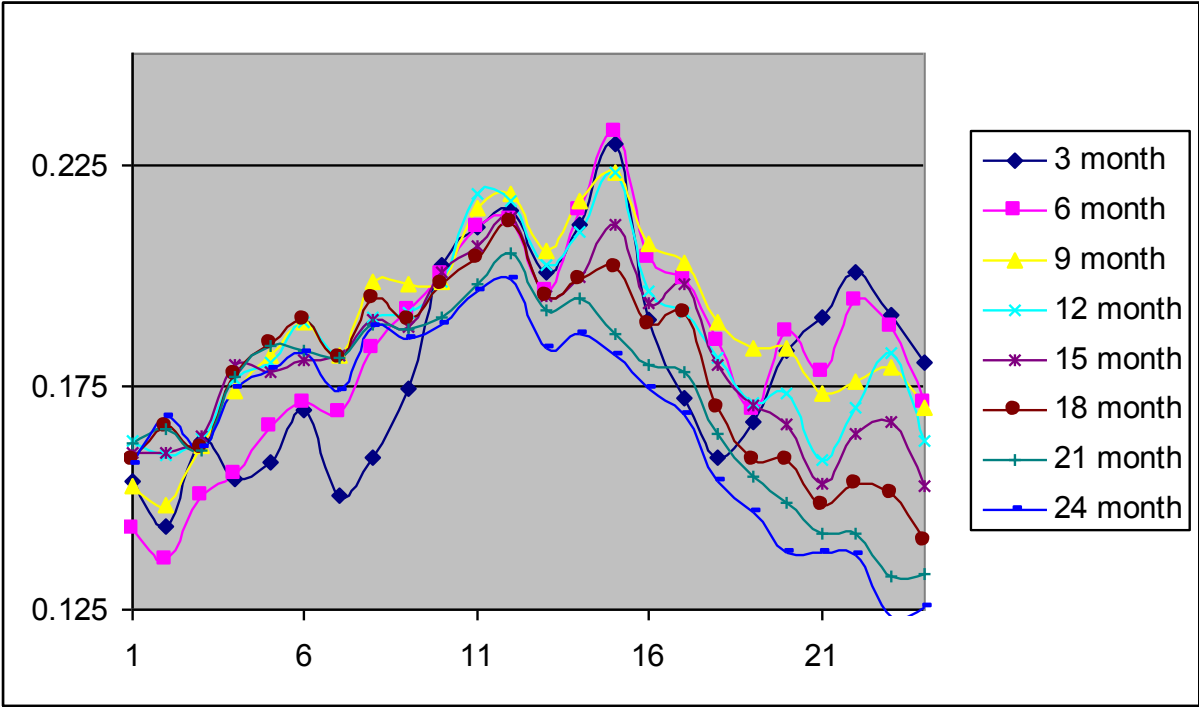




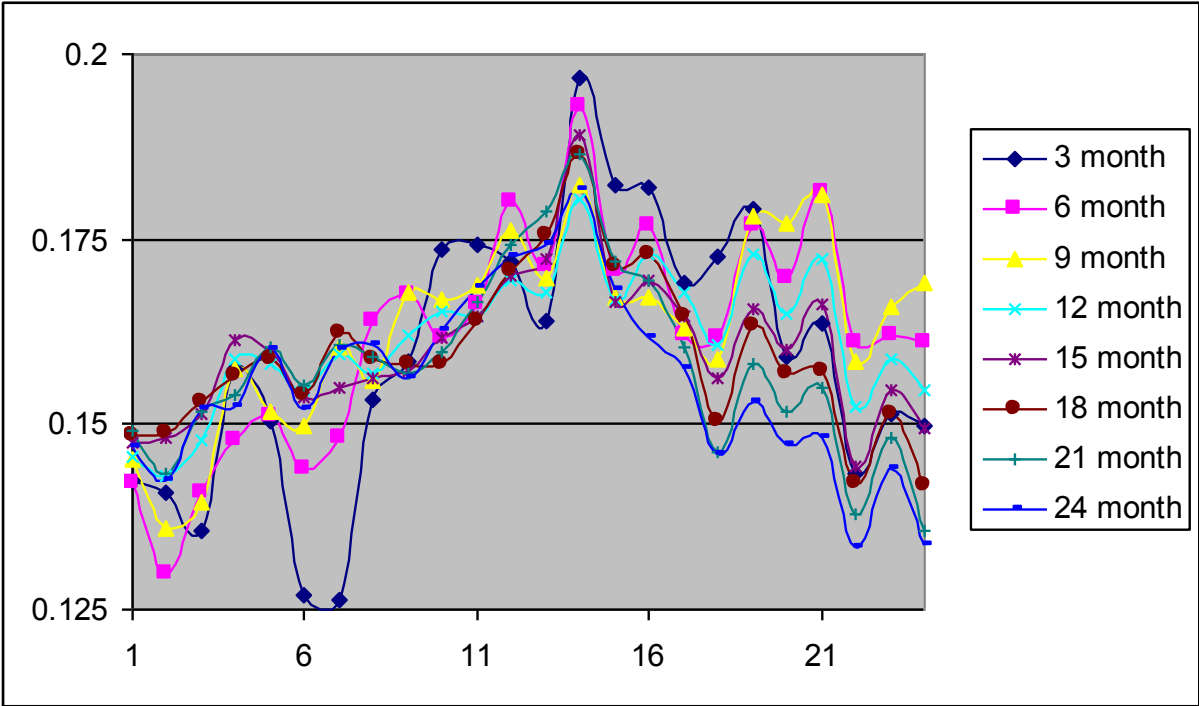
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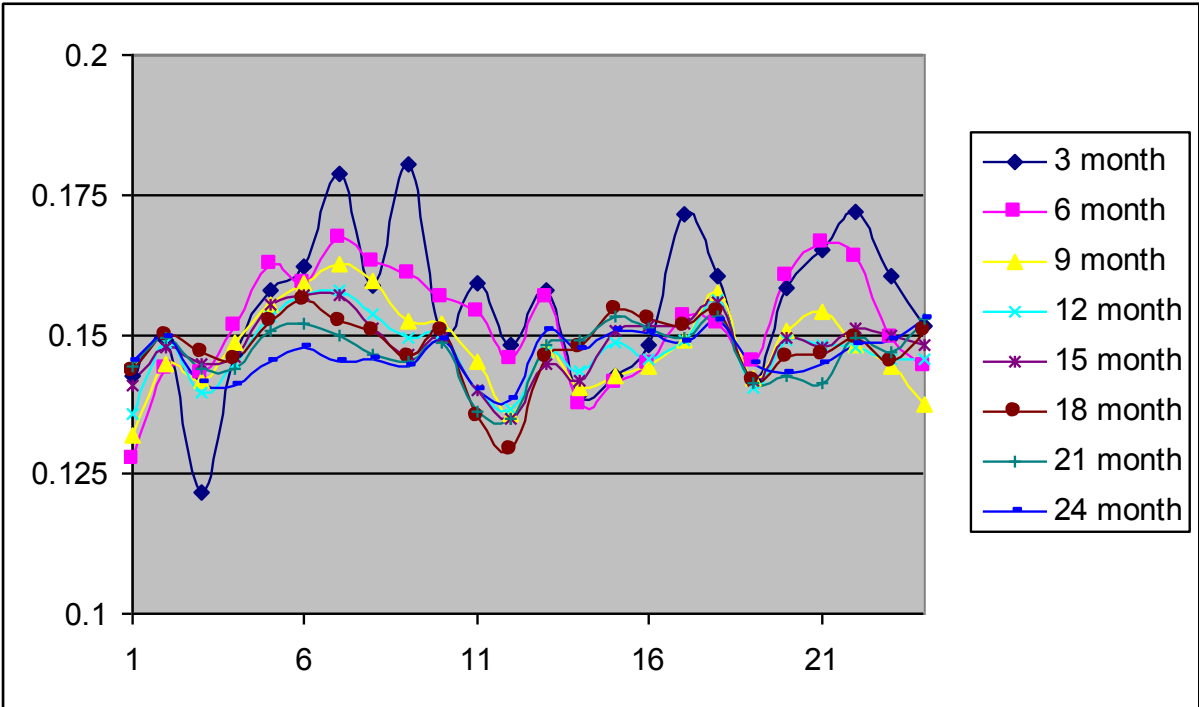
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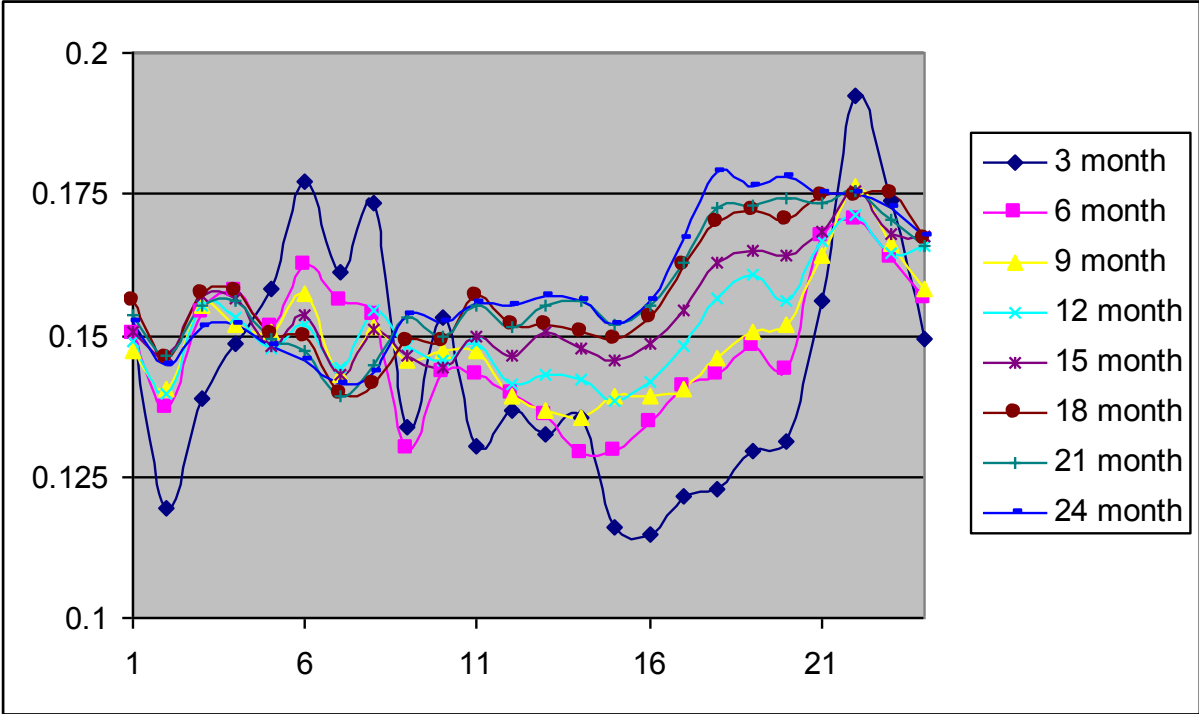
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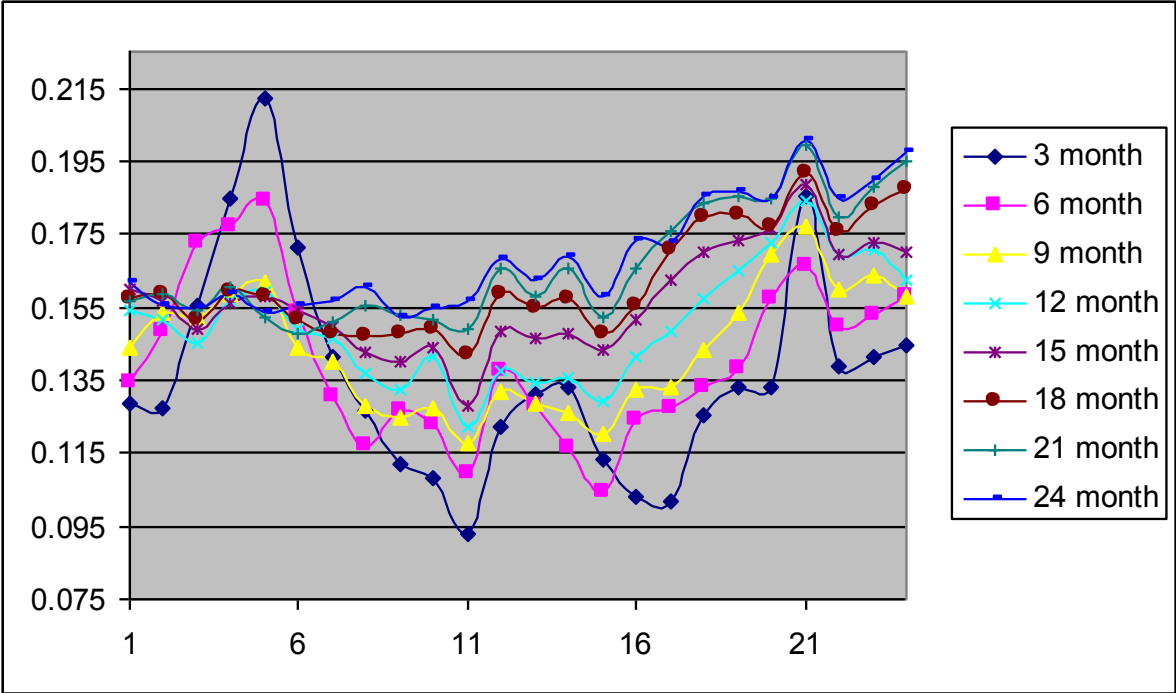
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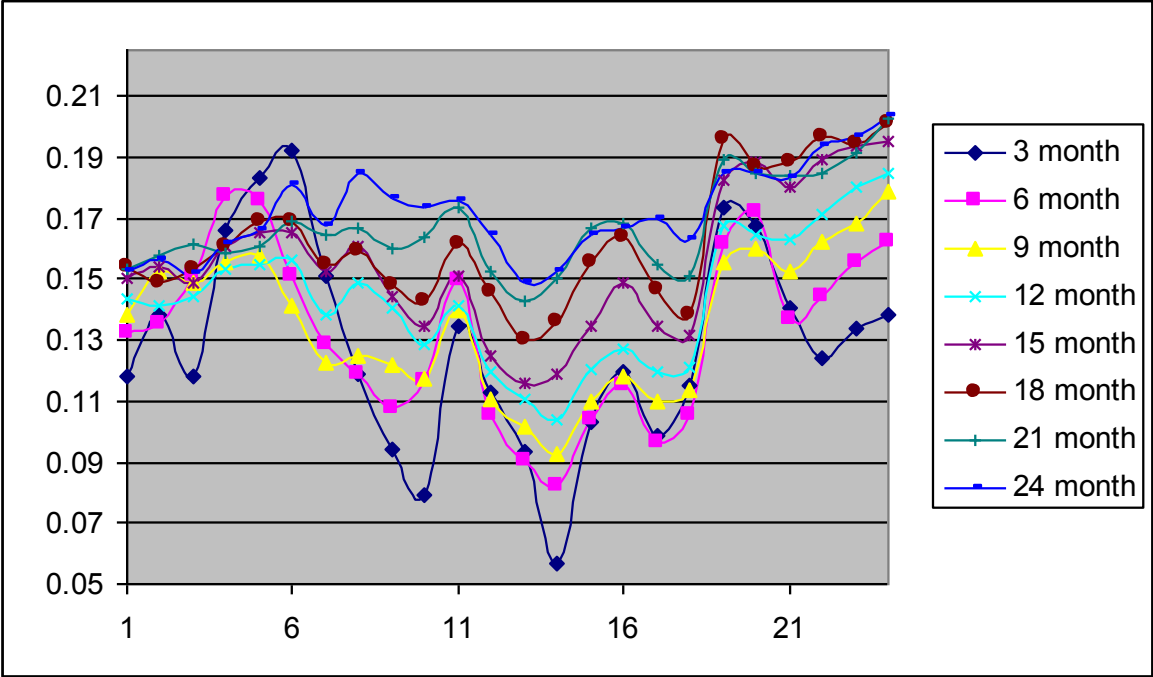
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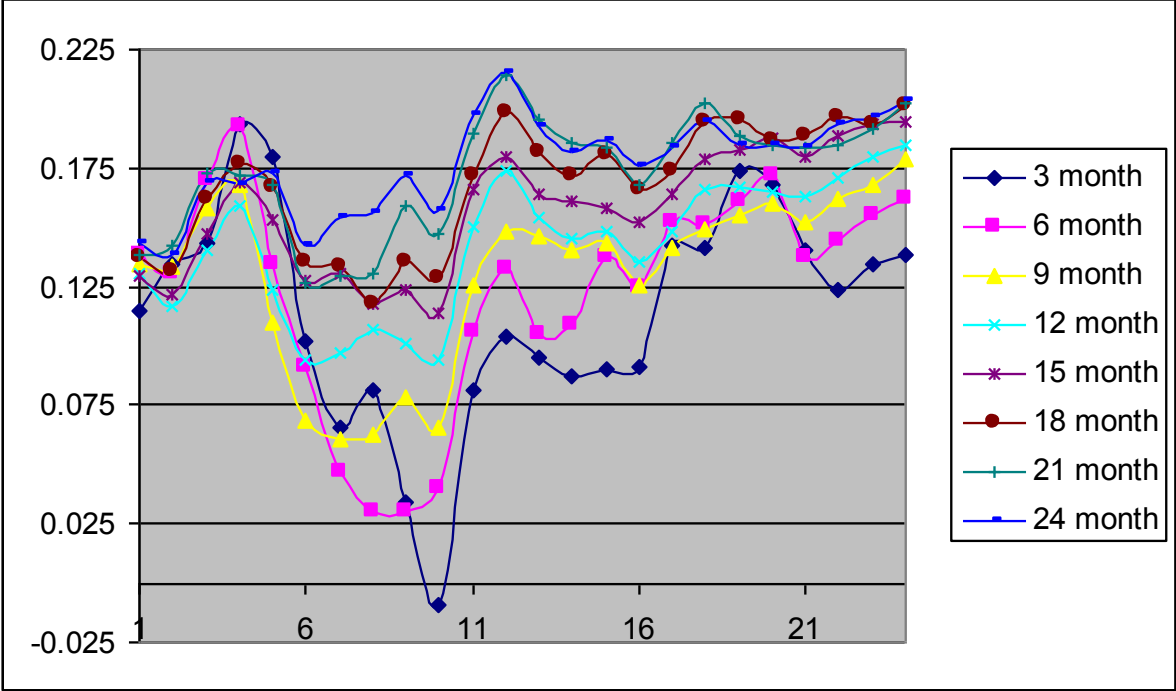
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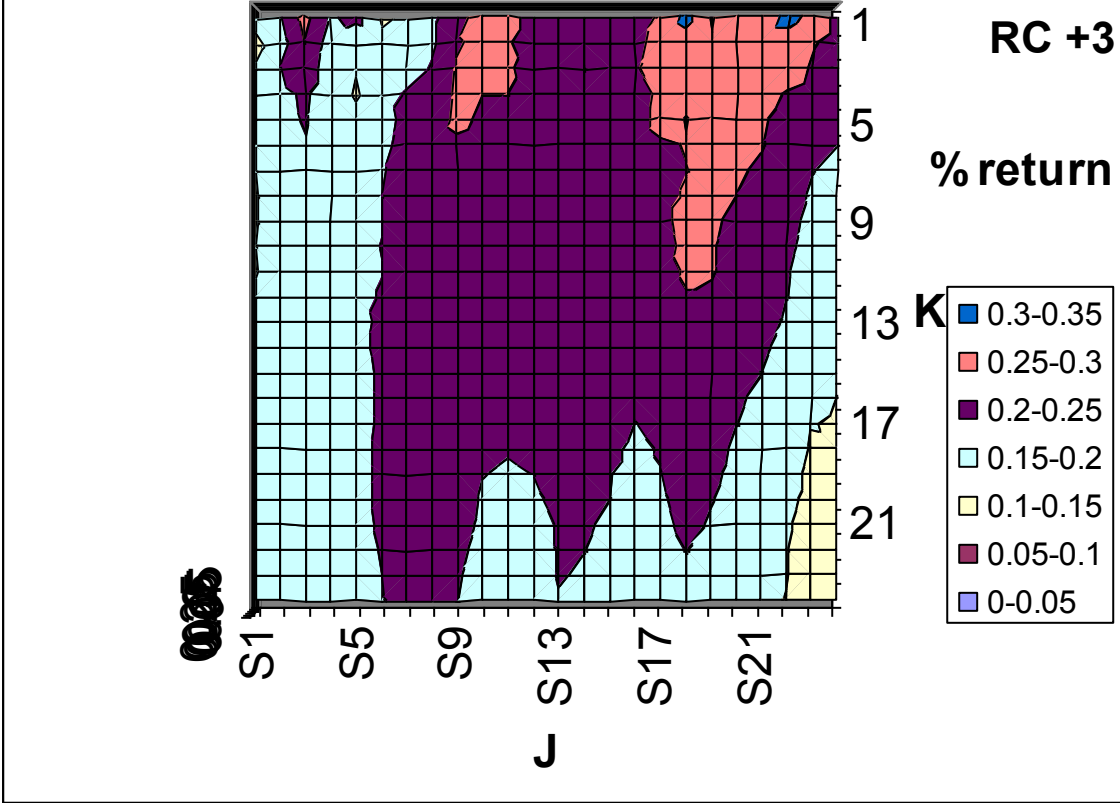
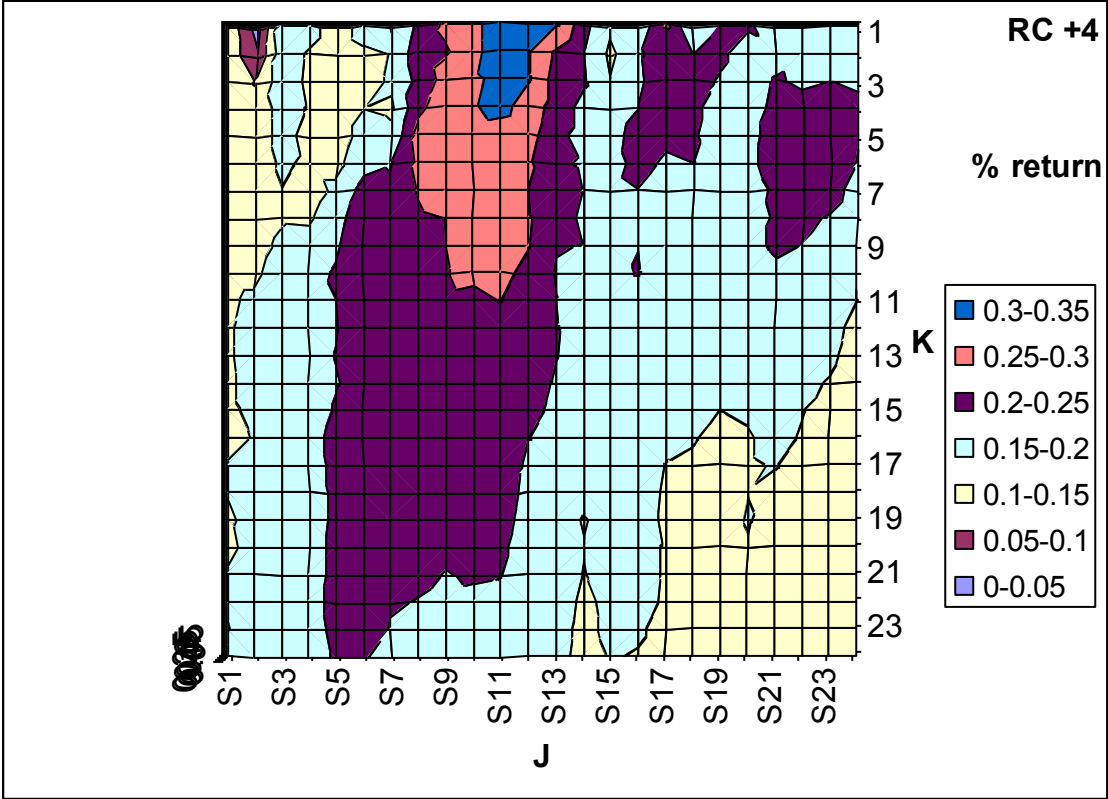
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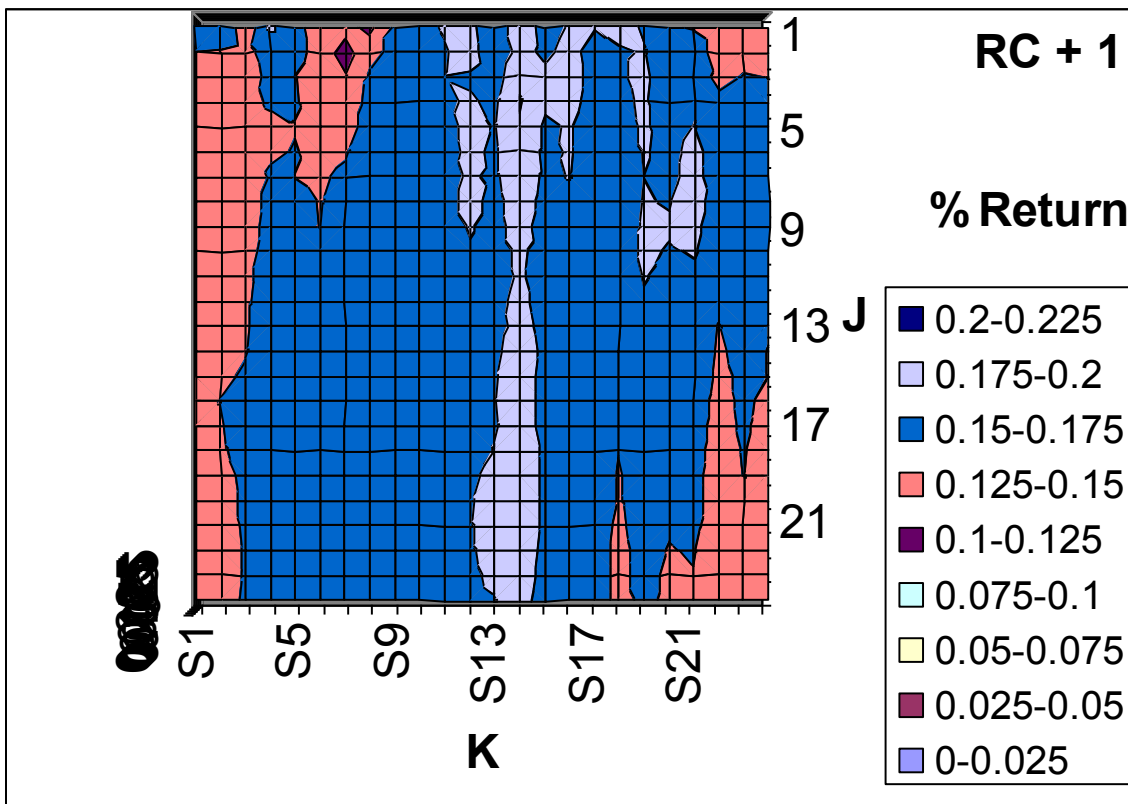
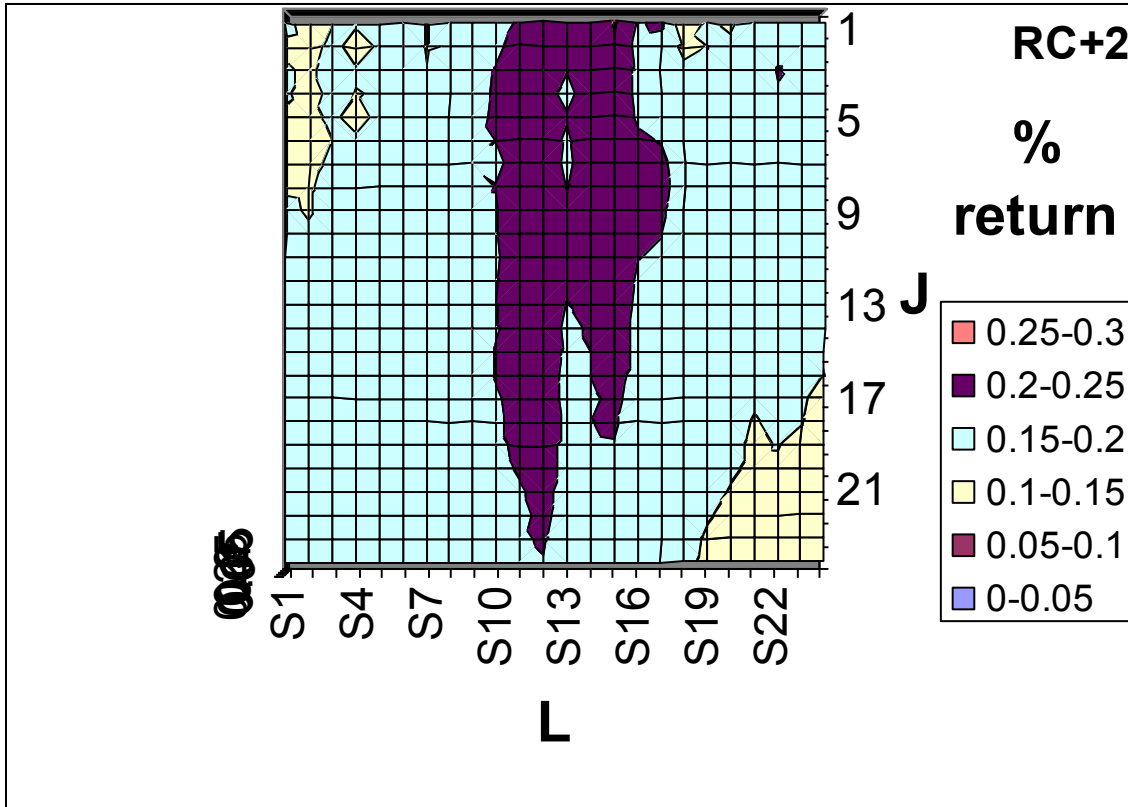


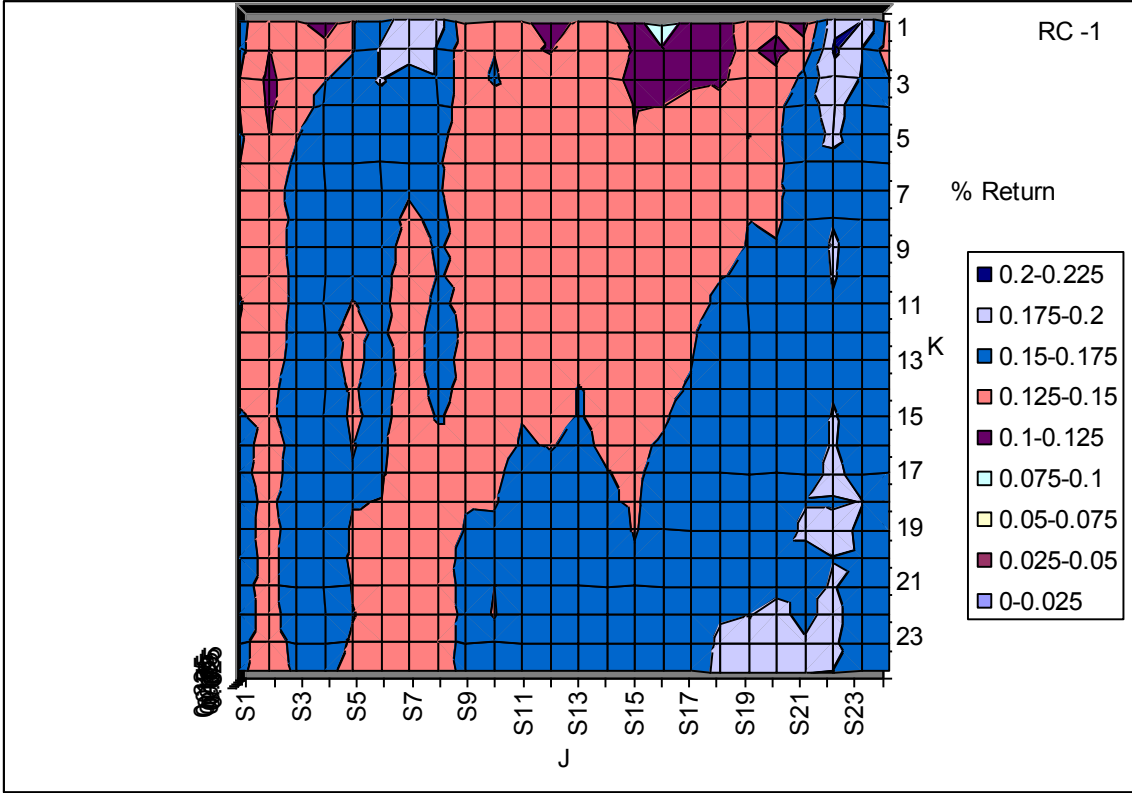
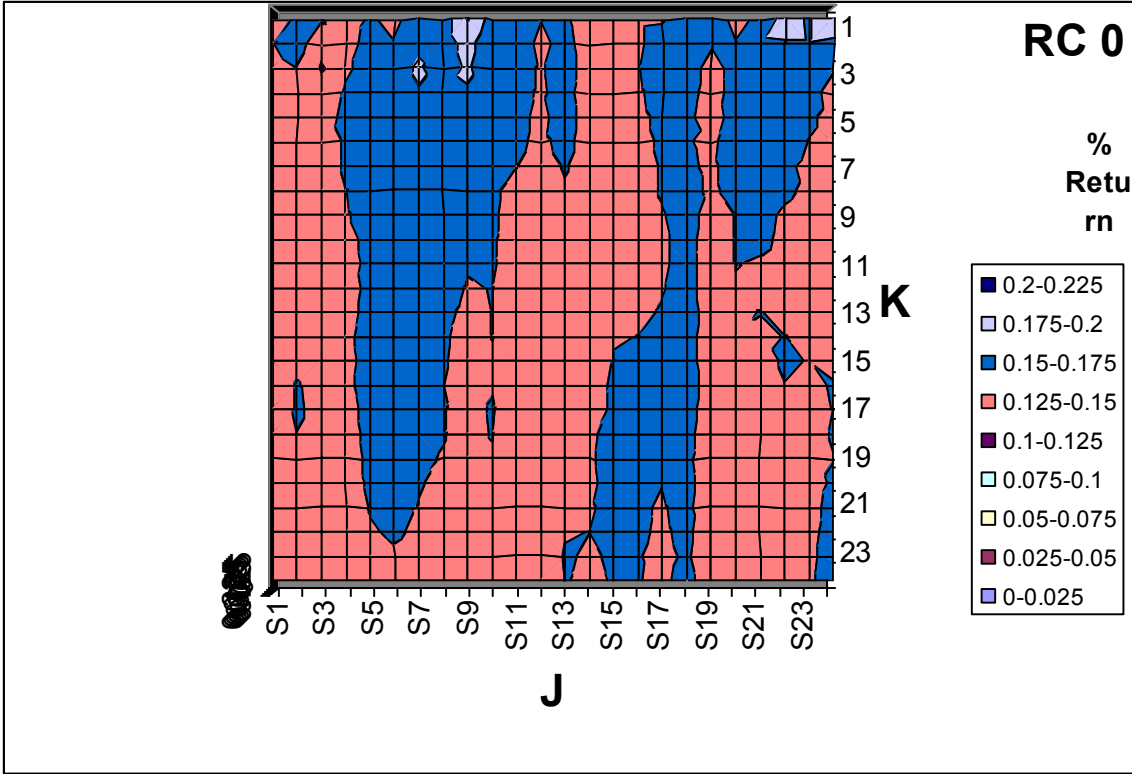
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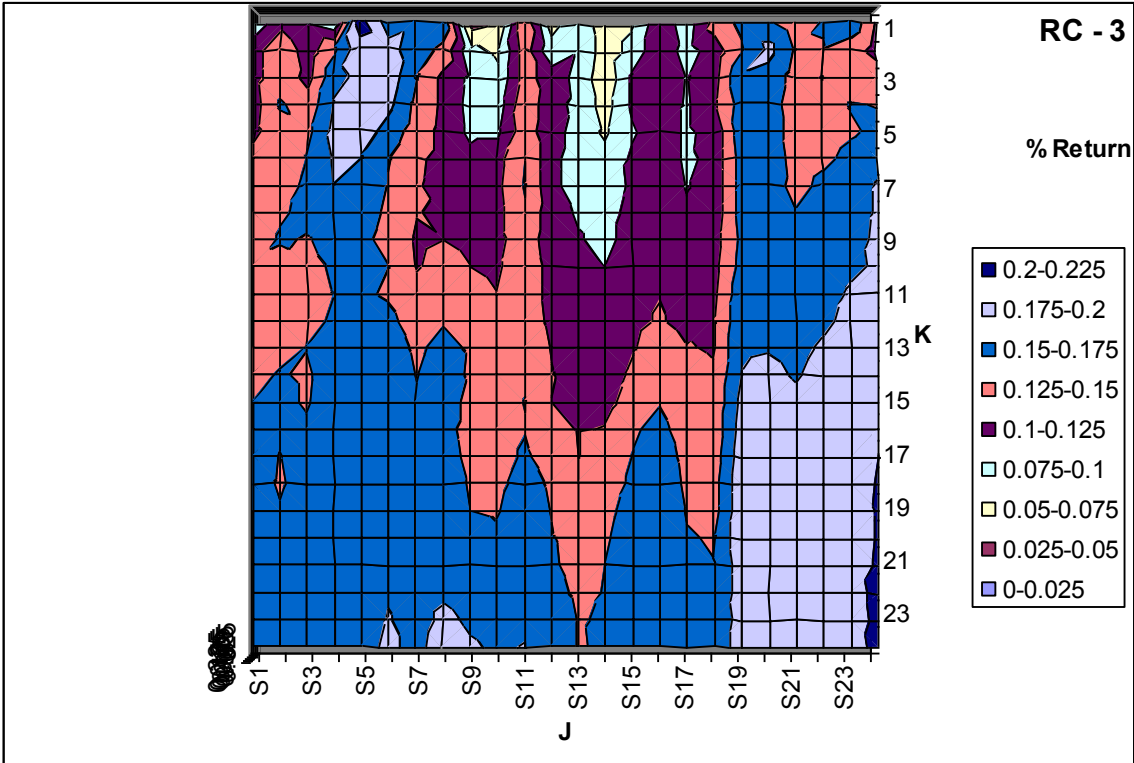
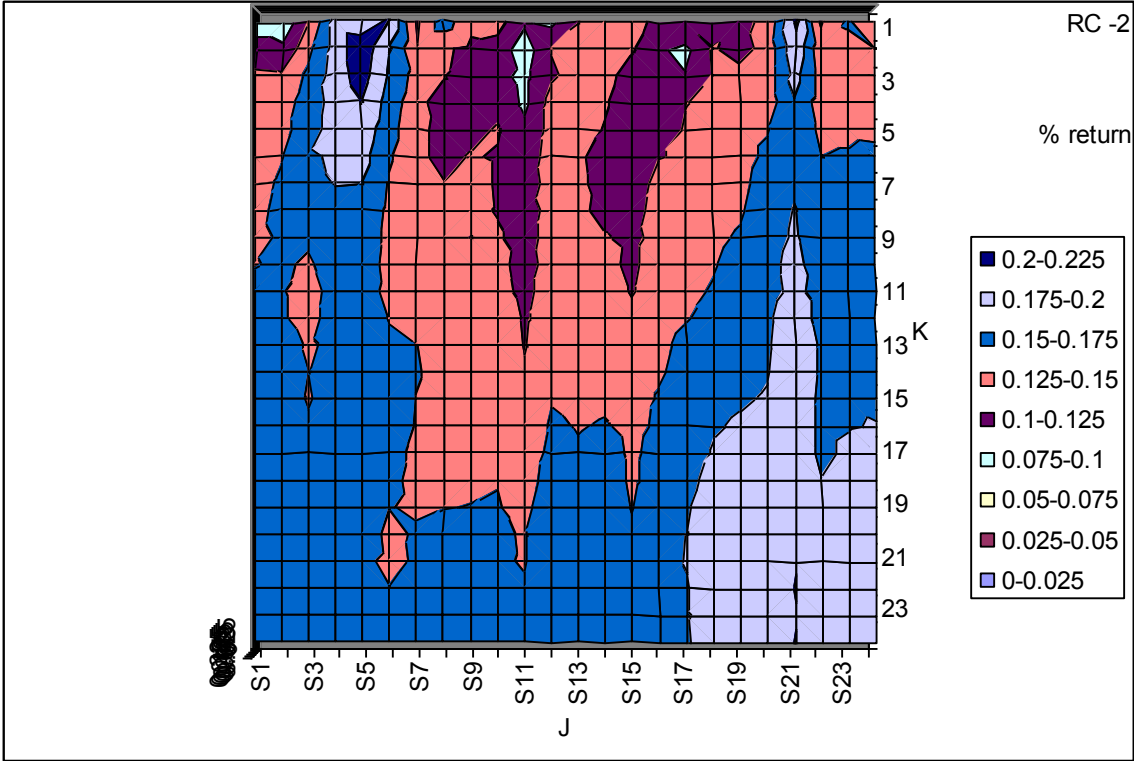


Rank Change (J,J){in chart J} , K Return Charts

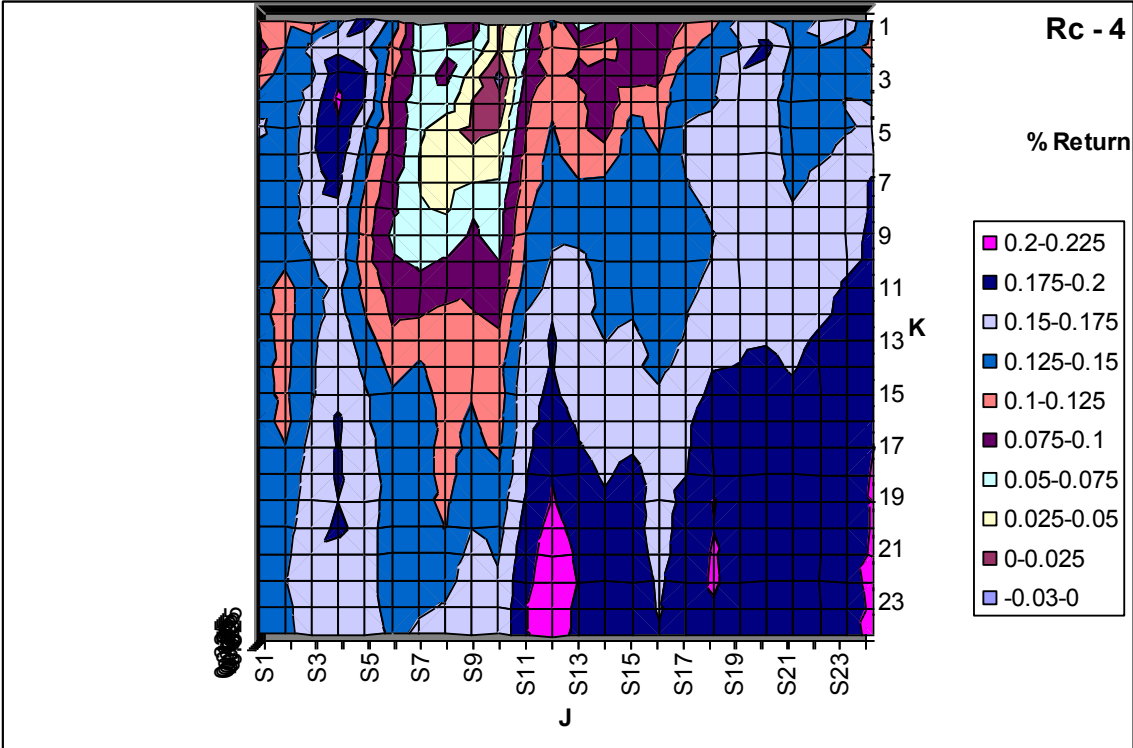












## Translocation of Mitogen-Activated Protein Kinase Pathways in Skeletal Muscle

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### **Abstract**

In subjects with Type 2 diabetes, the resistance of fat, muscle and liver to insulin is the central pathophysiological event in the development of this disease. Genetic and environmental factors play a major role in this process, although the precise pathogenesis of insulin resistance and Type 2 diabetes is still largely unknown. The factors regulating these changes are unknown; however, the mitogen activated protein kinase (MAPK) pathways have emerged as critical components for mediating numerous cellular responses including control of cell growth, differentiation and adaptation (3, 4, 11, 12). Here we compare the skeletal muscles of adult lean and obese Zucker rats for differences in the basal expression and exercise-induced regulation of the ERK1/2, p38 and JNK-MAPK proteins. Immunohistochemical analysis of the plantaris and extensor digitorum longus muscles of no exercise control and muscles from animals that had been sacrificed immediately, 1 hr or 3hr after completing an exercise bout (10 sets of 6 repetitions) was completed. We observe that insulin resistance in the obese Zucker rat is characterized by differences in the expression and exercise-induced regulation of MAPK proteins. Further investigation is needed to examine the molecular mechanisms underlying these alterations.

## Literature Review

When compared with other states, West Virginia, in 1999, had the third highest rate of death due to diabetes and ranked second in regard to prevalence of the killer. Obesity, a well-known risk factor of diabetes, is also a problem in the state, which currently has the second highest rate in the nation (8). Diabetes is a condition in which the body does not produce or properly use insulin. Insulin, a peptide hormone that regulates carbohydrate metabolism, is produced by the  $\beta$ -Islets of Langerhans cells in the pancreas. It has a role in the metabolism of fat (triglycerides) and proteins and affects other tissues. There are two types of diabetes, type I (juvenile) and type II, in which type II is the most common (90% to 95%). Type I diabetes is the result of the body not producing enough insulin. It is often referred to as juvenile diabetes because it is diagnosed in youth and young adults. Type II diabetes is characterized by cells that do not respond to insulin or do not produce enough insulin. Type II diabetes is more serious and is detected by having a fasting blood glucose level of 126 mg/dL or higher. Having type II diabetes increases the risk of having other life threatening diseases and conditions like heart disease, nerve damage, kidney damage, and blindness (5).

Syndrome X is the leading cause of type II diabetes (7). For research purposes, the Zucker rat model is used to demonstrate genetic obesity along with hyperatrophy and hypercholesterolemia (7). Syndrome X refers to a group of heart risk factors including: high levels of abnormal body fat, bad (LDL) cholesterol, high blood pressure, and abnormal glucose metabolism that can be life threatening (1, 10). Other disorders associated with Syndrome X are prothrombic and proinflammatory tendencies. This syndrome is initiated by poor eating habits, unhealthy lifestyle choices, genetic factors, and poorly controlled diabetes (10). It has been demonstrated that acute exercise and training increases insulin activity in skeletal muscles, which aids in the homeostasis of glucose levels (11). Animal models, such as the Zucker rat model, are used in place of human models for ethical reasons, but resemble the human condition of being overweight and diabetic.

Morbidly obese Zucker rats have two copies of the recessive allele, *fafa*, which make them destined to have diabetes and other diabetes-related health problems (1, 9). The Zucker rat exhibits

obesity as early as 3 weeks of age and by the age of 14 weeks, 40 percent of their body composition is lipid. In comparison with mice, Zucker rats tend to show very little sign of elevated hyperglycemia, which makes it easier to control the experiment and their skeletal muscle is highly insulin resistant (7). In contrast to the obese Zucker rat model, the lean rat models a healthy human and act as a control for comparison. The lean models are genetically similar to the Zucker rats, but possess at least one dominant allele, *fafa*.

The rat model body reflects that of a human, having both fast- and slow-twitch muscles. The body is made up of three types of muscle: cardiac, smooth, and skeletal. The skeletal muscle is what coordinates fingers to move and legs to walk and is broken down into the slow and fast twitch types. Slow twitch muscles, demonstrated by the soleus muscle, are labeled Type I and are associated with endurance exercises due to their slow contraction. The soleus attaches to the tibia, fibula and the calcaneus of the calf muscle. On the other hand, Type II, or fast-twitch muscles, are used in quick movements and involved a fast contraction. The plantaris muscle, located near the calf muscle, is a fast twitch muscle used in sprinting and quick movements (13).

As with any muscle, slow- and fast-twitch muscles rely on a system, mechanotransduction, to help bridge the gap between the environment and intracellular information. Mechanotransduction is how the body interprets mechanical stimuli, such as sound, through intracellular pathways. In the soleus and plantaris muscles, the mitogen- activated protein kinase (MAPK) pathway plays a large role in survival, proliferation, apoptosis (a form of programmed cell death) and differentiation in the cell by interacting with environmental stresses. The MAPK pathway works by phosphorylating and activating its three basic systems, MEKK (MAPK kinase kinase), MEK (MAP and ERK kinase), and MAPK (mitogen-activated protein kinase). MEKK begins the process by phosphorylating and activating MEK, while MEK phosphorylates MAPK. MAPK is currently under much scrutiny as to its role in the cell, either in encouraging or preventing apoptosis (6, 14).

MAPK is made up of three different families, ERK 1, 2 (extracellular signal regulated kinase 1, 2), p38, and JNK (Jun N-terminal kinase). The ERK 1, 2 pathways have been shown to stimulate growth

in the cell and oppose effects of the JNK and p38 pathways. All three pathways are stress activated, such as UV radiation, while the p38 and JNK pathways both simultaneously activate and play a critical role in transcriptional regulation. ERK 1, 2 plays a large role in the neural function in the cell cycling process. All three main proteins of each pathway, ERK 1, 2, p38, and JNK translocate to the nucleus to assist in the transcription process (6, 14).

In this study, we examined the possible differences of expression of the MAPK pathway associated proteins following acute exercise in soleus and plantaris muscles from both diabetic, obese Zucker rats and lean rat controls. As shown in previous research with obese Zucker rat model renal tubules, expression of p38 and ERK 1, 2 varied from that of the lean control rats (2). Diabetes is a life-threatening disease that affects the entire body including how it reacts to biochemical signals. With this study, we are investigating how diabetes really affects a person, not just the obvious, ubiquitous ailments. By distinguishing the differences between the two animal models, there could be more research to aid in the diabetic prevention field and possibly, one day, a cure for an ailment that affects 18.2 million Americans (5).

### **Methods and Materials**

Animal care: All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals as approved by the Council of the American Physiological Society and the Animal Use Review Board of The Marshall University. All procedures were conducted in strict accordance with Public Health Service animal welfare policy. Adult (6 wk. old male) Zucker and Obese Zucker rats were barrier housed two per cage in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved vivarium. Housing conditions consisted of a 12: 12 hour dark-light cycle with the temperature maintained at  $22 \pm 2$  °C. Animals were provided food and water *ad libitum* and allowed to recover from shipment for at least two weeks before experimentation began.

Treatment: Animals were randomly divided between control and exercise groups. Exercise animals were electrically stimulated to perform ten sets of six maximal repetitions. Exercised animals were sacrificed immediately (Time 0), 1 hr or 3 hr following the end of the exercise training.

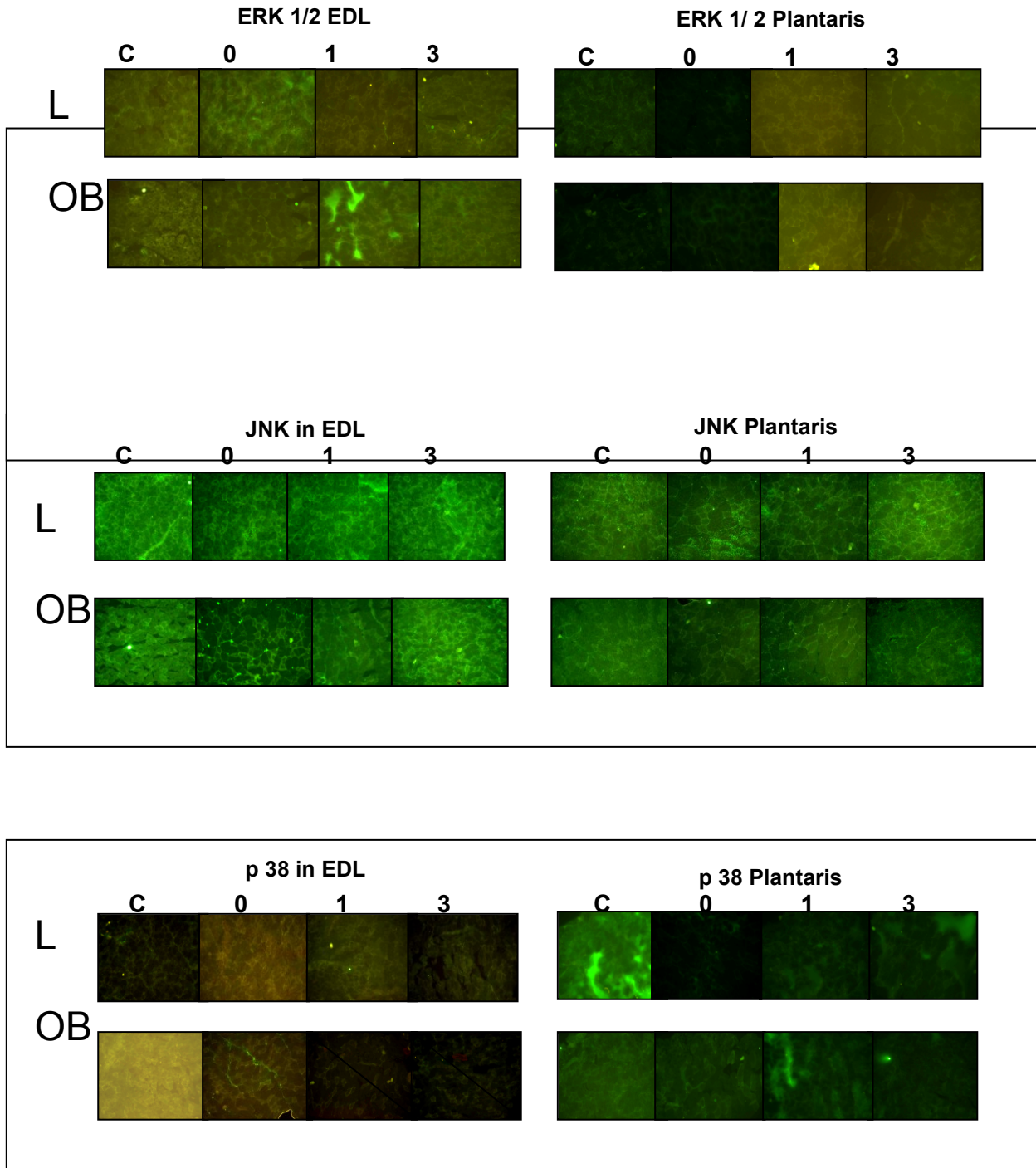
**Immunohistochemistry:** Muscle specimens were serially sectioned (8  $\mu\text{m}$ ) using an IEC Microtone cryostat and collected on poly-lysine coated slides. After fixing in acetone ( $-20^{\circ}\text{C}$  for 2 min), sections were stained with hematoxylin and eosin, mounted and a cover slip applied to the slide. Immunostaining for ERK 1/2, p38 and JNK was visualized by immunofluorescence as outlined by the antibody manufacturer. DAPI was also included in the secondary antibody solution at a concentration of 1.5  $\mu\text{g}/\text{ml}$  in order to visualize cell nuclei. Specimens were visualized by epifluorescence using an Olympus fluorescence microscope (Melville, NY) fitted with a 40X objective. Images were recorded digitally using a CCD camera and analyzed using Olympus MicroSuite™ Basic from Olympus America (Melville, NY).

## **Results**

In the slides of all three proteins, ERK 1/2, p38, and JNK, there was altered basal expression and muscle fiber type differences of regulation between the two models. In the case of the JNK protein, the normal model versus the Zucker model, the protein had translocated from the cytosol to the nucleus (Figure 1).

In comparison, the MAPK regulation altered between the normal model and the Zucker model with less expression of ERK 1, 2 in the Zucker models but an increase of expression of JNK and p38. ERK 1, 2 and p38 did not show translocation from the cytosol to the nucleus in the Zucker model. ERK 1, 2 expression in the normal model had a very low exposure time in comparison with the other slides while JNK in the normal model had the highest exposure time (Figure 1).

In contrast, JNK and p38 expression was relatively the same in both controls, with similar exposure times and increase exposure time in the Zucker model. When it came to muscle fiber types, there was more expression of ERK 1, 2 in the fast twitch muscles than in slow twitch muscles (Figure 1).



**Figure 1:** Lean and obese Zucker skeletal muscle tissue comparisons. C- Control; 0 – 0 hour; 1 – 1 hour; 3 – 3 hour; L – Lean; OB – Obese. Box 1 is ERK 1,2 in the slow and fast twitch muscles of EDL and plantaris. Box 2 is JNK expression in EDL and plantaris. Box 3 is p 38 expression in EDL and plantaris

**Discussion**

With any life-altering disease, there are more affects on the body than previously imagined. With diabetes, the underlying problems are significant and do help to answer some questions about obese

muscle tissues and why they do not operate like a normal model. In this study, the intercellular differences were observed between diabetic and non-diabetic muscle tissue. When the expression on ERK 1, 2 decreased in the Zucker model in comparison to the normal model, it indicates how the cell may not develop correctly and how little growth is being stimulated. Also, since ERK 1, 2 plays a large role in neural functions in cell cycling, the Zucker cell can not proliferate and cycle at the ease of a normal model. With less ERK 1, 2 to oppose the effects of JNK and p38, the cell cannot function correctly and will go through apoptosis (Figure 1). JNK and p38 increase expression in the diabetic model is due to the increase of apoptosis that occurs in the cells. The translocation of JNK to the nucleus as seen in Figure 1, Box 2 was harmful to the cell because it may have halted transcription of proteins that the cell needed and would therefore apoptose.

With the MAPKs, exercise elicits translocation to the nucleus, which mediates the cellular response of the cell. In fast- and slow twitch muscle fiber types, these proteins signal in different ways, suggesting that the observed differences are within the cell itself. These changes cause abnormalities not only in function but in the organism as a whole. The translocation of the MAPKs is significant because it suggests a molecular mechanism associated with insulin-resistance at the cellular level. With exercise, these pathways begin to resemble that of a normal model, which may be due to the increase in insulin mediating the body function in a more normal way. With this insulin activity, changes are being made in benefit of the cell mechanics mimicking that of a normal model instead of a defective one.

Future studies include the examination of molecular mechanisms that underlie these alterations and the phosphorylated MAPK family. Proteins in the cell work in a cascading mechanism, with some proteins altering the expression of other proteins and vice versa. Being able to fully understand other mechanisms and how MAPK proteins are affected will put another piece of the protein puzzle together. With such information, cellular pathways that cause harm in the cell could be altered while helpful pathways could be induced.



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**Perception of Color in Health-Related Food Packaging**

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### Perception of Color in Health-Related Food Packaging

Product packaging is a subject many consumers never consider. However, packaging plays an important role in each consumer's life on a daily basis. Product packaging serves many functions including storage, protection, and identification of a product. Packaging has also developed into a form of product marketing. When shopping for products in a store, the package can heavily influence the consumer's buying decision of one product over another. Much like a canvas is to an artist, the package has become an ideal medium for marketers to attract consumers.

One important aspect of packaging in regards to marketing is color. Color in packaging is influential to consumer choices. Consumer perception of color has been studied over the years to help the marketer better design product packaging. Marketers have found it necessary to learn about consumer reactions to color in order to use the information in product packaging to help sell their goods.

In today's society, consumers have become increasingly conscious about healthy food choices. A number of factors including consumer health issues, medical research and media interest have helped create a health trend. Along with this health trend have come hundreds of diets, including Weight Watchers, the Atkins low carb, and South Beach diets. As consumers began to become more health conscious and sought a more nutritious diet, food companies responded by developing healthier offerings of products and have even introduced new lines of products specifically targeted to the dieting groups, such as carb-friendly products associated with the Atkins low carbohydrate diet.

Companies have realized the need to branch off into these health-related lines of products to stay competitive in an ever-changing marketplace. In keeping with the development of the package as a silent salesman, it is important for these companies to develop packages to reinforce the health conscious aspects of the products that are being offered. Because color is an important aspect in consumer motivation, it seems that certain colors would imply, in the consumer's mind, that the contents of one package are healthier than others. Because color is an important aspect of product packaging, marketers need to be aware of those colors consumers perceive to be healthy in order to most effectively promote

their products. At this point, it seems necessary to ask which colors are best for promoting healthier products consumers are now demanding.

*Product Packaging as a Marketing Concept*

Packaging as a “silent salesman” is a relatively recent concept. Packaging has not always been looked at as a way to help market a product. The use of color in product packaging is a result of this modern marketing concept. To understand the modern concept of packaging, it is important to review some historical aspects of the package.

During the 1890s and early 1900s, a change occurred in the United States. Improvements in manufacturing and an increase in competition in the retail market caused a greater demand for consumer goods. As a result, manufacturers began producing more goods to keep up with the demand. Before this time, packaging mainly served as a means to protect, store, and ship products. Goods had also been packaged in bulk and shipped to stores where retailers would sell products out of a bulk package. Because manufacturers shipped products in bulk containers, retailers had to quickly sell these goods in order to avoid spoilage.

With advances in production technology, as well as the high demand for goods by retailers, overproduction occurred, and the seller’s market that had existed shifted to a buyer’s market. Products were no longer quickly consumed and consumers now had a selection of goods to buy. During this period, many marketing innovations and techniques occurred because of this shift. Consumers demanded higher quality goods at lower prices. Workers demanded higher pay and fewer working hours. As a result, the system of mass production was developed in the United States (Raphael, 1978), allowing more people to afford the goods that were being produced. Another result of mass production was the greater emphasis on unit packaging of goods as opposed to the sale of goods in bulk.

Mass distribution helped create package-marketing strategies. One such strategy was the use of the individual unit package tied to advertising and branded merchandise (Raphael, 1978). Producers discovered they could attract consumers and create demands for their products as well as customer loyalty through promotional efforts. Using the brand name implied that the product quality was guaranteed to be

the same each time a consumer purchased it. The package began to be used to carry the brand name to retail stores and consumers. Company good will and image were transferred from advertisements to the store by means of the package. This was one of the first large scale attempts to use the package as a means of marketing a product, rather than simply for storage and protection. Although the package was used to help market the product, it was not until the 1920s that it was regarded by management as a marketing tool.

During the Great Depression of the 1930s, the majority of companies' advertising budgets were drastically cut, and products were no longer extensively advertised as they were in the 1920s. With advertising budgets cut to a bare minimum, managers turned to the package as a way to help promote their products. The package was no longer only used to carry the company name, but was also used as a means to persuade consumers to purchase products. Attention was also given to the design of the package. Before the Great Depression, many food stores were also full service. Storeowners would retrieve products that the customer requested. To help reduce costs during the depression, most stores became self-service, where consumers shopped for the products they wanted. Thus the need for the package to adequately convey the contents and the company name grew. Managers realized the need for eye-appealing designs that would help consumers easily identify their products from those of their competitors. Package design, color, and shape were changed during the depression years to meet these new demands. These changes in design led to the creation of the package as a "silent salesman."

With the sales clerk eliminated, the package had to be able to sell itself. The package had to be able to present the product and persuade the consumer that it was the best choice. During the 1940s and 1950s, self-service grew from food stores to other retailers (Raphael, 1978). With this shift, the package as a salesman became even more significant.

By the 1960s, consumers were better educated and had more discretionary income that could be spent on goods. Consumers demanded attractive packages and convenience features. Consumers were able and willing to pay for better packaging and for convenience features that made the product easier to use (Raphael, 1978). As more and more competitors enter the market and dozens of different brands are

included in a category, the package becomes an important part of differentiating one's product from others. These events led marketers to research the design aspects of the package, including color, that would help them better promote their products over that of the competition.

### *Consumer Perceptions of Color*

Before beginning a discussion on the perception of color, it is important to define perception. Samuel H. Bartley, American physiologist and psychologist, suggests perception can be defined as "the experiencing of objects, activities of objects, and relations between objects," (Bartley, 1958, p.152). In other words, perception is the process by which a person interprets sensory input. Paul T. Young, American psychologist, uses a paradigm to further define perception. In his paradigm, to perceive is to see, hear, touch, taste, smell, or sense internally some thing, event, or relation (1961). These two definitions help give a general understanding of what is meant when discussing perception in the following paragraphs.

Color has the power to motivate and persuade consumers to perceive products in certain ways. Two people may react in different ways when analyzing a particular instance of color.

Research conducted over the years, however, suggests that, in general, consumers perceive color in similar manners. Marketers have used this perception of color to help imply certain characteristics they want the consumer to associate with their products.

In his book, *The Silent Salesman*, James Pilditch (1973) gives an example of the cigarette industry. When the negative health aspects of smoking cigarettes became publicly known, many cigarette companies around the world changed the color of their package. Many of the packages were changed to white. In this case, marketers hoped the color white would suggest or be associated with purity and cleanliness. This is an example of marketers using color to persuade consumers to perceive a product in a certain way, in this case as being a healthy choice or posing no serious risks.

One of the earliest studies on color perception was conducted by German poet Boethe (as cited in Sharpe, 1976) in the early nineteenth century. He found that red, yellow, and orange, colors that appear at the warm end of the color spectrum, were perceived as exciting, vital, and actionable. Colors such as blue,

green, and purple, which are found at the cool end of the color spectrum, were perceived as relaxing, comfortable, and soothing. Although his study dealt only with hues, the study did not deal with the brightness of the color, lighting conditions during testing, and other factors, Boethe's study is still considered valid. His study suggests that people do in fact perceive colors in certain ways.

Color has the ability to draw a customer's attention to a product. When consumers shop for products, the package that best draws the customer's attention will in most cases be the one the consumer selects if he or she does not already have loyalty to a particular brand. In his book, *Color Sells Your Package*, Dr. Jean-Paul Favre suggests that because of the large variety of offerings of a particular product, the average time a consumer takes to look at each individual item is between 1/25 and 1/50 of a second (Favre, 1969). It is important to point out this observation was published in 1969. Today there are even more products on the shelf competing for the attention of consumers. It is likely the time a consumer takes to look at each individual item is even shorter than Favre's observation. This observation suggests there is very little time for a package to make an impression in the mind of the consumer. Favre also suggests that shoppers usually remember the appearance of the packaging better than the name of the product because colors remain in the memory better than names. If a product is to be successful, the package must quickly make the impression the marketer has intended. Marketers can use color to help direct the consumer's attention to a particular product. However, the colors used must be appealing to the consumer. Because of the ability to use colors to attract the customer, it seems possible that a marketer could use a certain color or combination of colors to help give the appearance that a particular product is of a healthy or beneficial nature.

Color also has the ability to appeal to the emotions of the consumer. There are certain colors that have been shown to appeal to the need for food. Those colors that most stimulate the appetite are orange, pale shades of yellow, pale shades of green, brown and pale shades of brown (Favre, 1969). Favre further elaborates on these colors in his book. He points out that green is suggestive of nature, renewal, hope, and calmness. He also suggests that brown represents the qualities of a healthy life and everyday work. Yellow, Favre writes, is representative of youth, activeness, and when combined with a small

amount of red is pleasing to the eye, heartwarming, and suggests the feeling of satisfaction. From these observations, it would seem appropriate that such colors could be used in a way to suggest the contents of a package are of a healthful nature. A marketer could possibly use these colors in the design of a package to promote the customer's perception of healthy content.

Familiarity also plays an important role in a consumer's reaction to and perception of a product. Research has indicated that the average person is only familiar with and can name about 18-20 colors (Raphael, 1978). The colors that consumers are familiar with are basic colors such as blue, green, red, yellow, brown, black, and orange. Because these are the colors that the consumer is most familiar with, these are the colors that will elicit the most response from the consumer. The simple colors are most memorable, possibly because these are the colors that are taught to children at an early age and thus have the best power in producing sales. Colors that are not familiar or are eccentric may not elicit a response from the consumer and are easily forgotten. Because of this, these colors generally do not have much influence in promoting a product.

#### Summary of Literature

Material presented in this literature review suggests color can be used to draw attention to product packaging. Perception of color can persuade consumers to purchase products. A review of the literature also suggests familiarity plays an important role in the perception of color. Consumers are most familiar with the basic colors, including blue, green, red, yellow, brown, black, and orange (Raphael, 1978). These basic colors will elicit the most response from the consumer. Favre (1969) also reports certain colors, orange, pale shades of yellow, pale shades of green, brown and pale shades of brown, appeal to the appetite of the consumer. In light of this material, marketers would benefit from knowing the color and color combinations that best elicit the consumer perceived responses associated with healthy food choices.



## Method

### *Participants*

Participants for this research included full- and part-time students, enrolled in the fall 2005 academic semester at Concord University in Athens, West Virginia. According to the Admissions Office of Concord University, total enrollment of students, full- and part-time, for the fall 2005 academic semester was 3,015. Of 3,015 students, 161, or 5.3%, participated in the survey. This was the total participation after the initial email, as well as a follow up email, were sent asking for participation. All of the students who participated in the survey completed all of the questions provided on the survey. The response rate of 161 students, or 5.3%, was deemed adequate and the data was analyzed.

### *Design and Materials*

A web-based survey was created and administered to collect the data for this research project. An email was sent to participants with instructions on how to complete the survey. Students accessed the online survey using a link provided in the email. In an effort to allow for an anonymous sample, no identifying information was collected with the survey. The survey was created and hosted using the services provided by Insiteful Surveys ([www.insitefulsurveys.com](http://www.insitefulsurveys.com)). To help prevent duplicate responses from the same individual, the survey was configured to allow only one response per IP address. Although this measure is not foolproof, it helped to eliminate duplicate responses.

To explore consumer perception of color, the survey presented participants with an image of a plain box of a certain color. The participants were asked to rate how healthy they believe the contents of the box were based on the color of the box. The colors green, brown, white, yellow, and black were presented to the participant. The participants were asked to rate perception of color using a five point scale with choices of very unhealthy, unhealthy, somewhat healthy, healthy, and very healthy. The results of the survey were collected in a database, provided by Insiteful Surveys, that was later retrieved for analysis.

*Procedure*

An email was sent to participants using the university's student listserv with information about the research and a link to an online survey. The online survey was available for two months after the email was sent to students through the school listserv. After the two-month period, the survey website was closed and the results analyzed.

Limitations

One limitation affecting the research is the assumption that a survey participant will be able to view the survey images in the same manner as other participants. However, due to hardware, such as video cards, and software, such as operating systems and web browsers, different computers will likely display the colors of the images presented in the survey in a slightly different way. For example, the color green may appear different when viewed on two different monitors or on two different web browsers. A participant's answer to a question may be different if the colors of the images were presented on two different monitors or using two different web browsers.

A second limitation affecting the research is the assumption that survey participants will perceive the images in the same manner. In the survey, participants were presented with a picture of a plain box and asked to rate how healthy they felt the contents of that box to be based on the color alone. The assumption here was that consumers would rate the image based on color and not the contents. However, participants might also perceive the images as containing a particular product. One participant might perceive the box in the image as containing cereal, while another participant perceives the box as containing crackers or some other product. This difference in perception of the contents could change the outcome of the ratings. For example, if the color of the box presented to a participant was green, and the participant perceived the box as containing crackers, they might respond with a rating of "unhealthy." If that same participant, presented with the same image of a green box, perceived the box as containing cereal, they might respond with a rating of "healthy." Another possibility is that two participants who perceived the box as containing the same food, such as cereal, may have different responses. One

participant might perceive cereal as being healthy, while the other participant perceives the cereal as being unhealthy.

Research Question

One question was explored in this research dealing with the idea that certain colors are perceived as representing healthy food by consumers.

Research Question: Are there certain colors consumers perceive as representing healthy food?

*Hypothesis*

Green, brown, white, and yellow will be perceived by survey participants as representing healthy food, while black will not be perceived by survey participants as representing healthy food.

The sample mean of responses for green, brown, white, and yellow will be greater than or equal to a value of three. The value of three is calculated by assigning the numeric value 5 for a response of very healthy, 4 for healthy, 3 for somewhat healthy, 2 for unhealthy, and 1 for very unhealthy. Values of three or more are representative of the responses of somewhat healthy, healthy, and very healthy. The sample mean of responses for black will be less than a value of three. Values of less than three are representative of the responses of unhealthy and very unhealthy.

Results

Assigning the numeric value 5 for a response of very healthy, 4 for healthy, 3 for somewhat healthy, 2 for unhealthy, and 1 for very unhealthy, the mean of the responses for the color green was calculated at  $\bar{X}_1 = 3.615$ , brown was calculated at  $\bar{X}_2 = 3.155$ , white was calculated at  $\bar{X}_3 = 3.547$ , yellow was calculated at  $\bar{X}_4 = 3.056$ , and black was calculated at  $\bar{X}_5 = 1.888$ , as presented in table 1.

Table 1

*Participant Responses and Calculated Means*

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Color	Very Healthy Value: 5	Healthy Value: 4	Somewhat Healthy Value: 3	Unhealthy Value: 2	Very Unhealthy Value: 1	Total Responses	Mean $\bar{X}$
Green	21	78	44	15	3	161	3.615

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Brown	19	51	40	38	5	161	3.155
White	36	45	56	19	5	161	3.547
Yellow	10	53	45	42	11	161	3.056
Black	2	6	27	63	63	161	1.888

### Discussion

Upon analysis of the data collected, the colors green, brown, white, and yellow were found to be perceived by the survey participants as representing healthy food. The color black was perceived by survey participants as not representing healthy food. The colors green and white were found to have the highest perception rating as being healthy among the survey participants.

The implication of this study is that the findings could be used by marketers in targeting health conscious consumers. The colors presented in this study could be used in product packaging to elicit a favorable response from consumers. Further research could lead to a better understanding of the topic.

A possibility for future research could be for the researcher to have a controlled environment where all survey participants experience the same environmental conditions while taking the survey. Another possibility for future research could be to test additional colors that were not presented in this survey. Testing various shades of a particular color is another possibility for future study. Additional research could also test combinations of colors in packaging to see how they are perceived by consumers in regard to healthy food. Many other factors in product packaging also impact consumer's lives and some of these factors might also be added to the study to build a more comprehensive overview of the topic.

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**A Modeling and Simulation of Sensor Data for  
An Autonomous Ground Robotic Vehicle**

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## **1. Introduction**

### **1.1 Simulations**

The definition of a simulation can vary depending on the application and situations that are being evaluated. For instance, performing a test in a mock or created environment that differs from the actual environment it was intended for is considered a simulation [1]. Simulations can be created as a real-world physical experiment or as a computer generated one. For this paper we will apply Fishwick's definition of a computer simulation as a model of an actual or theoretical physical system with the ability to employ the model and evaluate its outputs [2]. The computer gaming industry uses simulation techniques to mimic real or fantasy situations such as driving a NASCAR racecar, or flying a spacecraft through a galaxy. Likewise, amusement parks use simulators along with motion platforms to enhance the "thrill ride" or entertainment value of a show. On the other hand, computer simulations have been used for many years as testing and design tools for pilot training as well as engineering applications for land, sea, air, and space vehicles [3]. Examples of this type of simulator can be found in the avionics industry to test new aircraft designs and the training of pilots. Furthermore, automotive companies such as Daimler-Benz and Deere and Company have used simulators to test vehicle design as well as human driving characteristics [3]. The advantages of using a computer simulation as compared to making a sophisticated prototype or performing real world experiments include: lower expenditures, saved time, increased safety, prototypes kept to a minimum, controlled testing environment, and easily re-created experiments.

Even though simulations and simulated environments vary depending on the applications they have been designed for, the focus of this research is the creation and implementation of a computer simulation that replicates actual sensor data for robotic systems which are currently being used in the robotics program at Bluefield State College, Bluefield, West Virginia. Our goal is to use this simulation as a testing tool that will provide a controlled testing environment as well as accurately repeatable experiments for debugging and testing various programs associated with Autonomous Ground Robotic Vehicles (AGRV).

## 1.2 Background and Motivation

Webster's New World Dictionary defines autonomous as "functioning independently without control of others"[4]. When applying this definition to a ground robotic vehicle, it could be defined as a land base means of transportation that functions independently without the aid of human assistance. AGRVs have received increasing attention from the U.S. military as well as academia in recent years. The Floyd D. Spence National Authorization Act (2001) states:

"It shall be a goal of the Armed Forces to achieve the fielding of unmanned, remotely controlled technology such that—by 2010, one-third of the aircraft in the operational deep strike force aircraft fleet are unmanned; and by 2015, one-third of the operational ground combat vehicles are unmanned" [5].

The Department of Defense (DoD) and its various resources has played a major role in the fielding of AGRVs through its involvements in projects such as the DEMO III and events like the Defense Advanced Research Projects Agency's (DARPA) Grand Challenge.

The DEMO III Experimental Unmanned Vehicle (XUV) was developed by the Army Research Laboratories, General Dynamics Robotics Systems, National Institute of Standards and Technology, and Jet Propulsions Laboratory among other contributors. This DoD project is aimed at advancing and demonstrating the technology required to develop future unmanned ground combat vehicles [6]. The DEMO III along with other autonomous vehicles platforms the Army has created, such as the Ground Standoff Minefield Detection System and the Accident Response Mobile Manipulation System are all projects developed for the Future Combat System mission [7]. These vehicles are among the many prototype test vehicles that continue to be a test bed for the Army's research on AGRVs.

The DoD's most recent involvement with AGRVs is with the DARPA Grand Challenge (DGC) events. The DGC is a field test aimed at advancing the research and development of autonomous ground vehicles. The DGC was started in response to a Congressional mandate, National Defense Authorization Act for Fiscal Year 2003, which authorizes DARPA to award prize winnings up to ten million dollars for technological breakthroughs in military research and development [8]. The first Grand Challenge was



held in March, 2004, and offered one million dollars to the team that could complete the 142-mile off-road course the fastest, within a ten hour time limit. The teams were faced with building an autonomous vehicle which could perform in the harsh desert environment while avoiding both natural and man made obstacles as well as other vehicles competing in the competition. The farthest any vehicle traveled in the 2004 DGC competition held on March 13, 2004, was 7.4 miles, by the Red Team - Carnegie Mellon, Pittsburgh, Pennsylvania. Because no one completed the 142 mile course, no prize was awarded [9]. On October 8, 2005, DARPA held the competition again, offering two million dollars to the team that could complete a 132 mile course in less than ten hours [10]. The number of participants and technological advancements grew from 106 contestants and traveling 7.4 miles in 2004 to 195 contestants and three teams completing the 132 mile course within the ten-hour time limit.

Other advancements in AGRV's are highlighted at the annual Intelligent Ground Vehicle Competition sponsored by the Army as well as other sponsors. This event is open to college and universities worldwide. It is aimed at advancing research and technology by involving both undergraduate and graduate engineering students in the area of AGRV. Competitors at this competition are judged on different areas of their robot, including vehicle design, autonomous navigation, and obstacle avoidance.

The robotic program at Bluefield State College (BSC), Bluefield WV, has been involved with AGRV research for over seven years. BSC combines both student and faculty in Mechanical Engineering, Electrical Engineering, and Computer Science together with local industrial leaders to produce highly prominent research and products for the southern West Virginia area. While predominantly an undergraduate institution, BSC has the knowledge and determination to compete against leading graduate institutions in competitions such as IGVC and DARPA Grand Challenge events.

In the past five years of competing in the IGVC contest, BSC has excelled among the top six contenders out of approximately twenty to thirty participants. In 2003, BSC won first place in Design Competition as well as the Most Intelligent Robot award. In both 2004 and 2005, BSC was awarded

fourth place in the Autonomous Challenge event. Also, in 2005, for the first time in the competition's history, BSC was one of two teams that completed the Autonomous Challenge course.

Our current research at BSC includes improving the design and software of the 2005 IGVC robot Anassa. Also, students are in the process of designing, fabrication, and programming software for a new robot, Segway Fox, expected to compete in the 2007 IGVC. Even though DARPA does not expect any more Grand Challenge event (<http://www.darpa.mil/grandchallenge05/qa.html>), BSC will continue its research on an off-road full-size autonomous vehicle. Despite the recent success at the 2005 DARPA Grand Challenge competition, there is still much to be learned about traveling autonomously in an off-road environment at realistic vehicle speeds. This along with other projects associated with BSC as well as the Center for Applied Research and Technology (CART) is our motivation and justification for this research.

### **1.3 Problem and Outline**

Traveling in an off-road environment at realistic speeds of twenty to thirty-five miles per hour can be very dangerous for a sensitive AGRV. Furthermore, the expense of these vehicles can typically exceed many thousands of dollars. This can make testing AGRVs in an off-road environment both difficult and costly. At the same time, the weather plays a major role in testing conditions for both the people conducting the testing as well as the robotic vehicle. The members of Team CART have seen the extremes of both hot and cold weather conditions such as the 115 degree heat of Tucson, Arizona in addition to the cold winter weather of Bluefield, West Virginia. Another factor that makes testing an AGRV difficult is arranging time in-between the schedules of faculty and students to actually conduct tests. This, along with many other factors gives justification for the need of a trouble-free method of testing the programs associated with AGRVs.

This paper explains the development and implementation of a computer simulation that simulates the sensor data for an AGRV. So far, the paper has introduced aspects and advantages of a computer simulation, supplied background information on previous research of AGRVs, and provided justification on this research. The remainder of the paper describes and explains the uses of sensors modeled in this

research. Then there is a discussion of programming language used and the simulation that was created. Next, there is an explanation of the simulation's execution and its outputs. After that is a debate on the analysis and validation of the simulation. Finally, there is a conclusion and mention about possible subsequent work related to this research.

## **2. Sensors Modeled**

This section will inform the reader on typical sensors used in current AGRV research and provide a brief description on the sensors modeled in this computer simulation. It must be noted that if the reader is already familiar with the types of sensors, then the reader may want to skip this section because it only provides basic information on each sensor as a means of informing readers who are unfamiliar with this type of research.

### **2.1 Global Positioning System**

A vital necessity for a AGRV is to know where the robot is located and to know where it is going. A commonly used method for determining position anywhere on the face of the earth is the Global Positioning System (GPS). This DoD project is used for military purposes, but is available to the public as a potentially free service. GPS uses a constellation of satellites orbiting the earth to pinpoint a user's position. A GPS receiver determines position by calculating the time it takes for the GPS signal from the satellites to reach a user's GPS antenna. At least three (usually four or more) satellite signals are needed to accurately determine the user's position on earth [11]. The user's location is given in geographic coordinates called latitude and longitude, which are measured in degrees and represent angular distances from the center of the earth. Latitude is the angular values north and south of the equator. Longitude is the angular measurement east and west of the prime meridian.

The GPS unit that is modeled in this research is a DGPS Max system made by CSI Wireless Incorporated. This system uses differential GPS (DGPS), which uses the aid of a local permanent land reference receiver that has a true measurement of each of the orbiting satellites to calculate out much of the measurement errors associated with mobile GPS receivers. The DGPS Max system uses differential corrections received by Wide Area Augmentation System (WAAS), Omni STAR, Worldwide DGPS

Services, and/or Radio Beacon DGPS. With the DGPS Max system we can obtain position accuracy of one to five meters [11].

To acquire and use the data received by the DGPS Max antenna and receiver we interface with the receiver via communications port to the robot's on-board computer. Even though the GPS signal has a multitude of information being received as a standard National Marine Electronics Association (NEMA) message, we are currently using only four different aspects of that data. Our current setup uses longitude, latitude, velocity, and heading information to determine the AGRV's position, speed, and direction.

## **2.2 Laser Measurement System**

Another crucial task for an AGRV is to sense obstacles around it. Many land based robotic vehicles today use a Laser Measurement System (LMS) to detect and measure obstacles. The LMS detects and measures the distance from an object by first sending a short pulse of infrared light. Milliseconds later, a portion of the light is reflected back to the LMS. It then calculates the time it takes the beam of light to be sent and received in order to acquire an accurate measurement of the objects distance from the LMS unit [12]. There are many different types and manufacturers of LMS units. This paper is focused on two separate LMS units made by SICK Incorporated. The remainder of this section will describe the uses and data obtained by each unit.

The SICK LMS 291-S14 unit is a 2-D laser scanner with a 180 degree field of view. This unit has a selectable angular resolution that is setup for 1.0 degree and a response time of 13 milliseconds. The 291-S14 unit has a maximum range of 80 meters (262.5 feet), with a measurement resolution of 10 millimeters (0.39 inches) [13]. This unit is modeled in the forward horizontal position at a predetermined height and provides a 180 degree swipe in front of the vehicle to detect positively protruding obstacles. Positive obstacles will be defined as any object protruding from the ground at a height that is greater than the preset height of the LMS. For instance, objects such as large rocks, barrels, fence posts, and other vehicles would be considered positive obstacles.

Data is transmitted via communications port to the robot's on-board computer. This data which arrives as a hexadecimal message is converted to a binary number format by the robot's computer

program. The information gives exact measurements (in inches or millimeters) of any obstacle that is within the LMS's view. If no obstacle is detected, then the number comes back as infinity or 9999.

The second LMS that is modeled is a SICK LMS 291-S09. This unit has many of the same characteristics as the 291-S14 unit, except that it has a 90 degree field of view. The 291-S09 unit is modeled in a horizontal position, on top of the vehicle at a preset height, with its field of view angled down to the ground at a predetermined angle. This LMS is used to detect both positive and negative objects in front of the vehicle. Negative objects are obstacles such as deep holes, ditches, and cliffs that protrude down below the initial ground surface. It must be noted that the 291-S09 unit is currently being used only on the DARPA vehicles.

### **2.3 Inertial Measurement Unit**

While traveling in an off-road terrain, a vehicle will often encounter situations where the ground is not ideally level. This can be a dangerous situation for anyone driving in this type of environment due to the possibility of a vehicle roll-over. An AGRV has an additional complication in that a change in the vehicle's orientation to the ground will change how the LMS sensor data will be interpreted.

The sensor used to determine a vehicle's orientation is an Inertial Measurement Unit (IMU). The system that is modeled in this research is a Sparkfun Electronics IMU 6DOF v2 unit which uses a combination of an accelerometer and three gyroscopes [14]. The accelerometer measure the acceleration and the three gyroscopes are used to obtain a vehicle's orientation of yaw (rotation about Z axis), pitch (rotation about X axis), and roll (rotation about Y axis).

### **2.4 Encoders**

Encoders are used on an AGRV as another means of obtaining information on the robot's position, speed, and heading. An encoder is an electro-mechanical device that acts as a counter for a rotating object. For example, an encoder is used on an AGRV's wheel to count the number of rotations the small shaft of the encoder makes. By knowing the number of encoder counts for one revolution of a wheel and the wheel's diameter, it is possible to calculate the distance the AGRV has traveled. Likewise,

one can calculate a robot’s heading by knowing a vehicle’s width and determining the difference between two adjacent wheel encoders.

### 3. Simulation Program

The programming language used for this research is Visual Basic 6.0 (Appendix A). Visual Basic is a Windows-based programming language that allows the user to interface with other Windows programs and applications [15]. This language was chosen because it is taught to all engineering students at Bluefield State College. Equally important is the fact that it is the computer language used on all the robotic vehicles at Bluefield State College.

#### 3.1 Simulation Created

This section describes, in a general format, the simulation and the sensor data created so that it can be applied to a computer simulation for any type of AGRV and implemented in any type of programming language. In order to simplify the calculations used throughout this paper, all dimensions will be in the units used in this research (pixels, degrees, feet, seconds, etc.). Nevertheless, all equations can be used with any type of units desired as long as unit conversions are appropriately used.

To describe the methodologies behind simulating data, will start by explaining the global map in which the simulated robot navigates. The term global map will be defined here as a map of the whole area in which the simulated robot can move around in. In order to design a simulation that is realistic to the real world environment, it is important that the simulated objects are to scale with the global map on which they are being placed. This can be accomplished by first creating an object in the computer simulation that has real-world dimensions that are already known such as, the robotic vehicle, a known obstacle, or even the global map itself. Taking the measurements of the object in the simulated world (in pixels) and dividing it by the dimensions of the same object in the real world (in feet), will produce a scale factor ( $\frac{\text{pixels}}{\text{feet}}$ ) that can be used for the sizing of the rest of the simulation.

$$\text{Scale Factor} \rightarrow \text{SF} = \frac{\text{Simulated Dimensions}}{\text{Real World Dimensions}} = \frac{\text{Pixels}}{\text{Feet}} \quad (\text{EQ 3.1})$$

Although the use of a scale factor is an important concept to incorporate into the design of the simulation, the level of detail used on simulated objects and the global map is typically only for aesthetic reasons. The simulated objects created in this research to represent things like the robotic vehicle, barrels, and roadways are simulated using basic shapes such as ovals, circles, and lines. Likewise, the amount of simulation controls and sensor data display depends on the programmer. The simulations created in this research have individual on/off control buttons for each of the sensors as well as control buttons for manual (human) and autonomous (computer) control of the simulated vehicle. Some of the sensor data such as latitude, longitude, speed, heading, pitch, and roll are displayed in real time in the simulation program. However, sensor data from the LMS systems is typically too large to be displayed on the actual simulation program. The LMS data along with GPS, encoder, and IMU data is written to a text file every cycle of the program. This data can be viewed by other computers and other computer programs like the navigation computer on-board the “real” robot. The navigation computer can in turn treat this simulated data as sensor data from a real world test.

### 3.2 Simulated GPS

In order to create realistic GPS longitude and latitude data, a location on earth needs to be chosen as the point of reference for the global map. The location that is chosen is somewhat irrelevant. However, the initial geographic coordinates of longitude and latitude are important in the simulation of GPS data. The longitude and latitude coordinates of any location on earth is readily obtainable through the use of an atlas, world maps, or the World Wide Web. The GPS coordinates that were chosen as a point of reference, are used as the starting values (zero position) of an X and Y Cartesian coordinate system of the global map. As the simulated robot moves around the global map, the X and Y coordinates of the simulated robot is converted first into real world dimensions and then further converted into GPS coordinates of longitude and latitude. This is accomplished by a conversion factor to convert nautical feet into degrees arc seconds (EQ 3.2).

$$\text{Degree Arc Seconds} = \theta_s = 6076.12 \frac{\text{Feet}}{\text{Deg. Arc Min.}} \left(60 \frac{\text{Sec.}}{\text{Min.}}\right)$$

(EQ 3.2)

For latitude, the X coordinate is divided by (SF) to convert into feet and then divided by ( $\theta_s$ ) to convert to degrees arc second (EQ 3.3).

$$\text{Latitude}_{\text{deg.arc sec.}} = \frac{X_{\text{pixel}}}{\left(\text{SF} \frac{\text{pixel}}{\text{feet}}\right) \left(\theta_s \frac{\text{feet}}{\text{deg.arc sec.}}\right)} \quad (\text{EQ 3.3})$$

Similarly, longitude is multiplied by (SF) and ( $\theta_s$ ), but is also multiplied by the cosine of the current latitude [ $\cos(\text{latitude})$ ] (EQ 3.4). The multiplication of [ $\cos(\text{latitude})$ ] is used to compensate for the slope of the latitude lines as they converge at the north and south poles.

$$\text{Longitude}_{\text{deg.arc sec.}} = \frac{X_{\text{pixel}}}{\left(\text{SF} \frac{\text{pixel}}{\text{Feet}}\right) \left(\theta_s \frac{\text{Feet}}{\text{deg.arc sec.}}\right)} [\cos(\text{Latitude})] \quad (\text{EQ 3.4})$$

In a “real” world situation, GPS calculates speed by taking the difference of the current and previous positions and dividing that by the time interval between the measurements. The simulated GPS is very similar except that it calculates speed instead of velocity (EQ 3.5 – 3.7). Velocity is different from speed because velocity gives information about a vehicles speed, direction and state [14].

$$\text{Speed}' = \frac{\Delta x}{\Delta t} = \frac{\text{Current position}(x_c, y_c) - \text{Previous position}(x_p, y_p)}{(\text{Current time} - \text{Previous time})} \quad (\text{EQ 3.5})$$

Much like the “real” GPS, the simulated GPS data must be converted into the proper units for speed (mph).

$$\text{Speed}'' \frac{\text{Feet}}{\text{sec}} = \frac{\text{Speed}' \frac{\text{Pixels}}{\text{mili sec}}}{\text{SF} \frac{\text{Pixels}}{\text{Feet}}} \left( \frac{1000 \text{ mili sec}}{1 \text{ sec}} \right) \quad (\text{EQ 3.6})$$

$$\text{Speed}''' \frac{\text{Miles}}{\text{Hour}} = \frac{\text{Speed}'' \frac{\text{Feet}}{\text{sec}}}{5280 \frac{\text{Feet}}{\text{Mile}}} \left( \frac{1 \text{ hour}}{3600 \text{ sec}} \right) \quad (\text{EQ 3.7})$$



To simulate heading, the direction of true north on the global map must be known. True north is defined as the direction pointing to the geographical North Pole. For most applications, true north can be chosen as the direction pointing to the top of the map or the map can be oriented to this position. The GPS heading can then be simulated based on where the robot is pointing on the global map in reference to the angle from true north. To determine the robot's orientation, there must be two X points of reference and two Y points of reference on the simulated robot (Figure 3.1).

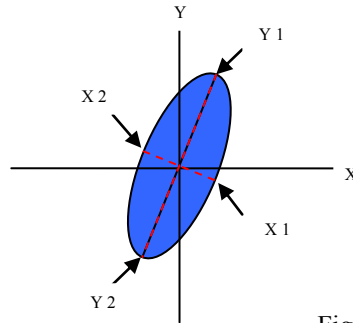


Figure 3.1 – X and Y reference points

Then, the trigonometric calculation that determines the vehicles heading ( $\omega_H$ ) is dependent on where the x and y points are positioned in the x-y plane of a Cartesian coordinate system. Equation 3.8 is for the situation in Figure 3.1.

$$\text{Heading} = \omega_H = \tan^{-1} \left( \frac{\text{Robot } X_1 - \text{Robot } X_2}{\text{Robot } Y_1 - \text{Robot } Y_2} \right) \quad (\text{EQ 3.8})$$

### 3.3 Simulated LMS

Many different methods of simulating LMS data was tested to find a technique that would give accurate measurements of surrounding obstacles and perform this action within a realistic cycle time. Some of the problems that had to be overcome were creating three-dimensional data in a two-dimensional simulated world. Likewise, creating measurements for the 291 - S14 LMS and the 291 – S09 LMS was a computationally intensive task. The ideal method for creating the LMS data would be to take measurements at each degree increment every complete cycle of the program. However, this method

caused a computational burden that slowed the simulation’s cycle time down immensely. In order to make the simulated data in a practical manner, the cycle time needed to create the simulated data should be very similar to the time taken to collect the “real” data.

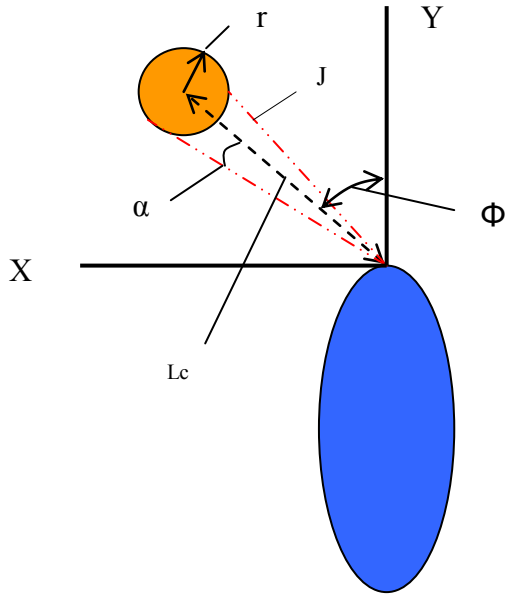
**3.3a LMS 291 – S14**

Two time-saving shortcuts were able to be implemented on the creation of the 291 – S14 LMS data due to its positioning and two-dimensional field of view in the “real” world. The first shortcut assumes the vehicle is traveling on flat ground and uses objects of known position, shape, and size. By knowing this information, the LMS data would only need to be created when the objects come within range (80 meters) of the LMS sensor. If the objects were within range of the LMS, then a procedure was performed to create the LMS measurements. Otherwise, the LMS data would default to the value of (9999), which is what the LMS obtains when it does not “see” any objects.

The second shortcut used to speed up the simulated 291 – S14 LMS was in the creation of the LMS measurements. Instead of taking measurements at every degree step, the objects distance and angle from the robot was found. Next, the distance of the object to the robot is found at each angle of the LMS (Figure 3.2). A ratio was setup by taking the angle from the center of the barrel to each side at 90

degrees from center  $\left( \frac{\pi}{2} * \frac{J - \alpha}{\phi} \right)$  and multiplying this by the LMS distance at the center of the barrel (EQ

3.9). The objects modeled in this research are of a round orange reflective highway barrel, approximately 3.3 feet (1 meter) in diameter and 4 feet (1.22 meter) high.



- $r$  = barrel radius
- $J$  = LMS distance  $J$  at one degree increments
- $L_c$  = length to the center of the barrel
- $\Phi$  = angle from true north
- $\alpha$  = angle from center of barrel to the edge of barrel

Figure 3.2 - LMS 291 – S14 view

$$291-S14 \text{ LMS}_{\text{Feet}} = (L_c - r) * \cos\left(\frac{\pi}{2} * \frac{j - \alpha}{\phi}\right) \quad (\text{EQ 3.9})$$

### 3.3.b LMS 291 – S09

The simulation of the 291 – S09 LMS revealed two more problems that needed to be overcome in order to simulate the data. This is caused by the technique used on the “real” 291 - S09 LMS to acquire three-dimensional data from a device that “sees” in a two-dimensional plane. This is accomplished by angling the LMS down-ward at a predetermined angle, which adds a third dimension (-z) to the data. Also, because the unit is angled in the direction of the ground, this causes the LMS to always “see” something in front of it. In fact, when there are no objects in the LMS’s field of view and it is on flat ground, the LMS data resembles that of a parabola (Figure 3.3). This is due to the length of the LMS beam gradually lengthening as the angle from center increases.

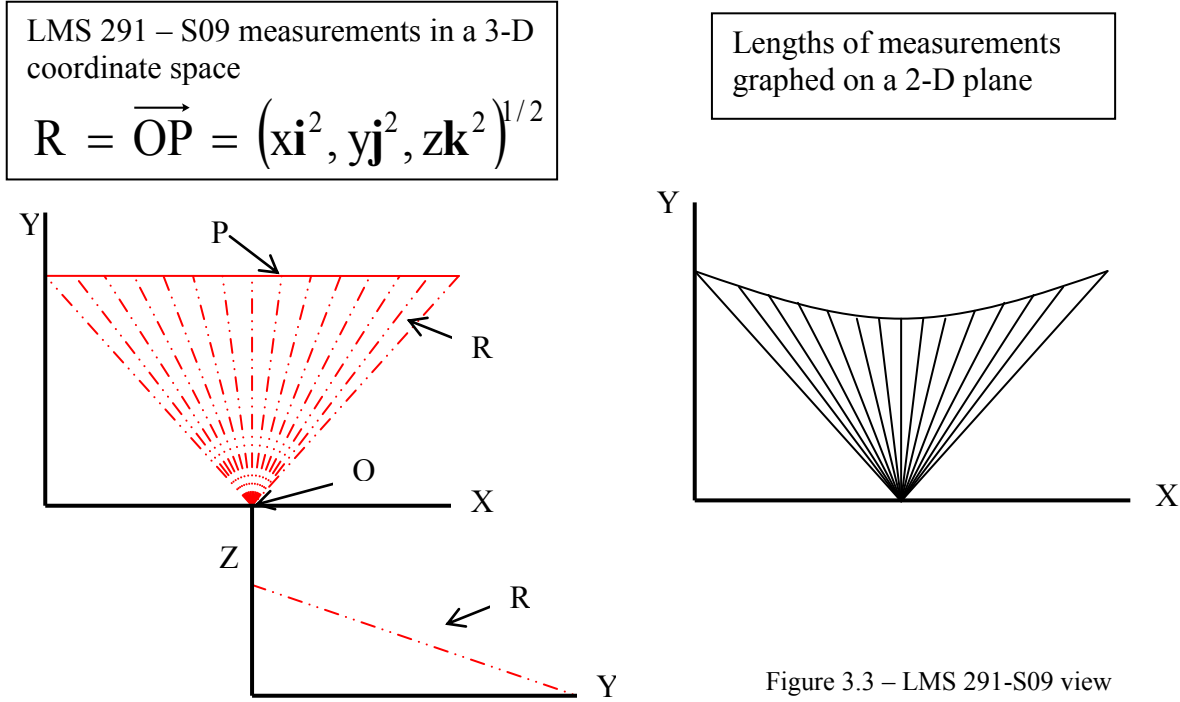


Figure 3.3 – LMS 291-S09 view

To simulate the data for the 291 – S09 LMS, the length at each angle was examined and measured during each cycle of the program. Likewise, the color was examined along the length of every LMS measurement to determine the height of any objects. By examining and converting the numerical representation of red, green, and blue (EQ 3.10), produces a value that can be used for the object’s height. It must be noted that the numerical representation of the RGB color is quite large (red =  $2^8$ , green =  $2^{16}$ , blue =  $2^{24}$ ). Therefore, the equation used to convert this number into a more realistic value (EQ 3.10), is of a general format and is only to change the size of the number and relate this to a height/depth value.

$$\text{Height} = H = \frac{\text{Current pixel color}}{\text{Color chosen to represent the ground}} \quad (\text{EQ 3.10})$$

If the height of an object (both positive and negative) is within view of the LMS, then the distance to that point is measured. To measure the distance of a point in space, a trigonometric formula is used, like the one in Figures 3.2 and 3.3 (EQ 3.11).

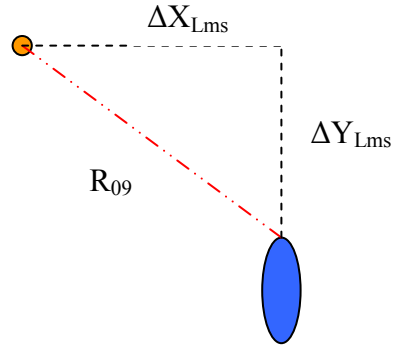
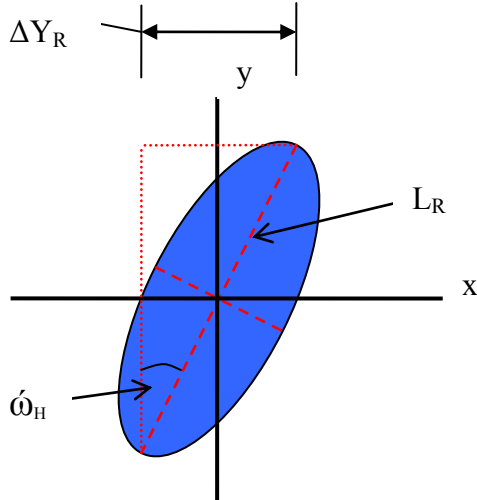


Figure 3.4 – view of equation 3.11

$$291 - S09 \text{ LMS } S_{\text{Feet}} = R_{09} = \frac{(\Delta x_{\text{Lms}}^2 + \Delta y_{\text{Lms}}^2 + H^2)^{1/2}}{\text{SF } \frac{\text{Pixels}}{\text{Feet}}} \quad (\text{EQ 3.11})$$

### 3.4 Simulated IMU

The creation of the IMU data used the same technique for making three-dimensional data from a two-dimensional image as the 291-S09 LMS. Each of the X and Y points in Figure 3.1 were examined for RGB color values. The robot’s pitch, which is the same as heading, is determined by the difference in Y position ( $\Delta Y$ ) of the (Figure



$\Delta Y_R$  = Difference in Y1 and Y2 of robot

$L_R$  = Length of simulated robot

$\omega_H$  = Robot heading (Yaw) in degrees

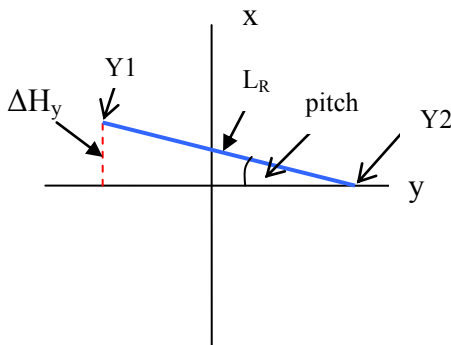
Figure 3.5 - View of the robot's yaw

$$\text{Yaw} = \omega_H = \sin^{-1}\left(\frac{\Delta Y_R}{L_R}\right) \quad (\text{EQ 3.12})$$

3.4)

(EQ 3.12).

For the robot's pitch, the difference in height ( $\Delta H$ ) of the two Y points (Y1, Y2) (Figure 3.1) were examined and used to find the angle of pitch (EQ 3.13).



$\Delta H_y$  = Difference in height of Y1 and Y2

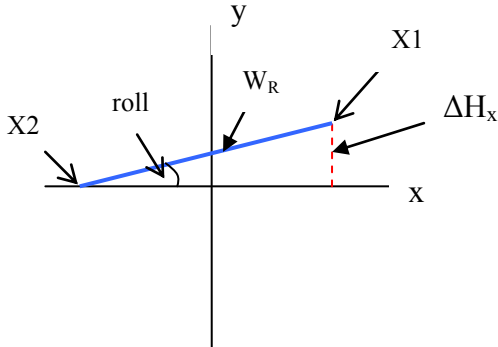
pitch = Angle of robot's pitch in degrees

$L_R$  = Length of simulated robot

Figure 3.6 - View of robot's pitch in the YX coordinate frame

$$\text{pitch} = \sin^{-1}\left(\frac{\Delta H_y}{L_R}\right) \quad (\text{EQ 3.13})$$

Similarly, the robot’s roll is calculated by taking the difference of the height at X1 and X2 (Figure 3.1) were used to determine the angel of roll (EQ 3.14).



$\Delta H_x$  = Difference in height of X1 and X2

roll = Angle of robot’s roll in degrees

$W_R$  = Width of simulated robot

Figure 3.7 - View of the robot’s roll in the XY coordinate frame.

$$\text{roll} = \sin^{-1} \left( \frac{\Delta H_x}{W_R} \right) \quad (\text{EQ 3.14})$$

#### 4. Validation of Simulation

The goal of this simulation is to mimic the “real” sensor data in a virtual world. This needed to be done to the extent that the “real” robot’s onboard computer would treat this simulated data the same as the actual data acquired during an authentic test. Obviously, the complexity of the real world environment is too large to be replicated in a computer simulation. Nonetheless, we can imitate this information by simplifying the real world into the basic information that is needed to create the sensor data. So, how do we know that the results in the simulated world will transfer into the real world? One method of validation would be to compare the behavior of the virtual robot to the robot in the real world. Currently, the students and faculty at Bluefield State College are working on re-writing software for the 2006 IGVC robot that will be able to utilize and test this computer simulation. However, if the two robots are independent of one another and exist in two separate worlds with different conditions, would the validation be accurate?

This paper will use the principles for validity as stated by Bishop and Potter, as a means of validation of the simulation [17].

#### 4.1 Principle 1

##### **Space should be represented and managed such that:**

**4.1a** *Dimensions are to scale with the real world.*

This is accomplished by the use of equation 3.1.

**4.1b** *The continuous nature of space is preserved or approximated at an arbitrary level of resolution that should be chosen to be very much smaller than any distance of interest.*

Our level of resolution was of the size of a individual pixel, which was much smaller than what is needed.

**4.1c** *No point in space may be occupied by more than one thing simultaneously.*

Even though the objects within the simulation could be place over top one another, this only happened at the users preference.

**4.1d** *Agents can be embodied in the sense of structural coupling.*

Bishop and Potter use Brooks explanation of embodiment as:

“The robots have bodies and experience the world directly – their actions are part of a dynamic with the world, and their actions have immediate feedback on the robot’s own sensation” [17][18].

This simulation uses a simulated robot that has a definite body or shape, which interacts with the global map that it is placed on. The robot’s actions have immediate feedback through the use of it’s various sensors (GPS, LMS, and IMU).

**4.1e** *Agents can be situated.*

Bishop and Potter again use works from other authors to describe what is meant by situated. However, their definitions will be paraphrased here. The robot(s) must be situated in it’s own predictable and stable world, responding in a timely fashion to the inputs that directly influence it’s behavior [18] [19] [20]. Examples of these



traits are seen in this research by the methods used to influence and control the robot by obstacles, GPS waypoints, and boundary lines. Similarly, the time in which the robot reacts to these controls was a major factor in the simulation design.

**4.1f** *Agents are all equally subject to a set of physical laws regardless of processing load on the simulation.*

Agents such as the robot and obstacles within the simulation perform alike, independent of the processor load. A large processing load can slow the simulation down and make the actions seem jerky, but this is true for all “agents” within the simulator.

**4.1g** *Behaviors can be observed in the same way (visually) as they could be in the real environment*

Most of the above statement holds true for this simulation. However, the method used to imitate a three-dimensional world in a two-dimensional environment, naturally would not be seen visually as it would in the real world. Nonetheless, the data created would in fact be seen the same as a real world test.

**4.1h** *Agents can be grounded in that all information on which a behavior operates internally is an abstraction of some aspect of the representation of the environment (including the agent’s representation), or derives from such abstractions via other behaviors.*

The sensors used in this simulation create data that is depends on the environment. As stated in the LMS Simulation section, the sensors create its data by the position, size, and depth of the obstacles within the environment. Similarly the GPS and IMU sensors hold true to the above statement because, they generate their data by depending on where the robot is positioned and what kind of environment it is in.

## **4.2 Principle 2**

**Time should be represented and managed such that:**

**4.2a** *Simulated time proceeds independently of any behavior.*

The simulated time in this simulator is determined through the internal clock of the processor and is indeed independent of any behavior.

**4.2b** *The continuous nature of time is preserved or approximated at an arbitrary level of resolution that should be chosen to be much smaller than any time intervals of interest.*

The level of time resolution is of the millisecond, which is adequate for this research.

**4.2c** *Relationships in simulated time are preserved independently of the amount of real time taken by the simulation to perform its processing.*

The programming language used in this research runs through each process as a sequence of events. If one event has a long processing time the rest of the events are unaffected because they will simply wait until the program has completed its cycle before restarting to the next cycle.

**4.2d** *Any number of behaviors may be active simultaneously without causing a distortion of spatial-temporal relationships due to processing load on the simulation.*

This subject was the very cause for the shortcuts implemented on the simulated LMS data. Due to the long processing time in calculating large LMS data files, the simulation would have a lag in events or appear jerky. By using shortcuts in creating this data, the simulation is now only slightly affected by the amount of processing load.

**4.2e** *The opportunity to interact with the environment afforded to a behavior is proportional to its response time and not the time taken for the simulation to perform processing associated with the environment.*

As mentioned earlier in section 4.2c, the program handles its processing as a sequence of events. Therefore, when an event causes a response, the processing

time associated with that response does not affect any other event because, the program is waiting for the processing of that response before it continues.

- 4.2f** *The response time of a behavior may be treated as being different from the time taken for the simulation to perform processing on behalf of that behavior.*

Again, the processing time is separate from the response time. For an extremely exaggerated example, it may take a full 10 seconds for the computer to process the entire sensor data used in determining an action, but the action taken is seen as happening in a fraction of a second.

- 4.2g** *The relative orders of magnitude of the time taken for an agent to move a percentage of its body length, and the response times of the behaviors involved, are the same in simulated time as they are in the real world.*

By utilizing a scale factor in the creation of the objects and global map on which they operate, enables us to obtain realistic units on the vehicle's speed, direction, and distance covered. This aided in creating a simulation with movements and actions that are closely related to the movements and actions of a real world robot.

## **5 Conclusion**

This paper has mapped out a methodology for creating a computer simulation to mimic the sensor data of an AGRV. The research performed here would be considered as the beginning stages of the creation of a computer simulator. This program, like many computer programs, are constantly updated and revised to produce a better version. Moreover, the research done here has opened many doors for the robotic team at Bluefield State College (BSC), in that another method can be used to create and test algorithms associated with controlling an AGRV. It is not expected that a navigation algorithm that has been created through simulation will work in a real world environment without any problems. All programs have their "bugs" to be worked out, especially programs that control a robot autonomously.

However, this simulation will provide BSC with a tool to develop and test programs without having an actual platform, or even test current programs without performing a real world test.

BSC has various ongoing work that is associated with this research. As mentioned earlier in the Validation of Simulation section, students and faculty of BSC are currently re-writing software for the 2006 IGVC robot by using a variation of the simulation created through this research. Also, a student is working on a program to interpret and use the data from the IMU sensor. This sensor was recently purchased and is expected to be used on the upcoming 2006 DARPA Grand Challenge vehicle. Even though time did not permit the implementation of errors into the simulated sensor data, it was evident that such an addition would greatly improve the realism of the simulation. Further research on this topic is need, because all of the sensors in a real world situation are subjected to errors. This could be just one of many changes that could be implemented to improve the simulation.

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**A Study of the Effects of Therapeutic Massage on Individuals' Stress Levels**

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**Abstract**

Massage therapy is increasingly being used in various areas of medicine and for stress relief. Studies have shown that massage therapy can reduce pain and anxiety and can help boost the immune systems of individuals' experiencing stress. The purpose of this study was to examine the effects of therapeutic massage on an individual's stress level. This was investigated by measuring the vital signs blood pressure, pulse, and temperature and a stress level assessment survey to record the perceived level of stress that each participant was experiencing. Measurements were taken pre-massage as well as post-massage and recorded. Various massage modalities were used for each session, but tailored for each participant as needed for the session. Temperature, pulse and blood pressure measurements were observed to decrease significantly post-massage ( $p < 0.001$ ). Perceived stress levels also significantly dropped from a marginally high stress level to a manageable level ( $p < 0.001$ ). These data suggest that massage therapy can help reduce individuals' stress levels.

### Introduction

The effects of massage therapy have been shown to decrease the amount of stress a person experiences and allow them to deal with stressful situations more effectively. Massage therapy is an ancient practice performed before recorded time and found in almost every culture in the world (2). A form of massage therapy is found in many native and folk cultures and it has been traced back to ancient civilizations through pictures and ancient writings. A clay tablet found from Sumer, dating back to 2100 BC, describes rubbing oil with a mixture of herbs on a diseased body part (2). The Chinese practiced massage; some of their ancient techniques still used today are acupuncture and acupressure. The Greeks used massage extensively; Hippocrates wrote about using friction rubbing to help sprains as well as the Greek philosopher Aristotle, who recommended that a person could remedy weariness by rubbing with water and oil (2). The Roman physician Galen wrote a description of massage done in the Greek gymnasia and his written observation preserved the Greek traditions and gave a detailed account of how these massages were performed (2). Because the Romans adopted many elements of the Greek culture, massage is found throughout the Roman Empire. The Romans used the concept of the Greek gymnasia to create their own Roman baths. Massage in these baths consisted of rubbing oil on the skin and using percussive type movements that have a stimulating effect on the body. Two men developed the foundation for the classic western massage performed today in the nineteenth and twentieth century. The first man, Pehr Henrik Ling from Sweden was a poet, playwright, teacher and fencing master who believed that body movement had the ability to protect and even heal (2). In the 1830's, Ling developed four different systems of movement that he called the general principles of gymnastics; these included military, educational, medical and aesthetic movements. Schools taught educational gymnastics and medical gymnastics, which are known as the Swedish movement cure, this combined both passive and active movements to help treat disease. Diseases commonly treated with this type of bodywork were asthma, chest congestion, gastritis, emphysema and other conditions such as epilepsy, paralysis and nerve pain. Ling's major contribution to modern day massage is the idea that both active and passive movements when applied to the skin in a skillful manner can help to restore balance and overall health in



the human body. His exercises of educational gymnastics were the forerunners to calisthenics used today and the Swedish movements that he used are found in the early practices of physical therapy.

The second man who contributed to western massage was Johann Mezger who believed that passive movements had the power to heal. Johann Mezger was a physician from Amsterdam who created four categories of techniques used for soft tissue manipulation (2). He named these categories effleurage, petrissage, friction, and tapotement. These techniques are tools still used by massage therapists today and taught in massage schools all over the world. Massage therapy continued to evolve with new techniques and new forms such as the Swedish massage, which became popular in the 1920's (2).

Today there are many different types of massage; these different types or modalities affect different layers of the body from the muscles to the connective tissue. A modality previously mentioned was Swedish massage. This modality includes gliding movements called effleurage, a lifting of the tissue called petrissage and rubbing one layer of tissue across another, referred to as friction (2). The last movement in the Swedish massage is tapotement, which is percussive and is used to stimulate the tissues. The benefits of Swedish massage are the improvement of the flow of blood and lymphatic fluid, relaxation of the muscles, to improve the movement of joints and can even make the skin healthier (2). Swedish massage is a common massage received in health clubs and spas around the world.

Another massage modality is connective tissue massage; this modality requires an extensive knowledge of anatomy. Elisabeth Dicke created this massage modality, which deals directly with the fascia in the body; it affects all the membranes that surround the muscles and the organs, along with bone (3). This is a more intense and deep massage that affects the body as a whole. The benefits of connective tissue massage are deep relaxation, release of restrictions in the fascia and muscles due to stress or trauma and restoring the integrity of the tissues in the whole body.

Neuromuscular therapy is another massage modality that can be used to help relieve myofascial pain. Stanley Leif originally developed it in 1925 in Hertfordshire England (2). However, Dr. Janet Travell was the pioneer that brought this therapy to the United States. Trigger points are small-constricted areas in muscles that radiate pain to another area of the body when the constriction is compressed with

static pressure. Trigger points occur because of an overload of the muscle or muscle fatigue. Repetitive movement will also cause a trigger point as well as poor posture and carrying too heavy of a load (2). Neuromuscular therapy uses a distinct amount of static pressure to cause the muscles in the area to unwind causing the constriction to release and reduce pain.

Some massage modalities are not applied to the entire body. Reflexology for example is a modality in which, pressure is applied to the feet in certain areas and is reflexive to the organs and parts of the body (2). This type of therapy dates back to 2587-2453 BC as seen on a wall painting found in an Egyptian tomb of two men rubbing each other's feet (2). The modern reflexology we use today originated from Eunice Ingham's work after the end of World War II. Reflexology is based on the ten zones of the body; this can be achieved by drawing an imaginary line through the middle of the body and dividing each half into five equal parts (2). These zones map out the organs and body parts on the feet as if the feet are the mirror image of the body. By applying pressure to a specific part of the foot, it will affect its corresponding organ or body part. Reflexology allows a therapist to give massage to a person for whom a classic western type massage may be contraindicated. These modalities are just a small sampling of some forms of massage, there are many more, however the aforementioned modalities were used interchangeably in this study.

The effects of massage are both physical and emotional. Massage affects the skin, by stimulating the sensory receptors in the skin and increasing oil secretions from the sebaceous glands (2). Massage helps to improve circulation and remove dead skin cells. It affects the connective tissue in the body by improving the pliability of the fascia and by separating the layers of tissue freeing restrictions. In addition, massage affects the circulatory system because it increases circulation enhancing the return of venous blood, and helps to reduce blood pressure and heart rate. Furthermore, it encourages the recycling of metabolic wastes from muscles through the bloodstream, causes relaxation of the muscles, and helps to relieve myofascial trigger points that can cause pain and increase the mobility of the joints and flexibility of the muscles. The parasympathetic response is stimulated by massage, which stimulates the release of endorphins to decrease pain. Massage also enhances the immune system by increasing the flow of

lymphatic fluid and improves immunity by decreasing stress, which has been previously shown to decrease immune function (2). The mental and emotional effects of massage include an increase in mental clarity, a reduction in anxiety and a general feeling of well-being (2). Massage therapy also encourages the mind-body connection and increases an individual's awareness. Although therapeutic massage is considered a non-invasive therapy, for some individuals therapeutic massage is contraindicated. Pathologies that are contraindicated include any condition or situation that could make receiving a massage unwise. General contraindications include endangerment sites at the anterior neck, axillary area, popliteal fossa, elbow, eyes, umbilicus, kidneys, and all major blood vessels (2). Massage is never applied in these areas regardless of an individual's health status. Therapeutic massage is never given when its application would aggravate a condition, could cause bleeding or spread an infection. This includes localized and systemic infections as well as inflammation, abnormal sensation or the use of certain types of medicines (2). A massage therapists' first rule is not to harm the client, in some cases the physician may need to be contacted for individuals with many underlying pathologies. Everyone experiences stress, but the causes of stress are different for each individual.

Stress is an individual's perception of a situation or event that may have been difficult, challenging or threatening (1). Hans Seyle was the first person to be able to categorize the stages of stress. He described these stages by observing patients in the hospital while he was in medical school. Seyle noted that regardless of cause, all of the patients seemed to have the same feelings of anxiousness and exhaustion; furthermore, he also observed that the longer they were in the hospital the more exhausted they became (1). These observations laid the foundation of the stages associated with stress syndrome. Seyle's general adaptation syndrome is composed of four steps, the first is the alarm phase, which is the awareness that a problem or danger exists and the release of stress hormones in the body (1). The second is the resistance phase, which gets the body ready for the "fight or flight" response to take affect and the third stage is the exhaustion phase where the body has no more energy to fight. All of the stages of the stress response can lead to the fourth stage, death. In most cases death does not always mean sudden

death, it can occur by disease that can result in death, for example autoimmune diseases. The stressor is different for everyone but the biological processes, which cause the stress response, are the same no matter what the stressor.

The origin of the stress response is deep within the brain and is caused by a cascade of hormones and the glands that are affected by them. When the body senses a stressor is present, the brain sends a message to the hypothalamus, which causes the release of corticotrophin releasing hormone or (CRH). CRH is quickly sent through the blood stream to the pituitary gland. This gland is responsible for secreting hormones that the body needs for regulation. Found in the anterior lobe of the pituitary gland is a hormone called adrenocorticotrophic hormone (ACTH). ACTH is then released into the blood stream to stimulate the adrenal glands to release stress hormones such as cortisol, from the adrenal cortex and epinephrine and norepinephrine from the adrenal medulla (4). These three substances mediate the stress reaction in the body. Cortisol levels affect sleep, mood, tissue repair and peristalsis, but are also toxic to brain cells. Epinephrine and norepinephrine facilitate the body to have energy to get away from the stressor. They increase the heart rate and blood pressure; epinephrine stimulates the liver to release glucose into the blood so that energy is readily available to the body. Although stress itself is not usually fatal, the response to stress may be.

Long-term stress has been shown to suppress the immune system and compromise the body's ability to heal itself. One disease that can be caused by stress is heart disease, which is the number one killer of men over the age of fifty and women over the age of sixty five (1). Excessive stress can lead to heart disease because of the vasoconstriction of blood vessels caused by the stress response and the overproduction of stress hormones in the body. Over long periods of stress, the body's natural balance can be disrupted; in this situation, stress can change the shape of the body, when glucose levels can no longer be controlled by the body's own insulin the body stores fat around the liver so that energy is readily available (5). As a result, people who are under large amounts of stress tend to accumulate fat around their liver making their abdomens large, even if the rest of their body is small. The strong effect that stress has on the immune system is mediated by the increase cortisol levels that can suppress the immune

system and make the body more vulnerable to illness (5). To counter these effects there are many different ways to help relax the body such as meditation, diaphragmatic breathing, and yoga as well as massage (6).

Although the causes of stress are different for every individual, massage has been shown to reduce stress and improve general health. One area currently being studied deals with the benefits of massage on disease. A study performed at the Sloan-Kettering Cancer Center examined the efficiency of massage therapy on the relief of pain, anxiety, fatigue and depression in cancer patients. The study lasted over a period of three years and 1,290 patients were treated. Of the 1,290 patients treated, approximately 50% of the patients' symptoms were reduced by massage therapy and the outpatients that participated in the study improved 10% greater than did the inpatient (9).

At the University of Miami School of Medicine, a comparison of the effects of both relaxation therapy and massage therapy was conducted to determine if massage alone was an effective cancer treatment. The numbers of circulating natural killer cells and lymphocytes were monitored. Fifty-eight women with early stage breast cancer participated in the study and received massage or relaxation therapy for three thirty-minute sessions per week for five weeks. The results from this study support massage therapy as an effective way to reduce the symptoms and many emotions experienced by cancer patients. Furthermore, the individuals who received massage therapy showed an increase in dopamine and serotonin levels as well as an increase in both circulating natural killer cells and lymphocytes (10).

Many studies have examined the application of massage to alleviate various effects of stress. One such study looked at the immunological effect that massage therapy has on stress cause by academics. In this study, female medical students between the ages of 21 and 25 where given a one-hour full body massage one day prior to a stressful test. Blood samples and vital signs were recorded before and after the sessions, as well as completion of a survey. The results suggested that massage not only reduced anxiety but also increased the blood circulation, as indicated by an increase of circulating white blood cells found following massage (7).

The effect of back massage on the immune system of the spouses of cancer patients was performed at Duquesne University School of Nursing. In this study, a group of 42 people participated and received three 20-minute back massages at three distinct points during their spouses' cancer treatment. Stress levels were measured by monitoring heart rate, mood, blood pressure and their stress. The data support the use of therapeutic back massage in aiding the spouses to cope with taking care of their sick spouse. Moreover, massage enhanced the care-giving spouses' mood and helped to reduce the stress they experienced (11).

Stress can also be caused by a person's everyday responsibility, such as their job. Stress in the work place was also a subject of study in which 52 employees of a company received a chair massage for fifteen minutes and their blood pressure was measured before and after the massage (8). Of the 52 employees, all of them showed a reduction in both systolic and diastolic pressure following the massage. Another study of the same type was performed using on-site chair massage to reduce anxiety and stress in the workplace (12). Eighteen people participated in this study and each of them received a chair massage once a week for six weeks. The results showed there was a decrease in the amount of anxiety that the participants felt following chair massage. The study could have very interesting implications as stress-related disorders cost fortune 500 companies approximately 25 billion dollars or more per year for healthcare. Not only does massage reduce stress in the workplace, but it also appears to increase morale and decrease the number of sick days taken by employees.

The objectives of this study were to examine the affects of massage therapy on an individual's stress levels. This study consisted of seventeen participants whose stress levels were measured using the vital signs of blood pressure, heart rate, and body temperature, as well as the completion of a stress assessment survey, both before and after each massage session. The participants received six individual, integrated massage sessions in order to assess the ability of massage to reduce an individual's stress level.

### **Materials and Methods**

Of the original sample size for the study of 18, 17 individuals completed the study. Of these, six were between the ages of 20-29, five between the ages of 30-39, and six in the age group 40 and above.

Each of the participants had different obligations in their lives, including different types of work such as secretarial work, physical labor and working in health care. Some of the participants were college students taking classes and all of the participants lived in different social settings. The participants were chosen on a first come, first serve basis and accepted as long as there were no contraindications toward them receiving therapeutic massage. Each participant completed a stress assessment survey before receiving a one-hour massage, as well as having his or her blood pressure, heart rate and pulse measured and recorded. After the massage, session was over the clients filled out the same survey that was given before the massage along with recording their vital sign measurements.

The stress assessment survey used to measure the participants' level of stress was a general survey used in stress awareness workshops, however it does not have a formal name, the stress assessment survey used in this study is found in Appendix A. The survey consisted of 20 questions, which were answered on a Likert scale with 1 never, 2 sometimes, 3 frequently and 4 always. Based on the total score, if the score was 20-30, "Chances are [the individuals are...] non-productive and [their...] life lacks stimulation". If the score was 31-50, "[The individuals...] life is in good balance and [they have...] the ability to handle and control stress". If the score was 51-60, "[The individuals...] stress level is marginally high and [they...] are bordering on being excessively tense". If the participants score was 60 or above "[They...] may be a candidate for serious illness, burn out or other major negative effects of stress".

The massage sessions were integrated sessions that were tailored to the needs of each participant, so that each person would receive a massage session that was unique and helpful to them as an individual. Each session was documented with the types of massage given to each participant as well as areas of constriction that were found during the massage session. Feelings and emotions as well as any other important information that may help to create a more purposeful massage session for the client were also recorded.

Each participant received six one-hour massage sessions in a private room on a massage table covered with a sheepskin cover on the table itself as well as a cover on the face cradle for increased comfort. The table was covered with a clean pair of twin size sheets to preserve the clients' modesty and a

blanket for warmth. The participants' were given privacy while undressing and the amount of clothing removed by the participants was determined by them individually. After the participant was comfortably positioned on the table, I returned to begin the session. The individual participant decided the areas that they were experiencing tension or pain in and those areas were addressed accordingly. The lubricant used was almond oil and apricot kernel oil and the participants had a choice of essential oils to add to the base oils, which included lavender, eucalyptus, and grapefruit, clove, jasmine, and ylang-ylang essential oils. Each of the essential oils created a different aromatherapy effect, lavender causes a relaxation effect, grapefruit, clove and eucalyptus have a detoxifying effect and jasmine and ylang-ylang have an anti-depressive effect.

The results were analyzed using the paired t-tests with SPSS statistical software 13.0, 2004 edition. With the use of these statistics, the vital signs of each participant were compared as well as their stress level assessment scores. A mean for each participant's physiological measures as well as their stress assessment scores for all six sessions were calculated and placed in Table 1.1. The graphical results of the statistical analysis are a calculation of the mean measurements among participants pre-massage and post-massage.

### **Results**

The individual measures of each participant's vital signs and stress test scores were averaged for all six sessions (Table 1.1). This table and other graphical data can be found in Appendix A. The individual participants' data were very distinct. Every participant had a decrease, some more significantly than others did, in their stress level, temperature and pulse. All participants, with the exception of one, experienced a decrease in both systolic and diastolic blood pressure. The rise in blood pressure experienced by participant #11 may have been due to an underlying physiological condition. There was also a very sharp drop in some individuals' blood pressure measures, for example participant #15 showed a dramatic drop in both systolic and diastolic blood pressure.

Body temperature is the one vital sign measure that did not decrease as much as other measures. The average drop in body temperature for the participants was approximately 1.5°F. The stress level



scores for all of the participants were variable. The scores ranged from a moderate stress level range or very high stress level range to a lower and healthier range. The mean for all participants stress level score dropped a total of 6 points from pre-massage to post-massage. All participants, regardless of pre-session score, demonstrated a decrease in the stress assessment score post-massage. A paired sample t-test was used to analyze the data.

Figure 1.1 shows the mean blood pressure among participants' pre-massage and post-massage. An overall decrease in systolic blood pressure was observed ( $t = 10.568$ ,  $N = 17$ ,  $p < 0.001$ ). The mean for pre-massage systolic pressure was 129.96 mmHg ( $SD \pm 11.97$ ) and the mean for post-massage systolic pressure was 112.33 mmHg ( $SD \pm 16.26$ ). A decrease in diastolic blood pressure was observed ( $t = 10.963$ ,  $N = 17$ ,  $p < 0.001$ ). The mean for pre-massage diastolic pressure was 83.69 mmHg ( $SD \pm 9.67$ ) and the mean post-massage diastolic pressure was 70.79 mmHg ( $SD \pm 11.69$ ).

Figure 1.2 shows the mean pulse among participants' pre-massage and post-massage. An overall decrease in pulse was observed ( $t = 15.840$ ,  $N = 17$ ,  $p < 0.001$ ). The mean for pre-massage pulse was 73 beats/minute ( $SD \pm 11.80$ ) and the mean for the post-massage pulse was 60 beats/minute ( $SD \pm 7.99$ ).

Figure 1.3 shows the mean temperature among participants' pre-massage and post-massage. A significant decrease in temperature was found ( $t = 7.724$ ,  $N = 17$ ,  $p < 0.001$ ). The mean for pre-massage temperature was 98.6° F ( $SD \pm 0.94$ ) and the mean post-massage temperature was 97.3° F ( $SD \pm 1.72$ ).

Figure 1.4 shows the mean stress level assessment score among participants' pre-massage and post-massage. A significant decrease in stress level was found ( $t = 11.943$ ,  $N = 17$ ,  $p < 0.001$ ). The mean for pre-massage stress test score was 55 ( $SD \pm 6.80$ ) and the mean for post-massage stress test score was 49 ( $SD \pm 5.71$ ). The pretest survey score of 55 indicated a marginally high stress level. Following treatment, the score of 49 suggests the clients perceived their stress levels to be more manageable.

### **Discussion**

Studies have previously shown that therapeutic massage is an effective way of reducing stress caused by many situations that are experienced in an individual's everyday life. Massage can be effective in reducing stress for those people who do not have physiological problems that would make therapeutic

massage a contraindication for them. The results obtained from this study support the use of therapeutic massage in stress reduction, particularly stress in the workplace. In Figure 1.1, the participants' blood pressure shows a decrease in both systolic and diastolic measures post-massage. One participant did not show an overall decrease in both systolic and diastolic blood pressure. It was concluded, with the assistance of the participants' physician that this fluctuation was due to medication being taken for an underlying pathology. A decrease in pulse post-massage was also observed (Figure 1.2) as well as a significant decrease in temperature (Figure 1.3). These vital sign measures were decreased which is consistent with previous studies (7, 8, 12).

A decrease in perceived stress for each participant was also observed using the stress assessment survey (Table 1.1). The stress assessment scores for the participants in this study all showed a decrease in stress level post-massage, also consistent with previous studies (7, 8, 12). Pre-massage stress level scores were marginally high, however these measures decreased to a more manageable level post-massage (Figure 1.4). The individual means of the participants stress assessment scores were variable; some participants showed a decrease in stress level to a greater degree than others did (Table 1.1). Because stress is a person's perception of a situation, this could account for the inconsistent decreases that were observed in the mean measurements. Another factor associated with the variable stress level results could be the time of day the participants' received their sessions and the obligations of each participant on that given day.

The implications for this study were alluded to in the previous studies on stress and the workplace (12). Companies could potentially save billions of dollars per year in health care costs as well as the number of sick days their employees take per year. Overall, these data suggest that massage therapy can help decrease stress, as evidenced by both the physiological and perceived stress methods evaluated in this study. This study could be extended over a longer period to determine if a greater number of sessions would be more effective. Additionally increasing the number of massage sessions to determine if greater effects on the level of stress a person experiences is observed. Another aspect of the study that could be

expanded upon is the emotional aspects of receiving therapeutic massage, which are experienced by the participants as well as the improved mind-body connection these individuals develop.

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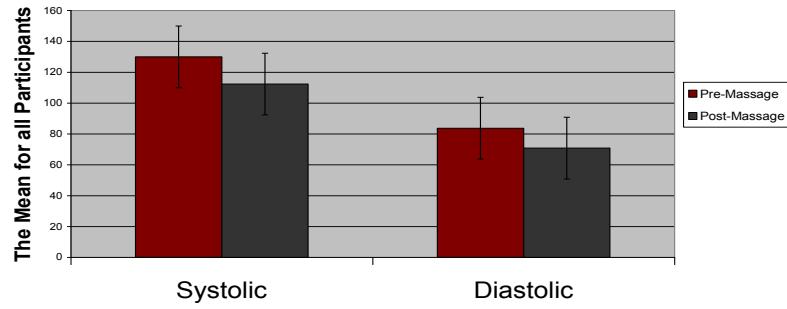
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Appendix A

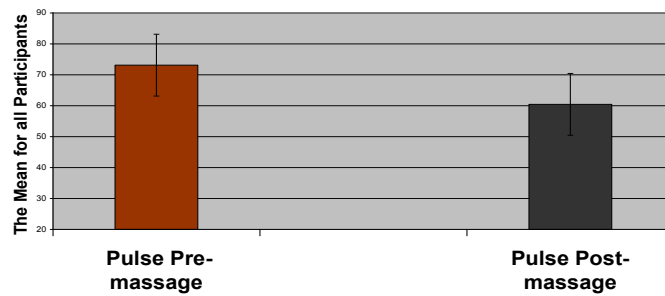
Data Table 1.1

Individual Means for each Participants' Vital Sign and Stress Measures

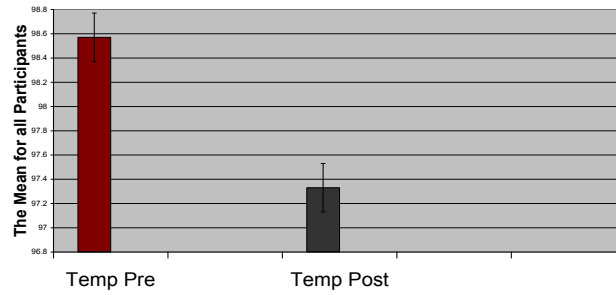
Participant Number	Blood Pressure		Temperature		Pulse		Stress Score	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
#1	131/89	129/86	98.6	98.2	70	61	57	49
#2	141/91	137/88	99.4	97.4	92	70	49	47
#3	131/83	101/67	98.6	97.5	73	63	66	54
#4	129/82	111/68	98.5	96.7	73	61	56	51
#5	134/91	111/73	98.4	97.1	73	61	50	49
#6	122/82	106/68	98.8	98.1	74	62	47	45
#7	139/90	121/76	98.2	96.1	71	61	61	55
#8	139/87	118/76	98.5	98.3	59	43	49	42
#9	122/74	105/65	98.6	97.9	63	60	58	54
#10	124/82	115/71	99.1	98.2	74	59	49	46
#11	129/80	144/84	98.9	98	87	66	54	50
#12	125/83	104/70	98.3	96	77	63	53	45
#13	116/74	100/60	98	97	73	60	44	38
#14	129/76	102/59	99	97.4	62	50	54	47
#15	136/88	97/59	97.6	96.7	72	61	59	48
#16	127/82	106/70	99.1	97.7	79	63	55	48
#17	136/90	105/64	98.1	96.3	72	57	62	52



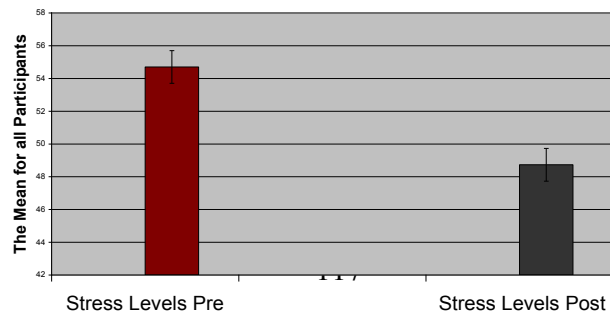
**Figure 1.1** Shows the mean systolic and diastolic blood pressure among participants pre-massage and post-massage in mmHg.



**Figure 1.2** Shows the mean pulse among participants pre-massage and post-massage in beats/minute.



**Figure 1.3** Shows the mean temperature among participants pre-massage and post-massage in °F.



**Figure 1.4** Shows the mean stress assessment score among participants pre-massage and post-massage.

## STRESS ASSESSMENT

Rate each item below on the following scale

- 4 = Always
- 3 = Frequently
- 2 = Sometime
- 1 = Never

- \_\_\_\_\_ 1. Do you try to do as much as possible in the least amount of time?
- \_\_\_\_\_ 2. Do you become impatient with delays or interruptions?
- \_\_\_\_\_ 3. Do you always have to win at games to enjoy yourself?
- \_\_\_\_\_ 4. Do you find yourself speeding up the car to beat the red light?
- \_\_\_\_\_ 5. Are you unlikely to ask for or indicate you need help with a problem?
- \_\_\_\_\_ 6. Do you constantly seek the respect and admiration of others?
- \_\_\_\_\_ 7. Are you overly critical of the way others do their work?
- \_\_\_\_\_ 8. Do you have the habit of looking at your watch or clock often?
- \_\_\_\_\_ 9. Do you constantly strive to better your position and achievements?
- \_\_\_\_\_ 10. Do you spread yourself "too thin" in terms of your time?
- \_\_\_\_\_ 11. Do you have the habit of doing more than one thing at a time?
- \_\_\_\_\_ 12. Do you frequently get angry or irritable?
- \_\_\_\_\_ 13. Do you have little time for hobbies or time by yourself?
- \_\_\_\_\_ 14. Do you have a tendency to talk quickly or hasten conversations?
- \_\_\_\_\_ 15. Do you consider yourself hard-driving?
- \_\_\_\_\_ 16. Do your friends or relatives consider you hard-driving?
- \_\_\_\_\_ 17. Do you have a tendency to get involved in multiple projects?
- \_\_\_\_\_ 18. Do you have a lot of deadlines in your work?
- \_\_\_\_\_ 19. Do you feel vaguely guilty if you relax and do nothing during leisure?
- \_\_\_\_\_ 20. Do you take on too many responsibilities?

\_\_\_\_\_ **TOTAL**

### **Score Result**

20-30 - chances are you are non-productive or your life lacks stimulation.  
31-50 - your life is in good balance and you have the ability to handle and control stress.  
51-60 - your stress level is marginally high and you are bordering on being excessively tense.  
60 and above - you may be a candidate for serious illness, burnout or other major negative effects of stress... (You need to learn to control stress and relax!!!)

**The Role of Contextual Components in the Generalization of Operant Behavior**

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## The Role of Contextual Components in the Generalization of Operant Behavior

Context has been shown to influence behavior in a variety of ways (see Balsam and Tomie, 1985). The current paper examines how individual components of the context may influence the generalization of operant behavior. This study demonstrates how one contextual component from a training context may lead rats to respond similarly to rats placed in the original training context. This apparent generalization in the presence of a single component suggests that some contextual components may have as much control over behavior as the entire training context. This paper also suggests that not all contextual components are able to produce generalization, which means that not all components have the same ability to acquire control over behavior.

The investigation of the role of contextual components in the generalization of operant behavior starts by defining context. The defining of context is followed by a discussion of some roles that context has been shown to play in influencing behavior, and then some approaches that have been used to explain how context is learned about are discussed. Finally, a description of the current experiment which deals with the role of contextual components in the generalization of operant behavior is presented. Context is the sum of internal and external conditions surrounding an organism at any given time (Gordon & Klein, 1994). External context consists of all stimuli that occur outside of the organism. As you read this paper, the sounds, odors, and images surrounding you comprise your current external context. Internal context consists of cues that arise inside the organism. Experiences such as hunger, anxiety, pain, anger, and different drug induced states of consciousness are some examples of internal context. The scope of the current paper will only address external context. More specifically, the static background cues that comprise the training environment will be the main focus and will be considered to be the context of interest. For the current paper, these static background cues (context) will be defined as any cue that persists for the entire duration that the organism is in a learning situation. That is, a discrete cue that has an onset, an offset, and is reliably paired with an event is not considered to be part of the context. For



example, a single tone that is reliably paired with a shock would not be included in this definition of context. However, a tone that is presented every two seconds for the full duration of conditioning, but is not reliably paired with some event would be considered a static background cue or part of the context. Context is defined this way to prevent it from being confused with a conditioned stimulus (CS). Balsam (1985) states that all learning takes place in a context. Students learn to do math in the context of their classroom, dogs may learn to obey commands in the context of a front yard, and rats can learn to press a bar for food in the context of an operant chamber. A better understanding of the role that context plays in generalization could lead to advancements in therapy and substance abuse rehabilitation, among other implications.

Both desirable and undesirable behaviors are learned in a context. Maladjusted behaviors such as aggression, depression, and anxiety can be learned in a specific context. Behaviors that are viewed more positively such as performing above average in a sport or learning good communication skills may also be learned in a context. Researching the role and function of context could lead to a better understanding of how both positive and negative behaviors are acquired, maintained, and generalized. It has been demonstrated numerous times that performance at the time of testing is typically better if testing occurs in the training context rather than a novel context (see Gordon & Klein 1994). A practical application and extension of this idea is suggested by Larson and Lochman (2002) who stress that when working with aggressive school children, it is important to move anger management sessions to different contexts; such as the classroom, playground, and home to help promote the use of the learned anger coping skills across various contexts. Larson and Lochman also suggest that practicing the anger coping skills in these different contexts is essential to the success of a good anger management program since performing the learned anger management skills are not always going to be tested in the training context. If their skills are trained in only one context, they will typically only be demonstrated in that one context.

The context may influence behavior in several ways, but only two are discussed here. One role that context may play in influencing behavior is that of an occasion setter (Holland, 1983). Holland suggests that context may indirectly influence behavior by informing the organism that a previously learned set of rules is in place. For example, when a person enters a church they often lower their voice. Holland (1983) would suggest that the church context in the above example functions as an occasion setter. After a child is exposed to the context of a church in which being quiet is reinforced, the child learns that the rule of being quiet in that context results in reinforcement. Once the rules of the church context have been learned, the child will be quiet upon entering that particular context.

A second role that context may play in influencing behavior is that of a CS. That is, the context may directly influence the behavior of an organism by producing a conditioned response, much like a CS would. For example, if a rat was placed in a black conditioning chamber and shocked, the next time the rat was placed in the black box it may exhibit the conditioned response (CR) of defensive freezing. In the absence of a discrete CS, the black box may become a reliable predictor of the US and thus come to act like a CS. Klein and Gordon (1993) demonstrated that context could directly influence behavior like a CS. In one of their experiments, rats were shocked in a black box containing an anise odor. The rats were then given a context-preference test. The tests revealed that the rats avoided the components comprising the original training context, the black chamber, and the anise odor. This experiment demonstrated not only that the context can function as a CS, but also that individual components of a context can influence behavior. Rescorla and Wagner (1972) suggest that the context and CS combine to make a compound CS. If a test is comprised of the same CS as used in training, but a context different from that used in training is used, then a decrease in the CR may occur. Some phobias can be explained using this paradigm. For example, an individual is robbed at gunpoint in a parking lot. If the victim later saw the robber (CS) and was able to recognize him or her, the victim may become fearful (CR).

Likewise, if the victim returned to the parking lot where the robbery occurred (context) the victim may become fearful (CR). These are just a few examples of how the context can function as a CS.

In order to understand the role of context on behavior it is useful to understand the three approaches that have emerged to explain how organisms learn about the context. One approach is the elemental view. It should be noted that there is not a single elemental theory that is universally agreed upon. Stimulus sampling theory (Atkinson & Estes, 1963) and the Rescorla-Wagner model (Rescorla & Wagner, 1972) are two prominent elemental theories. These theories suggest that when learning takes place, each component, or element, of the context is able to individually enter into an association with the learning contingency. For example, if learning occurred in a context comprised of a black visual cue and a vanilla olfactory cue, the vanilla cue could produce a learned response on a test even if the other element is not present at the time of testing. The strength of that response, according to an elemental approach, may depend on the number of contextual components from the training context present at the time of testing, among other factors. However, elemental theories have been criticized as being too simplistic, not able to fully explain generalization or retroactive interference, and as having a poor ability to predict outcomes of conditioning (Pearce, 1994).

Another approach to explain how organisms learn about the context is a configural approach. Much like the elemental approach, there is no universally accepted configural theory. One of the most current and comprehensive configural theories is proposed by Pearce (1994). This theory suggests that contextual learning does not consist of an association being formed between each individual element of a context, but that the entire context of a learning situation enters into an association as a unitary, or configural, representation of that context. The configural representation is a novel and unique cue that is different from the individual components that comprise the context. In configural theory, the extent to which a new context can produce a response depends on how closely that context matches an existing configural representation. The more similar a new context is to a preexisting configural representation the

more likely the organism will behave as if it were in the original context. However, configural approaches have difficulty explaining nonsymmetrical generalization of behavior when components of a context are added or removed. Gonzalez, Quinn, and Fanselow (2003) found that rats demonstrated contextual generalization when contextual components were added, but not when they were removed. However, configural approaches would predict that generalization would not occur in either test situation because the addition or removal of a component would be considered a change in the unique configural representation.

Though elemental and configural approaches are often presented as being conflicting views, some researchers have developed theories that incorporate both approaches. Fanselow (1999) proposes that animals have two independent processing mechanisms in the brain, one that is responsible for elemental information and one that is responsible for configural information. He suggests that some tasks are more appropriately solved using configural process while others are better solved using elemental processing. Wagner and Brandon (2001) suggest a replaced-elements model as an integration of the Rescorla-Wagner model (Rescorla & Wagner, 1972) and Pearce's configural approach (Pearce, 1994). The compromise between elemental and configural approaches proposed by the replaced-elements model can explain findings that are problematic for the two approaches alone and can help better explain contextual generalization.

A conditioned response can also occur in a context similar to that of the original conditioning context. This is referred to as contextual generalization, which is the tendency of an organism to respond in the same way to stimuli that are similar to those that have already been learned. Recall the occasion setter example from above that explained the contextual control that the church had over the child's behavior. If the same child were to enter a different church and behave the same way as he did in the original church where he learned to be quiet, then generalization would be said to have occurred. If generalization did not occur, then organisms would have to learn about every instance regardless of how

similar it was to previous learning situations. It is likely that most contexts that an organism experiences are similar, in some way, to a context that they have learned about previously. Thus, generalization plays an important role in the adaptation of behavior.

As stated earlier, the application of knowledge obtained about context may be able to help further advance the effectiveness of treatments and interventions for a variety of behavioral problems. In a recent study, Gonzalez et al. (2003) found that generalization to a context could occur if one or two components were added to the training context, but if the same component was removed from the training context the subjects did not generalize. Gonzalez et al. (2003) conducted two experiments to investigate how contextual manipulation affected generalization. In Experiment 1, 36 rat subjects were conditioned using a single, un signaled foot shock. The conditioning context consisted of a clear Plexiglas conditioning chamber, a room fan used for background noise, and one of three additional components; a flashing light, an odor, or a tone. The three additional components were counterbalanced across subjects. During testing the rats were divided into three groups: (1) tested in the same context as trained in, (2) tested in the same context as trained in plus an additional contextual component, or (3) tested in the same context as trained in plus two additional components. The additional components were also counterbalanced across groups. The freezing behaviors of the subjects were observed as a measurement of conditioning. The results from a mixed two-way ANOVA suggested that there were no differences in freezing behavior between the groups. Gonzalez et al. (2003) interpreted these findings as suggesting that generalization could still occur even when two components are added to a training context.

Experiment 2 was similar to Experiment 1 except that the training context was comprised of two additional components while the training context in Experiment 1 used only one additional component. For the test, three groups were again used: (1) tested in the same context as trained in, (2) tested in the same context but with one contextual component removed, (3) tested in the same context as trained in plus an additional contextual component. Freezing behavior was again measured and the results from a

mixed two-way ANOVA again found that adding a component resulted in no loss of responding, demonstrating that the subjects generalized when a contextual component was added to the original context. However, the results also suggested that removing a component decreased the freezing response, suggesting that the subjects did not generalize when a component was removed. Gonzalez et al. (2003) state that their findings have important implications in the field of behavior therapy. They suggest that if a therapist wants to alter contexts between sessions to increase the generalization of skills learned in the therapy room, then removing components from that context may influence the client to treat the therapy setting as a novel context. Conducting therapy sessions in this novel context may help to show the client that they can use the skills learned in therapy in different settings; which may help the client achieve greater generalization outside the presence of the therapeutic context. However, Gonzalez et al. (2003) do not discuss what the results of substituting components of a reinforced and a nonreinforced context might be. In such a procedure, one reinforced component could be substituted in the place of a removed nonreinforced component. For example, rats would be trained to press a bar in Context A, which is comprised of olfactory, visual, and auditory components, but are not reinforced for bar pressing in Context B, which is comprised of a variation of the same three components. When all other variables are held constant, how might the rats respond if the visual component from Context B were substituted for the visual component in Context A, or vice versa? It is possible that one component of the context may be disproportionately responsible for conditioning, or the strength of a response at the time of testing may depend on the number of components from the training context that are present at the time of testing. A pilot study in our lab suggests that an olfactory component may have a stronger ability to influence behavior in rats than visual or auditory components. In this pilot study, presenting an excitatory olfactory component with inhibitory visual and auditory components on a nonreinforced test resulted in only a 15% decrease in bar pressing when compared to a context comprised of components all trained to be excitatory.

It is important to investigate the individual components that comprise a context because their role in learning is not fully understood. The remainder of this paper describes an experiment that examined some possible roles of visual, olfactory, and auditory contextual components in the generalization of operant behavior. One aspect that the experiment examined was the strength that individual components may have in moderating behavior. The experiment suggests that not all components have the same influence and that rats learn primarily about olfactory and auditory components of the context. These two components can influence responding when presented in the absence of the other contextual components.

The current experiment trained rats to press a bar in an operant chamber for food in one context and trained them to withhold the bar pressing response in a different context using a discrimination procedure. The two contexts were made distinguishably different from one another by having different visual, auditory, and olfactory components. After the training period, the rats were randomly assigned to one of five test groups and the number of bar presses was recorded for each subject during two different tests. Two of the test conditions were comprised of either all three excitatory or all three inhibitory components. The other three test conditions had one excitatory component substituted for an inhibitory component. The other two contextual components of the inhibitory context remained the same. For example, in one group the excitatory visual cue was substituted for the inhibitory visual cue. In this example, the visual cue was the only excitatory component in the test context. The same was done for the olfactory and auditory components. The number of bar presses made by each subject was recorded and a suppression ratio was calculated and used as a measure of generalization. The hypothesis was that the substitution of the three different excitatory components; olfactory, visual, and auditory into the inhibitory context would result in different rates of responding at the time of testing. These differential rates of responding will be interpreted in regards to contextual stimulus generalization. The two groups that have all three excitatory or all three inhibitory components present at the time of testing are expected to

behavior as they would during training. That is, the excitatory group would be expected to respond more than the inhibitory group.

## Methods

### Subjects

The subjects were 25 female, Sprague-Dawley strain rats. The subjects were maintained at 85% of their normal body weight with water available ad lib throughout the experiment. The subjects were tested in two successive cohorts. The first cohort (Cohort 1) consisted of 15 subjects, which were approximately 65 days old at the start of the experiment. The second cohort (Cohort 2) consisted of 10 subjects, which were approximately 82 days old at the start of the experiment.

### Materials

The operant conditioning chambers were two identical operant chambers made by the Lafayette Instruments Company, model number 84022SS. The operant chambers measured 11 inches long, 8.5 inches wide, 8 inches deep, and had 18 stainless steel bars that comprised the floor. As reinforcers, 45 mg Noyes Precision Pellets made by Research Diets Incorporated were dispensed into the operant conditioning chambers by pellet dispensers made by the Lafayette Instruments Company, model number 80200. An operant conditioning console made by Lafayette Instruments Company, model number 81335, was used to generate reinforcement schedules and count responses.

Two contexts were used, Context White and Context Black. Context White was comprised of an operant conditioning chamber with Plexiglas inserts painted white and placed inside the operant conditioning chamber to cover three walls (all walls except the one the lever and food dish were on) to act as the visual component, one cotton ball saturated with anise extract was placed in the tray positioned under the floor of the operant chamber to act as the olfactory component, and a clicker from a multiple stimulus panel made by Foringer, model number 1166-4-M1 and a noncommercial clicker were used as the auditory component. The clickers were 63 dB, as measured from inside the operant chamber.



Context Black was comprised of an operant conditioning chamber with black Plexiglas inserts used as the visual component, lemon extract as the olfactory component, and a compact disc player was used to play a pure white noise CD at 63 dB, as measured from inside the operant box, for the auditory component. The operant chambers were housed in separate rooms. The subjects were transported from their home cages to the operant conditioning chambers in the hands of the experimenters.

### Procedure

Five days prior to the start of the experiment, all subjects were placed on food diets. Two days before the start of the experiment the rats were at 85% of their normal body weights and maintained there for the duration of the experiment. On each of the two days prior to training, all subjects were given 10, 45 mg food pellets. All subjects were trained in both of two contexts, Context Black and Context White. Context Black was trained as the excitatory context and Context White was trained as the inhibitory context for all subjects. That is, operant lever pressing was reinforced with food pellets in Context Black, but there was no food pellet reinforcer delivered for lever pressing in Context White.

The experiment consisted of a shaping phase, a training phase, and a test phase. The shaping phase consisted of one exposure to both Context White and Context Black for each subject. Each subject was placed into Context Black, which had 3, 45 mg food pellets in the food dish, and they were allowed to remain there until 50 lever press responses were made on a continuous, or fixed-ratio 1, schedule of reinforcement. After each subject made 50 responses in Context Black they were placed back into their home cage until Context White was available, at which time they entered that context. Subjects spent the same amount of time in Context White as they had in Context Black, but there was no food available and no mandatory number of responses.

The training phase consisted of a discrimination procedure where each subject was placed into each training context once a day for five minutes. Again reinforcement was available in Context Black, but not in Context White. During the training phase, the subjects were split into two groups. Cohort 1

had a group of 7 and a group of 8, Cohort 2 had two groups of 5. The training groups were placed into the two training contexts in alternating order. For example, a subject in Group A was placed in Context Black first on Day 1 of training and placed in Context White first on Day 2, while the opposite occurred for a subject in Group B. Once all subjects had finished their training session in the first training context, the training groups switched training contexts. This was done to prevent the subjects from learning to respond only in the first or second context they were placed in. The number of lever press responses each subject made in each context was recorded after they were removed from that context. The operant chambers were cleaned after each subject was removed.

The training phase for Cohort 1 lasted 17 days. The training phase for Cohort 2 lasted 21 days for most of the subjects. This phase was longer because some subjects did not clearly demonstrate discrimination. Three subjects from Cohort 2 did not demonstrate a clear discrimination after 21 days of training. These three subjects were given four extra training sessions on a variable-ratio 10 schedule of reinforcement. On Cohort 1's sixth day of training, the Foringer multiple stimulus panel failed to operate correctly and training did not occur. Training resumed the next day with the session scheduled for Day 6. The broken Foringer clicker was replaced with an alternate, but similar clicker. The schedules of reinforcement for the training sessions varied as shown in Table 1.

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The subjects were given two tests. Test 1 occurred approximately 24 hours after last training session for all subjects. Test 2 took place 48 hours after Test 1 for all subjects. The subjects were randomly assigned to one of the five test groups: Group Excitatory, Group Inhibitory, Group Olfactory, Group Visual, or Group Auditory. Group Excitatory was tested with all three components from Context Black and Group Inhibitory was tested with all three components from Context White. Group Olfactory, however, was tested in a context comprised of a lemon odor from Context Black and the clicker and white

wall inserts from Context White. Group Visual was tested in a context comprised of the black wall inserts from Context Black and the anise odor and clicker from Context White. Group Auditory was tested in a context comprised of the white-noise auditory component from Context Black and the anise odor and white wall inserts from Context White.

Test 1 consisted of exposing each subject to the appropriate test condition for five minutes and recording the number of bar press responses made during the session. No reinforcement for lever pressing was available during the test. All groups in Test 1 were tested in the room that housed Context White, the inhibitory context, during the training phase.

Test 2 occurred approximately 48 hours after Test 1. Test 2 was methodologically identical to Test 1 except that Test 2 took place in the room that housed Context Black, the excitatory context, during the training phase. For example, Test 2 for Group Inhibitory consisted of the white wall inserts, the anise odor, and the clicker being placed in the room that housed Context Black during training.

## Results

Results were obtained by first calculating the suppression ratio ( $A/(B+A)$ ) for each subject, where A equals the number of responses on the test and B equals the mean number of responses in the appropriate room over the last four sessions of training. That is, for Test 1, B equaled the mean number of responses made during the last 4 sessions in Context White and for Test 2, B equaled the mean number of responses made during the last 4 sessions in Context Black. A suppression ratio of 0 would suggest full suppression, that is, a complete withholding of responding. A suppression ratio of .5 would suggest no suppression, or no change in responding, and a suppression ratio  $>.5$  would suggest an increased rate of responding.

The mean suppression ratio for each group was calculated, the mean suppression ratio in Test 1 for Group Excitatory was .4529, for Group Olfactory was .5611, for Group Visual was .5475, for Group Auditory was .5791, and for Group Inhibitory was .5291.

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A One-Way ANOVA was conducted on the data from Test 1 and indicated that there was no significant difference between the groups,  $F(4) = .211$ ,  $p = .930$ .

The mean suppression ratios in Test 2 were .3786 for Group Excitatory, .3727 for Group Olfactory, .3480 for Group Visual, .2162 for Group Auditory, and .1928 for Group Inhibitory.

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Insert Figure 2 about here  
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A significant difference was found between the groups on Test 2,  $F(4) = 6.582$ ,  $p = .002$ . Planned contrasts were conducted to determine which groups differed from one another. These contrasts indicated that Group Excitatory, Group Olfactory, and Group Visual did not differ from each other, all  $t(20) \leq .635$ , all  $p$ 's  $\geq .532$ , and Group Auditory and Group Inhibitory did not differ from each other,  $t(20) = .471$ ,  $p = .643$ . However, Group Excitatory, Group Olfactory, and Group Visual each differed from both Group Auditory and Group Inhibitory, all  $t(20) \geq 2.656$ , all  $p$ 's  $\leq .015$ . To view all planned contrasts see Table 2.

### Discussion

The hypothesis that the substitution of the three different excitatory components into the inhibitory context would result in different amounts of responding was not supported according to Test 1, as no differences in responding between groups was found. However, a differential influence of contextual components on operant responding was found in Test 2. In Test 2, Group Excitatory did not respond significantly different from Group Olfactory or Group Visual. However, Group Auditory responded similar to Group Inhibitory, but both Groups Auditory and Inhibitory responded significantly less than the subjects in the other three groups. The results from the experiment suggest that, (1) individual contextual components may have an unequal influence on behavior; (2) inhibitory contextual

components may have more of an influence on behavior than do excitatory contextual components; and (3) that stimuli other than the contextual components that were manipulated in the experiment may have influenced how the subjects responded during testing. However, these three findings are not fully explainable and are both supported and limited by the data from the current experiment.

The data from Test 2 suggest that an olfactory or visual contextual component may have more of an influence on behavior than does an auditory contextual component. These findings are supported by the data that suggests Groups Excitatory, Olfactory, and Visual did not respond differently and the data that suggests that Group Inhibitory and Group Auditory responded in a similar way, but less than the other three groups. The symmetry in responding between Groups Excitatory, Olfactory, and Visual suggests that the presence of either an olfactory or visual contextual component can influence a rat to generalize a learned lever pressing behavior. The data from Test 2 also suggest that an auditory contextual component does not appear to lead the subjects to generalize, as is evident from the similarity in responding between Groups Inhibitory and Auditory, which was less than the responding from the other three groups. Thus, it appears not all contextual components have the same influence on behavior. However, differential influence of contextual components was not found in Test 1. This makes it difficult to definitively say that some contextual components have more influence on behavior than do others.

The data from the current experiment also suggest that inhibitory contextual components may have a stronger influence on behavior than do excitatory contextual components. In Test 1, Groups Olfactory, Visual, and Auditory all responded similarly to Group Inhibitory and in Test 2, Group Auditory responded similarly to Group Inhibitory and significantly less than the other three groups. In each of these cases, responding was not influenced by having one excitatory component present during testing, which suggests that the inhibitory contextual components may have more of an influence on responding than excitatory contextual components. However, other data from the current experiment are not in agreement with the notion that inhibitory contextual components have more influence on behavior

than excitatory components. In Test 1, Group Excitatory responded similarly to the other four groups despite no inhibitory components being present at the time of testing, and in Test 2, Groups Olfactory and Visual responded more than Groups Auditory and Inhibitory unlike in Test 1.

A third observation that could be made from the current experiment is that some stimuli other than the contextual components that were manipulated are having an influence on responding. This observation is supported by the responding of Groups Excitatory, Olfactory, and Visual in Test 1 versus Test 2. In Test 1 these three groups all responded similarly to Groups Inhibitory and Auditory, but in Test 2 these three groups responded more than Groups Inhibitory and Auditory. Since the room was the only variable that was different between tests, it is possible that the room itself was having some influence on responding. Also, in Test 1, Group Excitatory responded similarly to Group Inhibitory although all three excitatory components were present. This similarity in responding may indicate that in Test 1 the contextual components had no influence on responding. However, since Groups Inhibitory and Auditory responded differently from the other three groups in Test 2, it cannot be definitively stated that the non-manipulated stimuli in the rooms during testing had an influence on the subjects' responding.

The current experiment had a few methodological limits. One limit was that not all possible contextual components within the conditioning chamber were manipulated. The current experiment manipulated only the auditory, visual, and olfactory components of the context. These components were selected because together they appeal to three sensory modalities that humans commonly use to learn about the context. Limiting the number of contextual components manipulated also reduced the number of required subjects. The possible influence of other contextual components is unknown. However, this is a problem in all experiments, so it is not considered to be a weakness of the current study.

Second, the order of Test 1 and 2 were not counterbalanced. Because the order of the Tests were not counterbalanced it is not possible to know if Test 1 or the 48 hour interval between tests had an influence on the way the subjects responded in Test 2. However, since all subjects were treated the same,

it would be expected that any influence the 48 hour interval or the absence of counterbalancing had on of the tests would be the same for all subjects.

Finally, the components manipulated in the experiment were assumed to be equal. That is, lemon and anise odors, black and white inserts, and the clicker and white noise were thought to have equal salience and therefore equal influence on behavior. Since Context Black was the excitatory training context for all subjects and Context White was the inhibitory training context for all subjects it cannot be known if the components differed in behavioral influence due to the learning contingency they were paired with or their saliency.

Since the three claims above are not able to fully explain the pattern of results found in the current experiment, replication would be useful in clarifying the data. In future research on this topic is would be useful to control the variables limiting the current experiment by counterbalancing the order of the tests and counterbalancing the contextual components across groups so as to eliminate the limitations listed above. It would also be useful to test the three claims used to explain the results of the current experiment. The claim that the non-manipulated static background cues in the testing rooms may have had an influence on the subjects' operant responding could be tested by using the same training procedure used in the current experiment, but testing the subjects in a third (novel) room that is noticeably different from the two rooms used in training. Testing in a third room should remove any influence that the two rooms training took place in may have had, thus revealing the influence of the manipulated components. This would also allow for the testing of the claims that inhibitory contextual components have more influence on behavior than excitatory components and the claim that visual and olfactory components have more influence on behavior than an auditory component.

In an experiment methodologically similar to the current experiment, except that testing would take place in a third room, it would be expected that Group Excitatory would respond significantly more than the other four groups. The other four groups would be predicted to respond in a similar manner and

less than Group Excitatory. This is predicted because the other four groups would have at least an inhibitory visual or olfactory component. According to the findings from the current experiment, the presence of either of these components would result in the subjects responding in an inhibitory manner. That is, inhibitory contextual components were shown to have more of an influence on behavior than excitatory components and the presence of a visual or olfactory component has as much influence on behavior as all three components combined. Thus the presence of an inhibitory olfactory or visual component would result in the subjects responding as if they were in the inhibitory context.

The findings from the current experiment could have practical applications in treating individuals with substance abuse problems. Perhaps it is simplest to think of the proposed applications in a way that is analogous with the current experiment. In this analogy, lever pressing would be analogous to substance abuse, all excitatory contextual cues would be analogous to the context that the substance abuse took place in, and the inhibitory contextual cues would be analogous to a substance abuse rehabilitation center. According to the findings of the current experiment, if the goal was to decrease the use of a given substance it could be advantageous to replace some of the contextual components in the substance-taking context with contextual components from the rehabilitation center. For example, if a man routinely abused cocaine in his bedroom, it would be expected that when he returned to his bedroom after leaving the rehabilitation center his bedroom would still have an excitatory influence on his cocaine abusing behaviors. However, it could be possible to decrease these cocaine-abusing behaviors by changing the physical make-up (context) of the man's bedroom to resemble his bedroom at the rehabilitation center (assuming he did not use cocaine in that room). It is not suggested that the man renovate his bedroom to be identical to his room in the rehabilitation center, but some of the contextual components, such as the odor, colors of the walls, rugs, and bed sheet, could be manipulated to make his bedroom at home similar to his bedroom at the rehabilitation center. It is hypothesized that the proposed technique could be helpful despite the influence of the house itself. In the current experiment, the data suggested that placing



inhibitory components in the room that housed the excitatory context resulted in a decrease of the target behavior. Therefore, according to the findings of the current experiment it would be expected that the same thing happen in the substance abuse analogy, where the testing room is analogous to the house. The proposed technique would be intended merely to assist people recovering from substance abuse in the readjustment period that takes place just after they return from treatment. The proposed technique would not be expected to be the only component of a post treatment plan, but simply a tool within that plan.

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Table 1

Training Phase

Cohort 1		Cohort 2	
<u>Day of Training</u> <u>Schedule</u>	<u>Reinforcement</u>	<u>Day of Training</u> <u>Schedule</u>	<u>Reinforcement</u>
1	FR 1*	1	FR 1*
2	FR 1*	2	FR 1*
3	FR 5	3	FR 5
4	FR 5	4	FR 5
5	VR 3	5	VR 3
6	Clicker Failed	6	VR 3
7	VR 3	7	VR 5
8	VR 5	8	VR 5
9	VR 5	9	VR 7
10	VR 7	10	VR 7
11	VR 7	11	VR 10
12	VR 10	12	VR 10
13	VR 10	13	VR 10
14	VR 10	14	VR 10
15	VR 10	15	VR 10
16	VR 10	16	VR 10
17	VR 10	17	VR 10
		18	VR 10
		19	VR 10
		20	VR 10
		21	VR 10

\* Three 45 mg food pellets present in food tray at start of session.

Fixed ratio = FR  
Variable ratio = VR

Table 2

<u>Contrast</u>	<u>degrees of freedom</u>	<u>t-value</u>	<u>p-value</u>
Group Excitatory and Group Inhibitory	20	3.754	.001
Group Excitatory and Group Auditory	20	3.291	.004
Group Excitatory and Group Visual	20	.635	.532
Group Excitatory and Group Olfactory	20	.136	.893
Group Olfactory and Group Inhibitory	20	3.626	.002
Group Visual and Group Inhibitory	20	3.127	.005
Group Auditory and Group Inhibitory	20	.471	.643
Group Olfactory and Group Visual	20	.499	.623
Group Olfactory and Group Auditory	20	3.155	.005
Group Visual and Group Auditory	20	2.656	.015

Figure 1

### Test 1 Means

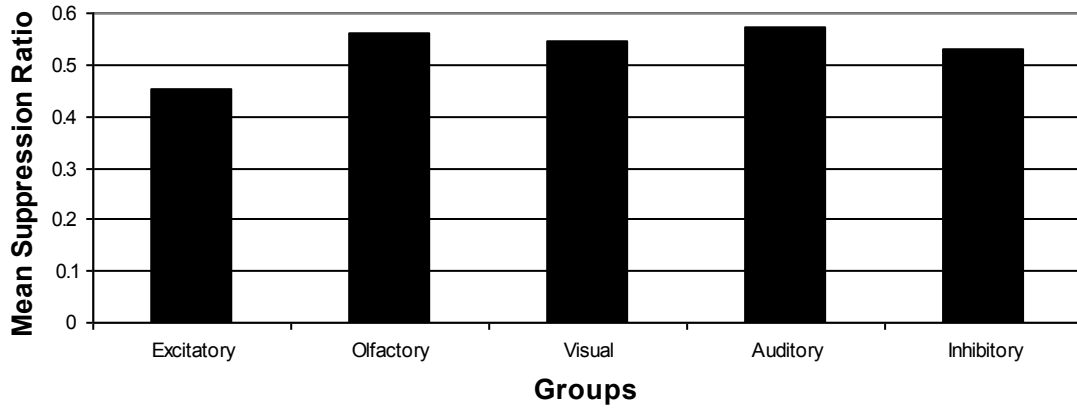
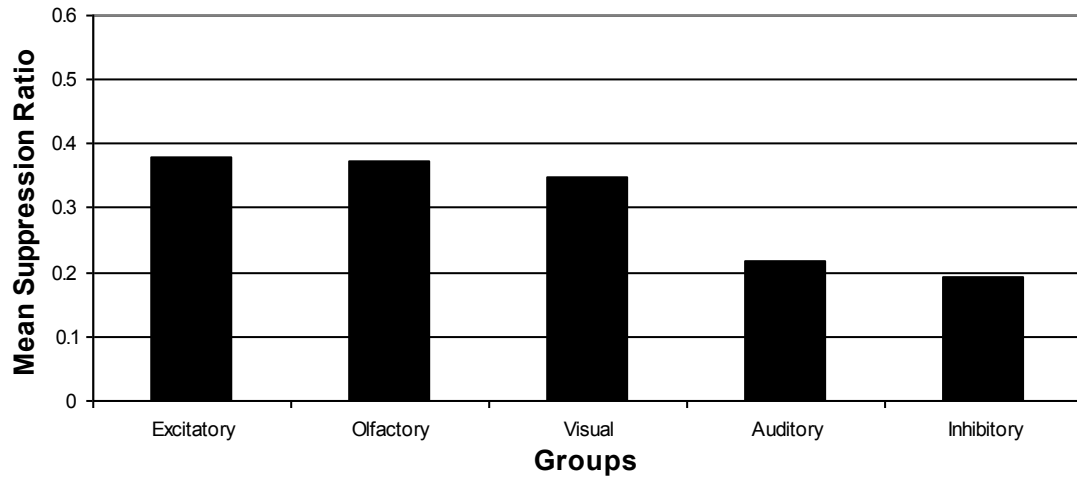


Figure 2

### Test 2 Means



## **Genetic Variation of Wood Cockroaches in Southern West Virginia**

Samantha Lane

Mentors: Dr. Darla Wise, Associate Professor of Biology

Dr. Roger Sheppard, Professor of Biology

### **ABSTRACT**

Genetic variation in wood cockroaches was examined between two different regions of the southern West Virginia area: Pipestem State Park and Concord University. Sixty-nine roaches were collected from six sites that differed in elevation and environment. The goal of the experiment was to determine if more than one species, *C. punctulatus*, is found in southern West Virginia. DNA was extracted from these wood cockroaches using a Qiagen DNeasy Kit (Qiagen Laboratories, CA) and subjected to polymerase chain reaction (PCR) amplification of the 16S and 12S ribosomal RNA genes. Preliminary data suggest that the predominant species is *C. punctulatus*. However, more roaches need to be examined before concluding that only one species occurs in the southern West Virginia region.

## INTRODUCTION

Wood cockroaches, *Cryptocercus* spp. from the family Cryptocercidae, are a highly investigated organism because of the number of newly identified species. *Cryptocercus* spp. are wingless, reddish brown colored cockroaches (Gordon 1996, Borror et al. 1976). They are about 23-29 millimeters in length and have a speckled back with many small pits in their sides (Gordon 1996). They act like termites by feeding on rotting wood and they possess symbiotic microbes that break down the cellulose in the wood so it is digestible. Furthermore, they are monogamous, meaning they stay with the same mate for many years and they display parental care. Additionally, they have only a single set of offspring that they remain with for their entire life span; it is then that the offspring are left to fend for themselves (Gordon 1996).

There appears to be nine species of *Cryptocercus* spp. worldwide, five of which are found in the United States (Park et al. 2002). Of these species, *Cryptocercus matielei* and *Cryptocercus primarius* are primarily found in China and *Cryptocercus kyebangiensis* and *Cryptocercus relictus* are primarily found in eastern Eurasia (Aldrich et al. 2004b). Out of the five species in the United States only one, *Cryptocercus clevelandi*, is found in the Pacific Northwest, more specifically in Northern California and Oregon (Houssain and Kambhampati 2001). The four remaining species, *Cryptocercus punctulatus*, *Cryptocercus garciai*, *Cryptocercus darwini*, and *Cryptocercus wrighti* are found in the Appalachian Mountain region of the eastern United States (Aldrich et al. 2004b, Houssain and Kambhampati 2001). The four different species in the Appalachian region have just recently been distinguished and they show a great deal of variation in their DNA sequence, morphology, habitat, and divergence.

The first observation that suggested there are four different species in the Appalachian region of the eastern United States was variation chromosome number and in DNA sequence (Steinmiller et al. 2001). This hypothesis of four karyotypes was initiated by Burnside et al. (1999) and Kambhampati et al. (1996). The studies done by Kambhampati et al. (1996) suggested that there was an evolutionary decrease

in the number of chromosomes, yet these results slightly varied from the results of Burnside et al. (1999). Burnside et al. (1999) found that the species with thirty-seven chromosomes were paraphyletic and species with forty-five chromosomes were basal. Burnside et al. (1999) used the variation in the mitochondrial rRNA genes and chromosome number to differentiate between the species (Aldrich et al. 2004a). Kambhampati and Houssain confirmed the studies of Burnside et al. by determining four monophyletic lineages in the Appalachian region, through the analysis of the DNA sequence of the nuclear rRNA genes (Aldrich et al. 2004b).

In 1970, Cohen and Roth found male cockroaches with a chromosomal number of 40 in Highlands, North Carolina (Nalepa et al. 2002). An analysis of the species done by Lucyx (1983) found males with 37 chromosomes in Highlands, North Carolina and also found males with 43 chromosomes at Mountain Lake, Virginia. Kambhampati (1996) found a fourth male chromosome number of 45 in the Asheville, North Carolina area. Therefore, the male chromosome numbers of the eastern species ranged from 37-45 (Steinmiller et al. 2001). The DNA sequence showed a divergence of 2.1-9.5% in the mitochondrial 12S ribosomal RNA and 16S ribosomal RNA genes.

These studies on DNA variation led to studies on the possible evolution of *Cryptocercus* spp. to estimate the time since divergence into four different species (Aldrich et al. 2004a). Based on this analysis, it has been estimated that *Cryptocercus punctulatus* and *Cryptocercus wrighti* diverged approximately 23-38 million years ago and *Cryptocercus garciai* and *Cryptocercus darwini* diverged approximately 13-20 million years ago (Aldrich et al. 2004a). An initial 12S rRNA and 16S rRNA phylogenetic analysis of the species in the eastern United States, showed an evolutionary decrease in chromosomal number (Nalepa et al. 2002). Nalepa et al. (2002) claim that the change in chromosomal number is due to Robertsonian changes, where fusion or fission of chromosomes occurs thereby increasing or decreasing chromosomal number. This information along with that of Kambhampati et al. (1996) suggested that the 37-chromosome karyotype was paraphyletic, meaning that it was composed of some



but not all members descending from a common ancestor, since it was the lowest chromosomal number found in the different species (Nalepa et al. 2002). Burnside et al (1999) concluded that the 45-chromosome karyotype, *Cryptocercus wrighti*, was basal in the eastern species because it was the highest chromosome number (Nalepa et al. 2002). However, other studies done by Kambhampati et. al. (1996) concluded that the 43-chromosome karyotype, *Cryptocercus punctulatus* was the basal lineage (Houssain and Kambhampati 2001). Thus, it is unclear which karyotype is basal and to which karyotype the sequence divergence estimates refer, but it is evident that there is a difference in the species (Nalepa et al. 2002).

The results of DNA sequence analysis and variation of chromosome numbers displaying different species is controversial (Nalepa et al. 2002). Nalepa et al. (2002) argues that the separation into species was premature and requires further evidence for a number of reasons. These include the fact that chromosome numbers are only known for part of the sample, the evolutionary relationships are uncertain, reproductive compatibility has yet to be investigated, and the morphological variation that has yet to be correlated with the karyotype results.

Another question is to address whether or not different species live in different habitats (Aldrich et al. 2004a). As previously mentioned, *Cryptocercus* spp. depend on cool, moist rotting wood for food and shelter during all life stages in mesic, mature forest (Nalepa et al. 2002). It was shown that many of the *Cryptocercus* spp. occupy primarily deciduous forests, but have been collected from both hardwood and coniferous trees (Nalepa 2003). Moreover, they were also found in regions at either extremes of altitudinal gradients, those in disturbed areas undergoing succession, or those with site-specific requirements like ridge lines and river bottoms. There were a large quantity of *Cryptocercus* spp. at higher elevation forests, but no particular species were found at a higher elevation more than the other species. Even though habitat did not help differentiate between species, it was shown that the reason for *Cryptocercus* spp. inhabiting logs at various elevations and habitats was to compensate for changes in

weather patterns (Nalepa 2003). During the glacial periods, cockroaches moved from higher elevations because of the lack of forest and host material (Nalepa 2003). Conversely, they are confined more to higher elevations during the warmer, drier periods. This may have led to variations in chromosome number by some species isolated in the higher elevations during these undesirable conditions via natural selection. This may also explain the random dispersal of the karyotypes in the eastern United States (Nalepa 2003).

All four of the previously mentioned studies of *Cryptocercus* spp. in the eastern United States also brought suspicion that there would be morphological variations (Aldrich et al. 2004a). Burnside et al. (1999) investigated many of the morphological characters of the four species in the Appalachian region including: the number of antennal segments, spines associated with the posteroventral and posterodorsal edges of the femora, tibial spines, shaped of the posterior margin of the eighth abdominal sternum, and structure of the terminal abdominal segments with their associated structures. This examination showed very little variation between species (Aldrich et al. 2004a). The only variation was in the shape of the epiproct and subgenital plate in *Cryptocercus wrighti*. Therefore, the male and female genitalia of the species were studied for morphological variation because morphological divergence in the genitalia is prevalent and may be induced by antagonistic coevolution between sexes (Aldrich et al. 2004a). Evolution in the genitalia seems to be more prevalent than other traits in many insects. Differentiation in the genitalia is also often used in taxonomy because speciation events are often preceded, accompanied, or followed by morphological changes in the genitalia, supporting reproductive separation (Aldrich et al. 2004a). There had also been previous studies that found morphological variation in regard to the genitalia of the species: *Cryptocercus relictus*, *Cryptocercus primarius*, and *Cryptocercus clevelandi*. This therefore increases the possibility that the Appalachian species display morphological variation between species.

This study investigates whether or not there is one species or two species in the southern West Virginia region, as two have been identified in nearby Wytheville, Virginia (Aldrich 2004b).

## **METHODS AND MATERIALS**

First, roaches were collected from forested areas around Concord University (Athens, WV) (Map 1) and Pipestem State Park (Pipestem, WV) (Map 2). Large, decaying logs were split open using a hatchet, a hammer, and a chisel. When roaches were found, they were placed in vials containing 80% ethanol. The vials were then stored at 4°C until used in DNA analysis of the roaches. Roaches were collected from June 7, 2005 to August 25, 2005.

One or two of the samples from each colony at all of the sites were used for DNA analysis. DNA analysis was done by extracting the DNA, amplifying the DNA using polymerase chain reaction (PCR), and gel electrophoresis of the amplicons. The DNA was extracted using a Qiagen DNeasy Tissue Kit (Qiagen Laboratories, CA) according to the manufacturer's instructions. The roaches were cut in half and using one half for DNA analysis and the other half stored at -20°C for later use. After the DNA had been extracted, PCR of the 12S rRNA and 16S rRNA genes was performed. The following primers were used: 16S forward- 5' TTACGCTGTTATCCTAA 3', 16S reverse- 5' CACCTGTTTAACAAAAACAT 3', 12S forward- 5' TACTATGTTACGACTTAT 3', and 12S reverse- 5' AAAGTAGGATTAGATACCC 3'. For each primer, a 100-uM stock was made. A working concentration from each stock was made by diluting 5-uL of the 100-uM stock with 95-uL distilled water. One microliter of the extracted DNA was placed in a 0.2 mL PCR tube with 1-uL of each the forward and reverse primers for either the 12S or 16S ribosomal RNA and 22-uL of the PCR Master mix (Invitrogen). The PCR tube was then placed in the thermal cycler. The temperature cycle was 95°C for 30 seconds, 40°C for 1 minute, and 72°C for one cycle followed by twenty-five cycles of 95°C for 30 seconds, 50°C for 1 minute, and 72°C for 1 minute (Kambhampati and Smith 1995). The amplicons were then separated by electrophoresis in a 1% agarose gel containing ethidium bromide for visualization.

In order to confirm observed variations, PCR was repeated with only the 12S primers that were used previously and the same cycle that was previously used. Gel electrophoresis was also repeated on a 1% agarose gel.

## RESULTS

Every gel except one showed a band approximately 388 base pairs in size and another band with a considerable smaller size of approximately 50 base pairs. The exception was from the site located in front of Concord University labeled CFD-2, seen in lane four of the gel (Figure 1). In this case, three bands were observed on the 12S rRNA gene with the largest being approximately 1250 base pairs in size, the middle one being about 560 base pairs in size, and the bottom one being about 50 base pairs in size (Fig. 1).

The experiment was repeated using only the 12S rRNA sample from Concord University showing variation and the other samples that had been previously tested on the gel with the variation as controls to verify that this result was indeed reflective of variation. However, all bands using the 12S rRNA primers were identical suggesting that all have amplicon products of 50 base pairs and 388 base pairs in size (Figure 2).

## DISCUSSION

All samples collected from Pipestem State Park (Pipestem, WV) and Concord University (Athens, WV) in southern West Virginia appear to be the same species. The 12S rRNA gene from *Cryptocercus punctulatus* should be a total of 438 base pairs in size, whereas the 12S rRNA gene from *Cryptocercus wrighti* should be a total of 436 base pairs in size. These numbers are very close in size and without restriction enzyme analysis or DNA sequencing, it is difficult to definitively determine which species was investigated in this study. However, Nalepa et al. (2002) examined samples in this area using DNA sequencing and identified them as *C. punctulatus*. Because of conclusions made by Nalepa et al., it is concluded that the predominant species in this studied area is most likely *Cryptocercus punctulatus*

(2002). The bands that were seen in lane four on the original gel were entirely too large to be pure *Cryptocercus* spp. DNA. This sample may have been contaminated, as a PCR hood was not used. Had a PCR hood been used, contamination could have been avoided. Alternatively, some nonspecific binding of primers may also have occurred.

However the controversy as to whether the species separation in the Appalachian region of *Cryptocercus* spp. was premature could lead to the availability of more genetic information and therefore this study would require that these results be re-examined. This controversy includes the fact that chromosome number was only previously tested by Burnside et al. (2000) for part of the sample and mitochondrial rRNA genes were tested for the other part of the sample. In order for this to be improved to give universal, accepted results all samples must be tested the same throughout the entire Appalachian region. Results found are also controversial because reproductive compatibility has yet to be investigated. Therefore, the specimens found in southern West Virginia and throughout the Appalachian region need to all be examined for reproductive compatibility confirming that the species found can reproduce with one another. There is also very little correlation between the morphological variation and the karyotype results. This makes it necessary to find correlations between these two factors. With the resolution of these controversial issues, more definitive results may be found and may change the results that were found in the area of southern West Virginia.

In order to fully complete this study, it is obligatory that further research be done to analyze the genetic variation in the southern West Virginia area. The sample size and site number would need to be increased in order to give more validity to the predominant species only being *Cryptocercus punctulatus*. If more sites were examined, there may be more variation in the results showing the presence of other possible species. It is also essential to examine all specimens found using restriction enzyme analysis. This will give more legitimacy to the species that was seen by confirming that *C. punctulatus* is the

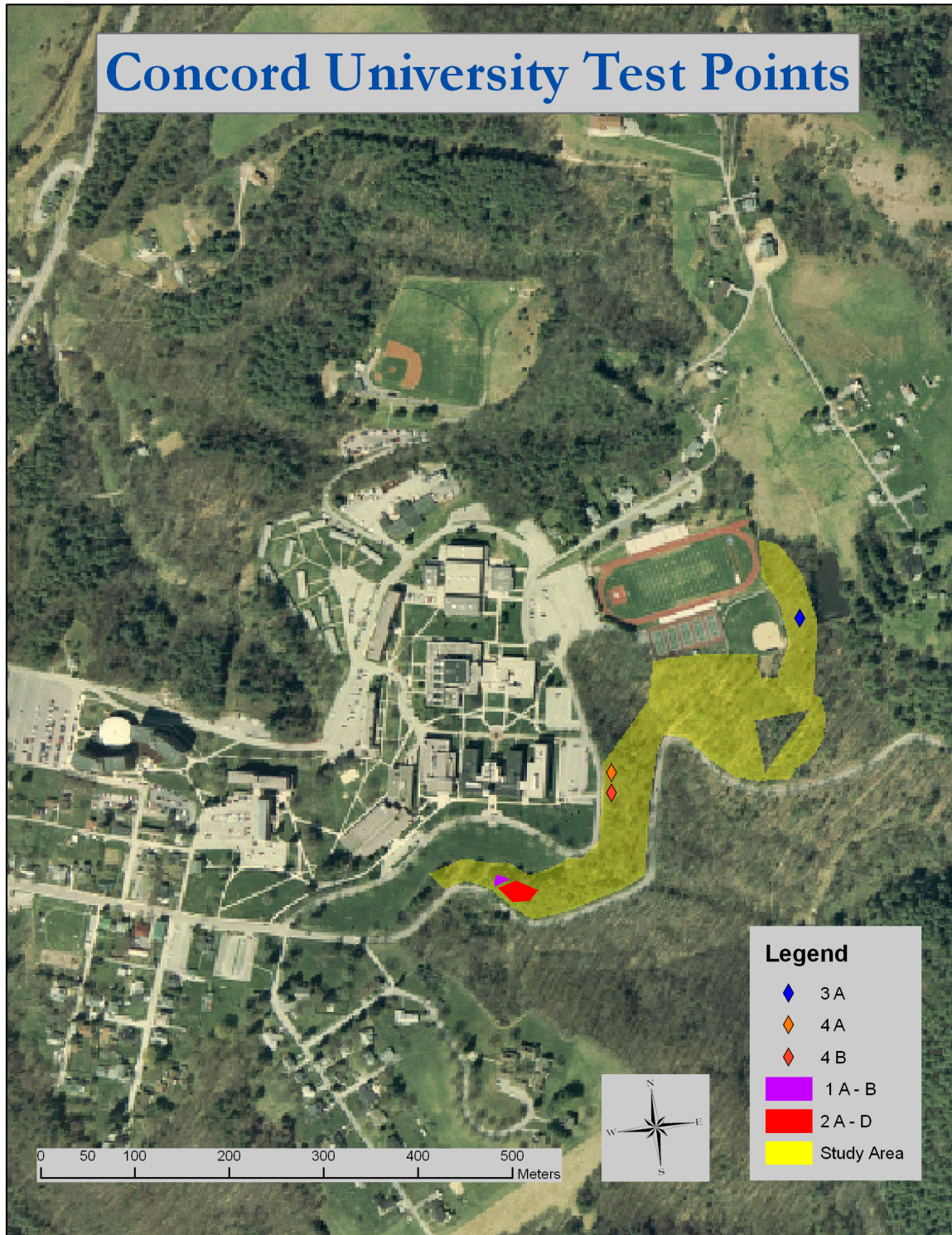
predominant species. Lastly, it may also be necessary to examine the morphological variation of the collected specimens in an attempt to find a correlation between the genetic variation and morphology.

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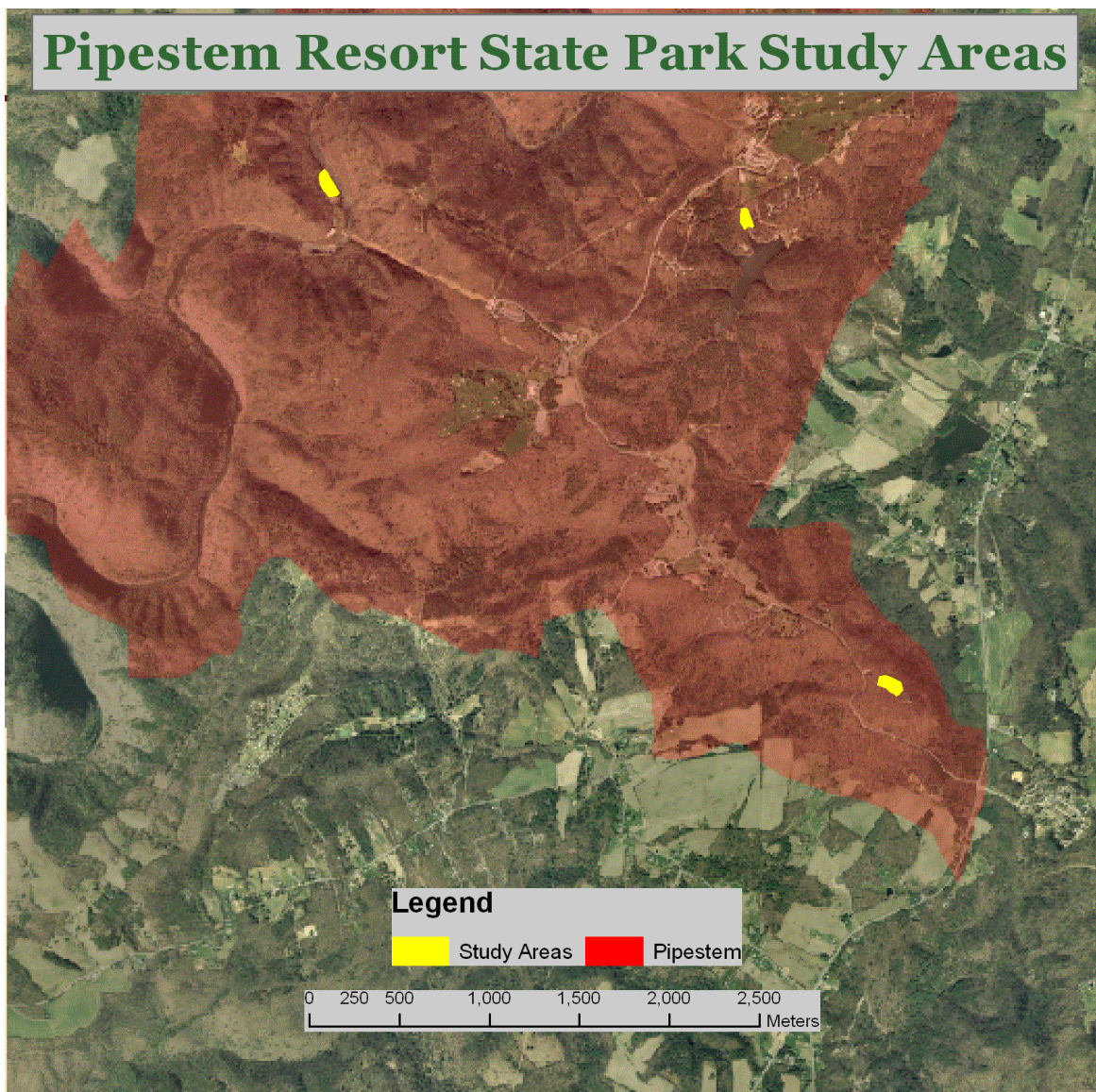
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FIGURES





**Map 1: Collection Sites at Concord University (Athens, WV):** The area in yellow is the study area and the other colored areas show where wood cockroaches were found.



**Map 2: Collection Sites at Pipestem State Park (Pipestem, WV):** The areas in yellow show the actual study areas

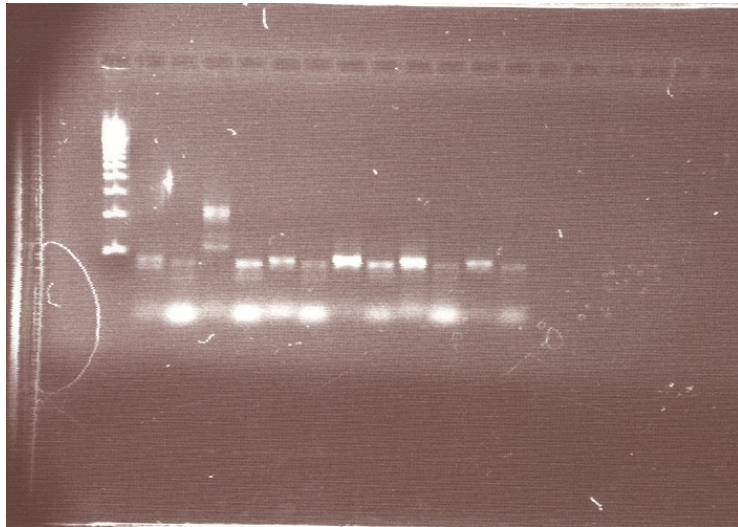


Fig. 1 Visualization of PCR amplicon by ethidium bromide following agarose gel electrophoresis. Lane 1: 500 bp DNA standard; Lane 2: 12S CFD-1; Lane 3: 16S CFD-1; Lane 4: 12S CFD-2; Lane 5: 16S CFD-2; Lane 6: 12S CFF-1; Lane 7: 16S CFF-1; Lane 8: 12S CFF-2; Lane 9: 16S CFF-2; Lane 10: 12S PBA-1; Lane 11: 16S PBA-1; Lane 12: 12S PBB-1; Lane 12: 16S PBB-1

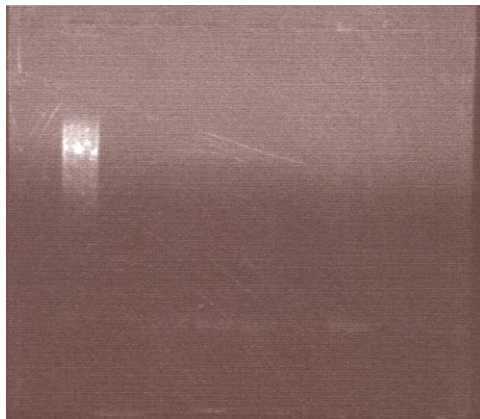


Fig. 2 Visualization of PCR amplicon by Ethidium Bromide following agarose gel electrophoresis. Lane 1: 500 bp DNA standard; Lane 2: Blank Lane 3: 12S CFD-1; Lane 4: 12S CFD-2; Lane 5: 12S CFF-1; Lane 6: 12S CFF-2; Lane 7: 12S PBA-1; Lane 8: 12S PBB-1

**The Effects of Inclusion in an Early Childhood Environment**

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McNair Scholars Program

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## **Introduction**

Inclusion in preschool classrooms is becoming more and more prominent in the United States. To discuss inclusion, there must first be a set vocabulary. Inclusion is a term which expresses commitment to educate each child, to the maximum extent appropriate, in the school and classroom he or she would otherwise attend. It involves bringing the support services to the child (rather than moving the child to the services) and requires only that the child will benefit from being in the class (rather than having to keep up with the other students) (Stout, 2001; p. 1).

Full inclusion is placing all children in regular education classrooms regardless of disability or severity. Inclusion is a very controversial topic in education today. It deals with our very sense of ethics and the worth of our children. Much research shows that inclusion holds many benefits for special needs children (Diamond & Carpenter, 2000; Guralnick, 1999; McCabe, Jenkins, Mills, Dale, Cole, 1999; etc.). We must ask ourselves if the benefits of inclusion for special needs children are worth the possible consequences for regular education children. Many evaluations of inclusive education programs report positive effects on academic, behavioral, and social outcomes for children with disabilities, while no negative consequences for the non-disabled children are reported (Karsten, Peetsma, Roeleveld, & Vergeer, 2001; from Bosworth & Bourdess).

### **Problem Statement**

I am conducting research in Mercer County as a McNair Scholar to determine if there are differences that can be noted between children who have participated in an early childhood inclusive program and those who have not. Data collected will result from surveys with parents and special needs and early education teachers.

### **Data Collection**

Data was collected through a literature review of past research on the effects of inclusion. Data was also collected through surveys completed by parents, special needs, pre-k, and third grade teachers in

Mercer County. There were thirty surveys sent out. Ten surveys were sent to parents, ten were sent to third grade teachers, five were sent to pre-k teachers, and five were sent to pre-k special needs teachers. These teachers and parents were selected from the Mercer Early Learning Centers, Sun Valley, Mercer School, and Athens School.

### **Literature Review**

The research on inclusion is somewhat inconsistent. Research shows that many family childcare providers do not want to serve children with disabilities due to liability and other various reasons (Buell, Gamel-McCormick, & Hallam, 1999). Other research shows that teachers do support inclusion and working with children with disabilities (Proctor & Niemeier, 2001). Many parents of regular education children saw inclusion as a risk for their child due to a lack of individualized attention for their child, yet 94% of these same parents said that they would place their children in an inclusive setting again (Rafferty, Boettcher, & Griffin, 2001).

These differences could result from differences in individualized service models. There are several of these models. The Itinerant Teaching-Direct Service Model is where services are given on a regular basis in regular classroom settings by special education teachers (Odom, Horn, Marquart, Hanson, Wolfberg, Beckman, Lieber, Li, Schwartz, Janko, & Sandall, 1999). The Team Teaching Model is where a regular teacher and a special education teacher share the teaching role at the same time (Odom, Horn, Marquart, Hanson, Wolfberg, Beckman, Lieber, Li, Schwartz, Janko, & Sandall, 1999). The Early Childhood Teaching Model is where just a regular education teacher takes care of a class with regular education and special education children, and the Early Childhood Teaching Model is the same but with just a special education teacher (Odom, Horn, Marquart, Hanson, Wolfberg, Beckman, Lieber, Li, Schwartz, Janko, & Sandall, 1999). The Integrated Activities Model is where the special education and regular education children stay separated for most of the day, but do some activities together (Odom, Horn, Marquart, Hanson, Wolfberg, Beckman, Lieber, Li, Schwartz, Janko, & Sandall, 1999).

Though, there is disagreement between teachers and parents about how worthwhile inclusion is, the consensus of most research shows that the effects of inclusion on early childhood special needs children are positive. These include effects on social development, language development, and cognitive development as well as developing a good self esteem (Bates, 1975; Garvey, 1986; Hartup, 1983; Howes, 1988, Rubin & Lollis, 1988; from Guralnick, 1999).

### *Social Development*

The most obvious positive effect on development from inclusion is on social development. The encouragement of creating relationships with children with and without disabilities paves the way for meaningful inclusive relationships in the future and throughout life and throughout the community (Guralnick, 1999). Parents agree that early childhood inclusive settings help children become accepting of those who are different and encourage relationships with all children regardless of these differences (Baily & Winton, 1987; Guralnick, 1994; Guralnick, Conner, & Hammond 1995; from Guralnick, 1999).

Inclusion also helps to create sympathy in young children. Research that involved thirty-three preschool children in inclusive classrooms and thirty preschool children in regular classrooms were interviewed about helping others. The children in the inclusive classrooms score significantly higher scores and were more likely to refer to disability than the children in the regular classrooms (Diamond & Carpenter, 2000). This study suggests that children are capable of showing empathy toward children with special needs if given the chance, and typically developing children who are in inclusive settings are more sensitive toward children with disabilities than typically developing children in a non-inclusive setting (Diamond & Carpenter, 2000). Children with disabilities have fewer difficulties interacting with typically developing children than with other children with disabilities. “They use fewer strong directives, share information more, have fewer disagreements, and are less negative and more positive during conflicts with typically developing children than with children with mild delays” (Guralnick, 1999).

Social inclusion also has an effect on the types of play special needs children engage in. A study was done that focuses on the effects of play group composition and play materials on how children with disabilities play. There are three types of play: functional, constructive, and dramatic, each higher than the last. The study involved twenty-four preschool children with disabilities. Studies showed that group composition had no effect on the amount of play, but mixed groups of typical and disabled children practiced higher levels of play as opposed to groups of only disabled children (McCabe, Jenkins, Mills, Dale, Cole, 1999). Studies also indicated that different types of toys were more cognitively stimulating (McCabe, Jenkins, Mills, Dale, Cole, 1999). Allowing children with developmental delays to play with regularly developing children gives children with delays a framework for social play (Guralnick, Conner, Hammond, Gottman, & Kinnish 1996; from Guralnick, 1999). There have been no negative effects of inclusive play groups on regularly developing children found (Buysse & Bailey, 1993; Guralnick, 1990).

#### *Language Development*

In a study, 96 preschoolers were given a test before and after being in either an inclusive or a non-inclusive classroom. The results indicated that children with non-severe disabilities showed no difference in posttest scores between segregated and inclusive classrooms (Rafferty, Boettcher, & Griffin, 2001), which coincided with previous research (Cole, Mills, Dale, & Jenkins, 1991; Jenkins, Odom, & Speltz, 1989; Mills, Cole, Jenkins, & Dale; 1998). However, children with severe disabilities showed significantly higher posttest scores in inclusive classes than those in segregated classes (Rafferty, Boettcher, & Griffin, 2001). These findings are consistent with a similar study done by Hundert and colleagues in 1998, but are inconsistent with other studies that found no difference in segregated and inclusive classrooms, or found higher scores from children in segregated classrooms than inclusive classrooms (Cole, Mills, Dale, & Jenkins, 1991; Mills, Cole, Jenkins, & Dale, 1998; from Rafferty, Boettcher, & Griffin, 2001).

#### *Cognitive Development*

Studies on the effect of inclusion on cognitive development on children with special needs are fairly consistent. Many studies report little or no beneficial effects of exclusively special needs classrooms (Kavale, & Glass, 1982; Madden & Slavin, 1983; from *Mainstreaming and Inclusion*). In fifty studies comparing the progress of academic performance of children in inclusive and non-inclusive settings, the children in the inclusive setting scored on average in the 80<sup>th</sup> percentile, and the children in the segregated setting scored on average in the 50<sup>th</sup> percentile (Weiner R., 1985; from Stout 2001). More recent studies also show that inclusive settings have beneficial effects on special needs children in academics (Carlberg, & Kavale, 1980; Baker, Wang, & Walberg, 1994-95; from *Mainstreaming and Inclusion*).

#### *From Preschool to a Third Grade Setting*

Much of the information discussed previously about preschool inclusion also applies to inclusion in the primary grades. There are some differences, however. In preschool, children without disabilities oftentimes do not notice the differences in children with special needs, especially if the child with special needs has a cognitive problem, but by the primary grades of school, after many social experiences between the children, the difference become more and more apparent (Tapasak & Walther-Thomas, 1999). This realization could result in inadequate feelings about academics and social areas for the special needs children (Tapasak & Walther-Thomas, 1999). In fact, research shows that self-esteem in special needs children in the primary grades is negatively proportionate to the time spent in a regular classroom (Wigle & DeMoulin, 1999). However, other studies have shown positive social effects on children in primary grades (Buysse & Bailey, 1993). Placing children with mild retardation in inclusive settings can decrease their feelings of loneliness and depression, and help them to gain social skills (Heiman & Margalit, 1998).

The ages of six to eight are considered to be critical in the development of social skills (Dodge, Jablon, & Bickart, 1994; Flavell, 1977). Failure to gain these social skills at this age can have dire results,



such as delinquency, dropping out of school, and adolescent drug use (Greene et al., 1999; Parker & Asher, 1987). Not gaining these skills can also be a good indication of mental health problems later in life (Strain & Odom, 1986).

To resolve these negative attitudes toward special needs children in primary grades from the special needs children themselves and from the other children, some research suggests the use of literature based on children with disabilities (Turner, 1997).

“Students with disabilities benefit by (1) having appropriate role models; (2) participating in the same inclusive, diverse communities that they will share as adults; and (3) establishing a network of friends and acquaintances that will increase the likelihood of their success in the community” (Farlow, 1996; p.55).

One researcher found that in 1980, the books that involved disabilities were about one child with one disability (Hopkins, 1980; from Turner 1997). However, newer research shows that there are more books today that portray children with disabilities and these books show a large variety of disabilities (Harrill, Loung, McKeag, & Price, 1993; from Turner 1997).

### *Conclusion*

Most of the research on inclusion points to a positive effect for early childhood special needs children. The only conflicting evidence was the study on language done by Cole and colleagues in 1991 and the study done by Mills and colleagues in 1998, which showed no difference in segregated and inclusive classrooms, or found higher scores from children in segregated classrooms than inclusive classrooms. The studies on social and cognitive skills, however, were consistent in finding beneficial results for children involved in inclusive settings. Some studies also indicated that inclusive settings had no adverse effects for typically developing children.

## **Hypothesis**

The researcher predicted that most teachers would see inclusion as an advantage for their children, but some would be apprehensive due to severity of children's needs and due to lack of training.

The researcher also predicted that parents of special needs children would see inclusion as an advantage for their children, but parents of typically developing children would see inclusion as a disadvantage for their children.

## ***Limitations***

In survey, results would be more accurate if there were more individuals involved in the survey. This survey was limited to Mercer County, West Virginia. It was also limited to only 13 (43%) surveys that were completed. Three surveys were returned from preschool special needs teachers, two were returned from preschool regular education teachers, four were returned from third grade teachers, and four were returned from parents. Another limitation could occur in that this survey is being conducted in a small area, a larger area and more school systems may have led to a different type of thinking and different opinions about this subject.

## ***Anticipated Benefits***

It is the goal of this research to provide other exerts with knowledge that can be used in starting or modifying a public classroom in order for it to benefit all children involved.

## ***Benefits to the Organization***

It is my hope that this research may act as a stepping stone to future McNair scholars who wish to pursue research in this same field of study. I hope it will allow these future researchers to discover new angles and theories. This research can also be used as proof of the profound benefits of inclusion in an early childhood setting and the thoughts and feeling of teachers and parents in this region.

## ***Results***

Most of the research on inclusion points to a positive effect for early childhood special needs children. I have found through my research that the teachers surveyed feel that inclusion is important to the success of special needs children. I have also found that parents of special needs and regular education children value inclusion as a part of their child's education.

When asked about their thoughts on inclusion, 86% of returned teacher surveys had only positive comments. Fourteen percent of teachers surveyed had mixed feelings about the subject. Zero percent had only negative comments. Teachers showing mixed feelings indicated concerns about the severity of the child's disabilities.

Of the teachers with experience with inclusion 100% of teachers reported seeing positive differences in children from inclusive classrooms as opposed to children in a strictly "special education" environment. When asked if special needs children would have a better success rate in an inclusive classroom compared to a special needs classroom 100% of teachers answered "yes".

When preschool teachers were asked to predict the overall developmental performance of children in inclusive classroom compared to those not in inclusive classrooms, 100% of teachers indicated that the children would do better.

When asked if they had it to do over again would they enroll their child in an inclusive program again, 100% of parents surveyed said "yes".

When asked their opinion on inclusion, parents made comments such as "Our son has matured mentally and emotionally through this program" and "I feel it has been excellent exposure for her to see all types of children". One parent who also indicated that she was a teacher said, "If the program has a tiny bit of structure, then it by far enables children to be on level when they start kindergarten."

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## Appendix A

### Introductory Letter



September 29, 2005

Nikki McCormick  
P.O. Box 1000  
Box C-634  
Athens WV, 24712

Dear Sir or Madam:

Hello, my name is Nikki McCormick. Thank you for taking time to answer some questions about your experiences with early childhood inclusive programs. I am conducting research as a Concord University McNair Scholar, with Dr. April Puzzuoli as my Mentor. My research is based on determining the success of early childhood inclusive programs, and I am interested in your opinions. It would be greatly appreciated if you could return the completed survey to me by October 21, 2005. There is a stamped, self-addressed envelope included with the survey.

If you have any questions, please contact me at [mccormickan@concord.edu](mailto:mccormickan@concord.edu) or 384-5831. Dr. Puzzuoli can be contacted at [apuzzuoli@concord.edu](mailto:apuzzuoli@concord.edu) or 384-5209.

Thank you again for your time.

Sincerely,  
Nikki McCormick  
McNair Scholar  
Concord University

## Appendix B

### Parent Letter



September 29, 2005

Nikki McCormick  
P.O. Box 1000  
Box C-634  
Athens WV, 24712

Dear Sir or Madam:

Hello, my name is Nikki McCormick. Your Child's teacher suggested that you might help me by answering some questions about your experiences with early childhood inclusive programs. I am conducting research as a Concord University McNair Scholar, with Dr. April Puzzuoli as my mentor, to determine the success of early childhood inclusive programs. I am interested in your opinions.

It would be greatly appreciated if you could return the completed survey to me by October 21, 2005. There is a stamped, self-addressed envelope included with the survey. If you have any questions, feel free to contact me by e-mail at [mccormickan@concord.edu](mailto:mccormickan@concord.edu) or 384-5831.

Thank you again for your time.

Sincerely,

Nikki McCormick  
McNair Scholar  
Concord University

## Appendix C

### Early Childhood Inclusion Survey Fall 2005

Position: \_\_\_\_\_

Location: \_\_\_\_\_

#### All Teachers Surveyed

1. How long have you been in your present position? What other positions have you held?
2. What are your thoughts on inclusion for young children?
3. Have you ever visited an (another) inclusive classroom? Yes/No, Explain
4. Do you see differences between students that have attended inclusive classrooms and students who have not? Describe:
5. How would you characterize children from inclusive classrooms compared with their peers without disabilities?  
  
\_\_\_\_\_ Socially Mature                      \_\_\_\_\_ Well Behaved  
\_\_\_\_\_ Active Learners                      \_\_\_\_\_ Creative  
\_\_\_\_\_ Other, Describe:
6. What do you consider to be the most important skills that can be given children, in order for them to be successful in their next placement?
7. In what direction do you see special education moving for young children in the next five years?
8. What (if any) are some inclusive teaching strategies that you have found to be effective?

Other comments you would like to make:



## **Appendix D**

### For Pre-K and Pre-K Special Needs Teachers

1. Do you have personal knowledge of how individual children, who have been in an inclusive classroom, perform in their next placement?
2. Do you know of children who received special education services in Pre-K that did not require them in kindergarten or beyond? Describe a couple:
3. Overall, how do you predict that children that have been in an inclusive classroom perform developmentally compared with their peers who have not had this experience?
4. ...and on standardized test scores in the third grade?
5. What transition services or information were provided for you before or when children with special needs are transferred to your classroom or program?

## **Appendix E**

### For Third Grade and Third Grade Special Needs Teachers

1. With children that have participated in an inclusive classroom compared with children who have not, have you found their cognitive skills to be age appropriate, delayed, or advanced?
2. ... Have you found their language skills to be age appropriate, delayed, or advanced?
3. ...have you found their gross motor skills to be age appropriate, delayed, or advanced?
4. ...have you found their fine motor skills to be age appropriate, delayed, or advanced?
5. ... Have you found their social skills to be age appropriate, delayed, or advanced?
6. What about their skills in attention, listening, and following directions?
7. Would you say that students that could be/or are already identified for special education services and have attended an inclusive classroom have a better success rate than students that did not?
8. Do you find that the children who attended an inclusive classroom are more/less frustrated in peer groups? At play? With their academics?
9. Does there appear to be a gap developmentally between children who attended an inclusive classroom and those who did not? If so, explain those differences and how you differentiate your instruction in order to individualize for them?
10. What transition services or information were provided for you before or when children with special needs were transferred to your classroom or program?

## **Appendix F**

### For Parents

1. What school is your child currently enrolled in?
2. What inclusive settings has your child attended?
3. If you had it to do over, would you enroll your child in an inclusive early childhood program? Y/N and why?  
Comments:
4. What teaching strategies have you found to be effective for your family and your child?
5. In what ways did teachers help you and your child make the transition from one setting to another?
6. What transition strategies have you found to be effective for your family and your child?

Other comments you care to make about inclusion for your child:

**Study of Factors Affecting the Distribution of**

**Wood Cockroaches (*Cryptocercus* spp.)**

Stacy Miller

**March 31, 2006**

Committee Members: Dr. R. Sheppard, Dr. T. Ford, and Dr. D. Wise

**ABSTRACT:**

Wood cockroaches of the genus *Cryptocercus* spp. were collected from seven different locations of wooded area in southern West Virginia around Concord University in Athens, and Pipestem State Park in Summers County. Different environmental factors of the wood cockroaches were examined to see if any had an effect on their distribution. Many parts of their habitats were examined and the list includes: the type of logs that the wood cockroaches were found in, what other species of insects were in the logs with them, the altitude, the moisture content, the number of adults and nymphs in each log, the number of colonies, and the class of nymphs. There are only nine species known of *Cryptocercus* spp. and one has been observed in the northern half of West Virginia, but southern West Virginia has not been studied. Twenty-one colonies were found and altitude was not a factor in their distribution. The type of the log was difficult to determine because of the state of decomposition. The sample size was too low to conclude if the size of the log affected the location of the colonies. Colonies with Class 1 and 2 nymphs had at least one adult. Class 3 and 4 nymphs had one or no adults present when they were observed.

## INTRODUCTION:

Cockroaches are insects with flattened oval shaped bodies. They are exothermic invertebrates without backbones. Cockroaches are in the order Blattaria (Gordon 1996). The genus we are working with is *Cryptocercus* spp. *Cryptocercus* spp. cockroaches are similar to termites in that the cockroaches have symbiotic, cellulose digesting protists living in the gut to help digest the wood (Gordon 1996). They are found in decaying moist wood of dead trees in the temperate areas.

*Cryptocercus* spp. are subsocial cockroaches that live in temperate forests. Subsocial behavior exists when there are family relationships with some parental care. Mated pairs of *Cryptocercus* spp. will stay together for their life time. The cockroaches are monogamous and they exhibit parental care (Hossain and Kambhampati 2001). The cockroaches live in galleries and will stay in the same log most of their life (Lumoa 1999). Cleveland et. al. (1934) thought that new colonies formed because the adults and nymphs moved to a new log. Nalepa (1984) classified the nymphal stages into four groups. Class 1 roaches are white to ivory and consist of the nymphs that had hatched the summer prior to colonization. Class 2 nymphs are ivory to gold and hatched two summers ago. Class 3 nymphs are gold to reddish-brown and born three summers ago. Class 4 nymphs are those that are not quite mature and are darker reddish-brown to black in color and are considered pre adults. After the 4<sup>th</sup> class, the cockroaches are considered adults and are black in color. The social unit consisted of a pair of adults and the offspring of one brood until they were mature enough to take care of themselves. When the nymph hatches it has no digesting protist in its gut. The nymph can only get the protist by proctodeal prophyllaxis, which involves feeding from the adults' anus (Lumoa 1999). The adult cockroach stays with the nymph its whole life and it protects it. The adult cockroach makes tunnels inside the logs so the nymph can get around. Termites and wood roaches are found in dead wood and have been found in the same logs. Both of them have protozoan in their hind gut that fixes nitrogen (Nalepa 1990).

Nine species are recognized in the genus *Cryptocercus* spp. worldwide, however that number is considered controversial (Steinmiller et. al. 2001). The cockroaches are found in two different regions, the Nearctic and Palearctic (Bei Bienko 1938, Nalepa et. al. 1997, Park et. al. 2002). The Nearctic consists of the species that are found in the United States, while the Palearctic consists of the species from China and Russia.

*Cryptocercus* spp. are found in the following locations: two species are in eastern Eurasia, two in China, one in the northwestern United States, and four in the Appalachian Mountains of the United States (Aldrich et al 2003). *C. clevelandi* is the only species found in the Pacific northwest of the United States. The four species that are found in the Appalachian Mountains are as follows: *C. punctulatus*, *C. darwini*, *C. garcia*, and *C. wrighti* (Nalepa 2003). *C. primarius* was found first in Sichuan Province, China (Bei-Bienko 1938) and it is hard to find the geographical range. A new species has been found in Korea called *C. kyebangensis* and exhibits morphological and molecular diversity (Hoassain and Kambhampati 2001). The last two species are in Eurasia; one is *C. matilei* and the other is *C. relictus*.

All of the *Cryptocercus* spp. in the Eastern United States were identified as one species until Kambhampati et. al. (1996) showed that *Cryptocercus* spp. had a different number of mitochondrial rRNA genes. Kambhampati et. al. (1996) also concluded that there was a variation in the number of chromosomes between the species in the west and the species in the east, as well as differences in the DNA sequences. Burnside et. al. (1999) and Kambhampati et. al. (1996) concluded that there were four karyotypes in the Appalachian mountains. The DNA sequence of nuclear rRNA was used to examine the four species that existed in the Eastern United States and to see if there was a compared relationship with the mitochondrial sequences (Hoassain and Kambhampati 2001). After doing the research Burnside et. al. (1999) concluded that one species had forty five chromosomes and was basal and the other species had thirty seven chromosomes and was paraphyletic. Species could be identified based on the mitochondrial rRNA genes and the chromosomes (Aldrich et. al. 2004). Many believe that the

distribution of these species is scattered; however, they have been found very close to one another. Steinmiller et al. (2001) concluded that *C. darwini*, *C. garciai*, and *C. wrighti* do overlap in the Appalachian Mountains. Furthermore, they exhibit divergence in mitochondrial DNA, chromosome number, nuclear DNA, and ribosomal DNA (Aldrich et. al. 2004).

There have been many different studies done on *Cryptocercus* spp. A previous survey was done in Oregon and California to see the geographic distribution of *C. clevelandi* and to find the genetic variation within the species (Steinmiller et al. 2001). Also 48 sites in the eastern United States were looked at. One species was found in the western United States and it is distributed in a more inconsistent manner than in the eastern United States species (Steinmiller et al. 2001). The Appalachian Mountains, where the other four species of *Cryptocercus* spp. are scattered, range from Pennsylvania to Alabama. *Cryptocercus punctulatus* was the only species found in Pennsylvania and it went through West Virginia and parts of Virginia. *Cryptocercus wrighti* occurred in Virginia, North Carolina and parts of Tennessee. *Cryptocercus garciai* was found primarily in Georgia and parts of Tennessee and southern North Carolina. *C. darwini* was observed in Kentucky, Tennessee, Alabama, and North Carolina. There were three potential zones of hybrid overlapping. One zone was in Virginia, one in Tennessee, and one in North Carolina (Aldrich et. al. 2004). The zone in Virginia was in Wytheville, Wythe County and *C. punctulatus* and *C. wrighti* were found 8km apart. The zone in Tennessee was in Monroe County and *C. garciai* and *C. darwini* were within 3km of each other. The last zone was south of Ashville, North Carolina and *C. wrighti*, *C. garciai*, and *C. darwini* were in close proximity. The species were very close to each other but none were found in the same log together (Aldrich et. al. 2004).

It has been suggested that *Cryptocercus* spp. is limited to higher elevations and has preferences for certain types of wood. Nalepa (2003) determined that these two factors do not have an effect on the distribution of the different species of *Cryptocercus* spp. She found cockroaches in evergreens and

deciduous logs. In her study she found no evidence to suggest that *Cryptocercus* spp. are found more at one elevation or another.

There have been other studies on their habitat; the elevation was again found to not be a factor because the roaches were found in low elevations (Park et. al. 2002). It was also shown that the winter had an effect on the *Cryptocercus* spp. because many nymphs did not make it through the bad weather. Since we will do most of our researching in the summer, the weather will not be a problem for us.

The distribution of *Cryptocercus* spp. has not been studied in southern West Virginia. I will be looking at what species of logs the wood cockroaches are found in and what other species of insects are in the logs with them. I will be taking the altitude into count; I will be going to low and high altitude locations to see if there is any difference in abundance of *Cryptocercus* spp. between them. The moisture content and size of logs will also be observed. I will be recording the geographical location and forest type for each colony. The number of adults and nymphs in each log, the number of colonies in each log, and the class of nymphs will also be recorded.

#### **METHODS AND MATERIALS:**

Wood cockroaches in southern West Virginia were sampled in the forested areas around Concord University and Pipestem state park (Figure 1). The forested areas chosen contained mature trees with logs in different stages of decomposition. One forested area on the east side of Concord University was surveyed as shown in (Figure 2). Three areas around Pipestem were surveyed (Figure 3); the first one was the tower at high elevation, the second one was the lake for medium elevation, and the third was at the bottom of the tram near the river at low elevation (Figures 4, 5, and 6). The survey was done to see where the wood roaches were found most frequently.

I used an assortment of tools during the investigation. The tools that I used were as follows: a hammer and chisel, a hatchet, a ruler, vials containing 80% ethanol, a moisture reader, and a topographic map. Only logs that were very moist and easy to break into were investigated for roaches. The logs were



examined to see if there were any exit holes that the wood roaches might have made. If the holes were found, then they could be traced and followed to see if they led to the wood roaches. The chisel and hammer, or hatchets were used to break into the wood to find the roaches. A camera was used to take pictures of the logs, the nymphs, and adults. The location where I found the roaches, the date, the species of tree, and the moisture content were recorded.

Once the wood cockroaches were observed, some were placed in 80% ethanol and refrigerated to later identify the species. I recorded how many adults and nymphs were in each log and how many colonies there were in each log. Nymphs were classified into one of five classes using a system developed by Nalepa (1984). If they are white to ivory they are Class 1, if they are ivory to gold they are Class 2, if they are gold to reddish brown they are Class 3, if they are darker reddish brown to black they are Class 4, and if they are black they are adults.

Other data were recorded including elevation of the sampling site, which was determined with topographic maps. I used the ruler to measure the diameter of each log to see if the size of the log had an effect on the distribution of the wood roaches. The logs were observed carefully to see what other insects were feeding on the wood with the wood roaches; for example, termites and beetles.

The molecular determination of the individual species of wood roaches present at each site will be determined later. If there is more than one species, then I will be able to look at the data from all of the above material and see if one species is found in a particular habitat that is different from the other species. After all of the research and data collecting had been done, there will be a better understanding of what habitat the wood roaches live in and what factors are affecting their distribution.

## **RESULTS:**

Twenty-one colonies were observed in seven different locations. All of these colonies were found in logs with 30% moisture levels or greater. Unfortunately, the moisture meter only measured levels to 30% so we were unable to ascertain how much moisture was in the logs we sampled. According

to other studies, only logs with high levels of moisture will contain roach colonies (Nalepa, 2002; Park et.al., 2002).

Figure 1 shows the main area of Concord University and Pipestem state park. Figure 3 shows the three areas of Pipestem State Park together. Four areas were surveyed at Concord University in front of the school and around the softball field (Figure 2). The Pipestem tower, river, and lake were the fifth, sixth, and seventh places observed (Figures 4, 5, and 6).

Results for colony composition are given in Table 1. The results for this study are very similar to Seelinger and Seelinger (1983) and Napela (1984). Many colonies that were found consisted of at least one adult and Class 1 and 2 nymphs. Most class 3 and 4 nymphs were found alone or with each other but occasionally they were found with an adult. They were never found with a pair of adults. Table 2 also has the data from the tree diameter of where the wood cockroaches were found. The mean diameter was 8.73 and the standard deviation was 3.629+/- 8.73.

Table 2 summarizes the results of the colony composition. Of the 21 colonies examined, 19% had two or more adults with no nymphs. One adult alone was found 19% of the time and Class 3 and 4 nymphs alone were found 19% of the time. The most frequent colony observed consisted of two or more adults with nymphs, which were found 28.6 % of the time. The last category was an adult alone with nymphs, which occurred 14.4% of the time.

Seven of the 21 colonies had Class 1 nymphs. Two of those seven had Class 2 nymphs with them. Class 1 nymphs were always found with 1 or more adults (Figure7). All but two of the colonies with Class 2 nymphs were found with at least one adult. None of the Class 3 and 4 nymphs were found with adults paired; however, (Figure 7) some were found with only one adult. Pre-adults were never found in colonies containing class 1 nymphs.

Other insects were found in the logs with roaches and in logs without roaches. There were millipedes, centipedes, beetles, ants, termites, and granddaddy longlegs. There were five beetles found

and collected. They were Carabid beetles, in the family Carabidae, and also an unknown larva, possibly from the same family. Termites and ants were not found in the same logs that had the roaches.

## **DISCUSSION:**

This study was conducted to look at many aspects of the habitat of *Cryptocercus punctulatus*. We went to different elevations to determine the altitude where *Cryptocercus punctulatus* was predominantly found. At the different altitudes there was no apparent difference found in the numbers of the roaches. For example we found around 60 at the highest elevation at the Pipestem tower and we found around 40 at Concord. In another study, it was also found that the elevation did not play a factor in where the roaches were found (Nalepa 2003).

The size and type of the logs were examined but the sample size was low and no reliable conclusions could be drawn. Some cockroaches were found in logs with diameters ranging from 5cm to 36cm but many cockroaches were found in logs that had been destroyed and just parts of the bark were remaining. The species of log was also hard to decide as most of the logs we sampled were decomposed. However, with the type of forest we have here in southern West Virginia we can assume that most of the cockroaches were found in hardwood logs. The wood cockroaches have been found in many different types of logs and from many different parts of the world (Nalepa 2003).

The results from Figure 6 showed that *Cryptocercus punctulatus* class one and class two nymphs were always found with at least one adult. In another study, all class one and two nymphs were with at least one adult and had both adults in most cases (Lumoa 1999). Nalepa (1984) also reported that class one and two nymphs were found with an adult present. Nalepa (1984) concluded that mated pair's stay together for life and raise their offspring. The results in my study showed that in half of the colonies paired adults were present.

*Cryptocercus punctulatus* has a long developmental time of six years for the cockroaches to reach maturity (Cleveland et.al 1934). Figure 7 shows that colonies with Class 3 and Class 4 nymphs had one

or no adults present. The colonies with older nymphs and pre-adults are several years older than the younger nymphs. There are several reasons why young nymphs are with the adults and older colonies had only one or no adults. The adults could have died off or they might have just been missed by my observation. Most adults die before their nymphs make it to pre-adults (Nalepa 1983). The class 3 and class 4 nymphs may be alone because they are capable of taking care of themselves (Nalepa 1984).

The young nymphs may be found with the adults because of the required parental care. The adults show great parental control care over them. The young nymphs are born with no protozoa in them and the adults transfer the protozoa to them by proctodeal feeding (Nalepa 1983). The adults also provide protection to the young nymphs. Furthermore, the adult cockroach makes tunnels inside the logs so the nymph can get around (Nalepa 2002).

A couple of the adult cockroaches were found alone. These cockroaches were probably looking for a mate so that they could start their own colony. The cockroaches also could have been the only ones that had survived, however it is more likely that they are looking for a mate.

The logs we examined had many animals, and insects in them like ants, beetles, bugs, and termites. When we found the cockroaches, there were no termites or ants in the logs with *Cryptocercus punctulatus*. Walker (1996) found them together but found that they are better apart so they can get more food. Nalepa et.al. (2002) also found subterranean termites but rarely in the same log as the wood cockroaches. Five adult Carabid beetles in the family Carabidae were found and collected while we were out sampling. Many insects were found in the logs where the wood roaches were found like millipedes, centipedes, and granddaddy longlegs. No other organisms were observed living in the wood along with the wood cockroaches. One unknown larva was found and is believed to be with the family Carabidae.

This research provides a foundation for future research. The life cycles of these wood cockroaches could be examined by bringing them into the laboratory and keeping them for observation. More cockroaches could be collected so that there will be a bigger sample size. I looked at the

cockroaches through the summer but someone could look at them through the winter and see how the temperatures affect them. I did not determine the sex of the cockroaches so someone could look to see which sex cares for the nymphs. A person could check the abundance of the wood cockroaches around here compared to other sites in Virginia and other parts of West Virginia. When I started my research, I wanted to know the exact moisture content of the logs to see how moist it really has to be for wood cockroaches. Someone could get a better moisture meter and check for the exact amount of moisture in the logs.

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Figure 1 Map showing the research vicinity of both Concord University and Pipestem Stat Park and their county lines.

Figure 2 Research study area at Concord University. The area in yellow is the area I studied at Concord University and the actual sample sites are marked.

Figure 3 Research study areas at Pipestem State Park, river, lake, and tower. The area in red represents Pipestem and the spots in yellow represent the research vicinity of all 3 places at Pipestem State Park.

Figure 4 Research study area at Pipestem State Park tower. The area in yellow is the area I studied at the tower at Pipestem State Park and the actual sites are marked. Each site had one colony.

Figure 5 Research study area at Pipestem State Park river. The area in yellow is the area I studied at the river at Pipestem State Park and the actual sites are marked. Each site had one colony.

Figure 6 Research study area at Pipestem State Park lake. The area in yellow is the area I studied at the lake at Pipestem State Park and the actual sites are marked. Each site had one colony.



Table 1: Composition of the colonies located including the number and classification of each wood roach along with the diameter of the tree they were f

	Classification	Adults	Class1	Class2	Class3	Class4	Tree (in) diameter	Tree (cm) diameter
6-7-05	<b>1A:</b> Colony 1	2	9				12	30.48cm
	<b>1B:</b> Colony 2 (same log as 1)	1		4	8	1	12	30.48
6-23-05	<b>2A:</b> Colony 1	3	3	14			10	25.4cm
	<b>2B:</b> Colony 2 (same as 6-7-05)			3	6		12	30.48cm
	<b>2C:</b> Colony 3	1					15	38.1cm
	<b>2D:</b> Colony 4	6					8	20.32cm
7-11-05	<b>3A:</b> Colony 1	2					14	35.56cm
7-19-05	<b>4A:</b> Colony 1	3	5	9			5	12.7cm
	<b>4B:</b> Colony 2 (same log as 1)	2		13			5	12.7cm
	<b>4C:</b> Colony 3	2					11	27.94cm
8-1-05 Pipestem Tower	<b>5A:</b> Colony 1				2		9	22.86cm
	<b>5B:</b> Colony 2			13			Unknown	
	<b>5C:</b> Colony 3	1	24				10.5	26.67cm
	<b>5D:</b> Colony 4 (same log as 3)	2	11				10.5	26.67cm
	<b>5E:</b> Colony 5	1					9	22.86cm
	<b>5F:</b> Colony 6	2	8				10	25.4cm
8-22-05 Pipestem River	<b>6A:</b> Colony 1	1					2	5.08cm
	<b>6B:</b> Colony 2	1					5.5	13.97cm
8-25-05 Pipestem Lake	<b>7A:</b> Colony 1	1			6	4	6	15.24cm
	<b>7B:</b> Colony 2				4	3	4	10.16cm
	<b>7C:</b> Colony 3 (same log as 2)	2					4	10.16cm
Total Number		33	60	56	26	8	Mean: 8.73	Standard Deviation: 3.629+/- 8.72

## **Comparison of the Antibacterial Activity of Extracts Prepared from *Ambrosia trifida* and *Ambrosia artemisiifolia***

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### **Abstract**

As part of a larger study of the bioactive constituents of local flora, the antibacterial properties of *Ambrosia trifida* and *Ambrosia artemisiifolia* were compared. A mid 20<sup>th</sup> century ethnobotanical study of the Cherokee Indians reported that both species have a history of use as antibacterial agents.<sup>xvii</sup>

Plant species were collected from southern West Virginia and southwestern Virginia. Several extraction methods were explored and the extracts obtained were tested for antibacterial activity against a nine-organism panel of bacteria. Activity was assayed by using the disc diffusion method for screening purposes and serial dilution to provide an estimated minimum inhibitory concentration (MIC).

Results show that different extraction methods produced extracts with differing antibacterial effectiveness. Exposure of the extracts to heat was correlated with decreased antibacterial activity. Both *Ambrosia* species showed similar antibacterial properties against a broad range of bacterial species.

## Introduction

The use of natural products offers many new and exciting possibilities in drug therapy and treatment. With the pharmaceutical field consistently in search of new medications, many are investigating novel sources of medicines. Rather than attempting to engineer a compound from scratch, more are turning to nature. Natural products have been used as medicinal agents by native peoples for centuries and are still being used by the modern pharmaceutical industry. The World Health Organization states that twenty five percent of modern medicines are made from plants that were used as “traditional medicines.”<sup>xviii</sup> Many of the drugs that are on the market today were derived from natural sources. Some examples of these products include aspirin, digitoxin, morphine, quinine, and pilocarpine. In the 1950s, the discovery of two plant derived antileukemic agent’s vinblastine and vincristine increased the interest in natural products.<sup>xix</sup> A more recent study found that almost 30% of the medicines launched in 2000 as well as many of the top prescription drugs dispensed from 2000-2002 were from natural products. In addition, there have been many new natural product and natural product derived drugs launched in the US, Europe, and Japan through 2000 – 2003.<sup>xx</sup>

Throughout history, the Native Americans offered much advancement in the lives of settlers, whether it was the development of crops or the treatment of newfound illnesses. Their culture has been developing the knowledge of traditional medicines for many years. These potential historic leads should be researched and could possibly yield a new modern treatment. This research project began by reviewing documents and books discussing forms of traditional medicines. A mid 20<sup>th</sup> century ethnobotanical study of the Cherokee Indians was the initial starting point for this study. The masters thesis discussed the use of Asteraceae that had a therapeutic reputation for infections and other medical conditions.<sup>1</sup> In addition to humans using Asteraceae other mammals appear to have stumbled upon their therapeutic importance. According to Wrangham and Nishida (1983), wild chimpanzees selectively pick and swallow leaves of certain species of the family Asteraceae, suggesting that they are consumed for a

medicinal purpose.<sup>xxi</sup> Asteraceae have the ability to produce many types of bioactive compounds such as polyacetylenes.<sup>xxii</sup> Dithiins, an extensively studied class of bioactive compounds, have a history of antibacterial uses and is present in the Asteraceae.<sup>xxiii</sup> In the mid 1960s, 1, 2 dithiins were first discovered in the Compositae (Asteraceae) family. 1, 2-dithiins are six-membered heterocycles that are characterized by a disulfide linkage between two adjacent benzene rings. The 1, 2 dithiins are deeply colored and are photosensitive.<sup>6</sup> All naturally occurring 1, 2 dithiin compounds isolated to date contain acetylene or polyacetylene side chains in the C-3 and C-6 positions. More specifically, thiarubrine A and thiarubrine B both show antimicrobial, antitumor, and nematocidal activity.<sup>4,xxiv,xxv,xxvi</sup> It has been hypothesized that the novel 1,2-dithiin ring system is the pharmacophore component, that may be responsible for the activity of the thiarubrines. This suggests that similar structures without the polyacetylene side chains may have activity.<sup>6</sup>

The genus *Ambrosia* (common name ragweed), a member of the Asteraceae family, is reported to have a variety of medicinal uses. Specifically, *Ambrosia trifida* (giant ragweed) has been used for folk medicinal purposes and has documented antibacterial properties.<sup>5</sup> Furthermore, herbal medicine has also referred to *A. trifida* as an antiseptic and as a treatment for intestinal worms.<sup>8</sup> It was said to aid in healing infected toes if the juice squeezed out of the leaves was applied to the affected area.<sup>1</sup> Additionally, an *A. trifida* leaf tea was reported to reduce fever. In the case of hives, a leaf of *Ambrosia* was rubbed on the sores as a cure.

Analysis of *Ambrosia trifida* components has indicated that the 3,6-disubstituted, 8-electron, antiaromatic 1,2-dithiin (1,2-dithia-3,5-cyclohexadiene) ring has antibacterial properties.<sup>8</sup>

This study will focus on determining if the antibacterial properties of *Ambrosia artemisiifolia* and *Ambrosia trifida* are similar. Additionally, it will examine the affect of various extraction methods on the antibacterial properties of the resultant extracts. This is designed to be a preliminary study that will lay

the foundation for more extensive and specific investigations into the potentially medicinally active components of *A. trifida* and *A. artemisiifolia*.

## Results and Discussion

Results show that both species of *Ambrosia* have antibacterial activities against several types of bacteria. Both *A. artemisiifolia* and *A. trifida* showed antibacterial properties against *Bacillus cereus*, *Corynebacterium xerosis*, and *Staphylococcus aureus*. Table 1 is a summary of the first screening test (Kirby Bauer Disc Diffusion Method). This test was the extraction of both samples DP-020 (*A. artemisiifolia*) and DP-019 (*A. trifida*). It consisted of two extraction techniques, Soxhlet extraction and 40°C shaker bath extraction. DP-020 (Soxhlet extraction) was tested against one type of bacteria as a comparison of extraction techniques with the same plant species. As shown below, (Table 1) DP-020 shaker extract had an inhibitory effect against *Bacillus cereus*, *Corynebacterium xerosis*, and *Staphylococcus aureus*. DP-019 shaker extract was effective against *Bacillus cereus*, and *Corynebacterium xerosis*. Extract DP-020 Soxhlet was effective against *Bacillus cereus*.

**Table 1 Kirby Bauer Screening Test 1**

Extraction:	Zone of Inhibition		
	DP-020-3 Shaker 40°C	DP-019-3 Shaker 40°C	DP-020-2 Soxhlet 60°C
Bacteria			
<i>Bacillus cereus</i>	12 mm	10 mm	9 mm
<i>Corynebacterium xerosis</i>	11 mm	8 mm	N/A
<i>Escherichia coli</i>	0 mm	0 mm	N/A
<i>Klebsiella pneumoniae</i>	0 mm	0 mm	N/A
<i>Mycobacterium smegmatis</i>	0 mm	0 mm	N/A
<i>Pseudomonas aeruginosa</i>	0 mm	0 mm	N/A
<i>Staphylococcus aureus</i>	12 mm	0 mm	N/A
<i>Salmonella typhimurium</i>	0 mm	0 mm	N/A
<i>Streptococcus epidermidis</i>	0 mm	0 mm	N/A

Table 2 shows the results from the trial, which tested different portions of both plant species. The test consisted of individual trials with the roots, stems, and leaves of each plant. All plant extracts were completed with the same extraction method. This particular screening method resulted in four zones of inhibition. An extract of *A. trifida* root against *S. aureus* yielded a 40 mm/mg zone. Three positive results of *Ambrosia artemisiifolia* were observed. An extract of *A. artemisiifolia* root yielded a 12.22 mm/mg zone against *B. cereus*. A leaf extract of the same plant yielded a 2.43 mm/mg zone against *S. aureus* and a 4.55 mm/mg zone against *B. cereus*.

**Table 2 Kirby Bauer Screening Test two Cold Extraction.**

	Zone of Inhibition (mm/mg)		
	<i>A. trifida</i>		
Bacteria	Root	Stem	Leaves
<i>B. cereus</i>	0	0	0
<i>C. xerosis</i>	N/A	N/A	N/A
<i>E. coli</i>	N/A	0	0
<i>M. smegmatis</i>	N/A	N/A	N/A
<i>P. aeruginosa</i>	0	0	0
<i>S. aureus</i>	40 mm/mg	0	0
<i>S. typhimurium</i>	0	0	0
	<i>A. artemisiifolia</i>		
	Root	Stem	Leaves
<i>B. cereus</i>	12.22 mm/mg	0	4.55 mm/mg
<i>C. xerosis</i>	N/A	N/A	N/A
<i>E. coli</i>	0	0	0
<i>M. smegmatis</i>	N/A	N/A	N/A
<i>P. aeruginosa</i>	0	0	0
<i>S. aureus</i>	0	0	2.43 mm/mg
<i>S. typhimurium</i>	0	0	0

Table 3 summarizes the last test, which used both plant species. Extractions consisted of whole plant (*A. artemisiifolia*) shaker and Soxhlet procedures. This test resulted in two positive hits a 1.02mm/mg against *B. cereus* and 0.81mm/mg zone again *S. aureus*.

**Table 3 Kirby Bauer Screening Test**

	Zone of Inhibition (mm/mg)	
	<i>A. trifida</i>	
Bacteria	Shaker-19	Soxlet-19
<i>B. cereus</i>	0	0
<i>C. xerosis</i>	N/A	N/A
<i>E. coli</i>		
<i>M. smegmatis</i>	N/A	N/A
<i>P. aeruginosa</i>		
<i>S. aureus</i>	0	0
<i>S. typhimurium</i>		
	<i>A. artemisiifolia</i>	
	Shaker-20	
<i>B. cereus</i>	1.02 mm/mg	
<i>C. xerosis</i>	N/A	
<i>E. coli</i>		
<i>M. smegmatis</i>	N/A	
<i>P. aeruginosa</i>		
<i>S. aureus</i>	0.81 mm/mg	
<i>S. typhimurium</i>		

## Conclusions

Both plant species showed inhibition against *Bacillus cereus*, *Corynebacterium xerosis*, and *Staphylococcus aureus*. It was also concluded that certain extraction processes have a negative affect on the extract. The extracts that used a higher temperature, appeared to degrade the active constituents of the extract, therefore decreasing its ability to inhibit bacteria (Table 4).

**Table 4 Summary of Positive Hits**

Plant	Extraction	Bacterium	Zone
<i>A. artemisiifolia</i>	Leaves - Shaker	<i>B. cereus</i>	12 mm
<i>A. trifida</i>	Leaves - Shaker	<i>B. cereus</i>	10 mm
<i>A. artemisiifolia</i>	Leaves - Soxhlet	<i>B. cereus</i>	9 mm
<i>A. artemisiifolia</i>	Leaves - Cold	<i>B. cereus</i>	4.55 mm/mg
<i>A. artemisiifolia</i>	Root - Cold	<i>B. cereus</i>	12.22 mm/mg
<i>A. artemisiifolia</i>	Leaves - Shaker	<i>B. cereus</i>	1.01 mm/mg

<i>A. artemisiifolia</i>	Leaves - Cold	<i>S. aureus</i>	2.43 mm/mg
<i>A. artemisiifolia</i>	Leaves - Shaker	<i>S. aureus</i>	12 mm
<i>A. artemisiifolia</i>	Leaves - Shaker	<i>S. aureus</i>	0.81 mm/mg
<i>A. trifida</i>	Root - Cold	<i>S. aureus</i>	40 mm/mg
<i>A. artemisiifolia</i>	Leaves - Soxhlet	<i>C. xerosis</i>	11 mm
<i>A. trifida</i>	Root - Shaker	<i>C. xerosis</i>	8 mm

The first four tests were against the same type of bacteria (*B. cereus*). The first two lines are shaker extractions of each plant species, which are at ~ 40°C they produced 12 mm (*A. artemisiifolia*), and 10 mm (*A. trifida*) zones of inhibition. When one compares the Soxhlet extraction (which has the highest heat) of *A. artemisiifolia* to the shaker and cold extraction of the same plant the Soxhlet has lower zones of inhibition. This allows us to conclude that the extraction procedure with higher amounts of heating lowered the activity of the plant extract. The fact that there is only one positive hit for bacterial inhibition using a Soxhlet extraction further illustrates this conclusion. It is hypothesized that several of the Soxhlet extractions degraded the antibacterial properties of the extract to the point of not being able to inhibit bacteria growth thus showing no inhibition. This is very important for future research because we now know that if we use a high heat extraction and get no inhibition the extracts ability to inhibit growth may have been degraded beyond the point of detection. The trend of similarities between the antibacterial properties and the heat degradation can be seen throughout the results.

Neither *Ambrosia trifida* nor *Ambrosia artemisiifolia* showed inhibitory affects for any of the following bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, or *Staphylococcus epidermidis*. Nevertheless, both *Ambrosia artemisiifolia* and *Ambrosia trifida* have similar antibacterial properties. Future research would be to retest shaker and cold extracts using all new stock plates of bacteria. One of interest would be to test the extracts using a Supercritical Fluid Extractor to observe the zones of inhibition compared to the previously collected shaker extractions.



## Experimental Section

*Ambrosia trifida* and *Ambrosia artemisiifolia* were collected in southern West Virginia and western Virginia during June – September 2005. A field notebook was used to record all essential information, which included site location, soil conditions, ecological habitat, date of collection, and plant identity. A GPS system was incorporated to identify the location and a digital camera was used to record the plants' original habitat. Each plant specimen and soil sample collected was given a unique identification code that was used to identify it through the entire project. Plant and soil collections were placed in Zip-Lock™ plastic bags on ice for transportation to permanent storage at -20°C to minimize enzymatic changes that may affect the naturally occurring metabolites.

Preparation procedures were identical for both plants regardless of the extraction method. The Soxhlet extractions were performed for an average of 7 hours a day. Plant samples were selected and chopped into small pieces. Leaves and stems on average were chopped into sizes no larger than 8 mm. Roots and larger stems were on average no larger than 16 mm. Masses of the samples were determined.

Samples, which used deionized water, were placed in a blender at room temperature on high speed for three, five-minute intervals. This slurry was filtered through a cellulose thimble, which allowed the plant material to be retained. The blender container was rinsed with deionized water and the cellulose thimble was carefully placed down into the Soxhlet. A round bottom flask was loaded with the remaining de-ionized water with approximately four boiling chips.

For a methanolic extraction, plant material was chopped manually into smaller pieces as previously described. This plant material was placed into a sonicator with a beaker filled with approximately 350 ml with methanol. The beaker was placed in the sonication device and deionized water was added around the beaker. The plant material underwent three; five-minute cycles allowing a five-minute soak in between each cycle. This plant material and solvent was poured through a cellulose

thimble and collected in a beaker. The thimble was placed into the Soxhlet and the remaining solvent was placed in a round bottom flask with a few boiling chips.

Following deionized water or methanol extraction, the round bottom flask was placed in the heating element, which was filled with sand and packed around the round bottom. The Soxhlet was placed down over the flask and the condenser down upon the Soxhlet extractor. The entire apparatus was wrapped in aluminum foil to provide some protection from ambient light. The Soxhlet extractor ran for the desired length of time, broken down and the extraction vessel was stored at 4° C or the extract was moved to the rotary evaporator to be dried.

A Rotary Evaporator was used to remove the extraction solvent. The solvent was filtered with an aspirator through a filter funnel and filter paper. The evaporation process was performed until the round bottom flask was completely dry. Extracts stored at 4° C were sealed with Parafilm and wrapped in aluminum foil.

Prior to the addition of deionized water or methanol, the mass of the round-bottom flask containing crude extract was obtained. The minimum amount of solvent was placed in the flask and swirled until the majority of the dried extract went into solution. Using sterile pipets, the solution of solvent and extract was drawn out of each flask and placed in a sterile test tube. Once the flask was completely dry, the mass of the flasks was obtained again and the amount of extract dissolved in the solvent was determined.

A nine-organism panel of bacteria (Table 5) was chosen to represent a wide diversity of organism types. The nine organisms selected were *Staphylococcus aureus*, *Streptococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Bacillus cereus*, *Corynebacterium diphtheriae*, *Mycobacterium smegmatis*, and *Pseudomonas aeruginosa*.

**Table 5 Nine-organism panel of bacteria**

Bacterium	Gram Stain	Description	
<i>Bacillus cereus</i>	positive	rod	food poisoning

<i>Corynebacterium xerosis</i>	positive	rod	diphtheria
<i>Escherichia coli</i>	negative	rod	UTI
<i>Klebsiella pneumonia</i>	negative	rod	UTI, pneumonia
<i>Mycobacterium smegmatis</i>	acid fast	rod	disease in animals
<i>Pseudomonas aeruginosa</i>	negative	rod	UTI
<i>Salmonella typhimurium</i>	negative	rod	salmonellosis
<i>Staphylococcus aureus</i>	positive	cocci	skin lesions
<i>Streptococcus epidermidis</i>	positive	cocci	skin lesions

All stock bacteria were purchased from Presque Isle Cultures, PA and grown in house for the antibacterial testing. Tryptic soy broth was prepared according to manufacture's instructions and autoclaved (121°C X 20min, 15psi). Bacterial culture tubes received three milliliters and turbidity test tubes received 2.5 mL of broth. For trypticase soy agar plates, 7.5 grams of agar and 15 grams of tryptic soy was added to 500 mL deionized water. The media was sterilized as previously described. The sterile medium was poured into sterile petri plates and was allowed to cool.

For the turbidity tests, an overnight culture of the bacterium to be tested was prepared and incubated overnight at 37° C. There was 2.5 mL of each extract added to nine test tubes containing 2.5 mL of broth. Each of these tubes received a 25-uL inoculum of the overnight culture. These tubes were then incubated overnight at 37° C and checked for growth (turbidity) the next day.

The Kirby Bauer disc diffusion method was used later in the research study as a screening method. Overnight cultures of the appropriate bacteria were prepared as previously described. Filter disks were then prepared by adding a methanolic plant extract drop-wise until a desired mass was obtained. Using a sterile cotton swab, the plates were inoculated with the appropriate culture and the extract-containing disc was placed on the plate using sterile forceps. Plates were incubated overnight and examined for a zone of inhibition. Estimations of the MIC (minimum inhibitory concentration) value for extracts that produced zones of inhibition were obtained as described below.

The MIC is an estimation of the concentration of extract it takes to inhibit growth of a particular bacterium. Two-fold serial dilutions of extract from 1:2 to 1:512 were prepared. The procedure varied from the turbidity tests in that 2.5 mL of extract was added to the first tube containing 2.5 mL broth (1:1), the tubes were mixed then 2.5 mL of tube number one was added it to tube number 2 containing 2.5 mL of broth (1:2) This procedure was repeated until reaching a concentration of 1:512.

### **Acknowledgements**

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Running head: PHEROMONES

Pheromones and Their Effects on the Sociosexual Behaviors of Lesbians

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**Abstract:**

Human pheromones can have various effects on human behavior. Past research has demonstrated that synthesized human sex-attractant pheromones increase sociosexual behaviors in heterosexual and homosexual men and in heterosexual women. However no research has been conducted on homosexual women. This study sought to determine whether a synthesized human female pheromone would increase the sociosexual behavior of 8 homosexual women (aged 18-40 yrs). Participants kept daily behavioral records over the course of eight weeks. During the last six weeks, participants applied either a pheromone or placebo solution three times daily, at least five times per week. The results demonstrated pheromone users increasing in stimulation by partner to orgasm over baseline more than placebo users.

## Pheromones and Their Effect on the Sociosexual Behaviors of Lesbians

In 1959, Karlson and Luscher discovered a class of biologically active chemical substances, and termed them 'pheromones.' They defined pheromones as "a substance excreted by an animal, to the outside of that individual, which is then received by another individual, classically of the same species, which then elicits some behavioral or developmental response related to the survival of the species." Today, most researchers more simply define pheromones as chemicals released by one individual of a species that, when detected by another individual of the same species, elicit a specific behavioral or physiological response.

Since the introduction of this term to scientific literature, numerous studies have been conducted to determine the physiological effects pheromones have on a number of species. Although research had demonstrated pheromones being present in animals for years, it was long believed that they were not present in humans because the organ responsible for detecting their presence was thought to be absent in humans.

The controversy over defining pheromones was a battle, but arguments over the functionality of the organ that detects them in humans became a war. Strong evidence suggests that animals have two different neural pathways that are sensitive to different types of chemicals: the olfactory epithelium used to detect odors and the vomeronasal organ (VNO) used to detect pheromones (Keverne, 1999). However, in humans the VNO had been thought to be vestigial for years. In 1991, Garcia-Velasco and Mondragon identified a VNO as present and functional in human anatomy. Further supporting this finding, Monti-Bloch and Grosser (1991) demonstrated an electrical potential in the human VNO by puffing putative pheromones into the nose. As a result, researchers now believe the human VNO acts as a "sixth sense" because most pheromones are odorless and therefore operate without detection by the olfactory epithelium.

Even before the existence of human pheromones and a functional VNO was established, research suggested the existence of pheromone like substances in humans. McClintock's (1971) classic study showed that women in frequent contact with one another tend to become synchronized in their menstrual cycle. Before this groundbreaking study, there was no evidence that social interactions could have an effect on women's menstrual cycles. Even then McClintock was unable to justifiably conclude what was causing the reaction.

Preti and his colleagues (1987) studied compounds derived from axillary secretions that influence menstrual timing based on concentration during each phase of menstruation. The levels of many compounds were measured in each phase of the women's menstrual cycle. He discovered that the concentrations in each stage were different and influenced menstrual timing. Because of their effect on menstrual timing, Preti assumed that certain compounds were acting as pheromones. In a later study on menstrual synchrony, Stern and McClintock (1998) discovered two distinct steroids that have physiological effects; one accelerates ovulation and shortens the menstrual cycle, and one that does just the opposite. Each steroid is present in different phases of women's menstrual cycle. The accelerating steroid is present during the follicular stage, while the delaying steroid is secreted during the ovulation stage. These steroids were the same that Preti (1987) had discovered years earlier. These steroids were termed human pheromones because of the effects they evoked. Although the same substance can act as a pheromone in a variety of species, it cannot evoke the same effect outside of that species. For example, Androstenol acts as a pheromone in boars. It is responsible for inducing the onset of puberty in boars (Kirkwood et al., 1981), while in humans it accelerates a woman's menstrual cycle (Stern and McClintock, 1998). Although this steroid acts as a pheromone in boars, it has no effect on human psychophysiology (Jennings-White, 1995; Jacob et al., 2002). Pheromones can have different types of effects on the human body ranging from biological to behavioral.



As more is discovered about the different types of pheromones and their effects, the mechanisms by which they are detected are also being explored. Clive Jennings-White (1995) studied the functional potential of the human VNO. Similar to Monti-Bloch and Grosser's (1991) work, White inserted a multifunctional miniprobe in the VNO of participants to determine if certain steroids collected from skin secretions acted as pheromones detected via the VNO. The sample steroids were collected from both humans and animals, and included those steroids studied before in Stern and McClintock's (1998) research on menstrual synchrony. Of all the steroids tested, only two were significant in affecting the VNO alone: androstadienone and estratetraenol. Male heterosexuals evidenced great potentials in the VNO to estratetraenol (EST), which is secreted by females, while female heterosexuals demonstrated great response to androstadienone (AND), which is secreted by males (Jennings-White, 1995). Although androstadienone is an odorous compound, the miniprobe prevents detection of the odor which disables the olfactory epithelium from detecting the odor. Therefore, any response to the substance would act via the VNO. On the other hand, estratetraenol is completely odorless. White determined that these human steroids acted as pheromones because the VNO was highly reactive to them. This research supports the claim that pheromones are species specific and elicit different responses in different species. This study gave rise to research on the behavioral effects of human pheromones.

Shortly after behaviorally effective, specifically effective as attractants, pheromones were found in humans researchers began to measure the sexual behaviors these pheromones affected. While Jennings-White (1995) studied the effects of pheromones on the VNO, Cutler, Friedmann and McCoy (1998) studied the effects of pheromones on sociosexual behavior in heterosexual men, and McCoy and Pitino (2001) studied those effects in heterosexual women. Both studies required the participants to keep a daily log of six sociosexual behaviors: "petting/affection/kissing, formal dates, informal dates, sleeping next to a romantic partner, sexual intercourse and masturbation." A synthesized form of androstadienone (AND) and estratetraenol (EST) was added to participants' aftershave and perfume. It was found in both

studies that subjects using pheromones significantly increased most of their behaviors. This data suggests that the pheromone used, acted as a sexual attractant and increased heterosexual men's attractiveness to heterosexual women.

Other studies examining the effects of AND and EST on subject's mood and sexual arousal demonstrated an increase in positive moods, reduced nervousness, tension and other negative feeling states after exposure to AND and EST (Grosser et al., 1999, Jacob et al., 2000, Jacob et al., 2001, Lundstrom et al., 2003). Another study demonstrated an increase in sexual arousal after exposure to the substances (Bensafi et al., 2003). These studies found similar results suggesting these specific pheromones evoke sexual behavioral effects on heterosexual men and women. However there was no research on homosexual exposure to these pheromones until recently.

Savic, Berglund and Lindstrom (2005) examined heterosexual men and women, and homosexual men to determine a possible link between hypothalamic neuronal processes and sexual orientation. The VNO navigates these processes on a neural path separate from the olfactory epithelium. After examining the PET scan and MRI results, they concluded that when heterosexual women and homosexual men were exposed to AND similar regions of the brain, the anterior hypothalamus, increased in activity. In heterosexual men, the same area was activated by exposure to EST. Thus, Savic, Berglund, and Lindstrom successfully demonstrated a link between a sexual attractant pheromone (AND) and sexual orientation in males. No research has yet been completed on the physiological effects of EST on homosexual women at this time although one could hypothesize similar findings.

The current study addressed that hypothesis by closely replicating the methodologies of Culter, Friedmann and McCoy (1998) and McCoy and Pitino (2001). It measured the effect a synthesized pheromone called estratetraenol (EST) on homosexual women.

## Methods

### *Participants*

Eight participants were recruited. Participants were female, predominantly homosexual, between the ages of 18 and 40, be in self-reported good health, and also actively involved in a romantic relationship(s), either monogamous or with multiple partners.

### *Materials*

*Symptom Checklist-90 Revised (SCL-90-R; Derogatis, 1975)*. Participants were asked to complete this measure of overall psychological distress which yields scores on 9 scales; somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. Participants who have a T-score of more than one and a half standard deviations from the mean of the group were excluded.

*Kinsey Heterosexual-Homosexual Rating Scale (Kinsey, 1948/1998)*. Participants were required to rate their sexuality on the Kinsey heterosexual-homosexual rating scale: a score of one being exclusively heterosexual and a score of six being exclusively homosexual. Those who rated themselves as below 5 (predominantly homosexual, only incidentally heterosexual) were excluded.

*Pheromones*. The pheromone used will be 1,3,5(10), 16-estratetraen-3-ol obtained from Steraloids (Newport, RI). Approximately 9 milligrams of estratetraenol was added to 15 milliliters of ethanol/clove oil. Contents will be poured into four ounce spray bottles. Approximately 0.0758 milligrams of pheromone will be delivered with each spray, totaling 0.22 milligrams per day.

### *Procedure*

*Subject Recruitment*. Flyers were posted on two university campuses, and neighboring nightclubs and bars. The two universities are demographically different, but are located in the South Eastern U.S. The flyers asked lesbians to participate in a study involving human pheromones and sociosexual behavior.

*Baseline Meeting.* Participants were required to meet individually with the experimenter for an initial meeting. In this meeting the experimenter described the experimental procedure. Participants who were willing to continue were asked to sign the consent form, complete the SCL-90-R, and complete the Kinsey heterosexual-homosexual rating scale which was included in a demographic questionnaire. They were then given instructions on recording occurrences of their sociosexual behaviors in the provided notebooks for the two week baseline period. A sheet listing definitions of the target behavior was provided with the notebooks (see Appendix F). They were instructed to record the occurrences of behaviors in their notebook and mail the hard copies (pages) of data to the experimenter for the first two weeks.

*Group Assignment.* In order to maintain a double blind a research assistant randomly assigned the participants to the placebo and the pheromone groups. This assignment was done by the research assistant writing each participant's number on a bottle of the assigned solution. The research assistant provided a list of the participant's group assignments to the experimenter in a sealed envelope after data collection was complete. The experimenter distributed the bottles out blindly at the treatment meeting according to number assignment.

*Treatment Meeting.* After the two week baseline period, participants met individually with the experimenter again to review the protocol for the study. Participants received bottles with either ethanol/clove oil (placebo) solution or pheromone/ethanol/clove oil solution. They were instructed to use one spray of the solution three times daily, at one of the suggested application times listed on the handout (see Appendix E) at least five times per week. Participants were instructed to stop solution application after four weeks. They were again instructed to record the behaviors in the same manner for six weeks, and were given envelopes to mail the data to the experimenter when collection was complete.

*Behavior Record.* The participants were instructed to record occurrences of each behavior daily in a pocket sized notebook for eight weeks; two weeks baseline; six weeks treatment; two weeks baseline.

Each page of the notebook was labeled with the number that identifies that participant. The six behaviors measured were: petting/affection/kissing, formal dates, informal dates, sleeping next to a romantic partner, stimulation by partner to orgasm, and self-stimulation to orgasm. On a scheduled day at the end of each week, participants were instructed to remove the sheets with data for that week, and place them in an envelope labeled by week number.

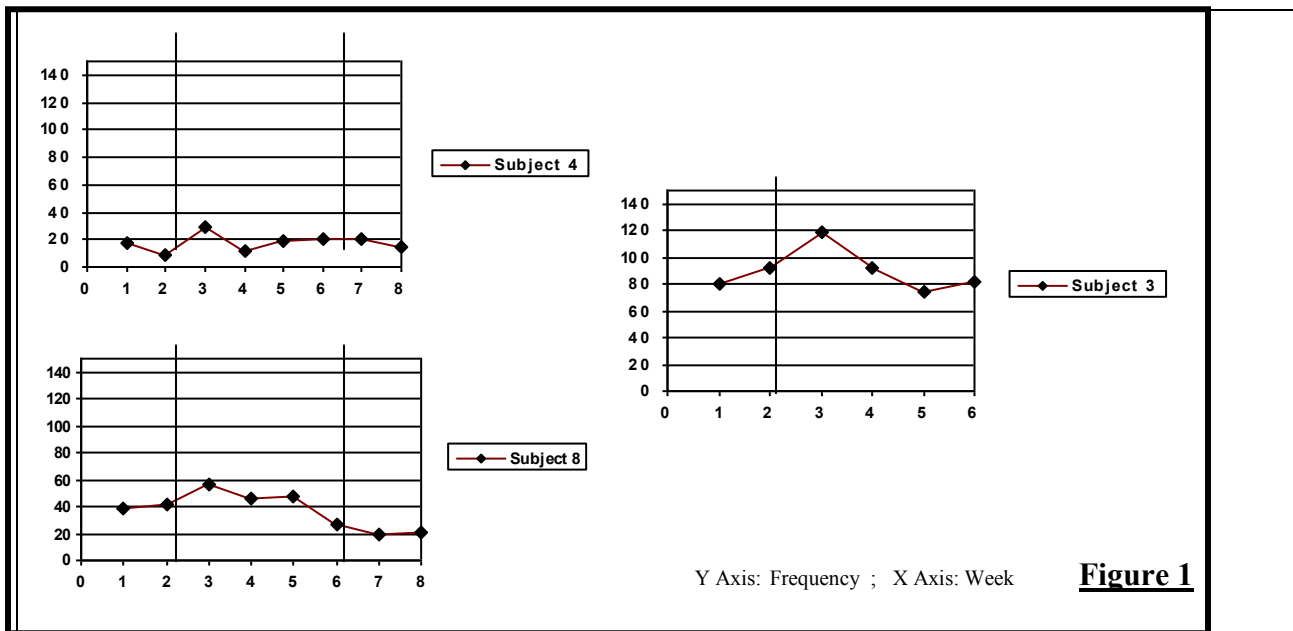
### Results

It was hypothesized that pheromone users would increase over baseline in petting/affection/kissing, formal dates, informal dates, sleeping next to a romantic partner, and stimulation by partner to orgasm. The hypothesis was partially supported in terms of behavior-specific increases. Y

Axis: Frequency

#### *Placebo Users*

Figure 1 illustrates petting/affection/kissing behavior for Placebo users.



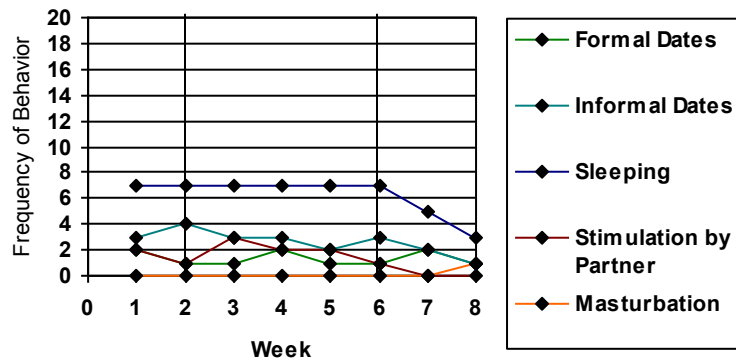
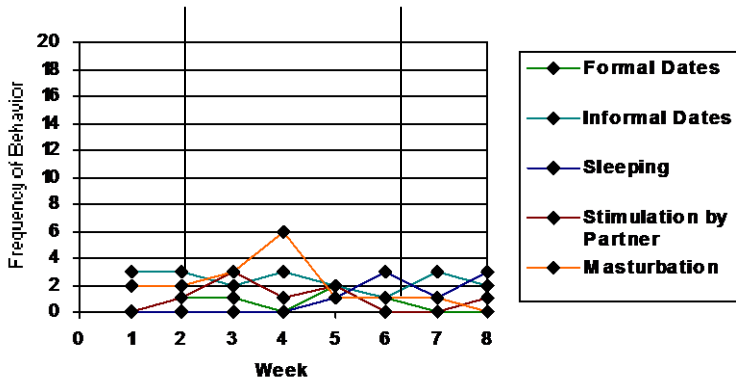
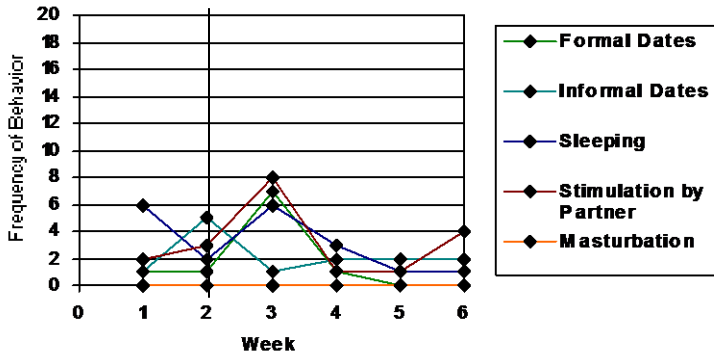
Participant 3 was a pilot participant who recorded behaviors for only six weeks while participants 4 and 8 recorded for eight weeks. Each participant peaked in frequency of occurrences during week three. This

can be explained by the placebo effect because participants began solution application during week three, presumably.

Figure 2 illustrates Placebo users' data for formal dates; informal dates; sleeping next to a romantic partner; stimulation by partner to orgasm and self-stimulation to orgasm.

**Figure 2**

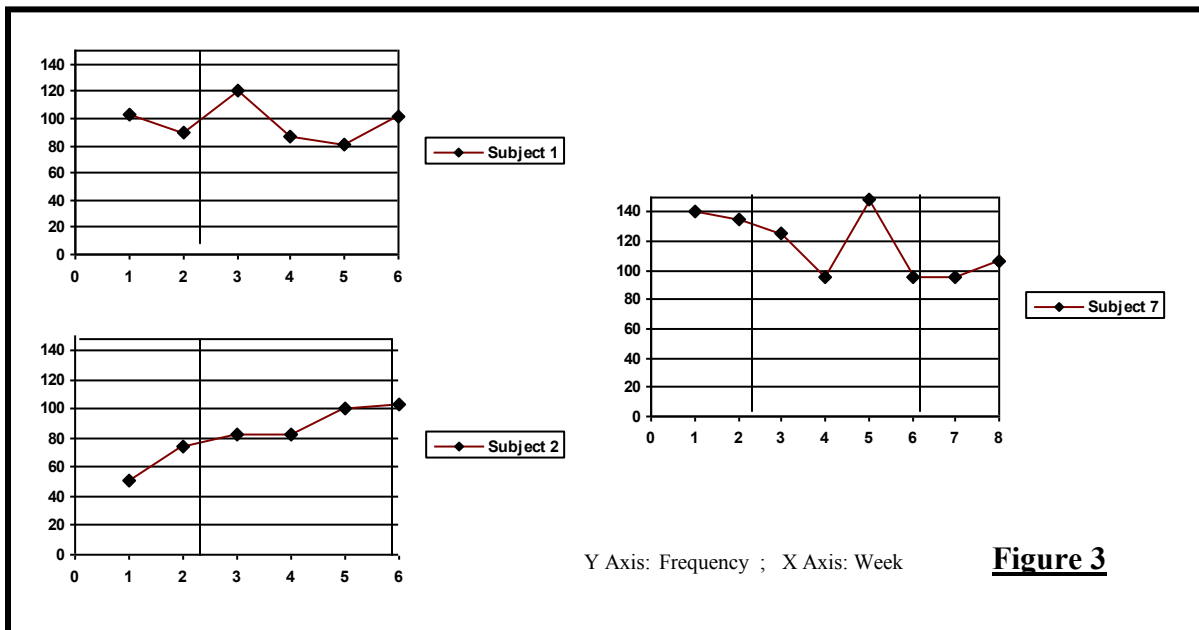
*Subject 3*



Participant 3 had a peak in sleeping next to a partner, formal dates, and stimulation to orgasm. This can be explained by the placebo effect. Participant 4 and 8 remained consistent in behaviors during week three which further supports the explanation of a placebo effect for participant 3. Participant 4 was the only participant who was honest in recording occurrences of self-stimulation to masturbation. The peak in masturbation during week four can be explained by a hormonal cycle specific to participant 4. If data collection would have continued past week eight, another peak in masturbation would be expected during the twelfth week. Participant 8 remained consistent in all behaviors excluding weeks seven and eight because her monogamous partner was out of town frequently. Overall placebo users demonstrated expected results by remaining predominately consistent in all behaviors.

*Pheromone Users*

Figure 3 illustrates pheromone users' petting/affection/kissing behaviors.

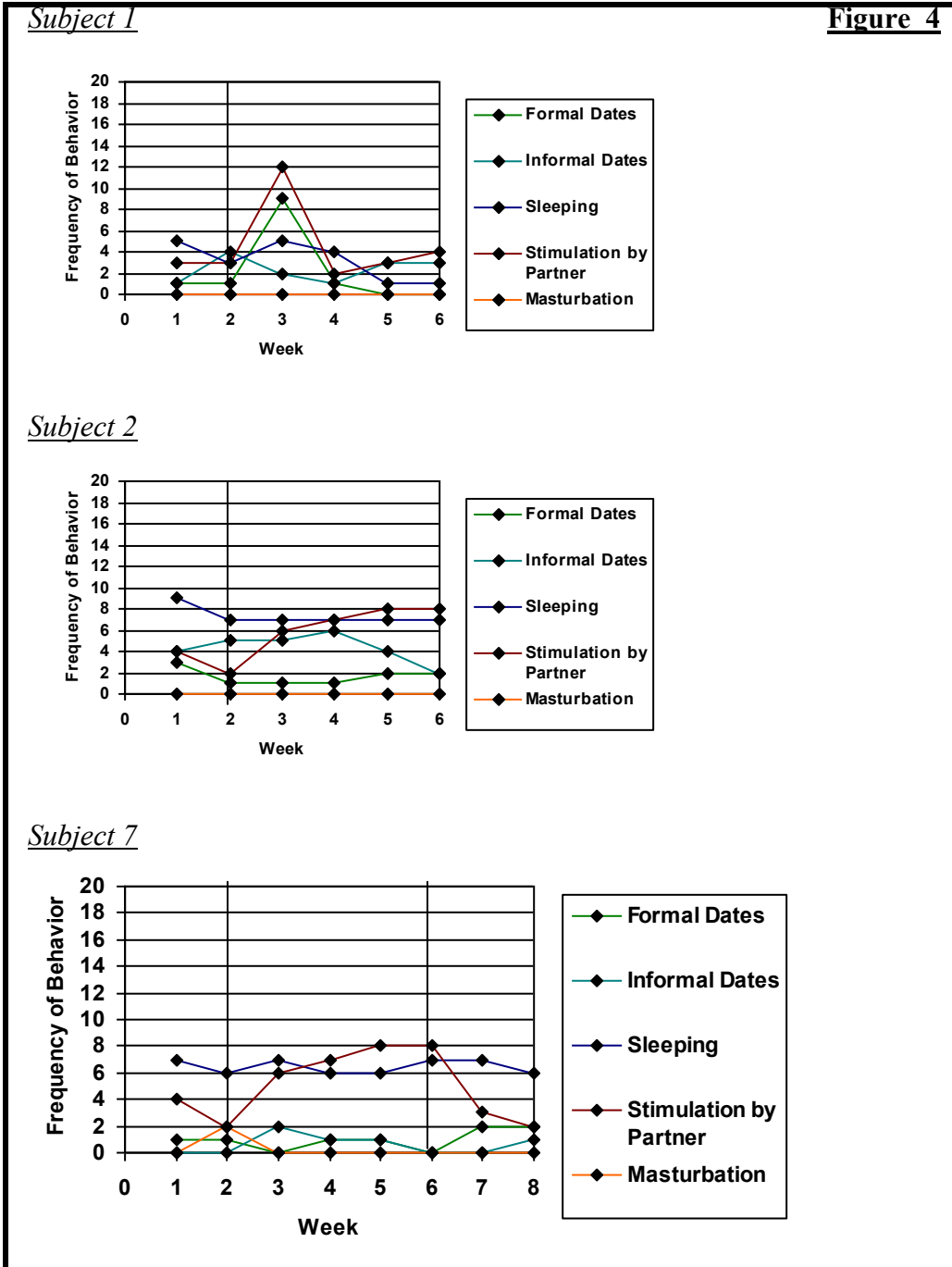


Participants 1 and 2 were pilot participants who recorded behaviors for only six weeks while participant 7 recorded for eight weeks. Participant 1 demonstrated a peak in week three, while

participants 2 and 7 did not. Pheromone users did not demonstrate an increase over baseline in the petting/affection/kissing behavior.



Figure 4 illustrates pheromone users' formal dates; informal dates; sleeping next to partner; stimulation by partner to orgasm and self-stimulation to orgasm behaviors.



Subject 1 increased greatly over baseline in formal dates and stimulation by partner to orgasm during week three of recording, but did not continue this pattern during the treatment period. Participant 2 and 7 greatly increased over baseline in the stimulation by partner to orgasm behavior during the treatment period. All other behaviors did not increase enough to suggest a pheromonal effect. The drop in Participant 7 stimulation by partner to orgasm behavior supports the increase in frequency due to the pheromone application. The same effect would be expected for participant 2 for the two weeks following the end of data recording.

### Discussion

It was hypothesized that Participants in the pheromone group would show an increase over baseline petting/affection/kissing, informal dates, formal dates, sleeping next to a romantic partner and stimulation by partner to orgasm compared to placebo users. The hypothesis was partially supported by the behavior of stimulation by partner to orgasm. Two out of the three pheromone users demonstrated a strong pheromonal effect in stimulation by partner to orgasm. The major effect EST has on users and their partners is prompting physical sexual behaviors. In turn, participants who increased in stimulation by partner to orgasm should have also increased in self stimulation to orgasm and petting/affection/kissing.

A possible explanation for the lack of increase in petting/affection/kissing is confusion about how to record occurrences of this behavior. Stimulation by partner to orgasm involves petting/affection/kissing behavior so it is apparent that while having “sex” participants would only document the number of orgasms and exclude petting/affection/kissing during that time.

An explanation for the few reported occurrences of masturbation is embarrassment. Because of the pheromonal effect on other behaviors, masturbation should have increased as well. It seems very possible that subjects simply failed to report this behavior.

There are a few implications for future research on EST and homosexual women. One needed revision of this study is the definition of petting/affection/kissing (Appendix F). There are simply too many behaviors, sexual and non-sexual, that fall into this category. It must be made clear that this behavior(s) can be documented simultaneously with stimulation by partner to orgasm. This should improve future results by clearing up any confusion about documentation.

A larger sample would demonstrate a more consistent effect on pheromone users. A statistical analysis would better demonstrate results the pheromone has on a much larger population.

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## Appendices

### Appendix A

#### STATEMENT OF INFORMED CONSENT

This is to certify that you agree to participate as a volunteer in a study conducted by Shelia R. Robinett and Christina M. Rock, M. S. of Concord University. This first phase of the study involves participation in an experiment involving frequencies of certain sociosexual behaviors. The procedure used for the first phase is described below.

1. Your participation is voluntary and you have the right to refuse to leave the study at anytime without penalty, even after signing this form.
2. Your confidentiality is guaranteed. Your name and e-mail address will be recorded and be seen only by the experimenter and her research assistant. One year after project completion, all information regarding your identity will be disposed of properly.
3. You will be asked to complete the SCL-90 (A self-reported questionnaire measuring your overall health), the Kinsey heterosexual-homosexual rating scale (A self-reported scale rating your sexuality), and a demographic questionnaire.
4. You will be asked to record each of the sociosexual behaviors daily in a notebook.
5. You will be asked to mail pages with data to the experimenter once weekly for eight weeks. You will not be responsible for postage costs.
6. You will be asked to attend another meeting at the end of the first two week period.
7. You will be asked to sign another informed consent at this meeting (the second and final meeting) and learn new procedure for the six week completion of the study.
8. You will be asked to apply a solution containing ethanol and clove oil, three times daily and at least five days per week.
9. You will be asked to apply a solution directly under the chin.
10. You will be asked to keep the solution refrigerated when not in use.
11. You will be given the opportunity to ask the experimenter any questions at the end of the experiment.
12. You will receive a handout to refer to regarding suggested application times.
13. You will be randomly assigned by number to one of two groups: Pheromone or Placebo. You will not be informed which group you belong to ensure the study remains blind.

14. Pheromones may impact your sociosexual behaviors.
15. You will be given the opportunity to ask the experimenter any questions at the end of the experiment.
16. You will have the opportunity to receive results of the data after the final analysis
17. Your statement of informed consent, results of the SCL-90 and the Kinsey heterosexual-homosexual rating scale will be kept for one year following the conclusion of the study, and will be destroyed at the end of that time period. (for legal purposes)

I have read and understand the above statements. All of my questions about the study have been answered to my satisfaction. I agree to participate in the study as a volunteer realizing that I may withdraw at anytime without penalty.

Signature:

Date:

Print Name Clearly:

E-Mail Address:

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## Appendix B

### **Script Read to Participants at Pre-Treatment Meeting**

This is a study that involves a human pheromone and its effect on six sociosexual behaviors. The behaviors are as follows: petting/affection/kissing, formal dates, informal dates, sleeping next to a romantic partner, stimulation by partner to orgasm, and self-stimulation to orgasm (masturbation). During the first two weeks the pheromone is not involved, and the purpose for collecting data here is to compare it to later data. While participating in this study, the frequency of these six sociosexual behaviors may increase.

#### **Pass out informed consent forms.**

Please take two copies of the same form so that you can turn one into me and the other you can keep for yourself. Read with me as I go through the consent form.

#### **Read informed consent forms to the group.**

Ask if there are any questions. If you are unable to answer those questions tell them you will get back to them with that information. If you are unable to tell them the answer to the question to ensure the accuracy of the results, then ask them if they are comfortable continuing with participation without that answer. If they agree ask them to continue. If they do not agree ask them to leave.

#### **Ask them to fill out the consent form.**

If everyone understands the information on the consent form and agrees with it, with a pen please sign and fill out the bottom of the form. Ask if everyone has a pen. Pass out pens to those who do not have them. Ask them to return the consent forms to the front of the room.

#### **Collect consent forms.**

Please keep one copy of the consent form for yourself. Ask participants turn in their consent forms. Place consent forms in the manila envelope. Please return to your seat so that you may fill out two questionnaires.

#### **Hand out the SCL-90-R and the demographic information form.**

The SCL-90-R questionnaire is to measure your overall health. The Kinsey scale questionnaire is to measure your level of sexual orientation and is included in your demographic information form. Make sure everyone has something to write with. Pass out writing utensils for those who do not have any. Read the directions for administering the SCL-90 to the participants.

#### **Collect the SCL-90 and the demographic information form.**

Collect both self-report forms individually and place them in the manila envelope.

#### **Pass out notebooks.**

Give each participant a notebook. This is what you will be writing occurrences of your behaviors in. Each page has the same number on it. Instead of using your name to identify yourself, this number will be your means of identification to preserve confidentiality. You can fill out tally marks or numbers beside of the listed behavior on each page. You must record behaviors daily. Do not wait until the end of the week and fill it in. At the end of each week you will be asked to mail tear out the used pages with recorded data, place it in the post-marked envelope and mail it to me. The address is already on the envelope. Please do not write your



name on any of this material. I want to keep your identity and the data separate to ensure confidentiality

**Pass out behavioral definition sheets.**

This is the list of the sociosexual behaviors being monitored. Read the definitions of the behaviors. Answer any questions they have about the clarity of the definition. Only provide examples listed on the definition sheet.

**Probable Questions**

**What is this study actually about?**

The study involves examining the frequency of these six specific behaviors before and after exposure to a human pheromone. It focuses on the Lesbian population because previous research has never looked at the homosexual community.

**Is this stuff going to make us horny, want to have more sex, more attractive, etc.?**

Form answers to these types of questions carefully and only tell them that it has the possibility to increase the frequencies or occurrences of these six sociosexual behaviors between themselves and another individual.

**Is this solution harmful to our body or skin?**

As long as you are not allergic to clove oil or ethanol you should be fine.

**What do you mean by \_\_\_\_\_?**

Answer the question within the guidelines of how much they can know about the research being conducted. Give definitions for the six behaviors. Answer questions about the SCL-90 and the Kinsey heterosexual-homosexual rating scale according to the manual for administering each test.

## Appendix C

### **Script Read to Participants at Treatment Meeting**

This is a study that involves a human pheromone and its effect on six sociosexual behaviors. The behaviors are as follows: petting/affection/kissing, formal dates, informal dates, sleeping next to a romantic partner, stimulation by partner to orgasm, and self-stimulation to orgasm (masturbation). These are the same behaviors that you have been recording for the past two weeks. During the first two weeks the pheromone was not involved, and the purpose for collecting data here is to compare it to later data. While participating in this study, the frequency of these six sociosexual behaviors may increase.

#### **Provide feedback of data recording.**

Mention how well/badly they are doing with recording and submitting data. Provide suggestions for improvement. Give praise for good recording habits.

#### **Demonstrate how to apply the solution.**

Ask everyone to direct their attention to the front of the room. You will all receive a bottle of solution to apply for the next six weeks. Some of you will receive a bottle containing pheromone and some of you will not. You must apply this solution THREE times daily and at least FIVE days per week. Show them where to apply the solution by spraying one spray of it on you.

Answer any questions.

#### **Distribute participant handouts**

Read over the handout of suggested times to apply the solution and how to store the solution.

Answer any questions.

#### **Distribute the bottles of solution.**

Call out the numbers of the participants and hand them the bottle marked with the corresponding number.

### **Probable Questions**

#### **What is this study actually about?**

The study involves examining the frequency of these six specific behaviors before and after exposure to a human pheromone. It focuses on the Lesbian population because previous research has never looked at the homosexual community.

#### **Is this stuff going to make us horny, want to have more sex, more attractive, etc.?**

Form answers to these types of questions carefully and only tell them that it has the possibility to increase the frequencies or occurrences of these six sociosexual behaviors between themselves and another individual.

#### **Is this solution harmful to our body or skin?**

As long as you are not allergic to clove oil or ethanol you will be fine.

#### **What do you mean by \_\_\_\_\_?**

Answer the question within the guidelines of how much they can know about the research being conducted. Answer all questions about when and where to apply the solution.

## Appendix D

### Demographic/ Sexuality Information Form

Participant Number \_\_\_\_\_

Today's Date:

Your Birth Date:

Age:

Race:

I grew up in a \_\_\_\_\_ environment.

Rural      Suburban      Urban

During the majority of my childhood and adolescence, I resided with:

My natural birth/ biological Parent(s)      **OR**      Adopted parents;  
Foster Care provider;  
Family member other than my parents  
(i.e., grandparents, uncle, aunt, etc.)

If you answered *My natural birth/ biological Parent(s)* to the above question, please choose from the selection below:

\_\_\_ Single Parent (Heterosexual)

\_\_\_ Single Parent (Homosexual)

\_\_\_ Married Heterosexual Parents

\_\_\_ Committed Homosexual Couple (Same as married heterosexuals except gay or lesbian)

\_\_\_ Divorced Heterosexual Parents

\_\_\_ Un-committed Homosexual Parents (Same as divorced heterosexual parents except gay or lesbian)

Please rate yourself on this scale.

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0                      1                      2                      3                      4                      5                      6

- 0- Exclusively heterosexual
- 1- Predominantly heterosexual, only incidentally homosexual
- 2- Predominantly heterosexual, but more than incidentally homosexual
- 3- Equally heterosexual and homosexual
- 4- Predominantly homosexual, but more than incidentally heterosexual
- 5- Predominantly homosexual, but only incidentally heterosexual
- 6- Exclusively homosexual

## Appendix E

### **Participant Handout**

Things to remember:

2. Please keep the bottle of solution refrigerated whenever possible. There is only a limited amount and it is expensive, so please be careful with it. If it is lost it cannot be replaced and you will no longer be able to participate in the study.
3. Keep recording your behaviors daily as they occur and e-mail them weekly.
4. Apply the solution directly under the chin **THREE** times daily and at least **FIVE** time per week
5. Your participation is **GREATLY APPRECIATED!!!**

Suggested times for solution application:

- After showering
- Shortly before informal or formal dates
- Shortly before attending a social gathering such as a party, nightclub, bar, etc.
- Shortly before attending class or work
- Shortly before engaging in social interaction with potential romantic interests or ongoing romantic partners

For any questions or comments please contact me anytime:

Shelia R Robinett

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Athens, WV 24740

Phone: (304)-921-0711

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## Appendix F

### **Sociosexual Behavioral definitions**

1. **Petting/Affection/Kissing-** romantic touching or flirting; public displays of affection; holding hands; hugs; kisses; making out; over the clothes rubbing or fondling; cuddling, etc.
2. **Formal Dates-** dates in which you were formally asked or made specific plans to do a certain activity or attend a certain event at a scheduled time. The type of date you would prepare yourself for way ahead of time. i.e.; going to the movies, out to dinner, to a concert, to a sporting event, etc.
3. **Informal Dates-** dates that you probably wouldn't consider a date normally. Planning to go spend time with someone of interest shortly before the "date" actually occurs. On these dates you will be doing activities that you wouldn't normally spend a lot of time preparing for. They include spontaneous decisions to engage in activity with another person. i.e. a walk in the park, hanging out at your house, listening to music, going out for a drink, etc.
4. **Sleeping next to a romantic partner-** this one is just how it sounds. It's all the same no matter the situation, environment or location.
5. **Stimulation by partner to orgasm-** this is what would be considered "having sex" to lesbians. It doesn't matter if body parts or other devices are used for stimulation.
6. **Self-stimulation to orgasm-** this is considered masturbation.

If you have any questions about how to classify a behavior PLEASE feel free to contact me!

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## **The Psychological Effects of the Vietnam War**

Kathy Stress

Mentor: Dr. Pat Mulvey

*I will show that Vietnam veterans have PTSD due to the events that happened while they were in the jungles of Vietnam, under severe combat conditions. I will do this by using secondary analysis from reports of Medical Staff from Veterans Hospitals.*

Why was it an unpopular war?

Robert Kennedy calls Vietnam an unwinnable war, in 1968<sup>5</sup> He said, "People must have a stake in the government in order to be willing to fight. We fight a government without supporters. He said we must realize that a military victory is not going to happen, and effort for such a victory will only cause more slaughtering of innocent people."

The citizens of the U.S. felt as if they had been deceived. They were told we could win this conflict; in as much, the leaders like Robert Kennedy knew this would never happen. The parents felt like their sons were sent to a slaughter, and they lost faith in the government. The young people protested the war. Some of the young men went to Canada and became draft-dodgers. The young men didn't have a chance, when they registered for the draft. They were 1-A ready for active duty. No one would hire them because the employers knew they would soon be drafted. Some men enlisted because they were unable to find work unknowing of what lay ahead of them. Young men 17 and 18 years of age, still think of war as G.I. Joe, and army men, and John Wayne on the movie screen. They didn't know the reality of battle. These are young men who were thrown into battle, hardly an adult, but yet not a man. Can you imagine the reality these men felt? The panic, stress and fear that hit them as soon as they were thrust into battle. Not really old enough or mature enough to make a decision, but had to make on the spot decisive

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<sup>5</sup> Footnote: Major problems in the history of the Vietnam War. Robert J. McMahon

decisions, which meant life or death. These lower and middle class people felt that their children were being discriminated against.

James Hollows describes his final year in college in the fall of 1969. He heard that his draft number was 45, and he said that it was unthinkable to answer the draft, and that he got a physical deferment. He was six foot-one inches tall, and 120 pounds. He knew his weight was close to an “underweight” disqualification. Remembering how he felt at his physical, and how he had used his wit to beat the physical. He commented about how he felt guilty, because four out of five from Harvard were being deferred, just the opposite was happening from Chelsea. He wondered why so many college men opposed the war, so few were willing to resist the draft, rather than simply evade it.<sup>6</sup> Why “we” that I refer to as the mainly white, mainly well-educated children of mainly comfortable parents. What makes them a class is that they all avoided the draft by taking care of the thinking man’s routes to escape.<sup>7</sup>

Television played a role in public opinion of the war. Every time you turned on the television, you would hear about how many soldiers were killed that day in Vietnam. The only thing that was on the news was about the brutal ness of the war. I hated for the television to be turned on at my house. It gave one such a feeling of death and desperation that you just couldn’t shake. The military did not censor the press in Vietnam. Reporters ‘killed’ the public support for the war. They relayed a feeling of pessimism to the public that this war might never be over. Here at home the protesting gained strength, as many students from colleges got involved in the Anti-War Movement. The students at Berkley began to demand the right to their ‘free speech.’ There were about 70% of them who protested against the war. In 1965 the anti-war movement expanded because of the increasingly bad situation going on in Vietnam and the way the government handled it here at home. Some Americans felt abandoned when Johnson ordered large scale bombing in Vietnam. Now protesters lead a crusade to end the war in Vietnam. This led to some students resisting the draft. College students received deferments because of health or occupation.

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<sup>6</sup> Major Problems: History Of Vietnam

<sup>7</sup> James Hollows: “What did You Do In The Class War Daddy?” pages 486-4487

Critics of the draft said that the burden fell on the poor, working class, and minorities. These students who were protesting the war, really didn't have the right to protest. These students dodged the draft and did not participate in the war; therefore they should have lost all rights to oppose what the soldiers were involved with over in Vietnam. If anyone were to protest the war it should have been those returning soldiers and their family members, because they were involved directly and had the right to oppose what was going on over there. But these soldiers and their families served their country. The war ultimately divided the nation because of this class separation between those who participated in the war and those who protested the war. People felt that the money that was spent on the war in Vietnam could have been used to help those in poverty. Later there were women, mothers of soldiers who died in Vietnam, who would protest the war. These women demanded to see the generals who had sent their sons to Vietnam.<sup>8</sup> Later some returning veterans joined the movement because of unfair treatment to some of the students at Kent State.

An account of one soldier detailed the way he felt when he reached Vietnam. He said, I had just turned 18 and some where 17 and then turned 18 on the way over there. They kept some of the soldiers on the ship until they turned 18.<sup>9</sup> The average age of a soldier was 22. The boys had no idea what Vietnam would be like. They had to watch from the ships as the artillery and the firefights were going on around them. They didn't know what to expect when they themselves got off of the ship. The soldiers endured harsh living conditions with unimaginable surroundings. The Vietnamese jungles were thick and unfamiliar. The weather was a harsh factor for the soldiers also. It would rain so hard that they couldn't keep anything dry and this led to a lot of health problems for the soldiers. The main thing they were told to do was to make sure they kept their feet dry; this would ensure that they could keep traveling through the jungle. If their feet were damp and wet for long periods of time this could produce athlete's foot, or

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<sup>8</sup> American Odyssey : The United States in the Twentieth Century, Author Nash Gary D.PP. 647-700

<sup>9</sup> Nam page 50



what the soldiers called 'jungle rot.' This was very painful for the soldiers and would have made travel through the dense jungles very unbearable.

The men on a particular transport saw two guys in green fatigues throwing grenades into the river. They capture them and take them in for interrogation; only to find out they were Green Berets, who were on their side. They were there throwing the grenades in the water to catch fish. It was very frustrating for soldiers not to know who was on their side. Sometimes they would end up killing their own men in 'friendly' firefights. This decreased the moral of the men. Many times soldiers would reach their assigned areas and see that it wasn't what they expected. One such account came from a marine who was assigned as a new soldier to a battalion located out in the jungle. He recalls his feelings when he reaches his assignment. He talked about how the eyes of the soldiers there looked so old and that he couldn't believe by looking at these other men that they were just kids. He himself was only 21 and they were putting him in charge of his own platoon. He was amazed at they were all just 'kids' and he was going to be in charge of them.

Soldiers were very afraid that they would suffer the fate of dying even while trying to sleep. They couldn't rest and this contributed to fatigue. One soldier's account said, I didn't sleep for the first two months that I was over there. I always volunteered for watch, because I didn't want to get killed in my sleep. Many of the soldiers who were there awhile developed an 'I don't give a damn' attitude. They felt that if they were to die then that was what was to be. They often tried to laugh off their fear and insecurities.

When the men first got to Vietnam, they wondered why there was wire mesh over the windows. This was to protect the soldiers from the 'Gook's' (little old Vietnamese), who would throw grenades through the windows of the transports. At night the men never saw the enemy very much. They would just open fire into the jungle, if they would here a scream or something gurgling then they would know that they had hit something. The reality never hit them until the next day when they would see the hair, blood, and tissue

of the persons they had killed lying all about. More often, the soldiers had to remove dead bodies from the vicinity. They would move V.C. or N.V.A. They would recall the images of people guts spilling out or their heads had been blown off. The soldiers would get sick and throw up the entire time they were having to remove these atrocious bodies. This was a form of training that would take place in order for soldier not to 'freak-out' during combat.

Infantry men would often be involved in what was called 'rat patrol,' this would require them to ride around in their jeeps clearing the roads of mines that were in the way.<sup>10</sup> They would be required to do this from morning until night, which was about 12 or 14 hours a day. Many of the soldiers would recall going into the villages looking for V.C. and confronting the men, women, and children of those villages. Some have said that it was very traumatic to have to go in and wipe out the people in entire villages. The soldiers were under orders to do so, because they never could really tell which of the Vietnamese people were on their side and would not try to kill them. A sergeant in a tank battalion recalls being on patrol and having a little child step into the roadway in front of his tank. He said, I got out of the tank to see if I could help the child. He was about 5 or 6 years old. He walked up to my tank and I could see he had a grenade, but by the time I dove to get out of the way, he had already blown up in front of me.<sup>11</sup> These type of actions were very devastating for American soldiers. They did not know how to handle these type of situations and became very disenfranchised from their feelings.

After the war when the Vietnam Veterans returned home they were not as well received as they would have hoped. When they returned home the soldiers were spat on, or called nasty names by the protestors of the war at home. They were called baby killers and such. They should have gotten a 'heroes welcome,' but instead they were met with hatred and vilification. This contributed to an increased amount of problems for returning veterans. They not only suffered symptoms from just being in the war,

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<sup>10</sup> We Were Soldiers Once...and Young page 59

<sup>11</sup> Major Problems In The History OF The Vietnam War, page 198

but they were unable to re-adjust to home, because of the way many people in the nation now perceived them.

In all actuality human beings are like animals and put in the right environment his primal instincts, according to Darwin, the survival of the fittest will emerge. This is what happened to our Vietnam Veterans. In a situation of kill or be killed, they chose to survive by killing. These men were in constant stressful situations. The veteran's eyes are still that of an old man. The experience he has had to engage in is hidden behind those eyes. Many of the veterans' experienced more death and destruction than any of us will ever see in a lifetime. The vets have said, the lucky ones died, because they don't have to live with ghosts each and everyday of their lives.<sup>1213</sup>

Combat veterans do not talk about their experiences. Along with the photographs they took, they also hide the memories within their mind. It is painful to remember and this is why they don't hold conversations about their experiences.

There were many illnesses that Vietnam Veterans suffered from after the war and they returned home. The most common of those was P.T.S.D. or Symptomatic Post Traumatic Stress Disorder. This disorder has been found in about 15% of Vietnam Veterans. Of those, 30% develop a chronic form that is persistent throughout their entire lifetime. This disorder is characterized by irritability, tension, exaggerated startle events, depression, nightmares, and flashbacks.<sup>13</sup> They face problems with their family relations, other interpersonal relationships, and with employment. They also have increased problems with crime. Headaches, gastrointestinal complaints, immune system problems, dizziness, chest pain, and discomfort in other parts of their body are some of the physical problems that veterans with P.T.S.D. are faced with. Many of the veterans who survived the Vietnam War were physically disabled and developed depression from the immense pressures they felt after their return. All of these things contributed to their

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<sup>12</sup> Nam, page 265

<sup>13</sup> Nightmares, page 52

severe mental trauma and lead them to abuse drugs and alcohol. Many of the veterans had trouble reestablishing relationships with their loved ones, because of the severe emotional trauma they were feeling. They complained of nightmares, insomnia, and feelings of edginess, which would cause them to want to drink. They succumb to these stressors and cannot cope, because their coping mechanisms have been exhausted by the stress of the war itself.

Many veterans live in remote areas in tents and cabins along Alaska's frigid Renai Peninsula.<sup>14</sup>

They are said to live in dark forests and high mountains to escape the contact of people. They may exist in the backwoods of every state and there is many other who hide in plain sight from their fellow man. They may have 60 jobs in 12 or 15 years, because they usually don't stay in one place for more than 3 or 4 months. They are not really considered troublemakers or lawbreakers. Those that suffer P.T.S.D. are so withdrawn, they feel the need to punish only themselves. Their problem is not that of a lack of control, but too much control and not letting anything out. Those counselors who have worked with soldier have reported a high cure rate, when society has reached out to these men. The problem is that they have a fear that people and society did not care about them or what they have went through for their country.

Our Vietnam Veterans have been treated with many methods in order to help these problems that they faced after returning home from the war. They have used psychotherapy, counseling, and medication to help control and improve the emotions and illnesses, which have afflicted them. There is no definite treatment or cure for the problems that these vets face, but some treatments appear to be quite promising. Some of these promising treatments include: cognitive behavior therapy, which seeks to try and change the way veterans feel about themselves and how they react to specific situations. Group therapy helps the veteran to talk about their feelings and problems within a group of their peers who have been through the same thing. Exposure therapy helps them by having the patient relive the frightening

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<sup>14</sup> Vets Living In The Wild, pages 25-26

experiences under controlled conditions to help him or her work through the trauma.<sup>15</sup> Medications help ease associated symptoms of depression, anxiety, and help them to sleep. The most selective drug treatments are the serotonin re-uptake inhibitors, such as Zoloft and Prozac. At the present time, most drug trials are in their early stages of development.

It is a continuing, lifelong battle for these veterans to overcome the tragedies and the atrocities they had to go through in Vietnam. Our veterans need all of the love and compassion that we as a nation can give them. Rather in concentrating on the particular actions of a war, we as citizens need to give our protecting soldiers the respect and dignity that they deserve. We now have soldiers involved again in a war that our country seems to be at odds with. Now is the time to look back and see the mistakes of the past and realize that these individuals need the support of their community and nation. They are susceptible to the same problems as those of the Vietnam Era and need to be dealt with in a more compassionate manor in order to ensure a better outcome for them. With the many breakthrough in treating veterans, there is no reason for any of them to suffer needlessly. Hopefully our society will be better to these veterans (especially the war protestors) than they were to those of the Vietnam War. These men and women are fighting for the U.S., as well as the Iraqi people. Now that there is more funding from our national officials, veterans are getting more money for treatments they may need. Funds and medical knowledge will be available to treat the new veterans faster and with better results.

The Vietnam War Memorial in Washington, D.C built November 11, 1982 stands to help reconnect and heal our separated nation of the policies and actions that went on during the war. It honors our fallen soldiers who gave their lives for their fellow soldiers and their country. There are more than 58,000 names on the wall of these courageous men and women. It stands as a place that loved ones can visit to feel close to those they lost during that war. It is still, after 20 years, the most visited memorial at our nation's capital.

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<sup>15</sup> Vets Living In The Wild, page 28

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