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### DISCOVERY

The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Vol. 11 Fall 2010







### DISCOVERY

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Cover: Beaver Lake is an impoundment of the White River that provides drinking water to more than 250,000 people, making up 9% of Arkansas' population (Beaver Water District, 2008). Two papers in this volume (pages 3 and 51) deal with water quality issues in Beaver Lake and its watershed areas. Cover design and photo by Judy Howard.

### Value-Added Learning

The *DISCOVERY* undergraduate journal is one of the ways Dale Bumpers College of Agricultural, Food and Life Sciences encourages students to engage in value-added learning experiences beyond the classroom. The student authors are reporting on the results of research projects they have conducted with faculty mentors.



Michael Vayda

The *DISCOVERY* journal provides a reporting outlet for our student scholars and scientists. It does not supersede publication elsewhere, but it does provide a forum for students and faculty to share their results and findings in a citable publication.

We encourage student research by awarding undergraduate research grants, and our students have been very competitive for research and travel grants awarded by the Honors College and the Arkansas Department of Higher Education.

Many undergraduate research projects are designed to meet the requirements of an honors thesis in the Bumper College Honors Program, which enables our students to enrich their educational experience and provide a very tangible service to society in the process.

We are proud to present these articles as examples of the work of our undergraduate students. I heartily congratulate the student authors and extend thanks to their faculty mentors and to the editors who reviewed their manuscripts.

Michael Vayda, Dean and

Associate Vice President-Academic Programs

### Sediment phosphorus flux in Beaver Lake in Northwest Arkansas

Taraf Abu Hamdan\*, Thad Scott $^{\dagger}$ , Duane Wolf $^{\S}$ , and Brian E. Haggard $^{\ddagger}$ 

### **ABSTRACT**

Internal phosphorus (P) loading may influence primary production in lakes, but the influence of sediment-derived P has not been well studied in Beaver Lake of Northwest Arkansas. Soluble reactive phosphorus (SRP), dissolved organic P (DOP), and total dissolved P (TDP) sediment-water fluxes were determined using intact sediment cores collected from deepwater environments in the riverine, transition zone, and lacustrine zones of Beaver Lake. The SRP, DOP, and TDP fluxes were also estimated from cores collected from shallow locations in the transition zone. There was a net positive SRP  $(0.001 - 0.005 \,\mu\text{g P cm}^{-2} \,\text{h}^{-1})$ , DOP  $(0.005 - 0.01 \,\mu\text{g P cm}^{-2} \,\text{h}^{-1})$ , and TDP  $(0.005 - 0.01 \,\mu\text{g})$ μg P cm<sup>-2</sup> h<sup>-1</sup>) flux from deepwater sediments into the water column. However, DOP and TDP flux in shallow sediments were net negative (-0.004 and -0.002 μg P cm<sup>-2</sup> h<sup>-1</sup>, respectively), suggesting that the majority of P was moving from water into sediment. The SRP flux from shallow sediments in the transition zone was similar to rates observed in deepwater sediments (0.002 µg P cm<sup>-2</sup> h<sup>-1</sup>). However, the variability among flux rates, sites and depths was high, and therefore no statistical differences were found. Sediment oxygen demand was positively correlated with SRP and DOP flux rates from shallow transition zone sediments suggesting that microbial biomass and activity may have influenced sediment P flux. The P flux from shallow sediments supports approximately 1% to 5% of the daily P demand of phytoplankton. When compared to other lakes, sediment P flux in Beaver Lake appears minimal and is probably not an effective avenue to manage eutrophication in this system.

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Taraf Abu Hamdan

I am an international student from Amman, Jordan. I graduated in 2010 with a Bachelor of Sciences in Agriculture with a major in Environmental, Soil and Water Sciences. I first came to the University of Arkansas while visiting Fayetteville, and I immediately decided that the U of A was the place for me to be. Being from the desert, I always had the affinity for water issues and quality.

With much help from Drs. Thad Scott, Duane Wolf and Brian Haggard, and the faculty and staff in the Crop, Soil, and Environmental Sciences Department I successfully defended my thesis and graduated with Honors. I am now a research intern in Columbia University Middle East Research Center in Amman, Jordan, and will be pursuing my masters degree next year.

### INTRODUCTION

Elevated nutrient levels in freshwaters are an ongoing water quality issue in Arkansas because of human activities. Increased phosphorus (P) in freshwater deteriorates water quality because P is the primary limiting factor in algal growth and P enrichment accelerates eutrophication (Sharpley et al., 2002). Eutrophication is the over enrichment of mineral nutrients that results in excessive autotrophic production, particularly, algae and cyanobacteria (Correll, 1998). The increased productivity and subsequent decomposition of dying algal blooms decreases dissolved oxygen (DO) creating water quality impairments. Phosphorus enrichment also increases the prevalence of harmful algae, such as Microcystis, a cyanobacterium that produces taste and odor compounds and even some toxic compounds as metabolic byproducts (Huisman et al., 2005). Moreover, the presence of algal blooms and the subsequent turbidity reduces the aesthetic value of the water body.

After input into lakes and reservoirs, soluble reactive P is generally assimilated biologically and deposited eventually in the bottom sediments (Correll, 1998). Additionally, P can enter lakes and reservoirs adsorbed to eroded soil material adding to the external P inputs to the water body, which is also deposited rapidly in bottom sediments. But P deposited in sediments can be released back to surface water in a process referred to as internal P flux. The entrapment and release of P by sediment is often linked to the

interactions between iron (Fe), oxygen ( $\mathrm{O_2}$ ) and P (Mortimer,1941). This model has been used to explain P flux from sediments to overlying water; it is referred to in this paper as the 'ferrous-wheel-model' (Fig. 1). In the presence of  $\mathrm{O_2}$ , SRP is bound by ferric iron (Fe III) in an oxidation-dependent reaction. When water overlying sediments become anoxic, Fe (III) is reduced to ferrous iron (Fe II), which causes P release. Therefore, P-driven eutrophication is a self-perpetuating process, where eutrophication increases bottom water anoxia, which in turn increases P release from bottom sediments (Fig. 1).

Some water quality problems in Beaver Lake of Northwest Arkansas have been attributed to increased P loads from the watershed (Sen et al., 2007). The Beaver Water District stated in their 2008 watershed report that the water quality concerns for Beaver Lake were algal blooms and turbidity caused by excess P in the water (Beaver Water District, 2008). Beaver Lake is an impoundment of the White River that provides drinking water to more than 250,000 people, making up 9% of Arkansas' population (Beaver Water District, 2008). Watershed management efforts have reduced P input into Beaver Lake. However, P concentrations in lake water have not declined (Sen et al., 2007). The internal P flux from sediments to overlying water may offset the decrease in external P loads (Mortimer, 1941; Böstrom et al., 1988). A study done on Lake Eucha Oklahoma, found that the internal P flux from bottom sediments accounted for 25% of the total P load (Haggard et al., 2005). Several other studies have shown that P loading from sediments has an effect on the trophic state of reservoirs (Larson et al., 1979; Ryding, 1981).

Several approaches in laboratory studies and *in-situ* have been utilized to quantify P loads from sediments (Holdren and Armstrong, 1980), many using intact sediment cores (e.g., see Anderson, 1975; Haggard et al., 2005; Moore and Reddy, 1994; Moore et al., 1998). In a previous study on Beaver Lake (Sen et al., 2007), sediment cores were incubated under static conditions in the laboratory to estimate P flux from the sediments. They found that that the highest P flux occurred in cores bubbled with N<sub>2</sub> gas amended with 300 ppm CO<sub>2</sub>, and P flux was lower in cores bubbled with air (Sen et al., 2007). Results of that study also showed that the highest P flux occurred in the riverreservoir transition zone, which is the location of Beaver Water District's water intake structures (Sen et al., 2007).

Although the sediment-P release rates reported by Sen et al. (2007) were a good initial estimate of the importance of sediment-P loading into Beaver Lake, the study focused on sediments from deep regions of the lake that were beneath anoxic waters. Phosphorus inputs from these deep sediments are cut off from the lake photic zone until lake turnover, which usually occurs in November or December each year. Therefore, P in deep waters is generally inaccessible by algae in the upper photic zone of the lake during the summer. Sediments in more shallow regions of the reservoir come into direct contact with the upper mixed layer where algae grow. But, P release from shallow sediments has not been well studied, and shallow-sediment P release has not been quantified for Beaver Lake. Some studies have indicated that P release from shallow sediments can influence lake water column P concentrations (Sondergaard et al., 1999). Therefore, more work is needed to quantify the role of shallow sediments as P sources influencing internal loading. Additionally, the previous sediment-P release studies on Beaver Lake have measured only SRP flux. No studies have attempted to quantify the total dissolved P (TDP) and dissolved organic P (DOP) in the lake, which may contribute a large portion of P flux and ultimately influence primary productivity.

The objectives of this study were to: (1) determine the differences in total, organic and inorganic dissolved P fluxes from Beaver Lake sediments, (2) determine the P flux from shallow transitional zones versus deep transitional zones in the lake, and (3) quantify the spatial variability of P flux between the three deep zones (riverine, transitional and lacustrine) in Beaver Lake. We expected to find SRP efflux from shallow transitional sediments, and that the rate of efflux could provide a substantial portion of phytoplankton P demand. I also expected to find that efflux of dissolved organic P from shallow sediments would be

higher than SRP efflux, making shallow-sediment organic P another important form of P supply to phytoplankton. Finally, we expected to find a positive relationship between SRP efflux from sediments and the sediment oxygen demand (SOD), with higher rates of SOD being a proxy for microbial metabolism and P mineralization.

### MATERIALS AND METHODS

Site Description. Beaver Lake is located in Northwest Arkansas and construction of the reservoir was completed in 1963. The Lake is an impoundment of the White River, managed by the U.S Army Corps of Engineers. Beaver Lake is a multipurpose reservoir used for flood control, hydroelectric power and most importantly water supply, along with recreational uses. Beaver lake is fed primarily by the White River, War Eagle Creek and Richland Creek. Beaver Lake, as expected from reservoirs (Kimmel et al., 1990), changes from more eutrophic conditions in regions close to the headwaters (riverine) and in the transitional zones to more pristine circumstances in the lacustrine zone (Haggard et al., 1999). These differences illustrate the importance in sampling all three zones within the lake.

Sediment Cores. Core collection sites were chosen following a previous coring study carried out on Beaver Lake (Sen et al., 2007). Eight cores were collected from the transitional zone (Fig. 2) at shallow depths between 0.5 and 2.0 m. Four cores were collected from each of the three zones (riverine, transitional and lacustrine) at depths of 12 m for the transitional and riverine, and 23 m for the lacustrine zones. Core collection occurred in September 2009 for the shallow zones and October 2009 for the deep zones. This time period was selected because it is the time of year when P release into anoxic hypolimnetic waters should be greatest, and it is also the time of the year when algal-derived taste and odor compounds are at their highest annual concentrations.

Shallow sediment cores were collected in 30-cm (7.5-cm diameter) clear polyvinyl tubes fitted with a one-way rubber valve on the upper end. Cores were pushed into the sediments using a 3-meter PVC pipe. The one-way valve ensured the integrity of sediment and the overlaying water during sample collection, which was confirmed through visual inspection of the turbidity of the overlying water. Cores from deepwater locations were collected directly into clear polyvinyl tubes by certified SCUBA divers.

Cores were sealed with rubber stoppers and insulation tape. Along with the cores, 60 L of lake water was collected from each coring zone. This was done in the deep zones using a pump lowered to the coring depth to pump out the anoxic water. After collection, the sealed cores were carefully transported to the lab.

Core Preparation and Incubation. In the lab, each core was fitted with a flow-through plunger sealed using an Oring seal and Teflon tape. The flow-through plunger was fitted with Teflon inlet and outlet tubes (Fig. 3). The inlet and outlet tubes help maintain a continuous flow in the chamber. A water column of approximately 7.5 cm in height was maintained above the sediment surface in each core at all times. Lake water was pumped over the surface of the core at approximately 1 ml min<sup>-1</sup>. Cores were incubated at ambient temperature. Inflow water for cores from shallow sites was continually aerated, whereas inflow water for cores from deepwater sites was not. Flow rates for each core were monitored daily over the entire experimental period of four days.

Sample Collection. The inflow and outflow water of each core was sampled daily for four days. Samples were collected in 40-ml beakers then filtered using a syringe filter (0.45-μm pore size) into a 20-ml scintillation vial. Filtered samples were then acidified with 2 drops of HCl for preservation. Samples designated for the measurement of TDP were digested using the acid-persulfate oxidation method. The digestion was carried out by mixing 15 ml of the filtered samples with 1.8 of the digestion solution (K<sub>2</sub>SO<sub>4</sub>) and autoclaved for one hour. Samples were analyzed for SRP and TDP spectrophotometrically using the ascorbic acid method (APHA, 1992). The dissolved organic P concentration of samples was calculated as the difference between SRP and TDP.

Phosphorus concentrations (SRP, TDP, DOP) from inflow and outflow measurements were converted into P flux estimates using the equation:

$$P_f = [(P_{out} - P_{in})^* Q]/45.4$$

Where  $P_f$  is the P flux ( $\mu$ g P cm<sup>-2</sup> hour<sup>-1</sup>);  $P_{out}$  and  $P_{in}$  are the P concentrations ( $\mu$ g ml<sup>-1</sup>) in the outflow and inflow water, respectively, Q is the flow rate in ml h<sup>-1</sup>, and 45.4 is the surface area of cores in cm<sup>2</sup>.

Samples from shallow cores were also collected as described above for the first two days of each experiment to quantify sediment oxygen demand (SOD). Briefly, O<sub>2</sub>/Ar ratios in inflow and outflow water were measured using a Membrane Inlet Mass Spectrometer (MIMS). Dissolved O<sub>2</sub> concentrations were determined from O<sub>2</sub>/Ar ratios by assuming Ar was saturated in water at the ambient temperature of the incubation. The SOD was calculated similar to that for P flux (see equation above), with dissolved O<sub>2</sub> rather than P as the variable of interest.

The results were analyzed used a one-way analysis of variance to determine phosphorus flux, the same method was used for DO analysis. The P-value was set at 0.05, for both the phosphorus and DO readings.

### **RESULTS AND DISCUSSION**

A net positive TDP and DOP flux occurred from deep sediments (0.0123 and 0.0098 μg P cm<sup>-2</sup> h<sup>-1</sup>, respectively), but a net negative TDP and DOP in shallow sediments from the transition zone (-0.0018 and -0.0034 μg P cm<sup>-2</sup> h<sup>-1</sup>, respectively) (Fig. 4). However, the SRP was always positive for all sites. This was because of relatively high variability observed in P flux rates among replicate cores at each site (reported as standard error of the mean in Fig. 4). The TDP flux appeared to be largely driven by the organic P fraction of TDP. Also noteworthy is that SRP efflux from shallow sediments was similar to the rate of SRP efflux from deepwater sediments. Sediment oxygen demand was positively correlated with SRP and DOP flux from the shallow transition zone sediments (Fig. 5).

There were no significant differences in SRP, DOP, or TDP sediment water flux between the reservoir zones or between water depths. These results did not support our hypotheses. The lack of statistical differences among sites was caused by large variability observed in P flux among cores from the same sites. A power analysis of the data (SAS 9.1) revealed that a minimum of 27 cores per site were needed to detect statistical differences ( $\alpha = 0.05$ ) in the SRP flux data, and 17 cores per site were needed to detect statistical differences ( $\alpha = 0.05$ ) in the DOP data. This level of sampling intensity was not feasible and likely will not be feasible in future studies. The high degree of error may have been caused by the short exposure time between water and sediments in the continuous flow core chambers. In this setup, water was exposed to sediments for a few hours as opposed to days worth of exposure time in static core incubations (e.g. Sen et al., 2007). Interestingly, power analysis confirmed that only 4 cores were needed per site to detect statistical differences in SOD. Therefore, continuous flow core chambers may be less appropriate for P flux studies even though they are widely used in nitrogen and O, flux studies (An et al., 2001, Gardner et al., 2006, Scott et al., 2008). Nevertheless, the P flux rates observed in this study were similar to those reported previously for deepwater areas in Beaver Lake using static cores (Sen et al., 2007), which were generally lower than sediment P flux compared to other lakes (Table 1).

Lake Eucha, Okla., had an anaerobic average SRP flux rate of 0.018 μg P cm<sup>-2</sup> h<sup>-1</sup> (Haggard et al., 2005). Other studies have reported P flux from anaerobic sediments to be as high as 0.11 μg P cm<sup>-2</sup> h<sup>-1</sup> (Freedman and Canale, 1977). The anaerobic P flux rates found in this study (~0.002 μg P cm<sup>-2</sup> h<sup>-1</sup> on average) were comparable to those of aerobic sediment P fluxes. For Example, Lake Eucha had an average aerobic SRP flux of 0.004 μg P cm<sup>-2</sup> h<sup>-1</sup> (Haggard et al., 2005), and deep Beaver Lake sediments that were oxidized

in the lab released P at a rate of  $0.003 \,\mu g \, P \, cm^{-2} \, h^{-1}$  (Sen et al., 2007). This suggests that even though the Beaver Lake deep water becomes anoxic, P release from sediments is not nearly as high as it might be based on other studies.

Even though the P flux rates of this study were not consistent with the hypothesis or previous models, they still bear an importance in the process of understanding the dynamics of P in sediments and overlying water. For example, it is important to consider DOP as well as SRP sediment-water interactions. According to Böstrom et al. (1988), the magnitude of P release rates from sediments to oxic waters are frequently the same in magnitude to rates detected from hypolimnetic bottom areas to anoxic waters. This study confirmed that analysis for SRP, but DOP flux in Beaver Lake was much higher in deepwater sediments.

Results of this study also allow us to estimate the proportion of primary production in Beaver Lake that may be supported by internal P loading. If Beaver Lake is mesotrophic, then phytoplankton productivity should range from 500-1500 mg C m<sup>-2</sup> day<sup>-1</sup> (Wetzel, 2001). Assuming a stoichiometric growth rate near optimum, phytoplankton P demand in the upper mixed layer would be approximately 10-35 mg P m<sup>-2</sup> day<sup>-1</sup>. If we also assume that only 10% of sediments in Beaver Lake are exposed to the upper mixed layer when the lake is stratified, then P flux from shallow sediments would support from 1% to 5% of the daily phytoplankton P demand. This indicates the importance of P recycling in the upper mixed layer and external P sources as the probable controls on annual productivity in Beaver Lake.

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Table 1. A comparison between soluble reactive phosphorus flux rates under aerobic and anaerobic conditions from several studies.

P release rate (μg P cm <sup>-2</sup> h <sup>-2</sup> )					
Location	Aerobic	Anaerobic	Source		
Beaver Lake					
Riverine Zone	nd*	0.001	This study		
Transition Zone	0.002	0.002	This study		
Lacustrine Zone	nd*	0.005	This study		
Beaver Lake					
Riverine Zone	0.003	0.002	Sen et al., 2007		
Transition Zone	0.004	0.006	Sen et al ,2007		
Lacustrine Zone	< 0.001	0.002	Sen et al., 2007		
Lake Eucha					
Riverine Zone	0.005	0.020	Haggard et al., 2005		
Transition Zone	0.004	0.010	Haggard et al., 2005		
Transition Zone	0.004	0.025	Haggard et al., 2005		
Clear Lake	0.041	0.036	Porcella et al., 1970		
Lake Mendota	0.204	0.221	Ahlgren, 1972		
Lake Norriviken	0.021	0.042	Bjork 1972		
Lake Trummen	nd*	0.063	Bjork ,1972		
Lake Erie	0.003	0.032	Burns and Ross, 1972		
Lake Ontario	0.002	nd*	Bannerman et al., 1974		
Lake Warner	0.005	0.108	Fillos and Swanson, 1975		

<sup>\*</sup>nd = not defined

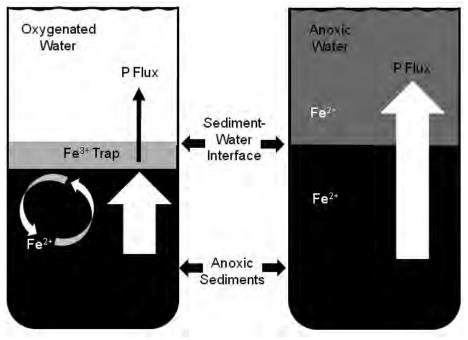


Fig 1. Ferrous wheel model in (left) oxic water, (right) anoxic water (Mortimer, 1941, Wetzel, 2001).

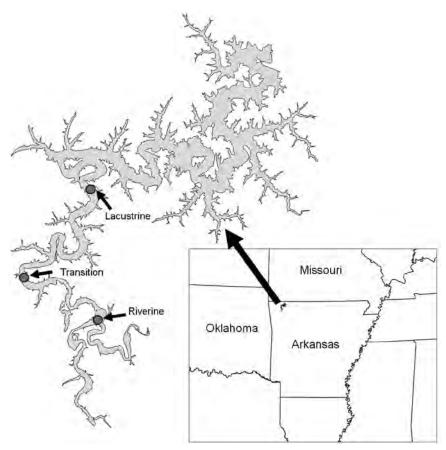


Fig 2. A map of the three different coring zones within Beaver Lake.

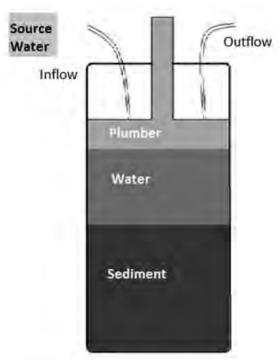
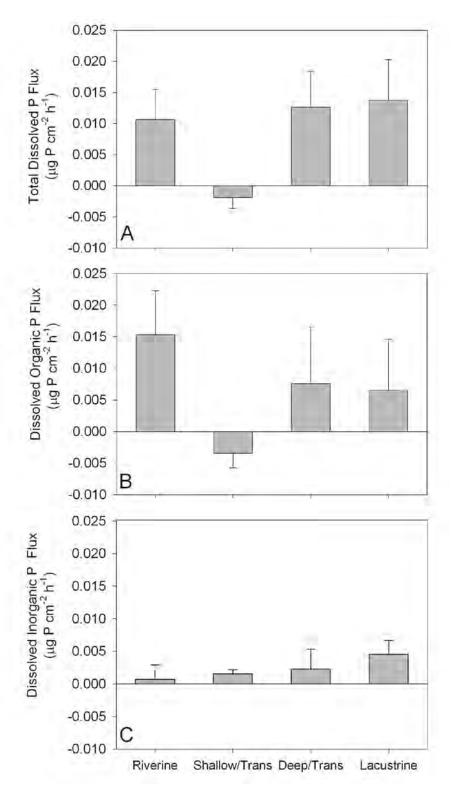


Fig 3. An illustration of the continuous flow-through core setup used in the experiment.



**Fig 4.** Total dissolved phosphorus flux (A), dissolved organic phosphorus (B), soluble reactive Phosphorus(C). The standard error of the mean is indicated by bars.

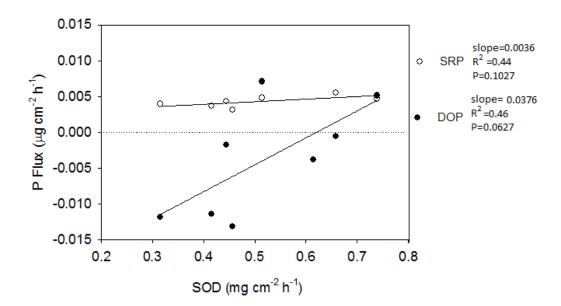


Fig 5. Relationship between sediment oxygen demand (SOD) and soluble reactive P (SRP) or dissolved organic P (DOP) flux in shallow sediment cores.

## Studies into cytauxzoon and helminth infections of bobcats (*Lynx rufus*) of Northwest Arkansas

Emily Hickman\*, David Kreider<sup>†</sup>, Chris Tucker<sup>§</sup>, Jana Reynolds<sup>‡</sup>, Jeremy Powell\*\*, and Tom Yazwinski<sup>††</sup>

### **ABSTRACT**

The purpose of this study was to determine the prevalence of *Cytauxzoon felis* and gastrointestinal helminth infections in bobcats (*Lynx rufus*) of Northwest Arkansas, an area known to have numerous cases of cytaux in domestic cats. Sixty bobcat carcasses were collected from trappers located in Mulberry and Decatur, Arkansas. Blood samples from the hearts were used to isolate *Cytauxzoon* DNA. Next, a polymerase chain reaction ( PCR) procedure coupled with gel-electrophoresis assay for the 18s region of extracted DNA were used to determine the presence of the protozoan in the bobcats at the time of harvest. Out of the 60 bobcats, 54 (90%) were positive for the protozoan's infection. These findings indicate a large reservoir of *Cytauxzoon* for possible infection of domestic cats. Along with the detection of *Cytauxzoon felis*, intestinal helminths of the bobcats were collected and identified. The isolated helminths included *Alaria marcianae*, *Ancylostoma* sp., *Molineus barbatus*, *Taenia* sp., *Spirometra mansonoides*, *Mesocestoides lineatus*, *Aonchotheca putorii*, *Physaloptera praeputialis* and *Toxocara cati*. All helminths found were previously shown to be common in omnivorous and carnivorous sylvatic as well as domestic mammals of the region. It is amazing that bobcats are able to withstand both parasitic infections concurrently, as they roam the forests of Northwest Arkansas.

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**Emily Hickman** 

Since graduating from Jonesboro High School in 2006, I have spent the past four years majoring in Animal Science at the University of Arkansas. I am an Honors College Fellow, and I was able to use funding from my scholarship to spend the summer after my sophomore year studying abroad in Florence, Italy. I am an active member in the St. Thomas Aquinas University Parish, leading the Seton's Women's group for two years, serving on the Peer Ministry Team for two years, singing in the choir, and acting as president of the organization last year. I currently work at Animal Medical Clinic in Fayetteville. I was a Pre-Vet club officer for two years, and enjoyed participating in the various hands on activities and lectures provided by the club. I am very grateful for the preparation I have received by being part of the Animal Science program. I will begin my first year of Veterinary School at LSU in Baton Rouge this fall.

I am very thankful to Dr. Jeremy Powell for helping me to find the topic for my honors project. With his assistance, as well as Dr. Yazwinski and Dr. Kreider's, I learned information that will be very valuable to me as I continue through my career.

### INTRODUCTION

Cytauxzoonosis is an infectious disease caused by the protozoan parasite *Cytauxzoon felis*. It was first discovered when a domestic cat was inoculated with blood from a Florida panther and in turn developed fatal cytauxzoonosis (Yabsley et al., 2006). Since then, the protozoan has been isolated from domestic cats throughout the southeastern United States. It is difficult to screen for the actual disease by standard veterinary procedures such as blood smears because of the pathogen's rapid switching between blood-borne and tissue phases.

The life cycle of organisms in the *Cytauxzoon* genus has two stages: a phagocytic cell phase followed by a red blood cell phase (Gardiner et al., 1998). The cycle in the cat (domestic or bobcat) begins with transfer of sporozoites from an attached, protozoa-infected tick to the cat. In the cat, the protozoa initially infect phagocytic cells (macrophages, reticuloendothelial cells, dendritic cells, certain leukocytes, etc). Rapidly, the infection spreads from the phagocytic cells to the red blood cells. The more pronounced symptoms of the infection in the domestic cat are due to restricted blood flow (because of distended phagocytic cells) and a lack of "efficient" blood (because of destroyed red blood cells). Ticks become infected by drawing blood from infected animals. It appears that the parasite reproduces sexually in the tick, and asexually in the cat.

Diagnosing *C. felis* infections in cats has proven to be difficult. An infected cat often goes undiagnosed or is in-

correctly diagnosed as having *Babesia felis* or *Mycoplasma haemofelis*. Cats infected with *C. felis* show nonspecific signs of infection such as anorexia, lethargy, shortness of breath, jaundice and pallor. Fever occurs early in the infection, while hypothermia indicates an animal close to death. Delirium, seizures and coma can also occur in the later stages of the disease. The disease works rapidly, with most domestic cats surviving for only a week after onset of signs. Postmortem examination of *C. felis* infected cats usually reveals enlarged and reddened lymph nodes, distended abdominal veins, hemorrhage in the abdominal organs, and a darkened spleen. Splenomegaly and/or hepatomegaly may also be observed (Shell and Cohn, 2005).

Because most of the symptoms of cytauxzoonosis are non-specific and shared with other diseases, the likelihood of misdiagnosis is high. As a result, the use of PCR testing has become accepted as the best diagnostic tool in documenting this disease (Birkenheuer et al., 2006a). Once the disease is diagnosed, treatment varies, but with no definite cures. There have been improvements with treatment regimes, with more cats currently surviving infections by the parasite than what was initially observed (Bondy et al., 2005). Treatment with the antiprotozoal drugs diminazene aceturate or imidocarb diproprionate, along with supportive care, has proven successful in some cases (Brooks, 2008). Better prognosis may also be due to a genetic change in the parasite, which has resulted in an attenuation of the protozoa over time (Merck Veterinary Manual, 2008).

Cytauxzoon felis is vectored to cats by the American dog tick (Dermacentor variabilis) with bobcats (Lynx rufus) as the primary reservoir host. Bobcats inhabit most of Canada and North America, as well as northern Mexico. The bobcats are not adversely affected by an infection, and only experience a mild phase of the disease. The bobcat infections have perhaps allowed the parasite to change to a less pathogenic strain over time. Brown et al. (2009) found that there were two genetically distinct strains of C. felis in both Georgia and Arkansas. The ability of C. felis to develop strain variation may explain why more cats are surviving today than previously.

The purpose of the following study was to determine the prevalence of cytauxzoonosis and gastrointestinal helminthiasis in bobcats obtained in Northwest Arkansas. The information gained from this study might help us better grasp the extent of possible exposure of domestic cats to bobcat-reservoired protozoan and helminth parasites.

### MATERIALS AND METHODS

Collection of Bobcat Specimens. Bobcat carcasses were obtained from two registered trappers located in Mulberry and Decatur, Ark. during the 2009 trapping season. The specific locations of the bobcat trappings were not recorded, but all bobcats were obtained in Northwest Arkansas. After the pelts were removed by the trappers, the carcasses were frozen and stored in a freezer at -20 °C. The total number of carcasses collected was 60: 57 from Mulberry and 3 from Decatur.

The carcasses were thawed after delivery to the University of Arkansas, Department of Animal Science, Parasitology facility. Immediately after thawing, blood and tissue samples were obtained from each carcass. The samples of clotted whole blood collected from the hearts were stored at -20 °C until DNA extraction. A sample of spleen tissue was also collected (and frozen) from each carcass in case the DNA extraction from the clotted blood was not successful.

Intestines were processed via standard protocol for isolation of helminth parasites. In brief, the entire intestinal tract from each bobcat was opened lengthwise, the total contents were collected and sieved (final screen aperture of 0.2 mm), and the sieve residues were examined microscopically (10-40 magnification) for helminth isolation and identification.

Performing DNA Extraction. A Qiagen DNA extraction kit for Blood and Tissues (QIAamp DNA Blood Mini Kit, catalog no. 51104, QIAGEN Sciences, Germatown, Md., 20874) was used to extract DNA from the clotted blood samples. In all cases, the DNA from the blood was of sufficient concentration to run the PCR and electrophoresis procedures. Hence, the spleen tissue samples were not used in this study.

To confirm that a sufficient amount of DNA was extracted from each sample, total DNA concentration was determined on a Hofer Dynaquant 200 flourometer using Hoest 33258 dye and calf thymus DNA as a standard (Cesarone et al., 1979). The goal was to have each sample contain a minimum concentration of 10 ng DNA/μL. If samples proved to contain less than the required DNA concentration after the first extraction, additional blood would have been extracted until an adequate amount of DNA was obtained.

Testing DNA Using Polymerase Chain Reaction. The presence of C. felis DNA in individual samples was determined by the use of a PCR assay that has previously been verified as specific for C. felis (Birkenheuer et al., 2006a). The specific forward and reverse primers used were 5'-GCGAATCGCATTGCTTTATGCT -3' and 5'- CCAAT-TGATACTCCGGAAAGAG -3', respectively. The assay amplifies a specific 284 bp segment of the C. felis 18s ribosomal DNA. Each assay mixture also included forward and reverse primers for the DNA region coding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a positive control; this was to detect any presence of PCR inhibitors in the extracted DNA. Glyceraldehyde 3-phosphate dehydrogenase is an enzyme that catalyzes the sixth step of glycolysis, and is present in tissues from all oxygen-breathing animals. All samples were also run with a negative control consisting of the complete reaction mixture with both sets of primers, but with water substituted for the DNA templates.

Each set of PCR reactions also included a positive control consisting of DNA obtained from a domestic cat that died from a *C. felis* infection. The positive control DNA was kindly provided by Dr. Mason Reichard of the Oklahoma State University College of Veterinary Medicine.

Conducting Gel Electrophoresis. A 1% agarose gel was prepared to process and view the DNA fragment material that was propagated by PCR and placed for electrophoretic separation. Gels were run at 85 V for one hour, and were then visualized with images recorded using a UVP Epi Chem II imaging system (UVP Inc., Upland, Calif.). A C. felis positive result was indicated by two bands at approximately 401 bp and 284 bp, and a negative result had only one band at 401 bp. The 284 bp band indicated the presence of the 18s region of C. felis, and the 401 bp represented the band for GAPDH and indicated that PCR inhibitors were not present in cytaux DNA-negative samples.

### RESULTS AND DISCUSSION

The assay was able to amplify cytaux-specific DNA when at a concentration >10 ng/ $\mu$ L (Table 1). Of the 60 samples, only 6 were negative for *C. felis*. This means that an astounding 90% of the bobcats tested were positive for

the presence of *C. felis* DNA and hence, infection. An example of a finished gel is given in Fig 1.

The above results have major implications. This study shows that the bobcat provides a considerable reservoir of the *C. felis* parasite. In other studies, infections with *C. felis* were shown to be far less common in the bobcat (Yabsley et al., 2006). This information is important for owners of cats living in Northwest Arkansas. Chance of infection from a tick would appear to be likely when there is close proximity or overlap of regions frequented by bobcats and domestic cats. Reichard et al. (2008) emphasized this point when he stressed that the presence of bobcats in a domestic cat's environment is a predisposing factor in the spread of this disease to the highly susceptible, domestic cat. It is therefore important that cat owners and veterinarians in the southeastern U.S., and especially in Arkansas, remain vigilant in detecting early signs of *Cytauxzoon* infection in cats.

It is also important to continue studying differences in strains from region to region to determine any connection between strain and pathogenicity. Brown et al. (2009) found 2 genotypes of the ITS region of DNA, indicating that there were two genetically distinct strains of C. felis in both Georgia and Arkansas. Changes in a strain could be important relative to protozoan virulence in domestic cats. Mutations might lead to the development of strains that are less (or more) virulent. Also, the rate of change in different geographic regions could vary. This would explain the lower incidence of disease in areas outside the southeastern U.S. It is important to determine if changes in this parasite affect its pathogenicity in the cat, and how to apply this knowledge for the future maintenance of healthy cats. The serious nature of C. felis infection was demonstrated by Birkenheuer et al. (2006b); a study wherein 32 of 34 infected domestic cats from North Carolina, South Carolina and West Virginia died or were euthanized because of a *Cytauxzoon* infection. The high incidence of *C*. felis infection in Northwest Arkansas bobcats, as demonstrated in this study, suggests a real and present threat to the well-being of area cats. Further studies are also needed to develop laboratory procedures that could determine the genotypes (strains) and pathogenicities of C. felis.

While the main focus of this project was to test for the presence of *C. felis*, discovering which intestinal helminths were present was also thought to be noteworthy given the range of alternate hosts that helminths routinely infect. The helminth parasites might be those that have a wide range of natural as well as dead end hosts, including humans. Nine different species of intestinal helminths were found: *Alaria marcianae*, *Ancylostoma* sp., *Molineus barbatus*, *Taenia* sp., *Spirometra mansonoides*, *Mesocestoides lineatus*, *Aonchotheca putorii*, *Physaloptera praeputialis*, and *Toxocara cati* (Fig. 2). All of the helminths can also infect domestic felines. Only two are considered to be of any

realistic threat to humans; *Ancylostoma* sp. and *Toxocara cati*, which precipitate cutaneous larval migrans (CLM) and visceral larval migrans (VLM) in people, respectively.

### **ACKNOWLEDGEMENTS**

I would like to extend many thanks to Hartz Mountain Industries, Inc. of Secaucus, New Jersey, who was the financial sponsor of this project, and Dr. Wesley Shoop of E.I. du Pont de Nemours & Company of Wilmington, Delaware, who provided supplemental helminth identifications. Thanks are also extended to Dr. Mason Reichard of Oklahoma State University College of Veterinary Medicine for providing positive test samples, and Kate Williams, DVM, who provided the negative control samples, and timely support and encouragement.

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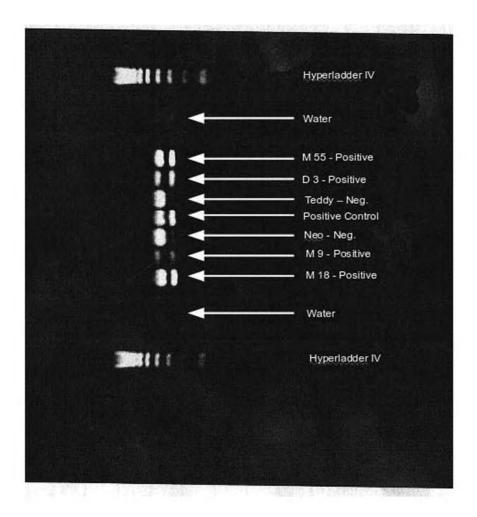
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Table 1. The initial DNA concentrations in the blood samples and the infection status results as determined by PCR and electrophoresis (Mulberry [M], Decatur [D]).

Sample # (bobcat)	[DNA] ng/µL	Pos	Neg	Sample # (bobcat)	[DNA] ng/µL	Pos	Neg
M-1	40	X		M-31	10	X	
M-2	300	×		M-32	110	X	
M-3	193		X	M-33	393	X	
M-4	163	X		M-34	270		X
M-5	137	X		M-35	69	X	
M-6	20	X		M-36	40	X	
M-7	87	X		M-37	80	X	
M-8	77	X		M-38	103	×	
M-9	50	X		M-39	143	X	
M-10	239	X		M-40	117	X	
M-11	20		×	M-41	60	X	
M-12	23	X		M-42	183	X	
M-13	125	×		M-43	283	X	
M-14	383	×		M-44	23	X	
M-15	13	×		M-45	63	X	
M-16	17	X		M-46	120	×	
M-17	123	×		M-47	196	×	
M-18	117	X		M-48	50	X	
M-19	83	X		M-49	50	x	
M-20	17	X		M-50	37	×	
M-21	53	X		M-51	37	X	
M-22	147	X		M-52	56	X	
M-23	77		×	M-53	420	X	
M-24	118	×		M-54	107		×
M-25	57	X		M-55	27	×	
M-26	196	X		M-56	67	X	
M-27	156	X		M-57	190		X
M-28	93	X		D-1	238	×	
M-29	246	×		D-2	330	×	
M-30	107	X		D-3	78	X	



**Fig. 1.** An example of a finished gel (Neo and Teddy were known negatives, M stands for Mulberry and D stands for Decatur as source regions of the bobcats and Hyperladder IV was a molecular weight marker).

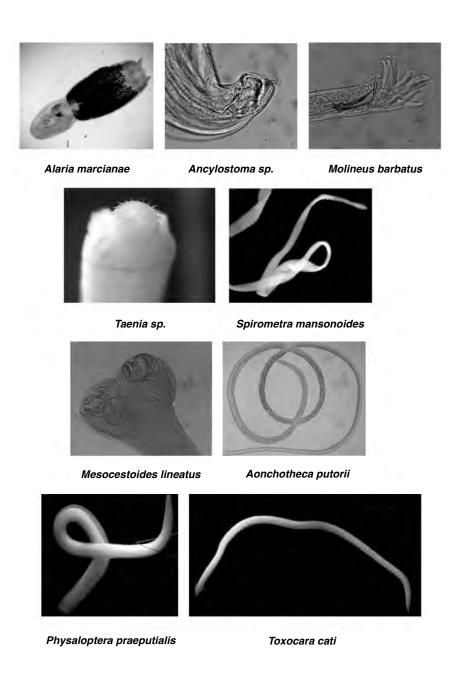


Fig. 2. Photographs of the helminths recovered in this survey.

## Effects of diesel and biodiesel blends on engine performance and efficiency

Christopher L. Hunt\*, Donald Johnson<sup>†</sup>, and Don Edgar<sup>§</sup>

### **ABSTRACT**

Tests were conducted during the summer of 2009 on a John Deere 3203 diesel tractor to determine differences in specific fuel consumption (sfc), power take-off (PTO) torque (Nm), and PTO power (kW), between ultralow sulfur No. 2 Diesel (D2), 20% biodiesel (B20), 50% biodiesel (B50), and 100% biodiesel (B100). Four 1-hr tests were conducted with D2, while three 1-hr tests were conducted with B20, B50, and B100. The results indicated that there was no significant (p < 0.05) difference between D2 and B20 for power or torque. Fueling with B50 resulted in significantly lower power and torque than fueling with D2 or B20, but significantly higher power and torque than fueling with B100. There were significant differences between each fuel in sfc; as the biodiesel blend increased, sfc also increased. Based on these data, B20 appears to be the optimal biodiesel blend for this and similar compact utility tractors since fueling with B20 resulted in no significant loss in power or torque (compared to D2) and only a slight increase in fuel consumption.

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Christopher Hunt

I am currently a senior majoring in agricultural education, communication and technology. I graduated from Paragould High School in 2007 and enrolled at the University of Arkansas that fall. I was awarded the Ring Scholar in 2008 and several college scholarships. I am a member of Collegiate FFA/4-H, Agricultural Mechanization Club, Alpha Zeta, and AEED REPS.

During the summer of 2009 I began working with Dr. Don Johnson and Dr. Don Edgar on a research project concerning biofuels and their effects on engine performance. Through this research, I have gained many valuable experiences. The research that was conducted has allowed me to compete in the Gamma Sigma Delta poster contest. I plan to continue my education in graduate school after receiving my B.S. degree.

### INTRODUCTION

With the increasing cost of petroleum-based fuels and U.S. dependency on them, the agricultural equipment industry is attempting to produce more compact utility equipment capable of operating on biodiesel and biodiesel blends (Cousins, 2006). Research is needed to determine the optimal blends of petroleum diesel and biodiesel for use in these vehicles.

Biodiesel is a renewable fuel manufactured from vegetable oils, cooking greases and oils, or animal fats (DOE, 2006). More technically, biodiesel is defined as "a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats, designated B100, and meeting the requirements of ASTM D6751" (NBB, 2007). ASTM D6751 establishes specific laboratory tests, methods, and minimum standards for biodiesel quality. Most current U.S. biodiesel is produced from the methyl ester of soybean oil, since this oil crop is available in sufficient quantities to supply the national market (Canakci and Van Gerpen, 2003).

Researchers (Proc et al., 2006; Canakci and Van Gerpen, 2003; and Schumacher et al., 2001) have found little difference in power performance, specific fuel consumption, or thermal efficiency between engines fueled with No. 2 petroleum diesel (D2) or with blends of up to 20% biodiesel and 80% D2 (B20). Decreased power and increased specific fuel consumption have been found in engines fueled

with neat biodiesel (B100), since it contains approximately 12.5% less energy than D2 (DOE, 2006).

This purpose of this study was to determine if there were significant (p < 0.05) differences in power take-off (PTO) power, PTO torque, or specific fuel consumption (sfc) for a compact diesel tractor fueled with D2, B20, B50, and B100 under variable load conditions. The findings from this study may be used in selecting the optimal biodiesel blend for compact diesel tractors similar to the one used in this study.

### **MATERIALS AND METHODS**

Test Fuels. One 18.93-liter container of each test fuel was provided by FutureFuel Chemical Co. (Batesville, Ark). FutureFuel is certified as a BQ-9000 producer by the National Biodiesel Board. BQ-9000 is a voluntary certification program of the National Biodiesel Accreditation Commission that ensures that accredited producers meet rigorous quality standards for production, sampling, storage, and testing of biodiesel. Samples of each fuel were collected and transported to the FutureFuel laboratory for analysis (Table1).

A John Deere 3203 compact utility tractor (Deere and Co., Moline, Ill.) with a Yanmar three cylinder, four-stroke compression-ignition diesel engine was used to test all fuels. Engine load was manually applied using an AW NEB-400 (Colfax, Ill.) PTO dynamometer. An auxiliary

fuel tank and Ohaus D-35 digital platform scale (Pine Brook, N.J.) were used to measure mass fuel consumption. The fuel system was drained and the fuel filter was replaced prior to testing each fuel. For each test, the engine was warmed up under moderate dynamometer load. After warm-up, the load was removed and the governor control was set to high idle speed (approximately 2970 engine RPM). Dynamometer load was then applied decreasing engine speed to 2900 RPM and in subsequent 100 RPM decrements. This process continued until the tractor operated at peak torque output (1600 engine RPM). The load was held at each RPM for three minutes. Fuel weight was recorded at the beginning and end of each three-minute period. PTO torque (Nm), PTO power (kW), and RPM were logged automatically at one second intervals (1 Hz). Specific fuel consumption was calculated as kg/kWh. Ambient environmental conditions were monitored to ensure tests were conducted in compliance with the OECD Tractor Test Codes (OECD, 2006).

Before switching fuels, the auxiliary fuel tank was drained completely and filled with the next test fuel. The engine was then operated for 10 minutes with all return line fuel collected in a separate tank. After the fuel system was purged, the fuel filter was changed and the bypass line was reconnected to the auxiliary fuel tank for the next test fuel. Data were analyzed using analysis of variance procedures.

### RESULTS AND DISCUSSION

There were significant (p < 0.0001) differences between fuels in PTO power, PTO torque, and specific fuel consumption when the effect of engine speed was held constant (Table 2). There were no significant differences in either power or torque between D2 and B20. However, fueling with B50 resulted in significantly less power and torque than either D2 or B20, but significantly more power and torque than B100. There were significant differences in specific fuel consumption between each of the four fuels. Specific fuel consumption increased as the percentage of biodiesel increased. This increase in specific fuel consumption was consistent with the decreased energy content of the fuels as compared to D2. Figures 1-3 present PTO power, torque and specific fuel consumption for each fuel across engine speeds. These figures indicate that differences between the fuels previously described were consistent regardless of tractor load.

The results of tests performed support previous research that little difference in power, torque, and specific fuel consumption results when an engine is fueled with D2 or B20 (DOE, 2006). Conversely, there are significant differences when engines are fueled with blends of biodiesel containing greater than 20% biodiesel compared to those with less than 20% biodiesel.

In this study the researcher did not examine or take into account the potential differences in engine wear, fuel system degradation, or start temperatures or other issues associated with biofuels. Consumers should take these issues into account and consult the manufacturer's warranty and recommendations in selecting biodiesel blends for use in diesel engines.

### CONCLUSION

Switching to B20 biodiesel for use in compression ignition engines could result in no significant difference in performance (power or torque) with only a slight (2.8%) increase in specific fuel consumption. However, use of B50 and B100 resulted in a significant decrease in torque and power and a significant increase in specific fuel consumption compared to D2. Thus, blends higher than B20 are not recommended for this or similar compact diesel tractors unless the decreased performance and increased fuel consumption are offset by reduced fuel costs.

### **ACKNOWLEDGEMENTS**

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Table 1. Chemical and Physical Properties of No. 2 petroleum diesel (D2), 20% biodiesel blend (B20), 50% biodiesel blend (B50), and 100% biodiesel blend (B100).

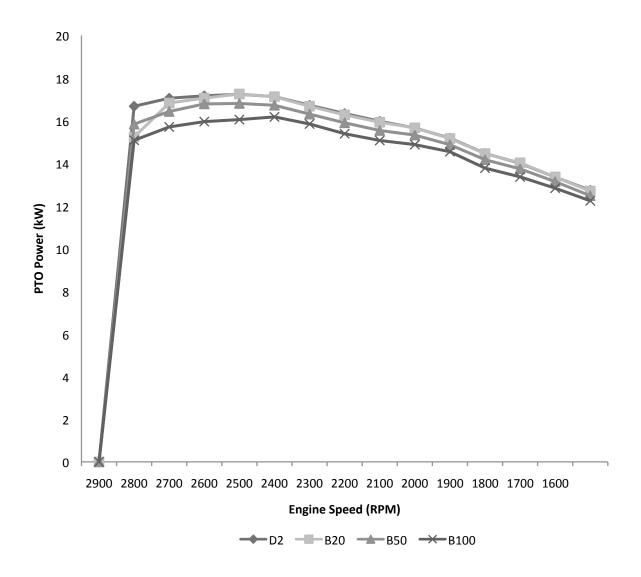
Fuel Property	D2	B20	B50	B100
Heat of Combustion (MJ/kg)	45.66	44.16	42.09	40.30
Specific Gravity (@15C)	0.85	0.85	0.86	0.88
Sulfur (ppm)	8.70	6.40	6.30	$NA^1$
Free Glycerin (%)	NA <sup>1</sup>	Not Detectable	Not Detectable	0.01
Total Glycerin (%)	NA <sup>1</sup>	0	0	0.01
lodine Number	1.60	13.40	27.10	48.90
Viscosity (CS@40C)	2.49	2.62	3.20	4.55

<sup>&</sup>lt;sup>1</sup>NA stands for not applicable because the fuel type does not contain these properties.

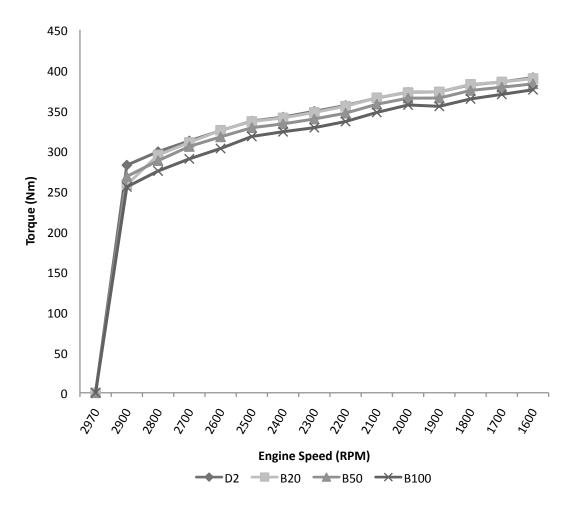
Table 2. Mean PTO power, torque, and specific fuel consumption for tractor fueled with No. 2 petroleum diesel (D2), 20% biodiesel blend (B20), 50% biodiesel blend (B50), and 100% biodiesel blend (B100).

		<i>j</i> ,	(= 100)
	Torque	Power	Specific fuel consumption
Fuel Type	(Nm)	(kW)	(kg/kWh)
D2	324.71 A <sup>1</sup>	14.64 A	0.286 A
B20	322.4 A	14.52 A	0.294 B
B50	316.75 B	14.27 B	0.303 C
B100	306.56 C	13.80 C	0.326 D

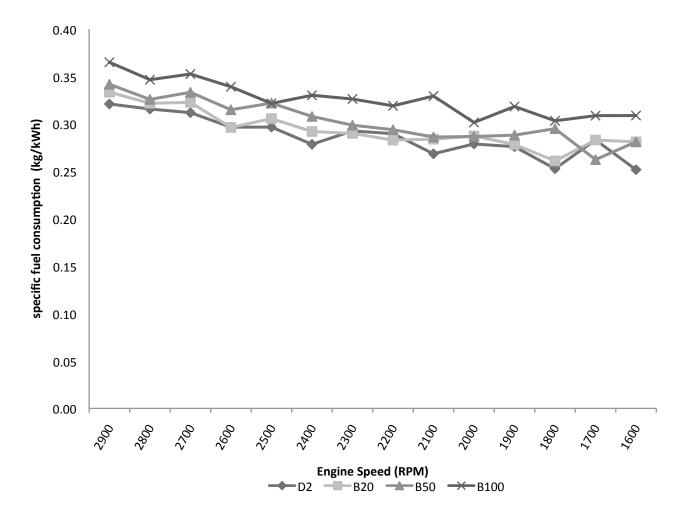
<sup>&</sup>lt;sup>1</sup> Means in the same column with the same letter are not significantly different at the 0.05 alpha level.



**Fig. 1.** Power take-off (PTO) power by engine speed for tractor fueled with No. 2 petroleum diesel (D2), 20% biodiesel blend (B20), 50% biodiesel blend (B50), and 100% biodiesel (B100).



**Fig. 2.** Power take-off (PTO) power by engine speed for tractor fueled with No. 2 petroleum diesel (D2), 20% biodiesel blend (B20), 50% biodiesel blend (B50), and 100% biodiesel (B100).



**Fig. 3.** Specific fuel consumption for tractor fueled with No. 2 petroleum diesel (D2), 20% biodiesel blend (B20), 50% biodiesel blend (B50), and 100% biodiesel (B100). Specific fuel consumption is calculated as kg/kWh.

# Narcissism, relationship satisfaction, and emotional intelligence among female college students

Chelsea D. Link\* and William C. Bailey<sup>†</sup>

### **ABSTRACT**

Emotional intelligence and narcissism have an influence on the overall relationship satisfaction people have with their significant others. Researchers have reported that as emotional intelligence increases, so does relationship satisfaction. However, researchers have also reported that as narcissism increases, relationship satisfaction decreases. No previous study has examined all three concepts together, which is the purpose of this study. Female college students (N=169) were given a questionnaire comprised of measures to assess emotional intelligence, narcissism, and relationship satisfaction. Correlation analysis determined there was a weak relationship between emotional intelligence and narcissism (r=0.28). Regression analysis found no relationship between emotional intelligence, narcissism, and relationship satisfaction with the student's significant others.

<sup>\*</sup> Chelsea Link received a B.S. degree in May 2010 with a major in Human Development, Family Sciences, and Rural Sociology. This paper is based on her undergraduate honors research project for which she received funding through a Student Undergraduate Research Fellowship from the Arkansas Department of Education.

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Chelsea Link

I am from Shawnee, Kansas, and a graduate of Shawnee Mission Northwest High School. I graduated from the University of Arkansas with a Bachelor of Science in Human Environmental Sciences. My major was Human Development, Family Sciences, and Rural Sociology with a concentration in Lifespan. This fall I will be attending graduate school at Kansas State University where I will obtain a master's degree in Marriage and Family Therapy.

During my four year career at the University of Arkansas, I was involved in several campus organizations. I was a member of Gamma Beta Phi for three years. During those three years, I was treasurer for one year and president for another year. I also was a member of Phi Upsilon Omicron and was treasurer for this organization for one year. During the summer semester of 2008, I studied in Paderno del Grappa, Italy.

This project was inspired by my desire to learn more about relationships. Since I plan on pursuing my goal of becoming a Marriage and Family Therapist, I was interested in discovering more about the dynamics of a relationship.

I would like to thank my committee, Dr. Jean Turner and Dr. Duane Wolf, for their support. I would also like to send a special thank you to my thesis mentor, Dr. William Bailey.

### INTRODUCTION

Many individuals strive to achieve high levels of relationship satisfaction in their important relationships, especially within one that is romantic. Those who study relationships have determined emotional intelligence (Keaten and Kelly, 2008) and narcissism (Twenge and Campbell, 2009) are two of many attributes that have a role in the satisfaction a person experiences in a relationship. Emotional intelligence and narcissism seem to have completely different influences on the levels of relationship satisfaction a person experiences. Keaten and Kelly (2008) found that high relationship satisfaction was related to high levels of emotional intelligence. Simultaneously, with the discovery of the role of emotional intelligence, Twenge and Campbell (2009) determined that people involved in relationships with narcissistic individuals have a low level of relationship satisfaction. Despite previous research findings that emotional intelligence has a positive influence and that narcissism has a negative impact on relationship satisfaction, no research has been done linking all three factors.

Research has shown that various factors influence relationships and the satisfaction people obtain from them. This research focused on female college students' satisfaction with their significant-other relationship. The research goal was to determine the role of the respondent's level of emotional intelligence and narcissism (Twenge and Campbell, 2009; Keaten and Kelly, 2008) in regard to her

satisfaction with her partner. This project examined the relationship between emotional intelligence, narcissism, and relationship satisfaction (Fig. 1).

Emotional Intelligence. One factor expected to influence relationship satisfaction is emotional intelligence. In this study, emotional intelligence is defined as a skill. The foundation for emotional intelligence is the concept of being able to understand one's own individual emotions (Schutte et al., 1998). Only a few studies have examined emotional intelligence and relationship satisfaction together. Keaten and Kelly (2008) found that relationship satisfaction is higher when a person has high emotional intelligence. Positive social interactions and better interpersonal factors like healthier relationships are more likely when someone has high levels of emotional intelligence (Mayer et al., 2004).

Narcissism. Another factor being examined in this study is narcissism. Narcissism is a "complex of personality traits and processes" (Ames et al., 2006). In a study that looked at the various aspects of narcissism across cultures, Munro et al. (2005) defined narcissism in a general way as "being unpleasant to others in the pursuit of one's own goals." Research has shown that narcissistic tendencies do influence overall relationship satisfaction. Twenge and Campbell (2009) found that relationships that involve a narcissistic individual are generally less satisfying. Boldt (2007) found that people in relationships with narcissistic individuals tend to have a less satisfying life overall. Recent research has also found that the rate of narcissism is increasing in

the population, especially among college students (Twenge et al., 2008). Twenge and Campbell (2009) predict that this generation of college students will have more problems with relationships than any previous groups of college students.

Relationship Satisfaction. The dependent variable in this study is relationship satisfaction. Vaughn and Baier (1999) claimed that relationship satisfaction is very subjective, making it difficult to have a definitive definition. The researchers stated that someone has achieved high relationship satisfaction if he or she evaluates the relationship as meeting or exceeding certain standards. Furthermore, Furman and Buhrmester (1985) stated that relationship satisfaction has to do with how happy a person is with the way things are in their relationship with another person.

### **MATERIALS AND METHODS**

A questionnaire was compiled using items from three existing, reliable and valid questionnaires. The Emotional Intelligence Scale, or the EIS (Schutte et al., 1998), was used to evaluate emotional intelligence. The scale was developed from Salovey and Mayer's model of emotional intelligence that included questions about appraisal, regulation, and utilization of emotions. Schutte et al. (1998) suggested that the EIS be used when exploring emotional intelligence and when examining the effects of emotional intelligence on other factors.

The 16-item Narcissistic Personality Inventory, or NPI-16 (Ames et al., 2006), was used to evaluate narcissism in the respondents. The NPI-16 is a valid alternative to the longer NPI-40, to be used when the longer inventory is not appropriate. The NPI-16 comprises several different measures in a shorter inventory to accurately measure various components of narcissism. Ames et al. (2006) report good reliability and high levels of validity on both the NPI-16 and the NPI-40.

The Network of Relationships Inventory, also called the NRI (Furman and Buhrmester, 1985), was used to evaluate relationship satisfaction. The NRI examines various characteristics of relationships as well as various types of relationships. Furman and Buhrmester (1985) have established the NRI's validity and reliability for accurately measuring relationship satisfaction. Demographic questions, such as age and relationship satisfaction, were also included at the end of the questionnaire.

The study used a convenience sample of 169 female students attending a large southeastern, public university. The data were collected using students who were enrolled in a variety of classes at the University Arkansas. The survey was placed online and an email with a hyperlink to the location of the survey was sent to the students in enrolled in the classes. In some of the classes, the students were given extra points for participating in the survey while oth-

ers were not, depending on the class. The survey remained open for two weeks in order to allow the respondents time to complete the survey. The data collected by the survey were downloaded and SPSS (SPSS Inc, Chicago) was used for correlation and regression analysis.

### **RESULTS AND DISCUSSION**

Respondents ranged in age from 19 to 25 (M = 20.64) (Table 1). More than half of the respondents reported being in a committed relationship, which included being in a serious dating relationship or marriage (64.5%, n = 109). Out of the 169 respondents, 35.5% reported they were casually dating (n = 60).

In order to make conclusions about the relationship between emotional intelligence, narcissism, and relationship satisfaction, the three variables were examined separately. The sample reported a mean score of 125.03 for EIS with a standard deviation of 10.122 (Fig. 2). On the NPI-16, the respondents had a mean of 4.85 and a standard deviation of 3.388. For relationship satisfaction, the sample had a mean score of 12.13 with a standard deviation of 3.296. The number of respondents in this inventory was 158 instead of 169 due to missing data from 11 respondents.

In order to determine any relationship between emotional intelligence, narcissism, and relationship satisfaction, a correlation was performed using the scores from the questionnaires. The results from the correlation analysis revealed that there were little or no relationships between the variables (Table 2). There was no statistically significant relationship between emotional intelligence and relationship satisfaction. In a similar manner, the relationship between narcissism and relationship satisfaction was nonexistent. However, the correlation revealed a weak relationship between emotional intelligence and narcissism (r = 0.28, p < 0.001).

A regression analysis was conducted. The regression used the Emotional Intelligence Scale scores as well as the Narcissistic Personality Inventory scores as the independent variables. The dependent variable was the score of each respondent on the Network of Relationships Inventory Relationship Satisfaction score. The results of the analysis were not significant (Table 3). It was determined that emotional intelligence and narcissism did not predict the relationship satisfaction for these respondents. Further research is needed to determine the predictors of relationship satisfaction.

Although previous research (Twenge and Campbell, 2009) suggests that narcissism does have an influence on relationship satisfaction, this study did not find one. However, the study did find that the level of emotional intelligence a woman has is slightly related to her narcissistic tendencies. This was an unexpected finding. More research

is needed to determine if this relationship between emotional intelligence and narcissism is an unusual finding or is more prevalent than would seem based on previous research. Based on this study, for which a very low percentage of variance could be accounted, it can be concluded that there are other factors besides emotional intelligence and narcissism that have a large impact on overall relationship satisfaction for college-aged women.

Future research should examine the same research question, but with a different sample. A limitation to this study was the lack of a diverse sample. A more diverse sample in all aspects, from gender to geographical location, could lead to different findings. It is logical to assume that emotional intelligence and narcissism are both impacted by development throughout adulthood. Therefore, varying the age range of the participants will provide an opportunity to explore the three characteristics from a developmental dimension. Also, exploration of additional personality characteristics and their relationship to narcissism and emotional intelligence should be examined in order to gain a better understanding of how narcissism and emotional intelligence function relative to relationship satisfaction. Increasing exploration of factors such as these can hopefully lead to the development of effective interventions leading to higher relationship satisfaction.

### **ACKNOWLEDGEMENTS**

I would like to thank Dr. William Bailey for all of his support, advice, and motivation throughout my thesis project. I would also like to extend a thank you to Drs. Jean Turner and Duane Wolf for their support and assistance. Financial assistance was provided by SURF.

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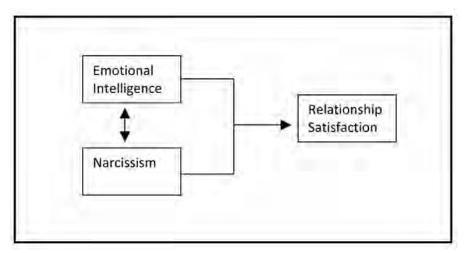
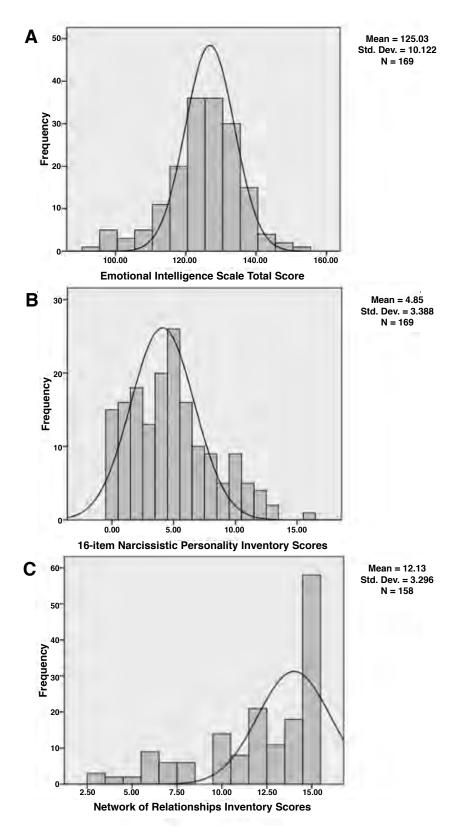


Fig. 1. Theoretical model of the association of emotional intelligence and narcissisism to relationship satisfaction.



**Fig. 2.** Charts and statistics of (A) emotional intelligence, (B) narcissism, and (C) relationship satisfaction of respondents.

Table 1. Demographic frequencies of female college students surveyed to explore relationship satisfaction, emotional intelligence and narcissism

Age	Frequency	Percent			
19	37	21.9			
20	56	33.1			
21	38	22.5			
22	20	11.8			
23	7	4.1			
24	8	4.7			
25	3	1.8			
Total	169	100.0			

Table 2. Correlation coefficients and sample size between emotional intelligence, narcissism, and relationship satisfaction

Concept	Emotional Intelligence	Narcissism	Relationship Satisfaction
Emotional Intelligence	1	0.28, N = 169	0.07, N = 158
Narcissism		1	-0.08, N = 158
Relationship Satisfaction			1

Table 3. Regression of Emotional Intelligence, Narcissism, and Relationship Satisfaction from Emotional Intelligence Scale and Narcissistic Personality Inventory

_	Unstandardized Coefficients		Coefficients	_	
Model	В	Standard Error	Beta	t	Significance
Constant	8.421	3.337		2.523	0.013
Emotional Intelligence	0.033	0.027	0.102	1.228	0.221
Narcissism Score	-0.100	0.079	-0.105	-1.262	0.209

# Industry professionals' perceptions of crisis communications educational needs for new professionals and best practices for Second Life® simulations

Kristin M. Pennington\* and Leslie D. Edgar<sup>†</sup>

### **ABSTRACT**

Crises impacting agriculture cost the nation billions of dollars in expenses and lost revenues annually. Organizations and governmental agencies continue to refocus energies on improving crisis communication plans in an effort to lessen economic impacts of unanticipated events. This study brought together an advisory team of agricultural communications professionals to gather perceptions of crisis communications educational needs for new professionals and to identify the best practices for using Second Life<sup>®</sup> (SL), a 3-D virtual world, simulations for training. Advisory team members represented the human, crop, animal, and environmental sectors of the agricultural industry. Perceptions were gathered during a roundtable, open-ended discussion using questioning techniques that progressed from comfortable, easy-to-answer questions to those that required analytical thought. Participants' comments and discussion remarks were analyzed using a technique to compress similar words into like categories and identify emergent themes. Four emergent themes were noted: 1) Pre-Planning; 2) During Crisis Communications / Actions; 3) Post-Crisis Communications / Actions; and 4) Individual Competencies Needed. Furthermore, multiple scenarios including environmental and product/food safety for SL simulations were noted. Findings from this study were used to identify educational objectives for training professionals in agricultural communications dealing with potential crisis situations.

<sup>\*</sup> Kristin M. Pennington is a 2010 graduate in from the Department of Agricultural and Extension Education.

<sup>†</sup> Leslie D. Edgar, the mentor for this project, is an assistant professor in agricultural communications in the Department of Agricultural and Extension Education.



Kristin Pennington

I am a native of Rogers, Arkansas, and graduated from Rogers High School in 2005. I transferred to the University of Arkansas as a junior Agricultural Education, Communication and Technology (AECT) major with an emphasis in Agricultural Education. Later, I declared a minor in Agricultural Systems Technology Management. I have been active in multiple student clubs including Ag Mech, AEED Reps, and Collegiate FFA/4-H. I am also a member of Alpha Zeta and Gamma Sigma Delta.

I plan to begin an M.S degree program in Agricultural and Extension Education in fall of 2010 under Dr. Leslie Edgar with continued research using Second Life<sup>®</sup>, a 3D virtual world for education purposes.

I would like to give a special thanks to Dr. Edgar, the mentor for this project, for her help and encouragement. She has opened many doors for me through this project, and I am forever grateful. In addition, I would like to thank all the faculty and staff of the Agricultural and Extension Education Department for their support.

### INTRODUCTION

We live in a society continually affected by natural disasters, such as hurricanes, tsunamis, and forest fires, and by organizational crises, such as food-borne illnesses, corporate malfeasance, and terrorism . . . No community and no organization, public or private, is immune from crises (Ulmer et al., 2007)

Crises have been called "predictably unpredictable" but effective managers know that crises can occur, they just do not know when (Heath and Millar, 2004). Unfortunately, the number of crises impacting citizens and the agricultural and life sciences areas is increasing. In the U.S., the number of Food and Drug Administration (FDA) product recalls, market withdrawals, and safety alerts have increased 10-fold from 36 FDA public notices in 1999 to 388 in 2007 (Buzby et al., 2008). These include food for human consumption, animal/pet food, and drug/personal care products.

The economic impact of recalls can be staggering. In 2008 the largest beef recall in U.S. history occurred when the USDA's Food Safety and Inspection Service (FSIS) stated that 143,383,823 pounds of raw and frozen beef products from the Westland/Hallmark Meat Company in Chino, Calif., were unfit for human consumption because of improper inspection by USDA food safety personnel (Eamich, 2008). Additionally, recent pet food recalls involved and impacted the human food supply (Paulman, 2008).

Agriculture-related crises are not limited to food recalls. Widespread pet food recalls consisting of many brands of cat and dog foods were initiated in 2007 (Paulman, 2008). The recalls in North America, Europe, and South Africa came in response to reports of renal failure in pets, and the economic impact on the pet food market was extensive, with Menu Foods alone losing \$42 million without taking into account reduced sales (Henderson, 2007). Animal diseases affecting humans have also been at the forefront of crises in recent times. The World Bank estimated in 2008 that the swine flu pandemic would cost \$3 trillion and result in a nearly 5 percent drop in world gross domestic product (Carey, 2009).

The ability to emerge from crises is dependent on an organization's ability to effectively and efficiently manage through the crisis event. Unfortunately, few organizations are prepared to deal effectively with crises. As of 2005, 60% of all major organizations had a crisis management plan, but only 38% of these organizations had trained key personnel in crisis management skills (AMA, 2005).

The ability to provide interactive, real-world scenarios can reduce the impact and cost of agricultural crises. A wide range of situations requires communicators to handle real and perceived crises involving public health, food safety, and environmental quality. New and experienced communicators need knowledge and skills to prepare for crises, rather than to react to them. Traditional classroombased instruction can provide a cognitive understanding

of case studies and a review of relevant theory, but it does not provide students with experiential opportunities to develop the competence, deep understanding, and positive attitude that will result in comprehensive communication plans to respond effectively to a crisis.

Second Life® (SL) (Linden Research, Inc., San Francisco, Calif.) is a 3-D virtual world that allows anyone to build an interactive experience or create educational training. In 2005, support systems were created to assist educators in SL. By August 2006, more than 80 SL islands (areas controlled by a specific organization or group) were dedicated to educational and academic use (Livingstone and Kemp, 2006). Educators have identified several characteristics that make SL a valuable educational environment: (a) strong sense of collaborative community, (b) interoperability, (c) interactive learning experiences that are hard and expensive to duplicate in real life, and (d) the ability for true collaboration (Bransford and Gawel, 2006).

The purpose of this study was to interview industry professionals to determine perceptions of educational needs for new professionals dealing with crisis communications and outline best practices for SL crisis communication simulations.

## **MATERIALS AND METHODS**

This exploratory, qualitative based research utilized an advisory team consisting of six professionals. The advisory team was comprised of representatives from production agriculture (livestock and crop), human sciences, and natural (environmental) resources. Participants were chosen to broadly represent the focus of the initial research. The advisory team participated in a roundtable, open-ended discussion where questions were provided by the researchers and conversations ensuing were recorded. Following the discussion, participants' comments and discussion remarks were analyzed.

A literature review by Creswell (2009) found that data from qualitative studies are descriptive and reported as words rather than numbers. General characteristics of this qualitative study reflect those identified by Dooley and Lindner (2002) as appropriate and acceptable methods for studying a phenomenon when:

The natural setting is the direct source of data (qualitative) versus a "snapshot" in time (quantitative); data are collected holistically from a participant's perspective (qualitative) versus relying on a participant's quantitative response (quantitative); the process (qualitative) as well as the variables of interest (quantitative) are considered; data is analyzed inductively (qualitative) versus deductively (quantitative); and data attempts to capture concern for a participant's behavior, attitude, reason, or motive (qualitative) (Dooley and Lindner, 2002)

With any research study, it is important to establish internal and external validity, reliability, and objectivity. In qualitative research, these quantitative based terms are referred to as credibility, transferability, dependability, and confirmability. For this study, credibility and dependability were established using triangulation. Member checks were conducted by providing respondents with a summary of the data to correct any misinterpretations. Transferability was established through the researcher's thick description of interpretations of the data allowing others interested in the study to draw conclusions. And finally, confirmability was established by conducting an audit trail. The researchers used a variety of qualitative methods to ensure truth value, applicability, consistency, and neutrality (Erlandson et al., 1993).

The study followed Krueger's (1998a, 1998b) method of questioning during the roundtable, open-ended discussion with the advisory panel. Researchers led participants through the discussion after the initial question "How does your company deal with crisis communication" was asked. The discussion progressed from comfortable, easy-to-answer questions to those that required analytical thought. Discussions were recorded and transcribed. Transcribed notes were then coded and themes, as outlined in the results, were identified (Creswell, 1998) to determine new crisis communications professionals' educational needs, best practices for crisis communications training, and possible SL simulations. For confidentiality reasons, quotes from team members have been coded using the group letter "A" and then each individual was assigned a number. For example, instead of a quote being noted to a person, it will be noted with quotations followed by "A#" in parentheses.

## **RESULTS AND DISCUSSION**

A set of best practices for training in crisis communication were identified based on emergent themes. Four emergent themes were identified: 1) Pre-Planning; 2) During Crisis Communications/Actions; 3) Post-Crisis Communications/Actions; and 4) Individual Competencies Needed (Table 1). The first three themes (Pre-Planning, During Crisis Communications/Actions, and Post-Crisis Communications/Actions) are necessary for an organization to be successful in both managing and responding effectively to a crisis. The final theme, Individual Competencies Needed, includes skills required for crisis communicators to support effectively and successfully an organization before, during and after a crisis situation. Within each theme, supporting evidence was identified. There were nine subcategories that supported the Pre-Planning emergent theme, two subcategories for the During Crisis Communications/Actions theme, two for the Post-Crisis Communications/Action theme, and eleven subcategories were identified for Individual Competencies Needed when dealing with a crisis.

Pre-Planning allows for organizations to take a proactive role in preventing and dealing with crisis situations. One participant of the advisory panel stated, "Communicators need an organized list of things to do and steps to take: analyze, identify options, have a plan of action, and analyze what you did" (A2). Effective analysis includes setting up a vulnerability assessment for the organization. A vulnerability assessment will identify possible/potential crises within an organization and also set a plan of action for communicators to follow during an agriculturally related crisis.

During Crisis Communications/Actions, communicators should act quickly and efficiently to "make people aware of what is happening to reduce the snowball effect" (A3). A plan of action established during Pre-Planning will delegate responsibilities during a crisis. Someone should be responsible for assessing media via multiple mediums (print, video, blogs, etc.). Tracking public perceptions and changes in perceptions will enable the company to repair or improve damaged perceptions in post crisis.

Post-Crisis Communications/Actions is the time to assess the organization's actions and the overall outcome from the crisis. This is the time to rebuild public perceptions and redevelop good media relations.

People on the front line of crisis communication must be able to communicate the message effectively. The advisory team clearly identified a set of individual competencies needed for crisis communicators. These competencies are needed in order for the pre, during, and post crisis themes to be successful. Individuals dealing with crises should write the crisis communication plan of action in the Pre-Planning stage. The plan of action will help communicators canvas all critical areas when a crisis occurs and know who to take their message to. Crisis communicators should be critical thinkers who are "able to assess if I do "?", what will happen" (A6). Additionally, individual competencies can be used for hiring or training agricultural communicators.

In addition to the four emergent themes, a set of guidelines preparing students and new professionals to use Second Life simulations were identified. It was noted that individual competencies needed should guide SL crisis communications training scenarios. Many possible SL crisis scenarios were outlined. The scenarios ranged from recent crises such as the Georgia peanut crisis to crises that occurred decades ago such as the Tylenol scare. Additionally, advisory panel members noted that getting students comfortable with "[video] gaming" (interactive technology skills) was a concern for students' ability to learn in the virtual world environment. One professional suggest-

ed, "Students with gaming experience will have increased adaptability and comfort with Second Life" (A4). After students are comfortable, both human and technological monitors will need to be available for additional assistance *in world* (a term used to indicate an individual is logged on to SL) for students to be successful.

Agricultural communications professionals can serve as effective instruments in identifying educational needs for new professionals dealing with crisis communications. This study identified educational needs and potential SL educational training scenarios for crisis communicators. Emergent themes from this study were used to identify educational objectives for training professionals in agricultural communications who deal with potential crisis situations. Dominant competencies from this study include developing and maintaining positive relationships with the media and knowing your audience specifically by needs to allow for quick crisis response. Along with personal competencies in crisis communications, the advisory panel indicated the need for all companies to have a preset plan, of which employees are aware, in place to implement as soon as a crisis occurs. Additional research should continue to identify crisis communication needs for new professionals and whether or not SL is an effective educational training platform.

Following this study, a needs assessment survey will be developed and administered to communication professionals dealing with crisis communications. These needs based studies will assist the researchers in creating a holistic crisis communications simulation in Second Life.

## **ACKNOWLEDGEMENTS**

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Table 1. Emergent crisis communication educational needs themes and supporting information.

Emergent Theme	Theme Subcategory	Supporting Quotes From the Discussion			
Pre-Planning	Build a positive image of your company/organization	"Unknown companies or companies without a name will find themselves behind the curve."			
		"Need to get back to building a trust bank."			
		"Take a proactive strategy, get to know agriculturalists."			
	Build support relationships and networks	"Is there someone that is better to take the information/message to the audience."			
	Complete an organization vulnerability study	"Organizations should complete vulnerability assessments to identify potential crisis plans of action for each."			
	Create a crisis communication plan for action	"Communicators need an organized list of things to do and steps to take: analyze, identify options, plan of action, ar analyze what you did."			
	Crisis team role identification and plan	"Every employee must know and follow their role."			
	Identify competency gaps with crisis	"Need to invest resources into training personnel."			
	communications team and look for training opportunities	"Communicators should be trained on good and bad ways handle crises."			
	Know how to identify warning signs	"Practitioners need to know when to act on a threat and w to ignore it."			
	Know your target audience and how to tailor the message	"There is a need to give audiences the information they need on sensitive topics and find the right way to present the information."			
		"Know the perception [of your audience] before a problem occurs."			
During Crisis Communications/Actions	Assess change in audience perceptions	"Assess what is happening in movies, print, blogs, etc."			
	Provide information immediately and continually	"Keep communicating with all sectors to keep relationship and get the message out."			
		"Make people aware of what is happening to reduce the snowball effect."			
Post-Crisis Communications/Actions	Ability to assess your audience(s) response / reaction to message(s)	"Keep communicating with all sectors to keep relationships used and get message out."			
		"Look to see what number of individuals got the messag believes us."			
	Improve audience perception of organization product	"There is a crisis every day. How do you spin and solve the problem."			
	Ability to create an effective crisis communications plan	"A protocol is essential because communication issues happen so we need to plan."			
	Ability to research	"Students need to have the ability to think and research."			

continued

Table 1. Continued.					
Emergent Theme	Theme Subcategory	Supporting Quotes From the Discussion			
Post-Crisis Communications/Actions (continued)	Ability to be an effective spokesperson	"Must go beyond talking points because they get old and need more general place to start. It is easy to get bogged down in the talking points."			
		"With audiences it is never safe to assume."			
	Adaptability	"Communicators need to deal with changes and plan for changes."			
		"Be able to deal with personnel."			
	Assess quickly and respond appropriately	"Communicators need to know if there is someone that is better to take the information, message to the audiences."			
Individual Competencies Needed	Create effective messages (educational pieces)	"When teaching students help them know what types of messages work and what can be said during a crisis.			
	Critical thinking	"Be able to assess if I do x what will happen. Need to develop a crisis instinct; what is a crisis and what can I do." "Being able to understand and respond to triggers."			
Individual Competencies Needed (continued)	Identify audiences(s)	"Being able to identify audiences is the most important."  "Communicators need to know influencer audiences, those that can cause increased impact on others."			
	Identify and gain media allies	"Identify your media allies and spin it [message] in a way they can help resolve and have scientists available for information."  "Know your allies, have a unified voice."			
	Media relationships	"Need media training to get out message."  "Training to help communicator: stay on target and cover key points."			
	Write efficiently and effectively under pressure	"Students need to be able to write efficiently, effectively under the gun."			

## Characterization and inheritance assessment of fruit and leaf shape in unique *Vitis* seedlings

Paul J. Sandefur\*, John R. Clark<sup>†</sup>, and Douglas Karcher<sup>§</sup>

## **ABSTRACT**

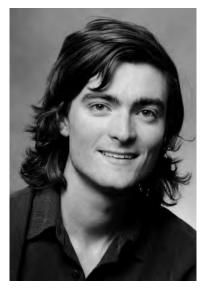
From August to October of 2009, two separate studies were conducted to assess fruit shape of Vitis section Euvitis hybrid bunch grapes using digital photography (Study 1) and evaluate inheritance of leaf shape in unique populations of V. rotundifolia hybrids (Vitis section Muscadinia) (Study 2). All vines studied were located at the University of Arkansas Fruit Research Station, Clarksville. Study 1 used SigmaScan® digital photography analysis software to calculate shape factor, major:minor axis ratio, and compactness of highly variable, unique-shaped fruit from a population of 182 Euvitis seedlings. SigmaScan® accurately characterized fruit shape elongation as had been recorded in previous studies. Although elongated shapes were measured accurately, the calculations used were unable to conclusively analyze ovoid or pear-shaped fruits. Study 2 evaluated the inheritance of leaf shape (lobing) in several populations of V. rotundifolia crosses within the University of Arkansas fruit breeding program. Based on previous studies, it was hypothesized that leaf lobing was a dominant trait. The two populations expected to segregate into a 3:1, lobed:standard, phenotypic ratio were successfully observed, while only two of the six expected to demonstrate a 1:1, lobed:standard, phenotypic ratio were observed. Previous studies suggest the unexpected ratios observed may be attributed to a lethal allele combination, where homozygous dominant progeny are not viable, or modifier genes impacted leaf shape of the seedlings. Further evaluation of these and other populations in addition to molecular analysis would be helpful in characterizing inheritance of leaf lobing in muscadine hybrids.

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D. Karcher is an Associate Professor, Department of Horticulture.

## MEET THE STUDENT-AUTHOR



Paul J. Sandefur

After graduating from Fayetteville High School in 2006, I began my undergraduate studies at the University of Arkansas. Throughout my time as an undergraduate, I received the Bob and Marilyn Bogle scholarship for horticulture students. While completing my degree, I had the chance to experience the horticultural sciences hands-on by working at the Fayetteville Farmers Market, hiking in the Ozarks, raising a vegetable garden, and participating in a three-month internship at the Scottish Plant Explorer's Garden in Pitlochry, Scotland. Thanks to funding from the Bumpers College undergraduate research program, I began work on an undergraduate special project course under Dr. John R. Clark at the Fruit Research Station, Clarksville, during my senior year. After completing my research and graduating with a B.S. degree in horticulture and a minor in global agriculture, food, and life sciences in December of 2009, I started my master's studies in the Department of Horticulture advised by Dr. Clark. I would like to thank Drs. John R. Clark, Douglas Karcher, and the staff at the Fruit Research Station, Clarksville for all of their help, as well as Drs. Curt Rom and Jon Lindstrom for their guidance during my undergraduate studies.

## INTRODUCTION

With over 7.2 million ha in production and 67.2 t being produced in 2007 worldwide, the grape is the world's most important fruit in terms of value and the second most important fruit crop in terms of overall production quantity (FAO, 2007). The history of grape production dates back to between 7000 BCE and 4000 BCE. Viticulture originated in the Middle East and quickly spread throughout the Mediterranean, reaching the North American coast by the late eighteenth century. Although the majority of grape production is processed for the wine industry, table grapes, raisins, and other processed products have been and continue to be major areas of production and research.

The grape family, Vitaceae, is made up of 14 genera, of which *Vitis* is the only genus utilized for food production. The genus *Vitis* is split into two sections, *Muscadinia* (2n = 40) and *Euvitis*, the bunch grape, (2n = 38). Although *Euvitis* heavily outweighs *Muscadinia* in its importance to world commerce, significant research has been conducted on both subgenera (Reisch and Pratt, 1996).

The grape breeding program at the University of Arkansas was started in 1964 by Dr. James N. Moore. In 1997, Dr. J.R. Clark replaced Dr. Moore as program director, and has continued and expanded the breeding objectives. The work at the University of Arkansas has resulted in the release of several table grape cultivars (*Euvitis*) including 'Reliance', 'Saturn', 'Venus', 'Mars', 'Jupiter', and 'Neptune' (Clark, 1997;

Clark and Moore, 1999a and b). Although muscadine grapes have been grown in Arkansas for many years, it was not until 2005 that a muscadine (*V. rotundifolia*) breeding effort was incorporated into the grape breeding program. The major focus of the muscadine program is to develop adapted cultivars with enhanced quality and winter hardiness for central and southern Arkansas (Clark, 2003, 2008).

Although the Arkansas muscadine breeding program focuses on environmental adaptation and fruit quality traits, one ornamental cultivar, 'Southern Home', has been used as a parent in the program. This hybrid release from the University of Florida was introduced for its unique ornamental value and potential disease resistance. 'Southern Home' is the only released hybrid between bunch grapes and muscadine grapes (Mortensen et al., 1994). The first population in Arkansas with 'Southern Home' as a parent was created in 2005, and this cultivar has since been crossed with other muscadine cultivars as well as a unique *Euvitis* hybrid, A-1665, developed in the University of Arkansas breeding program. The resulting populations included seedlings with unique-shaped leaves, exhibiting substantial lobing.

Fruit shape is also an important factor in the development of new grape cultivars, as high quality fruit with unique shapes can attract the attention of retail customers who are not enticed by standard oval grapes. Although fruit and leaf shape can be key traits in fruit breeding, little work has been done with shape as the specific breeding ob-

jective in the *Vitis* genus. Fruit with a unique shape and plants with unusual ornamental leaf characteristics have the potential to fill niche markets within fruit crop or ornamental plant production (Clark, 2003; Mortensen et al., 1994).

In 2005 Andrew Wycislo, M.S. student at the University of Arkansas, developed a simple method to characterize fruit shape using digital photography. The study compared digital findings to human raters and concluded that digital photography utilizing SigmaScan® software rapidly, simply, and accurately characterized fruit elongation with a strong correlation between the human subjective ratings and digital analysis. According to his report, SigmaScan® was able to differentiate elongation between berries in the population based on shape factor  $[(4 \times \pi^2)/(\text{perimeter})^2]$ , major:minor axis ratio, and compactness value [(perimeter) <sup>2</sup>/area]. This method was found to be more reliable than subjective visual assessment as variation in individuals in rating shapes was substantial (Wycislo et al., 2008). To further test the ability of digital photography to characterize fruit elongation and other diverse fruit shapes, a new population of hybrid table grapes was created and our study used this method to analyze this more diverseshaped population.

In breeding plants, the presence of genetic variation for traits, the ability to identify or classify this variation appropriately, and determining the type of inheritance are all very important components of an improvement program. Therefore, the two objectives of our study were to (1) characterize variation in fruit shape of a population of uniqueshaped bunch (*Vitis* section *Euvitis*) grapes using digital image analysis, and (2) evaluate variation in leaf shape in a series of populations derived from a muscadine grape cultivar ('Southern Home', *Vitis* section *Muscadinia*) and estimate the inheritance of this trait. Quantifying the type of inheritance exhibited and characterizing the variation in leaf and fruit shape should facilitate future breeding efforts attempting to develop the ornamental and commercial value of interspecific muscadine and bunch grape hybrids.

### MATERIALS AND METHODS

Study 1. The population studied was created from a 2005 cross of table grape selections A-2315 and V#1. The hybridization, seed collection, stratification, germination, and seedling production were all conducted at the University of Arkansas Fruit Research Station, Clarksville. During hybridization, emasculated female parent clusters were protected from contamination from foreign pollen by enclosure in a white, all-weather paper bag.

In August, 2009, 182 seedlings were examined and where possible, fruit was collected from each. Twenty five representative berries from each seedling bearing fruit and both

parents were collected, placed in freezer bags, and stored in a freezer until data were collected. Within the population, 79 seedlings either produced no fruit or could not be collected due to Green June beetle (*Cotinis nitida*) damage.

From early September to late October, ten representative berries from each seedling sample were removed from the freezer, cleaned of any frost, and placed on a fluorescentpink surface to be photographed. A Nikon D70S digital SLR camera (Nikon Corp., Tokyo, Japan) was suspended above the grape surface and set on the automatic 'A' mode with the flash turned off in order to prevent glare. One photograph of each seedling's 10 berries was taken, each using the same camera settings, downloaded to a computer, and labeled. The photographs were then uploaded onto a computer to be analyzed by SigmaScan® using the same macro and calculations used in Wycislo (2005), shape factor, major:minor axis ratio, and compactness. The data from each berry was placed in a Microsoft Excel® spreadsheet, and the minimum, maximum, and median of each variable were calculated. Once all data were collected, the results were compared to Wycislo's observations and visually assessed for accuracy of characterization of elongation (Wycislo, 2005).

Study 2. From 2005 to 2008, nine populations with over 1,000 seedlings collectively were produced and established at the Fruit Research Station, Clarksville, and all segregated for leaf shape. All nine populations were progeny of 'Southern Home' crossed with a variety of other muscadine parents and one *Euvitis* parent (Table 1). During hybridization, emasculated female parent clusters were protected from contamination with foreign pollen by enclosure in a white, all-weather paper bag.

From August through October 2009, each population was characterized for leaf shape. Based on an initial evaluation of variation, there were two distinct classes of shape, the lobed types (usually deeply lobed), and the standard muscadine shape (Fig. 1). Thus, characterization was for one type or the other. Leaf shape observations were based on the leaf shape of each seedling between the sixth and ninth leaves from a major shoot tip, which resulted in uniform mature leaf assessment on each seedling. From the data collected, the appropriate genetic analysis, chi-square, was used to evaluate the qualitative inheritance pattern found for leaf shape variation with a 95% confidence level. We hypothesized that the lobed leaf trait was dominant and the standard leaf was recessive within the populations, and would be represented by either a 3:1 or 1:1 ratio (Table 1).

## **RESULTS AND DISCUSSION**

Study 1. The Euvitis hybrid population studied in 2009 displayed a wide range of variation in fruit shape as indicated by the range in values for shape factor, major:minor

axis ratio, and compactness (Table 2). Some of the grapes within the population were highly elongated, almost banana-shaped, while others were very small and perfectly round, although the majority were oblong (Fig. 2).

According to Wycislo (2005) and based on my results, shape factor decreased with elongation, and major:minor axis ratio and compactness both increased with elongation. Even with our population containing more extreme shape variation, all three measurements utilized in the previous study and repeated in our study accurately characterized fruit shape. Overall, digital photography analysis utilizing SigmaScan® software is a simple, cost effective, and accurate means of characterizing fruit shape in grape. Although the major:minor axis ratio was unable to determine the exact major axis of the slightly curved, highly elongated berries, and was therefore less accurate at determining elongation than shape factor and compactness, it still provided a useful overall measurement for the population.

One of the limiting aspects of all my calculations was the inability of any of the ratings to judge ovoid or pear-shaped fruit. If the SigmaScan® macro can be programmed to measure the maximum minor axis and major axis as well as the minimum minor axis and major axis, there is strong potential for the program to more accurately measure even more unique fruit shapes. Future studies assessing the usefulness of SigmaScan® with additional calculations would be beneficial to plant breeders for characterizing fruit shape in a variety of crops. In Wycislo's concluding remarks, he hypothesized that because a majority of his grapes tended toward a round shape, "this might indicate a partial dominance toward round shape" (2005). Our results support Wycislo's hypothesis, as the majority (~70%) of the grapes observed were round or oblong.

Study 2. Overall, the leaf-shape segregation in the populations was easily classified into lobed and standard muscadine shapes. The only challenges in the classifications were that some seedlings had intermediate leaf shapes (Fig. 1). Although this might be attributed to a quantitative inheritance of leaf shape, or possibly some modifying genes, only approximately 10% of each population showed an intermediate nature making quantitative inheritance of the lobed trait unlikely.

Based on the chi-square analysis, the null hypothesis was rejected. Therefore, the proposed ratio of 3:1 in the two predicted populations, 'Southern Home' open pollinated and 'Southern Home' x A-1665; with the lobed genotype being dominant, was supported (Table 1). These populations were projected to segregate in this manner due to both likely being heterozygous for the lobed leaf trait. While 'Southern Home' open pollinated had the cultivar as the female parent, but an unknown pollen parent,

'Southern Home' x A-1665 was a cross of two lobed parents. One might question why the ratio was not closer to the projected 3:1 rather than the observed 2.1:1. A possible explanation for this was the likely presence of a homozygous dominant lethal in one-fourth of the progeny. If this were the case, the lobed allele is dominant with respect to phenotype, but behaves as lethal when homozygous dominant. Therefore, all progeny that are homozygous recessive would display a standard leaf shape, all heterozygous offspring would display a lobed leaf shape, and any homozygous dominant would abort before any plant development occurred. A 2:1 (lobed to standard leaf) segregation ratio has been reported in muscadine grape hybrids (P. Conner, pers. comm.). In saucer-shaped peaches, it was found that a homozygous dominant allele was lethal and was the cause of the unique observed ratios (Guo and Jiang, 2002).

The 1:1 null hypothesis ratio, standard shape being homozygous recessive and lobed being heterozygous, was supported by two of the six populations ('Black Fry' x 'Southern Home' and 'Southern Home' x 'Granny Val') (Table 1). The most obvious observations from the data were the unexpected ratios that predominated in the other four populations of 'Southern Home' crossed with standard muscadine cultivars. Further, two of the most extreme variations in segregations from the expected (1 lobed: 1 standard) of Ga. 33-2-1 x 'Southern Home' (1.0 lobed: 2.2 standard) and 'Southern Home' x 'Doreen' (1.7 lobed:1.0 standard) segregated in opposite directions. One might consider a maternal or paternal effect, but this was not consistent among the populations.

Overall, the hypothesized dominance of the lobed-leaf trait was successfully observed in the field and supported by half of the populations. To fully study the inheritance of the lobed-leaf trait, a crossing design such as a diallel, where all parents in the study are crossed with each other, would be appropriate. Also, molecular characterization of leaf shape would provide more precise information on this trait. Although our inheritance study leaves some questions unanswered, the lobed-leaf trait found within the University of Arkansas breeding program will be useful for continued fruit breeding work.

## **ACKNOWLEDGEMENTS**

Financial support for this project was provided by a grant from the Dale Bumpers College of Agricultural, Food, and Life Sciences undergraduate research program. I would like to thank Dr. John R. Clark for his advice and support, and Dr. Douglas Karcher for his technical expertise. Assistance from the staff at the Fruit Research Station, Clarksville, is also greatly appreciated.

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Table 1. Segregation of leaf lobing, expected and actual segregation ratios, and chi-square test results for crosses of 'Southern Home' lobed-leaf muscadine grape.

Population	Total seedlings	Lobed leaf %	Standard leaf %	Expected ratio	Actual ratio	Chi-Square Test
Southern Home (S.H.) x OP	84	67.8	32.1	3:1	2.1:1.0	Supports hypothesis
S.H x A-1665 (Euvitis)	153	66.0	34.0	3:1	2.1:1.0	Supports hypothesis
Supreme x S.H.	204	41.1	58.8	1:1	1.0:1.4	Rejects hypothesis
GA-33-2-1 x S.H.	107	30.8	69.1	1:1	1.0:2.2	Rejects hypothesis
S.H. x Doreen	181	63.5	36.4	1:1	1.7:1.0	Rejects hypothesis
Black Fry x S.H.	212	45.7	54.2	1:1	1.0:1.2	Supports hypothesis
Darlene x S.H.	156	34.6	65.3	1:1	1.0:1.9	Rejects hypothesis
S.H. x Granny Val	95	51.5	48.4	1:1	1.1:1.0	Supports hypothesis

Table 2. Overall distribution and median of fruit shape population for three SigmaScan® values.

SigmaScan® Calculations	Range	Median
Shape factor $[(4 \times \pi^2)/(\text{perimeter})^2]$	0.43 - 0.88	0.78
Compactness [(perimeter) <sup>2</sup> /area]	14.36 - 29.65	16.06
Axis ratio [major axis/minor axis]	1.17 – 4.10	1.72



**Fig. 1.** *Muscadinia* leaf types classified in Study 2. – Standard muscadine leaves lacked lobing on the leaf margins (a), while the lobed types (lobed and intermediate) had medium-deep lobing (b), which were found in a limited number of seedlings, to very deep lobing (c), the most common form of leaf lobing found.

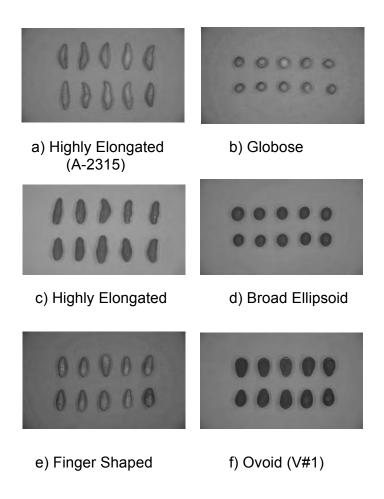


Fig. 2. Variation in characteristics of fruit shape in A-2315 (a) x V#1 (f) and some progeny.

# Effect of a direct-fed microbial (Eubios 1090) in the presence of antibiotics (Carbadox or CTC-Denagard) on post-weaning pig growth performance and immune response

Kimberly Santos\*, Charles Maxwell<sup>†</sup>, Elizabeth Kegley<sup>§</sup>, and Charles Rosenkrans<sup>‡</sup>

## **ABSTRACT**

A study was conducted to determine the effects of a probiotic (Eubios 1090), in the presence of two different antibiotics, on performance in nursery pigs. A total of 216 pigs were weaned at an average of 21 d, blocked by initial body weight (BW = 6.79 kg), and distributed into 32 pens of 6 to 7 pigs per pen in an offsite nursery facility. Pens were randomly assigned to 1 of 4 dietary treatments (8 pens per treatment) that were fed throughout post-weaning phase 1 (day (D) 0 to 10), phase 2 (D 10 to 20), and phase 3 (D 20 to 34). Dietary treatments were: 1) Carbadox without Eubios 1090; 2) Chlortetracycline + Tiamulin (CTC-Denagard) without Eubios 1090; 3) Carbadox + Eubios 1090; and 4) CTC-Denagard + Eubios 1090. There was no interaction observed between the two antibiotics and addition of the probiotic. There was a tendency for greater gain to feed ratio (G:F) in phase 2 when nursery pigs received Carbadox compared to CTC-Denagard (P = 0.08), and a tendency for greater average daily feed intake (ADFI) in the overall nursery period when pigs were fed CTC-Denagard compared to Carbadox (P = 0.10). Pigs that received the non-Eubios 1090 diets had greater average daily gain (ADG), G:F, and body weight (BW) during phase 2 compared to pigs that received diets containing Eubios 1090 (P = 0.05). In phase 3, pigs receiving the Eubios 1090 diet had increased ADG and G:F (P = 0.05). Between the Carbadox diet and the CTC-Denagard diet, the diet containing CTC-Denagard increased ADFI throughout the 3 phases. In summary, probiotic supplementation demonstrated negative effects in phase 2 and positive effects to growth performance in nursery pigs during the latter part of early post-weaning (phase 3).

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<sup>†</sup> Charles Maxwell, faculty mentor, is a professor in the Department of Animal Science.

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<sup>‡</sup> Charles Rosenkrans is a professor in the Department of Animal Science.

## MEET THE STUDENT-AUTHOR



Kimberly Santos

I grew up as the daughter of a United States Army infantryman and have lived in seven different states. After graduating from Shawnee High School in Shawnee, Oklahoma, I accepted a four-year Army Reserve Officer Training Corps scholarship along with other numerous departmental and third-party scholarships at the University of Arkansas. I decided to study Animal Science and Equine Science in preparation for veterinary school. In doing so, I have fulfilled my goal in being involved on campus, in the College of Agricultural, Food and Life Sciences, and in the Army Reserve Officer Training Corps. I have been a member of the Meat Science Quiz Bowl, Block and Bridle, and the Poultry Science Club. Also, I have served as the secretary and president for the Pre-Veterinary Club and completed an internship at the Royal School of Veterinary Studies at the University of Edinburgh in Scotland. Honor societies that I am a member of and have held officer positions are Golden Key, Alpha Zeta, and Gamma Beta Phi Arkansas Chapter.

As a contracted scholarship cadet, I have spent my time as the color guard commander at university athletic events and community events, the captain and member of the Arkansas Ranger Challenge Championship team, and the president and competitor of the Pershing Rifles Marksmanship Association. Throughout my achievements, I earned the position as the Razorback Battalion Cadet Commander and be-

came the national recipient of the General George C. Marshall Award.

During my time volunteering then working at the University Swine Research Farm, my interest for studying nursery pigs grew. The pig's performance and health concerned me as well as motivated me to conduct research in the field. Upon graduation, I will be commissioned as a second lieutenant in the United States Army Veterinary Corps. I would like to give special thanks to Dr. Charles Maxwell, Dr. Jason Frank and Benjamin Bass for their ongoing support over the past two years.

## INTRODUCTION

Early weaning is a common procedure causing physiological stress due to abrupt changes in diet and environment (Dybkjær et al., 2006). The pigs' transition from the diet of sow's milk to solid feed is usually associated with poor average daily feed intake (ADFI) during the first week after weaning (Spring, 1999). This can cause an adverse impact on the gastrointestinal microflora that can lead to the nursery pigs becoming more susceptible to high numbers of potentially pathogenic bacteria such as Escherichia coli and a decline in favorable lactobacilli (Bolduan, 1999). To ensure the gut has sufficient numbers and species of microbes that can benefit growth performance of the early-weaned pig, diets may contain probiotics, a direct-fed microbial (DFM) supplement. Direct-fed microbial supplements can result in a positive effect on the gastrointestinal microflora and subsequent growth performance of the animal.

A probiotic is a feed additive containing bacterial organisms that contribute to the health and balance of the

intestinal tract when ingested (Roselli et al., 2005). It has been reported that probiotic factors inhibit adhesion and viability of known enteric pathogens. This suggests that probiotics could be a rich source of new antipathogenic compounds (Howarth, 2009). Research indicates that dietary probiotics may serve as a partial replacement for dietary antibiotics (Roselli et al., 2005). The mechanisms by which dietary antibiotics or probiotics improve growth performance have not been completely clarified, but have been studied at the University of Arkansas. It was concluded that the supplementation of phosphorylated mannans (yeast cell wall) to the newly weaned pig can serve as a growthenhancing diet additive (Davis et al., 2004a). Because direct-fed probiotics have the ability to alter gastrointestinal microflora, this study was conducted to further evaluate the effect of Eubios 1090 (a probiotic) in nursery diets containing Carbadox or Chlorotetracycline + Tiamulin (CTC-Denagard). Carbadox is an antibiotic product that was approved in the 1970s to prevent and treat dysentery in swine and to maintain weight gain during periods of stress, such as weaning. In a previous study, it was found that Denagard Plus Chlortetracycline, an antibiotic premix, caused an increase in average daily gain (ADG), with Carbadox also improving ADG (Davis et al., 2004b). In recent studies at the University of Arkansas, probiotic supplementation with a *Bacillus* combination diet devoid of antibiotics resulted in improved final body weight (BW) at the end of the nursery period (Novartis Animal Health., 2007).

Probiotics and Antibiotics. One reason for the use of probiotics, instead of antibiotics, is to replace banned uses for antibiotics, such as the recent, April 2007, removal of fluoroquinolones from poultry production in the United States and the removal of growth-promotant use in many European countries (Singer and Hofacre, 2006). Another reason is the voluntary reduction in growth-promotant uses of antibiotics by some of the major poultry production companies and restaurant chains (Singer and Hofacre, 2006). The major driving force influencing these recent changes in antibiotic usage in poultry has been the perceived public health risks associated with this practice. There is a notion that antibiotic uses in agriculture may pose a risk to public health and animal health (Singer and Hofacre, 2006).

Probiotics are a major focus in today's society. They are live microorganisms that may produce a beneficial effect on the health of the animal. Probiotics replace harmful microbes with useful ones (Howarth, 2009). Recently, there has been a growing commercial interest in the probiotic food concept. Weaned pigs are usually chosen to carry out this concept because of the development phases they undergo (O' Hare and Wood, 2003). Because usual weaning procedures are accompanied by a general weakening of the immune system, implementing probiotics into the young pig's system at this time may help stabilize the microflora in the intestinal tract. Probiotic supplementation has been suggested to benefit the host animal by stimulating appetite and improving intestinal microbial population balance, digestion and growth performance of the animal (Dybkjaer et al., 2006).

There has been previous research studying the effects of antibiotics and probiotics on suckling pig and weaned pig performance to determine the effects of a probiotic on growth performance. The results of the study suggest that there is value to routinely administering a probiotic (*lactobacillus* and *streptococcus*) to neonatal pigs to improve pre-weaning performance. Under the conditions of their nursery study, the probiotic (*lactobacillus* and *streptococcus*) tended to enhance ADG and feed consumption in pigs that were weaned into pens with non-littermates (Estienne et al., 2005).

This study will further evaluate the effect of the probiotic, Eubios 1090, with antibiotics (Carbadox or CTC-Denagard) by measuring growth performance parameters in post-weaned pigs.

## **MATERIALS AND METHODS**

Animals and Housing. A total of 216 pigs from the University of Arkansas Research Swine Farm were transported to the University of Arkansas Offsite Nursery Facility. The pigs averaged 21 d of age at weaning and weighed an average of 6.79 kg  $\pm$  0.01. Pigs were individually weighed and divided into 5 weight blocks with stratification by sex and litter. Pens were randomly assigned to treatments and pigs were further subdivided into 32 pens with 6 to 7 pigs per pen. Housed in an environmentally controlled nursery, the pigs had *ad libitum* access to feed and water from a 3-head feeder and two nipple waterers.

Diets. Four dietary treatments were arranged as a  $2 \times 2$ factorial and were randomly assigned to pens (8 pens per treatment). Treatments included: 1) 50 g/ton of Carbadox antibiotic added to a basal diet; 2) 400 g/ton of Chlortetracycline antibiotic and 35 g/ton of Tiamulin antibiotic (CTC-Denagard) added to a basal diet; 3) 50 g/ton of Carbadox antibiotic and 0.45 kg/ton of Eubios 1090 probiotic added to a basal diet; 4) 400 g/ton of Chlortetracycline antibiotic, 35 g/ton of Tiamulin antibiotic, and 0.45 kg/ton Eubios 1090 probiotic added to a basal diet. The Eubios 1090 was obtained from Agtech Products, Inc. (Waukesha, Wis.). Pigs were fed the same dietary treatment throughout phase 1 (days 0 to 10), phase 2 (days 10 to 20), and phase 3 (days 20 to 34). Diets were formulated to meet or exceed the dietary nutrient requirements for nursery pigs as determined by the National Research Council (NRC, 1998).

Data Collection. Individual body weight was recorded on days 0, 10, 20, and 34. Feed disappearance was measured for each pen at the end of each phase. These measurements were used to calculate ADG (body weight  $\div$  number of days), ADFI (weight of feed disappearance  $\div$  number of days), and G:F (body weight gained to weight of feed disappearance) for each phase. Analyses of data were performed using the PROC GLM procedure of SAS with block and treatment in the model. The P value or probability is significant when ≤0.05, and the P value has a tendency to be significant when ≤0.10.

## RESULTS AND DISCUSSION

There were no interactions observed between the antibiotic and probiotic diets. Therefore, only the main effect of antibiotic inclusion and main effect of Eubios 1090 inclusion will be compared as shown in Table 1. There were no differences observed in ADG and BW between the Carbadox diet and the CTC-Denagard diet. In phase 1, ADFI was greater (P = 0.001) in pigs fed diets containing CTC-Denagard compared to those that were fed the diet containing Carbadox. During phase 2, there were tendencies for increased ADFI (P = 0.08) when nursery pigs were fed

diets containing CTC-Denagard compared to those fed a diet containing Carbadox but reduced G:F (P=0.08). Overall, there was a tendency for greater ADFI (P=0.10) when pigs were fed diets containing CTC-Denagard compared to diets containing Carbadox. Inclusion of Eubios 1090 had no effect on ADFI. During phase 2, pigs fed the non-Eubios 1090 diet had greater ADG (P=0.02) and G:F (P=0.03) compared to the pigs fed the diet containing Eubios 1090. Pigs fed the non-Eubios 1090 diet were also heavier (P=0.02) at the end of phase 2. However in phase 3, pigs fed the Eubios 1090 diet had an increased ADG (P=0.04) and G:F (P=0.04).

This tendency for increased ADFI is similar to previous experiments evaluating the effectiveness of Carbadox and CTC-Denagard. Novartis Animal Health reported that pigs fed diets containing CTC-Denagard had increased ADG and ADFI (Novartis Animal Health, 2007). There was a negative effect in pigs fed diets containing Eubios 1090 at an earlier age (phase 1 and 2) when compared to pigs fed diets without Eubios 1090, but once the pigs reached phase 3 their ADG and G:F was greater. One speculation could be that the developing gastrointestinal microflora reacted with Eubios 1090. This concurs with previous research that reported benefits from the addition of Eubios 1090 only during the last 14 days of post-weaning, increasing ADG and G:F (Estienne et al., 2005). However, for the entire 34 day post-weaning period, there was no effect of Eubios 1090 supplementation to pigs in this study.

## **ACKNOWLEDGEMENTS**

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Table 1. Nursery pig growth performance in response to four dietary treatments.

	<u>Antibiotic</u>		Probio	tic		<i>P</i> -value	
					Standard		
	0	CTC-	Non-	Eubios	Error	A (!!- ! - (! -	Decision (
	Carbadox	Denagard	Eubios 1090	1090	of the Mean	Antibiotic	Probiotic
Average Da	ilv						
Gain (ADG)							
Phase 1	110	124	121	113	6.7	0.15	0.39
Phase 2	389	397	409	378	9.3	0.54	0.02
Phase 3	620	625	607	638	10.4	0.73	0.04
Overall	401	409	404	406	6.6	0.41	0.83
_							
Average Da Feed Intake							
(ADFI), g	<u>-</u>						
Phase 1	194	215	207	201	4.1	0.001	0.30
Phase 2	518	545	541	521	11.0	0.08	0.20
Phase 3	872	887	874	884	14.8	0.46	0.63
Overall	568	588	579	577	8.4	0.10	0.87
Gain to Fee	<u>d</u>						
Ratio (G:F)							
Phase 1	0.568	0.577	0.585	0.560	0.029	0.83	0.53
Phase 2	0.752	0.728	0.755	0.725	0.009	0.08	0.03
Phase 3	0.708	0.699	0.691	0.716	0.008	0.44	0.04
Overall	0.707	0.695	0.698	0.703	0.006	0.14	0.51
Body Weigh	n <del>t</del>						
(BW), kg	<u>ıı</u>						
Initial wt	6.80	6.79	6.79	6.79	0.01	0.37	0.86
Phase 1	7.90	8.03	8.00	7.92	0.07	0.17	0.39
Phase 2	11.79	12.01	12.10	11.70	0.12	0.20	0.02
Phase 3	20.49	20.76	20.61	20.63	0.22	0.37	0.96

Phase 1 = day (D) 1 to 10; Phase 2 = D 10 to 20; Phase 3 = D 20 to 34.

## Assessment of total organic carbon concentrations in two streams of Northwest Arkansas: Town Branch and Brush Creek

Abigail N. Washispack\*, Jason A. McGinnis<sup>†</sup>, and Brian E. Haggard<sup>§</sup>

## **ABSTRACT**

Within a stream, changes in flow rate and local environment can affect the total organic content (TOC) concentrations in the stream water and TOC delivery downstream to water supply reservoirs. Disinfection by-products (DBPs) result from various chemical reactions between chlorine, bromine, and organic carbon in raw water during the drinking water treatment process; DBPs are potential carcinogens and are regulated by the U.S. Environmental Protection Agency. In this project, we measured the TOC concentrations in two streams in the Beaver Lake Watershed: Town Branch and Brush Creek. We then compared TOC concentrations between the two streams and to that observed in streams draining in forested areas to determine if differences in mean concentrations might be related to the streams' catchment. Finally, using instantaneous discharge at the time of sampling, we determined if TOC concentrations were significantly correlated to the volumetric flow of a stream. The data suggest that there is a positive linear relationship between the TOC concentration and the flow rate of a stream. While TOC concentrations did not vary between sites, TOC flux and yield were significantly different between the two streams.

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Jason A. McGinnis is a junior in the Department of Mechanical Engineering.

Brian E. Haggard is the faculty mentor and an associate professor in the Department of Biological Engineering.

## **MEET THE STUDENT-AUTHORS**



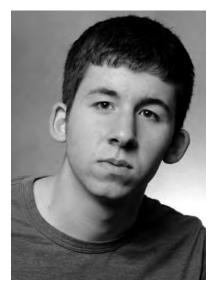
Abigail Washispack

I am from Conway, Arkansas, and graduated with honors from Conway High School in the spring of 2008. After being named a Bodenhamer fellow, I decided to attend the University of Arkansas for my undergraduate degree. I am currently pursuing a B.S. degree with a major in Biological Engineering and an emphasis in Biomedical Engineering. As a rising junior I am very involved in the campus ministry Campus Crusade for Christ and enjoy competing on various intramural teams, including soccer, softball, and ultimate Frisbee. I have also had the pleasure of being an Honors College Ambassador for the last two years.

I began this undergraduate research project with Dr. Brian Haggard in the spring of 2009 as part of the University of Arkansas Freshman Engineering Program's Honors Research Symposium. Upon graduation from the University of Arkansas, I plan to attend graduate school. After earning a doctoral degree in Biomedical Engineering, I hope to pursue a career in tissue engineering research.

I would like to thank Dr. Brian Haggard for all of his guidance and support throughout this project and give a special thanks to all the faculty of the Freshman Engineering Program. Finally, thank you to my wonderful family for all your love and support.

I grew up in Tomball, Texas, and graduated from Tomball High School in 2008. I came to the University of Arkansas to pursue an engineering degree, unsure which field to pursue. I participated in the Freshman Engineering Program's Honors Research Symposium and began this research with Abby. After completing this project I switched to Civil Engineering and went to the Community Development in Belize summer program where we constructed a 100,000 gal/day filtration system to provide clean water for the village of Steadfast. Now I have finally found my place in the Mechanical Engineering Department. This project showed me how stimulating research and interpreting data can be so I plan to pursue engineering research in the future.



Jason McGinnis

## INTRODUCTION

Disinfection by-products (DBPs) are the result of various treatment processes used to produce drinking water. The first DBPs identified were trihalomethanes (THMs), where presence of THMs was related to the concentration of total organic carbon (TOC) in source water. Shortly after, in 1976, the National Cancer Institute declared chloroform a carcinogen. Chloroform is a THM, so this discovery implied that the consumption of drinking water containing high concentrations of THMs could be correlated to the development of certain types of cancer (Singer, 1994). These findings led to various EPA regulations on DBPs and even TOC concentrations in source water.

The most common water treatment process today is chlorine disinfection, which produces DBPs when chlorine and bromide ions react with organic substances; the various DBPs produced include THMs and haloacetic acids (HAAs). In response to the discovery of different kinds of DBPs and the health risks associated with their presence in drinking water, new regulations on water quality have been passed in recent years. In 1996, amendments were made to the Safe Drinking Water Act (SDWA) of 1974 that required the EPA to set standards for harmful microorganisms and DBP concentrations in drinking water based on the risks associated with each (EPA, 2010). In response to the amendments, the EPA created Stage 1 and Stage 2 of the Disinfectants and Disinfection Byproducts Rule (EPA, 2010). Stage 1 sets regulations on maximum concentrations of three disinfectants and maximum concentrations of two common DBPs. Stage 2 builds on Stage 1 by first identifying treatment systems with high DBP concentrations and then requiring more stringent testing for THMs and HAAs.

The precursor for DBPs is organic carbon in the source water, which is separated into two categories: dissolved organic carbon (DOC) and particulate organic carbon (POC). Organic carbon can enter a stream through allochthonous inputs or autochthonous production; allochthonous inputs originate outside the fluvial channel and include land applied fertilizers, animal manure, and plant material. Leaf litter is the one of the main allochthonous inputs, particularly in forested catchments (Meyer et al., 1998). According to Meyer et al. (1998), up to 30% of DOC produced daily in a forested stream is generated from leaf litter stored in the fluvial channel. Autochthonous production occurs within the fluvial channel through autotrophs including macrophytes, phytoplankton, and periphyton.

Local and watershed scale land use also influences the delivery of organic carbon to streams, and the production of organic carbon within the stream. For example, catchments with more urban and/or agricultural area often have greater concentrations of TOC, as well as the poten-

tial to produce more autochthonous inputs. According to Goonetilleke et al. (2005), "catchment characteristics play the most significant role in urban stormwater runoff quality." Water chemistry is significantly impacted by land use and land cover and often catchment characteristics are used to predict water chemistry (Gergel et al., 1999).

Variation in TOC concentration occurs with both seasonal changes and discharge variation. Studies have shown that concentrations of organic carbon are greatest during autumn, mostly due to an influx of leaf litter (Meyer et al., 1998). Generally changes in fluvial channel discharge are related to storm events and coincide with higher organic carbon concentrations (Giovannetti, 2007). Storm events cause an increase in allochthonous inputs, particularly in urban catchments where increased runoff volume and peak discharge occur. Several studies show that there are higher concentrations of pollutants and, thus, organic carbon early in a storm event and preceding peak flow (Goonetilleke et al., 2005). Particulate organic carbon concentrations have also been recorded to be much higher during increasing flow rates than during the receding limb of the hydrograph (Meyer and Tate, 1983).

In order to limit the presence of DBPs in public drinking water, the amount of organic carbon in the raw water supplies needs to be minimal. The sources of organic carbon within drinking water supply reservoirs must be understood and measured. Then, management practices can be implemented to reduce the influx of organic carbon into the water supply. The first objective of this research was to determine the concentration of TOC in two streams located in the Beaver Lake Watershed: Town Branch and Brush Creek. Town Branch drains an urban area, whereas Brush Creek drains an agricultural and forested catchment. We compared TOC concentrations in the two streams to determine if there was a significant difference in mean concentrations that might be related to the streams' catchment. We also compared the TOC concentrations in these two streams to that observed in streams draining in forested areas that were sampled by Giovannetti (2007). The final objective was to determine if TOC concentrations were correlated to the volumetric flow of a stream using instantaneous discharge at the time of sampling.

## **MATERIALS AND METHODS**

We selected sites at two streams within the Beaver Lake Watershed: Town Branch 62 (USGS station ID 07048480), located at the bridge on Highway 62 and Brush Creek 45 (USGS station ID 07048890), located near the bridge on Highway 45. Town Branch has a catchment with 60% urban development and an area of 1.22 km², whereas Brush Creek has a catchment with 54% forest and 46% pasture land use with an area of 46.8 km² (Haggard et al., 2007).

Over a seventy day period from February 4th, 2009 to April 10th, 2009, we collected a total of twenty 250 mL water samples, nine at Brush Creek and eleven at Town Branch. Each sample was collected from the side of the fluvial channel, and the sampling equipment and bottles were field rinsed before each water sample was collected. The samples were then taken to the Arkansas Water Resources Center Water Quality Lab at the University of Arkansas Engineering Research Center in Fayetteville, Ark. where TOC concentration in the collected water samples was measured using a Skalar Wet Chemistry Autoanalyzer (Skalar Analytical B.V., The Netherlands).

The method used to measure TOC concentration was EPA Test Method 415.2 for Total Organic Carbon. This method is intended for samples with TOC concentrations between 0.05 mg/L and 10 mg/L and uses persulfate oxidation and ultraviolet illumination. In this method, 1 mL of acidified persulfate reagent is added to the sample, which is then placed in a sparger. The sample is then purged with helium and sent to a scrubber, which removes approximately 99.9% of  $\mathrm{CO}_2$  in the sample. Purgeable organics then progress through a reduction system. Hydrogen is added to the gas stream, which then passes over a nickel catalyst. This catalyst converts the purgeable organic carbon to methane. A flame ionization detector then measures the concentration of purgeable organic carbon.

The sample is then moved to a quartz ultraviolet reaction coil. In the presence of the acidified persulfate reagent, the nonpurgeable organics are exposed to ultraviolet illumination. This process converts the nonpurgeable organics to  $\mathrm{CO}_2$ , which progresses to a second sparger where it is purged with helium and transferred to the reduction system and then the methane detector. The measured concentration of nonpurgeable organic carbon is then added to the concentration of purgeable organic carbon. This sum is the TOC concentration of the sample (EPA, 1982).

The discharge of the water at each site is available on the World Wide Web through the United States Geological Survey (USGS) real-time stream flow-monitoring program. The discharge and gage height (i.e., depth of water) at the time of sampling was recorded from the USGS website (http://waterdata.usgs.gov/nwis/rt). The gage height (m), volumetric discharge (m³/s), and the TOC concentration (mg/L) at the time of sampling were recorded for each sample taken at Town Branch and Brush Creek (Tables 1 and 2). The flux (mg/sec) of each sample was calculated by multiplying the discharge and TOC concentration. The yield (mg/sec/km<sup>2</sup>) of each sample was calculated by multiplying the flux and the catchment area. Statistical analysis was performed for the TOC concentration (mg/L), TOC flux (mg/second), and TOC yield (mg/sec/km2) at each site (Table 3) using Microsoft Excel® 2007 software (Microsoft Corporation, Redmond, Wash.).

## **RESULTS AND DISCUSSION**

A Student's *t*-test with unequal variances on log-transformed data showed that TOC concentrations were not significantly different at the two sites, where geometric means were 2.18 mg/L at Brush Creek and 2.73 mg/L at Town Branch. Nine sampling dates overlapped between the two sites, and a paired T-test also showed that TOC concentrations were not significantly different between sites. Overall, the range in TOC concentrations was similar between sites, except that one sampling date at Town Branch had a concentration over 15 mg/L. Unfortunately, Brush Creek was not sampled on this date because of logistical constraints.

The water samples generally had lower TOC concentrations (<2 mg/L), when it had not rained for a few days. The water samples taken during or right after a rainstorm had TOC concentrations higher than those measured during periods of low flow. For example, the last three samples collected at Town Branch were taken before, at the beginning of (i.e., first flush), and after a single storm (i.e., receding limb of the hydrograph). The TOC concentrations for these samples were 1.58 mg/L, 15.8 mg/L, and 3.70 mg/L, respectively (Table 1). This suggests that TOC concentrations increased during storm events relative to the concentrations measured [prior to the rain] during periods of low flow.

TOC concentrations were graphed as a function of volumetric discharge at the time of sampling at Brush Creek and Town Branch (Fig. 1). Both graphs showed a positive linear relationship between TOC concentration and flow rate ( $r^2 = 0.80$  and 0.94, respectively). The linear relationship remained strong ( $r^2 = 0.74$ ) for the Town Branch site even without the data point where TOC concentration was 15.8 mg/L. This indicated that TOC concentrations increase with increasing flow rate, and that storm events can significantly increase TOC concentrations in these streams.

The average TOC concentration for sites in different catchment types were computed based on data collected by Giovannetti (2007), which sampled twenty different streams throughout Northwest Arkansas including the two streams in this study. Forest watersheds were defined as a catchment with greater than 80% forested land use; agricultural watersheds were defined as a catchment with greater than 30% pasture land area; mixed forest watersheds were defined as a catchment that had less than 2% urban-suburban land use and did not fit into the forest on agricultural classifications; and, mixed urban watersheds were defined as a catchment that had 2% or more urban-suburban land use and did not fit into other categories (Giovannetti, 2007). The TOC concentration for agriculture, forest, mixed forest, and mixed urban catchments were compared to that measured in this study at Brush Creek and Town Branch. This study had geomean TOC concentrations numerically higher than the forested streams (0.84-1.33 mg/L) sampled by Giovannetti (2007), whereas the other streams (agricultural and mixed land use catchments) had geomean TOC concentrations ranging from 0.84 to 3.45 mg/L (Fig. 2).

The data we have collected showed that the TOC concentrations for Town Branch draining an urban catchment was statistically not different than that from Brush Creek draining the mixed agricultural and forested catchment. However, there were significant differences between the streams based on flux and yield (Table 3). The TOC flux was greatest at Brush Creek (Student's t-test for unequal variances on log-transformed data, P < 0.03), likely because stream discharge was greatest at this site. On the other hand, TOC yield for Town Branch was significantly higher than that from Brush Creek (P < 0.08), indicating that the urban landscape of the Town Branch catchment produced more organic carbon per unit area than the agricultural catchment of Brush Creek. Thus, it is best to look at more than just concentration when trying to understand TOC transport from different catchments.

This study shows that TOC concentration was similar between streams draining agricultural and urban catchments, whereas transport on a unit area basis was greater from the urban stream. This supports the conclusion of previous papers (Goonetilleke et al., 2005; Gergel et al., 1999), which indicated that urban catchments had higher TOC concentrations and transport than other catchment types. However, the urban stream in this study had concentrations similar to Brush Creek and within the range of that measured in streams draining agricultural and mixed land use catchments (data from Giovannetti, 2007).

Our sampling data showed that there was a positive linear relationship between TOC concentrations and flow rates at these two streams (P < 0.05). However, previous research indicates that TOC might be lost as a first flush during storm events. Since most of our high flow rate TOC samples were taken after the peak flow rate, we might not have adequately characterized this relation. In order to confirm this conclusion, more samples need to be taken at the beginning of peak flow events. Other research studies (Goonetilleke et al., 2005; Meyer et al., 1983) and the one sample taken during these conditions indicate that the TOC concentration may be considerably higher prior to peak flow rate. The overall TOC data displayed as a function of flow rate shows that first flush of organic carbon might occur in these watersheds, because the slope of the linear relation was steep for Town Brach compared to Brush Creek. Future studies should conduct an in-depth assessment of TOC concentrations during storm events to determine if a specific trend is followed and if the trend is the same for different catchment types.

## **ACKNOWLEDEGMENTS**

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Table 1. Measured Gage Height, Volumetric Discharge, and TOC Concentration for Water Samples Collected at Town Branch.

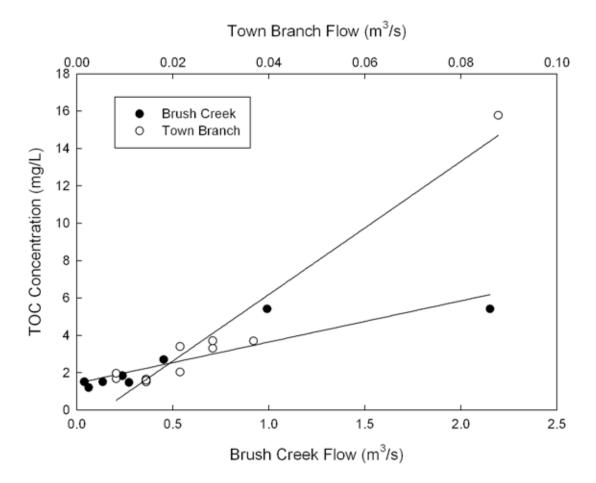
Date	Time	Gage Height (m)	Discharge (m³/s)	TOC (mg/L)
2/4/09	16:05	0.532	0.008	1.67
2/11/09	17:20	0.537	0.037	3.69
2/18/09	16:00	0.536	0.014	1.51
3/4/09	16:05	0.536	0.014	1.65
3/11/09	16:45	0.533	0.008	1.96
3/25/09	14:50	0.543	0.028	3.3
3/27/09	14:50	0.539	0.022	3.39
4/1/09	16:50	0.539	0.022	2.03
4/8/09	17:35	0.536	0.014	1.58
4/9/09	16:20	0.536	0.088	15.8
4/10/09	12:50	0.543	0.028	3.7

Table 2. Measured Gage Height, Volumetric Discharge, and TOC Concentration for Water Samples Collected at Brush Creek.

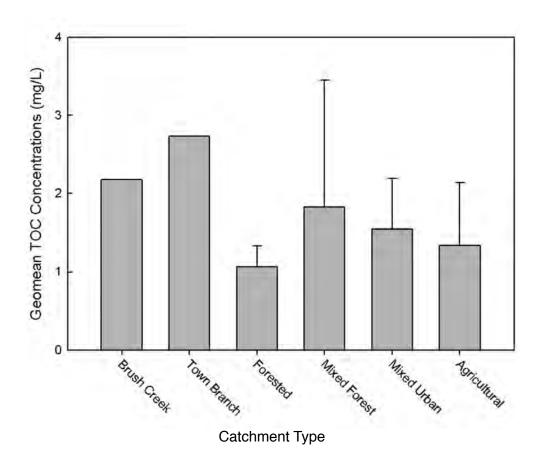
Date	Time	Gage Height (m)	Discharge (m³/s)	TOC (mg/L)
2/4/09	16:45	0.884	0.272	1.47
2/11/09	16:45	1.073	2.152	5.41
2/18/09	16:45	0.872	0.207	1.92
3/4/09	15:45	0.835	0.062	1.20
3/11/09	16:15	0.826	0.040	1.51
3/25/09	16:00	0.975	0.991	5.41
4/1/09	17:45	0.911	0.453	2.70
4/8/09	16:30	0.856	0.136	1.51
4/10/09	14:00	0.878	0.238	1.83

Table 3. Number of samples (n), Minimum, Median, Maximum, Mean, Standard Deviation (S), and Geometric Mean (GeoMean) for TOC Concentration, Flux, and Yield at Brush Creek and Town Branch.

	TOC Concentration (mg/L)						
Site	n	Minimum	Median	Maximum	Mean	S	GeoMean
Brush Creek	9	1.20	1.83	5.41	2.55	1.68	2.18
Town Branch	11	1.51	2.03	15.8	3.66	4.12	2.73
	TOC Flux (mg/s)						
Site	n	Minimum	Median	Maximum	Mean	S	GeoMean
Brush Creek	9	59.8	399	11600	2200	3910	553
Town Branch	11	13.7	43.7	1380	176	403	55.5
	TOC Yield (mg/s*km²)						
Site	n	Minimum	Median	Maximum	Mean	S	GeoMean
Brush Creek	9	1.28	8.53	248	47.0	83.6	11.8
Town Branch	11	11.2	35.8	1130	144	83.6	45.5



**Fig. 1.** Total organic carbon concentrations (mg/L) as a function of flow rate (m³/s) for samples taken at Town Branch and Brush Creek.



**Fig. 2.** Comparison of average TOC concentrations for different catchment types. Stream from this study or catchment type from Giovannetti (2007).

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