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The Effects of Violence and Competition in Sports Video Games on Aggressive  
Thoughts, Feelings, and Physiological Arousal

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Abstract

Research over the past few decades has indicated that violent media, including video games, increase aggression (Anderson, 2004). The current study was investigating the effects that violent content and competitive scenarios in video games have on aggressive thought, feelings, and level of arousal in male college students. Four video games were used in this study, two violent and two nonviolent. One of the games in each pair was competitive and the other game was noncompetitive.

It was hypothesized that participants who played the violent competitive game would have higher levels of arousal, as measured by heart rate and blood pressure, aggressive thoughts, as measured by the Word Completion Task (Anderson, et al., 2004), and aggressive feelings, as measured by the State Hostility Scale (Anderson & Morrow, 1995) than those who played any of the other games.

The data shows that heart rate decreased in participants playing the violent competitive video game significantly more than for the participants playing the nonviolent competitive game, which did not support the hypothesis. Blood pressure decreased among participants in all groups. No other significant effects were found for heart rate and no significant effects were found between any of the groups on the State Hostility Scale or the Word Completion Task.

## The Effects of Violence and Competition in Sports Video Games on Aggressive Thoughts, Feelings, and Physiological Arousal

The media tends to exert an unnatural and often unhealthy influence on our thoughts and actions. People are likely to mimic everything portrayed in the media from diets to fashion to how to get a date. Unfortunately, no behavior is out of the realm of replication, and this is especially worrisome when the behaviors are violent and aggressive. Numerous studies over the past few decades have documented the effect that the violent content in various forms of media can have on behavior (Anderson, 2004; Thomas, 1982). Although the conclusions are not well-accepted, or even well-known among the public, the research indicates that violent media increases aggressive thoughts (Anderson & Dill, 2000), feelings (Carnagey & Anderson, 2005), and behavior (Bartholow & Anderson, 2002).

Video games are one specific example of media influence, and may be particularly powerful due to their interactive nature. As more realistic games are created it becomes an important task to understand how they affect behavior. In the wake of an unsettling number of school shootings, society is faced with the possibility that violent video games may have a negative influence on behavior. Social scientists have sought to understand if and how these seemingly harmless games influence behavior.

It is helpful to define certain terms when discussing the effects of media violence on aggressive or hostile behavior. Violence, as it is represented in video games, involves using a body part, weapon, or any other object to wound or kill an opponent (Jurica, Alanis, & Ogletree, 2002). One study reported that 71% of video games featured in arcades contained some form of violence (Lachlan, Smith, & Tamborini, 2005).

Similarly, Dietz (1998) reported that 80% of the most popular Nintendo and Sega Genesis video games required the use of violence to attain goals.

Aggression is defined by most researchers as behavior intended to harm another individual who is motivated to avoid that harm (Anderson & Bushman, 2001). There are two important points this definition suggests. First, that intention is necessary to label a behavior as aggressive. Unintentionally harming someone would not be considered aggressive behavior. Second, the victim or intended victim is motivated to avoid the harm. Some instances of violent behavior, such as sadistic and masochistic practices and “fight club” groups, by this definition, would not be considered aggressive because the people involved are not trying to avoid the harm.

While researchers agree on how to define the important terms, there has been no such agreement on the specific video games used in studies or even a systematic analysis of which video game genres to study. Lachlan, Smith, and Tamborini’s (2005) analysis of the top 60 most played video games did look for violent content across genres. They included sports, first-person shooter, fantasy, fighter-style, and racing video games. Violent characters and themes were presented in many of the games they analyzed.

The basic method used in experimental studies was to have a fighter-style or first-person shooter game for the violent scenario and a puzzle game for the nonviolent scenario (Anderson, 2004; Anderson & Dill, 2000). Beyond this general formula there is no consistency in which specific titles are used. While this would suggest that several games in these particular genres have the effect of increasing violence, the results cannot be generalized to other video game genres or to the differences between fighter-style/first person shooter games and nonviolent games that are not puzzles.

A few studies have extended the literature to look at online role-playing games, both first-person shooter and fantasy. Schneider, Lang, Shin, and Bradley (2004) used a first-person shooter computer game that they found increased aggression, but the same was not true of a violent fantasy personal computer (PC) game used by Williams and Skoric (2005). While these studies branched out a little further to incorporate a different game medium, the PC, there are still a large number of video game genres that have received little, if any, experimental attention.

In addition, the effects of several different methodological factors have not been clearly determined. The amount of time spent playing a game in the study is one of those factors that differ between the methodologies of video game studies. Within the short-term exposure studies there is some variability in how long participants play the game before being tested for aggression. The time interval generally falls somewhere between ten and thirty minutes (Deselms & Altman, 2003; Anderson & Dill, 2000). Although these studies reported similar findings, the nuances of time effects have not been completely worked out. For many researchers, twenty minutes of game play is the general rule to follow (Anderson, 2004). Whether there are differences between playing for 20, 30, 50, or more minutes has received little attention.

The literature shows that aggressive behavior, aggressive thought, aggressive affect, and physiological arousal have been used most often to measure video game effects. These variables have been studied in both correlational and experimental research (Anderson & Dill, 2000; Bushman & Anderson, 2002). Short-term and long-term exposure to violent video games has also been shown to affect these variables (Deselms & Altman, 2003). The effects of time seem to differ depending on the age group, however, with children being more affected by short-term exposure to video game

violence and adolescents and adults showing more aggression after long-term exposure (Bushman & Huesmann, 2006). Long-term exposure has had various meanings depending on the study; it has been used to describe the effects of playing a video game for an hour as well as playing for hours each day for months (Anderson, 2004).

In regards to aggressive behavior, the evidence from the research shows that violent video games can have a negative impact on children, adolescents, and young adults and that the measures used in these studies translate into aggressive behavior outside of the laboratory (Anderson & Bushman, 2001). Anderson and Dill (2000) for instance, used both correlational methods and an experimental design to support the connection between violent video games and aggressive behavior in undergraduate students. Long term exposure to violent video games was highly correlated with delinquent behavior and people who played a violent game displayed more aggression immediately afterwards than those who played a nonviolent video game. Another correlational study revealed that adolescents who reported more exposure to violent video games were found to act more aggressively in the classroom, evidenced by a higher instance of arguments with teachers and physical fights with other students (Gentile, Lynch, Linder, & Walsh, 2004)

Exposure to violent video games appears to correspond to more aggressive behavior within and outside of the video game scenario. In Anderson and Morrow's (1995) study aggressive behavior within the video game was assessed. For example, given the option of killing an enemy or going around the enemy, participants were more likely to kill when they had been given competitive directions.

The most common measure of aggressive behavior in video game research is the reaction time task that involves hitting a button faster than an imaginary opponent



(Anderson & Dill, 2000). Participants are allowed to set the duration and intensity of a white noise blast that they have been told will punish their opponent for losing. Higher intensities and longer durations indicate higher levels of aggression. Bartholow and Anderson (2002) found that males set higher noise levels to punish opponents after playing a violent video game. Carnagey and Anderson (2005) using the same noise task found that participants who were rewarded rather than punished for violent actions in the game behaved more aggressively during the task.

Although increases in aggressive behavior are certainly one of the more dangerous outcomes of exposure to violent video games, researchers have found that thoughts become more aggressive following game play as well (Anderson & Dill, 2000; Carnagey & Anderson, 2005). These studies have used different methods ranging from simple questionnaires to more subtle story stem completions to measure aggressive cognition. Tamborini, et al. (2004), for example, found that participants who played violent video games scored higher on the Buss and Perry Aggression Questionnaire than participants who played a nonviolent game. Participants in another study self-reported having more aggressive thoughts and traits after playing a graphically violent video game (Uhlmann & Swanson, 2004).

Additionally, Carnagey and Anderson (2005) reported that participants created more aggressive words on a word fragment task after being rewarded for using violence in a video game. Anderson and Dill's (2000) study using reaction time to aggressive words as an indicator of aggressive thoughts suggests that aggressive thoughts are more accessible to people immediately after they have been exposed to violent video games. A study by Bushman and Anderson (2002) provides further support for this conclusion by showing that participants had higher expectations that conflicts would be resolved

aggressively after playing a violent video game. The researchers attributed this to more readily accessible aggressive thoughts.

Violent video games negatively impact emotions as well, resulting in players feeling more aggressive and hostile after playing. Whether the aggressive emotions are directed towards another person, a situation, the world, or even their own self-concept, the violence in video games can create and heighten aggressive affect. Arriaga, Esteves, Carneiro, and Monteiro (2006) measured state hostility as an indicator of aggressive affect and found that participants reported higher levels of hostility after playing violent video games. Furthermore, the study by Carnagey and Anderson (2005) reported that participants felt more hostile after playing a violent game regardless of whether or not they were rewarded for their violent acts. Other studies have found that hostility and aggressive feelings were increased more if a story line was present in the video game (Schneider, Lang, Shin, & Bradley, 2004) and that regardless of how high or low a person scores on hostility prior to playing a game their hostility will increase after playing a violent video game (Gentile, et al., 2004).

Physiological arousal, another frequently used indicator of the effects of violent video games, is a double-edged sword. Arousal is ambiguous and must be interpreted within a specific context. In many cases it is difficult to say that arousal equals aggression rather than frustration or excitement. This has in fact been an argument used against the findings of violent media research; critics have suggested that the effects seen in these studies are not due to a difference between violent versus nonviolent content but rather to a difference in arousal level inherent in the games (Freedman, 2001).

Nevertheless, video game researchers have studied physiological arousal both as an effect of the video games and as a factor to hold constant between nonviolent and

violent games (Anderson & Bushman, 2001). The results have shown that violent video games do increase arousal as measured by heart rate, blood pressure, and skin-conductance response (Bushman & Huesmann, 2006; Arriaga, et al., 2006; Schneider, Lang, Shin, & Bradley, 2004). This increased arousal is interpreted as the output of aggression because the responses on the behavioral and affective measures that were administered after the video game exposure support that interpretation of the physiological data.

To test the claim that frustration and excitement are responsible for increased arousal following exposure to violent video games, studies have been conducted that control the level of difficulty and perceived excitement across games. Bushman and Anderson (2002) for instance, used multiple video games in their study and both the violent and nonviolent games were all rated equally exciting and frustrating by independent raters and the participants who played them. Only the amount of violent content differed between the video games, so the difference in participants' hostility was attributed to the violence in the game.

Although most of the research supports a link between violent content and aggression, some studies have failed to find a connection between violent video games and real-life violence (Scott, 1995). People in and outside of the psychological field have cited many reasons why the evidence for the negative effects associated with violent video games is insubstantial. Most of the criticism focuses on the measures of aggression used in the studies being inadequate or irrelevant to real-life violence (Freedman, 2001). Furthermore, people often think that laboratory experiments have no resemblance to real-life situations and that correlational studies are useless because they cannot reveal causal relationships (Anderson, 2004).

The relevance of aggressive measures to real-life violence may be an unavoidable shortcoming in video game literature. Measures of aggressive affect and cognition will not predict exactly who will engage in violent behavior or when they will do so, however, these measures do provide some insight into how the violent content influences participants' thoughts and feelings. Freedman (2001) also questions the validity of the behavioral measures used, particularly the reaction time task. Delivering white noise blasts is not the same as punching or shooting someone. While the current measures may be far from perfect, it would be unethical to study aggressive behavior by actually giving the participant the chance to hit someone. For now, these measures are the safest and most ethical way to study aggression, despite their possible flaws.

Both correlational studies and experimental research have shortcomings. The combined findings of both types of research, however, can enhance and support each other so the deficits of either method are lessened. Video game researchers have purposely used both methods to study the issue and the agreement among the findings has been that violent content does increase aggression in participants' thoughts, feelings, and behavior (Anderson & Bushman, 2001).

There may be an alternate explanation for the lack of results in some studies. Desensitization, or habituation, which refers to the decreased psychological or physiological arousal to a stimulus after repeated exposure to the stimulus, has become a concern in video game research (Bartholow, Bushman, & Sestir, in press). A small number of studies have looked at desensitization to video games. Bartholow, et al. (in press) found that violent video game players had reduced brain response to violent images, but were more likely to behave aggressively. This study looked at event-related brain potential, specifically, amplitudes associated with violent behavior. Decreased

amplitudes were present in violent video game players, but these participants performed more aggressively later during the reaction time task. Williams and Skoric (2005) ran a month long study on a large group of online game players. All first time players, the participants played a violent online computer game for several hours a day over the course of the month, yet no increases in aggression were found based on several self-reported questionnaires. The researchers sited habituation to the game violence as a possible explanation for the lack of results.

Research is still needed to understand why desensitization might occur among some game players and not others. Habitual gamers may be likely candidates for the effects of desensitization because they have played video games for several years and play for several hours a day on average. This over-exposure to violent content could be responsible for a lack of physiological responsiveness to the violent content in the game. Currently, research has not been done to see if there are differences between habitual gamers and the average video game player.

The vast majority of research supports the idea that violent content in video games increases aggressive thoughts, feelings, behavior, and arousal. Researchers have utilized a few different theories to explain the relationship between violent media and aggression. Social learning theory, excitation transfer theory, and the General Aggression Model (GAM) have been discussed in the literature (Arriaga, et al., 2006). Recently, the GAM has been applied most often to the video game scenario, but each theory has helped further the understanding of how violent video games affect aggression.

The social learning theory of aggression, first proposed by Bandura (1973), suggests that people learn behaviors by observing and modeling others. Through

observation, people learn how to perform behaviors as well as the situations where the behavior is appropriate. Many factors have been found to increase the likelihood that the observer will learn the behavior. One such factor is greater similarity between the model and observer. A second factor, rewarding the behavior, shows the observer that the behavior is an appropriate way to achieve a goal and receive reinforcement.

The research does support the predictions of the social learning theory (Tamborini, et al., 2004; Bartholow & Anderson, 2001). Participants are more likely to act aggressively after playing violent video games when they relate to the characters in the games and those characters have been rewarded for their violent behavior (Schneider et al., 2004; Carnagey & Anderson, 2005). Most video game characters are young and attractive males who are rewarded with more points, praise, or affection from an opposite sex character for their violent behavior (Lachlan, et al., 2005). These characters are readily relatable models for video game players, the majority of which are young men and boys. Dietz (1998) noted that the emphasis on male aggressiveness and dominance contained in most video games teaches harmful gender roles as well as violent behavior.

Unlike the social learning theory which accounts only for the situational factors leading to aggression, the excitation transfer theory includes the affect physiological arousal has on the expression of violent behavior. Zillmann (1983) suggested that arousal leads to aggressive behavior when it is misattributed to an aversive event. Exposure to violent video games, or any form of media, raises the person's level of physiological arousal. The arousal does not immediately dissipate; a person who recently played a video game and is further provoked by an aversive event may attribute all of the arousal they experience to the event. The person may then react more aggressively than if they had not been previously exposed to the video game.

It is important to note that excitation transfer theory does not suggest that the arousal must be caused by violent content in the game. It could be due to any other factor that would raise arousal levels, for example frustration or excitement. If the arousal is misattributed to a provocation soon after game play it is interpreted as aggression toward the current situation. Whether or not violent content would cause more arousal to later be misattributed is an empirical question that had not yet been answered (Bushman & Anderson, 2002).

Whereas social learning and excitation transfer theories look at external and internal events respectively, the GAM incorporates person and situational variables. Just as a person's internal state, affectively and cognitively, will impact their behavior in a situation, the situation itself exerts influence on their future emotions, thoughts, and behavior (Anderson & Bushman, 2001).

Violent media exerts its influence initially as a situational variable that influences the person's internal state (Bushman & Anderson, 2002). Aggressive cognitions become more prevalent in the form of scripts and schemas, the patterns a person uses to understand and interact with the world around them, learned through the game. In conjunction with the aggressive thoughts and emotions, the video game may increase physiological arousal. Behavior may be immediately impacted if an appropriate situation in which to act out the aggression occurs (Carnagey & Anderson, 2005).

The GAM suggests that, over time, exposure to violent media will teach people new ways of perceiving, interpreting, and behaving in situations in an aggressive manner (Anderson & Bushman, 2001). The repeated exposure will make the aggressive scripts, schemas, emotions, and behavioral patterns readily accessible. Therefore, the GAM accounts for effects of external and internal variables, as well as their interactions, on

aggression, and describes the development of short-term and long-term aggressive behavior.

The literature on video game effects is suggestive of a link between violent content and aggression, but it is not comprehensive. There are many questions left to answer, not only about violent content but other factors that may have detrimental effects on players. The increases in all measures of aggression appear general in their scope, however, only some people are led to extreme violence similar to what is illustrated in some games. There are several important links still missing in order to gain a complete understanding of the effects of violent video games on behavior.

One missing link is sex differences in video game exposure. Most researchers have noted that boys and men are far more likely to play video games than girls and women (Jurica, et al., 2002). Video game characters and scenarios are geared more toward males than females, but it is difficult to know whether this is the cause of more male players or a result of there being more male video game players (Lachlan, et al., 2005). There are also differences in how males and females behave after playing video games. For example, on the reaction time task, males delivered louder noise blasts to opponents than females, but females delivered longer noise blasts than males (Bartholow & Anderson, 2002). Women also assigned harsher punishments to criminals after playing a violent game than men did (Deselms & Altman, 2003). Differences in acceptable behavior for males and females in Western culture could account for these results. Males may be more likely to play violent video games because they see violent behavior as more acceptable than do females. Further research is needed to understand the differences between males and females regarding video games.



Another variable that has been virtually ignored is the different types of video game players. Studies rarely distinguish between first time gamers and habitual gamers (Anderson, 2004). Habitual gamers might be responsible for some of the desensitization that has been noted (Bartholow, et al., in press). Years of playing violent video games may explain decreased arousal to similar stimuli. Players may also have learned and internalized the aggressive thought and behavior patterns so thoroughly that they view aggression as appropriate, acceptable, or even natural behavior.

Additionally, there is a lack of research on possible differences between playing against the video game's computer, other human players, or multiple players online. Who people play video games with or against could affect the learning and reinforcement of aggressive behaviors. Interacting with real people may enhance the effects of the violent video games, although it could be that playing alone creates a more immersive learning environment because there are no distractions from the fantasy world in the game. The dynamics of social interactions within video games may be an important key to understanding their effects on behavior (Bailey, 2006b).

Finally, differences in personality variables among players are yet to be carefully examined. Many studies include pre-tests to rule out significant differences in trait aggression among the participants in different groups (Anderson & Bushman, 2001; Bartholow, Bushman, & Sestir, in press), but few studies have looked at any pre-existing differences in personality characteristics that could account for more aggressive behavior from some violent video gamers over others. Arriaga, et al. (2006) concluded that aggressive personality was a mediating variable for the effects of playing a violent video game on current hostile feelings.

While there have been a few extreme cases of video game players acting out the violence learned from a first-person shooter game, most players do not engage in this type of behavior even if their aggression levels do increase after playing (Freedman, 2001). The extreme violent behaviors modeled in most of the games studied may be too extreme for the average player to ever repeat. There is another type of popular video game, however, that models violent behaviors that are socially acceptable: sports.

Although some studies have found no difference between athletes and non-athletes (Lemieux, McKelvie, & Stout, 2002), there is some evidence that athletes behave more aggressively than the average person. Wann (2005) found that athletes commonly use aggression during games to achieve goals and to harm other players. A study by Nucci and Young-Shim (2005) suggested that poor leadership and unhealthy competitive environments model aggressive behavior for athletes. The behavior is effective in the game, as well as encouraged, so athletes may learn and use them outside of the sport.

Based on the predictions of the GAM, situations that promote the development of aggressive scripts and schemas will lead to more aggressive behavior (Bushman & Anderson, 2002). A study by Anderson and Morrow (1995) showed that people consider competition inherently more aggressive than cooperation. This finding suggests that competitive situations are more likely to result in the development of aggression than cooperative or noncompetitive situations. Athletic events are almost always competitive, and often involve violence, so they represent the type of situation that the GAM predicts would result in more aggressive cognitions, affect, and behavior.

In fact, many athletic events and the video games based on them can serve to model aggressive behavior. Due to the cultural acceptance of violence in sports and the competitive nature of society in general this genre of video game may be an even more

powerful influence on behavior. The current study focused on the effects that sports video games have on players, when the games are violent and competitive.

This study used sports video games because they are a more relevant model of aggressive behavior than the traditional first-person shooter and martial arts games that researchers typically use. This study also looked at the effects of competition in a game, as well as violent content. Four sports video games with varying levels of competition and violence were used.

It was hypothesized that participants who played the violent-competitive game would have higher levels of arousal as measured by heart rate and blood pressure, aggressive thoughts as measured by the Word Completion Task (Anderson, et al., 2004), and aggressive feelings as measured by the State Hostility Scale (Anderson & Morrow, 1995) than those who played any of the other games. It was further hypothesized that the participants who played the nonviolent-noncompetitive video game would have the lowest arousal, aggressive thoughts, and aggressive feelings. The other two video games, violent-noncompetitive and nonviolent-competitive would result in arousal levels, aggressive thoughts, and aggressive feelings that fall between the other two types of games.

## Method

### Participants

The participants for this study were primarily Concord University students enrolled in Introductory Psychology and Sociology classes. Three of the participants were students responding to signs advertising the study, which were posted around the campus. The ages of the participants ranged from 18-27 years. There were 40 participants in this study, all males.

## Materials

A PlayStation 2 game system, a standard PS2 controller, and a 13-inch television set were used. Participants played one of four games, each representing a different combination of violence and competition. *Tiger Woods PGA Tour 2004* was used as the nonviolent-competitive game and *Fight Night 2004* was used as the violent-competitive game. The nonviolent-noncompetitive game was *Tony Hawk Pro-skater 4* and the violent-noncompetitive game was *Cabela's Deer Hunt: 2004 Season*. All of the games had comparable graphics quality and controls.

All participants signed an informed consent. They filled out a survey on their video game experience, which asked questions about how much they play video games and what types of games they play (see Appendix A). A typed list of standard controls for each game was provided for each participant. Heart rate and blood pressure was measured using the Omron Automatic Extra Compact Wrist Blood Pressure Monitor.

The State Hostility Scale (Anderson & Morrow, 1995; see Appendix B) was used to assess aggressive affect. This is a list of 35 statements describing how one might feel, such as “I feel angry”. The scale is labeled Current Mood, so as not to bias the participants’ responses. The participant must rank how much each statement describes them currently on a 5-point Likert scale. To measure aggressive thoughts the Word Completion Task was used (Anderson, et al., 2004; see Appendix C). This task requires the participant to create words from a list of 98 word fragments.

## Procedure

Participants were randomly assigned to Group Golf, Group Boxing, Group Skateboard, or Group Hunt. After reading and signing the informed consent, the participant completed the video game experience survey. Following the completion of the

survey, the experimenter immediately measured blood pressure by placing the cuff on the participant's right wrist. The blood pressure cuff was then removed and the researcher informed the participant of what game they would be playing and gave them the appropriate control sheet. The researcher then started the video game and left the room.

Participants played the game for 20 minutes, after which the researcher turned the game and television off. The blood pressure cuff was placed on the right wrist again and the measures were taken immediately. Then the participant completed the State Hostility Scale. Afterwards, the participant was given three minutes to generate words on the Word Completion Task. The researcher then fielded any questions the participant had about the study and thanked them for participating.

## Results

The dependent variables in this study were heart rate and blood pressure before and after playing a video game, level of hostility after playing a video game as measured by the State Hostility Scale, and the ratio of aggressive words to total words attempted on the Word Completion Task.

The mean difference in heart rate before and after playing the video game for Groups Skateboard, Hunt, Golf, and Boxing were 4.9, 2.0, 5.5, and -4.0 bpm (beats per minute), respectively (see Figure 1). Planned pairwise comparisons were conducted and a significant effect was found on difference in heart rate (pre-post) between Group Golf and Group Boxing,  $t(36) = 2.057$ ,  $p = 0.047$ . A marginally significant effect was also found on this measure between Group Skateboard and Group Boxing,  $t(36) = 1.927$ ,  $p = 0.062$ . The comparison between the nonviolent video games (Group Skateboard and Group Golf) and the violent video games (Group Hunt and Group Boxing) also revealed

a marginally significant effect,  $t(36) = 1.899$ ,  $p = 0.066$ . No other significant effects were found for difference in heart rate, all  $t$ 's  $(36) \leq 1.299$ , all  $p$ 's  $\geq 0.202$ .

Blood pressure was measured before and after participants played a video game. The mean pre-game systolic blood pressures for Groups Skateboard, Hunt, Golf, and Boxing were 119.1, 121.2, 128.3, and 119.2 mmHg, respectively. Post-video game mean systolic blood pressures for these groups were 113.9, 119.8, 120.4, and 118.4 mmHg, respectively (see Figure 2). Multivariate tests were conducted and a significant difference between pre and post systolic blood pressures was found,  $F(1, 36) = 1510.966$ ,  $p < 0.001$ , but no significant group interaction was found,  $F(3, 36) = 1.798$ ,  $p = 0.165$ .

For diastolic blood pressure, pre-video game means for Groups Skateboard, Hunt, Golf, and Boxing were 76.2, 76.5, 79.3, and 76.5 mmHg, respectively, and the post-video game means were 74.8, 70.1, 76.8, and 76.8 mmHg, respectively (see Figure 3). Multivariate tests revealed a significant effect between pre and post diastolic blood pressure,  $F(1, 36) = 7.652$ ,  $p = 0.009$ . But again, there was no significant group interaction effect,  $F(3, 36) = 0.841$ ,  $p = 0.480$ .

On the State Hostility Scale, the mean hostility levels for Groups Skateboard, Hunt, Golf, and Boxing were 77.3, 80.4, 72.2, and 87.7, respectively (see Figure 4). Planned pairwise comparisons were conducted on the State Hostility Scale and found no significant effects, all  $t$ 's  $(36) \leq 1.540$ , all  $p$ 's  $\geq 0.132$ .

For the Word Completion Task the mean ratio of aggressive words to total words for Groups Skateboard, Hunt, Golf, and Boxing were 21.23, 18.80, 18.69, and 22.48, respectively (see Figure 5). On the Word Completion Task, pairwise comparisons were also conducted and no significant effects were found, all  $t$ 's  $\leq 1.028$ , all  $p$ 's  $\geq 0.311$ .

Further analysis of the Word Completion Task, however, revealed some interesting findings. The mean number of aggressive words for Groups Skateboard, Hunt, Golf, and Boxing were 8.0, 8.4, 7.0, and 10.5, respectively. Post hoc pairwise comparisons were conducted and found a significant effect between Group Golf and Group Boxing,  $t(36) = 2.300$ ,  $p = 0.027$ . A marginally significant effect was also found between the combined violent video games and the combined nonviolent video games,  $t(36) = 1.812$ ,  $p = 0.078$ . The mean total words produced for Groups Skateboard, Hunt, Golf, and Boxing were 38.1, 46.0, 37.6, and 48.1. Post hoc pairwise comparisons revealed a significant difference between Group Golf and Group Boxing,  $t(36) = 2.363$ ,  $p = 0.024$ , Group Skateboard and Group Boxing,  $t(36) = 2.250$ ,  $p = 0.031$ , and violent games compared to the nonviolent games,  $t(36) = 2.928$ ,  $p = 0.006$ . Marginally significant differences were also found between Group Golf and Group Hunt,  $t(36) = 1.890$ ,  $p = 0.067$ , and between Group Skateboard and Group Hunt,  $t(36) = 1.778$ ,  $p = 0.084$ .

Post hoc analyses also examined the effects of competition in the video games. Pairwise comparisons between the noncompetitive games (Groups Skateboard and Hunt) and the competitive games (Groups Golf and Boxing) found no significant effect on heart rate, blood pressure, the State Hostility Scale, or the Word Completion Task, all  $t$ 's  $(36) \leq 0.827$ ,  $p \geq 0.414$ .

## Discussion

It was hypothesized that playing a violent competitive video game would result in more aggressive thoughts, feelings, and higher arousal than playing a video game with any other combination of violence and competition. This hypothesis was generally not supported and the results do not provide further support for the GAM (Anderson &

Bushman, 2001) or for past research on the effects of violent video games (e.g. Bushman & Anderson, 2002).

Furthermore, the significant effect between Group Golf and Group Boxing on heart rate was going in the wrong direction. Instead of increasing heart rate, which would have been consistent with past research (Bushman & Huesmann, 2006), the current study found that the violent video game *Fight Night 2004*, played by Group Boxing, decreased heart rate. Both of the nonviolent video games tended to increase heart rate. The other violent video game, *Cabela's Deer Hunt*, increased heart rate, but not as much as either of the two nonviolent games.

Blood pressure also yielded a pattern opposite from what was expected. Following exposure to the video games, participants showed a general decrease in both systolic and diastolic pressure that was significant and not related to which game was played. The only deviation of this pattern was in Group Boxing's diastolic blood pressure; a slight increase occurred after exposure to the video game.

Although it is not clear why the results did not reveal the expected pattern, a possible explanation is that the participants' had increased physiological arousal at the beginning of the experiment for any number of reasons. Anxiety about participating and recent exposure to certain substances, such as nicotine and caffeine, may have caused the participants' baseline blood pressure to be artificially high. The county in which Concord University is located has a higher rate of cigarette smokers than the national average according to the West Virginia Department of Health and Human Resources (2004). West Virginia is also one of the leading states in prevalence of obesity, another cause of high blood pressure (Centers for Disease Control, 2006). Without having health



information on the participants it is difficult to know how much these factors played a role in their physiological measurements.

The scores on the State Hostility scale are closer to the predicted pattern, but no significant effects were found. The Word Completion task added little information about any of the groups. One issue with the Word Completion Task is the ambiguity of some words and the fact that sometimes non-words can take on meaning. For instance, a majority of the participants responded with the word “muggle” to one of the incomplete words, which may have meant nothing when the scale was originally designed, but now has meaning because of its use in the popular Harry Potter books and movies.

The measures themselves may have been a problem due to lack of sensitivity. These measures may not have been sensitive enough to detect differences between the groups with such small sample sizes. If the effects of violent content in video games are relatively small for any one individual, more sensitive measures and much larger sample sizes may be necessary to detect differences.

Competition in the video games used in this study appeared to have no effect on the participants. Post hoc analysis confirmed this. One possible explanation for the lack of impact on the participants is that the competition was not salient enough in these scenarios. For instance, playing against a computer may not have the same effect as playing against another person. Past research in this lab (Bailey, 2006b) found no difference between playing against another player or the computer, but different hostility measures were used, so further research is still needed in this area.

Differences between the video games used that were not accounted for by the researcher may also have affected the results. *Cabela's Deer Hunt*, for instance, may have had less of an effect because the violence was against deer, not human characters. In

addition, the participants were students from a location where deer hunting is a prominent pastime. Some of the participants may not even view hunting as violent.

The results of this study do not support the majority of findings on video game effects (Anderson, 2004) however the findings are consistent with some past research (Scott, 1995). Of all the possible explanations for the lack of significant effects found here, sample size may be the most relevant. Anderson (2004) reports the effect size as relatively large compared to other effect sizes, such as the effects of second-hand smoke on cancer. The effect size is still arguably small though, and the vast majority of published research has reported sample sizes around 200 participants when significant effects are found (Anderson & Bushman, 2001).

Another important difference between this study and previous research is the use of a different genre of video games. Little research has been collected to understand the effects of sports video games specifically, although it seems that the GAM would suggest violent sports games would have an impact on players' aggression (Bushman & Anderson, 2002). Past studies have focused on fighter-style games, such as *Mortal Kombat* (e.g. Deselms & Altman, 2003), or first-person shooter games, like *Doom* (e.g. Uhlmann & Swanson, 2004), whereas this study looked at the effects of four different sports video games. Another study performed in this lab found some evidence that as the use of violence to win the sports video game increases, the players' hostility increases (Bailey, 2006a). The popularity of sports video games supports the effort to find out how the violence and competition in those games affects behavior.

Aside from violent content and level of excitement, the many differences between individual video games have been largely unexplored (Anderson, 2004). This study sought to broaden the scope of the literature on violent video games. Much more research

is needed in the area of sports video games to understand if and how they affect players. Judging from how much other types of video games influence behavior, such as learning and aggression, it is likely that sports video games impact players in some form.

The GAM suggests that many different pathways can lead to increased aggression (Anderson & Bushman, 2001). The pathways through which video games specifically affect players may prove to be equally complex. Many people play video games, but only a few have acted out in an extremely aggressive manner. The focus of this and future studies should be on other factors that may mediate the effects of video games, not just violent content. In order to explain the differences between people who play video games as well as predict the effects all video games may have on behavior, it has become necessary to look at these other factors.

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## Appendix A

Video Game Experience Survey      Age: \_\_\_\_

1. Have you ever played a video game?    \_\_\_\_ Yes    \_\_\_\_ No
2. If yes, how old were you when you first played a video game? \_\_\_\_
3. On average, how many hours per week do you play video games?
 

0
0-1
2-4
5-7
8-10
> 10
  
4. Do you have a home video game system?    \_\_\_\_ Yes    \_\_\_\_ No
5. What video game system(s) do you have? (Check all that apply)
 

____ PC/Mac	____ Nintendo GameCube
____ PlayStation 2	____ Nintendo DS
____ PlayStation 3	____ Xbox
____ PSP	____ XBox 360
____ Other: _____	
6. What video game system do you play on most often? (Choose only one)
 

____ PC/Mac	____ Nintendo GameCube
____ PlayStation 2	____ Nintendo DS
____ PlayStation 3	____ Xbox
____ PSP	____ XBox 360
____ Other: _____	
7. What is your favorite type of game? (Choose only one)
 

____ Sports	____ Role playing games
____ Puzzle	____ First person shooter
____ Action/Adventure	____ Strategy/Simulation
____ Fighting	____ Racing
8. List your top three favorite video games:
  1. \_\_\_\_\_
  2. \_\_\_\_\_
  3. \_\_\_\_\_
9. How much action do you like in a video game?
 

1	2	3	4	5	6	7	8	9	10
Low			Medium				High		
10. How much violence do you like in a video game?
 

1	2	3	4	5	6	7	8	9	10
Low			Medium				High		
12. How difficult do you like video games to be?
 

1	2	3	4	5	6	7	8	9	10
Low			Medium				High		



## Appendix B

### “State Hostility Scale”

#### Current Mood

Please indicate the extent to which you agree or disagree with each of the following mood statements. Use the following 5 point scale. Write the number corresponding to your rating on the blank line in front of each statement.

Strongly Disagree	Disagree	Neither Agree Nor Disagree	Agree	Strongly Agree
1	2	3	4	5
___				___
I feel furious.				I feel like I'm about to explode.
___				___
I feel willful.				I feel friendly.*
___				___
I feel aggravated.				I feel understanding.*
___				___
I feel tender.*				I feel amiable.*
___				___
I feel stormy.				I feel mad.
___				___
I feel polite.*				I feel mean.
___				___
I feel discontented.				I feel bitter.
___				___
I feel like banging on a table.				I feel burned up.
___				___
I feel irritated.				I feel like yelling at somebody.
___				___
I feel frustrated.				I feel cooperative.*
___				___
I feel kindly.*				I feel like swearing.
___				___
I feel unsociable.				I feel cruel.
___				___
I feel outraged.				I feel good-natured.*
___				___
I feel agreeable.*				I feel disagreeable.
___				___
I feel angry.				I feel enraged.
___				___
I feel offended.				I feel sympathetic.*
___				___
I feel disgusted.				I feel vexed.
___				
I feel tame.*				

**\*Item was reverse scored. Asterisks were not present on the scale when presented to the participants.**

## Appendix C

### “Word Completion Task”

- |              |            |                |
|--------------|------------|----------------|
| 1. b_h____   | 29. bl_nd  | 57. fo_e_t     |
| 2. in__re    | 30. sn_re  | 58. off____    |
| 3. ex_e__    | 31. b_e    | 59. l__on      |
| 4. mu__er    | 32. h_t    | 60. cr__l      |
| 5. pr__e     | 33. g__pe  | 61. c_e_te     |
| 6. spea_     | 34. sm_ck  | 62. st_r_y     |
| 7. fli__er   | 35. sm__e  | 63. m_tc_      |
| 8. expl__e   | 36. kn____ | 64. f_r__      |
| 9. w__m      | 37. t_ne   | 65. t__te      |
| 10. ki__     | 38. s__b   | 66. n__t_      |
| 11. t_p_     | 39. sh_r_  | 67. w__d_w     |
| 12. h_r_     | 40. dr__n  | 68. w__ked     |
| 13. a_t_r    | 41. p__ne  | 69. vis__n     |
| 14. cho_e    | 42. ang__  | 70. en_age     |
| 15. s_mp__   | 43. fl__t  | 71. scr__n     |
| 16. att_c_   | 44. fi__t  | 72. h_tr_d     |
| 17. c_mp__t  | 45. p_ck   | 73. t_l_ph____ |
| 18. des_____ | 46. ha_e   | 74. dis__s_ed  |
| 19. sh_l_    | 47. a_t    | 75. c_nt__l    |
| 20. sho_t    | 48. c_t    | 76. prov__e    |
| 21. r_p__t   | 49. w_n    | 77. p_nb_ll    |
| 22. str__e   | 50. a_e    | 78. out____e   |
| 23. l__e     | 51. _ry    | 79. c_ll       |
| 24. b_rn     | 52. wa_    | 80. r_de       |
| 25. st_r_o   | 53. f_m_   | 81. m_n_ge     |
| 26. p__son   | 54. sl_p   | 82. ins____    |
| 27. p_st_r   | 55. b__k   | 83. s_d_       |
| 28. m__gle   | 56. r_pe   | 84. b__t       |
| 85. br__ze   |            |                |

- 86. r e v \_ \_ t
- 87. c o o \_
- 88. s \_ \_ y
- 89. d \_ \_ r
- 90. s m \_ c k
- 91. f r \_ \_ t
- 92. \_ u n c h
- 93. s h \_ r e
- 94. a \_ u s e
- 95. c l \_ \_ r
- 96. h \_ n t
- 97. w \_ t \_ r
- 98. s \_ a s h

**The Use of Benthic Macroinvertebrates to Assess Water  
Quality in an Urban WV Stream**

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Major: Biology  
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## **Abstract**

Biodiversity is an important indicator of a healthy stream ecosystem. Pollutants can often eliminate sensitive macroinvertebrate taxa. Brush Creek, in southern West Virginia, begins in a rural, suburban landscape, continues flowing through the town of Princeton, and finally flows through a forested area before joining the Bluestone River. By sampling the macroinvertebrate community, water quality can be assessed and the impact of different land uses can be evaluated. Five reaches were sampled within the Brush Creek watershed. Macroinvertebrates were collected from each reach by using a 500  $\mu\text{m}$  kick net. Samples were placed in labeled jars for later identification. Two hundred macroinvertebrates were chosen randomly from each sample and identified to the family level. Percent EPT was 52.4, 34.4, 2.34, 20.5, and 75.1 for Glenwood, Willowbrook, Princeton, Brickyard, and the Falls, respectively. HBI was 5.81, 6.74, 7.95, 7.27, and 4.57 for Glenwood, Willowbrook, Princeton, Brickyard, and the Falls, respectively. The diversity of taxa was lower in the urban reaches of the stream, with fewer pollution sensitive macroinvertebrate taxa present. However, the stream does seem to recover after flowing through the forested area, having more pollution sensitive taxa present. Previous studies on Brush Creek also support this trend. Action needs to be taken in the Princeton area to prevent future urbanization from further degrading the water quality of Brush Creek.

## **Introduction**

Streams and rivers worldwide are being continuously degraded due to alteration of their hydrology and water chemistry as a result of pollution from mining, urbanization, industrialization, and many other human activities. In a healthy stream ecosystem there is usually a very diverse macroinvertebrate community present. Macroinvertebrates are bottom-dwelling organisms that are visible to the naked eye. Macroinvertebrates include aquatic insects, such as mayflies and stoneflies, snails, mussels, aquatic worms, and crayfish (West Virginia Department of Environmental Protection 2006). Benthic macroinvertebrates are not only important to the ecosystem as a food source for fish, but they also facilitate nutrient cycling through the break down of larger particulate matter into forms that are more usable by stream life (Gomi et al. 2002). A low diversity and/or abundance of macroinvertebrates in a stream usually indicates that water quality is poor.

Streams can often be negatively impacted by a variety of factors. Urban storm water runoff can greatly alter the habitat and cause excessive sedimentation in streams (Pedersen and Perkins, 1986). Eutrophication due to organic pollution is also a growing problem due to excessive fertilizers, animal wastes, and untreated sewage (Nedeau et al. 2003). These organic pollutants cause unnatural nutrient enrichment and decreased levels of oxygen. Organic wastes also cause habitat degradation because the solid waste settles to the bottom, altering the substrates of the stream. Not all macroinvertebrate taxa can tolerate the decreased levels of oxygen (Dahl et al. 2004). Many anthropogenic activities, including mining, agriculture, and industrial processing, can lead to elevated levels of arsenic, zinc, lead, and other minerals that are potentially harmful to the biodiversity of a stream (Marques et al. 2003; Valenti et al. 2005). Acid mine drainage can have adverse effects on the pH of a stream. This often eliminates many sensitive taxa (e.g. Ephemeroptera, Plecoptera, and Trichoptera), leaving only tolerant ones (e.g. Diptera).

Valenti et al. (2005) examined the effects of an abandoned arsenic mine on an Appalachian headwater stream. They found that the density of macroinvertebrates and percent of sensitive taxa was substantially lower at sites downstream of the mine, as compared to the upstream reference sites. The mean density of aquatic insects was five to ten times higher at the upstream sites and sensitive taxa were almost completely absent from the mine influenced area. Poor water quality can also be due to warmer water temperatures, resulting from industrial effluent (Leblanc et al. 1997). Warmer water holds lower concentrations of oxygen, which negatively impacts macroinvertebrates. Organic pollution mixed with warmer water temperatures can be particularly detrimental because of the drastic oxygen depletion. Higher water temperatures also facilitate eutrophication and an elevated growth of vegetation, ultimately leading to even less oxygen available in the water. However, eutrophication is more predominant in lakes or stagnant bodies of water.

It is important to assess water quality and determine the level of pollution in our streams and rivers. Macroinvertebrates are often chosen as a bioindicator, because they are widespread, provide a variety of responses to disturbances, and can act as continuous monitors of stream quality (Rosenberg and Resh 1993). Macroinvertebrate communities can be sampled from year-to-year, comparing populations over time and thus assuring the integrity of a stream. Assessing benthic macroinvertebrate communities is known as one of the most reliable measures for evaluating water quality (Valenti et al. 2005). More so than fish communities, macroinvertebrate communities can respond to slight changes in aquatic environments due to human activities (West Virginia Department of Environmental Protection 2006). Monitoring macroinvertebrates is easy to conduct, and is also very cost effective (Valenti et al. 2005). One of the most commonly used biotic

indices to evaluate the macroinvertebrate community structure is the Ephemeroptera + Plecoptera + Trichoptera (EPT) index (Valenti et al. 2005). This index is the percent of the community composed of the pollution sensitive macroinvertebrate taxa combined. According to Wallace et al. (1996), the EPT index is especially useful for evaluating chemically induced disturbances in southern Appalachian headwater streams.

Macroinvertebrates can be sampled in a variety of different ways. One is to use a kick net and disturb an area upstream, allowing the benthos to flow into the net (EPA 2004). The macroinvertebrates are then preserved and identified at a later time. Another method is to plant artificial substrates in a stream, allowing the macroinvertebrates to colonize them. The substrates are then collected and the macroinvertebrates are counted and identified (Nedeau et al. 2003). To assess water quality, pollution sensitive macroinvertebrates can be placed in enclosures within a stream to assess their mortality rate (Pesacreta 1997).

Urbanization of watersheds has many effects on streams. According to the U.S. Census Bureau, an 'urban area' is defined as a densely settled area with a population between 2,500 and 49,999, and more than 1,000 people per square mile (US Census Bureau 1930-1980). An 'urbanized area' is defined as having a population greater than 50,000 with more than 1,000 people per square mile. Even a small, urban area can have a great impact on local streams. A stream's channel can be altered by excessive storm water runoff. This often changes the flow and can widen the stream channel. Excessive erosion can increase sediment in the stream, altering amounts of sunlight and altering the bottom substrates of a stream once it settles out (Pedersen and Perkins 1986). Urban runoff can contain chemicals that are harmful to the biota of the stream. Elevated levels of phosphorus, nitrogen, and other ions are generally much higher in urban catchments



(Paul and Meyer 2001). An increase in fecal coliforms increases the risk of disease within the human population of the watershed (Paul and Meyer 2001). Urbanization generally decreases algal species diversity, which has been attributed to the alteration of water chemistry (Chessman et al. 1999). The presence of metals or herbicides can also reduce their numbers; however, algae can flourish in an urban environment if excessive nutrients, such as nitrates and phosphates, are introduced (Paul and Meyer 2001).

It is not unusual for the density of macroinvertebrates to be the same in rural and urban streams. However, rural streams often have a higher diversity of macroinvertebrate taxa than that of urban streams (Dahl et al. 2004; Pedersen and Perkins 1986; Pesacreta 1997). Studies that have sampled forested headwaters and urbanized or industrialized areas downstream, have reported higher diversity in the headwaters (Nedeau et al. 2003; Robson et al. 2006; Valenti et al. 2005). Nedeau et al. (2003) sampled a rural upstream area along with an industrialized area downstream. Fewer EPT taxa, especially caddisflies and mayflies, were found in the downstream area. Robson et al. (2006) sampled a stream in different locations, noting changes in water quality with the onset of urbanization. A significant drop in water quality was found downstream of the urban area. The rural upstream areas had a much higher EPT score. Only Baetidae, a relatively tolerant Ephemeroptera family, was found at all sites.

Our main objective for this study was to assess the water quality of Brush Creek using benthic macroinvertebrates as a bioindicator. Brush Creek, in southern West Virginia, begins in a rural, suburban landscape, continues flowing through the town of Princeton, an urban area, and finally flows through a rural, forested area before discharging into the Bluestone River.

By sampling different points along the stream, we were able to evaluate the impact that different land uses have on the macroinvertebrate community of Brush Creek. Princeton, WV is not a large, urban city. Therefore, we were able to examine the impact that a smaller, lower density urban area has on a stream. Urbanization tends to occur in the downstream areas of a watershed. In the Brush Creek watershed, the urban area is near the headwaters of the watershed and the rural area is downstream. We determined whether or not the stream recovers after the urban area, once it flows into a rural, forested area.

## **Methods**

Five different reaches along the Brush Creek watershed were sampled (Figure 1). These included, from upstream to downstream, the Glenwood Reservoir/Dam, Willowbrook, the Princeton Fire tower, Brickyard, and the Falls. The Glenwood and Willowbrook areas, near the headwaters of Brush Creek, were in a suburban landscape (Figure 2). There was some development and residential areas, but a forested landscape was still present. The Princeton area was the heart of the urban landscape (Figure 3). One of the sampling sites within this landscape was located behind the Kroger Plaza on Stafford Drive, near parking lots, roads, and other development. The Brickyard sampling site was in more of a suburban landscape, with residential and commercial development evident. The last reach was near Brush Creek Falls, which was the furthest downstream before the confluence with the Bluestone River. This area was a completely forested area, except for agricultural and residential areas on the ridge tops.

At each site, an accessible part of the stream was chosen. A random spot was picked every 10-15 m by walking upstream, in a zigzag pattern, taking around five samples. The substrate was kicked up and debris was allowed to flow downstream into a

500 µm net. Organisms caught in the net were placed in a labeled jar for later processing. Basic chemical water quality tests were also performed using HACH test strips, including pH, nitrate/nitrite, phosphate, total alkalinity, and hardness. Various physical conditions were also documented, along with a description of the surrounding terrain.

In the lab, water was drained from the jar through a 500 µm sieve, so no organisms were lost. Ethanol was then added to double the volume of liquid, so that the organisms would be preserved. At a later time, the liquid was drained so that the organisms could be poured into a large pan. The pan was marked into a grid with sixteen squares. Random squares were picked by a probability simulation program on a TI-83 calculator. All the organisms from the chosen square were picked out, identified down to the family level, and documented on WV Save Our Streams Macroinvertebrate data sheets. All organisms were removed and identified from the chosen square, before another square was chosen. Random sampling continued until 200 organisms had been identified.

The Hilsenhoff Biotic Index (HBI) Score and %EPT were calculated. The Hilsenhoff Index gives each family of macroinvertebrates a tolerance number from 1 to 10 (1=most sensitive, 10=most tolerant). Tolerance scores were assigned according to Merritt and Cummins (1996). The higher the HBI score, the lower the water quality. HBI was calculated as follows:

$$\text{HBI Family Score} = \# \text{ Individuals of Family} \times \text{Tolerance Score of Family}$$

$$\text{HBI} = \text{Total HBI Score} / \text{Total \# Organisms in Sample}$$

% EPT is the percent of Ephemeroptera (Mayflies), Plecoptera (Stoneflies), and Trichoptera (Caddisflies). A higher % EPT indicates higher water quality. % EPT is calculated as follows:

$$\%EPT = (\# \text{ Ephemeroptera} + \# \text{ Plecoptera} + \# \text{ Trichoptera}) / \text{Total} \# \text{ Organisms} \times 100$$

Percent Chironomidae was also calculated for each sampling site. The functional groups composition (shredders, scrapers, etc.) was also analyzed from at each sample reach. A Chi-square test was performed to compare percent EPT among the five sample reaches.

## **Results**

The Glenwood area at the reservoir/dam was sampled on 1/27/07 (Table 1). The pH, nitrate, nitrite, phosphate, total alkalinity, and hardness of the stream were 6.5, 0 ppm, 0 ppm, 30 ppm, 20 ppm, and 50 ppm, respectively (Table 2). The macroinvertebrate sample was composed of 36.5% chironomidae, and had a 52.4% EPT abundance (Table 3). The HBI for the site was 5.8. Functional group composition included 58.7% scrapers, 0.8% shredders, 8.7% collector/filterers, and 1.6% predators (Table 3).

The Willowbrook area was sampled on 3/23/07 (Table 1). Nitrate, nitrite, phosphate, total alkalinity, and hardness of the stream were 0 ppm, 0ppm, 15 ppm, 40 ppm, and 120 ppm, respectively (Table 2). The macroinvertebrate sample was composed of 59.6% chironomidae, and had a 34.4% EPT abundance. The HBI for the site was 6.7 (Table 3). Functional group composition included 65.1% scrapers, 0.0% shredders, 28.9% collector/filterers, and 0.0% predators (Table 3)

The Princeton area was sampled on 4/4/07 (Table 1). Nitrate, nitrite, phosphate, total alkalinity, and hardness of the stream were 0 ppm, 0ppm, 30 ppm, 80 ppm, and 120 ppm, respectively (Table 2). The macroinvertebrate sample was composed of 89.7% chironomidae, and had a 2.3% EPT abundance. The HBI for the site was 7.95 (Table 3). Functional group composition included 95.3% scrapers, 0.0% shredders, 2.8% collector/filterers, and 0.0% predators (Table 3).

The Brickyard area was sampled on 3/25/07 (Table 1). Nitrate, nitrite, phosphate, total alkalinity, and hardness of the stream were 0.5ppm, 0ppm, 5 ppm, 80 ppm, and 120 ppm, respectively (Table 2). The macroinvertebrate sample was composed of 74.7% chironomidae, and had a 20.5% EPT abundance. The HBI for the site was 7.2. Functional group composition included 75.3% scrapers, 0.5% shredders, 18.4% collector/filterers, and 3.2% predators (Table 3).

The Falls area was sampled on 11/4/06 (Table 1). The pH, nitrate, nitrite, phosphate, total alkalinity, and hardness of the stream were 6.8, 1.0 ppm, 0.15 ppm, 5 ppm, 30 ppm, and 120 ppm (Table 2). The macroinvertebrate sample was composed of 0.0% chironomidae, and had a 75.1% EPT abundance. The HBI for the site was 4.59 (Table 3). Functional group composition included 12.9% scrapers, 0.5% shredders, 81.3% collector/filterers, and 5.3% predators (Table 3).

Overall, percent chironomidae and HBI were highest at Princeton and Brickyard, and was lowest at the Falls. Percent EPT was highest at the Falls and lowest at Princeton and Brickyard. Functional group diversity was also highest at the Falls. Chi-square analysis showed that % EPT was significantly different among the sites ( $X^2= 280.13$ ,  $df = 4$ ,  $p < 0.01$ ).

	Glenwood	Willowbrook	Princeton	Brickyard	Falls
Date	1/27/07	3/23/07	4/4/07	3/25/07	11/4/06
Description of Site	-Flows under small bridge -small trees, grasses and wetland area -sampled b/n bridge & dam, & other side of bridge -flowed from dam to bridge	-sampled on both sides of the bridge -swift current -rocky bottom -surrounded by some trees & brush, a church & some trailers	-site directly behind industrial park -small brush on banks -very shallow -trash on both banks	-water very deep -water had an odd odor -evidence of dumping (tires, microwave, etc) -surrounded by thick foliage -flows parallel to road	-very forested -flows under small bridge -sampled at least 100 yds. from bridge -swift current
Water Temp.	4.9 °C	13°C	17°C	13.5°C	5.7°C
Water Level	Normal	Normal/High	Normal	Normal	High
Weather Over Past Week	½ in. of snow	Warm	Warm & Dry	Dry/Warm	Cold/no rain

**Table 1: Conditions at Sampling Sites**

	Glenwood	Willowbrook	Princeton	Brickyard	Falls
pH	<6.5	n/a	n/a	n/a	6.8
Nitrate/Nitrite	0 ppm	0 ppm	0 ppm	0.5ppm/0 ppm	1 ppm/0.15 ppm
Phosphate	30 ppm	15 ppm	30 ppm	5 ppm	5 ppm
Total Alkalinity	20 ppm	40 ppm	80 ppm	80 ppm	30 ppm
Hardness	50 ppm	120 ppm	120 ppm	120 ppm	120 ppm

**Table 2: Results of Chemical Water Quality Tests**

	Glenwood	Willowbrook	Princeton	Brickyard	Falls
% Scrapers (Grazers)	58.7%	65.1%	95.3%	75.3%	12.9%
% Shredders	0.8%	0.0%	0.0%	0.5%	0.5%
% Collector/Filterers	8.7%	28.9%	2.8%	18.4%	81.3%
% Predators	1.6%	0.0%	0.0%	3.2%	5.3%
<b>% Chironimidae</b>	<b>36.5</b>	<b>59.6</b>	<b>89.7</b>	<b>74.7</b>	<b>0.0</b>
% Dominant Taxa	36.8	59.6	89.7	74.7	48.8
<b>% EPT Abundance</b>	<b>52.4</b>	<b>34.4</b>	<b>2.3</b>	<b>20.5</b>	<b>75.1</b>
EPT Taxa Richness	6	4	2	3	6
% Tolerant	39.2	59.6	95.3	74.7	10.5
<b>Hilsenhoff Biotic Index (HBI)</b>	<b>5.8</b>	<b>6.7</b>	<b>7.95</b>	<b>7.2</b>	<b>4.59</b>
Total Taxa Richness	11	9	7	9	10

**Table 3: Stream Condition Index Metrics****Discussion**

Brush Creek has been negatively impacted by urbanization in the Princeton and Bluefield area of southern West Virginia. Stoneflies, mayflies, and caddisflies are much more sensitive to pollutants than other macroinvertebrate taxa. The presence of these sensitive taxa indicates better water quality and the absence of pollutants. According to the EPA (2004), a percent EPT above 65% indicates that a stream is not impaired. EPT abundance drastically fell from Glenwood to Princeton, and Princeton to Brickyard, and drastically increased from Brickyard to the Falls (Figure 5 and Table 3). The Falls area

had 75.1% EPT abundance and is therefore the only unimpaired site out of the five sampling sites, according to the EPA standard. The absence of sensitive taxa at the other four sites indicates that there are pollutants present. The unimpaired site at the Falls is the last site before the confluence with the Bluestone river, indicating that the stream was able to remediate after the impaired sites.

The HBI Index was also used to analyze water quality at each sampling site (Table 4). The HBI scores increased from the number at Glenwood to the number at Willowbrook, and then the number at Princeton. This indicates that water quality deteriorates as the stream flows from the headwaters through the urban area. The HBI scores of Princeton and Brickyard were both above 7.00, indicating that those sites are polluted (Figure 6). The HBI score fell drastically from the number at Brickyard to 4.59 at the Falls.

Percent chironomidae was also calculated for each sampling site. Chironomids are a pollution tolerant taxa, and high densities usually indicate poor water quality. The percent chironomidae drastically increased from Glenwood to Princeton, barely decreased from Princeton to Brickyard, and fell to zero at the Falls (Figure 7). The decrease in percent chironomidae indicates an increase in water quality at the Falls. The percent chironomidae present in the Princeton and Brickyard samples is consistent with the HBI score and percent EPT, indicating that these two sites are the most impaired.

Functional group diversity was also analyzed in each sample. Functional groups represent different levels of the food web. The absence of one or more functional groups can also be an indicator of stream impairment. Functional group diversity was dominated by the collector/filterers in each sample (Figure 8). There was a higher percentage of scrapers and predators in the Falls sample than any of the others. The shredders were

only found in the Glenwood and Falls samples. The presence of collector/filterers in the first four samples is consistent with the lack of tree covering at the sampling sites. Only fine particulate matter would be available in these areas, which collector/filterers utilize for food. At the Falls, the presence of shredders is consistent with the tree covering surrounding the stream. The trees are a source of coarse particulate matter, which shredders use for food.

The results of the water chemistry tests showed relatively low nutrient levels at all of the sampling sites. Alkalinity was on the low end of the spectrum for buffering capacity at all of the sampling sites. These results show that there were not outstanding abnormal conditions at any of the sampling sites. However, the HACH test strips used in this study had very low resolution and accuracy.

There have been two previous unpublished studies conducted on the water chemistry of Brush Creek in West Virginia. Both assessed the water quality by testing for pH, dissolved oxygen, and phosphate levels at various sampling points along Brush Creek. Roark (1997) found that dissolved oxygen was very low directly after the stream flowed through Princeton, but levels recovered upon reaching the rural Falls area. Phosphate levels were high directly after Princeton, and decreased as the stream flowed through the forested Falls area. Low dissolved oxygen and high phosphate levels both indicate a presence of sewage in the stream. Roark (1997) commented that water quality had improved since a USGS study in 1976, but that water quality was still low, compared to national water quality standards. Bowman (2006) also found high phosphate and chemical oxygen demand levels, indicating low dissolved oxygen concentrations directly after Princeton. But levels did recover as the stream flowed into the rural Falls area. However, it is unclear if water quality improved or declined from 1997 to 2006. The



overall trends in water quality are similar to those of this study. Water quality declines through Princeton and Brickyard. However the stream is able to self-remediate upon reaching the more rural Falls area.

Robinson (2006) also conducted a study on Brush Creek that used macroinvertebrates to assess water quality. This study sampled four sites along Brush Creek and used the Hilsenhoff Biotic Index to analyze the water quality of the stream. The HBI scores for Willowbrook, Stafford Drive, Brickyard, and the Falls were 4.9, 7.3, 7.8, and 2.0, respectively. The HBI score is high at Princeton and Brickyard, but is low at the Falls area. The data of this study also follows this trend (Table 1)

Overall, previous studies support the data of this study. The water quality of Brush Creek decreases upon flowing through the urban area of Princeton and through Brickyard, but water quality improves at the Falls area, just before the confluence with the Bluestone River. The percent EPT and percent chironomidae found in this study also supports this trend. While Brush Creek seems to be able to remediate from Brickyard to the Falls, action needs to be taken to prevent pollutants from entering the stream in the Princeton area. Urbanization of the Princeton, West Virginia will only continue in the coming years, and with urbanization comes more sources of pollutants. If pollutants entering the stream increase, Brush Creek may no longer be able to self-remediate as it nears its confluence with the Bluestone River.

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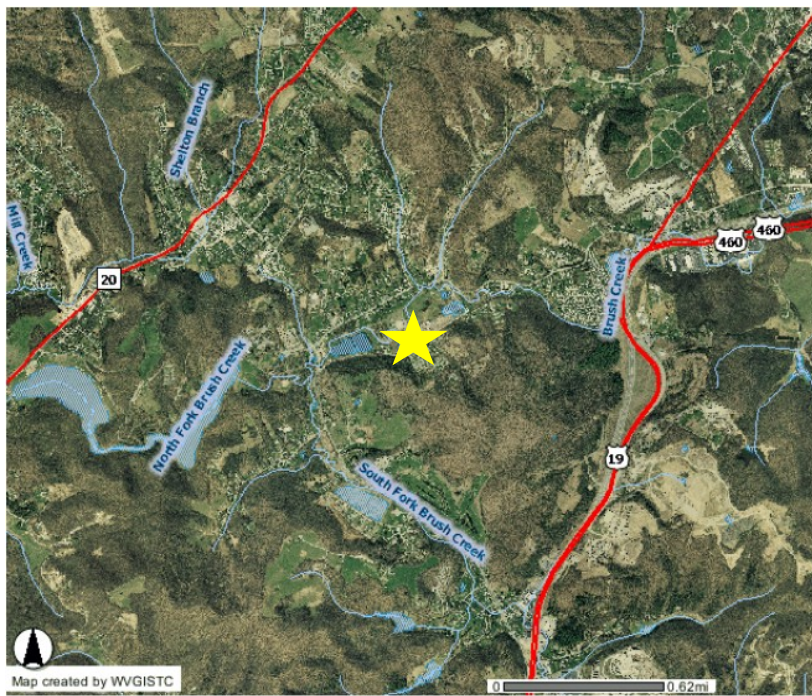
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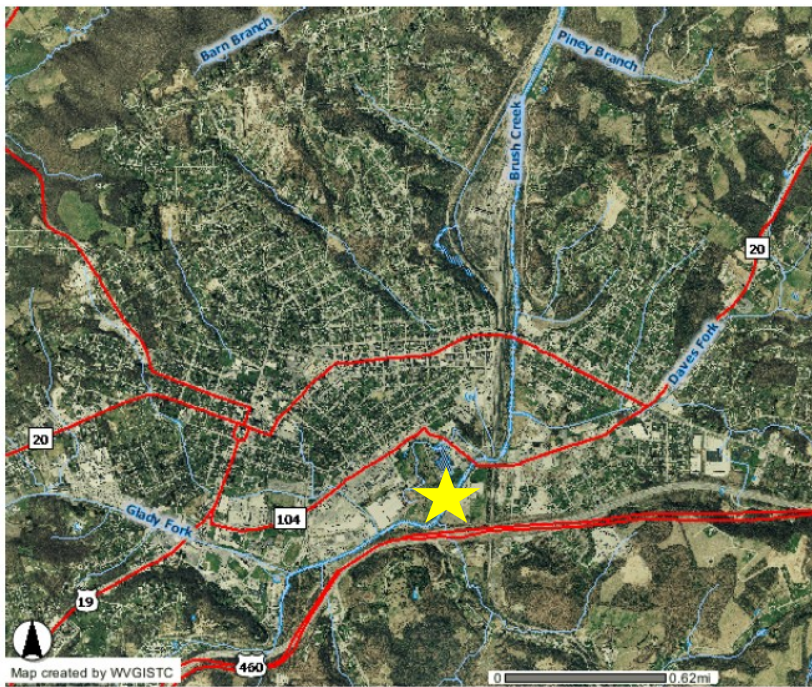
**Figures and Tables**

**Figure 1: Areas sampled along Brush Creek**

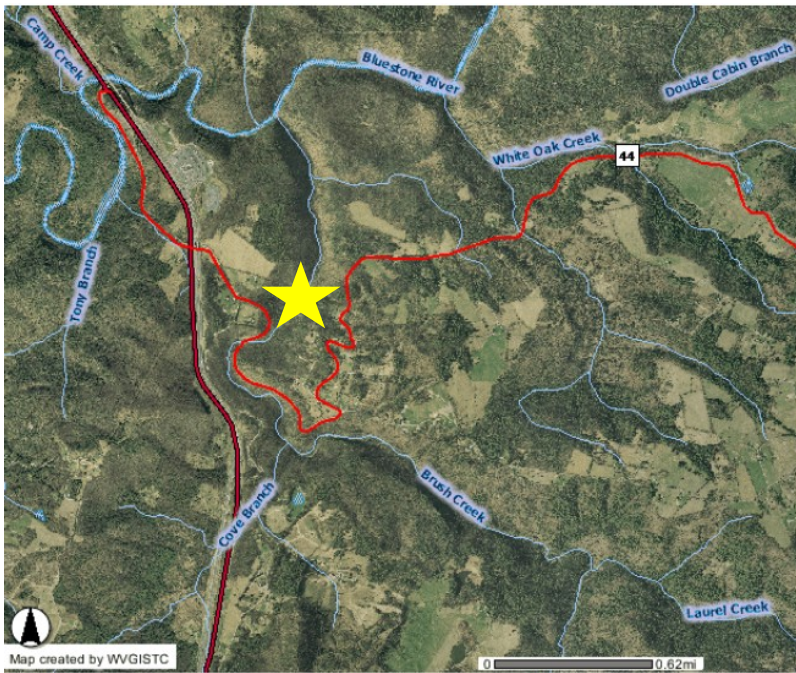




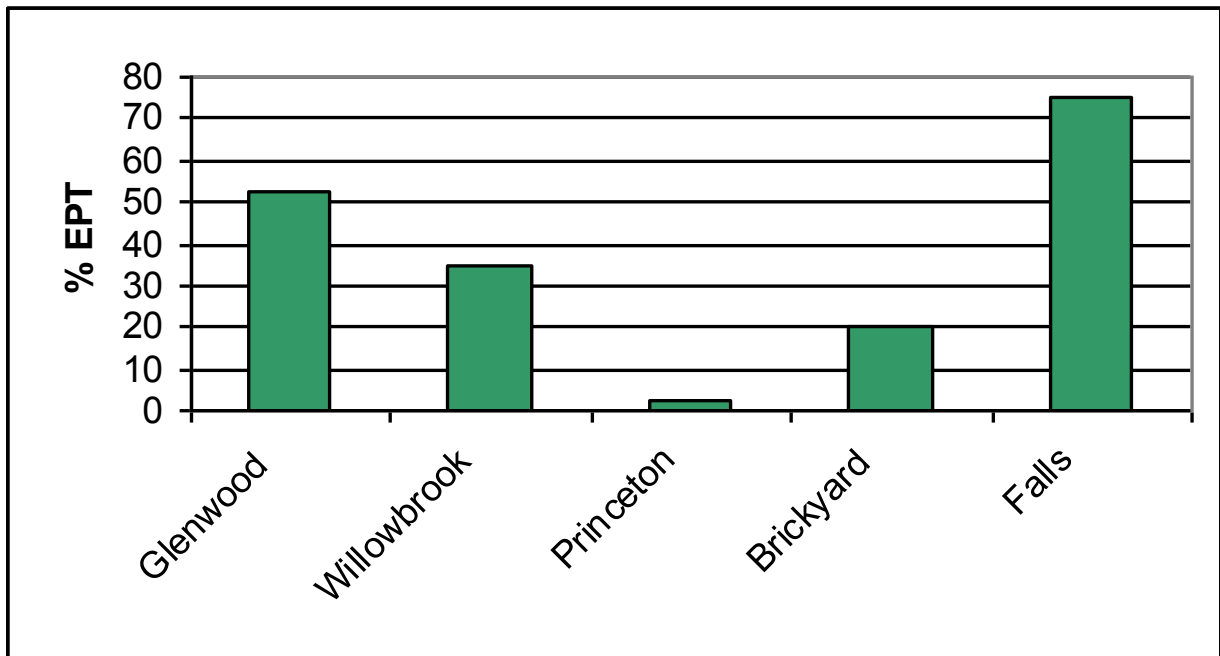
**Figure 2. Glenwood Sample Area**



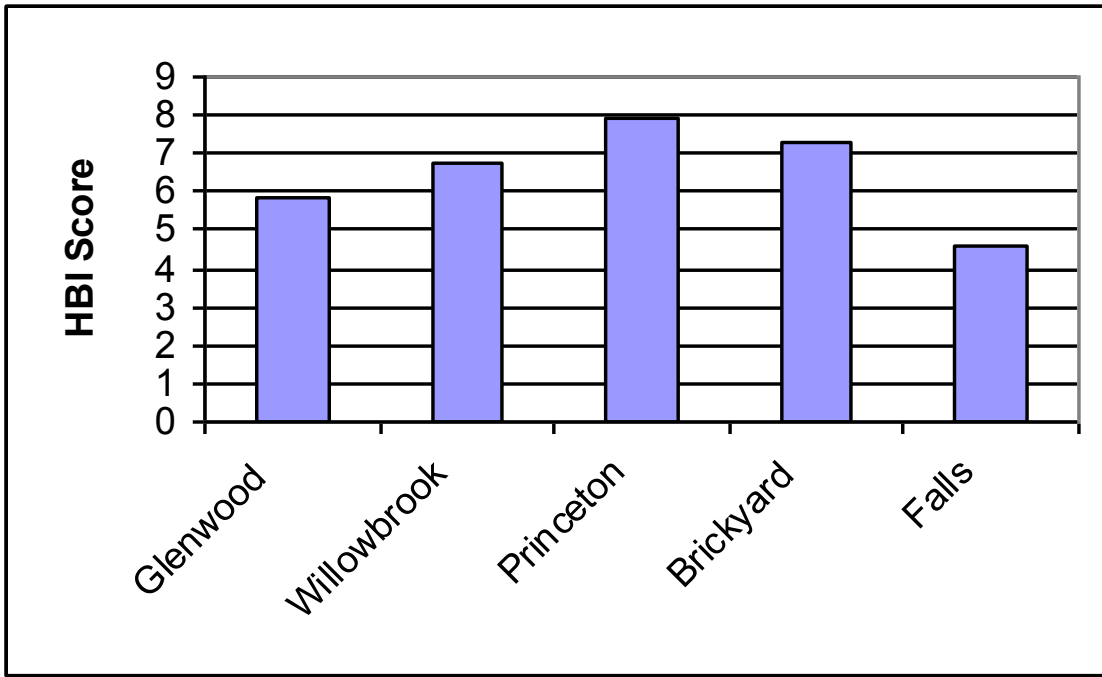
**Figure 3. Princeton Sample Area**



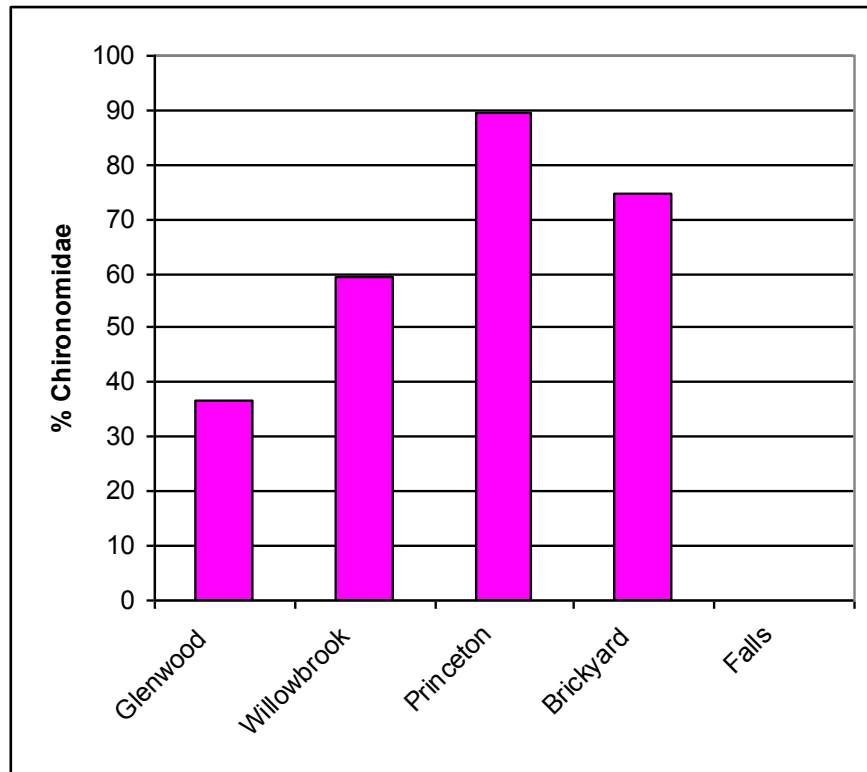
**Figure 4. Brush Creek Falls Sample Area**



**Figure 5: Percent EPT Abundance Present at each Sampling Site**

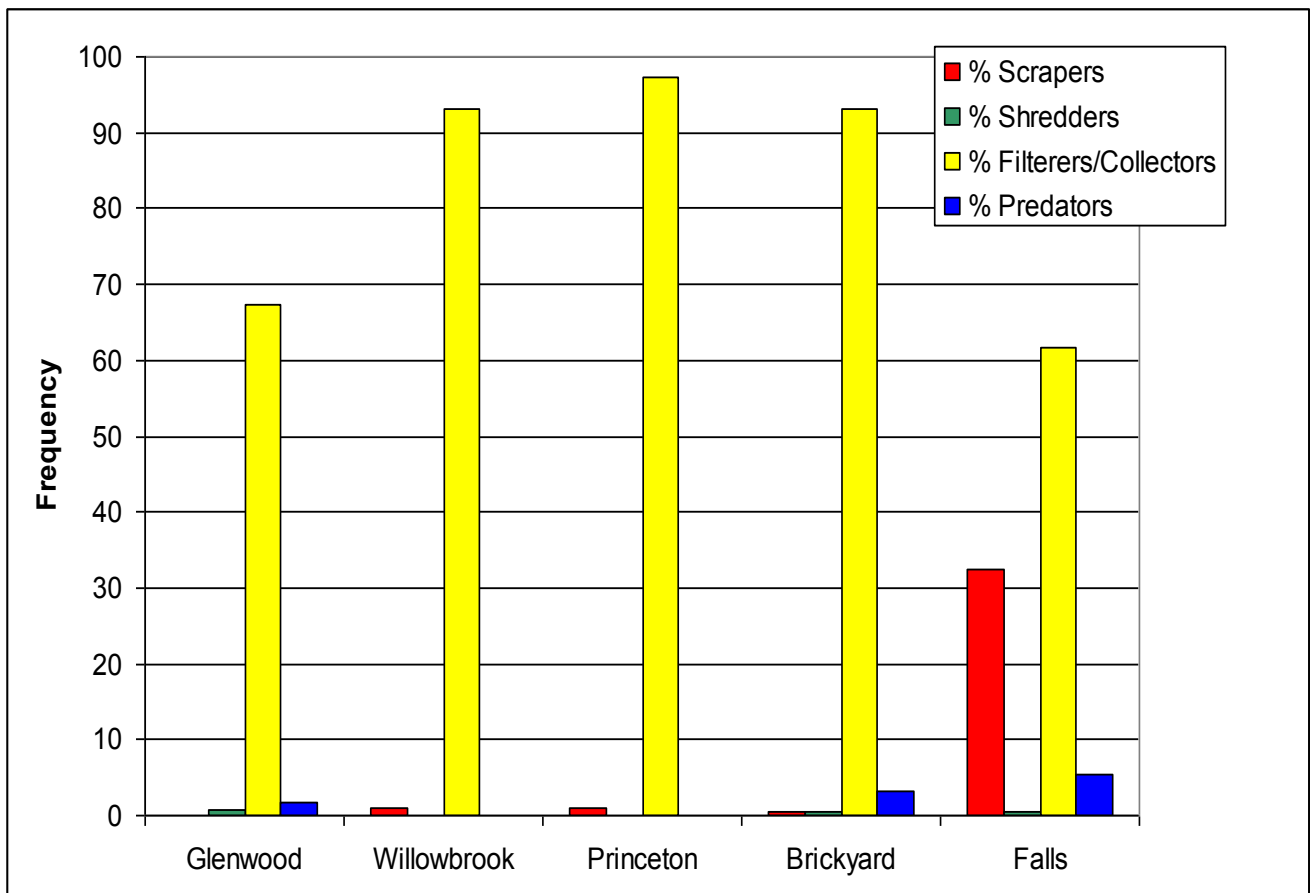


**Figure 6: HBI Score for each Sampling Site**



**Figure 7: Percent Chironomidae Present at each Sampling Site**





**Figure 8: Functional Group Diversity for each Sampling Site**

Location	HBI (2006/2007)	HBI (2007/2008)
Willowbrook	4.9	6.7
Princeton	7.3	7.95
Brickyard	7.8	7.2
Falls	2.0	4.59

**Table 1: Comparison of HBI Scores between 2006/2007 and 2007/2008 Studies**

	Glenwood	Willowbrook	Princeton	Brickyard	Falls
% EPT	52.4	34.4	2.34	20.5	75.1
HBI	5.81	6.74	7.95	7.27	4.57
% Chironomidae	36.5	59.6	89.7	74.7	0.0
Diversity	Low	Very Low	Very Low	Moderate	High
Water Quality	Fair	Poor	Very Poor	Very Poor	Good

**Table 2: Summary of Data Collected from Macroinvertebrate Study**

	GLENWOOD	WILLOWBROOK	PRINCETON	BRICKYARD	FALLS
<b>EPHEMEROPTERA</b>					
BAETIDAE	27	1			
EPHEMERELLIDAE	1				5
HEPTAGENIIDAE		2		1	22
OLIGONEURIDAE					13
<b>PLECOPTERA</b>					
NEMOURIDAE	1				
PERLIDAE					6
TAENIOPTERYGIDAE	2				
<b>TRICHOPTERA</b>					
HYDROPSYCHIDEA	11	58	1	33	102
HYDROPTILIDAE	24	14	4	5	9
<b>GASTROPODA</b>					
LYMNAEIDAE			2		22
<b>MEGALOPTERA</b>					
CORYDALIDAE					5
<b>COLEOPTERA</b>					
DYTISCIDAE	1				
ELMIDAE		3	2		
PSEPHENIDAE					24
<b>DIPTERA</b>					
CHIRONOMIDAE	46	130	192	142	
EMPIDIDAE				6	
SIMULIIDAE	10	1		1	
TIPULIDAE				1	1
<b>BIVALVIA</b>					
CORBICULIDAE		3	3	1	
<b>CRUSTACEA</b>					
CAMBARIDAE		6			
<b>MISCELLANEOUS</b>					
HEMIPTERA	1				
OLIGOCHAETA			10		

**Table 3: Orders and Families of Macroinvertebrate Taxa**

<b>Hilsenhoff Biotic Index</b>	<b>Water Quality</b>	<b>Degree of Organic Pollution</b>
0.00-3.75	Excellent	Organic Pollution unlikely
3.76-4.25	Very Good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution likely
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very Poor	Sever organic pollution likely

**Table 4: Standards for Water Quality Using the HBI Index**

**GLENWOOD:**



**WILLOWBROOK:**



**BRICKYARD:**



**PRINCETON:**



**FALLS:**





Gene Expression Profiles of Toll-Like Receptors (TLRs) 2 and 4 during *Chlamydia*  
Infection in a Mouse Stress Model

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Abstract

The role of Toll-Like Receptors (TLRs) 2 and 4 in the recognition of pathogen associated molecular patterns (PAMPS) and activation of the immune inflammatory response in humans and mice has been elucidated. However, the effect of stress against the innate immune system, particularly with TLRs activity, during the early phases of *Chlamydia* infection remains unknown. The goal of the present study was to examine the gene expression profiles of TLR2 and TLR4 in the genital tract of mice, when under stressful conditions, during *Chlamydia* infection using RT-PCR analysis. Quantitative analysis of TLR2 and TLR4 at different time intervals of early infection showed higher expression of the TLRs in the non-stressed mice compared to the stressed. A decreased expression of TLRs 2 and 4 was observed in stressed mice compared to non-stressed mice during *Chlamydia* infection. The greatest difference in the gene expression profiles of the TLRs between the non-stressed infected and stressed infected mice occurred at 4h-8h post infection. This data suggests that stress does alter the expression of TLR2 and TLR4 during *Chlamydia* infection. Our findings may contribute to our present understanding of the association of TLRs and the innate immune system in relation to *Chlamydia* disease, particularly under stressful conditions.

## Introduction

Chlamydia genital infection caused by *Chlamydia trachomatis* is the most common bacteria sexually transmitted disease (STD) worldwide. Its complications include pelvic inflammatory disease and ectopic pregnancy that may lead to infertility in women (7,23). In addition, Chlamydia infection is known to increase susceptibility to HIV infection by 3-5 times. Approximately 2.8 million cases are reported annually in the United States with health care costs exceeding \$3.5 billion. The CDC estimates that for every dollar spent on Chlamydia testing and treatment saves \$12 in complications arising from untreated infections. Approximately three-quarter of the women and half of the men infected with Chlamydia experience no symptoms which has made it known as the “silent epidemic”.

*Chlamydia trachomatis* is a small gram-negative bacterium. It is an obligate intracellular parasite that attacks epithelial and endothelial cells (38). *C. trachomatis* is composed of a lipopolysaccharide cell wall, making it a potent stimulator of innate immunity. It is unclear which chlamydial structures are involved in triggering the cellular responses during infection (30). However, the focus of chlamydial studies has been on the role of the innate immune system on regulating the early response to infection (10). A fairly new discovery of a family of proteins that share homology with the toll antimicrobial proteins of *Drosophila* have been associated with the innate immune response to Chlamydia infection. These proteins, called Toll-like receptors (TLRs), are receptors that recognize pathogen associated molecular patterns (PAMPs). TLRs are a component of the innate immune system. They are found on epithelial cells. TLRs are composed of a leucine-rich extracellular domain and an intracellular tail that shares homology with the intracellular domain of the Toll/interleukin-1 receptor (IL-1R) (24). After a host is infected with a pathogen, the activated IL-1R binds to the adaptor protein MyD88, which initiates a signaling cascade, leading to activation of inflammatory leukocyte NF- $\kappa$ B and the subsequent production of proinflammatory and immunoregulatory cytokines, chemokines, and costimulatory molecules (29). There are 13 known TLRs that recognize a wide variety of pathogens. Among those, TLR2 and TLR4 have been associated with several bacterial pathogens, including *Chlamydia trachomatis*. TLR2 recognizes and signals peptidoglycans from gram-positive bacteria and bacterial lipoprotein. TLR4 recognizes LPS from gram-negative bacteria (1).



Stress is known to influence the actions of the immune system. Naturally, the brain and the immune system interact in order to maintain health and stability in the body (12). When the brain becomes stimulated due to a stressor, neurotransmitter norepinephrine is released from the sympathetic nerve terminals in lymphoid (or immune) organs. In response, immune cells targeting the norepinephrine express adrenoceptors. Under stimulation of these receptors, the released norepinephrine or epinephrine affect lymphocyte trafficking, circulation, and proliferation, as well as alter cytokine production and the sensitivity of the lymphatic cells. When the stressor is negative, such as pain or aggregation, enhanced inhibition of the lymphoid cells occur. Study shows that stimulation of the  $\beta_2$ -adrenoceptor-cAMP-protein kinase A pathway by norepi may inhibit the production and actions of Th1 proinflammatory cytokines, such as IL-12, IL-1, IFN- $\gamma$ , and TNF- $\alpha$ , and enhance the actions of the Th2 anti-inflammatory cytokines, such as IL-10 and TGF- $\beta$ . The Th1 proinflammatory cytokines is associated with T-cell mediated immunity. This immune response occurs during cell-mediated immunity or acquired immunity, where the pathogen invasion has been present in the body for more than one day. The Th2 anti-inflammatory response occurs during innate immunity, or the first few hours of pathogen invasion. My research focused on the innate immune system. This study may provide some insight as to the immune response during Chlamydia infection within 2-24 hours after invasion. Overall, stress does impact the response of the immune system.

The goal of the present study was to examine the gene expression profiles of TLR2 and TLR4 in the genital tract of mice when under stressful conditions during Chlamydia infection. To date there is enough literature showing that stress can suppress various aspects of the innate and adapted immune responses. However, the role of stress to invasion and the response of the innate immune system during exposure to *Chlamydia trachomatis* have not yet been established. Our approach in this study was to stress mice with cold water, then intravaginally infect them with *Chlamydia trachomatis* (mice pneumonitis). The genital tract was extracted from stressed and non-stressed mice and harvested at 2, 4, 8, and 24 hour intervals following infection for RNA isolation. Patterns of gene expression were determined using RT-PCR analysis. Data obtained from this study may aid in our understanding of Chlamydia infection and expound upon knowledge of the effects of stress on the innate immune system.

## Materials and Methods

**Cell lines and *Chlamydia trachomatis* stock cultures.** HeLa-229 cell line and McCoy cells for tissue culture and the *Chlamydia trachomatis* mouse pneumonia (MoPn) biovar (strain Nigg II), (new name: *Chlamydia muridarum*), obtained from American Type Culture Collection (ATCC) was used in this study.

**Animals.** Five to seven-week-old female BALB/c mice were purchased from Harlan-Sprague Daley (Indianapolis, IN). Mice were given food and water ad libitum in an environmentally controlled room illuminated 24 hours. All animal experimentation was preapproved by the IACUC of Bluefield State College.

**Cold water stress protocol.** We used an 8 day cold water stress paradigm that has been previously found to be optimal for detection of certain stress-induced immune response and hormonal level changes in previous studies (3,8). Briefly, mice were placed in white shallow tub filled with 5cm of ice water ( $1\pm 0.5^{\circ}\text{C}$ ) for 5 minutes each day for 8 days. The water level was deep enough to cover the backs of the mice while their heads remained above as they swam. The animals were allowed to move freely within the tub. The non-stressed mice were not subject to cold water stress.

**In vivo infection of mice and isolation of genital tract.** Twelve stressed and non-stressed mice were infected intravaginally with  $10^7$  IFU of MoPn per mouse in a volume of 30ul of phosphate-buffer saline (PBS) while under isoflurane-induced anesthesia. Uninfected control mice received 30ul of PBS. Euthanasia was carried out by CO<sub>2</sub> inhalation at 2, 4, 8, and 24 hours after the infection. A portion of genital tract was harvested for total RNA extraction using Tri Reagent from Sigma following the manufacturer's instructions.

**RNA Isolation and reverse transcription-PCR.** Total RNA was isolated from the genital tract by sequential separation using the Tri Reagent and protocol. RNA concentration was determined by RNA quantitation. All RNA samples were treated with Rnase-free DNase I to prevent DNA contamination. With 5ug of RNA as the template for each reaction, first-strand cDNA was synthesized in a 20ul reaction with random primers, RT-reagents, and Stratascript first strand synthesis system (Stratagene). PCR analysis was performed using the Mx3000P QPCR system. The IFU values in stressed, non-stressed and normally caged control mice was determined by staining of the infected monolayer

of McCoy cells with fluorescein isothiocyanate-labeled, genus-specific antichlamydial antibodies to detect Chlamydia inclusions by direct immunofluorescence.

**Real-time PCR.** We used an Mx3000P QPCR system using Brilliant SYBR Green QPCR (Stratagene). For each reaction, 1ul of cDNA of target TLRs were subjected to PCR amplification in a 25ul final volume containing 1ul of each primer and 12.5ul of 2X SYBR green master mix. The following conditions were used for amplification: 1 cycle at 90°C for 5 minutes, 40 cycles at 95°C for 15 seconds, and 1 cycle for 60°C for 1 minute.

#### **Primer Sequences of TLRs for PCR analysis**

TLR2 F: 5'-TGCAAGTACGAACTGGACTTCT-3'

R: 5'-CCAGGTAGGTCTTGGTGTTTCATT-3'

TLR4 F: 5'-TAGCCATTGCTGCCAACATCAT-3'

R: 5'-AAGATACACCAACGGCTCTGAA-3'

**Detection of chemokines from HeLa cells.** Tissue cultures of HeLa cells were grown and analyzed by ELISA 2, 4, 24, and 48 hours after infection to detect the secretions of various chemokines: RANTES, IP-10, MCP-1, MIP-1alpha. The supernatant was removed from the culture well and assayed using the ELISA kit according to manufacturer's instructions.

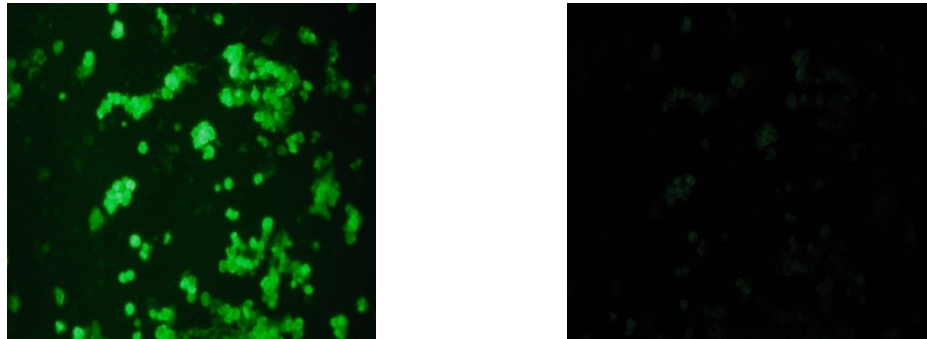
**Data Analysis.** Data analysis was carried out by use of quantitative real-time PCR and multiplexing applications of Mx3000P system.

### **Results**

TLRs are recognized for their primary and significant role in the early detection of infection and the subsequent signaling of a cytokine pathway that results in an immune defense. We examined whether the host's stress level would impact the immediate response of the TLRs following infection.

Before measuring the mRNA levels, i.e. the expression of the TLRs, the Chlamydia infected mice cells were stained to verify successful infection. Figure 1 shows micrographs of stained infected and non-infected McCoy (mice) cell lines. The McCoy cells were stained with antichlamydial antibodies. These antibodies would attach to any Chlamydia infected cells and fluoresce. Cells that have no Chlamydia infection would not bind the antibodies, and therefore would not fluoresce.

Figure 1: Micrographs of Stained McCoy Cells  
Chlamydia Infected                      Chlamydia Non-Infected



The picture on the left shows infected McCoy cells. The micrograph clearly shows fluorescence. The picture on the right shows green McCoy cells but no fluorescence, indicating that there is no Chlamydia present in these cells.

DNA samples from stressed and non-stressed mice at each time interval after infection was amplified to measure the mice mRNA levels (Figure 2). The mRNA is representative of the DNA (specifically the synthesized cDNA molecule). These measured mRNA levels were a measure of the amount of immune cells responding to the infection. Higher mRNA levels would indicate more immune cells present during infection, therefore the response of the immune system was not inhibited. Lower mRNA levels would indicate less immune cells present during infection, showing an inhibition of the immune system. The inhibition of the immune system would be attributed to stress. This research primarily focuses on the expression of TLRs in the immune response to Chlamydia infection. Therefore, TLRs expression would be decreased when stress is present. A decreased expression of the receptors would lead to the stimulation of fewer immune cells. Data was analyzed by use of comparative critical threshold (Ct) method from DNA amplification through quantitative PCR. The Ct value is based on a threshold that identifies the amount of DNA present in a sample depending on when the sample crosses the threshold during amplification. The assay would recognize how many cycles occur before the sample of DNA fluoresces. It is at that time point that the assay generates a Ct value. A lower Ct value would indicate a sample with more DNA. A sample with more DNA would take less cycles to fluoresce, generating a smaller Ct value. A higher Ct value would be indicative of less DNA in the sample. This means that

more cycles occurred before the DNA sample fluoresced, generating a lower Ct value. This comparative method was used to analyze data throughout the experiment.

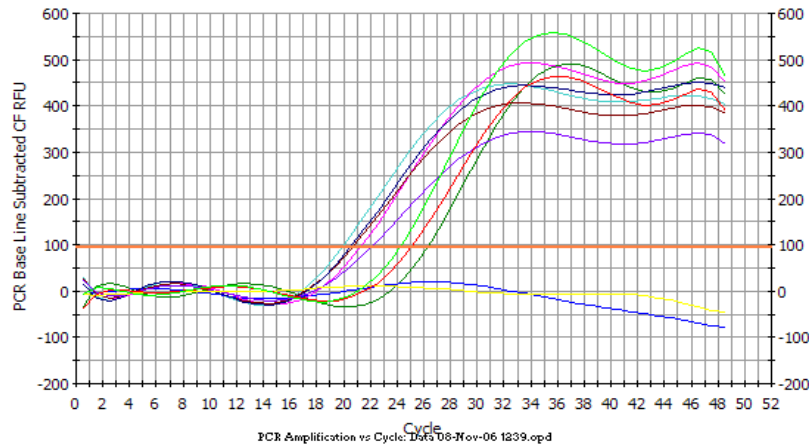


Figure 2: DNA Amplification Chart

**The Ct values of TLR2 showed increased mRNA levels present in non-stressed mice compared to stressed mice during Chlamydia infection.** DNA amplification showed lower Ct values for the non-stressed infected mice compared to the stressed infected mice (Table 1a). There was a significant difference in the Ct values of the non-stressed infected compared to the stressed infected mice from 4h-8h post infection. A minimal difference between the non-stressed infected and the stressed infected mice was detected at 2h after infection. The differences between the two groups increased as time post infection increased. At 24h the Ct value of the stressed infected mice was significantly lower than that of the non-stressed infected mice. This cause of this reversed result is unknown.

**Ct values for TLR4 showed higher levels of mRNA in the non-stressed infected mice compared to the stressed infected mice.** TLR4 is especially important for its role in recognizing Chlamydia for its gram-negative characteristic. Our results show lower Ct values in the non-stressed infected mice compared to the stressed infected mice (Table 1b). A marked difference in mRNA levels between the two groups was observed at 4h post infection where the Ct value of the non-stressed mice was significantly lower than that of the stressed mice. There was a steady increase in the Ct values for both groups as the time post infection increased.

Toll-Like Receptor 2				
Hours After Infection	Non-Infected	Non-Stress Infected	Stress Infected	Difference in Ct value
2	44	45.20	45.59	0.37
4	-	42.31	45.15	2.84
8	-	43.44	46.92	3.48
24	-	43.48	39.90	-3.58

Figure 1a: Ct values for TLR2

Toll-Like Receptor 4				
Hours After Infection	Non-Infected	Non-Stress Infected	Stress Infected	Difference in Ct value
2	29.96	24.40	25.83	1.43
4	-	25.25	28.46	3.21
8	-	25.55	27.29	1.74
24	-	28.63	30.59	1.96

Figure 1b: Ct values for TLR4

To examine other immune responses to the Chlamydia infection, we observed the actions of some chemokines; RANTES, IP-10, MCP-1, and MIP-1 $\alpha$ . ELISA assay was used to quantitate the production of chemokines in Chlamydia infected and non-infected HeLa cells. We observed that Chlamydia infection induced early production of the chemokines.

**Chlamydia infection in HeLa cells lead to increased production of chemokine RANTES in non-stressed mice compared to stressed mice.** Infection by *Chlamydia trachomatis* resulted in a marked increase in RANTES production at 4h post infection, with the secretions reaching a peak at 24h post infection and slight decrease at 48h (Figure 3). Non-infected HeLa cells showed no significant levels of secretion until 24h after infection, continuing with a slight increase in production at 48h.

**Chlamydia infection resulted in an increased production in chemokine IP-10 as early as 4h post infection.** Marked secretions of chemokine IP-10 occurred at 4h post infection with a steady rise until 24 h (Figure 4). There was a significant increase in secretion level at 48h post infection. In comparison, secretions in non-infected HeLa cells remained minimal until 24h. At 48h after infection a decrease in secretion was observed in the non-infected cells.

**Secretions of chemokine MCP-1 showed a steady rise in both the infected and non-infected HeLa cells beginning at 2h.** A marked increase of MCP-1 production was observed as early as 2h (Figure 5). At each time point, secretions by the infected cells were significantly higher than that of the non-infected cells.

**Observed secretions of chemokine MIP-1alpha beginning at 2h reached its peak at 24h and 48h in the infected and non-infected HeLa cells.** Chlamydia infection resulted in an increase in production at 4h post infection for infected HeLa cells (Figure 6). At 24h secretions rapidly increased and remained high with a slight decrease at 48h. The non-infected cells had minimal secretions until 48h where there was a rapid increase in production level of the chemokine.

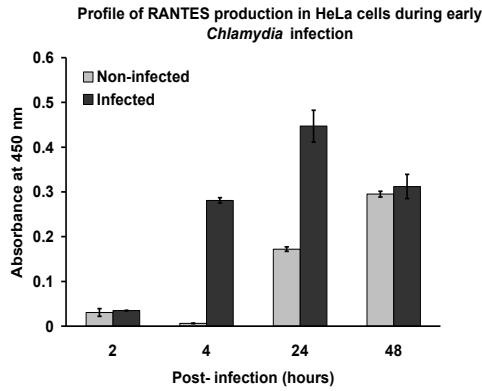


Figure 3: RANTES production  
Chlamydia infection resulted in an increase in secretion of RANTES over the constitutive levels after 4h infection and remained at the same level by 48h post infection with a slight decrease at 48h.

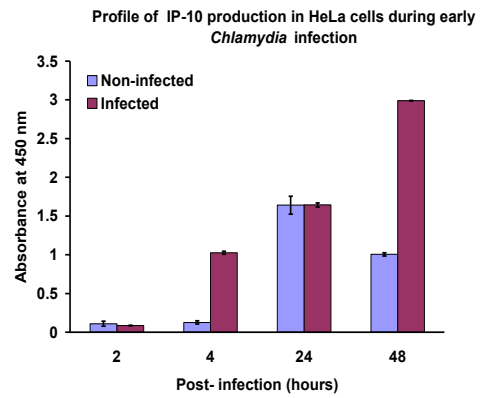


Figure 4: IP-10 production  
Chlamydia infection resulted in an increased expression of IP-10 with a marked rise 48h post infection, compared to the non-infected HeLa cells.

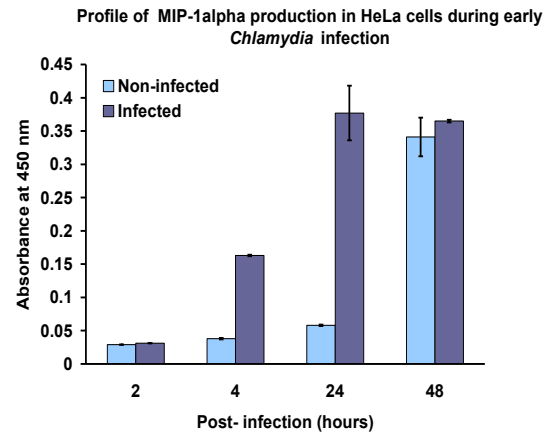


Figure 6: MIP-1 $\alpha$  production  
Chlamydia infection resulted in a rapid increase in production of MIP-1 $\alpha$  as early as 4h post infection. The highest peak in production of the chemokine occurred at 24h post infection. The constitutive level and the induced production remained at the same high level by 48h post infection.

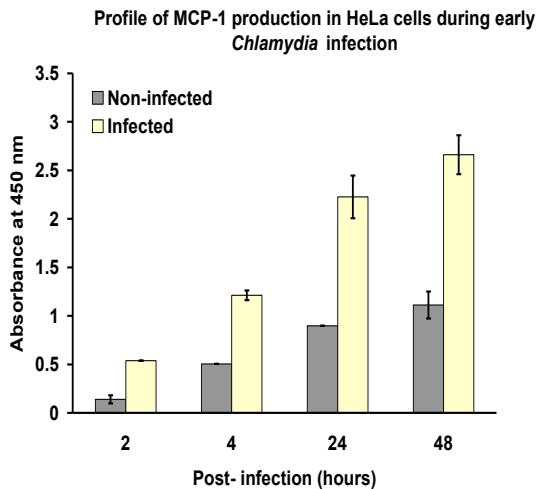


Figure 5: MCP-1 production  
Chlamydia infection resulted in a steady increase in production of MCP-1 as early as 2h post infection. This rise was continuous throughout 48h post infection.



## Discussion

Studies of the innate immune system have linked the membrane bound Toll-Like Receptors (TLRs) to the Chlamydia genital tract infection (10,29). These studies suggested that the TLRs do play a role in the innate response to infection by *Chlamydia trachomatis*. In previous studies, these receptors were shown to be expressed in the tissues of the female reproductive tract during immune surveillance and were also shown to be involved in the production of certain cytokines following chlamydial infection (28,10). Although the expression of the TLRs during Chlamydia infection has been elucidated, the involvement of the TLRs during Chlamydia infection under stressful conditions has not been studied. In this study, we examined the roles of TLRs 2 and 4 in the Chlamydia genital tract infection when the host was under stressful conditions. The results of this in vivo experiment indicated a link between the stressed conditions of the mice and the expression of the TLRs.

Analysis of TLR2 showed a higher expression of the receptor when the infected mouse was non-stressed versus a lower expression in the infected stressed mice. The level of TLR expression was evident in the production of mRNA, shown by higher mRNA levels in the non-stressed mice, indicating a higher expression of the TLR compared to a lower mRNA level in the stressed infected mice, indicating a lower expression of the TLR. The Ct value for the non-stressed mice was low, but increased as the time post infection progressed. This observation indicated a decrease in the expression of TLR2 as the time after infection increased. TLR are recognized as a component of the innate immune system. The innate immune system is active within the first few hours after infection. The decreased expression of the TLR2 as time after infection increased satisfies the known behavior of the receptor. The higher Ct values of the stressed mice showed an increase until 8h post infection. At 24h, the Ct value was significantly lower than that of the non-stressed mice. This result may indicate some unknown response of the immune system at 24h post infection or a testing error. The differences in the values of the non-stressed infected mice and the stressed infected mice suggest that stress does have an impact on the expression of TLR2 during Chlamydia infection. When the mice were free of stress, their TLRs were expressed at a normal level by signaling a substantial amount of immune cells to the infection. On the other hand, when the mice were subject to stress, there was an inhibition in the expression of their TLRs. This decrease in receptor expression lead to a decreased amount of immune cells signaled and presented against the infection.

Observations of TLR4 showed a higher expression of the TLRs in the non-stressed infected mice compared to the stressed infected mice, implying that stress did alter the response of the immune system to chlamydial infection. Ct values were lower for the non-stressed infected mice compared to the stressed infected mice. This indicates that expression of the TLRs was normal when the mice were not under stress. When the mice were under stress, expression of the TLRs was inhibited. The largest difference in the secretion levels of the TLR was seen at 4h post infection where the Ct value of the non-stressed mice was significantly lower than the stressed mice. This suggests that at 4 hours, infection is high and has been recognized by the TLRs. It is at this time that the non-stressed mice show its greatest response to the infection with very low Ct values. The stressed mice, on the other hand, respond less to the infection. Our data suggests that stress does play a role on the expression of TLR4 during Chlamydia infection.

In this study, stress has shown to modulate the expression of TLRs 2 and 4 during Chlamydia infection. Both receptors showed an increase in Ct values for both the non-stressed and stressed mice. This result may imply that as time after infection grew, expression of these two receptors declined. This may be due to the fact that other immune receptors and cells have become active later in the infection. We also observed significantly lower overall Ct values in TLR4 compared to TLR2. This observation allows us to understand a significant difference in the roles of the TLRs in recognizing the Chlamydia infection. The significance of the receptors cannot be compared because they each identify different structures in the Chlamydia bacterium. However, we were able to suggest that one TLR, TLR4, has greater expression and signals more immune cells than TLR2. Overall, both receptors showed inhibition when the mice were subject to stress.

In conclusion, this study examined the response of the immune system to the Chlamydia genital tract infection in relation to TLRs when the body is under stress. We were able to gather from this study the impact of stress on the immune system. Stress significantly alters the expression of the two receptors studied. Looking at the epidemic of Chlamydia genital infection, more complications arise from the disease than deaths. A cure exists, however infectious cases continue to rise. Therefore, the focus of Chlamydia prevention is not curing the disease but controlling and minimizing its complications, as well as developing awareness. Prolonged exposure to the Chlamydia genital infection can eventually destroy the reproductive organs. In this study, when the host was subject to stress, the response of the immune cells was decreased.

Less immune cells fighting the infection allows greater damage to be done. If the individual is able to control stress, they can decrease the amount of destruction caused by Chlamydia. This in vivo study provided further insight to the behavior of TLRs and the immune system when under stress. This study will have to be repeated in order to make correlations between the data. Future studies would involve analyzing the stress hormones of mice and the response of the chemokines and cytokines that are secreted after infection. Much information was established in this study to promote further research on TLRs and emphasize its significance and influence on the immune system.

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## **Fecal Coliforms in Brush Creek**

**Amanda Lawrence**

**Mentor: Tom Ford**

### **ABSTRACT**

We examined how human activity and land use impacted fecal coliform concentrations in Brush Creek, in southern West Virginia. We examined three segments of the stream: the suburban headwaters, the urban middle reaches, and the downstream forested reaches using the membrane filtration method. Characteristics that might have impacted the number of fecal coliforms examined included impervious surface runoff, septic systems, and farmland runoff. This preliminary study is intended to examine the potential level of coliform exposure in the recreational Brush Creek area. In this study, the urban landscape had greater mean colony forming units (CFU) per 100 ml than the suburban area, with 630 and 272 CFU per 100ml respectively. The forested landscape however, had greater mean CFU per ml than both the suburban and urban landscapes with a mean of 5,666 CFU per 100ml. The CFU for all three landscapes exceeded standard water quality regulations for drinking and recreational use. The problem with fecal coliform contamination could be solved or decreased by minimizing parking lots, installing filtration systems, and the inflow of untreated sewage.

## **Introduction**

Water quality is of great importance when it comes to people and their health; as a result it is essential to understand the impact that human land use has on bodies of water in urban areas (Roark 1997). A major impact that urban areas have on streams and rivers is elevation in concentration of fecal coliforms. Fecal coliforms are bacteria that live in the digestive tracts of warm-blooded animals, and are excreted in the feces (EPA 2006). Some fecal coliforms include disease causing bacteria that can be hazardous to human health (EPA 2006). Diseases such as dysentery, typhoid, and cholera, are all caused by bacteria that are members of the fecal coliforms (EPA 2006). Respiratory infections, liver disease and potentially fatal gastrointestinal disorders may also be caused by fecal coliform infections (Mallin 2006).

Fecal coliforms are commonly found in aquatic habitats and are usually more abundant within urban areas (Paul and Meyer 2001). An urban area, as defined by the US Census Bureau can include both urban clusters and urbanized areas. An urban cluster is a densely settled area with a population of 2,500 to 49,999, and more than 1,000 people per square mile. An urbanized area is a population size that is greater than 5,000 people and 1,000 or more people per square mile. In 2001, more than 75% of the U.S. population lived in urban areas (Paul and Meyer 2001). This percentage shows the large number of people that could be affected by fecal coliform contamination in water used to bathe, drink, and swim (EPA 2006).

Fecal coliforms come from many different sources that can feed into nearby streams, creeks, and bays. These sources include impervious surface runoff, septic systems, and farmland runoff (Mallin 2006). The construction of buildings with large parking lots degrades the natural drainage system around aquatic areas (Mallin 2006). Septic systems that are overloaded result in high concentration of fecal bacteria in urban streams (Young and Thackston 1999). Farmland



runoff contributes manure, pesticides and fertilizers to streams; thereby causing increased fecal coliform levels.

### **Impervious Surfaces**

Impervious surfaces are areas covered by pavement, rooftops and sidewalks (Albanese and MatLack 1998). These impervious surfaces do not let water penetrate into the soil, thus when it rains the water picks up substances like oil, fuel, and animal feces, and washes them into ditches and drains that usually lead directly to urban waterways (Mallin 2006). The Environmental Protection Agency has reported that this type of pollution is the leading cause of water quality problems in the U. S. (EPA 2006).

In Hattiesburg, Mississippi, pollutants such as oils, fuels, radiator fluid, and heavy metals were found to have entered streams from parking lots (Albanese and MatLack 1998). Although parking lots are essential for people to park their cars, there are ways in which much of the runoff could be minimized. Parking lots tend to be much larger than needed, and are usually used below capacity (Wilson 1995). Mallin and others examined the relationship between land use and fecal coliform concentrations within several watersheds (Mallin 2006). They found that fecal coliform concentrations were higher in creeks with high human population and developed land in their watersheds. Bacterial counts associated with fecal coliforms were highest in areas with a high percentage of impervious surfaces (Mallin 2006).

There are ways in which much of the runoff from impervious surfaces could be minimized. Parking lots can actually be paved with porous concrete that allows water to pass into the soil below, and still provides enough support for vehicles (Mallin 2006). Parking lots that are already made can install filtration systems to filter out the pollutants, and thus cut down on the bacterial counts in nearby streams (Albanese and MatLack 1998).

## **Septic Systems**

Failing septic systems and sanitary sewer overflows can lead to dangerous conditions for surrounding streams. During heavy rainfall the water quality of many urban streams tests positive for high levels of fecal coliforms (Young and Thackston 1999). Bacteria counts are usually higher in urban streams, especially after heavy storms (Paul and Meyer 2001). This is usually due to septic systems that have not been taken care of properly and overflow causing unsafe conditions. Some communities have their storm drains connected to septic systems, and a heavy rain can cause major overflows into nearby watersheds.

It is not uncommon for septic systems to cause potential threats during the dry season as well. High values during a dry season are a good indication that there is chronic sewer leakage (Mallin 2006). The Florida Keys are one good example of how septic systems can cause major problems. In 1995 Paul and Rose found that the fecal microbes from septic tanks in the Keys pass easily through the soil and within hours can enter coastal waters (Mallin 2006). The Florida Keys are not the only location with this problem. Parts of North Carolina share the same septic tank concerns. Septic systems are not only causing problems in the US, but they are presenting problems in tropical regions as well (Isobe 2004). Sewage pollution is a serious health risk for people who live near waterways in tropical regions. Direct discharge from waste coming from poorly maintained septic tanks are one of the major causes of waterborne disease. It is an important task for people in tropical regions to pay close attention to their septic systems because high temperature and rainfall lead to soil erosion and eventual sewage leakage into nearby watersheds.

The fact that septic tanks can cause high fecal coliform levels in waterways could be solved with careful planning and better sewage treatment. Septic tanks need to be pumped

regularly, and replaced when necessary. Conversion of septic tanks to a municipal sewer system can also improve water quality (EPA 2006).

### **Farmland**

Storm runoff carries pesticides, fertilizers, and manure from farmland to streams. Pesticides are usually high in urban streams and the concentrations usually exceed guidelines for the protection of aquatic life (USGS 1999). Pesticides are frequently applied around homes, lawns, and businesses; and without drainage or buffer zones they can be carried to nearby streams. The major concern is the feces carried from farmlands to streams. The bacteria, viruses, and protozoa from feces pose the greatest threat to human health (Mallin 2006). Mallin reported that a single gram of dog feces contains an estimated 23 million bacteria. Diesch reported that diseases could be spread from one warm blooded organism to another by water (Diesh 1970). Contamination of water is sometimes caused by feedlots. In 1970 a bacterial outbreak from fecal matter known as *Leptospirosis* occurred in several people in Iowa (Buckhouse 1976). These people were infected because water from a cattle farm had access to a nearby swimming hole. A similar incident happened in Columbus, Georgia where cattle, pigs, and dogs all were infected by *Leptospirosis*, and had access to a stream that led to a swimming hole (Buckhouse 1976). This type of incident is life threatening to the human population surrounding the streams. Streams in urban areas receive a lot of farmland runoff. Disposal of the waste in areas far away from waterways or installation of filters that catches runoff and cleanses it before it enters waterways can decrease the concentration of fecal coliforms from this source.

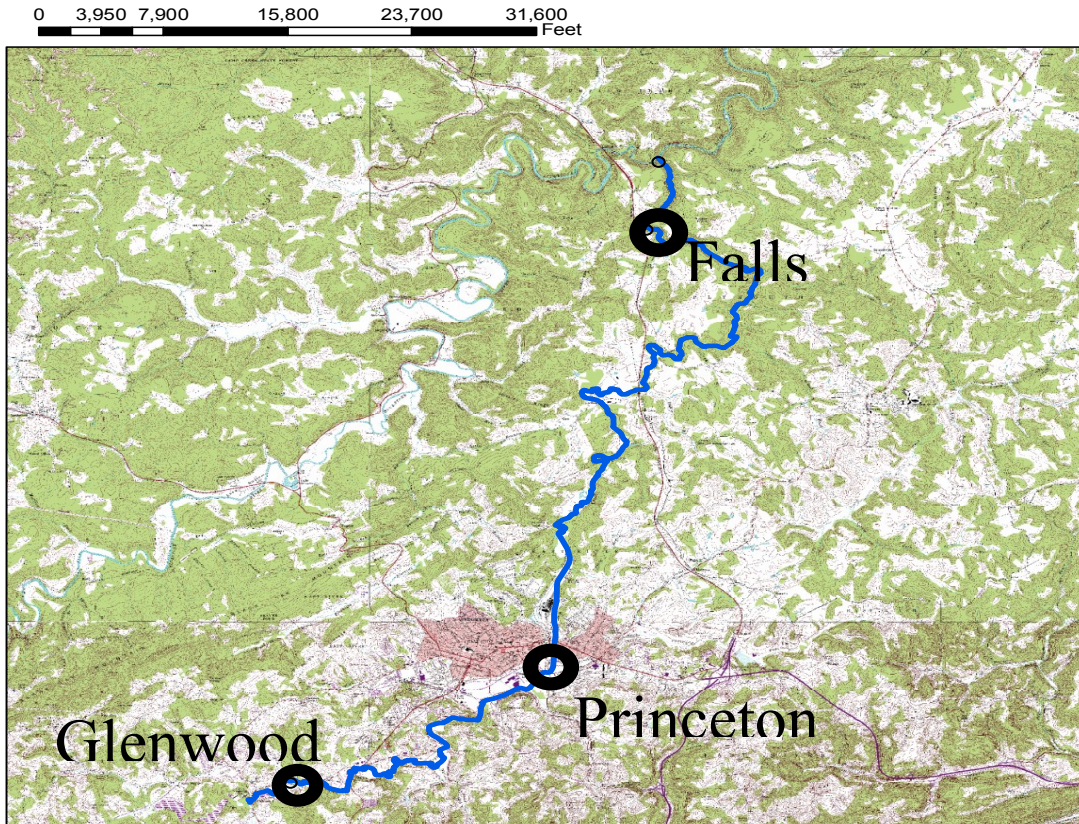
### **Objective of Research: Brush Creek in southern West Virginia**

In this study we examined how human activity and land use impact fecal coliform concentrations in Brush Creek, located in southern West Virginia. Brush Creek's headwaters are

near Bluefield, WV; and the waters run through Princeton, WV where it is heavily affected by humans and poor land use (Bowman 2006).

We examined three different segments of the stream: the suburban headwaters, the urban middle section of the stream, and the downstream forested area. The suburban area was located in the outlying area of Princeton, and we expected to find fecal contamination from septic systems, and farmland. The second area we examined was the actual urban cluster. This area runs through Princeton and should have significant impervious surface runoff as well as septic tank, and parking lot contamination. The last area we studied was a more forested area, and is known as Brush Creek Falls. This area runs downstream into the Bluestone River. In this area we expect to find some traces of farmland runoff and possible impervious surface runoff due to the forest.

After obtaining water samples at the three different locations in Brush Creek along its entire extent, we were able to measure the fecal coliform concentrations using the membrane filtration method. This allowed us to discuss what specific sources might have impacted the levels of fecal coliforms in Brush Creek. Using this information we can assess what possible health hazards people around the Brush Creek area may be exposed, and the extent to which they are impacted by fecal coliform contamination. Below is a map that identifies the sampling sites of Brush Creek (Bowman 2006)



**Figure 1. Map of the sites sampled along brush creek (Bowman 2006).**

**Methods and Materials**

In order to conduct this research, water samples of Brush Creek from different regions were collected. Three different segments of the stream from three different landscapes were examined: the suburban headwaters, the urban middle section of the stream, and the downstream forested area. Presterilized NASCO Whirl-pak bags were used to obtain three single samples from each of the three regions. Below is a table including the longitude and latitude for each of the three sampled sites within each of the three landscapes, as well as a brief description of the location.

Landscapes (Sample Sites)	Longitude	Latitude	Brief Description of Location
<b>Suburban Landscape</b>	Glenwood, WV		
Waterplant	81° 8' 39" W	37° 20' 11" N	located on Pepsi Plant Road just off 460
Pepsi Plant Rd.	81° 8' 35" W	37° 20' 13" N	20 m downstream from the water plant on Pepsi Plant Rd
End Reservoir	81° 11' 2" W	37° 19' 42" N	just off Rt. 20 on Brush Creek Reservoir Rd
<b>Urban Landscape</b>	Princeton, WV		
Exxon	81° 5' 48" W	37° 21' 29" N	behind Exxon located 50 m from 460 west
Princeton Mart	81° 5' 5" W	37° 21' 51" N	behind Princeton Mart located 10 meters from Roger's Street
Willowbrook Rd.	81° 6' 29" W	37° 20' 55" N	along Willowbrook Rd located 65 m from 460 west
<b>Forested Landscape</b>	Brush Creek Falls, WV		
Bridge	81° 3' 48" W	37° 21' 53" N	near the bridge on Brush Creek Falls Road that passes over Brush Creek
Midway	81° 3' 43" W	37° 27' 54" N	100 m midway between the bridge and the falls
Falls	81° 3' 38" W	37° 27' 58" N	80 m from the midway collection site to the falls

Table 1. Location of sampling sites in suburban, urban, and forested landscapes within the Brush Creek watershed.

The samples were all taken from the middle of the creek, and were collected between November of 2006, and March of 2007 (Appendix I, II, and III). Once in the middle of the creek the Whirl-pak bag was dipped about a foot into the water, and water was collected from the bottom of the creek up in a seeping motion (Harley 2005). After the bag was full of water, it was then dumped out, and a second sample was collected in the same manner. The bags were then tied off and placed on ice until reaching the lab. The samples were then placed in the refrigerator at 4°C until filtered; within 48 hours.

### **Filtration Procedure**

The membrane filtration method, as described in Standard Methods for the Examination of Water and Wastewater by the American Public Health Association, was used to conduct this research (APHA 1999). Pre-sterilized membrane filters with a pore size of 0.45µm. were used (APHA 1999). Samples were all diluted using dilution water composed of magnesium chloride, and potassium dihydrogen phosphate (HACH dilution water – product #21431-66). One hundred milliliters of each sample was filtered. Samples at each site were all analyzed at 1% dilutions (99% dilution water). The suburban and urban areas were also analyzed at 2% (98% dilution water), 3% (97% dilution water) and 5% (95% dilution water) dilutions.

Sterile forceps were then used to place the filter for each sample on M-Endo medium with a rolling motion to avoid entrapment of air. The petri dishes were then inverted and incubated for 20-24 hours at 37 degrees Celsius. After the incubation period the colonies were counted.

### **Colony Count and Data Analysis**

A low-power (10-15 magnifications) dissecting microscope was used to determine the number of colonies on a filter (APHA 1999). Colonies that appeared pink to dark red in color

with a metallic surface sheen were classified as fecal coliforms (APHA 1999). The fecal coliform density was calculated by using the following equation:

$$\text{Total coliforms/100mL} = \frac{\text{coliform colonies counted}}{\text{mL sample filtered}} \times 100$$

If there were no colonies observed, the sample was reported as < 1 coliform/100mL.

For verified coliform counts the initial count based was adjusted on the positive verification percentage. For example, if the volume of sample filtered was 1ml, then the positive verification percentage would have been 1% or 1% of verified coliforms. This was then reported as verified coliform count/100mL.

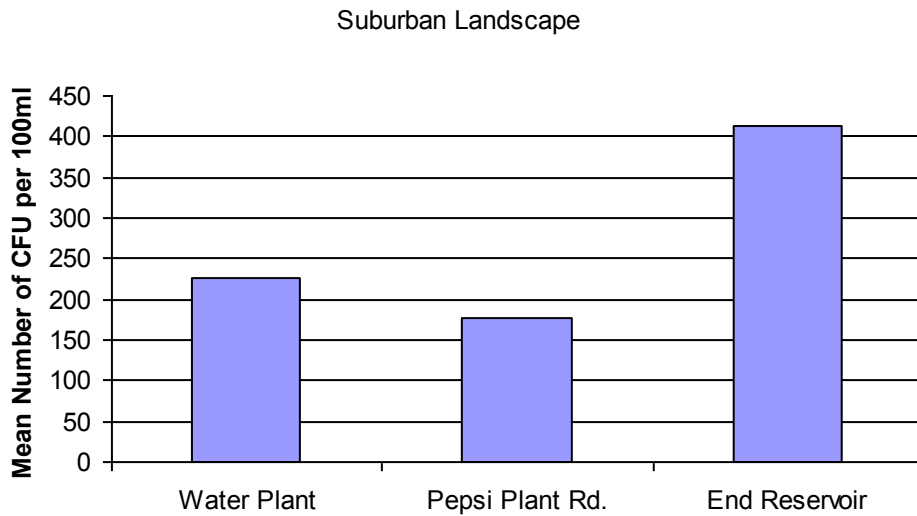
$$\% \text{ verified fecal coliforms} = \frac{\# \text{ colonies observed}}{\text{total \# coliform per 100ml}} \times 100$$

## **Results**

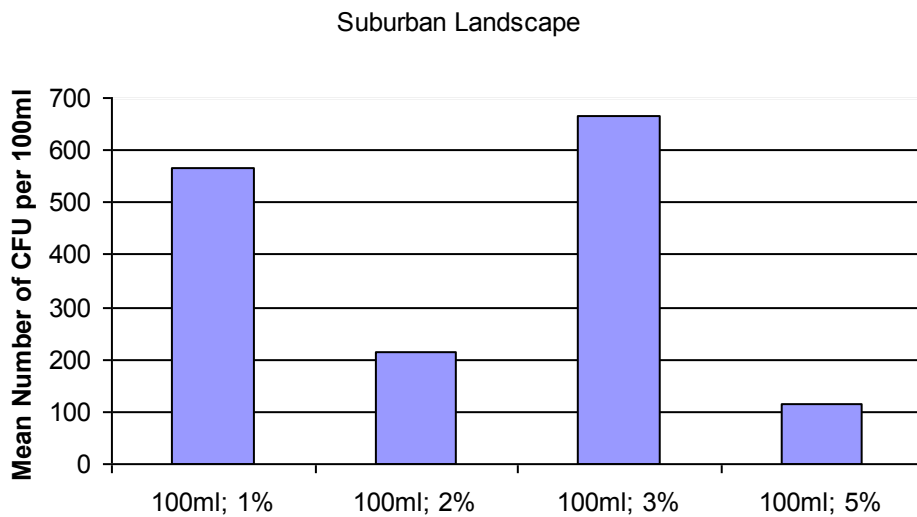
### **Suburban Landscape**

Samples were collected from three different areas within the suburban landscape of Glenwood, WV. Samples were collected near a water treatment plant located on Pepsi Plant Road, 20 meters downstream from the water plant on Pepsi Plant Rd., and at the end of the Brush Creek reservoir just off Rt. 20 on Brush Creek Reservoir Road (Table 1). The mean colony forming units (CFU) per 100ml at these three sites were 226, 178, and 412, respectively (Figure 2). The data collected was filtered at four different dilutions. The mean CFU per 100ml for 1%, 2%, 3%, and 5% dilution was 566, 216, 666, and 113, respectively (Figure 3; Appendix I).





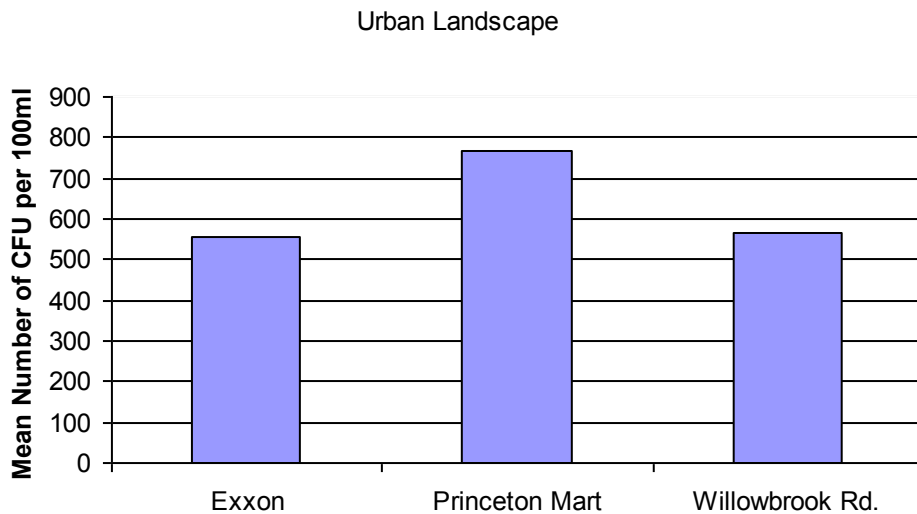
**Figure 2.** The mean number of CFU from three different areas within the suburban landscape in Glenwood, WV. The mean is of 1%, 2%, 3%, and 5% dilutions per 100ml for each of the three areas sampled. The data was collected January through March of 2007.



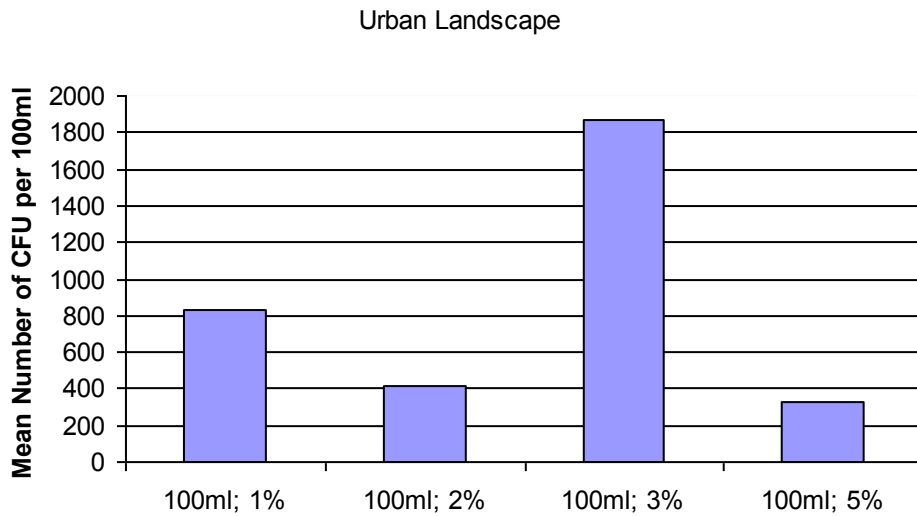
**Figure 3.** The mean number of CFU per 100ml for the suburban landscape filtered at 1%, 2%, 3%, and 5% dilutions. The samples were collected January through March of 2007.

## Urban Landscape

Samples were collected from three different areas within the urban landscape of Princeton, WV. Samples were collected behind Exxon located 50 meters from 460 west, behind Princeton Mart located 10 meters from Roger's Street, and along Willowbrook Road located 65 meters from 460 west (Table 1). The mean CFU per 100ml at the three sites was 556, 767, and 567, respectively (Figure 4). The data collected was filtered at four different dilutions. The mean CFU per 100ml for the 1%, 2%, 3%, and 5% dilution was 833, 416, 1866, and 323, respectively (Figure 5; Appendix II).



**Figure 4.** The mean number of CFU from three different areas within the urban landscape in Princeton, WV. The mean is of 1%, 2%, 3%, and 5% dilutions per 100ml for each of the three areas sampled. The data was collected January through March of 2007.



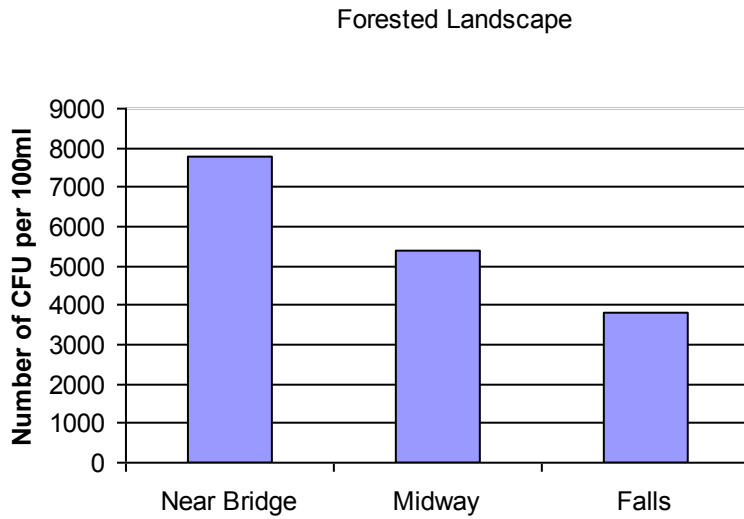
**Figure 5.** The mean number of CFU per 100ml for the urban landscape filtered at 1%, 2%, 3%, and 5% dilutions. The samples were collected January through March of 2007.

### **Forested Landscape**

Samples were collected from three different areas along Brush Creek Falls, located in WV.

Samples were collected near the bridge on Brush Creek Falls Road that passes over Brush Creek, 100 meters midway between the bridge and the falls, and 80 meters from the midway collection site to the falls (Table 1). The mean CFU per 100ml was 7,800, 5,400, and 3,800, respectively

(Figure 6; Appendix III). All samples were filtered at a 1% dilution.

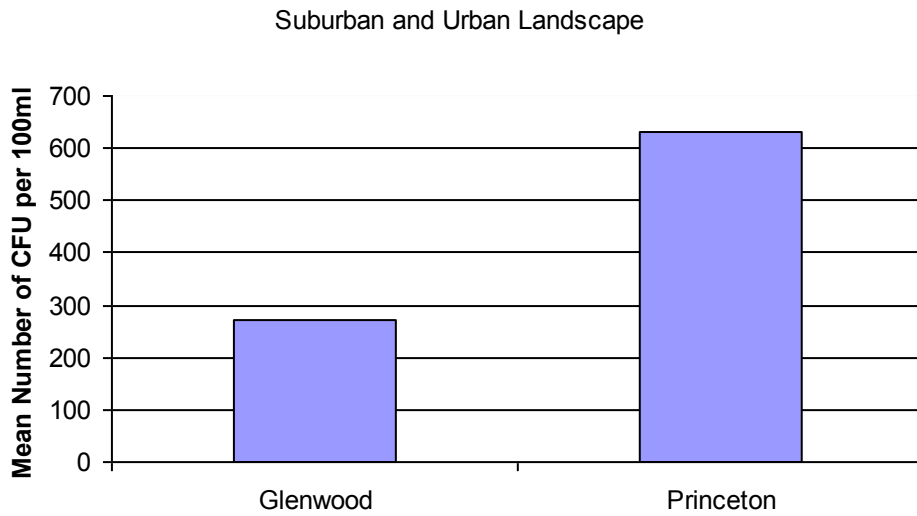


**Figure 6.** Number of coliform forming units per 100ml at a 1% dilution from the three different areas collected within the forested landscape near Brush Creek Falls.

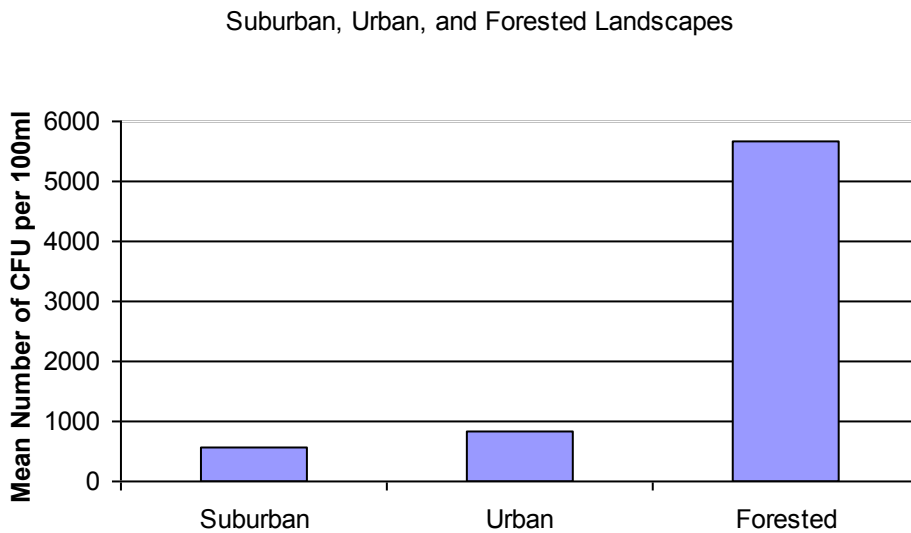
### **Differences Among Suburban, Urban, and Forested Landscapes**

The suburban and urban landscapes had a mean CFU per 100ml of 272 and 630 respectively (Figure 7). All three sites in the suburban landscape had a lower number of CFU per 100 ml than the sites in the urban landscape.

At a 1% dilution the mean CFU per 100ml was 566, 833, and 5,666 in the suburban, urban, and forested landscapes, respectively (Figure 8). All three sites within the forested landscape had higher CFU per 100 ml than the sites in both the suburban and urban landscapes.



**Figure 7.** Mean number of CFU within the three sampled areas in both Glenwood and Princeton. The mean number for each landscape includes dilutions at 1%, 2%, 3%, and 5% per 100ml. The data was collected January through March.



**Figure 8.** Mean CFU of the three areas sampled within the suburban, urban and forested landscapes all filtered at a 1% dilution per 100ml.

## Discussion

The level of fecal coliform contamination in the suburban and urban areas did not coincide with the hypothesis that they would both be higher in fecal coliform levels than the

forested landscape. The mean colony forming units (CFU) per 100ml at a 1% dilution for the suburban, urban, and forested landscapes were 566, 833, and 5,666 respectively (Figure 5). The presence of nearby highways, businesses, impervious surface runoff, and septic runoff all contributed to the higher levels of contamination in the urban landscape. The forested area was predicted to have a lower concentration of fecal coliforms than in the upstream suburban and urban landscapes. This area had little to no impervious surfaces and few residential areas within the watershed. However, the forested area had the highest concentration of fecal coliforms (Figure 5).

Based on land use in the Brush Creek watershed, we expected the fecal coliform levels to be higher in the suburban and urban areas than in the forested area of the watershed. The suburban landscape at the headwaters of Brush Creek may be impacted by septic tank runoff and farmland runoff. The urban landscape downstream of the headwaters may be impacted by impervious surface runoff as well as septic tank runoff. The forested landscape at the confluence of Brush Creek with the Bluestone River may be affected by farmland runoff as well as some impervious surface runoff, but not as much as the suburban and urban areas.

The accepted level of fecal coliform contamination for drinking water is 1 CFU per 100 mL, with a limit of 4 CFU per 100 mL before appropriate action must be taken (Harley 2005). For recreational activity, the maximum concentration of fecal coliforms is 200 CFU per 100ml (WVDEP). The fecal coliform levels for all three landscapes surpassed the accepted levels of fecal coliform levels for both drinking water and recreational use.

Because the fecal coliform concentrations exceed the water quality standards in all three landscapes there is an implication for health risks for the people associated with this watershed. The concern is often that disease causing bacteria can be associated with high fecal coliform

bacteria. Fecal coliforms by themselves do not generally pose a danger to people or animals, but are used as indicators for the presence of other disease-causing bacteria (EPA 2006), which are associated with various gastrointestinal disorders.

There are possible health hazards for people associated with the Brush Creek watershed. The fecal coliform concentration for all the sites exceeded standard water quality regulations for drinking and recreational use. This level of contamination could be a possible health concern for those that choose to use the creek for kayaking, swimming, canoeing, and other recreational activities.

One factor that could have attributed to the differences among the three landscapes is that the samples were collected at different times of the year. The samples from the forested landscape were collected in November, while the samples from the urban and suburban areas were collected during January-March, when the water was significantly colder. Water temperature would exhibit an effect on the growth levels of fecal coliform levels and would be lower during the January through March time period. Thus, future research should measure temperature levels of water at each collection site and collect samples during the same time of the year. Future researchers should also look at other parameters associated with water quality, such as the chemical composition within each of these three landscapes. In the future, more sampling sites should be studied, as well as several replicates within each area should be obtained.

Four different dilutions were used to determine the fecal coliform levels in the suburban and urban landscape, including 1%, 2%, 3%, and 5%. The results showed no dilution effect (Appendix I and II). This could be attributed to error in collection technique or error during the

membrane filtration. The inconsistencies in the dilutions indicate a lack of accuracy in the processing of the samples using the membrane filtration technique.

The problem with fecal coliform contamination could be solved or decreased by minimizing parking lots, installing filtration systems, and sewage treatment. Future research should look to further examine the fecal coliform contamination of the Brush Creek as well as other contamination that affects Brush Creek such as fuel, trash, and other pollutants.



## Appendix I

### The Results from the Three Samples Collected from the Glenwood Area Filtered Using Four Different Dilutions.

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
1-11-07	Pepsi Plant Rd. (near water plant)	1 mL	5	500	1%
1-11-07	Pepsi Plant Rd.	1mL	2	200	1%
1-11-07	End Reservoir	1mL	10	1000	1%
1-16-07	Pepsi Plant Rd. (near water plant)	2mL	5	250	2%
1-16-07	Pepsi Plant Rd.	2mL	3	150	2%
1-16-07	End Reservoir	2mL	5	250	2%
2-2-06	Pepsi Plant Rd. (near water plant)	5mL	0	< 1 coliform/100mL	<1%
2-2-06	Pepsi Plant Rd.	5mL	0	< 1 coliform/100mL	<1%
2-2-06	End Reservoir	5mL	0	< 1 coliform/100mL	<1%

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
2-6-07	Pepsi Plant Rd. (near water plant)	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Pepsi Plant Rd.	5mL	0	< 1 coliform/100mL	<1%
2-6-07	End Reservoir	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Pepsi Plant Rd. (near water plant)	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Pepsi Plant Rd.	5mL	0	< 1 coliform/100mL	<1%
2-6-07	End Reservoir	5mL	0	< 1 coliform/100mL	<1%
3-23-07	Pepsi Plant Rd. (near water plant)	5mL	20	400	5%
3-23-07	Pepsi Plant Rd.	5mL	18	360	5%
3-23-07	End Reservoir	5mL	30	600	5%
3-23-07	Pepsi Plant Rd. (near water plant)	3mL	13	433	3%
3-23-07	Pepsi Plant Rd.	3mL	16	533	3%
3-23-07	End Reservoir	3mL	31	1033	3%

## Appendix II

**The Results from the Three Samples Collected from the Princeton Area Filtered Using Four Different Dilutions.**

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
1-11-07	Exxon	1mL	8	800	1%
1-11-07	Princeton Mart	1mL	10	1000	1%
1-11-07	Willowbrook Rd.	1mL	7	700	1%
1-11-07	Exxon	2mL	3	150	2%
1-11-07	Princeton Mart	2mL	19	950	2%
1-11-07	Willowbrook Rd.	2mL	3	150	2%
2-2-07	Exxon	5mL	0	< 1 coliform/100mL	<1%
2-2-07	Princeton Mart	5mL	1	20	5%
2-2-07	Willowbrook Rd.	5mL	0	< 1 coliform/100mL	<1%

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
2-6-07	Exxon	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Princeton Mart	5mL	1	20	5%
2-6-07	Willowbrook Rd.	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Exxon	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Princeton Mart	5mL	1	20	5%
2-6-07	Willowbrook Rd.	5mL	0	< 1 coliform/100mL	<1%
3-23-07	Exxon	5mL	67	1340	5%
3-23-07	Princeton Mart	5mL	63	1260	5%
3-23-07	Willowbrook Rd.	5mL	61	1220	5%

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
3-23-07	Exxon	3mL	48	1600	3%
3-23-07	Princeton Mart	3mL	63	2100	3%
3-23-07	Willowbrook Rd.	3mL	57	1900	3%

### **Appendix III**

#### **The Results from the Three Samples Collected From Brush Creek Falls**

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
11-16-06	near bridge	1 mL	78	7800	1%
11-16-06	midway	1mL	54	5400	1%
11-16-06	Falls	1mL	38	3800	1%

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## Senior Research Paper

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### **Abstract**

The Avis Limestone of the Upper Mississippian Hinton Formation is an important regional, subsurface marker bed, and is commonly used as a datum for stratigraphic cross-sections and structural contour maps. Yet, it is uncertain that the Avis represents a specific time interval. This investigation was examined at two separate outcrops around Athens, WV: Laurel Creek Rd. and Old Dairy Rd. This investigation examined the faunal distribution of these limestones that ranged from highly fossiliferous zones to zones where fossils were scattered in an attempt to determine causes for depositional and environmental processes. Lithologic data included limestones that were mainly grainstone/packstone, but varied from shaly mudstone to wackestone, packstone, and grainstone to coquinite. Fossil data from this outcrop includes brachiopods, such as *Productus* n.sp., *Orthotetes* n.sp., *Ovatia elongata*, *Echinoconchus punctatus*, *Productus* n.sp., *Lingula* n.sp., *Composita subquadrata*, and *Martinia contracta*, pelecypods, such as *Palaeolima* sp., *Punctospirifer transversus*, and bryozoans, *Fenestrellina* sp. and *Pseudohornera* sp.?. The brachiopods and pelecypods were found in various abundances throughout the outcrop. The *Pseudohornera* bryozoans occurred in the middle of the Laurel Creek outcrop and may serve as an internal marker to trace the Avis over a lateral extent.



## INTRODUCTION:

### The Avis (Little Stone Gap)

Limestone Member of the Hinton Formation is located in the Mauch Chunk Group of the Upper Mississippian (Fig. 1). Because the Avis member has been found to have a wide lateral extent in the subsurface, it has been used in the past as an important marker bed for the Upper Mississippian (Barlow, 1996). However, the internal stratigraphy of the member itself is relatively unknown. This is due to the fact that it does not outcrop to the extent of many other marker beds, such as those of the Greenbrier Limestone (Fig. 1). Previous work has subdivided the Avis into three parasequences: lower, middle, and

upper (Place and Beuthin, 1998). The purpose of this study is to document the internal stratigraphy of these three subdivisions. Correlating information from this study to those previously done will be an important project for future research as well as the member's lateral extent at the surface.

The Avis Member as be measured and described at two locations (Fig. 2). The outcrops are approximately 1.50 miles (7900ft) apart, one of which, Old Dairy Rd., has been weathered

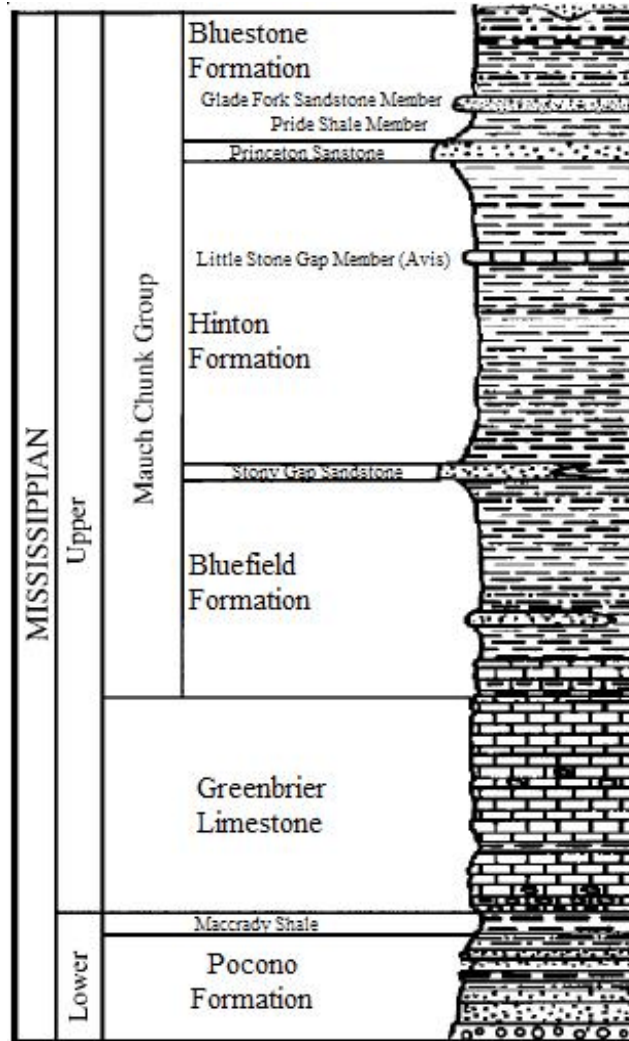
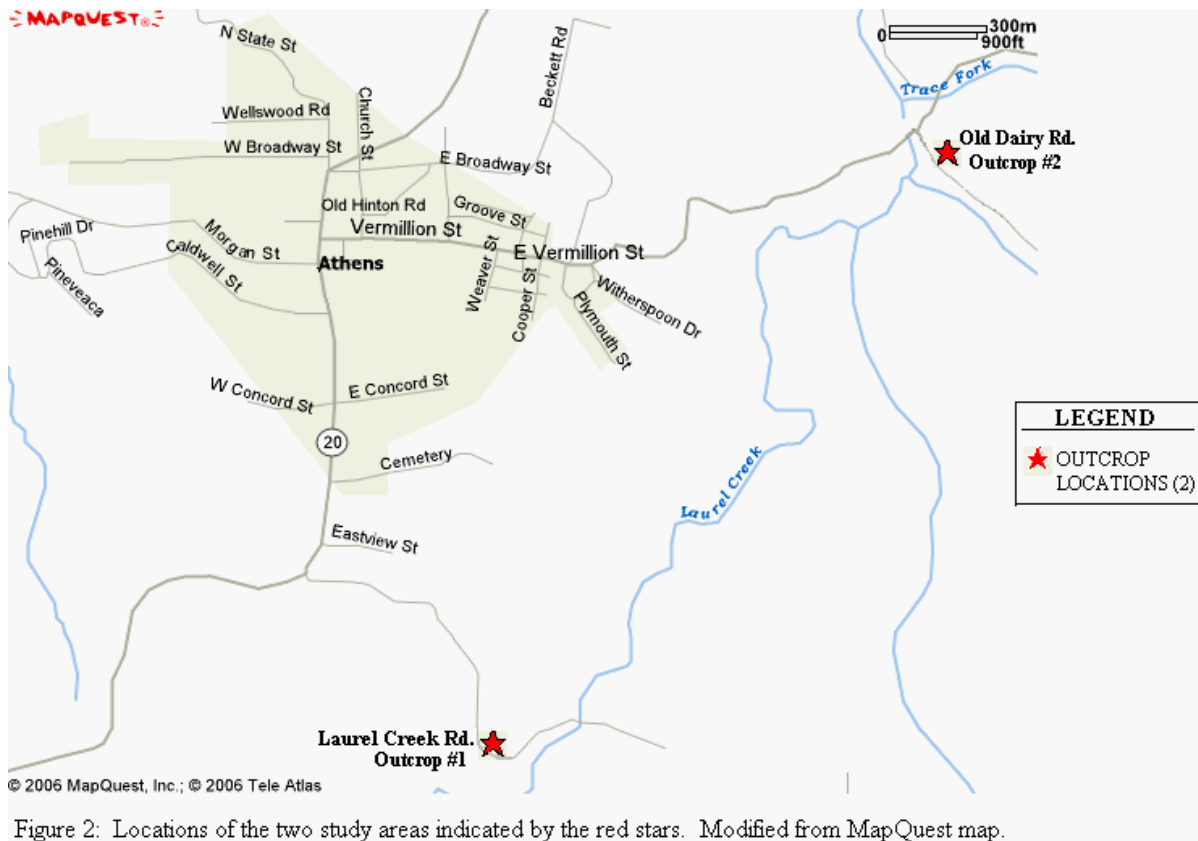


Figure 1: Location of the Little Stone Gap Limestone (Avis) of the Hinton Formation found in Upper Mississippian strata, modified from Stewart et al (2000).



extensively and has also been used by Concord University geology students for laboratory studies. The second outcrop, Laurel Creek Rd., is relatively unknown and is well preserved. The two outcrops were compared, and the stratigraphic units were correlated and compared to Place and Beuthin (1998).

**GEOLOGIC SETTING:**

The study area is located in the central part of the Appalachian foreland basin north of the Appalachian Plateau /Allegheny Front boundary with the Valley and Ridge Province to the east. The Hinton Formation comprises siliciclastic sediments that come from sources in the north and east and created a “southward prograding clastic wedge” within the basin. These deposits were part of a wide-ranging transgression of the "Little Stone Gap" epeiric sea that formed a low-dipping carbonate ramp on which the Avis is located (Place and Beuthin, 1998).

## **METHODS:**

Specific methods are required for the different types of research performed: qualitative and quantitative information. Qualitative information describes the outcrops. Pictures were taken for visual references for data collection and for visual cues during future presentations. Sequence and internal stratigraphy were described via lithology, structures, colors, fossils, and upper and lower contacts. Transgressive and regressive cycles were described from the changes in the bed compositions and paleontological variations (Tucker, 2003). The differences in the size, type, and abundance of mesoscopic fossils were recorded and identifications of each type of fossil were documented (Kummel and Raup, 1965; Case, 1992; Pinna, 1990; Lehmann and Hillmer, 1983; Moore et al, 1952). Quantitative information refers to the measurements taken. Thicknesses of individual beds and the outcrop on a whole were measured using a folding ruler, tape measure, and step measurements. Individual beds were measured on a using a foot-long ruler. Both large and small samples of rock were extracted for fossil description, identification, and measurements in a laboratory setting. Large samples of rock containing mesoscopic fossils were extracted using a sledgehammer and pick (Macfall and Wollin, 1972; Kummel and Raup, 1965). Individual mesoscopic samples were extracted using paleontological tools.

Table 1: Fossil Assemblages for Laurel Creek (LC) and Old Dairy (OD) Outcrops.\*\*\*

Sequences	<i>Brachiopods</i>	Bivalves	Bryozoans	Others
PS1 (LC)*	A,B,C,D,G?,H?	~	I	N
PS2 (LC)*	A,B,C,D,E	L?,M?	I,J?	N,O
PS3 (LC)*	A,B,C,F,K	L?	I	N
PS1 (OD)**	A,B,C,D,E	L?,M?,P?	I,J?	N,O

\* **Laurel Creek (LC): Fossils**

- A – *Productus* n.sp.
- B – *Orthotetes* n.sp.
- C – *Ovatia elongata*
- D – *Lingula* n.sp.
- E – *Composita subquadrata*
- F – *Martinia contracta*
- G – *Echinoconchus* sp.?
- H – *Anthracospirifer* sp.?

- I – *Fenestrellina* sp.
- J – *Pseudohornera* sp.?
- K – *Eumetrica costata*
- L – *Palaeolima* sp?
- M – *Edmondia* sp?
- N – *Crinoid* sp.
- O – *Nautiloid* sp.

\*\* **Old Dairy (OD): Fossils**

- A – *Productus* n.sp.
- B – *Orthotetes* n.sp.
- C – *Ovatia elongata*
- D – *Lingula* n.sp.
- I – *Fenestrellina* sp.
- J – *Pseudohornera* sp.?
- M – *Edmondia* sp?
- N – *Crinoid* sp.
- O – *Nautiloid* sp.
- P – *Punctospirifer* sp?

\*\*\*See Column 1 and Column 2; See Fossil Plate 1

Outcrop #1: Laurel Creek Rd.



Outcrop #2: Old Dairy Rd.



## **RESULTS/INTERPRETATIONS:**

### *Outcrop #1: Laurel Creek Road*

The first parasequence begins with a sharp contact above the red beds of the Bluefield Fm. It starts with a clayey, shaly mudstone until a sharp contact where the limestone becomes brittle, interbedded fossiliferous grainstone and clayey packstone where fossils are absent. Fossils range from small brittle fragments to large intact fossils, and include the following assemblages: *Productus* n. sp., *Orthotetes* n. sp., *Ovatia elongata*, and *Lingula* n. sp. The interbedding of grainstone and packstone suggest intermittent supratidal environments where the clayey packstone represents intervals where limestone creation experienced a lag due to detrital influxes, sea level changes, and possible climate changes. The intact fossils in the grainstone suggest an intertidal location. Parasequence is approximately 16-19 inches.

The second parasequence is marked by a change in lithology and fossil assemblages. It consists of mudstone where fossils are absent with intermittent coquinite lags where fossils are fragmented. Fossils within these lags consist of: *Productus* n. sp., *Orthotetes* n. sp., *Ovatia elongata*, *Lingula* n. sp., *Echinoconchus* n. sp.(?), and *Anthracospirifer* sp.(?). A sharp contact is indicated by the presence of two bryozoan beds. Nodular limestone lenses are located between the two. A mudstone riddled with calcite veins and a lack of fossils lies above the bryozoan beds. Fossils among the bryozoan beds are: *Productus* n. sp.(?), *Orthotetes* n. sp., *Lingula* n. sp., *Fenestrellina* sp., *Nautiloids*, and *Crinoids*. *In situ* fossils among the veins suggest intertidal conditions and consist of: *Productus* n. sp.(?), *Orthotetes* n. sp., and *Ovatia elongata*. This parasequence ends with a solid mudstone riddled with calcite veins where fossils are only present at the top among the veins. The coquinite lags

suggest an intertidal environment, where as the mudstones are subtidal. Parasequence is approximately 47-51 inches.

The third parasequence is marked by a dark mudstone where fossils are very scarce but intact. Fossil scarcity prevails as the limestone lightens and becomes moderately brittle. The limestone then becomes very solid mudstone where fossils are abundant, larger, (1.5-2.8 in.), intact, and *in situ*. The limestone grades from a mudstone to a clayey packstone and finally to a clayey siltstone. Where gradation begins and to the top of the section, fossils are absent. Fossils assemblages consist of: *Productus* n. sp., *Orthotetes* n. sp., *Ovatia elongata*, *Fenestrellina* sp., *Martinia contracta*, *Eumetria costata?*, and *Crinoids*. The gradation from mudstone to siltstone suggests an intertidal environment where siliciclastic influxes become prominent. Parasequence is approximately 33-36 inches. Total outcrop is approximately 99-106 inches.

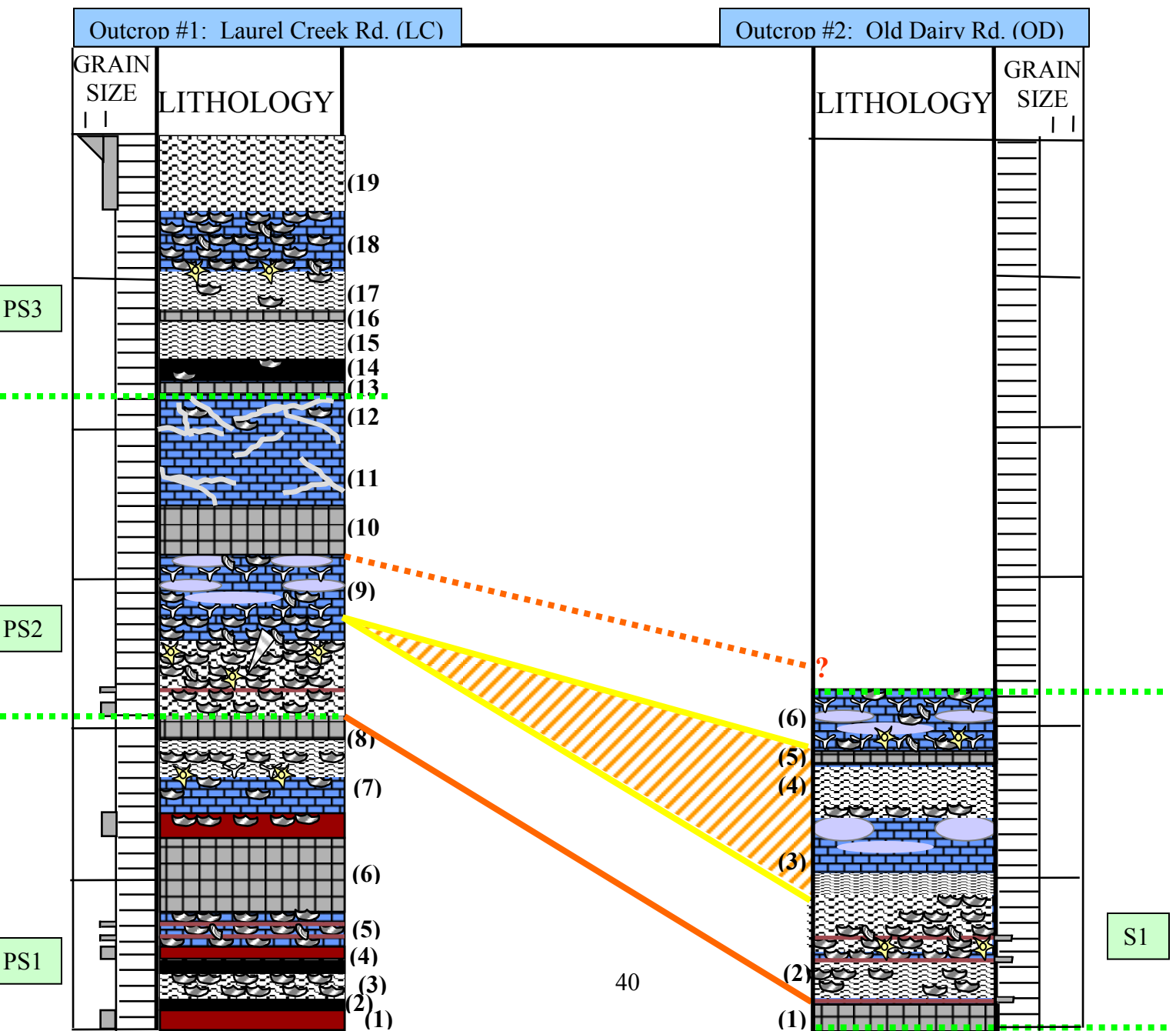
#### Outcrop #2: Old Dairy Road

This sequence begins with a section dark mudstone where intact fossils are very scarce. The mudstone lightens in color and intact fossils appear to be abundant, large (1.5–2.8in.), intact, and *in situ*. This limestone grades from a mudstone to a clayey packstone and finally to a clayey siltstone. Where gradation begins and to the top of the section, fossils are absent. Fossil assemblages consist of: *Productus* n. sp., *Orthotetes* n. sp., *Ovatia elongata*, *Fenestrellina* sp., *Martinia contracta*, *Eumetria costata?*, and *Crinoids*. The gradation from mudstone to siltstone suggests an intertidal environment where siliciclastic influxes become prominent. Parasequence is approximately 33-36 inches. Total outcrop is approximately 99-106 inches.

**CORRELATION:**

A correlation exists between the 2<sup>nd</sup> parasequence of Outcrop #1 and the sequence in Outcrop #2 (Figure 3). The bryozoan beds and surrounding fossil assemblages remain constant in the sections below. The limestone lithology within the correlated areas also remains constant. However, the second outcrop displays a growth pattern of limestone absent in the first outcrop and is completely devoid of fossils.

**Figure 3:** Correlation of PS2(LC) and S1(OD).



## **CONCLUSIONS:**

Now that the internal stratigraphy has been documented, the data collected may be used to trace the Avis Limestone Member over a lateral extent. However, further correlations among other outcrops across the state will be needed to prove the theory of a lateral extent.



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Mentor:

### **Abstract**

We examined how human activity and land use impacted fecal coliform concentrations in Brush Creek, in southern West Virginia. We examined three segments of the stream: the suburban headwaters, the urban middle reaches, and the downstream forested reaches using the membrane filtration method. Characteristics that might have impacted the number of fecal coliforms examined included impervious surface runoff, septic systems, and farmland runoff. This preliminary study is intended to examine the potential level of coliform exposure in the recreational Brush Creek area. In this study, the urban landscape had greater mean colony forming units (CFU) per 100 ml than the suburban area, with 630 and 272 CFU per 100ml respectively. The forested landscape however, had greater mean CFU per ml than both the suburban and urban landscapes with a mean of 5,666 CFU per 100ml. The CFU for all three landscapes exceeded standard water quality regulations for drinking and recreational use. The problem with fecal coliform contamination could be solved or decreased by minimizing parking lots, installing filtration systems, and the inflow of untreated sewage.

## **Introduction**

Water quality is of great importance when it comes to people and their health; as a result it is essential to understand the impact that human land use has on bodies of water in urban areas (Roark 1997). A major impact that urban areas have on streams and rivers is elevation in concentration of fecal coliforms. Fecal coliforms are bacteria that live in the digestive tracts of warm-blooded animals, and are excreted in the feces (EPA 2006). Some fecal coliforms include disease causing bacteria that can be hazardous to human health (EPA 2006). Diseases such as dysentery, typhoid, and cholera, are all caused by bacteria that are members of the fecal coliforms (EPA 2006). Respiratory infections, liver disease and potentially fatal gastrointestinal disorders may also be caused by fecal coliform infections (Mallin 2006).

Fecal coliforms are commonly found in aquatic habitats and are usually more abundant within urban areas (Paul and Meyer 2001). An urban area, as defined by the US Census Bureau can include both urban clusters and urbanized areas. An urban cluster is a densely settled area with a population of 2,500 to 49,999, and more than 1,000 people per square mile. An urbanized area is a population size that is greater than 5,000 people and 1,000 or more people per square mile. In 2001, more than 75% of the U.S. population lived in urban areas (Paul and Meyer 2001). This percentage shows the large number of people that could be affected by fecal coliform contamination in water used to bathe, drink, and swim (EPA 2006).

Fecal coliforms come from many different sources that can feed into nearby streams, creeks, and bays. These sources include impervious surface runoff, septic systems, and farmland runoff (Mallin 2006). The construction of buildings with large parking lots degrades the natural drainage system around aquatic areas (Mallin 2006). Septic systems that are overloaded result in high concentration of fecal bacteria in urban streams (Young and Thackston 1999). Farmland

runoff contributes manure, pesticides and fertilizers to streams; thereby causing increased fecal coliform levels.

### **Impervious Surfaces**

Impervious surfaces are areas covered by pavement, rooftops and sidewalks (Albanese and MatLack 1998). These impervious surfaces do not let water penetrate into the soil, thus when it rains the water picks up substances like oil, fuel, and animal feces, and washes them into ditches and drains that usually lead directly to urban waterways (Mallin 2006). The Environmental Protection Agency has reported that this type of pollution is the leading cause of water quality problems in the U. S. (EPA 2006).

In Hattiesburg, Mississippi, pollutants such as oils, fuels, radiator fluid, and heavy metals were found to have entered streams from parking lots (Albanese and MatLack 1998). Although parking lots are essential for people to park their cars, there are ways in which much of the runoff could be minimized. Parking lots tend to be much larger than needed, and are usually used below capacity (Wilson 1995). Mallin and others examined the relationship between land use and fecal coliform concentrations within several watersheds (Mallin 2006). They found that fecal coliform concentrations were higher in creeks with high human population and developed land in their watersheds. Bacterial counts associated with fecal coliforms were highest in areas with a high percentage of impervious surfaces (Mallin 2006).

There are ways in which much of the runoff from impervious surfaces could be minimized. Parking lots can actually be paved with porous concrete that allows water to pass into the soil below, and still provides enough support for vehicles (Mallin 2006). Parking lots that are already made can install filtration systems to filter out the pollutants, and thus cut down on the bacterial counts in nearby streams (Albanese and MatLack 1998).

## **Septic Systems**

Failing septic systems and sanitary sewer overflows can lead to dangerous conditions for surrounding streams. During heavy rainfall the water quality of many urban streams tests positive for high levels of fecal coliforms (Young and Thackston 1999). Bacteria counts are usually higher in urban streams, especially after heavy storms (Paul and Meyer 2001). This is usually due to septic systems that have not been taken care of properly and overflow causing unsafe conditions. Some communities have their storm drains connected to septic systems, and a heavy rain can cause major overflows into nearby watersheds.

It is not uncommon for septic systems to cause potential threats during the dry season as well. High values during a dry season are a good indication that there is chronic sewer leakage (Mallin 2006). The Florida Keys are one good example of how septic systems can cause major problems. In 1995 Paul and Rose found that the fecal microbes from septic tanks in the Keys pass easily through the soil and within hours can enter coastal waters (Mallin 2006). The Florida Keys are not the only location with this problem. Parts of North Carolina share the same septic tank concerns. Septic systems are not only causing problems in the US, but they are presenting problems in tropical regions as well (Isobe 2004). Sewage pollution is a serious health risk for people who live near waterways in tropical regions. Direct discharge from waste coming from poorly maintained septic tanks are one of the major causes of waterborne disease. It is an important task for people in tropical regions to pay close attention to their septic systems because high temperature and rainfall lead to soil erosion and eventual sewage leakage into nearby watersheds.

The fact that septic tanks can cause high fecal coliform levels in waterways could be solved with careful planning and better sewage treatment. Septic tanks need to be pumped

regularly, and replaced when necessary. Conversion of septic tanks to a municipal sewer system can also improve water quality (EPA 2006).

## **Farmland**

Storm runoff carries pesticides, fertilizers, and manure from farmland to streams. Pesticides are usually high in urban streams and the concentrations usually exceed guidelines for the protection of aquatic life (USGS 1999). Pesticides are frequently applied around homes, lawns, and businesses; and without drainage or buffer zones they can be carried to nearby streams. The major concern is the feces carried from farmlands to streams. The bacteria, viruses, and protozoa from feces pose the greatest threat to human health (Mallin 2006). Mallin reported that a single gram of dog feces contains an estimated 23 million bacteria. Diesch reported that diseases could be spread from one warm blooded organism to another by water (Diesch 1970). Contamination of water is sometimes caused by feedlots. In 1970 a bacterial outbreak from fecal matter known as *Leptospirosis* occurred in several people in Iowa (Buckhouse 1976). These people were infected because water from a cattle farm had access to a nearby swimming hole. A similar incident happened in Columbus, Georgia where cattle, pigs, and dogs all were infected by *Leptospirosis*, and had access to a stream that led to a swimming hole (Buckhouse 1976). This type of incident is life threatening to the human population surrounding the streams. Streams in urban areas receive a lot of farmland runoff. Disposal of the waste in areas far away from waterways or installation of filters that catches runoff and cleanses it before it enters waterways can decrease the concentration of fecal coliforms from this source.

## **Objective of Research: Brush Creek in southern West Virginia**

In this study we examined how human activity and land use impact fecal coliform concentrations in Brush Creek, located in southern West Virginia. Brush Creek's headwaters are



near Bluefield, WV; and the waters run through Princeton, WV where it is heavily affected by humans and poor land use (Bowman 2006).

We examined three different segments of the stream: the suburban headwaters, the urban middle section of the stream, and the downstream forested area. The suburban area was located in the outlying area of Princeton, and we expected to find fecal contamination from septic systems, and farmland. The second area we examined was the actual urban cluster. This area runs through Princeton and should have significant impervious surface runoff as well as septic tank, and parking lot contamination. The last area we studied was a more forested area, and is known as Brush Creek Falls. This area runs downstream into the Bluestone River. In this area we expect to find some traces of farmland runoff and possible impervious surface runoff due to the forest.

After obtaining water samples at the three different locations in Brush Creek along its entire extent, we were able to measure the fecal coliform concentrations using the membrane filtration method. This allowed us to discuss what specific sources might have impacted the levels of fecal coliforms in Brush Creek. Using this information we can assess what possible health hazards people around the Brush Creek area may be exposed, and the extent to which they are impacted by fecal coliform contamination. Below is a map that identifies the sampling sites of Brush Creek (Bowman 2006)



**Figure 1. Map of the sites sampled along brush creek (Bowman 2006).**

**Methods and Materials**

In order to conduct this research, water samples of Brush Creek from different regions were collected. Three different segments of the stream from three different landscapes were examined: the suburban headwaters, the urban middle section of the stream, and the downstream forested area. Presterilized NASCO Whirl-pak bags were used to obtain three single samples from each of the three regions. Below is a table including the longitude and latitude for each of the three sampled sites within each of the three landscapes, as well as a brief description of the location.

Landscapes (Sample Sites)	Longitude	Latitude	Brief Description of Location
<b>Suburban Landscape</b>	Glenwood, WV		
Waterplant	81° 8' 39" W	37° 20' 11" N	located on Pepsi Plant Road just off 460
Pepsi Plant Rd.	81° 8' 35" W	37° 20' 13" N	20 m downstream from the water plant on Pepsi Plant Rd
End Reservoir	81° 11' 2" W	37° 19' 42" N	just off Rt. 20 on Brush Creek Reservoir Rd
<b>Urban Landscape</b>	Princeton, WV		
Exxon	81° 5' 48" W	37° 21' 29" N	behind Exxon located 50 m from 460 west
Princeton Mart	81° 5' 5" W	37° 21' 51" N	behind Princeton Mart located 10 meters from Roger's Street
Willowbrook Rd.	81° 6' 29" W	37° 20' 55" N	along Willowbrook Rd located 65 m from 460 west
<b>Forested Landscape</b>	Brush Creek Falls, WV		
Bridge	81° 3' 48" W	37° 21' 53" N	near the bridge on Brush Creek Falls Road that passes over Brush Creek
Midway	81° 3' 43" W	37° 27' 54" N	100 m midway between the bridge and the falls
Falls	81° 3' 38" W	37° 27' 58" N	80 m from the midway collection site to the falls

Table 1. Location of sampling sites in suburban, urban, and forested landscapes within the Brush Creek watershed.

The samples were all taken from the middle of the creek, and were collected between November of 2006, and March of 2007 (Appendix I, II, and III). Once in the middle of the creek

the Whirl-pak bag was dipped about a foot into the water, and water was collected from the bottom of the creek up in a seeping motion (Harley 2005). After the bag was full of water, it was then dumped out, and a second sample was collected in the same manner. The bags were then tied off and placed on ice until reaching the lab. The samples were then placed in the refrigerator at 4°C until filtered; within 48 hours.

### **Filtration Procedure**

The membrane filtration method, as described in Standard Methods for the Examination of Water and Wastewater by the American Public Health Association, was used to conduct this research (APHA 1999). Pre-sterilized membrane filters with a pore size of 0.45µm. were used (APHA 1999). Samples were all diluted using dilution water composed of magnesium chloride, and potassium dihydrogen phosphate (HACH dilution water – product #21431-66). One hundred milliliters of each sample was filtered. Samples at each site were all analyzed at 1% dilutions (99% dilution water). The suburban and urban areas were also analyzed at 2% (98% dilution water), 3% (97% dilution water) and 5%(95% dilution water) dilutions.

Sterile forceps were then used to place the filter for each sample on M-Endo medium with a rolling motion to avoid entrapment of air. The petri dishes were then inverted and incubated for 20-24 hours at 37 degrees Celsius. After the incubation period the colonies were counted.

### **Colony Count and Data Analysis**

A low-power (10-15 magnifications) dissecting microscope was used to determine the number of colonies on a filter (APHA 1999). Colonies that appeared pink to dark red in color with a metallic surface sheen were classified as fecal coliforms (APHA 1999). The fecal coliform density was calculated by using the following equation:

$$\text{Total coliforms/100mL} = \frac{\text{coliform colonies counted}}{\text{mL sample filtered}} \times 100$$

If there were no colonies observed, the sample was reported as < 1 coliform/100mL.

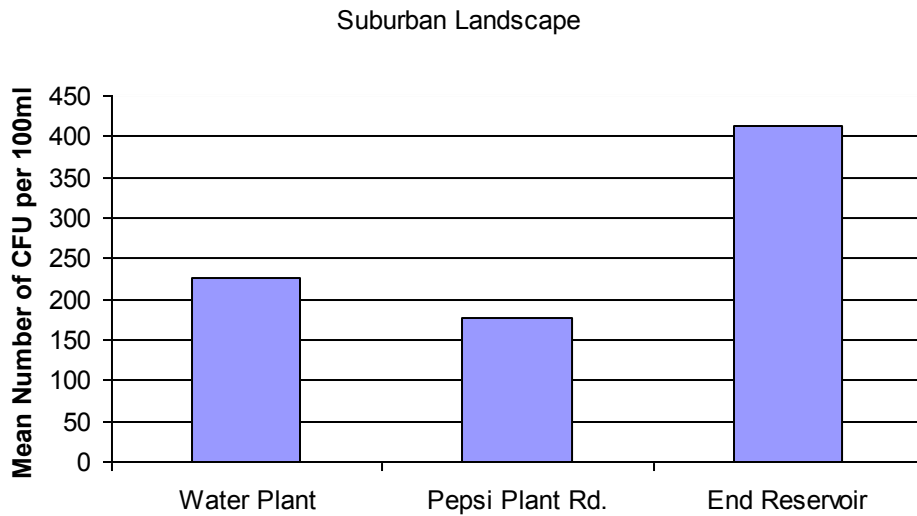
For verified coliform counts the initial count based was adjusted on the positive verification percentage. For example, if the volume of sample filtered was 1ml, then the positive verification percentage would have been 1% or 1% of verified coliforms. This was then reported as verified coliform count/100mL.

$$\% \text{ verified fecal coliforms} = \frac{\# \text{ colonies observed}}{\text{total \# coliform per 100ml}} \times 100$$

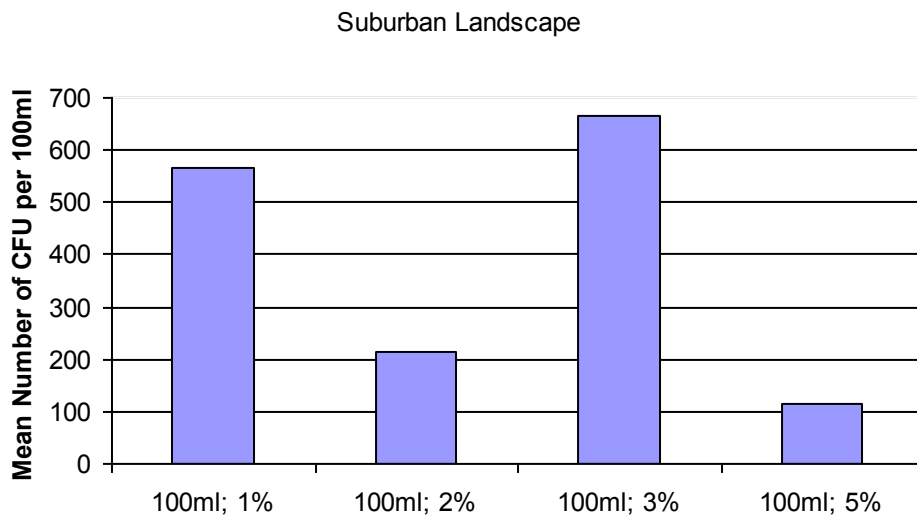
## **Results**

### **Suburban Landscape**

Samples were collected from three different areas within the suburban landscape of Glenwood, WV. Samples were collected near a water treatment plant located on Pepsi Plant Road, 20 meters downstream from the water plant on Pepsi Plant Rd., and at the end of the Brush Creek reservoir just off Rt. 20 on Brush Creek Reservoir Road (Table 1). The mean colony forming units (CFU) per 100ml at these three sites were 226, 178, and 412, respectively (Figure 2). The data collected was filtered at four different dilutions. The mean CFU per 100ml for 1%, 2%, 3%, and 5% dilution was 566, 216, 666, and 113, respectively (Figure 3; Appendix I).



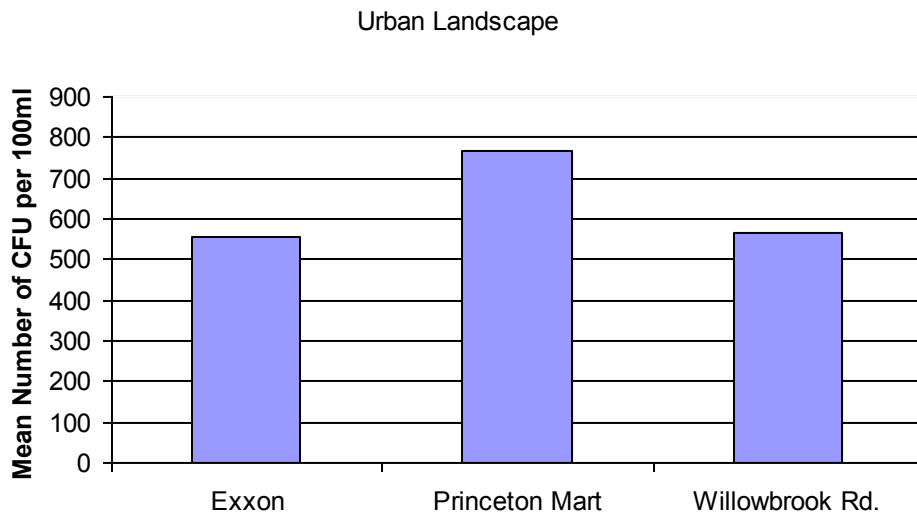
**Figure 2. The mean number of CFU from three different areas within the suburban landscape in Glenwood, WV. The mean is of 1%, 2%, 3%, and 5% dilutions per 100ml for each of the three areas sampled. The data was collected January through March of 2007.**



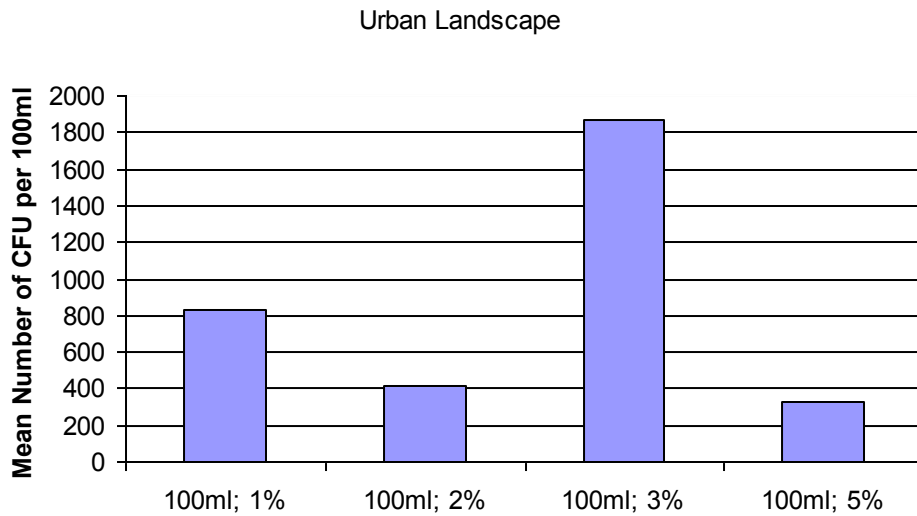
**Figure 3. The mean number of CFU per 100ml for the suburban landscape filtered at 1%, 2%, 3%, and 5% dilutions. The samples were collected January through March of 2007.**

## Urban Landscape

Samples were collected from three different areas within the urban landscape of Princeton, WV. Samples were collected behind Exxon located 50 meters from 460 west, behind Princeton Mart located 10 meters from Roger's Street, and along Willowbrook Road located 65 meters from 460 west (Table 1). The mean CFU per 100ml at the three sites was 556, 767, and 567, respectively (Figure 4). The data collected was filtered at four different dilutions. The mean CFU per 100ml for the 1%, 2%, 3%, and 5% dilution was 833, 416, 1866, and 323, respectively (Figure 5; Appendix II).



**Figure 4. The mean number of CFU from three different areas within the urban landscape in Princeton, WV. The mean is of 1%, 2%, 3%, and 5% dilutions per 100ml for each of the three areas sampled. The data was collected January through March of 2007.**



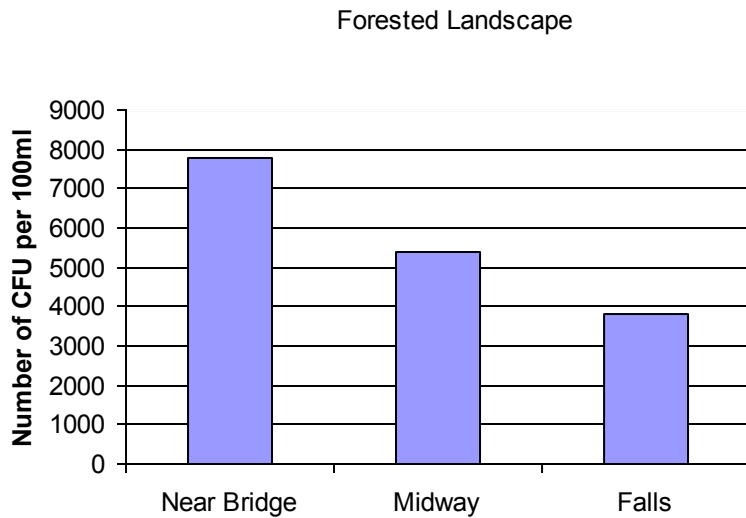
**Figure 5. The mean number of CFU per 100ml for the urban landscape filtered at 1%, 2%, 3%, and 5% dilutions. The samples were collected January through March of 2007.**  
**Forested Landscape**

Samples were collected from three different areas along Brush Creek Falls, located in WV.

Samples were collected near the bridge on Brush Creek Falls Road that passes over Brush Creek, 100 meters midway between the bridge and the falls, and 80 meters from the midway collection site to the falls (Table 1). The mean CFU per 100ml was 7,800, 5,400, and 3,800, respectively

Figure 6; Appendix III). All samples were filtered at a 1% dilution.



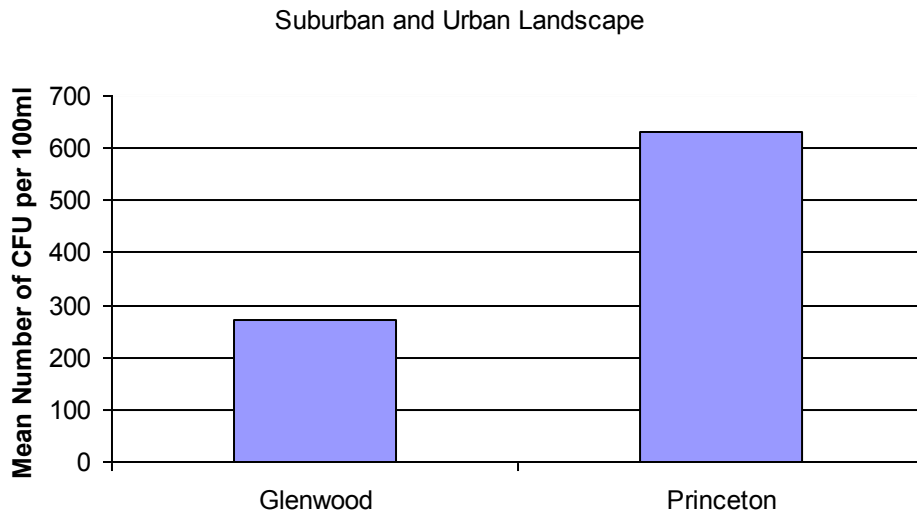


**Figure 6. Number of coliform forming units per 100ml at a 1% dilution from the three different areas collected within the forested landscape near Brush Creek Falls.**

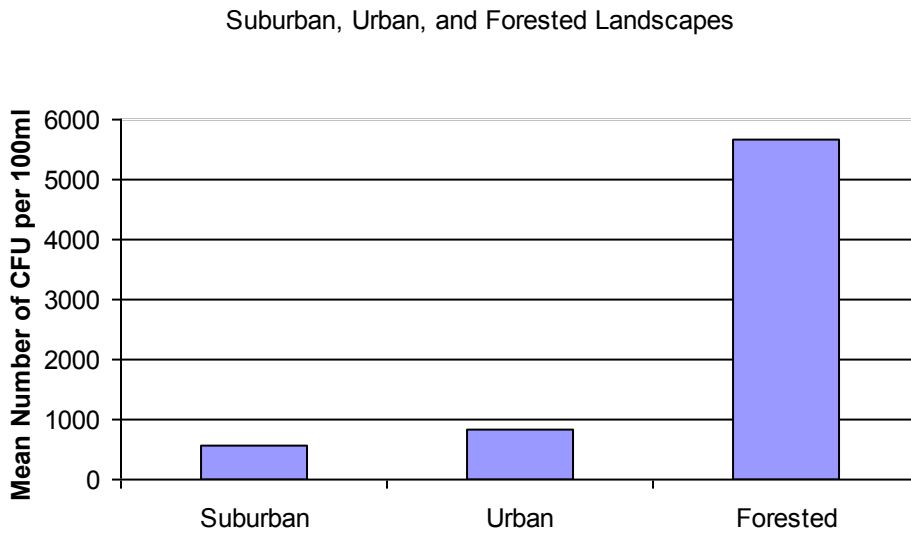
### **Differences Among Suburban, Urban, and Forested Landscapes**

The suburban and urban landscapes had a mean CFU per 100ml of 272 and 630 respectively (Figure 7). All three sites in the suburban landscape had a lower number of CFU per 100 ml than the sites in the urban landscape.

At a 1% dilution the mean CFU per 100ml was 566, 833, and 5,666 in the suburban, urban, and forested landscapes, respectively (Figure 8). All three sites within the forested landscape had higher CFU per 100 ml than the sites in both the suburban and urban landscapes.



**Figure 7. Mean number of CFU within the three sampled areas in both Glenwood and Princeton. The mean number for each landscape includes dilutions at 1%, 2%, 3%, and 5% per 100ml. The data was collected January through March.**



**Figure 8. Mean CFU of the three areas sampled within the suburban, urban and forested landscapes all filtered at a 1% dilution per 100ml .**

## **Discussion**

The level of fecal coliform contamination in the suburban and urban areas did not coincide with the hypothesis that they would both be higher in fecal coliform levels than the forested landscape. The mean colony forming units (CFU) per 100ml at a 1% dilution for the suburban, urban, and forested landscapes were 566, 833, and 5,666 respectively (Figure 5). The presence of nearby highways, businesses, impervious surface runoff, and septic runoff all contributed to the higher levels of contamination in the urban landscape. The forested area was predicted to have a lower concentration of fecal coliforms than in the upstream suburban and urban landscapes. This area had little to no impervious surfaces and few residential areas within the watershed. However, the forested area had the highest concentration of fecal coliforms (Figure 5).

Based on land use in the Brush Creek watershed, we expected the fecal coliform levels to be higher in the suburban and urban areas than in the forested area of the watershed. The suburban landscape at the headwaters of Brush Creek may be impacted by septic tank runoff and farmland runoff. The urban landscape downstream of the headwaters may be impacted by impervious surface runoff as well as septic tank runoff. The forested landscape at the confluence of Brush Creek with the Bluestone River may be affected by farmland runoff as well as some impervious surface runoff, but not as much as the suburban and urban areas.

The accepted level of fecal coliform contamination for drinking water is 1 CFU per 100 mL, with a limit of 4 CFU per 100 mL before appropriate action must be taken (Harley 2005). For recreational activity, the maximum concentration of fecal coliforms is 200 CFU per 100ml (WVDEP). The fecal coliform levels for all three landscapes surpassed the accepted levels of fecal coliform levels for both drinking water and recreational use.

Because the fecal coliform concentrations exceed the water quality standards in all three landscapes there is an implication for health risks for the people associated with this watershed. The concern is often that disease causing bacteria can be associated with high fecal coliform bacteria. Fecal coliforms by themselves do not generally pose a danger to people or animals, but are used as indicators for the presence of other disease-causing bacteria (EPA 2006), which are associated with various gastrointestinal disorders.

There are possible health hazards for people associated with the Brush Creek watershed. The fecal coliform concentration for all the sites exceeded standard water quality regulations for drinking and recreational use. This level of contamination could be a possible health concern for those that choose to use the creek for kayaking, swimming, canoeing, and other recreational activities.

One factor that could have attributed to the differences among the three landscapes is that the samples were collected at different times of the year. The samples from the forested landscape were collected in November, while the samples from the urban and suburban areas were collected during January-March, when the water was significantly colder. Water temperature would exhibit an effect on the growth levels of fecal coliform levels and would be lower during the January through March time period. Thus, future research should measure temperature levels of water at each collection site and collect samples during the same time of the year. Future researchers should also look at other parameters associated with water quality, such as the chemical composition within each of these three landscapes. In the future, more sampling sites should be studied, as well as several replicates within each area should be obtained.

Four different dilutions were used to determine the fecal coliform levels in the suburban and urban landscape, including 1%, 2%, 3%, and 5%. The results showed no dilution effect (Appendix I and II). This could be attributed to error in collection technique or error during the membrane filtration. The inconsistencies in the dilutions indicate a lack of accuracy in the processing of the samples using the membrane filtration technique.

The problem with fecal coliform contamination could be solved or decreased by minimizing parking lots, installing filtration systems, and sewage treatment. Future research should look to further examine the fecal coliform contamination of the Brush Creek as well as other contamination that affects Brush Creek such as fuel, trash, and other pollutants.

## Appendix I

**The Results from the Three Samples Collected from the Glenwood Area Filtered Using  
Four Different Dilutions.**

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
1-11-07	Pepsi Plant Rd. (near water plant)	1 mL	5	500	1%
1-11-07	Pepsi Plant Rd.	1mL	2	200	1%
1-11-07	End Reservoir	1mL	10	1000	1%
1-16-07	Pepsi Plant Rd. (near water plant)	2mL	5	250	2%
1-16-07	Pepsi Plant Rd.	2mL	3	150	2%
1-16-07	End Reservoir	2mL	5	250	2%
2-2-06	Pepsi Plant Rd. (near water plant)	5mL	0	< 1 coliform/100mL	<1%
2-2-06	Pepsi Plant Rd.	5mL	0	< 1 coliform/100mL	<1%
2-2-06	End Reservoir	5mL	0	< 1 coliform/100mL	<1%

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
2-6-07	Pepsi Plant Rd. (near water plant)	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Pepsi Plant Rd.	5mL	0	< 1 coliform/100mL	<1%
2-6-07	End Reservoir	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Pepsi Plant Rd. (near water plant)	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Pepsi Plant Rd.	5mL	0	< 1 coliform/100mL	<1%
2-6-07	End Reservoir	5mL	0	< 1 coliform/100mL	<1%
3-23-07	Pepsi Plant Rd. (near water plant)	5mL	20	400	5%
3-23-07	Pepsi Plant Rd.	5mL	18	360	5%
3-23-07	End Reservoir	5mL	30	600	5%
3-23-07	Pepsi Plant Rd. (near water plant)	3mL	13	433	3%
3-23-07	Pepsi Plant Rd.	3mL	16	533	3%
3-23-07	End Reservoir	3mL	31	1033	3%

**Appendix II**

**The Results from the Three Samples Collected from the Princeton Area Filtered Using  
Four Different Dilutions.**

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
1-11-07	Exxon	1mL	8	800	1%
1-11-07	Princeton Mart	1mL	10	1000	1%
1-11-07	Willowbrook Rd.	1mL	7	700	1%
1-11-07	Exxon	2mL	3	150	2%
1-11-07	Princeton Mart	2mL	19	950	2%
1-11-07	Willowbrook Rd.	2mL	3	150	2%
2-2-07	Exxon	5mL	0	< 1 coliform/100mL	<1%
2-2-07	Princeton Mart	5mL	1	20	5%
2-2-07	Willowbrook Rd.	5mL	0	< 1 coliform/100mL	<1%



<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
2-6-07	Exxon	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Princeton Mart	5mL	1	20	5%
2-6-07	Willowbrook Rd.	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Exxon	5mL	0	< 1 coliform/100mL	<1%
2-6-07	Princeton Mart	5mL	1	20	5%
2-6-07	Willowbrook Rd.	5mL	0	< 1 coliform/100mL	<1%
3-23-07	Exxon	5mL	67	1340	5%
3-23-07	Princeton Mart	5mL	63	1260	5%
3-23-07	Willowbrook Rd.	5mL	61	1220	5%

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
3-23-07	Exxon	3mL	48	1600	3%
3-23-07	Princeton Mart	3mL	63	2100	3%
3-23-07	Willowbrook Rd.	3mL	57	1900	3%

**Appendix III**

**The Results from the Three Samples Collected From Brush Creek Falls**

<b>Date</b>	<b>Location</b>	<b>mL sample filtered</b>	<b># coliforms</b>	<b>(Total) coliforms/100mL</b>	<b>Percentage verified coliforms</b>
11-16-06	near bridge	1 mL	78	7800	1%
11-16-06	midway	1mL	54	5400	1%
11-16-06	Falls	1mL	38	3800	1%

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# Screening of Sorrel (*Oxalis* spp.) for Antioxidant and Antibacterial Activity

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## **ABSTRACT**

Sorrel (*Oxalis* spp.) is a common plant found in lawns and roadsides throughout the United States. A recently published ethnobotanical study of the Eastern Band of the Cherokees indicates that the plant was used as a treatment for cancer among other medicinal uses. *Oxalis* spp. plants were collected from local flower beds and preparation of the extract was then conducted via supercritical fluid and solvent extractions. Bioactivity was assayed by evaluation of antioxidant activity (DPPH method) and antibacterial activity (Kirby-Bauer disk diffusion test).

## **INTRODUCTION**

### **History**

Plants are currently one of the most widely used resources; they are a fundamental nutrient source for life and are central to many people's livelihoods. Everyday, plants are used to build shelter, make clothes, provide food, and treat various medical ailments. The medicinal use of plants has been recorded since the early Egyptian pictographs and the ideographic writing of the Babylonians on clay tablets. The secrets of plant medicine were also been verbally passed down from the early Greek physicians, such as Hippocrates, who were known for their secretive behavior (1).

### **Current Uses**

Today, plants are used worldwide as a form of complementary and alternative medicine (CAM) in richer countries, while poorer countries continue to use them as a form of traditional medicine (TM) (1). TM refers to the indigenous traditions of health care globally, while CAM refers to the practice of medicine and treatment outside of modern medicine (2). In a recent study conducted in Brazil, 24% of participants reported using herbal medicines indigenous to the area to aid in the treatment cancer, while 63% of the participants reported using some form of TM. In this particular study, there was no reported use of any of the industrialized CAM treatments, such as aromatherapy, homeopathy, osteopathy, and chiropractic (1).

The use of TM is best documented in China, and until recently was widely practiced and accepted there (3). Their TM techniques, known as traditional Chinese medicine (TCM) involve combining herbology and acupuncture with conventional medicine (3). TCM is authorized by the National Institutes of Health as an acceptable form of CAM in the United States and its use is growing daily. In contrast, TCM use is decreasing in China due to a shift toward conventional

medicine by the younger people (3). However, despite the decrease in popularity of TCM, many doctors still incorporate it into modern medicine and many people still choose to use it (3).

Unlike China, TM in lesser developed countries is usually ignored by the physicians who choose to practice only modern medicine (4). In certain African countries nearly 90% of the population rely on TM, yet when questioned at a doctor's office or hospital only 1% admitted to using TM for fear of receiving a lesser quality of care by the physicians who largely disrespect the trade of traditional healers (4).

Despite the lack of respect from some medical professionals, TM using plant species is the only trusted form of medicine in many countries. A recent study revealed the use of 39 medicinal plant species across 37 genera representing 27 families by indigenous tribes in India. The above ground portion of the plant was reportedly used more frequently for curing various ailments than the underground plant parts. The leaf was used more often than the fruit of the above ground portion and the root, tuber, rhizome, bulb, and pseudobulb were all used as the underground portion. Collectively, there are over 2400 ethnomedicinal uses for plants in the country of India demonstrating the importance of it in their culture. The majority of the data previously reported was collected from the village chief and the medicine man of various tribes in India (5).

### **Native American Ethnobotany**

Although many cultures have their own "medicine men" such as the shamans of Asia and the herbalists and witch doctors of Africa, the Native American medicine men are the most well known (5). The Native American culture has always used the elements of nature, such as plants and animals, for their survival and continues to use the elements to enhance their lives. Many Native Americans believe in using modern medicine for surgeries and diseases, while only the



medicine man can heal the problems of the mind and spirit. Today, there is a renewed interest in using the ancient traditions of the Native American religion, language, and other aspects of their culture, including ethnobotany (1). The Cherokee medicine man treats disease according to four “life values” rather than the shape of the plant (6). The four life values are East medicine, South medicine, West medicine, and North medicine. East medicine addresses the importance of family life; south medicine is used when the person has been exposed to the elements of nature; west medicine focuses on internal conditions and diseases that influence the physical body, while the north medicine refers to the four winds, cold weather, and calm surroundings. The Cherokees also believe in the Rule of Opposites that does not differentiate between good and bad energies, but rather says that both are part of the harmony of life. This rule aids the Native Americans in deciding which plants to use to help maintain the balance of harmony in the body (2).

### **Plant Botany**

*Oxalis* spp. are common plants found in lawns and roadsides throughout the United States and other various parts of the world. They belong to the Oxalidaceae family that includes over 800 different species (7, 8). Other common and Latin names for these plants include creeping woods, yellow wood sorrel, sourgrass, *Acetosella corniculata*, *O. corniculata*, *O. langloisii*, *O. pusilla*, *O. repens*, *O. villosa*, *Xanthoxalis corniculata*, *X. langloisii*, and *X. repens* (9). *Oxalis* spp. are difficult plants to control, particularly *O. corniculata*, which grows uncontrollably in gardens and container nurseries (10). *O. corniculata* (Fig. 1) is physically characterized by three heart-shaped, yellow-green leaves, often times with a yellow or white flower. The plant is commonly found during the summer months.



**Fig. 1.** Picture of *O. corniculata*.

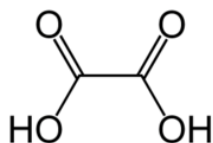
### **Medicinal Uses of the Plant**

The use of this plant for medicinal purposes dates back to the Cherokee Indian nation. As reported by William H. Banks Jr., *O. corniculata* is useful in the treatment of vomiting, removal of hookworm, and treatment of cancer when it is first diagnosed (11). The whole plant has also been used as an anti-inflammatory agent, halt secretion of fluids associated with diarrhea, control bleeding, reduce fevers, and destroy kidney and bladder stones (12, 13). The juice from the leaves of *O. corniculata* has been used to treat insect bites, burns, and skin infections and is highly effective due to its known antibacterial activity (7, 12, 14). Other controlled studies have investigated the use of *O. corniculata* for relief from canker sores (15). Studies regarding the cytotoxicity of *Oxalis* spp. have not been documented.

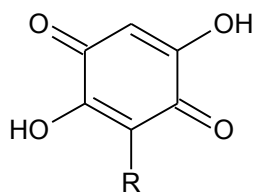
### **Chemistry of the Plant and Related Species**

The chemistry of the family Oxalidaceae has been widely studied with the main constituent being oxalic acid (Fig. 2), which contributes to the sour taste of the plant (10). The roots of *O. pes-caprae* typically contain other carboxylate anions such as acetate, lactate, succinate, fumarate, malate, citrate, isocitrate, and aconitate (16, 17). Bioassay-guided fractionation of *O. erythrorrhiza* isolated benzoquinone embelins as the main active constituent of the plant, which is the first reported occurrence in the *Oxalis* species (10). Benzoquinone-

embelins (Fig. 3) possess biological activities related to anti-tumor, anti-inflammatory, antimicrobial, antihelminthic and analgesic properties (10).



**Fig. 2.** Chemical Structure of Oxalic Acid



**Fig. 3.** Chemical Structure of Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone).

### Related Plants

Many plants related to *Oxalis* spp. have also been studied for their biological properties. The whole plant of *O. erythrorrhiza* is used in San Juan, Argentina, to treat heart and liver problems, while *O. peduncularis*, *O. amara*, *O. chrysantha*, *O. tuberosa* are useful in treating skin infections and other unspecified microbial infections (18, 19). In particular, *O. erythrorrhiza* demonstrated valuable antimicrobial properties in the ability to inhibit the growth of methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*, dermatophytes, and bacteria (10).

### Hypothesis

Based on ethnobotanical uses, I hypothesized that extracts of *Oxalis* spp. would exhibit bioactivity. As a starting point, the antibacterial and antioxidant properties of *Oxalis* extracts were evaluated using the Kirby-Bauer disk diffusion test and 1,1-diphenyl-2-picrylhydrazyl radical quenching assay, respectively.

### MATERIALS AND METHODS

## Plant Collection

Plant collection occurred during June, July, and August from lawns and flower beds in Princeton, WV and surrounding areas including Pipestem, WV. The plant was collected by digging around the base of the plant and then gently pulling the plant out of the ground, trying to include as much of the root as possible. The plant was immediately placed in a labeled Ziploc bag and stored in a freezer until it could be transported and stored at -20°C at Concord University. The label on the Ziploc bag included the collector's initials, and the number of the collection that increased numerically (i.e. JP-012 for collection number 12). Information regarding the collection was placed in the field manual and labeled with the same number found on the Ziploc bag. This information included the date, time, physical location, weather, GPS coordinate, and any other information regarding the soil such as the use of fertilizers. In addition, a soil sample was also collected and the nitrogen, phosphorus, and potassium levels will be determined.

Due to the similarities in the varieties of *Oxalis* spp. several varieties of *Oxalis* were collected.



Figure 4A



Figure 4B

**Fig. 4A and 4B.** Pictures of Plant Collection. Figure 1A shows the inclusion of as much of the plant as possible when collected. Figure 1B shows the plant being pulled from the ground and placed in a labeled plastic bag.

## Extraction Methods

The collected plant was removed from the freezer and laid out to dry on newspaper for two days. The plant was also cut into smaller pieces and then ground using a coffee grinder.

#### Soxhlet Extraction:

The soxhlet extraction method exposes the plant extract to fresh hot methanol (MeOH) over a period of several hours. For the soxhlet extraction, 7.0086g of the ground plant material was packed into a cellulose thimble so that the thimble was slightly over half full. The extraction was conducted for eight hours. The extract was placed into a labeled vial.

This procedure was repeated with ground material from all of the remaining collections grouped together. For this extraction, a total of 7.5524g of plant material placed into the thimble and the extraction was conducted for approximately seven hours. This extract was placed into a separate vial.



**Fig. 5.** Picture of a Soxhlet extraction.

#### Room-Temperature Solvent Extraction:

Approximately 500 mL of MeOH was added to a clean Erlenmeyer flask along with 7.0535g of ground material from JP012 and a magnetic spin bar. The flask was wrapped in

aluminum foil and placed on a stir plate at a medium speed and allowed to stir for three days. Plant material was removed by vacuum filtration. The filtered extract was placed into a vial labeled JP01-02.

The entire process of room temperature extractions and vacuum filtration was repeated on pooled plant collections. The filtered extract was placed in a vial labeled JP01-08.

#### Supercritical CO<sub>2</sub> Fluid Extraction:

Supercritical CO<sub>2</sub> extraction was performed using a model SFT-1000 Supercritical Fluid Technologies, Inc. extractor. Three different supercritical CO<sub>2</sub> extractions were conducted at varying temperatures and pressures. All three extractions used the same 50 g of plant material from JP003-JP012. Each extraction used a total of 250 mL of CO<sub>2</sub> over a period of five static and dynamic cycles. The first extraction, labeled JP01-11a, was performed at 40°C and 2000 PSI. The second extraction, JP01-11b, was performed at 40°C and 4000 PSI while the third and final extraction, JP01-11c, was performed 60°C and 6000 PSI with 5mL of MeOH as the cosolvent.

#### **Rotary Evaporation**

Following the soxhlet (JP01-01 and JP01-05) and room temperature extractions (JP01-02 and JP01-08), the individual extracts collected were evaporated by rotary evaporation (rotovap) (Fig.6).



**Fig. 6.** Picture of the Rotovap apparatus.

### **Vacuum Liquid Chromatography**

Vacuum Liquid Chromatography (VLC) was conducted on extracts JP01-05, JP01-06, and JP01-11a, b, and c (Fig.7). VLC was performed on the room temperature and soxhlet extractions following rotary evaporation and was performed directly on the supercritical CO<sub>2</sub> extractions.

Silica gel was used to pack the column and a 50 or 100 mL round bottom flask was attached to the bottom. The column was rinsed with MeOH and the vacuum was applied to pack the column.

For VLC, various mixtures of hexane, ethyl acetate and MeOH acted as the solvent to redissolve the dried extract (Table 1). Fifteen mL of the solvent mixture was added to the dried extract and swirled around in the vial or flask. The extract plus solvent was then added to the top of the silica gel column using a sterile pipet. The extract was pulled through the column by applying a vacuum to the apparatus. After the extract was pulled through, another 15 mL of the same solvent mixture was added to the top of the column and pulled through. This process was repeated for each of the twelve solvent mixtures using a different flask for each of the twelve mixtures.

Following VLC, each of the twelve fractions for each extraction was then subjected to rotary evaporation and the dried extract weight was recorded.



**Fig. 7.** Vacuum Liquid Chromatography Apparatus

**Table 1.** Table of the ratios of the different solvents for each fraction.

<u>Number</u>	<u>Hexane</u>	<u>Ethyl Acetate</u>
1	100	0
2	99	1
3	98	2
4	96	4
5	94	6
6	90	10
7	80	20
8	60	40
9	20	80



10	0	100
	<u>Methanol</u>	<u>Ethyl Acetate</u>
11	50	50
12	100	0

### Redissolving of Extract for Assays

Soxhlet extraction JP01-05, room temperature extraction JP01-06, and all three supercritical CO<sub>2</sub> extractions, JP01-11a, b, and c were redissolved using dimethyl sulfoxide (DMSO) following VLC and rotary evaporation to a concentration of 10 mg/mL. Each fraction was then placed in a labeled amber vial and stored at 4°C.

### Redissolving for Kirby-Bauer Antibacterial Assay

Extracts JP01-01 and JP01-02 were used to initially screen for antibacterial properties. These extracts did not undergo VLC and remained in their dried form from rotary evaporation and therefore had to be redissolved to a known concentration. A 3:2 volume to volume ratio of MeOH to hexane was used. All dissolved extract was removed and placed in a labeled vial.

### Kirby-Bauer Antibacterial Assay

For the Kirby-Bauer Antibacterial Assay, ten different bacterial species were used to determine antibacterial activity of JP01-01 and JP01-02 (Table 2). Each disk for the assay was spotted three times with a micropipette set at 20 µL. For JP01-01, this was approximately 4.3 mg/disk and for JP01-02 this was approximately 4.7 mg/disk. Nutrient agar plates (150mm x 100mm) were streaked with the appropriate bacterial species using calcium alginate swabs, from bacterial cultures grown overnight at 37°C in nutrient broth. The plates were incubated at 37°C and examined 24 hours later.

**Table 2.** List and properties of bacteria used for the Kirby-Bauer assay.

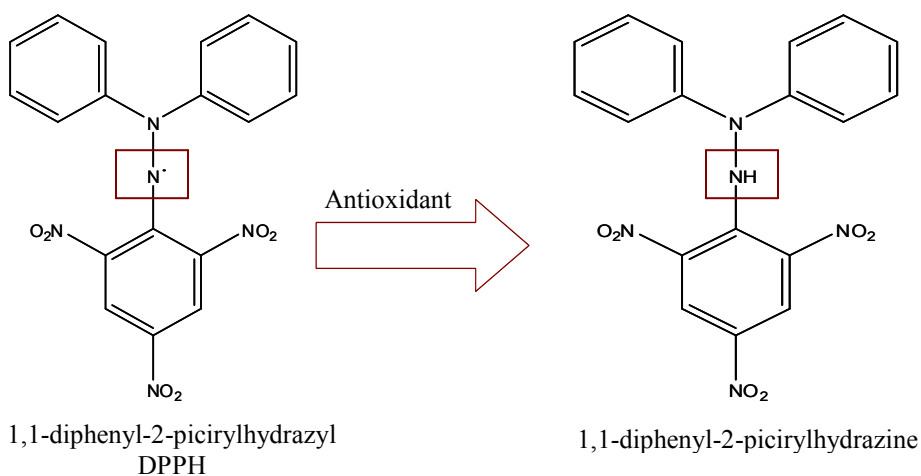
<u>Species</u>	<u>Gram Stain</u>	<u>Shape</u>	<u>Causes</u>
<i>Bacillus cereus</i>	+	Rod-shaped	Food poisoning
<i>Corynebacterium xerosis</i>	+	Cocci	Bacteriemia, Skin infections

<i>Escherichia coli</i>	-	Rod-shaped	Urinary Tract Infections
<i>Klebsiella pneumoniae</i>	-	Rod-shaped	Pneumonia, Urinary Tract Infections
<i>Mycobacterium smegmatis</i>	Acid-Fast Stain		Disease in animals
<i>Pseudomonas aeruginosa</i>	-	Rod-shaped	Urinary Tract Infections
<i>Salmonella typhimurium</i>	-	Rod-shaped	Food Poisoning
<i>Staphylococcus aureus</i>	+	Cocci in clusters	Skin lesions, Boils, Toxic Shock Syndrome
<i>Staphylococcus epidermidis</i>	+	Cocci in clusters	Skin lesions
<i>Streptococcus faecalis</i>	+	Cocci in clusters	Endocarditis

### 1,1-diphenyl-2-picrylhydrazyl (DPPH) Antioxidant Assay

Antioxidant activity was tested using the DPPH antioxidant assay. If an antioxidant is present in the extract, a hydrogen atom will bond to the free radical on DPPH and the color of the solution will turn from dark purple to yellow (Fig. 8).

Extracts JP01-05, JP01-06, JP01-11a, b and c were placed in the wells of a 96 well plate. The rows represented the six different extracts being tested, including a positive and negative control (Fig. 9). The columns represented the twelve different fractions that were produced by VLC for each of the extracts. Wells contained 20  $\mu$ L of DMSO dissolved extract, 80  $\mu$ L of Tris buffer, and 100  $\mu$ L of DPPH. The control wells did not contain extract. The first six wells of the control contained 20  $\mu$ L of thymol, a known antioxidant agent, and 100  $\mu$ L of DPPH only; while the last six wells contained 20  $\mu$ L of DMSO and the 100  $\mu$ L of DPPH. The seventh row contained another sample, while the eighth row was filled only with DPPH. After the wells were filled, they were placed in a dark area and visually analyzed thirty minutes later.



**Fig. 8.** Illustration indicating the quenching of the free radical on the nitrogen of DPPH by an antioxidant compound.

	1	2	3	4	5	6	7	8	9	10	11	12
JP01-05 (SOX)	○	○	○	○	○	○	○	○	○	○	○	○
JP01-06 (RTE)	○	○	○	○	○	○	○	○	○	○	○	○
JP01-11a	○	○	○	○	○	○	○	○	○	○	○	○
JP01-11b	○	○	○	○	○	○	○	○	○	○	○	○
JP01-11c	○	○	○	○	○	○	○	○	○	○	○	○
CONTROL	○	○	○	○	○	○	○	○	○	○	○	○

**Fig. 9.** Chart demonstrating the set-up of the DPPH assay

## RESULTS

Collection Number	Extraction Procedure	Extract Number
JP012	Soxhlet	JP01-01
	Room Temperature	JP01-02
JP003-JP012 (Grouped together)	Soxhlet	JP01-05
	Room Temperature	JP01-08
	Low Temperature/Low Pressure Supercritical CO <sub>2</sub> Fluid Extraction	JP01-11A

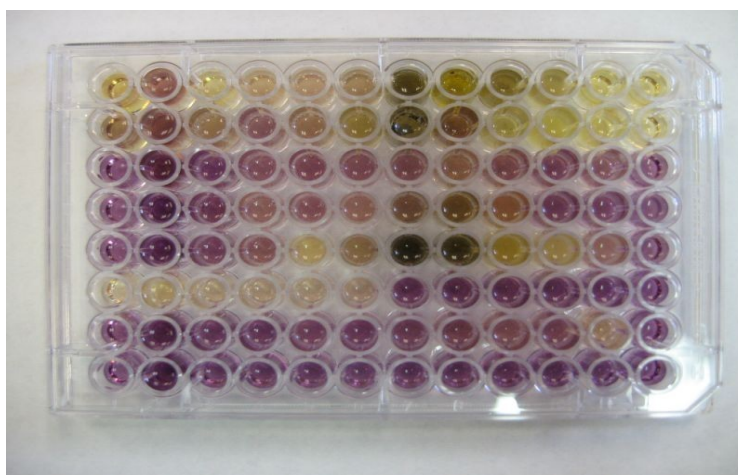
	Medium Temperature/Medium Pressure Supercritical CO <sub>2</sub> Fluid Extraction	JP01-11B
	High Temperature/High Pressure with MeOH cosolvent Supercritical CO <sub>2</sub> Fluid Extraction	JP01-11C

**Table 3.** Summary of the plant collection numbers, extractions performed, and the final extract number.

### Kirby-Bauer Antibacterial Assay

Extracts JP01-01 and JP01-02 demonstrated no observable antibacterial activity against all ten bacteria following the twenty-four hour incubation period.

### DPPH Antioxidant Assay



**Fig. 10.** DPPH assay results of various extracts as indicated in the template below

**Table 4.** Chart showing the strength of color change indicating DPPH activity.

	1	2	3	4	5	6	7	8	9	10	11	12
JP01-05	++	+	++	++	++	++	++	++	++	++	++	++
JP01-06	++		++	+	++	++	++	++	++	++	++	++
JP01-11a				+	+	+	+	+	+			
JP01-11b				+	+	+	++	++				
JP01-11c				+	++	+	++	++	++	++	+	

++, Strong antioxidant activity; +, weaker antioxidant activity.

Of the sixty wells that contained extract, 33 of them demonstrated some color change, indicating DPPH activity as shown in Figure 10 and Table 4. The wells that turned a yellow color and demonstrated a complete loss of the purple color had more antioxidant activity and are indicated by the ++ in Table 4. The wells that turned a light purple color demonstrated some antioxidant activity and are indicated in Table 4 using a +. Fractions 7, 8, and 9 of JP01-05 and fractions 7 and 8 of JP01-06, JP01-11b, and JP-01-11c turned green (the color of the initial extract) rather than yellow. These extracts were decided to be strong positives since they were void of any purple color.

The soxhlet extraction (JP01-05) and room temperature extraction (JP01-06) demonstrated more DPPH activity than each of the remaining three rows containing the three supercritical CO<sub>2</sub> fluid extractions. Similarly, the rows containing the soxhlet and room temperature extractions show stronger antioxidant activity than the remaining three rows. In addition, the strength of antioxidant activity as well as the number of wells containing antioxidant activity was higher in the third supercritical CO<sub>2</sub> extraction (JP01-11c) than in the other two supercritical extractions.

## **DISCUSSION**

Based on the results of the antibacterial test, the whole plant of *Oxalis* spp. does not exhibit any observable antibacterial activity by the Kirby-Bauer Disk Diffusion test. The plant will later be divided into the different parts and reexamined using more sensitive antibacterial assays.

The DPPH antioxidant assay shows that *Oxalis* spp. contains antioxidant activity in the whole plant extract as well as in individual fractions. The soxhlet extraction and the room temperature extraction both demonstrated antioxidant activity over the majority of the

fractionations produced through VLC. One reason for this wide range of activity is the unselective nature of the soxhlet extraction and the room temperature extraction. There was less antioxidant activity found in the supercritical CO<sub>2</sub> extractions because they extraction procedure is more selective. Furthermore, the amount and strength of the antioxidant activity increased as the temperature and pressure increased in the supercritical CO<sub>2</sub> extraction, indicating an increase in polarity.

These data suggest the antioxidant compounds in *Oxalis* spp. are polar in nature since the amount of antioxidant activity increased with increasing polarity.

### **Future Research**

Future research for this project includes the conclusion of the DPPH assay through reevaluation of the positive antioxidant wells in order to quantitatively measure the amount of antioxidant activity. This will then allow the compounds in *Oxalis* spp. to be compared to ascorbic acid, a known antioxidant, and determine the relative strength. Cytotoxicity testing for *Oxalis* spp. will also begin in the near future. More long terms goals include the complete isolation and identification of compounds from *Oxalis* spp.

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Running head: Social Impacts of Housing Development at the New River Gorge National River

Social Impacts of Housing Development at the New River Gorge National River

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## INTRODUCTION

In the article *Affluent moving in droves to woods*, Susan Williams (2006) writes, “Many advertise that they are next to a protected wilderness or national park as a way to capitalize on the natural settings and views. But people worried about this trend fear that the beauty and solace people seek in parks will be compromised from the pressure these developments exert outside park boundaries.”

The New River Gorge National River was established in 1978 to conserve and protect 53 miles of one of the oldest waterways in the world that runs north. There is 70,000 acres within the boundaries of this national park from Hinton to Fayetteville, West Virginia for the National Park Service (NPS) to manage. Like many of the national parks, the New River Gorge National River is facing encroachment from private land development. Two major land developers, Land Resource Companies (LRC) and New River Lodges Associates are ready to begin housing development in and around the New River Gorge National River. There has been an ongoing battle among local government, the National Park Service and local residents for nearly two years concerning the possible impacts these proposed houses could have on the national park and the community (Williams, 2006).

The Roaring River development is owned by a Georgia-based Land Resource Company, which will eventually build approximately 2000 homes on property surrounding the New River Gorge National River (Williams, 2006). Those homes will be very expensive homes in gated communities. The first phase of development, which could soon begin will consist of about 484 homes. Park officials are concerned that about 80 of these homes overlooking the gorge will negatively impact the park’s viewshed. A viewshed is an area of land, water, and other environmental elements that is visible from a fixed vantage point. They also worry about the

effect the cutting of roads and the trees will have on the forest. According to the park officials, this forest is “globally significant” because it is part of the largest remaining stand of mid-latitude forest in the world (Williams, 2006).

Gary Driggs bought his property, New River Lodges, from a whitewater rafting company that had already been zoned for housing and a commercial building such as a hotel. He could begin his housing development; however, he is seeking to have the property annexed into the town of Fayetteville to connect to their existing water and sewage systems. Cal Hite, Superintendent of National Park Service, is concerned with the houses being seen from the Canyon Rim Visitors’ Center, Hawks’ Nest State Park, Long Point Hiking Trail and the New River Gorge Bridge itself. Driggs admits they will probably be seen in the winter when the leaves are gone from the trees. However, he plans to protect from the New River Gorge and Hawks Nest (AP, 2005).

Tourism and recreation development is a very fast growing industry with many controversial issues over the positive and negative impacts that come along with trying to provide a better quality of life. Often it is unclear what is meant by quality of life; there is no clear definition for it. A review by the Centre for Urban and Regional Studies at the University of Birmingham (CURS) shows that quality of life is something personal and can have different meanings for different individuals. The meaning of quality of life is often determined by how it is measured. Governments try to measure the impact of their policy making on things which they feel will improve the quality of the community (Galloway, 2006). One politician may feel different from another on what is best for the community; therefore, depending on which party is in office and what their particular views of quality of life are determines the policies they propose or endorse.

Beginning in the 1950's many people fled the cities in search of the American dream of quiet peaceful places like Tyson's Corner, Virginia, the San Fernando Valley, California, Aurora, Colorado, and Federal Way, Washington (Howe, McMahon, Prospt, 1997).

After more than forty years of poorly managed growth, these communities have become places worse than where the people fled. The highways have become highly congested, the towns have miles and miles of malls that all look alike, the houses and architecture are built on the same design with no character (Howe, McMahon, Prospt, 1997).

Again, many of the American people are leaving the suburbs trying to get farther and farther out into the country. They are drawn to areas with beautiful scenery such as majestic mountains, beautiful lakes, grassy meadows and open countryside. Many of these people are choosing to live in the small towns or open land just outside our national parks, state parks, wilderness refuges, forests, historic sites, and other public lands (Howe, McMahon, Propst, 1997). By doing so, communities are being developed with possible endless opportunity, which can have both positive and negative impacts on its residents.

Looking at the positive side, these gateway communities can offer a clean environment, safe streets, and a friendly small town atmosphere. Many people feel they will provide new jobs. On the other hand, these jobs are usually assumed to be low paying, such as hotels and restaurant work. With gateway communities attracting more people, it can also be harder to find work. However, when ERS researchers conducted a study and measured the residents' total income including the second jobs (seasonal and part-time) they concluded that recreation development does seem to increase residents' earnings (Reeder, Brown, 2005).

Another negative impact is a rise in real estate values, and high property taxes. Due to the increase in demand for housing this could force lifelong residents from their home

communities and prevent their children and grandchildren from remaining in the area (Howe, McMahon, 1997). High cost of rent can force workers to move out and have to commute long distances to work. An example of displacement due to the demand for land and housing forcing locals and families that had lived there for generations to move away is in Tremont, Maine. According to George Lawson, a retired fisherman, places that were selling for \$10,000 rose to \$80,000 to \$90,000 on Mount Desert Island, the gateway to Acadia National Park (Howe, McMahon, Propst, 1997). Higher cost of living may be true; however, it is unknown how widespread it is.

According to the Economic Research Service (ERS) median household income in recreation counties are \$3,185 higher than any other county, while the median annual rent is only \$1,080 higher in these recreation counties opposed to other counties. This indicates that about a third of the higher rent offsets the recreation county advantage (Reeder, Brown, 2005).

An Associated Press (AP) review finds the National Parks are facing tremendous problems outside their boundaries as well as within the parks, which includes population growth, homeland security, drug trafficking, and some of the conveniences that many Americans feel they cannot live without such as hotels, restaurants, stores, cell phones, and vacation homes (Bass, Beamish, 2006).

Inside the parks 30 cell towers have been erected, one in view of Yellowstone's famed Old Faithful geyser. Above the Civil War cannon at Georgia's Kennesaw Mountain an emergency radio communication tower has been constructed. In an attempt to slow illegal immigrants and drug trafficking, a 30-mile long steel and concrete vehicle blockade has been build along Arizona's Organ Pipe Cactus National Monument (Bass, Beamish, 2006). These things have definitely had a negative impact on the view of the parks. Have the benefits

outweighed the cost? These are only a few examples of the pressures the park service deals with within the borders of the parks; the pressures mounting from outside the boundaries are another story.

According to the Associated Press (AP) census data analysis, more than 1.3 million people have moved into counties surrounding Gettysburg, Everglades, Glacier, Yellowstone, Shenandoah, and Great Smokey Mountain National Parks since 1990 (Bass, Beamish, 2006).

The average number of people per square mile in these counties has grown by one-third. The four urban counties around the Florida Everglades show the most dramatic gains. But even in the remote areas of Glacier, the number of people per square mile has raised from eight in 1990 to eleven in 2005 (Bass, Beamish, 2006).

With more than 2000 houses being built along the New River Gorge National River there has to be both positive and negative impacts on the gateway communities that surround it. Will these impacts balance and improve the quality of life for the community as a whole? The residents' quality of life will depend on many factors. It will depend on how well the process is planned and perceived. Change is good if it is properly planned; however, this is quite a magnitude of change for such a small area. Will the area be sustainable with such change? The residents' attitudes can play an important part on how well change is accepted in the community.

## METHODS

This study on the social impacts of the housing development at the New River Gorge National River will consist of a written survey. The survey questions will consist of seven standard background questions along with four development issue questions. Two of the

development issue questions will address quality of life issues, one having ten items, the other one having five items. Survey questions can be found in Appendix A. Next, a report to the Human Subjects Review Board at Concord University was submitted for approval.

Once the approval of the HSRB was received, the data collection process began. The sample size consisted of 116 Fayette County residents in the Oak Hill-Fayetteville area. The surveys were conducted on site in various parts of Fayette County, as well as, at a midget league football game in Oak Hill and another game in Fayetteville. Once the surveys were completed the results were entered into SPSS on the computer to be analyzed.

## RESULTS

Of the 116 respondents, 69% were female and 31% were male. Their ages ranged from 18 years to 78 years old. About 78% of the respondents had graduated high school, attended some college or vocational training, or graduated college. While 17% of the respondents were homemakers, 62% were employed full time, nearly 8% were part time employees, 6% were retired, and only 2.6% reported they were unemployed.

**Table 1: Respondent Characteristics (Age)**

	N	Minimum	Maximum	Mean	Std. Deviation
Age	115	18	78	38.02	11.266
Valid N (listwise)	115				

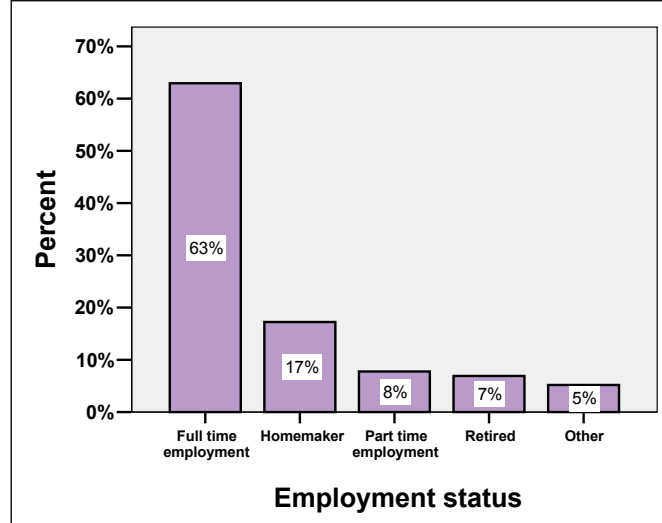
**Table 2: Respondent Characteristics (Gender)**

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
Female	80	69.0	69.0	69.0
Male	36	31.0	31.0	100.0
Total	116	100.0	100.0	

**Table 3: Residency Characteristics**

<b>Years of Residency</b>	
Mean years lived in Fayette County	29
<b>Type of Residency</b>	
Own home	86%
Vacation home/used for retirement or seasonal	1%
Own rental property	2%
Rent	11%
Other (Live with parents)	1%
<b>Employment Status</b>	
Full time employment	62%
Part-time employment	8%
Homemaker	17%
Retired	6%
Student	2%
Unemployed	3%
Other	2%
<b>Primary Source of Income</b>	
Construction	10%
Finance, insurance, or real estate	1%
Government	11%
Mining	17%
Transportation, communication or utilities	9%
Travel/tourism industry	4%
Wholesale or retail	11%
Service	13%
Other	24%

**Table 10: Graph Employment Status**



The majority of respondents had a great deal of attachment to the area. They had lived in the area from 1 to 67 years with a mean of 29.41. And 86% reported they own or are buying their home; approximately 47% strongly agree they would rather live in Fayette County than anywhere else and 42% strongly agree they would be sorry if they had to move away. There



were about 54% agree that the Future of Fayette County looks bright while 35% disagree and another 11% don't know.

**Table: 3 Level of Support of the Development**

	<b>N</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Mean</b>	<b>Std. Deviation</b>
Future of Fayette County looks bright	116	1	5	2.52	1.342
Support new housing development at New River Gorge National River	116	1	5	2.97	1.450
Support new development of hotels/restaurants in Fayetteville area	116	1	5	2.47	1.392
Valid N (listwise)	116				

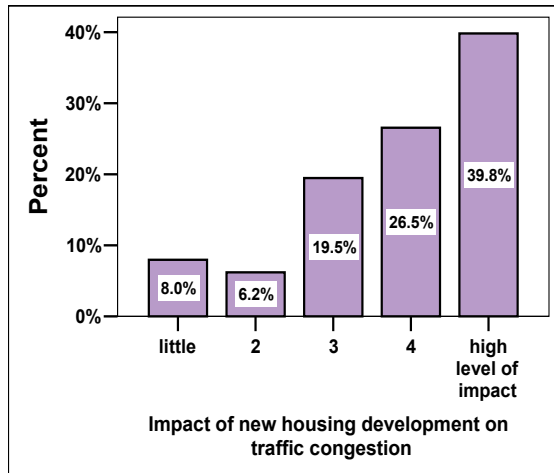
The factors the respondents feel the housing development will affect the most are traffic congestion, cost of living, and parks and recreation areas. Next, they felt parking would be affected. Then equally weighed were the condition of roads and highways, and overall cleanliness and appearance... However, they feel the other factors, such as education system, parks and recreation, as well as the emergency systems will be moderately affected.

**Table 4: Factors impacted by New Housing Development**

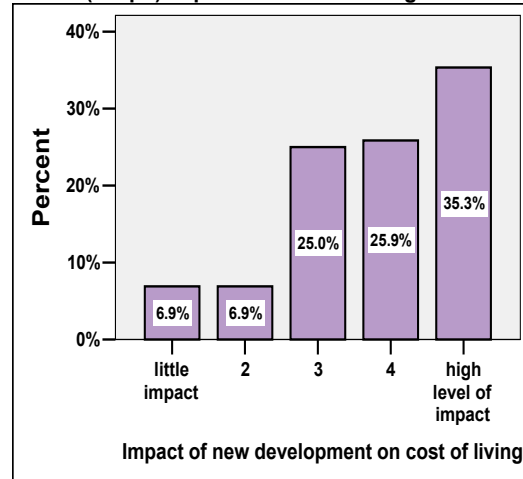
	<b>N</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Mean</b>	<b>Std. Deviation</b>
Condition of roads and highways	112	1	5	3.53	1.301
Education system	114	1	5	3.11	1.155
Traffic congestion	113	1	5	3.84	1.243
Overall cleanliness and appearance	114	1	5	3.53	1.146
Safety and security	115	1	5	3.31	1.202
Parking	115	1	5	3.57	1.365
Cost of living	116	1	5	3.76	1.206
Parks and recreation areas	114	1	5	3.62	1.200
Water and sewer	115	1	5	3.50	1.307

Surveyed 116 respondents

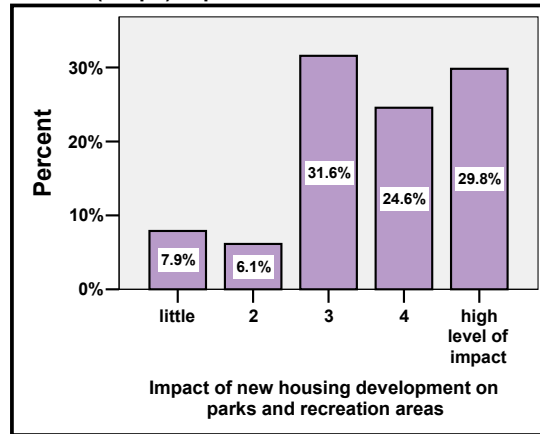
**Table 5: (Graph) Impact on Traffic Congestion**



**Table6: (Graph) Impact on Cost of Living**



**Table 7: (Graph) Impact on Parks & Recreation**



**Table 8: How much contact do you have with tourists visiting in Fayette County?**

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Frequent	26	22.4	22.4	22.4
Some Contact	42	36.2	36.2	58.6
Infrequent	30	25.9	25.9	84.5
No contact	18	15.5	15.5	100.0
Total	116	100.0	100.0	

**Table 9: Statement that best describes your interaction with tourists in Fayette County.**

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Enjoy meeting and interacting with tourist	38	32.8	32.8	32.8
Indifferent	29	25.0	25.0	57.8
Do not enjoy	16	13.8	13.8	
No opinion	33	28.4	28.4	100.0
Total	116	100.0	100.0	

## DISCUSSION

The biggest concern among respondents that voiced an opinion was the loss of forest and natural beauty of the New River Gorge National River. Many said that it would be like lining the Grand Canyon or Yellowstone National Park with houses and they could not see that happening. The quality of life in the Fayetteville area will be affected one way or the other. Many feel that it will improve economic growth, while it will take from the beauty of the New River Gorge National Park and the recreation they enjoy so much. Diminishing quality of parks and recreation in the area are of great concern to most. The majority feels the traffic congestion will be a huge problem. The next major concern is with the cost of living rising and not being affordable. This is consistent with other research on the social impact of housing development in gateway communities.

## Appendix A

### *(Background questions)*

1. Which best describes your type of residence?
  - A. Primary residence (own house here)
  - B. Vacation home / property used for recreational or seasonal purposes
  - C. Rent home / apartment
  - D. Own rental property
  - E. Other (specify)\_\_\_\_\_
  
2. How long have you lived in Fayette County? \_\_\_\_\_ Years
  
3. What is your age? \_\_\_\_\_
  
4. What is your gender?    A. \_\_\_ Male            B. \_\_\_ Female
  
5. What level of education have you completed?
  - A. Elementary School
  - B. Some high school
  - C. Graduated high school
  - D. Some college / vocational training
  - E. Graduated college
  - F. Graduate student
  
6. What is your employment status? (Please check one.)
  - A. Full time employed
  - B. Part time employed
  - C. Retired
  - D. Homemaker
  - E. Student
  - F. Unemployed
  - G. Other (specify)\_\_\_\_\_
  
7. What is the primary source of your household's income?
  - A. Construction
  - B. Finance, Insurance, or real estate
  - C. Government
  - D. Mining
  - E. Transportation, communication or utilities
  - F. Travel/tourism industry
  - G. Wholesale or retail
  - H. Service (specify)\_\_\_\_\_
  - I. Other (specify)\_\_\_\_\_

***(Development issue questions)***

1. With more than 2000 houses being built in or around the New River Gorge National River, indicate how you think the following elements of the quality of life will be affected in Fayette County. *(Rate from 1 to 5 with 1 being least affected and 5 being the greatest.)*

a. Condition of roads and highways	1	2	3	4	5
b. Cost of Living	1	2	3	4	5
c. Education System	1	2	3	4	5
d. Emergency Systems	1	2	3	4	5
e. Traffic Congestion	1	2	3	4	5
f. Parking	1	2	3	4	5
g. Overall Cleanliness and appearance	1	2	3	4	5
h. Parks and recreation areas	1	2	3	4	5
i. Safety and security	1	2	3	4	5
j. Water and sewer systems	1	2	3	4	5

2. Rate the following statements – **(1 strongly disagrees, 2 disagree, 3 agree, 4 strongly agree, and 5 don't know.)**

a. I'd rather live in Fayette County than anywhere else.	1	2	3	4	5
b. If I had to move away from Fayette County, I would be very sorry to leave.	1	2	3	4	5
c. I think the future of Fayette County looks bright.	1	2	3	4	5
d. I support the development a new houses in and around the New Gorge National River in Fayette County.	1	2	3	4	5
e. I support the development of hotels and restaurants in the Fayetteville area.	1	2	3	4	5

3. How much contact do you have with tourists visiting in Fayette County?

- a. Frequent contact
- b. Some contact
- c. Infrequent contact
- d. No contact

4. Which of the following statements best describes your attitude toward tourists in Fayette County?

- a. I enjoy meeting and interacting with tourists
- b. I am indifferent about meeting and interacting with tourists
- c. I do not enjoy meeting and interacting with tourists
- d. No opinion

Additional Comments:

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Rembrandt's Path to Master Painter

Sandra L. Rodgers

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“Artists are not created in a Vacuum”  
Rembrandt’s Path to Master Painter

Art is not created in and of itself, but rather the reflection of the artist’s view of outside influences. Influenced by the painting techniques of his predecessors, Caravaggio, Rubens, and Lastman, Rembrandt added his own interpretation of technique, and semiotics, which he passed on to his students: for example, Gerrit Dou, Govert Flinck, Carel Fabritius, and Arent de Gelder (Benesch, 1957; Schama, 1999). Rembrandt’s art reflects a succession of artistic, economic, intellectual, emotional, and cultural influences of the time.

As an artist, Rembrandt belongs to the Baroque period. The Baroque period encompassed the seventeenth century, emerging in the late sixteenth century and ending in the early eighteenth century. The Baroque style is distinctive in paintings, music, and architecture. It is a style characterized by strong color, elaborate ornamentation, dramatic lighting, and strong emotional content (McCarter, 1985). The Baroque style emerged as a result of the Counter-Reformation in which the Roman Catholic Church sought to defeat Protestantism. The Catholic Church and Catholic nobility used visual means to glorify their religious importance. In doing so, they used elaborate and large religious paintings, buildings, and sculptures that would appeal to the vast majority of people, increasing the fellowship. Seeking a rich visual display modified the direction of art to the Baroque style which captured the moment and challenged artists to capture an emotional subject and an emotional audience (Stokstad, 1999; Stearns, 1967; History World International, 2006). In this manner, Rembrandt sought the inspiration of an early Baroque artist, Caravaggio.

Caravaggio had a strong influence on the Baroque style and an indirect influence on Rembrandt. Caravaggio is recognized as the main contributor of dramatic lighting known as “tenebrism”, which is a term signifying the use of strong “chiaroscuro”, or the contrast of dark



and light. He used tenebrism to magnify his naturalistic painting style in compositions of still life, genre, and religious paintings as he interpreted them (Stokstad, 1999). Breaking away from tradition, Caravaggio experimented with harsh light. If Caravaggio had used the traditional soft light of the sixteenth century Mannerism period for which he is associated, other painters, such as Georges de La Tour, Jan Vermeer, and Rembrandt, would not have had the foresight to manipulate light within their own creations.

Rembrandt started his life in Leiden, Netherlands, in the year 1606. He was the son of Harmen Garretszoon van Rijn and Neeltje Cornelia Willemsdochter. His father was a miller who added “van Rijn” which means “by the river” as his surname for commercial purposes. His mother came from a family of bakers, which in turn made their marriage a prosperous union, the mill provided flour for the bakery. At the age of seven, Rembrandt was enrolled at the Latin School and at the age of fourteen he entered the University of Leiden. Perhaps his parents believed that Rembrandt exhibited perception and knowledge that placed him above trade work, and they decided to send him to formal education (Stearns, 1967). His enrollment at the University of Leiden, however, did not last long and soon he left to study art.

According to Stearns (1967), Rembrandt studied with local artists, learning the basic techniques of mixing paints, preparing canvases, and brushes, before accepting a formal apprenticeship with the local artist Jacob van Swanenburgh. From Swanenburgh, Rembrandt learned perspective and to study the effects of light as it falls upon an object creating shadows (Stearns, 1967; Solman, 2004). Rembrandt’s study of light at an early apprenticeship foreshadowed his life-long study of manipulating light. Rembrandt’s early life influences were crucial in setting his path as a master painter.

Rembrandt was apprenticed to Pieter Lastman in Amsterdam, by the recommendation of Swanenburgh. With Lastman, Rembrandt studied the composition of popular subject matter of the time: history and religion. Rembrandt left his mentor Pieter Lastman after six short months and arrived back at Leiden a young inspired artist embarking on a life journey (Stearns, 1967).

In Leiden, Rembrandt collaborated with a friend, Jan Lievens. Sharing a studio, ideas, and models, Rembrandt and Lievens' works were often mistaken for each other's work. Rembrandt sought to find a unique quality in his work which he did in portraying thought and emotion through the eyes in his portraits. Rembrandt outgrew Lievens as an artist but not as a friend, for their friendship would last over six years (Stearns, 1967).

Finding himself back in Amsterdam, Rembrandt faced artistic challenges, economic considerations, connections, and inspiration. In Amsterdam, Rembrandt compared his work to fellow artists, this competition allowed him to further his talent and creativity which resulted in his unique style. Rembrandt's artistic talent brought him portrait commissions and students to apprentice, enabling him to gain moderate wealth.

Rembrandt took residence with Hendrick van Uylenburch, a painter as well as an art dealer. Through this friendship, Rembrandt was introduced to wealthy patrons. These connections lead to portrait commissions that in turn furthered Rembrandt's career and boosted his popularity among the wealthy status-quo. Hendrick van Uylenburch also introduced Rembrandt to Saskia, whom he married and became the subject of several paintings. His marriage inspired his painting as well as influenced his social status. Saskia was also influential with the upper class and made connections for which Rembrandt reaped financial rewards (Benesch, 1957).

Rembrandt's apprenticeships led to independent work which propelled him to become a master in his own right, encompassing all that he had learned and all that had influenced him. Rembrandt shared his knowledge with his own students that include Jacob Backer, Ferdinand Bol, Johannes Victors, Philips Koninck, Van Den Eeckhout , Gerrit Dou, Govert Flinck, Carel Fabritius, and Arent de Gelder (Benesch 1957; Schama, 1999). Extraordinary in his ability to teach students to replicate his style, he also pushed them to explore their own individual artistic freedom (Benesch 1957). After mastering one style an artist is more able to apply the same principles to various other styles of interest.

"Semiotics," the study of signs, is most relevant in art combining meaning and value (Chandler, 2005; Bal, 1996). The use of signs to represent an idea has been used many times in paintings throughout the years. Semiotics includes the value for which the audience places on the painting. Semiotics personalizes the representations made within a painting and makes a statement about the subject, for example, religious, personal, political, economical, or emotional.

Rembrandt's sensitive religious upbringing is portrayed in his work. A painting titled *St Paul*, 1630, Germanisches Museum, Nuremberg, depicts St. Paul as a thin-faced elderly man having a high forehead, receding hairline and long beard, a common description of St. Paul as seen throughout historical paintings. The semiotics of the painting addresses the figure as St. Paul, or the way in which the audience is to perceive St. Paul, stripped of elaborate dress wearing humble clothing. Within the layout of the painting are the swords hanging, representing his previous slaughter of Christians and the foreshadowing of his own death. The pen in his right hand depicts the strength of the written scripture. His facial expression is deep in thought reflecting his maturity. The semiotic content reveals his past and future. Rembrandt leaves an

image and idea which is both explicit and implicit, a semiotic view showing the importance of St. Paul and the value of his presence in the religious community.

An early milestone for Rembrandt was the commission of a life-size group portrait by patron Dr. Tulp, entitled *The Anatomy Class of Dr. Tulp* 1632, Mauritshuis, The Hague (The Great Masters, 2003; Schama, 1999). More significantly, this painting placed emphasis on the cadaver, the subject of the painting and the subject within the painting. It is successful in regards to the movement from figure to figure through facial expression and in Rembrandt style, invites the audience to participate. The semiotics of this painting can be drawn from the unknown. The cadaver, once a living person capable of learning, is now the teacher, or that there is a purpose in life as well as death.

Another painting, *Young Woman Bathing* 1655, in the National Gallery, London, captures a moment of private time inhibited of innocence. The semiotic structure of the painting addresses the woman in grace and the beauty of her inner character that is symbolic to every woman. The luxury of bathing lends to the idea of being renewed, both physically and spiritually. She is dressed in a white undershirt illuminated by a single light source creating an angelic, heavenly feel. Rembrandt portrays the emotional innocence that is woman. The semiotic confines of the painting are the fragile structures imposed upon a woman. The aforementioned paintings are examples of semiotics used within Rembrandt's paintings, and key to the thought process when creating a painting.

Rembrandt used a limited color palette, usually consisting of only ten colors, with which he mixed a variety of hues. Colors of his palette included black, brown, red ocher, transparent browns, vermillion, lead tin yellow, azurite, smalt, greens, and lead white (*The Great Masters*, 2003). Oil paint was actually made by the artist or the artist's apprentice. The process of

grinding pigment and combining with oil took careful attention and time. Not mixing the paint properly could end in disaster; the outcome could mean discoloration and unstable adherence to the canvas. The type of oil used and the amount was crucial in the longevity of a painting (Mayer, 1991).

Rembrandt's use of paint was almost sculptural, created by applying many layers of paint. The layering process enabled Rembrandt to create depth within the painting, textural and visual. Layers of color allow for a richness that mimics the spectrum found in nature. After some of the paint dried, he was able to scratch and carve away paint for greater detail which allowed the layers of colors to emanate in a jewel-like fashion. The thickness of the paint did not inhibit an object from appearing light, for the draping of a dress or fold of fabric was never confused with an immovable object. Rembrandt used no harsh lines, but his illusion of great detail is captured in the way light bounces off objects. Light dominated his work, for Rembrandt worked a painting from the back coming forward as if his subjects emerged. Starting with a rather dark background of greens and browns, he layered his paint from dark to light, which lent to the depth he was able to create (Danby, 1999). He would use thin washes or layers that melted and merged into successive layers. Rembrandt's *Self-Portrait* of 1640, in the National Gallery, London, demonstrates how he uses light. The succession of layers allows for even the moisture in the eyes to be seen (Landsberger, 1946). These details provide an interest for the audience at a distance, the overall image, as well as up close, the fine lines of the eyes. Such a technique marks Rembrandt's personifying the emotional context that he sought to evoke from himself and his subjects.

The strong yet subtle way in which Rembrandt uses light classifies him as a Master Painter. He employs light to create dimension. For most of his paintings, a single source of light

enters from either the left or center left of the canvas and sweeps across the subject. There is a balance of light and dark within the paintings. There are many variances of value within the shadows adding to the dimensional structure. With light, Rembrandt was able to emphasize and de-emphasize areas within his works which gave more life and an in-the-moment feel for his audience. In this way he was able to draw the audience in and move them through the painting to a focal point. Even in the shadows Rembrandt emphasized the presence of life and structure (Danby, 1999).

His early years depicted allegorical scenes that were small and rich in color like that of *The Presentation In The Temple*, 1628. Kunsthalle, Hamburg. Rembrandt draws the audience in as a presence to experience a holy tranquility that explores what is important to him as a person and as an artist.

Rembrandt used rich colors again in the 1650's in a self-portrait, *Rembrandt self-portrait*, 1658. New York, Frick Collection. In this self-portrait, Rembrandt is pulled from the dark background into his signature lighting. His heavy use of paint exemplifies his full body; subtle layering of the rich golden hues enthrones him to a sense of royalty. His expression is one of presence, not to be ignored but acknowledged, again showing a quality that is inviting yet painted for self. The final year of Rembrandt's life produced yet more self-portraits showing the artist marked with the grief and sorrow that he had come to know.

His final year of painting produced the painting, *Simeon in the Temple with the Christ Child*, 1669. Stockholm, National museum, which is exuberant with color, emotion and personification of birth and death (Benesch, 1957). One of his most painterly styled paintings, Rembrandt again touches on the roots of his religious upbringing, showing more emotion through color and light and less detail of line.

The techniques and style of Rembrandt have been influential to many artists from the seventeenth century to the twenty-first century. Adriaen van Ostade, a seventeenth century painter, used Rembrandt's chiaroscuro lighting in depicting peasant life (Encarta 2006). Nineteenth century French artists, such as Degas, Manet, and Vincent van Gogh, looked to Rembrandt's technique and emotional content as inspiration. In a letter from Vincent van Gogh to his brother Theo, van Gogh referred to Rembrandt as most of all a magician (Stein, 1986). Other artists of the nineteenth century influenced by Rembrandt include Gustave Courbet, depicting realism; Jean-Francois Millet, dignifying peasant life; and Thomas Eakins, who was influenced by the realism and psychological intensity that Rembrandt held in so many paintings especially portraits of older subjects portraying deep and reflective thought (Encarta 2006). Artists of the nineteenth and twentieth century influenced by Rembrandt include Paul Cezanne, Henri Matisse, German expressionists, and artist Francis Bacon. Contemporary artists influenced by Rembrandt include, Charles Matton, and Lucian Freud (Frankel, 2002: Ford-Wille 2006). Those influenced by Rembrandt used the application of chiaroscuro light, semiotics, cultural, and economic content, and added their own thoughts and values of composition to create their own style.

An artist is able to see his surroundings as new frontiers yet reflect upon the true nature of the subject. The path of a master painter is ever-changing and the passion to create compels an artist to push forward the boundaries set by artistic predecessors. Rembrandt's genius was his ability to portray a variety of emotions in his work: joy, sorrow, peace, and turmoil. Rembrandt was an artist sensitive to his environment and reflected the artistic, economic, intellectual, emotional, and cultural influences of his time while portraying his subjects in overpowering grace and beauty.

The path of a master painter cannot be truly duplicated but it can be followed closely. Using equivalent materials, techniques, settings, expressions, and semiotic values, I attempted to mimic the chiaroscuro lighting and the changing brush styles of Rembrandt. Placing myself as painter and model, I simulated five of his paintings, consisting of two self-portraits and three group portraits. I relied on previous knowledge of basic techniques, and added elements indicative of Rembrandt: layering paint and use of chiaroscuro. The project at worst produced a better painter; at best, the project produced a painter capable of inviting an audience to share an experience. Much like Rembrandt's students become better painters yet were denied the title Master Painter for which Rembrandt holds.

The process for this project consisted of simulating the placement of figures and the direction of light as set in each of the five paintings by Rembrandt that I chose. Each painting that I created is comparative in size, color, and arrangement. Other noticeable attributes included size of brushstrokes, refinement of brushstrokes, and the gradual change to a painterly brushstroke.

## Method

### Participants:

I painted a series of self-portraits in the manner that Rembrandt painted. I substituted my self and used other models as well.

### Materials:

The materials I used were similar to those used by Rembrandt. With the exception of the paint which Rembrandt would mix as it was not readily available at the time, I purchased paint of similar quality. The brand of paint I used was Winsor & Newton, which consisted of these colors: black, brown, red ocher, transparent browns, vermillion, yellow, azurite, blues, and



greens. For building the frames I used a pine wood and construct with staples and nails. They were covered by a one hundred percent, bleached cotton canvas stretched and held in place by staples. For the painting that called for a panel, I used a solid wood panel and attach to a constructed frame as aforementioned. Brushes consisted of variety of sizes with a mix of blunt and fine point ends. The brushes were hog's-hair as was the type used by Rembrandt. Linseed oil and turpentine was used to thin the paint and clean the brushes. Acrylic gesso was used to prime the canvas making the surface ready to paint. I used simple suggestive backgrounds and interiors simulated from Rembrandt's original paintings. Due to time constraints, sketches and photographs were used as reference when working on a larger canvas, in contrast to Rembrandt who would have used sketches rather than photographs. Rembrandt's paintings for which I simulated consist of the following:

*Self-Portrait with a Gorget*, Ca. 1629. Mauritshuis, The Hague.

*The Anatomy Class of Dr. Tulp*, 1632. Mauritshuis, The Hague.

*Simeon in the Temple with the Christ Child*, 1669. Stockholm, National Museum.

*Hendrickje Bathing*, 1655. London, National Gallery.

*Self-Portrait with Maulstick, Palette and Brushes*, 1663. London, Eveagh Bequest, Kenwood House.

#### Procedure:

The process to begin a painting starts with building a frame for which canvas material is stretched and secured in place with staples. Each canvas was built according to the size of the painting referenced to Rembrandt. Once the canvas was completely constructed, it was then covered with an acrylic gesso in order to prime the surface before painting. If Rembrandt's painting was originally on a panel, I also constructed a frame with a panel board attached to the frame with nails. After a thorough study of the reference painting, I arranged the light and costuming in a fashion similar to Rembrandt's painting. Sketches along with photographs, made

for reference due to time constraints. Two consistent elements to emphasize from Rembrandt were the use of light and the layering of paint. As Rembrandt worked a painting from the background forward, I began the background with layers of dark greens and browns. Emerging from the background I painted the figures in a succession of layers using the paint in a sculptural sense layering from dark to light. In this way, I demonstrate the chiaroscuro lighting that Rembrandt used so effectively. I used several natural-hair brushes which made underlying colors vulnerable to showing through, helping to create a translucent effect. Concentrating on the eyes and facial expressions was important in order to present a life quality in the painting. Along with the facial expressions, I concentrated on proportions and hand gestures that allowed for movement within the paintings as portrayed in paintings by Rembrandt. The variety of props combined with facial expressions and costuming tied the painting to a contextual semiotic meaning.

## Results

The results for this project have been favorable. Obtaining background information as to the process of Rembrandt's paintings helped to consciously articulate each painting. I found success in bringing my painting skills to a new level that stemmed from the concentration of trying to mimic the skills of Rembrandt. Though I obtained light in my paintings, it did lack the warm glow which is so evident and characteristic of Rembrandt. I was able to embark on the emotional semiotics of the paintings yet I was not able to disclose full emotion through the eyes as I had hoped. Each painting began with the same process of background to foreground and each painting became more comfortable in that process. Tying all elements together was difficult and challenging. Where Rembrandt would allow layers of paint to dry before applying another layer taking months to complete, I was working with minimal drying between layers.

Rembrandt's method allowed him to go back in and at times scratch and scrape through successive layers which I believe helped to pull the figure from the background.

### Discussion

The apprenticeship approach to becoming a master painter allows for experiential learning even without the master present. The textbook research allowed for the knowledge of Rembrandt's process with key elements to consider: chiaroscuro lighting, layering of paint, movement within the painting, emotional value, and semiotic content.

Rembrandt's students were fortunate to have first hand experience with a master painter. I compensated for not having Rembrandt's direct teachings with textbooks and mentoring by Jack Sheffler.

Difficulties of this project were the inability to completely match colors and obtain a candlelight glow within the figures. In thought this does not seem a difficult process to duplicate but in practice it is the difference between a painter and a master painter.

My strengths can be seen by the painter I started as and the painter I am now, embarking on new textures and striving to complement the light with the subject of the painting. Embracing the blank canvas and manipulating the paint in a new way shows my growth as a painter and allows me to think of semiotic content in every creation. I obtained success through a favorable audience and the sale of two paintings. The audience inevitably determines the success or failure of an artist.

The process of painting from the guidance of a master painter allows for the conception of art to begin within another painter. Rembrandt himself worked in successiveness; the application of many layers of paint, and the application of many paintings. Rembrandt depicted

both in his many self-portraits, painting for more than half of his life. The path to becoming a master painter is truly contingent upon patience, dedication to skill and time.

Because of Rembrandt, my path thus far has allowed me to embark on new materials and new techniques, yet holding to the core elements influenced by Rembrandt. My recent works are figurative paintings ranging in size from small to life size figures. I begin my work on sheet-rock and apply texture either from a commercial spackling compound with the figure in mind or a random texture from a commercial spray. Once the texture has dried, I apply acrylic gesso or a Kilz primer. The figure is sketched with contour lines using chalk until I apply washes of oil on the surface to begin more definition. Along with using oil paint I also use paint sticks, which are oil based, that allow me to skim the surface of the texture. At times I blend colors using brushes, but also I find that rubbing the colors into the surface allows for better layering and depth. The colors manipulate the light which allows for movement in the painting though the figure itself depicts movement on the basis of human content. The figures are allowed to provide an emotional content through the mood of color and the abstracted figure. Semiotics of the paintings provides the viewer a glimpse of an emotion, a passing of time and the connection of seeing oneself in a vulnerable moment. My style and techniques are changing as I learn more and express more. Rembrandt started as a realistic painter and in his latter years become more painterly yet was always aware of the figure and the light. It is my hope to continue with what I have learned from my research on Rembrandt and to achieve the status bestowed upon Rembrandt, that of Master Painter.

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Appalachian Females: Catalysts to Obtaining Doctorate Degrees

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**Abstract**

In this hermeneutic qualitative study, I explore the lived experiences of three Concord University McNair Scholar Graduates. These women were born, raised, and educated in West Virginia, and have obtained or are currently completing their doctoral degrees. My question for this investigation asks, “What are the catalysts that lead to West Virginia Appalachian Females obtaining their Doctoral Degrees?”

The literature suggests that gender differences, role models, Appalachian values, and socioeconomic status are four variables that influence the education obtained by Appalachian women. In studies, income has been linked to the amount of education obtained; however literature also suggests that females make less income than males with the same Doctoral Degree.

The data used was collected through multiple interviews conducted by e-mail and over the telephone. Member checks and personal analysis were used to clarify the findings and analyze the results. The findings were in agreement with the literature. Appalachian women’s education is influenced by gender differences, role models, values, and socioeconomic status. All three of the women were challenged by values, gender differences and monetary availability; however the women had strong positive role models that encouraged them to overcome and obtain higher education. Additional research concerning first generation college students and females is needed to further investigate this issue.

According to the U. S. Census Bureau the average earned income for a woman in the United States is 33,000 dollars a year. A man living in the United States on average brings home 17,000 more dollars than a female; on average the male living in the United States brings home 50,000 dollars a year (U.S. Census Bureau, 2004). Education is a large determinant in the amount of income a person will be able to obtain in their lifetime. Women and men differ in their education accomplishments due to the gendered image placed upon different subjects taught (Breakwell, 2003). Research shows that males and females are taught differently, hired differently, and treated differently during the whole process of establishing a career. Men have always had a fair number complete college degrees. It was not until World War II that women's degree achievement began to rise (Haaga, 2004) and more women graduated from college. Therefore, what are the catalysts in enabling females to obtain advanced degrees?

In comparison to past generations, more women are completing degrees. For example, in the 1970s the female college enrollment numbers increased and by 1980 more women were attending college than men (Glazer-Raymo, 1999). The college enrollment rates increased 60 percent during the time period of 1970 and 1994, from 8.6 million to 14.3 million (Snyder, Huffman, and Geddes as cited in Glazer-Raymo, 1999). Females and part-time students made up for this increase. In 1995 more girls than boys were graduating from high school and receiving bachelor's degrees, but 60.7 percent of doctorate degrees were still going to men (Rimm, 1999). The increases in women's degree attainment include Associate Degrees; men still dominate post-graduate attainment of degrees (Haaga, 2004). Regardless of the change, men are ahead of women 10 to 8 percent on holding advanced degrees (Bauman and Graf, 2003).

These changes could be due to the way women are educated. For women to be offered the same opportunities as men in post secondary education and job options, they need to be well



prepared academically (Freeman, 2004). The importance of female role models being present in schools is found to be as important as the women being educated there. It has been found that liberal-arts colleges with a higher number of female faculty members send more females on to get their Ph. D's due to the presence of females, than most doctoral institutions or Universities ("A hothouse", 2006).

Regardless of the growth throughout the nation, the Appalachian Region continues to have fewer college graduates as compared to the whole United States. According to the U. S. Census the United States has an overall 24.4 percent of its population having a bachelor's degree or higher compared to the 14.8 percent of West Virginia's that have the same status (Bauman and Graf, 2003). Regardless of these grim statistics, what has encouraged some West Virginian Females to succeed in obtaining terminal degrees? The presence of a support network, a positive role model, and positive influence from programs that assist the underprivileged have been shown to promote achievement. For Ronald E. McNair said "Before you can make a dream come true, you must first have one." (McNair Handbook, p. 5)

The McNair Scholars program gives students who come from a background of families without a higher education a chance to explore and obtain guidance to a path of higher learning. The program has given the women I interviewed a better life and a career. "When you come from a home where you are the first to graduate from college you have to succeed or you have not changed your family's view of college as a good way to go into debt" (Ryan, Pseudonym, November 11, 2006). Working is the only way to make a living for families who have not gone to college. My research shows that first generation college students have to push their own way through college. Often these women had to educate their families about the university process while they are learning the steps themselves. They spend their time explaining why they are

going to college if they have already found a husband. It is still believed that if we go to long we will educate ourselves right out of a husband. This is what our Appalachian values force upon us. Previous research has shown if Appalachian women have good positive role models, a strong personality, and a determination to be happier and live more comfortable; they have more opportunities to pursue higher education. They will obtain their doctorate degrees despite the Appalachian barriers Marie, Pseudonym, November 13, 2006.

### Definitions

Appalachian Region- defined by the Appalachian Regional Commission (Hicks, 2002) as the region including all of West Virginia and parts of 12 other states, stretching from the Appalachian Mountain Range, from New York to Mississippi.

### Gender Differences

Numerous studies have tried to demonstrate that gender differences account for the wide margin between female and male achievement. For this reason, gender differences in education have been studied many times. Men have been shown to dominate classrooms by using a more aggressive style (Tayebi and Johnson, 2004; Wilson, Peterson, and Wilson, 1993). With this women become silenced and stop participating in the class discussion. To study this Tayebi and Johnson (2004) felt that taking the students out of the formal classroom and only allowing the class to interact through the use of computers would solve the gender roles and silencing that would typically go on. When the men and women took part in the computer classroom with only typed communication, women used expressions that gave away their sex, thus putting the researcher's right back where they started. Herring (1993) believes that for women not to be noticed as being female they must learn to use men's expressive language to be taken seriously in the academic world. If the females and males would have refrained from bringing their

genders online with them the computer classroom might have been a success. This is one of the ways women are forced to change who they are to be competitive in a man's world. Some students have even shown that women are not as likely to be encouraged by teachers and mentors as men (Haaga, 2004). Women may view hostile learning environments less favorable thus making them uncomfortable; so they are shy about participating in the class discussions (Watson, 2005). In addition women often favored teachers who are caring and give good examples and explanations while teaching (Watson, 2005). Research done by Connell (1975) showed that boys often demonstrate higher self-concept than girls (as cited in Hicks, 1995).

David and Myra Sadker, former education professors, at American University, studied gender differences in education for more than 20 years. They state that things have changed in the 20 years, like girls math scores have improved but science is still a man's subject. When girls do not want to speak in class there is no push for making them participate and explore the material (Sadker and Sadker, 1994). They believe that girls have self-esteem issues that can result in disengagement, hindering the learning process. If teachers were aware of this problem, it can be corrected. Girls need to be motivated and pushed to build confidence (Sadker and Sadker, 1994). Females have to be told they can further their education. Some women still feel they do not have the right to an education. Terminal degrees are valuable to the people who hold them; they are one accomplishment that no one can take away.

Gender studies also show that females in college are not supported by faculty when they apply for degrees in the fields of mathematics, science, technology or engineering. Women have low participation and success rates in these fields (Atkin, Green, McLaughlin, 2002). Studies also show that females experience less encouragement from parents and teachers to go into science majors (Eccles and Jacobs, 1986; Maccoby and Jacklin, 1974; Fox and Tobin, 1980;

Hanson and Ginsburg, 1988; Crowley, Shapiro, 1982; as cited in Atkin, Green, and McLaughlin, 2002). Women can and do come across negative attitudes about their positions as science or non traditional gender role majors. These attitudes may come from classmates, family, professors, or others (Atkin, Green, and McLaughlin, 2002). In addition once women succeed and obtain the Ph. D. it is believed that it is harder for them to have families and tenure. While men can have it all, a marriage and as many children as they want (Mason and Goulden, 2004) women must choose. According to Chenoweth and Galliher (2004) gender roles for women are housekeeping and being a mother. Professional careers are often not offered for women in the Appalachian area due to the limited job market and the Appalachian values that are attached. Why hire a woman who will need maternity leave over a man who can work all 365 days a year. There has been such a push for more equality between males and females that a group called AAUW has worked to improve the differences (“Envisioning the future...”, 2005). Now that most girls have shown an increase in college completion their goal is to make it last a lifetime.

### Role Models

Another possible deterrent to a female’s pursuit in education is they are less likely to be encouraged to continue their education despite having better grades than their male counterparts. Studies show that father’s educational attainment predicted the daughter’s attainment in education (Wilson, Peterson, and Wilson 1993). The parents’ aspirations for education of their children also played an important role in the educational accomplishments of their children (Wilson 1993). The women had a need for the support from their parents to succeed in school. In Rimm, Rimm-Kaufman, and Rimm’s study (1995) most of the women stated that their mothers supported them and motivated them to get a higher education. Most of their mothers were homemakers until their daughter’s started school, and then they joined the work force or

furthered their own education. In LePage-Lee's book (1997) Michelle described the love and support she received from her father "he gave me confidence and acted like I could do no wrong" (p. 98). In LePage-Lee's book (1997) the women also talked about their husband's or significant others that had encouraged them throughout their education. However, the females did not credit the male supporters with their success. Role models for women are hard to come by in Appalachia. The only place you find an abundance of females is in teaching, nursing, or secretarial work (Spatig et al., 2001). Female teachers became important to the women because they encouraged and supported the women and were living examples that degrees could be obtained. The role models that were the most beneficial to successful females were the ones that helped build confidence, had high expectations, and motivated the women to succeed (Rimm, Rimm-Kaufman, and Rimm, 1999). When faculty members were assessed to see what male to female proportion were found in majors that women did not usually select, Digest of Educational Statistics (1997) found that only 2 percent of faculty in engineering and physics were female (as cited in Atkin, Green, and McLaughlin, 2002). According to Conklin and Dailey (1981); and Murphy (1981) influences on family values of children's enrollment in college are families as providers of resources, role models within the family, and encouragement for college success (as cited in Chenoweth and Galliher, 2004).

#### Socioeconomic Status

Socioeconomic status (SES) also affects the options open to the women in Appalachia. Along with socioeconomic status is the low-income that places females in the poverty bracket. This puts education against eating. The U.S. Census Bureau states that 16.3 percent of West Virginia's were below poverty in 2003 compared to 12.5 percent below poverty in the nation. LePage-Lee (1997) says that "being poor can be a major stress in and of itself, and people who

are poor are more likely to face other kinds of stresses” (p. 103). This adds to the complex schedule of an already stressed college student. Branscome (1970) stated that “the records of Appalachian students revealed that they were from homes where low economic standing may be one of many barriers to higher education” (as cited in Hicks, 1995, p. 7). The West Virginia girls studied by Ewen (1996) had family incomes of less than 21,301 dollars (Spatig et al. 2001). The people of the Appalachian region did not always fall below the poverty level. During World War II the area benefited from the mountain resources such as coal for economic growth. However, in current times when females must work for food or go to school, school usually loses out. This does not say that all women do not succeed, but few overcome the obstacles (Wilson, Peterson, and Wilson, 1993). The family’s physical size that the women come from also has a role in how resources are distributed within the family. The male siblings are given first choice of the extra funds available (Peterson et al., as cited in Wilson et al., 1993). Tickameyer and Tickameyer (1986) in their discussion of dynamic regional differences such as a families composition changes, economics, and gender interactions claimed that Appalachian poverty is due to local economy structure, types of employment available, and the isolation that makes income opportunities low (as cited in Oberhauser, 1995). According to the United State Census Bureau in 1992 “of more than 320,000 West Virginians about 18 percent of the states population lived in poverty, compared to the national average of 13.5 percent in 1990” (as cited in Oberhauser, 1995, p. 6). The Appalachian region according to Couto (1994) and Mencken (1997) is “defined by low incomes, high unemployment, underdevelopment, and high poverty rates despite state and federal interventions to help economic growth” (as cited in Latimer, 2000, p. 3). A fathers and mothers educational achievement, as well as family earnings, play an important role in affecting parents education goals for their children as reported by Stage and

Hossler (1989) (as cited in Chenoweth and Galliher, 2004). Household incomes and per capita incomes in Appalachia are much lower than those reported from the United States as a whole (Chenoweth and Galliher, 2004).

Inexpensive state colleges were the only option for some women who have obtained degrees. In addition to school, most of them worked and some received financial aid. In order for some of the women to be able to attend master and doctorate programs they had to spend their little bit of savings. This was their last option to make the goal of education obtainable. To make matters worse, there is little equality in salaries according to Weinberg (2004) and the U.S Census Bureau. In 2004 a women who worked year around made 77 cents for every dollar earned by a man according to the U.S Census Bureau (Weinberg, 2004).

West Virginia's mountains are beautiful, but they make traveling and accessibility difficult. Travel takes twice as long because roads have been built around the mountains. The rugged terrain has prevented universities from coming into the remote areas. Until a few years ago, Huntington and Morgantown were the only options for obtaining doctorate degrees in West Virginia.

In LePage-Lee's book (1997) most of the females had to be asked to define low-income because if you have never had money then you never missed it. Most grew up not knowing they were any different or that they were poor. Coming from a low-income family made the women in LePage-Lee's study grow up faster and accept responsibility sooner. Some women had to help raise siblings and keep the house on their own to help the family survive. This role becomes common and comfortable to the females. Instead of exploring something new she sticks with what she knows and marries young. This eliminates stress of the unknown and contributes to her new family. This issue brings up values.

## Appalachian Values

Characteristics of the unique Appalachian Culture as described by Chenoweth and Galliher (2004) are localism, historicism, and familism. Localism can be described as feeling a sense of home and comfort. Historicism refers to an understanding of what happened before and wanting to be there for future happenings. Familism is a strong commitment to and devotion to family. With these characteristics or values the people in Appalachia value their families, hold their land very close and stay in the area where they were born and raised. The people of Appalachia are stereotyped as being “hillbillies” who are undereducated and non-working (Chenoweth and Galliher, 2004). This pushes the Appalachia natives to stay away from high populations of people, because they believe the stereotypes to be true.

Motherhood is the main goal for females in this Appalachian area. Teenage motherhood is not as threatening to the girls because that is what the female had as a main goal in her lifetime (Chenoweth and Galliher, 2004). Events that have effects on women’s educational attainment are marriage and childbearing at young ages (Wilson, Peterson, and Wilson, 1993). These life happenings make not only getting a higher education more difficult, but also make competing for jobs after the Ph. D. harder. Sewll states, husbands may also discourage women by forcing the roles of wife or mother on their wives (as cited in Wilson, 1993). Women must live up to the expectations of being mothers and or wives, but also be able to responsibly handle the professional job as well. Family values in the Appalachian region create a unique culture that focuses on family as being the center of life, and the most important aspect of your life. With low-incomes the members usually follow traditional gender role occupations (Hennon and Photiadis, as cited in Wilson et al., 1993). Mothers in the past did not encourage their daughter’s to go to college because it was not a possibility for them or an option (LePage-Lee). Other



mothers who have raised children on their own encouraged their daughter's to go get the college education. Toni from LePage-Lee's book states "My family thinks I am insane; I've been in graduate school for eight years" (LePage-Lee, p. 99). This is how families with little education view those who get higher education. This is due to lack of experience and understanding. Due to the values in Appalachia and according to a father of four girls "The women have even less opportunities than the men do." A mentor commented "The only female professionals the girls are able to see in their own communities are teachers, just a few nurses, and secretaries." (Spatig, Parrott, Carter, et al., 2001, p. 68). Females in Rimm, Rimm-Kaufman, and Rimm's book (1999) had issues with their mothers saying that they would educate themselves out of a husband. This is a common statement made because men in the Appalachian area are afraid of women who are educated and can think for themselves. The women in LePage-Lee's book talked about being single mothers and how their children motivated them to succeed. Most of the women who went on to college and were Ph. D. holders avoided marrying too young. In a study Ford conducted in 1962 he found four traits linked to Appalachian people: individualism and self-reliance, traditionalism, fatalism and religious fundamentalism. These traits and life deep within the mountains has left the Appalachian people isolated and has resulted in "superstition, backwardness, and clannishness" as Weaver states (as cited in Hicks, 1995). Females involved with majors in mathematics, science, technology, or engineering are faced with traditional values of what women roles are in society (Atkin, Green, and McLaughlin, 2002). Epstein found (1983b) that "family influences were found to be stronger in the developmental process than classroom influences. However, school influences were more important to children who were not permitted such opportunities at home. These effects were more substantial than those produced by socioeconomic status or ethnicity" (as cited in Chenoweth and Galliher, 2004). Goodenow

and Grady (1993) discussed the nature of motivation to succeed by noting that achievement in academics comes from the development of personal values that are influenced by people close to the students and their culture (as cited in Chenoweth and Galliher, 2004).

### Methods

This study focused on Appalachian females and catalysts to why they succeed. A qualitative study was conducted using interviews as the primary collection process. The interview questions were original created by the author. The interview questions can be found in Appendix A. A report was submitted to the Human Subjects Review Board at Concord University for approval. Once the approval was received a search to locate the participants that met qualifications was preformed. The eligible women were then contacted to set up and discuss the study and guidelines thereof. The interviews were conducted in two ways: telephone and email. A recorder was used for the telephone interviews. Three Appalachian females were interviewed. Each female was interviewed three times and took no more than one hour to complete. These women were born and raised in West Virginia. The women completed both their B.A. or B.S. degrees in West Virginia. The females have their Ph. Ds or are currently working on their Ph. D. These women were also McNair Scholars. The completed interviews were transcribed using a transcription device. Member checks were used to ensure data accuracy. Once the data was complete, an analysis examined the research focusing on patterns from the responses. Once the analysis was complete, the results were compiled in a narrative report using names selected by the participants to protect their identity.

### Ryan

Ryan was born and raised in Alderson, West Virginia, a rural area with little commerce. She lived with her parents and two siblings, an older brother and younger sister. Ryan is close to

her family and wanted to stay close to them during her higher learning opportunities. Her family had limited resources. Ryan stated “we definitely did not have a lot of money. I think this made me respect money as something you had to work hard for”. Ryan worked at her mother and father’s business when she was young. She was already tired of working and knew that there was so much more for her than working at the family business. She wanted to prepare herself for a career she could enjoy and respect herself for.

Ryan wanted to attend college, with a close family there was pressure for her to stay close. Ryan stated “This could have been a big problem if I did not tell myself that being gone for a while, for school, would not be intolerable”. Her family did not have the experience to help her make decisions about college, so Ryan went by the advice of acquaintances and teachers that she sought out. Ryan sought out professors and opportunities to gain knowledge that would help her succeed. Female professors and teachers that Ryan had who had accomplished the goals of higher education gave excellent examples that higher education could and had been done by women. A teacher who was tough but fair and who wanted students to succeed were the type of educators that Ryan found as positive role models and ones she could look up to and aspire to be. Attitude and personality helped Ryan decide which teachers or professors to work with and become close to as mentors. Ryan liked teachers who challenged her. Ryan stated “There’s a lot more joy in the accomplishment of having to work hard for a good grade.”

The McNair Program help Ryan learn about possibilities of further education and what she would need to do to obtain those different careers options. Ryan said what motivated her to obtain her doctorate degree was “the desire to do well, desire for recognition of hard work through attaining my degrees, and wanting to be a good professor.” To Ryan success is defined

by happiness. “What is the point to work so hard if you have no time to spend time with your loved ones?”

Women earning less than men with equal degrees has been witnessed by Ryan, as well as, women working harder to be recognized for the same accomplishments. Over all women have to work harder and accomplish more to be well known in their field while men can publish one paragraph and be well known and accomplished. Ryan commented that “there is definitely a bias towards women in academia”.

Ryan’s family did not understand why she was in college so long. They did not understand the difference between undergraduate and graduate school. Her parents were very supportive if she needed assistance with items such as helping moving her belongs to different schools. Her family was supportive in many ways and others filled in the gaps that her parents could not help. Ryan states her support network like “undergraduate professors, fellow graduate students, and determination” helped her achieve her degrees.

Marie

Marie grew up in Shady Spring, West Virginia with both parents until she was five. They then moved to Cool Ridge, West Virginia. Cool Ridge is a small rural area with many churches, a gas station, a few salons and one general store. Marie was an only child. Marie’s family influenced her education decisions. She had strong role models who encouraged her continuously. Marie had a father who encouraged her independent thinking. He pushed her to think outside the box because of his experience as a Green Beret. He had traveled, attended the JKF School of Special Warfare, and experienced more than most of the people who influenced Marie’s life.

Marie's paternal grandmother had been a great role model by leaving a place she had always known to pursue a nursing career after losing her husband and with two sons involved in war. She exposed her granddaughter to women's rights. Marie's grandmother completed her GED and attended nursing school at the age of forty-eight.

Marie's father also stood for what he believed. He went on strike in the coal mines when it hurt his family because to him "money meant nothing when you have to sell out what you believe in". Marie's mother was a role model by graduating from high school and working for thirteen years before marrying. She showed Marie that it was acceptable to go against the Appalachian value of marrying young. Her parents held the typical coal miner and homemaker occupations of West Virginia and the Appalachian Culture, but they encouraged their daughter to learn and value education.

Teachers that Marie had did not challenge her like she thought they should throughout grade school. Once she entered Concord College she was challenged. A male professor once told Marie that she was "one of the very few female students he had ever truly respected because she relied on her brains and not her blonde hair to do well in his class." To Marie "The best teachers had a sense of humor and went out of their way to teach them to be good citizens, not just good students". Marie commented "once in college, the professors and friends encouraged me to go to graduate school and get my Ph. D".

The McNair program gave Marie the opportunity to be exposed to graduate work and careers without spending the time to find that it was not for her. Marie sees success as "caring for other people and standing up for what is important- your value system or whatever you believe in." Marie's father encouraged her to pursue a graduate degree when most of her friend's parents were lucky to mention college to their children.

Marie expanded her support network by joining a sorority her second year at Concord College. Marie stated “I have a wonderful network of smart, professional, educated young women that I can call on anytime for support. And for a female growing up in Appalachia where I knew few professionals of any sort, these connections were absolutely invaluable.” She goes on to say “if I had not joined the sorority, then I would have lost out on some great female role models.”

Marie stated “I think the main motivation was myself, I am very ambitious, confident, and a self motivated person”. The main reason Marie went to graduate school and to get her Ph.D. was her father, college professors, the self motivation, ambitions, and confidence she found within herself. When asked about her definition of success Marie commented “real success is caring for other people and standing up for what’s important”. “In other words, when I die no one will care about how many times I’ve taught U.S. History or how many articles I’ve published. The only lasting things that matter are your family and what you do for God.”

Denise

Denise grew up in Princeton, West Virginia as an only child with both parents until they divorced when she was sixteen. Her parents were blue collar laborers. Her mother worked at a lumber factory, then cleaned houses. She then worked for Council on Aging. Denise’s father worked for a construction company. He is now a custodian.

Denise’s family did not have money to send her to college. They lived comfortably, but did not have extra money to spend on unnecessary items. Her mother was a big influence in her education life and never gave up hope that Denise would attend college. Denise had teachers that believed in her enough to suggest higher learning opportunities. Denise had to search on her own for information on colleges and how to apply. She had no help from school guidance counselors.

Denise takes pride in being the first member of her family to graduate from college not to mention obtaining her Master's Degree.

The teachers and professors at Marshall University who encouraged Denise were kind and sympathetic. When Denise went to Marshall she found her first female role model that had attended college. This professor encouraged Denise continually. Denise found good mentors if they showed an interest in her academically like if they mentioned an upcoming scholarship or introduced her to the McNair Program. McNair help Denise by introducing her to graduate schools and the types of requirements such as oral presentations.

Her extended family thinks she has been in school too long and cannot understand why she continues to attend. They think it is time for her to seek employment.

Watching family work hard labored jobs and her mother struggle after the divorce gave Denise an impulse to get a better education. She was encouraged by her mother, a Master's degree professor, and her own ambitions to succeed, and get her Ph.D. Success to Denise is whatever makes her happy.

Perseverance and stubbornness helped her to achieve her degrees. Personal experiences change who we are sickness, family sickness, or death increase stress and how we deal with people and events in our lives. Denise's PhD advisor turned on her when she got sick and her mother got sick and she needed some time off to recover. A professor's attitude changed Denise's personal drive to succeed. A mentor's professionalism, attitude, and demeanor can help or hinder a student's life. Denise when asked what had pushed her to succeed commented "My mother, my MA thesis director, my fear of failure, my drive to succeed, and my desire not to end up in a job with no intellectual payoff".

## Conclusion

Role models were important to all three of the women I interviewed. Denise had a mother and thesis director who were both very encouraging. Most of Denise's high school teachers were women, but in college men took over which agrees with the literature in that more men have professional degrees than women. Marie's parents and grandmother were all supportive of her learning and experiences in life. Ryan's teachers and professor were role models to her.

One of the women I interview, Ryan, stated that "high school was even a ways from home" so once again inaccessibility made education harder. Marie states that schools were in bad condition and it gave the impression that education was not as important as it should be. I know money is not abundant in West Virginia, but if we don't spend it on the education and betterment (building business) in our area then how can we be upset when our cities are turned into ghost towns and our children are moving away to obtain employment? Marie comments that the "sports program got all the money". She was in band and had worn out uniforms and they did not get the monetary support like the sports programs received. In the schools that Marie attended there were all white students and teachers so this was used to unite the students into one way of thinking. They were not exposed to other cultures or ways of thinking.

Ryan is close to her family and that "has influenced my desire to attend schools and work close by" this is a way that Appalachian values have proven that they affected Ryan's decisions. "Values of not being in debt, not wasting money on things that were not needed" were also taught when Ryan was young. Ryan's parents owned their own business so Ryan worked a job or had working experience from a young age. Ryan's big factors in her decision to attend Concord College were that she "wanted to stay close to home and needed somewhere that wasn't too expensive". Ryan states "she was fairly clueless going into her undergraduate degree". I felt this



same way and everyone assumed that you knew everything about getting an undergraduate degree, but to someone who has never experienced it or had a family member to talk to, it made the experience very difficult. The values Marie's parents bestowed upon her life were attending church, honesty, hard work, tolerance, and integrity. These are values that I have come in contact with in Appalachia, but with little exposure to other cultures tolerance is one value hard to teach. Ryan's beliefs were similar to those expressed in the literature. In Appalachia men want women in the house. Wives surely can not have brains, an education, or work. The husbands feel threatened by being out smarted by women because men are to be the bread winners and ahead of the household this is due to such a masculine society in Appalachia.

Gender bias was something that Ryan stated "there is definitely a bias towards women in academia". Marie stated that women are discriminated based on appearance such as blonde hair, blue eyes, clothes and bra size. Men are not judged by underwear size when they go for a job interview then why does this happen to women? Many people feel girls go to school and college to find a husband and nothing more.

Ryan states "female teachers were a real inspiration because it was good to see females in that position" proving it to be possible. In obtaining her graduate and Doctor of Philosophy degree Ryan states she finds herself wanting to "focus more on her personal life" since it has been on hold for some time. Denise stated that a female professor got highly upset when she had to leave school to take care of her sick mother and another student had a baby. This to Denise was a direct gender bias towards the students and it was not just the males throwing the punches.

Success is defined by all three of the girls differently. Ryan thinks being able to support yourself and family is important, but that happiness is success, Marie thinks "success is caring

for others and standing up for what is important”. Denise thinks success is what you do that makes you happy.

The catalysts that help Appalachian women to achieve their professional degrees include positive role models like Marie’s grandmother who was an independent thinker and went back to get her GED and became a nurse. Another example is Marie’s mother who did not marry young so Marie had another way of approaching life. Marie’s father also had experience outside of West Virginia through being a Green Beret, and he taught Marie about U.S. Government and how to think independently. Marie’s father was also a leader in the UMWA as he was a West Virginia Coal Miner. Ryan’s drive to be a professor helped her succeed along with role models and good mentors. Denise stated “her perseverance, support, hard work, and faith in herself helped her achieve her degrees”.

#### Summary

From this study, the Appalachian females’ main catalysts to obtaining a professional degree include positive role models, good support networks, self motivation and determination.

Women who are self driven, have to work for everything, and study to get good grades are the ones that are more likely to obtain Ph.D.s and overcome the obstacles that exist. Women have to work harder; therefore those women are usually motivated by success and at times have been judged due to male and female injustices.

#### Further Research

In concluding my project a few questions came up that sparked my interest. I feel these could be investigated further to form an answer. Are most first generation college students in the 21<sup>st</sup> century female? Is it due to job availability for females that they are now seeing the need to attend college? Does maternity leave make women less popular for hire? Why were the first two

McNair Scholars at Concord to achieve their professional degrees females? Why were the three women I interview not math and science majors? Was it because they are females and math and science are still viewed as a man's subject? These and other questions should be further explored to shed light on this subject and eradicate future injustices on females in Appalachia.

## Appendix A

### Transcribed Interviews

Interview A

Pseudonym \_\_\_ Marie \_\_\_\_\_

Any elaboration would be helpful to the study.

1A. Can you tell me about where you were born and raised?

I was born in Raleigh, County in Beckley WV. Until I was five, I lived with both parents in a small neighborhood outside of Beckley, called Shady Spring. Then I moved with both parents to another area outside of Beckley that adjoins Shady, called Cool Ridge. It is a very rural area and most people in the community either work in Beckley or are retired. There are hardly any businesses in Cool Ridge, except for a gas station, a small convenience store, and an occasional hair salon that is locally run and owned. There are quite a few churches, however, and a nearby elementary school.

2A. Tell me about the schools you attend from Kindergarten thru college and their locations?

I first attended Ghent Elementary school for grades K-6. Until I was in 2<sup>nd</sup> grade, our classrooms were old, metal trailers that were cold, drafty, leaky, and at times mouse-ridden. Of course, when you are 6 or 7 you think that having mice in the trash can by the chalkboard is funny. I remember that we had very few games and learning materials, but our teachers worked incredibly hard to make up for the deficiencies. Finally, when I entered third grade our school got a new building and more supplies.

My elementary school was very small, at the time it was the second smallest in the county. During my fifth grade year my class was divided into about 15 fifth graders and 6 sixth graders. It was great. The sixth graders would sometimes help us and they never treated us any different. That year was one of the best ever. I recently heard some parents worriedly talking about this "split grade" system, but I think that it worked great.

As for the work in elementary school, by the time I entered fifth grade I was finishing assignments faster than my teacher could give them to me. (I had the same teacher for both fifth and sixth grade.) She would often give me additional "creative" assignments to keep me busy, like creative writing, art projects, or letting me read books. I also remember that once every week we would be given a list of spelling words that we had to alphabetize and look up in the dictionary. After a few weeks, I was handing in my list well before the other students. I had finished first because I hadn't looked the words up in the dictionary. Instead, I just wrote down what I thought they meant. Because I got them all correct without a dictionary, my teacher allowed me to quit looking them up. After that, I would alphabetize them, define them in my own words, and then I would get an advanced spelling list. This extra work is probably why I won the Spelling Bee every year. At the end of sixth grade, we had an awards day and I was the Valedictorian. On the last day of school we took a trip to the state capitol at Charleston. While other schools went to amusement parks and Washington, DC, our little school was so poor that we were lucky just to go to Charleston for the day. Many of the kids who went to my elementary school were poor and my own family was by no means wealthy. In fact, I can't remember even one classmate whose family was wealthy when I was in elementary school. Sure, some were comfortable, but in southern West Virginia, and especially in my rural community, no one was anywhere near being rich.

I entered junior high at Shady Spring Junior High School next. It was grades 7-9. It was a whole new world, because the other 3 elementary schools that fed into it had students who

were financially better off than us Ghent kids, for the most part. The school itself had been condemned several times, but rumors had it that the Board of Education had pleaded with the state not to close it because the county could not afford a new one. (In fact, a new one wasn't built until about three years ago.) I remember that you could spill water on the third floor, and by the time you ran down to the art room in the basement you could take a cup and catch it as it trickled down. Of course, my own grandfather had graduated from high school there in the 1920s, so it was old by the time I got there in the late 1980s and early 1990s. The steps from one floor to the next were worn down at least a few inches in the middle from all the traffic. It was so crowded! It was all you could do not to get trampled going from class to class. And there were only restrooms on the first and third floors.

Some of the teachers there were abysmal. I doubted if they had high school degrees. I remembered thinking that it was like a giant daycare and that the point seemed to be to keep us corralled until the buses came to take us home again at the end of the day. But on the other hand there were a few star teachers. My algebra teacher was fantastic. He was smart, fun, and could teach you three chapters of algebra before you realized you were actually doing work.

I was elected to student council every year, not that it did anything. And I joined the marching band. In retrospect, I think that the band experience was similar to the Hitler Youth program. We were 'strongly encouraged' not to engage in any other activities and virtually had to swear allegiance to the Master, I mean, band director. In the beginning it was fun for me because I was always first chair and I won first chair at the county-wide competition. And then I joined the flag corps and that was not fun. Practice for band and flag corps consumed all of my extra time. I still got good grades, but I wanted to quit because I wanted to participate in other activities. So, at the end of 9<sup>th</sup> grade, I quit playing the saxophone and I quit the flag corps. (Actually, I had to quit the flag corps, because at Shady they had this insidious little plot in which you couldn't be a flag corps member if you didn't play an instrument in the band as well.) While in junior high, though, I remember that we would go to band competitions and *always* win first place in *everything*. But who wouldn't come in first when you had sold your soul to the band and spent every waking moment absorbed in it? We must have really been good, though, because our uniforms were really old and crummy, and we didn't have the best equipment. We weren't the poorest school, but sports programs got all the money.

For grades 10-12 I went to Shady High School. Socially, it was great. I was on student council every year, in the Honor Society, and involved in a ton of other clubs. It seemed like I was President or Vice President of everything. I had a good time. As for learning anything, well, let's just say that it's a good thing I was encouraged to be a self-directed learner. I think I learned more from my dad, documentaries, and books that I read on my own than I did in high school. Academically, it was a joke. I remember in 12<sup>th</sup> grade Advanced Placement (AP) English that we were assigned *Wuthering Heights* in October. No problem, I thought, I read this in 7<sup>th</sup> grade. Well, it turned out that I didn't have to re-read it all. Our teacher, *our advanced placement teacher*, read it *to us*, a little every day, from October through February! I was so insulted. The year before in 11<sup>th</sup> grade AP English we read a novel a week. And we wrote a paper every week. That 11<sup>th</sup> grade AP class was the only useful academic thing I got from high school.

I remember going to college and telling people that I had graduated from Shady High School. They all thought I was rich. I thought they were crazy. But I started to think; maybe we were pretty well off. I mean, so many people had their own cars to drive to school that the school had to expand the parking lot every year. And they weren't clunkers, either. Even I had a new car to drive to school. When I first got to college, I thought about high school a lot. Every student at Shady had been white. No Asians, no Latinos, no African Americans. There was one Catholic girl and she was the most "exotic" person there, made so only by her Catholicism. (No one persecuted her for it though, she was quite popular.) And there were very few visibly poor kids. And there were certainly no openly gay or lesbian students. We were all the same. It was as if someone had taken a cookie cutter and cut out the same little person from a loaf of white

bread a hundred times. It was like that movie "Pleasantville." We made up for the lack of diversity with an abundance of conformity, though. Independent thought of any kind was not tolerated. Any questioning, not defiance mind you, but mere questioning of authority would not be tolerated. I found out the hard way.

There were two instances that introduced me to the authoritarianism that is public school, or at least my public school. First, the school board had installed televisions into every classroom in the school. They wanted them to be used to get students acquainted with current events. Teachers were supposed to let us watch anything of national or international significance – space shuttle launches, elections, etc. Well, when Bill Clinton was inaugurated in January 1993, every teacher turned on their TV's to let students watch the event. (Looking back, I'm almost certain that this is the only inauguration that many of those students had seen or will ever see.) My Calculus teacher, however, refused to let us watch the historic event because she did not like President-elect Clinton. I was furious! How dare she impose her political views on us and rob us of watching an important national event. This had been the first election I had ever been interested in (I was only 16) and now I wouldn't get to see the culminating event. I was especially angry on the one hand that my dad had worked so hard with me in following the election and teaching me about US government, and now I wouldn't get to finish our "study." (He later said that if he would have known I wouldn't be allowed to watch it he would have let me stay home.) On the other hand, I was outraged that that my fellow classmates were so influenced by our teacher. Let me explain. Teachers are viewed as authority figures with, supposedly, superior amounts of knowledge. At least that's how my classmates viewed all of their teachers, with a boundless optimism in their teachers' abilities. So when Ms. Calculus used class time to "bash" Clinton, I think that many of my classmates became Republicans on the spot! Surely, they thought (and some of them later told me), that because she was a teacher she would know best about things like politics! And when you go to school where you are brainwashed into thinking that your teachers are never wrong and that they are some sort of demigods, then of course students blindly accepted her views, because they were not taught to think for themselves. We were only taught to regurgitate the "accepted" viewpoints. It was like growing up during the McCarthy era.

The other event was perhaps even worse. As part of student council, I was on the graduation committee. The student body had voted that they wanted to exit the graduation ceremony to the then-popular Garth Brooks song "We Shall Be Free," rather than the traditional "pomp and circumstance" arrangement. Our school administration flatly rejected the idea. And if they needed any support, they got it when members of the community made threatening phone calls to the school stating that if we played that song, something "bad" would happen. (Bomb threats were made among other things.) The problem was this: first, Brook's sister was a lesbian, and he wrote the song in support of her lifestyle. So we couldn't play the song because the singer had a gay sister. The other problem was the actual message of the song – that someday discrimination of all sorts will stop and we'll have a better world. When you live in a community of all white, middle class people, who actually benefit from discrimination, well, I guess I shouldn't have been surprised. I cemented the conclusion that I had come to a year earlier – public school stinks. I thank the Lord that I had one good teacher. He was an Advanced Placement English teacher who taught us not what to think, but how to think. In my book, he is a hero.

I graduated fifth in my class of about 150 students. A few months before graduation I overheard the school librarian and the yearbook advisor (I was on the staff) saying that I would never make it through the first six weeks of college because I was an independent thinker. Now that I have my Ph.D., I think I will send them my college transcripts. I earned a perfect 4.0 GPA at Concord College, where I got my bachelor's degree in history with psychology and English minors, and I had a perfect 4.0 gpa at Marshall University where I earned my master's degree

and I had a perfect 4.0 gpa at the University of Tennessee where I just earned my PhD. I guess that 12 years of straight A's proves that being an independent thinker is not so bad after all.

Concord College was fantastic. I not only received a good liberal arts education, but I also learned a lot about myself, the world, and my place in it. I had outstanding professors and terrific friends. I pledged a sorority my second year there and it is one of the best decisions I have ever made. I have a wonderful network of smart, professional, educated young women that I can call on anytime for support. And for a female growing up in Appalachia, where I know few professionals of any sort, these connections are absolutely invaluable. I treasure them for both personal and professional reasons. If I had not joined the sorority, then I would have lost out on some great female role models. Out of my immediate circle of sisters, we ended up with 2 PhD's, a dentist, a doctor, and many more young women who have earned advanced degrees. I'm extra proud that we are all West Virginia women who have obtained good educations and are chipping away at both stereotypes of women and Appalachians.

3A. Can you tell me about the members in your family?

My family has always been incredibly small, just my mom, dad, and me. I never knew either grandfather, but I knew both grandmothers. My Mom grew up on a farm in Cool Ridge and her family was very poor. Her father had his leg crushed in the coal mines when she was three, so her family had little money. She graduated high school and worked for 13 years, then got married and became a homemaker.

My dad graduated high school and entered the military. He was a highly decorated Vietnam Veteran. He was in the 101<sup>st</sup> Airborne Division as a paratrooper and then he attended the JFK School of Special Warfare and became a Green Beret, the most elite unit in the Army. (They are the Army's equivalent of the Navy's Seals.) He earned 2 Purple Hearts, a Silver Star and a Bronze Star. (The latter two are the nation's second and third highest honors any soldier can get.) He also earned many more honors and medals in the military. In short, he was a war hero. If his unit would not have been such an elite outfit, the Silver Star would have been a Congressional Medal of Honor. But more is expected from a Green Beret than a regular soldier. He served two combat tours in Vietnam and left the military in the early 1970s. Two books have been written and published about his experiences in Vietnam as a Green Beret.

He finally became a coal miner. Every male in my family has been a coal miner – my great grandfather, both of my grandfathers, my cousin, my uncle, and my father. My father and grandfather were very active in the union. In fact, my grandfather risked his life to start the UMWA in the 1910s and 1920s. My father worked as a coal miner until his death in 1994.

My grandmother on my father's side is the only other family member that I knew well. She passed away this past April at the age of 92. Her husband (the union coal miner) passed away when she was only 48. So, at the age of 48, she went back to school to get her GED, went to nursing school, and worked as a nurse for 20 years. In her first years as a nurse, she had both sons in the Vietnam War. She moved to Beckley when she became a nurse, leaving the rural area she grew up in for the "city." She is the strongest female role model I ever had, and even though she probably never thought of herself as a feminist, she taught me everything I know about feminism and women's rights.

4A. Tell me about the jobs and the money that was available to the family members in your home?

My mother was a homemaker so she only worked inside the home. My father worked as a coal miner. He made good money for someone with no college degree, BUT the good years were often canceled out by the lean ones in which he was on strike and no money was coming in. I used to wonder why he just didn't go get another job. But now I know it wasn't about the money. He was committed to the idea of the union and was convinced of its importance. He

went on strike, even when it hurt us financially, because he was standing up for his beliefs. What I learned from watching him do this is worth more than all the money in the world now. All of the money he would have made as a “scab” would have been earned at the expense of oppressing his fellow coal miners who would have to work in unsafe and exploitive conditions. And all that money he could have made could have never bought things like integrity, character, and determination – qualities that he had that he taught me to have, too. In short, I learned real fast that money means nothing when you sell out what you believe in. I am thankful now for those years when he was on strike and we didn’t have any money.

After he died, my mom and I had a really hard time. She had had three strokes, so working was out of the question for her. We almost lost our house and we were only weeks away from being homeless. That’s why today I get so angry with people who think that all poor people are just lazy bums who waste their money. My mom and dad worked like crazy and we were still just getting by sometimes. And believe me, we didn’t waste money. Poverty is not always a failure of the people; it’s a failure of the system. I guess the aftermath of my father’s death really influenced the way I feel about poverty.

When my dad was working, at least we had good healthcare. When he died we had nothing. So I had to watch helplessly as my mom had three strokes, because we couldn’t afford to go to the hospital or even the local doctor. I wish that everyone in America that opposed universal healthcare could feel what I felt watching my mom lay in bed, almost dying, because we had no money.

5A. Tell me about your siblings.

I don’t have any – thank goodness!

6A. What influences or experiences affected which colleges you attended?

My parents didn’t have the money to send me too far away, so I chose Concord. I’m grateful I went there, though.

As for my graduate education, I went to Marshall because I received a graduate assistantship that paid for my tuition. I absolutely loved Marshall, though. Some of the best people I’ve met worked in the history department there.

I applied to three PhD programs and was accepted to all three. Each of them offered me a tuition waiver and a stipend. So I had the luxury of just choosing the one I liked best. All through college, no matter where I attended, though, I knew I had to get the best grades so that I could get scholarships, etc. I couldn’t afford to pay for school, so I *had* either had to get good grades or go home.

7A. What were important or unique family traditions, values, or experiences to you?

My family always attended church and I am incredibly grateful that I was brought up in church. I am also thankful that although I grew up in a very spiritual home, it did not make me or my parents close-minded. My father always encouraged me to refrain from using religion as a way to oppress others. I think that the most important thing that my parents have done for me is to take me to church and to teach me values like honesty, hard work, tolerance, and integrity. I know that my liberal political and social beliefs are a direct outgrowth of the Christian teaching I received growing up.

8A. Anything you would like to add please feel free to do so.

I don’t know if more personal information is necessary, but I am married, to a man I met in college.

Pseudonym/Name \_\_\_\_\_ Marie \_\_\_\_\_



## Interview B

1B. Can you tell me about the teachers you had? In school what teachers inspired you?

Most of my teachers seemed to have an adequate grasp of the material that they were teaching us, but only one really stands out in public school – my 11<sup>th</sup> grade Advanced Placement English teacher. He was great because he taught us *how* to think, not *what* to think. The writing and critical thinking skills I learned from him have been incredibly useful. He also let us read books that had been “banned” from public school, like *Catcher in the Rye*. I learned more from this act of rebellion than I did from the book. The lesson? Don’t blindly follow and accept what others try to tell you – think, and in this case read, for yourself!

2B. What characteristics did the teachers have that attracted you?

The best teachers had a sense of humor and went out of their way to teach us to be good citizens, not just good students.

3B Did any family members encourage your education in anyway? Can you tell me about your family’s view of college?

My father always encouraged me to go to college. I think that the primary reason that the rest of my family thought that college was a good idea came down to economics. They thought it would help me get a good job. But a good liberal arts education does so much more than that – it prepares you to be a life-long learner and to develop skills that are useful no matter what job you get. My father really encouraged me not only to go to college, but also to pursue a graduate degree of some kind.

4B. Who do you feel communicated with you best – male or female teachers?

I have had some great male and female teachers in college. I think that the best female teachers were the ones who were committed feminists that had a real interest in helping me develop my full potential as a woman. They understood the special challenges that women face in academia and they helped me to prepare for them. They have been a great support network.

As for the male teachers, they were a mixed bag. I actually had one of my graduate professors say that I was “one of the very few female students that he had ever truly respected because I relied on my brains and not my blonde hair to do well in his class.”

5B. Tell me about the jobs around where you grew up. Were there many female professionals where you grew up?

In southern West Virginia in the 1980s and early 1990s there weren’t any jobs for anyone, male or female. There weren’t even a lot of union coal mining jobs. So, no, I didn’t really know any female professionals. And of those who were around, they certainly were not visible. There were no women in my community that I could look to as role models. In fact, most people looked down on women who worked, unless they held jobs that were considered traditional female jobs, like nurses or secretaries. And those women still weren’t held up as paragons of achievement. I personally know people from my old neighborhood that think that women should remain in the home as homemakers

and mothers. (Of course I have my own theory about these men. Because of the poor economic conditions, they are insecure about their masculinity. So they do not want women who are their “equals” much less their superiors. A woman who works, has a career, or heaven forbid a brain and an education, are just too much of a threat to them. So their solution is to keep her in the house!)

I do think that popular culture helped to fill in that gap for me, though. My two favorite TV shows growing up had principal female characters – one was an advertising executive and one was a lawyer. Ironically enough, those two professions were my top two career choices before I decided I wanted to be a professor. In my case, then, pop culture had a good influence. (Of course who knows what pop culture’s influence did to all those girls who watched half-clothed women on MTV all day and grew up playing “house” with their Barbie’s, baking in their Easy-Bake ovens and learning to be good, submissive, consumers. I think, twenty-five years after the fact, I am beginning to understand why my parents would not buy me the elusive Easy Baker gear and Barbie dolls.)

6B. Who or what pushed you to attain your Ph. D. or professional degrees?

I would have to say that my father encouraged me to become a professional. And once in college, the professors and friends I met there encouraged me to go to graduate school and get my PhD. I think that the main motivation was myself, though. I am a very ambitious, confident, and self-motivated person. I knew I could do the work and do it well, if only I could find the financial means to get through graduate school.

In addition, I was always fascinated by books and learning. My father had to teach me how to read before I started kindergarten because I was just driving him crazy wanting him to read to me all the time. He finally said he was either going to have to quit his job to stay home to play library all day, or he would have to just teach me how to read. He taught me to read. And once I learned how to read, my desire for the written word was insatiable. I have always loved words, ideas, and books, so getting a PhD was a natural fit.

7B. What is your definition of success?

Professional success is important. After all, in my case I have invested 12 years of my life in college and in pursuing a PhD. In addition, I believe that I really make a difference in the classroom everyday and through my research. But when it comes down to it, real success is caring for other people and standing up for what’s important – your value system or whatever it is that you believe in. In my case, I care about my job, but the most important thing in life is my husband and serving God. In other words, when I die no one will care about how many times I’ve taught US History or how many articles I’ve published. The only lasting things that matter are your family and what you do for God. No one wants to be remembered only for their curriculum vitae.

8B What are important goals or ideas to you?

Professionally, my goals are to publish my research and to improve my teaching. In addition, I want to challenge students to think about new things in new ways and use the classroom to combat ignorance and discrimination of all kinds. Personally, I want to

be a person that always stands up for what is right and I want to be a good wife, friend, and servant for God.

I am also politically active and passionately committed to issues of social justice. I am a dyed-in-the-wool New Deal liberal. (Meaning that I believe, in the spirit of President Franklin Roosevelt, that the federal government has a responsibility to use its power to care for citizens and to promote social justice, fairness, and equality for all. Contrary to popular belief, this description is the *real* definition of liberalism.) I am really dedicated to my political beliefs because of my firm commitment to my Christian principles. In other words, it is my Christian beliefs that provide the foundation for my political philosophy. Unfortunately, most people are beginning to associate Christianity with conservatism and narrow-mindedness. Thus, I work hard to explain to people that liberals not only can be Christians, but also that they are moral, caring people. I could say a lot more on this issue, but I think I better stop.

9B Anything you would like to add?

Interview C Marie

Q: What do you feel helped you achieve your degrees?

A: I was just really determined; I knew that is what I wanted to do. It was kinda what I was meant to do, I didn't have any other career options I was interested in, I was really determined, and I had a lot of support from my immediate family and from my husband, he was great, he really...you know, financial support, mental support every time, so that and I wasn't pressured by him or by my immediate family to get out of school, so I had a lot of support to do it, and I was really determined to do it.

Q: Think about your dreams from your past, and aspirations, and think about what your goals are for the future and just kind of tell me how they were, and how they changed?

A: I always knew I was gonna go to college, in the beginning because my parents just really wanted me to go to college, especially my dad. In the beginning I wanted to be a lawyer, and we have a close family friend is a attorney, and he loves being an attorney but for some reason he just didn't think that I should be one, so he kinda talked me...kinda got me thinking about something else besides being an attorney, and after I talked him and really realized what his day to day work as a lawyer was, especially from southern West Virginia. I didn't want to do, social security claims or workers comp claims, and I didn't want to do like, divorce or family law. If I would have pursued that I would have wanted to do like, civil liberty and there's not a good \_\_\_\_\_ for that in southern West Virginia, and I really didn't know anybody that did that, so that just became less appealing. At the same time I got involved in the McNair scholars program, and I kinda wondered if I don't want to be an attorney then what do I want to do. Once I got in McNair I knew exactly, I wanted to be a professor. I loved school and I thought well...this is the way to kinda never leave school and to continue doing this kinda work, because there just wasn't anything else out there that appealed to me at all. I just kinda had my mind on being a professor after that.

Q: Well you mentioned McNair, so how do you think McNair influenced you and helped you get to where you are today?

A: It was kinda a trial run. I knew it was a chance to experiment without actually getting a degree and getting out in the world and saying uh-oh, I've spent 4 to 10 years in college and now I'm finally on the job and I hate this. I had the leisure of having a trial run kinda being a professor and doing the work as graduate school and doing presentations, public speaking. Once I did my project and proved that I did, I knew I wanted to be a professor because it was kinda like a trial run being a professor while being an undergrad, so it was great, it was a great experience without an investment of my time and money. I actually went to school with quite a few people that go to school for x number of years and get out and actually get the job they thought they wanted only to find out, oh no this is not what I wanted to do but by that time your already in debt and already committed to that career track and can't turn back. McNair was great, it was a test run at grad school and test run at what professors actually do, I kinda got to see behind the scene early on what was going on, so it really helped a lot.

Q: So you're wanting to be professors probably what bugged (?) you to get your PHD?

A: Yes, absolutely.

Q: So what do you think helped you be who you are today?

A: I guess, I always loved books, I loved to read and I always loved school, so I think part of it is just natural inherent inclinations. My parents really, my dad really encouraged me to get in school. I never had the attitude that well your a girl and you should get a girl job like a nurse or secretary, traditional female job, so I always felt like I could really honestly do anything I wanted to do, no matter. I never saw it in terms of; well I'm a female, so I'll kinda steer towards this path. I just \_\_\_\_\_ these are the jobs, I want to go to college and I can do anything I want to do. I just had, I was encouraged to have good role models, like women that had jobs, successful women had careers, and I thought Hillary Clinton was the greatest person ever. There was finally a First Lady that didn't stay in the White House and bake cookies all day, she had a brain and she wasn't afraid to use it. My dad was really good about saying, you need to have good female role models like, Hillary Clinton or Eleanor Roosevelt, just because you're a girl doesn't mean that you have to be like a nurse or something. I think early on, you know, having like really strong examples in my family too. My Grandma always worked and was really, you know I don't think she knew what feminist meant but she definitely was one. (Ashley: Yea you mentioned that in the first interview and I thought that was just amazing). Yea, yea it's really cool. I just have really good role models and I loved school and I'm a Perfectionist, just naturally type a personality, I want to be the best at everything. I was never athletic, so but I was always pretty good at school and that's what I was good at and stick with what you know and do what your good at.

Q: Do any school experiences, good or bad stand out in your mind?

A: Yea definitely, I think I said in my first interview, High School was pretty much a joke. It was very sexist looking back at it, very racist there were no African Americans at my school, no Asian kids and that wasn't I guess the community's fault but our teachers never encouraged us to have any sense of the world, it was just little tiny southern West Virginia town to form or you'll be a freak. They never encouraged us to think about things that really matter like, politics, what your really gonna do with your life like, community service to give something back to the community or it's kinda giving the message that well, girls go to school, go to college to find a husband. I mean thank God for my family; if school would have been my only influence then

God only knows where I would have ended up because the messages were terrible. College was great, I loved Concord, it's a wonderful school and I loved Marshall and just finished at UT. It was great to get somewhere where you did stuff I felt really mattered and that what went on in the classroom academically really mattered, it wasn't about sports or all these extra circular activities. You were there to get an education and if you were serious about that you were rewarded for it, so in college was fabulous after High School.

Q: So you went to Concord as an Undergrad and Marshall as a Graduate and then where did you go to get your PHD?

A: University of Tennessee at Knoxville. (Ashley: Because I don't think I have that written down anywhere because I'm trying to talk about the Appalachian women get educated mostly in West Virginia but usually to get your PHD you have to leave). Yea I got accepted at West Virginia University and they offered me \_\_\_\_\_ and \_\_\_\_\_ but the history of \_\_\_\_\_ at UT was more appealing to me nothing against West Virginia but UT offered more of what I wanted, broader. They had a world history concentration that WVU didn't have, so it really came down to a matter of economics, I felt I would be more marginable once I got a degree from UT than I would be at WVU. (Ashley: I understand that. Sometimes you have to go where you fit in the best and where their going to give you the best kinda money). Yea well actually University Of Kentucky offered me a lot more money than UT but I turned them down because again I didn't want to come out being really specialized, I wanted something a little more generalized because if you're too specialized you'll never get a job. I was really worried about at least in my field, now in other fields it's not like that. I always loved UT and I love the area and it worked out great, I had a really good experience there.

Q: So did you have any particular professor, man or women that really influenced your life? Maybe even an advisor that really.

A: When I was at Marshall the people there were fantastic. I had...just everyone there were wonderful even professors that I didn't necessary have for class were still really supportive and interested in what I was doing and was willing to help. They were great. At UT my advisor, I had a female advisor, she was great. It was great to....there was only one female professor at Marshall well there was one active female professor but at UT there were more and that was really nice.

Q: If you could do or say one thing to an Appalachian female wanting to be successful, wanting to get a higher education. What would it be?

A: Ha-ha. Don't listen to anybody. If that is what you want to do, do it because specially an Appalachia. First of all the deck is stacked against you. People automatically think you're stupid because you are women. Even in places that should know better like University's there is always that thought, so I would just say be warned about that and also be warned if you have 2 strikes against already, your a women and your from West Virginia. I mean even though there are hundreds of thousands really intelligent successful people from West Virginia, it's not the image that you carry with you. I would say know that but totally ignore it, don't listen to all the people that say that your going to be in debt or when you get out your not gonna make a lot of money, do what you want to do and forget everybody else because your the one who has to live with it. Especially coming from Appalachia I feel like women aren't supported, I actually feel your looked down on if you go and in my case a PHD, people think well you think your too good

to live in West Virginia, you left here, your a women you know why in the world would you want to be a professor, why can't you just get married, why can't you just have kids, I heard all of that. Ignore it and do what you want to do. Work really hard, I mean the harder you work. I was so blessed; I never had to pay a dime of tuition because I worked really hard. Just make your mind up to do it and do it, treat it like a job and anybody can do it.

Q: Saying all that, what motivates you?

A: Just, I'm really self motivated. (Ashley: Ok I can relate). I guess a really proud person and probably over confident. I don't want to say egotistical but...I had a lot of support too, my husband was really great never got tired of me being in school all the time and never got tired of me having to do homework all the time, you know, never have any free weekends, he was really, really great, so if your going to get married before you finish make sure you marry someone like him that's not, you know, makes sure you marry somebody supportive of women. One that doesn't expect you to go to school and cook and clean and do every thing else to. I'm just naturally a motivated, really probably over ambitious person.

Q: Who or what has been the most significant event in your life?

A: Oh gosh, I don't know. There has just been so many, like I would change anything because I probably would be here right now, but gosh, I just don't know. It would have to be going to college to begin with I guess and getting married, that's probably the best thing I ever did. Not that I'm promoting marriage it's just I happen to marry somebody that really great. I don't necessarily think marriage is a good thing all the time. (Ashley: I understand that too. There are a lot of things that go with that to but it's like you said your marriage has been supportive and let you be the women you want to be with or without that). Yea and I have really great friends too and I think I said that in the first interview, I mean it's been really great to have, you know, like I joined a sorority and that's all my friends because this is the people I was around but I mean out of just small little group we have two PhD's a ton a Masters degree's and dentist, a doctor and it's nice to have that kinda, you know, at least I can always pickup the phone and then there's somebody that knows what I'm talking about, where as the people I went to High School with, their in a whole different world, they have no idea, you know, about the challenges or what it like to go to school and go get a Graduate Degree or anything so at least I have some friends that we can \_\_\_\_\_ together and we understand each other. (Ashley: Ok, so support is definitely important, has been very helpful). Yea, I mean larger schools sororities aren't like that because I know, I've been there too. At Concord it's great because that really is great support group that sticks with you and we all went on a really cared about educations and we all turned out just about the same or had the same experiences.

Q: Is there anything you would like to add to anything that I've asked you or anything you would like to add to the study.

A: I don't think so. I don't know if I am like the...I guess in some ways I am typical person from Appalachia but then again I'm not because my dad really raised me to, you know, kinda look around and see this should not be your whole world and you should travel and leave here one day. Like one thing I told you about my High School experience, I knew that that was ridicules, I mean thank goodness for my parents influence because if...I grew up in Appalachia but I don't think I have an Appalachia outlook on things, especially for a girl. (Ashley: But that's good because I need a broad range, so it definitely helps everything). Yea.

## Interview A

Pseudonym: Ryan Griffith

Any elaboration would be helpful to the study.

1A. Can you tell me about where you were born and raised?

I was born and raised in Alderson, WV. This is a very rural place with very little commerce, you have to drive about an hour to go to the mall, see a movie, basically do anything other than outdoorsy stuff. This was nice because I liked the seclusion and the beauty of the area, but also annoying b/c of having to drive so far to get anywhere. I still appreciate rural areas though, I think it gives you more peace of mind, allows you to relax more.

2A. Tell me about the schools you attend from Kindergarten thru college and their locations?

Up through college I attended schools in the area. High school was a ways from home, so socializing outside of school pretty much wasn't an option b/c I never had transportation. Less socializing definitely left more time for school work, so I'm sure that contributed to making good grades throughout. I commuted to college and so that was actually a similar experience.

3A. Can you tell me about the members in your family?

My mother, father, and brother were it for a while, and then when I was a teenager my little sister came along. Her presence really kept me closer to home, wanting to be there for her and to see her grow up. I'm close with my family and that has influenced my desire to attend schools and work close by. This can be really tough sometimes b/c it cuts down your options drastically, but I feel like I know what is really important to me, and that's to be as close to home as I can.

4A. Tell me about the jobs and the money that was available to the family members in your home?

We never really went without anything that we needed, but we definitely didn't have a lot of money. I think this made me respect money as something that you had to work hard for, and something to not take for granted. We had values of not being in debt and not wasting money on things that you didn't really need. This view has helped me stay away from credit cards and blissfully out of debt (scholarships and graduate assistantships helped a lot too). Mom and dad ended up owning and operating their own business, where I had to work as well. So I was pretty sick of working a job from a young age, something that probably led to me being ok with being in school for so long, I was in no rush to get a job b/c I felt like I'd already been working forever.

5A. Tell me about your siblings.

My brother is master of all things mechanical but, never had formal schooling. He could build a house but would never read a book. So were different in terms of types of knowledge, but as far as practical intelligence goes I think he has me beat. My little sister is in middle school. When she was young I really tried to get her interested in reading and learning, she struggled when she was younger b/c her reading skills developed slowly. But now is doing great, she is actually giving me books to read, which blows me away. We read books in a series together, I'll buy one and read it and give it to her to read and then we'll talk about them.

6A. What influences or experiences affected which colleges you attended?

Wanting to stay close to home and needed to go somewhere that wasn't too expensive were big factors. I was fortunate to have great professors, and I sought out the opportunities to gain the experience outside of class that I thought I needed. I tried to have a personal relationship with my professors, so that they could let me know if my work was ok/if I was on the right track, and I tried to get advice from them about my future.

7A. What were important or unique family traditions, values, or experiences to you?

We're a close family so there was pressure to stay close. This could have been a big problem if I didn't tell myself that being gone for a while, for school, wouldn't be intolerable. I just tried to visit as much as I could (grad school was about 3 ½ hrs away). My family never really put pressure on me to do anything academically, but they always helped me when I needed it (moving, cars, getting furniture). I think we all feel like there are much more important things in life than work, and I think that led to me being a little less ambitious than I might have been otherwise, I wanted to be a professor, but I never wanted to be a well known scholar, or strive to be at the top of my field—I noticed pretty quick that those people don't have time for much else, and I knew I wanted time for family and fellowship.

8A. Anything you would like to add please feel free to do so.

I think reminding yourself that nothing lasts forever is helpful when planning your future. Sometimes you just have to investigate and figure out what you really want to do, and the best way to do that is to track down people who do it and watch them. Once you figure it out you have to attain whatever degree/training you need. There were times in grad school when I really wanted to quit for a lot of reasons, but I stuck it out b/c I know it wouldn't last forever and I knew it would get me to



the career that I really wanted. At times it was really hard to have to work so much and be so far away from home. But now I have my degree and that is something that I will always have no matter where I end up.

Pseudonym/Name Ryan Griffith

## Interview B

1B. Can you tell me about the teachers you had? In school what teachers inspired you?  
Teachers that inspired me, were one's who seemed to enjoy and appreciate the work that they did, as opposed to just seeming like they were going through the motions. In particular, female teachers that I had were a real inspiration because it was good for me to see females in that position, where they were assertive and used examples from their own lives to help the material make more sense.

2B. What characteristics did the teachers have that attracted you?  
Teachers that seemed to genuinely want their students to do well always made a good impression on me. Also, I appreciated it when a teacher was tough but fair. I liked to feel challenged and a tough teacher made you really feel like you earned your grade as opposed to being handed it. There's a lot more joy in the accomplishment of having to work hard for a good grade.

3B Did any family members encourage your education in anyway? Can you tell me about your families view of college.  
My family was pretty hands off when it came to my education. I pretty much went by the advice of acquaintances and teachers that I sought out.

4B. Who do you feel communicated with you best male or female teachers?  
It really depended on the teacher, not the gender.

5B. Tell me about the jobs around where you grew up. Were there many female professionals where you grew up?  
I grew up in a pretty rural place (not a lot around), but I saw that my mom worked really hard, so I had the impression that males and females contribute.

6B. Who or what pushed you to attain your Ph. D. or professional degrees?  
I was fairly clueless going into my undergraduate degree, so I can't say that I had a good plan for my future at that point. But after interacting with my professors and discussing my options with them, and with getting into the McNair program ☺ I learned about the possibilities and discovered what I wanted to do. I decided that I wanted the life of a professor, based much on seeing their lives, and I knew that I would have to attain a Ph.D. in order to be able to have that profession.

7B. What is your definition of success?  
Happiness. I think that being able to support yourself and your family is important, so money is certainly an aspect of this, but happiness is success and I don't think that there is any one path to

that, it can be different for everyone. But I do think that you have to be independent and strong and hardworking, if you're the type of person that quits, any goal will be hard to attain.

8B What are important goals or ideas to you?

During graduate school I was very focused on finishing successfully, and since then I'm feeling a need to focus more on my personal life. So, though I'm sure a lot of people in my position would be more ambitious, but my personal goals are also very focused on family and my relationships.

9B Anything you would like to add?

Seeking out good advice from people who are experts/doing what you want to do, is by far the best way to discover what you want and what your options are.

Interview C Ryan

Q: You said you have your mom, dad, brother and sister is your brother older?

A: He is.

Q: What do you feel has helped you achieve your degrees?

A: Well, I think kinda going back \_\_\_\_\_

Q: In what way did McNair help you? I know you mentioned research and stuff, is there anything in particular that helped you?

A: Well, with the kinda programs I was interested in, I think that was the biggest part of it. (Ashley: I can see where it has already helped me in some aspects.) That's great, it's a program that a lot of people are familiar with and they know it's a great program.

Q: Part of my study is on women who were educated in West Virginia. Now I know you attended Concord, as an undergrad, now graduate, where did you attend?

A: West Virginia University

Q: Then PHD, was it the same?

A: Yes. The program that I went into was a PHD program. The way that it was structured, you could attend a 2 year \_\_\_\_\_, if you pass those and you're all finished with your Masters \_\_\_\_\_, \_\_\_\_\_ program wasn't like that. It was, you get your Masters, pass your prelims, your \_\_\_\_\_. I liked it, I like the way it was layed out. (Ashley: Well it kinda gives you a milestone too.) Right. I know at Virginia Tech there PHD programs aren't right.

Q: Looking at your goals in your College years, High school. Your dreams and aspirations from the younger time. What are your goals for the future? What they were and how they have changed now?

A: I can pretty much say that I didn't have any goals. (Ashley: Well, that makes me feel better because when I started out in undergrad, I had no idea.) I think that happens a lot. You have to kinda get out there. It did take me a while to kinda figure out oh \_\_\_\_\_.

Q: So is it basically you wanted to be a profession that drives you to get your PHD.

A: Oh certainly, yea 100%. The life of a professor.

Q: Looking at your PHD, what was your family's view of you being in college or the amount of time you spent in college?

A: Well, I guess, this might sound a little strange. They probably thought I was some horrible student who's been an undergrad for like 10 years. I would say no, don't tell them I'm in college, tell them I'm in graduate school, it's very different. My mom would talk to people and they would tell her, that what I'm doing. They didn't have a clear idea of what I was doing. (Ashley: I get the same thing from my parents. I think it's just from a lack of understanding.) Yea, that's the thing, but it doesn't bother me at all.

Q: Were they supportive though?

A: I would say that they were. Like I said at first \_\_\_\_\_ health insurance and stuff like that but nothing major, but that wasn't the case. So they were pretty supportive.

Q: Who do you think and what do you think helped influence who you are today?

A: I think once I did kinda start \_\_\_\_\_.

I thank you for answering all of my questions.

Follow up questions

Puedonym Ryan Griffith

1) Do any school experiences (good or bad) stand out in your mind? Can you describe the situation?

I had bad experiences in terms of being very overwhelmed and burned out during graduate school—those are the times when you just have to push yourself.

Good times were when I was nearing the completion of my masters degree and especially my doctoral degree, it really was worth it. If it wasn't for the struggle, I don't think attaining the goal would have been as sweet.

2) If you could do/say one thing to an Appalachian female wanting to succeed, what would it be?

Seek out good advisors and mentors.

Find those who do what you think you want to do and shadow them, so you can be sure that it is for you.

Build supportive relationships with your fellow students, their help will make a huge difference. Put the time in, there will be times when all you will be doing is eating, sleeping, and working—and that's ok, just keep reminding yourself that all the work will be worth it.

3) What motivates you?

The desire to do well.

The desire for recognition of hard work through attaining my degrees.

Wanting to be a good professor.

4) Who/What has been the most significant event in your life?

Career wise, the most significant events would have to be being accepted into a doctoral program and subsequently graduating.

5) Do you see male and female job differences in the same profession?

There is definitely a bias towards women in academia. I think its less likely that they will be paid as much, less likely they will be promoted, and perhaps less likely their work will be respected. It does mean that we have to work that much harder. Its hard to know exactly if/when this happens since so many other factors come into play, but hopefully the differences diminishing. That's overall though, in my experiences males do seem to have to work less to be respected, though I've seen examples of women on the same par with men and excelling in their field.

Interview C                      Pseudonym Ryan Griffith (done due to recorder cut out)

1C. What do you feel helped you achieve your degrees?

As an undergraduate I had great instructors who motivated me to do the best I could. My fellow grad students were a great source of support during grad school. Beyond the people around me, I just had a lot of determination that I would finish. Often times I felt discouraged, but I just tried to not let anything get me down, I just kept focusing on attaining the degree.

2C. What were your goals, dreams, aspirations from the past? What are you goals for the future?

I had no idea what I wanted to do out of high school. As an undergrad I talked to my professors and sort of tried to get a sense of what they do, and decide that I wanted to try to be a professor as well, knowing that a Ph.D. was required for this led me to apply to doctoral programs. For the future, I plan to focus more on teaching than research in my field.

3C. What in your opinion lead to or drove you to get your Doctor of Philosophy or professional degree?

Knowing that I would need it to be able to be a professor, and also just loving the field and wanting to learn more about it and be around scholars working in it.

4C. What do you think helped you be who you are today?

Seeing examples of what I did and did not want to become in other people.  
Being persistent all the way through grad school.  
Having good supportive friends and mentors.

5C Anything you care to add?

Being determined and reminding yourself that working hard now pays off later, really does help in getting through tough times. Also, my grad program was very strict in terms of completing milestones on time, looking back I would have preferred a different situation, I wouldn't recommend a similar program to anyone who wants a decent pace.

Interview A

Pseudonym: Denise Helton

Any elaboration would be helpful to the study.

1A. Can you tell me about where you were born and raised?

Princeton, West Virginia. I always thought Princeton was an okay place to grow up, albeit a bit short on entertainment/educational opportunities.

2A. Tell me about the schools you attend from Kindergarten thru college and there locations?

Oakvale High School in Oakvale, West Virginia. I attended this school from Kindergarten through my sophomore year in high school. Oakvale was a very small school with poor resources and little money. We even had to disband the high school football team due to lack of funding.

PikeView High School in Athens, West Virginia. I went to PikeView through my last two years of high school. PikeView came into existence when school board members decided to consolidate Oakvale, Athens, Matoka, and Spanishburg high schools. PikeView allowed me a boarder depth of educational opportunities.

Concord University in Athens, West Virginia. Concord is a very adept school, especially in the field of Liberal Arts. I have a BA in History with a minor in English from Concord.

Marshall University in Huntington, West Virginia. I received my MA from Marshall in the discipline of History.

University of Kentucky in Lexington, Kentucky. I am currently working on a PhD in Modern European History.

3A. Can you tell me about the members in your family?

My mother is the core member of my family. My parents divorced when I was sixteen.

4A. Tell me about the jobs and the money that was available to the family members in your home?

My mother worked in a lumber factory until I was a toddler. She then performed house cleaning services for various families in Princeton and eventually worked for Council on Aging for several years. My father was a foreman for a construction company until my parents divorced. He now works as a custodian. I did not grow up with a lot of money at our disposal. We were

always comfortable, but not much more. My parents could not afford to send me to college. I had to have grants, loans (which I believe the payments will follow me the rest of my life), and work study jobs to pay tuition.

5A. Tell me about your siblings.

I am an only child.

6A. What influences or experiences affected which colleges you attended?

My mother has always been the biggest influence on my educational career. She encouraged me to attend college. She never gave up that hope for me. I also had teachers who believed in me enough to suggest I apply to higher education institutions. Yet I never had guidance counselors or the like help me choose colleges or even give me suggestions where to look for further information. I was on my own for that process. I have an innate desire to learn and education is very important to me, so I found a way to attend college. I really also cannot state enough how my mother's belief in me and my abilities gave me the courage and drive to succeed.

7A. What were important or unique family traditions, values, or experiences to you?

I am the first member of my family to graduate college, much less get an MA. Frankly, I did not want to stay in the Princeton area and after high school felt I no longer belonged there. I did not "fit in" anymore. Members of my family asked when I was going to get a job after high school without understanding how college could – or even should – fit into my plans. To this day I believe they think I am wasting my time in school.

8A. Anything you would like to add please feel free to do so.

I think that in my case family and environment is the key to understanding why I chose the educational path that I now follow.

Pseudonym/Name Denise Helton

Interview B

1B. Can you tell me about the teachers you had? In school what teachers inspired you?

Most of my teachers in high school were women but the exact opposite is true for college and even graduate school. My teachers had so many students to contend with that none of us got a lot of extra time and attention. I found my first female role model when I worked on my MA degree at Marshall. She is the only professor I have had to encourage me almost as much as I motivate myself.

2B. What characteristics did the teachers have that attracted you?

Strength of character, unafraid, well-read and very articulate, independent, charismatic, intelligent, kind and sympathetic.

3B Did any family members encourage your education in anyway? Can you tell me about your families view of college.

My mother always encouraged me beyond my wildest expectations. The other members of my family, while proud of me, always thought I should get a job right out of high school. They do not fully understand the importance, role, or inner mechanism(s) of higher education. Also, my family (excluding my mother) thinks I have been in school too long.

4B. Who do you feel communicated with you best male or female teachers?

Female teachers in high school, male professors in college, and back to women in graduate school. For whatever reasons, most of my teachers/professors in higher education have been males. I think it is because History is conventionally seen as a white man's discipline. I communicated equally well regardless of the sex of my teachers. What mattered most was my relationship with them.

5B. Tell me about the jobs around where you grew up. Were there many female professionals where you grew up?

There were no female professionals in my immediate orbit where I grew up. The jobs were minimum wage, manual labor type deals. Working class all the way. However, the skills I learned from watching my family work these jobs provided me with the impulse to work hard at my education and life. I never to this day look down upon such jobs. I respect my family.

6B. Who or what pushed you to attain your Ph. D. or professional degrees?

My mother, my MA thesis director, my fear of failure, my drive to succeed, and my desire not to end up in a job with no intellectual payoff.

7B. What is your definition of success?

Nowadays it is making sure my family members are healthy and happy. The academic world often is one in which professors sometimes forget that students have lives outside of graduate school. They get annoyed and often downright rude if you have other issues that get in the way of your life as a student. My dissertation advisor at the University of Kentucky has a habit of turning on female students when they do not only focus on graduate school. For instance, when my mother got sick and I had to take a leave of absence from school – as well as when one of her

other students had a child – my advisor was extraordinarily unsympathetic and toxic. So, even female professors sometimes do not offer female students the support they need.

8B What are important goals or ideas to you?

My goals are to stay in a field where I can gain intellectual satisfaction and always have the chance to keep learning. I also think it is important to encourage others (especially women) who have aspirations of higher education. Too often groups of people look at women as “bitchy” for expressing opinions in the educational realm.

9B Anything you would like to add?

Not at this time.

Transcribed Interview C with Denise H.

Q: You think that in your case that family and environment is the key to understanding why you chose the educational path you did. What part of your family or what part of the environment helped chose the educational path?

A: I guess it would be my mom who really pushed me to get an education. Not push in the negative stance, but always supported me. I think it's just most of my immediate family, like my aunts, uncles and even my father was...I think they just don't, and I'm not being rude or anything, but they just don't understand the point of higher education, so they never really thought about it in my case but my mom said, as soon as I was old enough to walk, she was saying you need to go to college. I think when I say environment, it's just where I'm from in Princeton, it's just nobody talked about it with me, except my mom.

Q: You talked about a mentor that you had in getting your Masters at Marshall, and you said that she encouraged you. How did she encourage you?

A: She just really wanted me to apply to PhD schools and she just always kept that in the forefront of my mind and let me know that I could do it. She just didn't want me to stop at the Masters level. She was always very encouraging to me and gave me contacts that I probably wouldn't get if I didn't know her and she wrote me excellent recommendation letter. She was just always the sounding board for me and gave me, like I said, the contacts that I needed, that I don't think, that I, as a graduate student could have got with out her.

Q: Now also you say, that in High School you were, women teachers encouraged you, then you got to college there was more males in your education. What do you think made certain ones stick out mind as people who you could go to as mentors?

A: I guess it was just the personal relationship I developed with them. I guess if I were to go to their office and they would show particular interest in me, as a student, and tell me "hey, maybe you should look at this scholarship that's coming up" or it was one of my professors actually who introduced me to the McNair program, for instance, so it was just if they were willing to put effort into me and they recognized something in me as a student, I think made them stand out for me.



Q: Now you mention McNair. How do you think McNair personally helped you?

A: It definitely opened a lot of doors and it made me more comfortable. The first public presentation I ever gave as a student was with McNair and so it's really helped me know that circuit. I just really don't think that if I hadn't been in the McNair program I would have even thought about graduate school. It helped me better my research skills, interview skills everything that I think you need if you are going to go on to the graduate level.

Q: So what do you think helped you achieve your degrees?

A: Perseverance, you gotta have that. You have to have people that support you, not only family but your professors because you can't, or it's very difficult to if their not on your side, and you have to work hard, and you just have to have faith in yourself.

Q: Just sit there and think about your goals, your dreams from your past, and compare them to what your goals are today. How have they changed?

A: Well I still want to get my PHD but my mother has had some health problems and I've had some health problems and I think that it's put things in perspective for me that now I look beyond just, you know, if I don't get an A on this paper it's the end of the world or if I don't keep a 4.0, my life is over, and I think I'm more family oriented than I was when I was younger and I don't know that I would have been if it hadn't been for my mom getting sick, so I don't know if you had asked me that question 2 years ago, if I would have said, my goals would be exactly the same they were when I was younger, so I think it was just personal experience that sorta flipped it for me.

Q: In your opinion what drove you to decide to go for that PHD?

A: I just always wanted to keep learning, I think that is one thing and to be honest I just didn't want to stay in West Virginia. I just wanted to move away from there, after High School I never really felt like I fit in, so I think it's that and I just have a keen desire to keep learning my whole life, so I guess it was those two factors.

Q: When you say you don't feel like you fit in, what exactly do you mean?

A: Well I just always felt like I was talking subjects that members of my family didn't, I don't want to say they didn't understand, but they just didn't...they wasn't interested in it and the same way with some of my friends, other friends of mine that went off to college and everything, I really felt like I related to them and felt that's where I should be. I think that probably it.

Q: So what do you think has made you who you are today?

A: I guess part of it is, my mom really shaped my character and I guess just my own \_\_\_\_, I believe. I think those are the two big factors. (Ashley: I can understand that, you had that model in your life.) If not for her I definitely don't think I would have ended up where I am or who I am.

Q: Do any school experiences good or bad stand out in your mind?

A: Well I've had lots of positive role models who have been very helpful and want me to succeed, and have gone out of their way to help me like my mentor at Marshall, and also I have fellow graduate students who push me forward, and by there example I want to do better as a scholar. As for negative experiences, my PHD advisor, I guess would be the worst example that

I have, she kinda flip-flopped on me. When I got sick and my mom got sick she just sorta turned on me, I didn't feel like I did anything it just seemed to me that she's the type of professor that if your not truly centered on school and the academic life, then somehow you are less of a person, that was very surprising to me because I went to the University of Kentucky to work with her, she was very helpful at first and then when things changed in my life, she just turned her back on me. So I guess that is the most negative experience I've ever had.

Q: So do you think that's hurt you as far as pushing for that PHD as much as you used to?

A: Yea, because now I'm thinking about switching programs and not having to deal with her, and I have friends, that one of my friends had a baby, and another who had some issues in their life, these are two girls that are my friends, and she was also on their committee, and she flipped on them. One of them actually left the program totally and she was one of the smartest girls I've ever met, so it's very kinda degrading, you know, you just get to the point that you just kinda shut down a little bit. I'm so used to having positive roll models that this just threw me for a loop.

Q: If you could do or say anything to an Appalachian female wanting to succeed, what would it be?

A: I would just say you have to surround yourself with positive people. You've got to have faith in yourself, but never let anybody tell you, you can't do it, and it doesn't matter where you are from. Just because you are from Appalachian that doesn't mean anything in the larger scheme of things. It matter where you want to go.

Q: So what motivates you?

A: I guess people motivate me, the people around me. My friends in graduate school, most particularly motivate me because they do such interesting work and they are...like my roommate she's incredibly articulate and bright and she motivates me to do better, so I guess it would probably be right now, I see what my goals used to be, you know, my professors, and now there my fellow graduate students. Academically that's what motivates me now.  
\_\_\_\_\_ they managed to get through it so it leads me to think that I could do it to.

Q: I think you have kinda touched on this but what or who has been most significant event in your life?

A: I guess again my mother is the most important person, as far as almost everything, shaping me as a person and academically. I guess the most important academic event in my life is my whole experience at Marshall getting my Masters degree because then I realize I really can do this, I can right that Masters thesis, I can get into these PHD schools, I can \_\_\_ myself as a academic.

## Questions asked during Interviews

### Interview A

1A. Can you tell me about where you were born and raised?

2A. Tell me about the schools you attend from Kindergarten thru college and there locations?

3A. Can you tell me about the members in your family?

4A. Tell me about the jobs and the money that was available to the family members in your home?

5A. Tell me about your siblings.

6A. What influences or experiences affected which colleges you attended?

7A. What were important or unique family traditions, values, or experiences to you?

8A. Anything you would like to add please feel free to do so.

#### Interview B

1B. Can you tell me about the teachers you had? In school what teachers inspired you?

2B. What characteristics did the teachers have that attracted you?

3B Did any family members encourage your education in anyway? Can you tell me about your families view of college.

4B. Who do you feel communicated with you best male or female teachers?

5B. Tell me about the jobs around where you grew up. Where there many female professionals where you grew up?

6B. Who or what pushed you to attain your Ph. D. or professional degrees?

7B. What is your definition of success?

8B What are important goals or ideas to you?

#### Interview C

1C. What do you feel helped you achieve your degrees?

2C. What were your goals, dreams, aspirations from the past? What are you goals for the future?

3C. What in your oppinion lead to or drove you to get your Doctor of Philosophy or professional degree?

4C. What do you think helped you be who you are today?

5C Anything you care to add?

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## Bioactivity of Extracts Prepared from *Hieracium venosum*

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### **Abstract**

*Hieracium venosum*, commonly referred to as Rattlesnake weed, has been used by the Eastern Band of the Cherokees to control bleeding and as a remedy for gastrointestinal and respiratory illnesses. As part of a more comprehensive study underway at Concord University, the antibacterial and antioxidant activity of extracts prepared from Rattlesnake weed were evaluated. Whole plant specimens were extracted using supercritical carbon dioxide or methanol under a variety of conditions. Crude extracts exhibited little to no antibacterial activity against the bacteria examined. Extracts were partially fractionated using normal-phase vacuum liquid chromatography and the relative antioxidant activity of each fraction evaluated via a DPPH free-radical-scavenging assay. More polar fractions exhibited stronger antioxidant activity.

## Introduction

Various extractions and infusions of plants have been traditionally used throughout the world as remedies for many ailments in folk and Native American medicine.<sup>i, ii, iii, iv</sup> Plants have a vast ability to synthesize bioactive components, most of which are secondary metabolites such as terpenes or substituted phenolics. These substances often serve as defense mechanisms against predation. Early medicines, such as morphine, aspirin, and penicillin were mainly derived from natural resources.<sup>1, 4</sup> The use of natural products in drug discovery is not a thing of the past. In the year 2000, almost 40 percent of the pharmaceuticals sold were derived from natural products.<sup>1</sup> Bioactivity assays are continuously being developed and conducted in an attempt to find potentially medicinal compounds from natural sources.<sup>1, 2, 4</sup>

Over a dozen different species in the genus *Hieracium* are found throughout the Appalachian region, and thousands more in the world. These are a very prolific and invasive species of plants that are often categorized as weeds by farmers. Hawkweeds are given their name because it was believed that the milky juices produced by this weed were ingested by hawks in order to improve their eyesight.<sup>v</sup>

With few exceptions, the flowers of the genus *Hieracium* are yellow and the stems, leaves, and under parts of the flowers are covered with fine to coarse hairs ranging in color (Figure 1).<sup>vi, vii, viii, ix, x</sup> Most species are alien to America, having been brought over by folk doctors from Europe to treat eye diseases.<sup>5</sup> Hawkweeds are typically found along roadsides, in dry, open woods, and in pastures or fields.





**Figure 1.** *Hieracium venosum* whole plant

Few prior studies of the bioactivity or chemical constituents of *Hieracium* species have been conducted. Weak antimicrobial activity against eight species of bacteria was exhibited by a species of *Hieracium* indigenous to Lebanon.<sup>3</sup> Chlorogenic acid, 3, 5-dicaffeoylquinic acid, luteolin 7-O- $\beta$ -D-glucuronopyranoside and apigenin 4'-O- $\beta$ -D-glucuronopyranoside were discovered in the leaves of five apomictic, or genetically identical, species of *Hieracium*.<sup>xi</sup> The coumarin umbelliferone has also been found in the genus *Hieracium*.<sup>2</sup> The presence of coumarins is correlated with anticoagulant activity.<sup>xii</sup>

*Hieracium venosum* is a very distinctive hawkweed noted for its purple-red veins prominent in its basal rosette of light green leaves (Figure 2).<sup>xiii</sup> It is known by several names including: Hawk plant, Vein-leaf hawkweed, Rattlesnake plantain, Snake-plantain, Adder's tongue, Bloodwort, Striped bloodwort, and Hawkbit; with Rattlesnake Weed and Poor Robin's plantain being the most common.<sup>10, xiv, xv</sup> The patterned leaf suggested its potential use as a Native American remedy for rattlesnake bites, in which the leaves were chewed and then the juice spit onto the wound.<sup>13</sup> A tea made from the whole plant was consumed to stop hemorrhages, spitting up of blood, diarrhea, and coughs.<sup>xvi</sup> Dried leaves and roots were crushed into a powder and were used as an astringent and expectorant.<sup>13, 16</sup> External warts were treated using the juice from fresh leaves. Rattlesnake weed is a familiar sight in dry, open woodlands

(pine woods specifically), on shaded banks, and along roadsides. The flowers, which bloom from May to October, are often mistaken for dandelions.<sup>13</sup> This plant is found in the Appalachian, Blue Ridge, and Alleghany Mountains, as well as the Shenandoah Valley.<sup>12</sup>



**Figure 2.** *Hieracium venosum* leaves

The ethnobotanical uses of *Hieracium venosum* suggest that it is a potentially bioactive plant. Together with a lack of prior studies and an easily accessible local supply, this plant is considered worthy of inclusion in the more comprehensive ongoing study of local flora at Concord University.

The general strategy used to identify bioactive compounds is to begin with a bioassay of the crude plant. If the plant exhibits bioactivity using the assay of choice, then the myriad compounds are separated, primarily by chromatography, into smaller subsets of compounds (referred to as fractions). Each fraction is then analyzed and active fractions are subjected to additional separation. The process is repeated until single, bioactive compounds are isolated. These so called “lead compounds” can then be optimized by chemical modification to produce useful drugs. Good examples of this process are found in the development of the anticancer compounds etoposide and taxol.<sup>xvii</sup> This study reports the initial screening of crude extracts and initial fractions for antibacterial and antioxidant activity.

## **Methods and Materials**

All solvents and reagents were used as received without further purification. Solvents were HPLC grade and were received from Fisher Scientific unless otherwise noted.

### Plant Collection:

Whole plant specimens (including roots) were collected in June 2006 during peak bloom. The plants were collected from a single site located on private property in Mercer County, West Virginia with permission from the owners. A soil sample was also obtained from the plant collection site, labeled and stored at -20°C. The collection code, location, date, GPS coordinates, weather, temperature, and time were recorded for the plant collection site. Plants were placed in air-tight plastic freezer bags labeled with an individual code and stored at -20°C. An entire plant was air-dried under constant pressure to be retained in the Concord University herbarium as a voucher specimen.

### Extraction Methods:

#### *A. Soxhlet Extraction*

Whole plant material stored at -20°C was air dried before being extracted using boiling methanol in a Soxhlet apparatus. The plant material was contained within a cellulose extraction thimble and the solvent was cycled through the apparatus approximately seven times. Solvent was removed under vacuum at 40°C using a rotary evaporator. Rotary evaporation of the solution prepared from 11.0 grams of dried plant material yielded 1.68 grams of crude extract. The crude extract was then dissolved in sufficient 3:2 hexane:methanol mixture (vol:vol) to give a final concentration of 10 mg/ml. The extract (sample number SD0102) was stored at -4°C.

#### *B. Room Temperature Extraction*

A second 11.0 gram sample of air-dried plant material was extracted at room temperature. The plant material was stirred in 500 mL of methanol for 76 hours after which the dark green solution was vacuum filtered three times using Whatman No. 1 filter paper. Following removal of solvent by rotary evaporation, the crude extract (2.72 grams) was redissolved in sufficient 3:2 hexane:methanol (vol:vol) to again yield a final concentration of 10mg/ml. The extract (sample number SD0103) was stored at -4°C.

### *C. Supercritical Extraction*

Air dried plant material (20.0 grams, previously stored at -20 °C) was extracted using carbon dioxide in a supercritical fluid extractor (Model SFT100, Supercritical Fluid Technologies, Newark, DE). The sample was extracted using three different combinations of pressure, temperature, and co-solvent which resulted in three different effective solvent polarities. In order of increasing polarity, the three sets of conditions were a) 40°C/2000 psi/no co-solvent, b) 40°C/4000 psi/no co-solvent, and c) 60°C/6000 psi/10% methanol co-solvent. The extraction vessel was filled to 80% capacity with plant material and extracted with seven volumes (each 20% of the vessel capacity) of carbon dioxide using three 30 minute soak/30 minute dynamic flow cycles. During the dynamic flow portion of the cycle, carbon dioxide is allowed to exit the extraction vessel at a rate of approximately 5 ml/min into an amber EPA collection vial. Crude plant extract was deposited in the EPA vial as the carbon dioxide evaporated. Masses of extracts obtained under the three sets of extraction conditions were, in order of increasing polarity of solvent, 211 mg, 13 mg, and 11 mg. The extracts were labeled SD0104A, SD0104B, and SD0104C, respectively, and stored at -4°C.

An additional supercritical fluid extraction was performed in which 65.26 grams of air-dried, ground whole plant material was exposed to the same parameters as previously described

to yield 337 mg of extract. The extracts SD0114A (288 mg), SD0114B, and SD0114C, were stored at -4°C.

#### Fractionation of Extracts:

Vacuum liquid chromatography (VLC) is a simple, inexpensive column chromatography method in which silica gel is used as the stationary phase. The material is eluted through the column using small batches of mobile phase of increasing polarity which are pulled through the column using gentle vacuum.

A vacuum-packed chromatography column was prepared using silica gel (Sigma-Aldrich, 70-230 mesh) as the stationary phase.<sup>xviii</sup> The mobile phase consisted of a solvent gradient of increasing polarity. Mobile phase compositions were as follows: 1) 100% hexane; 2 through 9 hexane:ethyl acetate vol:vol as indicated 2) 99:1 3) 98:2 4) 96:4 5) 94:6 6) 90:10 7) 80:20 8) 60:40 9) 20:80; 10) 100% ethyl acetate; 11) 50:50 ethyl acetate: methanol; and 12) 100% methanol. Each mobile phase was eluted through the column into a tared round-bottom flask. Solvent was removed under vacuum at 40°C using a rotary evaporator. Extracts were redissolved in dimethylsulfoxide (DMSO; Alfa-Aesar, HPLC-grade) to give a final concentration of 10 mg/ml.

#### Antibacterial Activity:

##### *A. Kirby-Bauer disk diffusion*

A modified Kirby-Bauer disk diffusion protocol was used to determine antibacterial activity of SD0102, SD0103, SD0104A, and SD0104B. Three 10 µl aliquots were used to impregnate a paper filter disk with 300 µg of crude extract. The impregnated filter disc was placed on a nutrient agar plate (Bacto™ Tryptic soy broth). Following incubation at 37°C for 24 hours, the zone of growth inhibition surrounding the disk was measured to determine

antibacterial activity. Efficacy of the crude extract against the following bacteria was examined: *Salmonella typhimurium*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Corynebacterium xerosis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

#### B. Microplate Assay

Inoculum was prepared by culturing each bacterium in Bacto™ Tryptic soy broth and incubated at 37°C for 24 hours. No effort was made to standardize the concentration of bacterium in the broth. The bacterial suspensions were prepared in 96-well round-bottom microtiter plates. Test wells contained 5µl of each extract, 190µl of Tryptic soy broth, and 5µl of inoculum. Controls consisted of bacterial broth (blank), broth control with no extract and no inoculum (negative growth control), inoculum with no extract (positive growth control), and inoculum containing a saturated sodium chloride solution (negative growth control). The extract was tested against the previously mentioned bacteria by incubating at 37°C for approximately 24 hours. The amount of turbidity in the wells was rated visually, where +++ indicated no growth, ++ moderate growth, and + very little or no inhibition of growth.

#### Antioxidant Assay:

DPPH (2, 2-diphenyl-picryl-hydrazyl) is a stable free-radical that serves as an easily used color indicator of antioxidant activity. The purple DPPH radical changes to yellow when it accepts a hydrogen atom from an antioxidant molecule. The disappearance of the purple color can be measured with a spectrophotometer and used to quantify the antioxidant power of an extract or fraction. Preliminary screening consisted of placing 80 µl of 0.1 M Tris (Eastman) buffer (pH 7.4) into each well of a 96-well microtiter plate, along with 20 µl of plant extract in DMSO, and 100 µl of 0.3 mM DPPH (in methanol). Thymol (10 mg/ml) was used as a positive

control. Samples were allowed to react in the dark in at ambient temperature for 30 minutes, after which wells were either visually inspected or their absorbance at 595 nm measured using a BioRad Model 550 microtiter plate reader. Visual evaluation yielded a semi-qualitative measurement, which is summarized in Table 1.

All samples rated +++ and ++ for antioxidant activity were reexamined in an attempt to measure IC<sub>50</sub> values (concentration of extract that quenches 50 % of the DPPH radical) using the procedure described but with a two-fold serial dilution of the extract. L-Ascorbic acid was used as the positive control. Serial dilutions of the positive control were also conducted to provide a baseline curve against which extract activity could be compared.

It should be noted that the measurement at wavelength 595 nm was chosen based on the filters available for the microtiter plate reader. Inconsistencies in the readings and low absorbance values prompted the purchase of filters that allow measurement at wavelengths much closer to the  $\lambda_{\text{max}}$  of DPPH (515-520 nm).

## **Results and Discussion**

### Antibacterial Activity:

The soxhlet-methanol (SD0102), room-temperature methanol (SD0103), supercritical CO<sub>2</sub> extraction at 40°C, 2000PSI (SD0104A), and supercritical CO<sub>2</sub> extraction at 40°C, 4000PSI (SD0104B) samples tested for antibacterial activity using Kirby-Bauer disk diffusion showed minimal or no zones of bacterial growth inhibition against all species tested.

The same bacterial panel was tested using the fractions of the supercritical CO<sub>2</sub> extraction at 60°C, 6000PSI, with 10% methanol (Fisher, HPLC-grade) co-solvent (SD0104C) using an antibacterial microplate assay. Antibacterial activity was evaluated by visual inspection of

turbidity (compared to the negative bacterial growth control). As with the Kirby-Bauer disk diffusion assay, the microplate assay showed little to no activity.

The redox cell growth indicator AlamarBlue™ is currently being evaluated. The AlamarBlue™ based assay shows great promise for sensitive measurements of antibacterial activity in 96-well microtiter plates. *Hieracium venosum* may be reexamined using this assay at some point. However, the lack of activity observed in the experiments described here puts a lower priority on reexamination.

There was limited to no antibacterial activity observed with the extracts of this plant against the ten-organism bacterial panel. The lack of antibacterial activity was unexpected based on the ethnobotanical uses of the plant. While it may indeed be true that *H. venosum* does not have antibacterial properties, there are several reasons why an inactive plant collection may be obtained from an otherwise active species. Note that all plants tested were collected from the same site on the same day therefore the absence of activity could possibly be attributed to the unique characteristics of that site at the time of collection. The time delay from plant collection to freezing (1 hour), as well as air-drying instead of freeze-drying, may have decomposed active compounds. A pioneer in the research of natural products recently advised that one collect five different samples of species before abandoning the search for bioactivity.<sup>xix</sup>

#### Antioxidant Activity:

Preliminary antioxidant results of supercritical fluid extraction at low temperature and pressure showed a non-selective extraction of antioxidant compounds that have moderate activity.



**Table 1.** Semi-qualitative Antioxidant Results of Fractionated Extracts.

EXTRACTION METHOD	1	2	3	4	5	6	7	8	9	10	11	12
Supercritical fluid 2000 psi, 40°C					++		+	+	+		+++	
Supercritical fluid 4000 psi, 40°C	++	++			+		+	+	+	+++	+++	
Supercritical fluid 6000 psi, 60°C, 10 % Methanol co-solvent					++		++	+++	+++	+++	+++	++
Soxhlet Methanol 60°C				+	+	+	++	+++	+++	+++	+++	+++
Room temperature Methanol	++	+		+	+	++	++	+++	+++	+++	+++	+++

Antioxidant activity was evaluated on a scale in which +++ equals strong antioxidant activity (no purple color visible), ++ equals moderate antioxidant activity (very little purple color visible), and + equals very little or no antioxidant activity (purple color very visible).

Attempts to measure IC<sub>50</sub> values for the VLC fractions were complicated by both the limited solubility of extracted compounds in the buffer/methanol matrix and by lack of assay sensitivity due to the measurement of absorbance far from the  $\lambda_{\max}$  that was required by the then currently available filters. Solubility issues lead to falsely high absorbance readings at higher extract concentrations due to light scattering caused by the precipitate. Lower buffer concentrations were briefly explored without improvement in the results. Alternate solvent systems for the extract may be examined in the future to reduce solubility issues.

It was possible to obtain semi-quantitative results relative to the 1 ppm L-ascorbic acid positive control. Comparison of absorbance vs. concentration curves reveals that samples with a qualitative rating of +++ are at least as active as 1 ppm L-ascorbic acid.

In conclusion, the more polar extract fractions of *Hieracium venosum* at a concentration of 10 mg/ml show antioxidant activity comparable to that of 1 ppm L-ascorbic acid. However, there is very little antibacterial activity present in the extracts prepared from the current samples.

### Future Research

Future studies include evaluation of cytotoxicity assays and coagulant assays of the extract versus human cancer cell lines and sheep erythrocytes, respectively. Those fractions that exhibit bioactivity will be subjected to high performance liquid chromatography (HPLC) to

better separate compounds until an active compound or compounds are identified. Recollection of this species from other sites followed by reevaluation of antibacterial properties is also warranted.

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