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**COMMENTARY:**  
**THE SMaRT SCHOLARS FOR SUCCESS PROGRAM– PREPARING COMMUNITY COLLEGE STUDENTS FOR CAREERS IN SCIENCE, TECHNOLOGY, ENGINEERING, AND MATHEMATICS**

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**INTRODUCTION**

Science, technology, engineering and mathematics (STEM) talent is in high demand with changes in the global workforce and economic growth trends. Community colleges can serve as a bridge of opportunities by recruiting, retaining and preparing students for careers in the STEM fields. However, different financial, personal, and academic challenges exist for community college students. The SMaRT Scholars for Success Program at Northampton Community College is an example of how to help students achieve academic success, engage in a community of learners, transfer to 4-year institutions, and explore STEM career pathways.

Many reports have indicated the need for more students, especially under-represented groups such as women and minorities, to enter the STEM (Science, Technology, Engineering, and Mathematics) pipeline to keep the United States competitive in a global marketplace. Predictions for job growth show that STEM jobs will grow at a higher rate than non-STEM fields. The Economics and Statistics Administration of the Department of Commerce (2011) states “Over the past 10 years, growth in STEM jobs was three times as fast as growth in non-STEM jobs.” According to the National Science Board in 2010 only 10% of U. S. science and engineering workers were members of a historically under-represented racial or ethnic group, even though they comprised 26% of the population (National Science Board, 2014). In addition, most STEM disciplines do not attract women in the ratios equal to their numbers in the population as a whole. In 2010 women comprised 28% of the science and engineering workforce, even though they represented half of all college-educated workers according to the National Science Board. Many businesses have to look abroad for employees with the education and skills they need. These businesses have to pay the government for the right to bring in these foreign employees. The National Science Foundation (NSF) uses these monies to fund the Scholarships for Science, Technology, Engineering, and Mathematics (S-STEM) program. As a funding opportunity with the Division of Undergraduate Education (DUE), the

S-STEM program “provides grants to selected institutions of higher education to support scholarships for academically talented students demonstrating financial need, enabling them to enter the STEM workforce or STEM graduate school following completion of an associate, baccalaureate, or graduate-level degree in science, technology, engineering or mathematics disciplines.”

The goal of the S-STEM program is to increase the recruitment and retention of STEM majors, especially among under-represented groups. Northampton Community College received a five-year S-STEM grant in 2007, the SMaRT (Science, Mathematics, and Related Technologies) Scholars for Success Program with DUE Grant 0728464. This grant had three objectives centered on (1) Recruitment, (2) Retention, and (3) Career and Transfer Services. The lessons learned from this grant were used to apply for and receive a second S-STEM grant with a start date of March, 2012. The second grant has a stronger focus on recruitment of under-represented groups and utilizes new approaches to retain students in the program with DUE Grant 1154236. Interested candidates for the SMaRT scholarship contact our institution’s Office of Financial Aid and can submit an online application. In addition to managing the S-STEM project, we select the SMaRT scholars and submit requested demographic information about our student scholars through periodic NSF reporting. This commentary describes highlights of these two grant programs and looks at our successes and challenges.

**THE SMaRT SCHOLARS FOR  
SUCCESS PROGRAMS**

Northampton Community College (NCC) is a comprehensive, two-year, publicly supported community college with a main campus in Bethlehem, Pennsylvania, and a campus further north in Monroe County, (Tannersville, PA). The college is accredited by the Middle States Association of Colleges and Secondary Schools. NCC offers 17 transfer programs leading to the Associate in Arts (A.A.) or Associate in Science (A.S.) degree, 35 career programs leading to the Associate in Applied Science (A.A.S.) degree, 5 certificate

programs, and 38 specialized credit diploma programs. As an open access college, NCC provides an excellent opportunity for academically talented and financially needy students who wish to start their higher education career in an affordable, local two year institution. From NCC, a student can transfer to no fewer than 9 local four year colleges or universities or gain employment in a vibrant regional workforce.

The SMaRT (Science Math and Related Technologies) Scholars for Success grant's goal is to increase the number of academically talented students in the STEM disciplines. The first grant required implementing an intensified, coordinated and targeted recruitment program with the collaborative contributions of various faculty, staff, and administrators maximizing available campus resources. Also team building cohort activities and student support services were integrated to increase academic success of the scholars. The objectives for the second grant are currently focused on recruiting more under-represented scholars, with retaining them into the second year of their two-year program, and with keeping them in the STEM pipeline after they leave the program. The objectives of both of our National Science Foundation (NSF) funded S-STEM grants are described in Table 1.

*The initial recruitment activities of the first grant are expanded in the second grant. These recruitment strategies of both grants include the following:*

1. **Widely advertise the SMaRT Scholars program throughout NCC's service area:** As part of the first grant, the SMaRT Scholars Advisory Committee implemented informational sessions with guidance

counselors, teachers, and parents and participated in school career fairs with the help of the NCC Admissions. Program information packets were disseminated to the following: all area high school/ Vo-Tech math, science and technology teachers, all students applying to or enrolled in a STEM program, homeschooled and cyber schooled students, and participants in STEM-focused summer programs for middle and high school students offered through NCC's Horizons for Youth program. In addition, NCC faculty and the offices of Financial Aid and Institutional Advancement assisted in the recruitment and identification of potential scholars.

2. **Promote the scholarship program through the internet and social media:** The home page of the college website periodically highlights the SMaRT Scholars program through news articles and posting a link to our scholarship page. The NCC's SMaRT Scholars website <http://www.northampton.edu/smart-scholars.htm> provides information about the scholarship program (e.g., qualifying programs, benefits of being a SMaRT Scholar, frequently asked questions) along with the contact information for the principal investigator (PI) and the Co-PI. Interested candidates can also print a program application or complete it online. The SMaRT Scholars Facebook page provides additional exposure for the program and creates a sense of community within the cohorts.

Table 1. Description of the S-STEM Grant Objectives of the SMaRT Scholars for Success

Objectives	SMaRT Scholars 2007 - 2012	SMaRT Scholars 2012 - 2017
1. Recruitment	NCC will provide two-year scholarships to 54 (3 cohorts of 18 students each) financially disadvantaged, academically talented students; and to increase the number of full-time science, technology, engineering and math majors seeking an associate degree by 15%.	NCC will provide two-year scholarships to 72 (4 cohorts of 18 students each) financially disadvantaged, academically talented students of which 75% will be from under-represented groups in STEM (women and minorities).
2. Retention	At least 80% of SMaRT Scholars will successfully complete the first semester required math course (College Algebra or Calculus I) with a grade of "C" or better.	NCC will retain 94 % of SMaRT Scholars in the program from Fall to Fall semesters.
3. Transfer and Career Placement	NCC will provide 100% of SMaRT Scholars who successfully complete coursework with either transfer or job placement, depending on the individual scholar's goals	85% of scholars will transfer to a four-year institution or obtain employment in a STEM field within 6 months of graduation.

3. **Broaden targeted recruitment efforts by actively recruiting from community groups and agencies that serve the target population and with which the college has existing relationships:** These target organizations for recruiting new scholars include SkillsUSA Council, a non-profit created to ensure America has a skilled workforce. NCC has community relations with the Latino Leadership Alliance of the Lehigh Valley and the AmeriCorps Vista agency partners, the Children's Home of Easton and the Boys and Girls Club of Bethlehem. NCC's annual Math Summit brings together over 50 local school district and college math faculty, providing an excellent venue for promoting the SMaRT Scholars program.
4. **Provide personal outreach to individual under-represented students:** To expand our initial recruitment strategies, prospective SMaRT scholars are provided an explanation of the benefits of the program and any needed assistance in filing the FAFSA and scholarship program application forms. We also invite local STEM practitioners, who are from diverse career backgrounds and underrepresented groups, to speak at scholar seminar events as successful role models. Connecting scholars to professional organizations and their representatives is also a priority activity such as the American Chemical Society, Society of Women Engineers, the Association for Women in Science and the American Association of University Women.
5. **Increase the visibility of the program at the college and in the community:** As another expanded recruitment strategy, more recognition efforts of student achievements are underway such as a SMaRT Scholars' graduation ceremony, which include presenting certificates of completion, a reception, and distinguished speakers at NCC. College-wide promotion of the program and events are being coupled with widening dissemination of their scholar activities, such as internships, graduation, job placement and transfer successes, with the assistance of NCC Marketing and Publications and the NCC Public Info Office.

*These initial and expanded recruitment strategies of both grants include the following:*

1. **Provide academic and career support:** The STEM Student Success Specialist plays an integral role by directing scholars to the various campus resources (Learning Center, Career Services, Advising and Transfer Office) depending on the specific needs of the students. The STEM Student Success Specialist has the following roles: develops and presents workshops for the scholars on building college success skills; provides assistance with attaining graduation, career and transfer goals; communicates with faculty on methods for improving retention and graduation rates; and develops relationships with local industries to stay informed on regional job market trends and to assist students with job opportunities.
2. **Assign scholars to sections of gateway math courses (college algebra or calculus) that use a project-based approach to learning:** Research done as part of our Achieving the Dream initiative, a national non-profit, non-governmental reform movement in higher education, showed that success rates in developmental courses were significantly higher in sections that used a project-based approach (76% versus 58%) over a five-year period. The SMaRT Scholars are scheduled gateway math courses that integrate project based learning strategies to increase their math completion success rates.
3. **Institute an academic early warning system:** A new early warning system was developed five-week point of each semester to provide early identification of scholars' academic progress and allow for early intervention by faculty and/or the STEM Student Success Specialist, as appropriate. Scholars exhibiting a lack of academic progress will be required to meet with the STEM Student Success Specialist to develop a success plan, which will be monitored by the Specialist and may include weekly meetings with Specialist, additional study skills training and mandated tutoring hours, as appropriate.
4. **Create a community of learners by implementing a two-day, on-campus retreat:** A team building activity in the form a two-day retreat is planned to generate student excitement about their academic program and familiarize them with faculty and staff to whom they can turn for assistance. The retreat agenda includes team-building activities and improving study habits. This community of learners will be developed further by enrolling SMaRT Scholars in common sections of first semester required math courses (college algebra or calculus). SMaRT Scholar program events, speakers and social events add to the Scholar's sense of community and belonging.
5. **Strongly advise Scholars to maintain 20 or fewer hours of work per week:** Students who are working more than the recommended maximum are required to undergo a one-on-one counseling session and then a form is signed to indicate the reasons they need to work the additional hours.

6. **Enhance Scholar's leadership skills and self-awareness:** To expand our initial retention strategies, scholars are required to attend NCC's Fall Leadership Institute, a series of workshops and seminars that will assist students in identifying and understanding personal strengths and how they apply to leadership. Scholars discover their core values, take a Myers Briggs Personality Indicator assessment, and learn critical communication, teamwork, and conflict resolution skills through the leadership training experience.
7. **Strongly encourage scholars to complete a capstone activity:** Also new to the second grant activities is to introduce and promote active participation in extracurricular activities beyond the classroom. For examples, scholars are encouraged to participate in a practicum or internship, a service learning project, or completing NCC's Student Leadership Program is encouraged to scholars before they graduate, transfer or seek employment.
8. **Further develop the Community of Learners by assigning SMaRT Scholars to a Learning Circle, based on major and facilitated by a faculty or industry mentor:** The Learning Circle provides scholars with a peer support network under the supervision of the mentor. Scholars develop self-confidence and effective academic coping strategies, set goals, and explore potential career choices in a combination of one-on-one and small group activities. Recruited mentors of diverse genders, ethnicities, and STEM expertise undergo mentor training and keep a record of activities completed with scholars. NCC faculty, faculty from regional 4-year colleges, and industry can serve as mentors.

*The initial and expanded strategies used for the Career and Transfer objective are also follows:*

1. **Provide role models to Scholars that will offer a "real world" perspective on career choices and paths available to STEM graduates.**
2. **Educate Scholars about effective job search strategies, resume writing, the interview process, and professional work expectations through workshops and individual sessions offered by NCC's Office of Career Services.**
3. **Provide assistance in choosing a transfer institution and help ensure that Scholars take all courses required for transfer:** Regional colleges, including East Stroudsburg University (the top transfer destination of NCC students), Lehigh University, and Wilkes University, have agreed to assist with completion of transfer and financial aid applications, present at seminars, host campus tours, and attend SMaRT Scholar events. Wilkes University has offered guaranteed admission and a merit scholarship of at least \$7,000 to Scholars transferring with a GPA of 3.0 or better. Moravian MCS2 program provides \$10,000 scholarships for Scholars wishing to pursue a bachelor's degree in mathematics or computer science. Also the STEM Scholars Program at Immaculata University can provide transfer scholarships funds of \$17,000 to \$25,000 to eligible community college students.
4. **Enhance Scholars' "soft business skills,"(i.e., strong work ethic, good communication skills, time management abilities, problem solving, teamwork, and self-confidence) by requiring scholar participation in the NCC Emerging Student Leadership workshops in the fall semester and providing additional seminars.**
5. **Provide Scholars with the hands-on experiences valued by employers, including practicum experiences, internships, service learning, and community science projects.**
6. **Facilitate a seamless transfer process:** During the spring semester of their first year, all Scholars in a transfer degree will attend a transfer workshop in which top transfer four- year institutions (East Stroudsburg University, Moravian College, Lehigh University, Wilkes University) will present on scholarship opportunities and transfer requirements. The STEM Student Success Specialist, working with NCC faculty, will facilitate introductions of transferring scholars to faculty or current STEM students at their targeted transfer institution. The project will expand on existing S-STEM transfer scholarship pipelines with Moravian College, Lafayette College, and Wilkes University by creating joint social and educational events for the students. For example, the NCC Engineering majors and SMaRT Scholars are invited to attend the annual Engineering Open event at Lafayette College each fall semester. The SMaRT Scholars in the NCC Honors Program are informed about transfer and articulation agreements with Lehigh University and other transfer institutions.

## RESULTS SUMMARY

*The first grant SMaRT Scholars for Success 2007 – 2012 was very successful in meeting its objectives. The results by objective are as follows:*

1. **Recruitment:** *NCC will provide scholarships to 54 financially disadvantaged, academically talented students so as to increase the number of full-time science, technology, engineering and math majors seeking an associate degree by 15%. NCC provided scholarships to 62 financially disadvantaged, academically talented students (scholarship funds were freed up for 8 additional scholars when scholars transferred early or were dropped from the program). During the project period, enrollment at the college increased 17%, while enrollment in STEM majors increased by over 58%. This project helped to raise the profile of STEM programs at the College, resulting in part of this substantial increase. However, we were not satisfied with the number of under-represented scholars during the first grant.*
2. **Retention:** *At least 80% of SMaRT Scholars will successfully complete the first semester required math course (College Algebra or Calculus) with a grade of "C" or better. 86.7% of Scholars in the third cohort of scholars passed their gateway math course, College Algebra or Calculus I. Only the first cohort of scholars did not meet the benchmark. Average success rates for the college population as a whole in these courses are 58% for College Algebra and 62% for Calculus I. In addition, 100% of the minority scholars and 79% of the female scholars successfully completed their first math course. In addition, eleven scholars chose to take Calculus I as an additional math course, even though it was not required in their major. Of these eleven, five were members of a minority and four were females (duplicated count).*
3. **Career and Transfer:** *NCC will provide 100% of SMaRT Scholars who successfully complete coursework with either transfer or job placement assistance, depending on the individual scholar's goals. We exceeded expectations for this objective with 100% of Scholars receiving career and/or transfer services. In addition, several mandated seminars were devoted to career and transfer planning and all scholars were made aware of the resources available to them on campus and on the web. The STEM Student Success Specialist helped each of the scholars create and improve their resumes and worked with them to find either employment in their field of study or a suitable transfer institution. Like most community colleges, relatively few students graduate within two*

years, but 60% of the SMaRT Scholars graduated within two years. Our success rate (scholars graduated in a STEM major, transferred to a STEM program at a four-year college or started employment in a STEM career) after two and one-half years is close to 90%. We strengthened relationships with regional transfer institutions that resulted in two SMaRT Scholars receiving S-STEM transfer scholarships and scholars participating in undergraduate research projects at Lehigh University, Wilkes University, and Moravian College

As for the second grant SMaRT Scholars for Success 2012 – 2017, periodic evaluations and assessment of collected data collection are in progress. It is too early in the second grant to have many results. However, we are making progress on the new objectives. In the first two cohorts of scholars, 64% are females or minorities, so we are getting closer to the 75% goal. Also, over 70% of the first cohort of scholars graduated in a STEM program in two years. We expect to meet the 94% retention from fall-to-fall with the second cohort of scholars. We will get close to the 85% success rate with the first cohort of scholars in the second S-STEM grant. To enhance opportunities for STEM career exploration, we have expanded our network of STEM industry contacts for guest speakers, company visits, and potential mentors. In response to the fall 2012 and spring 2013 survey results, scholars requested more field trips to universities and businesses. The company representatives from Sanofi Pasteur, Air Products, FLSmidth, Inc, and B. Braun Medical, Inc. were invited seminar speakers with SMaRT Scholars this past academic year 2013 - 2014. The SMaRT Scholars toured the following locations in October 2013: the corporate headquarters of ABEC Integrated Solutions, the Coca-Cola Bottling Company of the Lehigh Valley, and the Northeast Wildlife DNA Laboratory at East Stroudsburg University. Of the 33 scholars surveyed, 66% "strongly agree" or "agree" to the statement "attending the field trip or presentation experience has brought a new or different perspective (insight) in applying for jobs/internships in the future." We had SMaRT Scholars successfully completed internships at the Lehigh Valley Zoo, ABEC Integrated Solutions, and the NCC Fab Fabrication lab this past academic year. We will continue to meet with our SMaRT Scholars Advisory committee to discuss strategies, evaluate results and monitor the impact of implemented activities on our project objectives.

## CONCLUSIONS &amp; FUTURE PLANS

Northampton Community College SMaRT Scholars for Success grants has increased the number of academically talented students with financial need in the STEM disciplines by offering scholarships. Interdisciplinary and

intradepartmental collaborations across multiple NCC college campuses are in place to implement and monitor activities of the SMaRT Scholars program.

As for the most difficult challenges of the SMaRT Scholars program thus far, we need to start the scholars' STEM career explorations early on and focus on their preparedness to undertake the rigorous college coursework, especially with the completion of math requirements. New college students also need training sessions on effective time management, study skills, and soft skills important for developing young professionals. Scholars are often reminded about important seminar dates, scholarship renewal requirements, and important campus resources to seek out; however, some struggling or unsuccessful scholars did not follow through with recommended academic (or career) plans or were overwhelmed with unexpected personal situations (e.g., family obligations or financial challenges). Nonetheless, the SMaRT Scholars program recommends that all scholars visit their specific course instructors, faculty advisors, Learning Center, Career Services, Counseling Services, Advising and Transfer, Scholarship Office and the STEM Student Success Specialist, as appropriate. Scholars are encouraged and advised based on their individual goals.

As for future plans, the current brochure of the SMaRT Scholarship program is being updated and will be used to explain the benefits, eligibility and application process. The revised brochure will be sent to high school counselors, STEM high school teachers, and prospective NCC students. Using social media and campus events to promote the scholarship program will be explored to increase recruitment of eligible STEM majors. More seminar topics focused on effective time management, stress management strategies, and how to take tests will be planned early in the semester in response to students' recommendations from previous surveys. Future field trip destinations to visit local companies and STEM professionals in the community will also be identified with the help of our STEM mentors, NCC Alumni Office, and the NCC Business and Technology advisory board. Discussion of current literature will further inform our mentors about the challenges of recruiting and retaining talented STEM students. A future mentor meeting topic will be to discuss Eileen Pollack's article "Why Are There Still So Few Women in Science," which was published on October 3, 2013, in the New York Times. Other related articles will be forwarded to the STEM mentors to read. Future plans also include providing a seminar topic on resume writing, using social media for career networking and exploring the new NCC Career Coach website (<http://www.northampton.edu/career-coach.htm>). To help us monitor and improve the quality of the SMaRT Scholars program, the NCC Office of Institutional Research has provided many invaluable resources. Their staff has reviewed our proposed survey instruments to help us align the questions with the project goals. They have created surveys which can be scanned by a computer using Remark Survey Software, which allows

more efficient data analysis for disseminating key findings in a timely manner. They have recommended that we make changes to the Likert scale in order to evaluate the semester events (i.e., change to excellent, good, fair, or poor). Thus, we will review and modify implementation strategies based on their recommendations and the survey results collected over time.

We are enthusiastic about our progress and results thus far with the S-STEM grants. Our efforts are ongoing to implement a sustainable, replicable, and scalable program that prepares students for employment or further academic study in the STEM disciplines by leveraging college and community resources. Future findings and insights will be disseminated as we continue the SMaRT Scholars Program at Northampton Community College.

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For more information about the SMaRT Scholars Program or how to apply for a scholarship, contact Dennis Ebersole, Professor of Mathematics & Computer Science; Principal Investigator of the SMaRT Scholars Program



Website: <http://www.northampton.edu/smart-scholars.htm>  
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 DUE Grant 1154236 Smart Scholars for Success 2012 - 2017



Northampton Community College SMaRT scholars at commencement with Robert Colletta, Student Success Specialist, May 2014.



Northampton Community College SMaRT scholars with two seminar speakers at the Monroe Campus.



Northampton Community College SMaRT scholars in a team building exercise.





Northampton Community College SMaRT scholars with guest speaker Terry Hart (center, front row), former NASA astronaut.



Northampton Community College SMaRT scholars taking a walking tour of the Poconos.

## EFFECTS OF TRANSPORT ON FECAL GLUCOCORTICOID LEVELS IN CAPTIVE-BRED COTTON-TOP TAMARINS (*SAGUINUS OEDIPUS*)<sup>1</sup>

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### ABSTRACT

The relocation of animals can induce stress when animals are placed in novel environmental conditions. The movement of captive animals among facilities is common, especially for non-human primates used in research. The stress response begins with the activation of the hypothalamic-pituitary-adrenal (HPA) axis which results in the release of glucocorticoid hormones (GC), which at chronic levels could lead to deleterious physiological effects. There is a substantial body of data concerning GC levels and reproduction, and rank and aggression in primates. However, the effect of transport has received much less attention. Fecal samples from eight (four male and four female) captive-bred cotton-top tamarins (*Saguinus oedipus*) were collected at four different time points (two pre-transport and two post-transport). The fecal samples were analyzed using an immunoassay to determine GC levels. A repeated measures analysis of variance (ANOVA) demonstrated that GC levels differed among transport times ( $p = 0.009$ ), but not between sexes ( $p = 0.963$ ). Five of the eight tamarins exhibited an increase in GC levels after transport. Seven of the eight tamarins exhibited a decrease in GC levels from three to six days post-transport to three weeks post-transport. Most values returned to pre-transport levels after three weeks. The results indicate that these tamarins experienced elevated GC levels following transport, but these increases were of short duration. This outcome would suggest that the negative effects of elevated GC levels were also of short duration. [ J PA Acad Sci 88(2): 84-88, 2014 ]

### INTRODUCTION

The movement of animals to different enclosures or facilities can be stressful, but frequently occurs with non-human primates used in research (Kim et al. 2005). The physiological stress response begins with the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which causes the release of glucocorticoids (GC) from the adrenal cortex. At chronic levels, elevated GC may lead to deleterious physiological effects. Hormone profiles can be accurately monitored through the use of fecal steroid assays, offering a noninvasive means of assessing GC hormones in primates and other animals (Ziegler and Wittwer 2005). Feces can be collected without restraint or immobilization, making GC analysis increasingly popular in studies of both captive and free-ranging animals (Whitten et al. 1998). A substantial amount of research conducted by primatologists has compared the influence of reproductive status, and rank and aggression to GC levels in primates (Czoty et al. 2009; Hoffman et al. 2010). However, few studies have investigated the effects of transport on GC levels in primates. The intent of this study was to measure fecal GC levels during pre- and post-transport periods in cotton-top tamarins (*Saguinus oedipus*) transported between captive facilities as an assessment of stress levels.

### MATERIALS AND METHODS

#### *Animal husbandry*

This study involved eight cotton-top tamarins (four females and four males) obtained from the Department of Psychology at the University of Wisconsin, Madison, WI. The tamarins were born into a captive colony at the University of Wisconsin and were used in non-invasive hormonal,

<sup>1</sup>Accepted for publication May 2014.

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behavioral and cognitive studies. Animal age varied from three to thirteen years (Table 1). The tamarins were transported to Frostburg State University (FSU), Frostburg, MD on June 25, 2008 for continued involvement in similar non-invasive research. Facilities and animal husbandry methods were similar at both institutions. Animals were housed in the same male-female pairs in 1.60 x 2.36 x 0.93 m cages made of black polyurethane-coated steel mesh on anodized aluminum framing. Cages were spaced 0.46 m apart and opaque fabric sheets prevented physical and visual contact among the pairs. Each of the cages contained natural tree branches, platforms, and a nest box. Cage floors were covered with pine shavings, which were replaced weekly. Cage platforms were sanitized once a week. Temperature was maintained between 25.5-28.3 °C, and full-spectrum overhead fluorescent lighting was set on 12 hour light/dark cycle. Water was given ad libitum and animals were fed three times per day. No physical restraint or handling of the tamarins occurred in fecal sample collection for this study. This study was conducted in accordance with the guidelines laid down by the U.S. Office of Laboratory Welfare and the research was approved by the Institutional Animal Care and Use Committee from each respective University.

#### *Transport*

On the night prior to transport to FSU, the tamarins were removed from their cages and placed in medium-sized animal carriers (36.8 x 58.4 x 29.2 cm). Transit time was approximately 12 hours—two and half hours by vehicle to the airport, a flight of six hours, and another three and a half hours by vehicle from the airport to FSU. A tree branch and platform were placed in the carrier so the tamarins could remain elevated. Tamarins were given grapes, New World Monkey Chow (Zupreem, 10504 W 79th St., Shawnee, KS) prior to the flight, and water (in the form of a gelatin) in flight. A sheet covered the cage at most times to reduce the amount of visual stress.

#### *Fecal collections*

Samples were collected from each animal between 9:00 and 10:00 AM immediately after defecation. Date, collection time, freeze time, and unusual circumstances (e.g., medication given or any disturbances to their schedule) were recorded during sampling. Samples were frozen at -20° C within two hours of collection. Samples were collected at four different time points. The first (Time 1) was 17-18 days pre-transport. The second (Time 2) was one to two days pre-transport, the third (Time 3) was three to six days post-transport and the final (Time 4) was 9-21 days post-transport. Fecal samples were stored at -80 °C within three weeks of collection and prior to processing. Also, all of the

samples were processed within eight months of final sample collection.

#### *Steroid extraction*

Steroids were extracted from the scat following Wasser et al. (2000) with modifications. Briefly, samples were lyophilized using a VirTis™ Bench Top K Series Freeze Dryer (SP Industries, Inc., Gardiner, NY). Samples were pulverized thoroughly using a mortar and pestle, then sifted through a screen sieve (#60) to remove vegetative and other large particulate matter (Millspaugh et al. 2002; Washburn and Millspaugh 2002). Five mL of 95% ethanol was added to each 15 mL glass screw cap centrifuge tube (Kimble Glass, Inc., Vineland, NJ) containing 0.1 g of sample. The glass tube was placed in a beaker of boiling water and heated for 20 minutes with additional ethanol added as needed to maintain a volume of 5.0 mL. The extracted sample was centrifuged for 20 minutes at 500 x g and the supernatant fluid removed to a new 15 mL tube. The pellet was resuspended in 5.0 mL of 95% ethanol, vortexed for 1 minute, and centrifuged as before. The supernatant fluids were pooled for further processing. The ethanol was removed and the extracts were dried using a CentriVap Mobile System (Labconco; Kansas City, MO). Dried samples were stored at -20 °C prior to assay.

#### *Assay procedure*

The Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit (Assay Designs, Inc.; Ann Arbor, MI) was used to determine the GC levels in the extracted fecal samples. Manufacturer's instructions were followed with the following modification. The extracted samples were dissolved in 2.0 mL of assay buffer and diluted 1:20 before performing the assay. A standard curve for each plate was created using corticosterone concentrations of 20,000, 4,000, 800, 160, and 32 pg/mL. All samples were assayed in duplicate. A SPECTRAMax Microplate Spectrophotometer (Molecular Devices Corporation; Sunnydale, CA) was used to determine absorbance at 405 and 650 nm. If a sample was outside the linear portion of the standard curve, the sample was further diluted an additional 1:10 and re-assayed.

#### *Data analysis*

The suitability of the Correlate-EIA™ Corticosterone assay in determining GC levels in cotton-top tamarins was evaluated by initially performing a parallelism assay (Zar 2010). The difference between the slope of a serially diluted kit standard and the slope of a serially diluted pooled sample of five randomly chosen fecal samples was determined using

a Student's *t*-test. Validation would be demonstrated by failing to detect a significant difference between the two slopes indicating the assay as being appropriate for assessing GC levels in tamarins.

A repeated measures Analysis of Variance was performed to compare average GC levels among the four transport periods by sex, and to assess interactions between these two factors. Tukey HSD pairwise comparisons were examined at a studentized range critical value of 4.02 (0.05, 4, 17) (Zar 2010). STATA (StataCorp LP; College Station, TX) was used for all statistical comparisons. Statistical tests were considered to be significant at an alpha level  $< 0.05$ .

## RESULTS

### *Kit validation*

There was no significant difference between slopes of the serially diluted standard and the pooled sample. Therefore, the Correlate-EIA<sup>TM</sup> Corticosterone Enzyme Immunoassay Kit was validated for use with cotton-top tamarins ( $p = 0.350$ ) (Fig. 1).

### *Transport and GC levels*

GC levels differed among transport times ( $p = 0.009$ ), but not by sex ( $p = 0.963$ ). Tukey HSD pairwise comparisons demonstrated significant differences between Times 1 and 3, Times 2 and 3, and Times 3 and 4 (all  $p < 0.05$ ). Five of

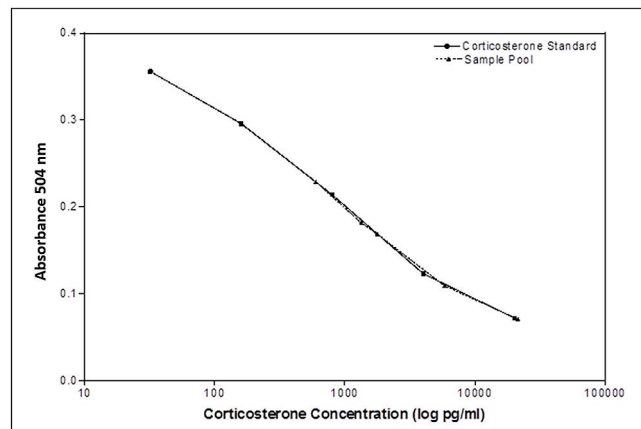


Figure 1. Parallelism between the Correlate-EIA<sup>TM</sup> Corticosterone Enzyme Immunoassay Kit (Assay Designs, Inc.; Ann Arbor, MI 48108, USA) standard curve and serially diluted pooled random sample conducted at Frostburg State University, 2010.

the eight tamarins had increased GC levels after transport, one had decreased GC levels, and two showed relatively little change (Table 1). Seven of the eight tamarins showed decreased GC levels from Time 3 to Time 4, with most values returning to pre-transport values by Time 4. The mean post-transport level of 952.70 (SE =241.50) ng/g dried feces and was about four times that of Time 1 and Time 2, and about three times that of Time 4.

Table 1. Glucocorticoid (GC) concentrations (ng/g dried feces) for eight cotton-top tamarins transported from the University of Wisconsin-Madison to Frostburg State University on 6/25/08. Times 1 and 2 represent pre-transport GC levels (about 17-18 days and 1-2 days, respectively, prior to transport) and Times 3 and 4 represent post-transport GC levels (about 3-6 days and 9-21 days, respectively, after transport).

Tamarin	Sex	Cage mate	Age	Time 1 GC concentration (ng/g dried scat)	Time 2 GC concentration (ng/g dried scat)	Time 3 GC concentration (ng/g dried scat)	Time 4 GC concentration (ng/g dried scat)
GA	F	QE	13	80.61	232.92	107.64	235.55
QE	M	GA	3	238.63	270.67	778.95	N/A
IY	F	WN	4	74.60	59.02	1072.54	524.76
WN	M	IY	8	107.23	147.22	287.72	237.74
ZT	F	YI	11	10.64	126.59	1955.31	413.15
YI	M	ZT	5	31.96	442.73	1149.85	336.06
WA	M	VO	11	898.05	408.15	443.90	268.94
VO	F	WA	8	185.27	206.62	1825.68	218.94
F mean				265.98	206.71	894.85	360.60
M mean				140.77	266.81	1010.55	264.25
Overall mean				203.37	236.74	952.70	319.31

## DISCUSSION

Non-human primates represent a small (estimated at <0.3%) but critical proportion of animals used in laboratory research in the United States (Capitanio et al. 2006). Of the approximately 1,800 cotton-top tamarins in captivity, 64% are housed in research laboratories (Savage et al. 1997). Many such research laboratories obtain animals from domestic breeding sources or other facilities which require shipment, often over large distances. Transport, along with environmental and social change, can constitute a long-term stressor for non-human primates, potentially leading to increases in GC levels. Chronically increased GC levels have been linked to deleterious physiological effects (Pride 2005; Sapolsky 1989).

This study demonstrated that the use of the Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit is an appropriate technique for determining GC levels in cotton-top tamarins. This was demonstrated using a parallelism assay in which there was no difference between the slopes of a serially diluted standard and a serially diluted pooled sample.

Average GC concentrations exhibited an approximately four-fold increase from pre-transport to immediate post-transport levels (Time 3). These results are consistent with studies in other species (e.g., domestic pig (*Sus domesticus*), greyhound (*Canis familiaris*), and mouse (*Mus musculus*) (Kim et al. 2005; Nyberg et al 1988; Leadons and Mullins 1991; Tuli et al. 1995) that have detected higher GC concentrations following transport. Although tamarins showed a post-transport elevation in GC levels, this increase was transient. The immediate post-transport mean (Time 3) was significantly greater than all other times (Time 1, 2, and 4). However, GC levels returned to or near pre-transport level within three weeks. The large increase in GC levels directly after transport is presumed to be a stress response.

GC concentrations varied among individuals. Although the difference in GC levels between two individuals (WN and ZT) was large (Table 1), primates naturally exhibit two to 10 fold variation in GC levels, with daily fluctuations of  $\geq 2.2$  fold, depending on the species (Sapolsky 1992). Individual variation could due to previous exposure to stressors (Sapolsky et al. 2000). Although none of the subject animals had been transported across facilities before, previous experience with such stressors (e.g., movement to a new cage or room or veterinary procedures) may have altered the effects of transport for some individuals. For example, GA was moved to a new cage prior to transport and exhibited lower GC levels following transport than during baseline data collection.

Glucocorticoid levels may have been higher if the animals had not been transported in male/female pairs. Close proximity of a heterosexual partner has been shown to reduce the physiological consequences of novel-cage housing for black tufted-ear marmosets (*Callithrix kuli*) and other New

World monkeys (Hennessy et al. 1995; Smith et al. 1998). In the callitrichid primates (marmosets and tamarins), there is a strong, long-term socio-sexual pair bond that exists between the breeding male and female in a social group (Evans 1983; Anzenberger 1993; Schaffner et al. 1995). If this close bond provides a form of support during stressful events, paired tamarins and marmosets may exhibit a reduced stress response. All of the tamarins in this study were transported with an established mate; therefore their physiological stress responses may have been socially mitigated. Regardless, there was clear evidence that transport induced increased GC levels among the majority of tamarins in the study.

## ACKNOWLEDGEMENTS

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## EFFECTS OF ROADS ON MOVEMENT OF DISPLACED WHITE-FOOTED DEERMICE (*PEROMYSCUS LEUCOPUS*)<sup>1</sup>

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### ABSTRACT

**Although roads are a major part of modern landscapes, the impacts of roads on small mammal behavior and population dynamics are still poorly understood. In a study over the summers of 2003 and 2004 in Nolde Forest near Reading, PA, we examined the impacts of roads on the behavior of white-footed deermice (*Peromyscus leucopus*). Mice were trapped near the center of the park using Sherman traps. Half of the mice were released near roads that they would have to cross in order to return home, and half were released in the park interior. Using a combination of powder tracking and radio tracking, we were able to investigate the impacts of roads on the movement of displaced individuals. Although our mice avoided roads immediately after release, longer tracking through radio-telemetry showed that they did cross roads. Our findings suggest that small roads (i.e., two-lane paved roads) do not impede movement of white-footed deermice. [ J PA Acad Sci 88(2): 89-94, 2014 ]**

### INTRODUCTION

Many recent studies have focused on the impacts of habitat fragmentation whereby a contiguous habitat is partitioned into smaller and more isolated patches (Crooks and Sanjayan 2006, Ewers and Didham 2005, Fischer and Lindenmayer 2007, Horskins et al. 2006, Lindenmayer and Fischer 2006). Roads are a major cause of habitat fragmentation (Forman and Alexander 1998), and roads influence the movement of animals through the landscape mosaic (e.g., Adams and Geis 1983, Clark et al. 2001, Forman and Alexander 1998, Oxley et al. 1974). Given the high density of roads in the modern landscape (Forman and Alexander 1998), understanding how roads impact wildlife is a critical issue.

Multiple studies have demonstrated taxon-specific responses to roads. For example, Benítez-López et al. (2010)

showed that the effect of roads is greater on mammals than on birds, and greater on large mammals than on small mammals. Similarly, Goosem (2001) found that species size, behavior, and dispersal ability affect the impacts of roads on small mammals. Furthermore, while brown hares (*Lepus europaeus*) are negatively affected by road density and avoid areas with roads (Roedenbeck and Voser 2008) and west European hedgehogs (*Erinaceus europaeus*) avoid crossing large roads (Rondinini and Doncaster 2002), many smaller mammals appear to readily cross roads near their home territory (Clark et al. 2001, McGregor et al. 2008, Richardson et al. 1997). However, habitats closer to roads have lower population densities of some small mammal species (Adams and Geis 1983), and therefore roads can still negatively impact small mammal populations.

Some studies suggest that roads act to fragment small mammal populations (Adams and Geis 1983, Gerlach and Musolf 2000, Merriam et al. 1989, Oxley et al. 1974, Spellerberg 1998, Swihart and Slade 1984). While these studies demonstrate that resident animals do not often cross roads (Adams and Geis 1983, Oxley et al. 1974, Spellerberg 1998, Swihart and Slade 1984), the subjects of these studies are usually residents rather than dispersing individuals. In a study using multiple tracking methods and both spontaneous movements and displacement methods, Clark et al. (2001) showed that a variety of rodent species will readily cross roads, but are more likely to do so when they are displaced rather than spontaneously. Similar to most of the other studies cited, however, Clark et al. focused on animals whose home range is close to the road and therefore may be habituated to the road.

Genetic studies suggest only very large roads have a significant effect on the genetic connectedness of populations (Gerlach and Musolf 2000). High genetic connectedness indicates high rates of gene flow and thus high migration rates. Thus, while residents may use roads as convenient home range boundaries (Merriam et al. 1989), dispersing individuals may treat roads very differently allowing for high genetic migration rates (*sensu* Gerlach and Musolf 2000).

In an effort to determine how roads and associated vehicular traffic affect dispersing individuals, Ford and

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Fahrig (2008) examined movement patterns of displaced eastern chipmunks (*Tamias striatus*) near roads. They found that animals avoid the roadside verge and road surface rather than avoiding traffic noise and motion. They argued that roads will therefore act to subdivide populations through behavioral effects rather than by being a significant cause of mortality for chipmunks (Ford and Fahrig 2008).

Although some studies (e.g., Clark et al. 2001, Ford and Fahrig 2008, McGregor et al. 2008) have examined the effects of roads on displaced individuals who may or may not be familiar with the area and therefore perceive the roads differently, we still lack a basic understanding of the potential behavioral impacts of roads on the dispersal of small mammals across longer distances. To address this question, we performed a study similar to Ford and Fahrig (2008) but focused on white-footed deermice (*Peromyscus leucopus*). Our objective was to test what effect, if any, the presence of a road has on the behavior of dispersing white-footed deermice and their ability to cross roads when they are unfamiliar with their surroundings. Specifically, we hypothesized that deermice would behave differently when released near roads than when released further from roads.

## METHODS

This experiment took place in Nolde Forest near Reading, PA, USA (Figure 1) from 10 June through 30 July 2003 and from 1 June through 29 July 2004. The park consists of a closed-canopy forest with a mixture of deciduous (primarily red oak – *Quercus rubra*, tulip tree – *Liriodendron tulipifera*, and green ash – *Fraxinus pennsylvanica*) and coniferous trees (primarily Norway spruce – *Picea abies*, white pine – *Pinus strobus*, and red spruce – *Picea rubens*). The understory

consists primarily of spice bush (*Lindera benzoin*), wine berry (*Rubus phoenicolasius*), Christmas fern (*Polystichium acrostichoides*), and mapleleaf viburnum (*Viburnum acerfolium*). The park has numerous walking/biking trails allowing easy access to all areas, and there are paved roads surrounding the park but minimal paved surfaces within the park and these are restricted to the eastern edge of where the park buildings are located (Figure 1). The traffic estimates of the paved roads range from fewer than 200 vehicles per day along the 8 m wide road on western edge of the park through 1500 vehicles per day along the 9 m wide road on the northern edge to 4300 vehicles per day along the 11 m wide road on the eastern side of the park (traffic data from 2009, <http://www.dot.state.pa.us>).

We established a 135 m by 135 m small-mammal trapping grid in a mixed deciduous and coniferous stand near the center of the park (Figure 1). The grid consisted of 100 Sherman traps (Model LFA, H. B. Sherman Traps, Inc. Tallahassee, FL) with 15 m spacing. The traps were baited with rolled oats and peanut butter, and checked every morning and evening (2003) or checked and closed in the morning and reopened in the evening (2004). Each animal captured was weighed, sexed, and permanently marked by toe clipping. All methods were approved by the animal care and use committee of Albright College (ACUC protocol 2003-SGM1).

We selected adult male white-footed deermice from the trapping grid for experimental displacement. We used males to avoid potential effects of sex-biased dispersal and removing pregnant or lactating females from their home ranges. We held each selected individual for 12 h before release (captured in the morning and held until the evening for release). In 2003, we displaced five mice at each of six release points for a total of 30 displaced mice. Half the release points were located across the roads surrounding the park (hereafter referred to as road releases) and the other half were located at similar distances from the trapping grid but within the park (hereafter referred to as control releases; Figure 1). Release point habitat was visually matched to be similar to the trapping grid habitat (closed canopy deciduous forest with similar understory). The release points covered a range of distances from the original trapping grid: road releases at 500 m, 942 m and 1090 m; and control releases at 680 m, 880 m, and 1075 m. Release points were also located at varying distances from roads: Road releases at 16 m, 18 m, and 30 m; and control releases at 179 m, 388 m, and 174 m.

Mice were held for 8-12 hrs and then transported in the evening to the release site in Sherman traps so they were unable to see their surroundings. In 2003, mice were thoroughly brushed with fluorescent powder (Lemen and Freeman 1985) and placed inside a 10.16 cm diameter PVC pipe that was open on one end and capped on the other end. The pipe was then placed open-side-down on an 'L'-shaped platform with a 50 m string attached from the closed end of the pipe over the high-point of the 'L' and stretched parallel to the road (and perpendicular to the direction the mouse

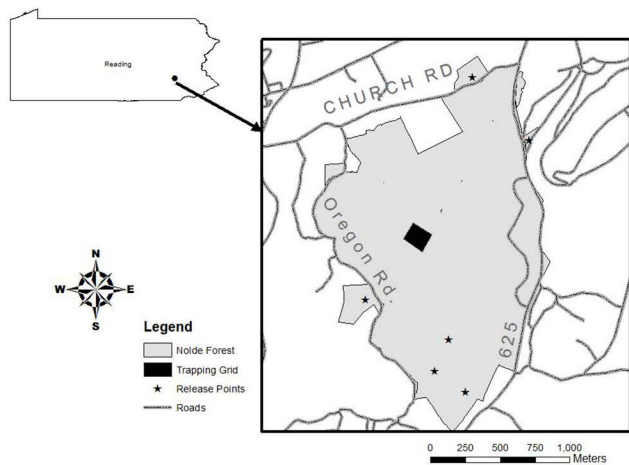


Figure 1. Map of Nolde Environmental Education Forest near Reading, PA including trapping grid and release points. The three roads surrounding the park are labeled.

would travel 'home'). The mouse was released from the mechanism by pulling the string to raise the pipe allowing the mouse to move freely. Two hours after the release, we used a 4.5 W handheld black light to trace and mark the powder trail with survey flags. The following day we recorded the trail by plotting bearing and net distance moved from the release point for every 1 m of trail distance. Sample trails are presented in Figure 2A.

In 2004, we radio-collared (Holohil Systems, Inc. Model BD-2C, Ontario, Canada) and displaced nine adult male mice. Mice were released in the same manner as for the powder-tracking study except they were not brushed with fluorescent powder. Using yagi antennas connected to Merlin 12 receivers (Custom Electronics of Urbana), we located each radio-collared male every 12 h for 3-8 days via triangulation from three fixed points near each release site (White and Garrott 1990). In 2004, five mice were released across the paved roads surrounding the park (road releases), while the other four individuals were released within the roads surrounding the park (control releases). We recorded locations of the trapping grid and release sites using a global positioning system (Trimble Company, Model TSC1) and used ArcView (ESRI Corp. Redlands, CA, Version 10.0) to map the movements of released individuals.

We used a Watson-Williams test (Zar 2009) to test for differences in movement direction between the road and control releases. We compared directions after the mice had traveled 5, 10 and 15 m net displacement from the release point (Figure 2B). To compare the overall tortuosity of the paths for road and control releases, we calculated the ratio of the trail distance (total distance travelled) to the net distance (distance from the release point). A value close to one indicates a nearly linear path whereas a larger value indicates a more curved or tortuous path. We then tested for differences in the ratio using a repeated measures ANOVA with the between-subject factor being presence/absence of road and the within subject factor being trail distance. A significant main effect of the presence of roads would indicate a difference in the response to roads, whereas a significant interaction between trail distance and presence of road would indicate that the presence of a road may affect behavior differently as the trail lengthens (e.g., the animal develops an awareness of the road after the immediate flight response).

## RESULTS

We discarded six powder-tracking trails (two road releases and four control releases) that did not extend more than 5 m from the release point (for three of these trails there was clear evidence that the animal stopped to clean itself before moving further, and the other three made several small loops around the release point). For the remaining trails, results are summarized in Table 1. At 5 m and 10 m net displacement, the two groups had significant differences in their directions

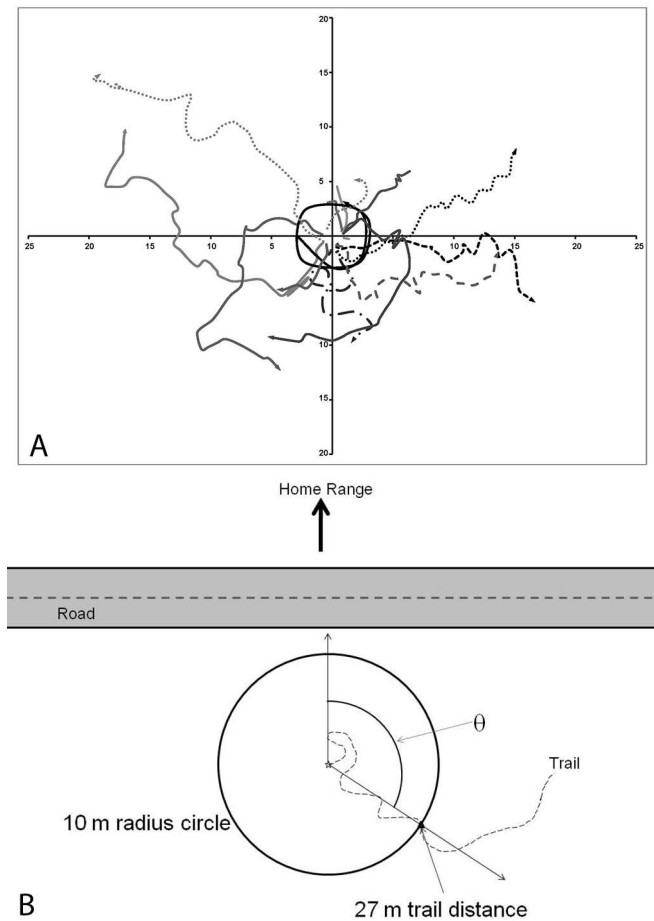


Figure 2. (A) Example data from powder tracking. Each line represents a unique path from a tracked individual. Trails were mapped at 1-m intervals. (B) Schematic representation of trail for circular analysis. The angle ( $\theta$ ) between home and the point on the 10-m radius circle was used in the circular analysis. Note that although this does not represent an actual trail, the tortuosity used for the trail in this representation is 2.7.

with the mice near roads moving away from the road while the control mice tended to move more towards home (Figure 3); at 15 m the difference between groups was not significant. The two groups were not significantly different in their tortuosity ( $F_{1,22} = 0.05$ ,  $P = 0.820$  for the main effect) but there was a significant interaction between treatment and travel distance ( $F_{39,523} = 1.75$ ,  $P = 0.004$  for the interaction).

Radio tracking results were assessed qualitatively due to small sample sizes. In 2004, the radio-tracked individuals all failed to return to the original trapping grid; however, they all demonstrated some degree of exploration. All radio-tracked mice eventually established new home ranges, which we arbitrarily defined as more than three successive locations ( $>36$  hours) in the same general area. Interestingly, all radio-collared individuals released on the opposite side of a road from the main park crossed the road before establishing a new home range, despite there being no qualitative differences in

Table 1. Summary of analysis of movement paths in white-footed deermice. Distance refers to the net distance from the release point. Theta is the mean angle relative to the original capture point and nearest road (if release was near a road).  $\bar{R}$  is the mean length of the vector (ranges 0 to 1) and is directly related to the variance estimate of the angle.

Distance	Road?	N	Mean Trail Length	Theta	$\bar{R}$	F* (df)	P
5	N	11	7.50 ± 0.75	329.94	0.22	8.37	0.009
	Y	13	8.27 ± 0.74	205.46	0.16	(10,12)	
10	N	7	18.43 ± 1.85	278.47	0.18	4.684	0.0482
	Y	9	17.11 ± 2.71	154.32	0.28	(6,9)	
15	N	4	24.75 ± 1.75	80.82	0.29	0.171	0.6917
	Y	5	22.60 ± 1.86	121.82	0.98	(3,4)	

\* F-ratio for Watson & Williams test for differences in directionality.

the habitat on either side of the road. Radio-tracking data showed that mice moved about 100 to 200 m from the release points before establishing new home ranges. Mice released at the same point tended to establish overlapping home ranges. Examples of radio-tracking data are presented in Figure 4.

## DISCUSSION

Our results demonstrate that white-footed deermice behave differently when released within 30 m of roads compared to when they are released more than 100 m from roads. Mice released near roads tended to move away from

the road rather than towards home, and their paths became more tortuous as they moved further from the release point as indicated by the significant interaction between tortuosity and distance. In contrast, mice released at the control sites appeared to have a stronger homing drive as indicated by their initial tendency to move towards the original trapping grid, although this should be interpreted with caution as no mice actually returned to the original trapping grid. Due to the shape of the park and the location of the control release points, these mice may have been moving towards home simply to avoid the roads surrounding the park. However, we do not believe this is the case because the closest road was more than 100 m away and the topography was such that vehicular noise was minimal at the release points.

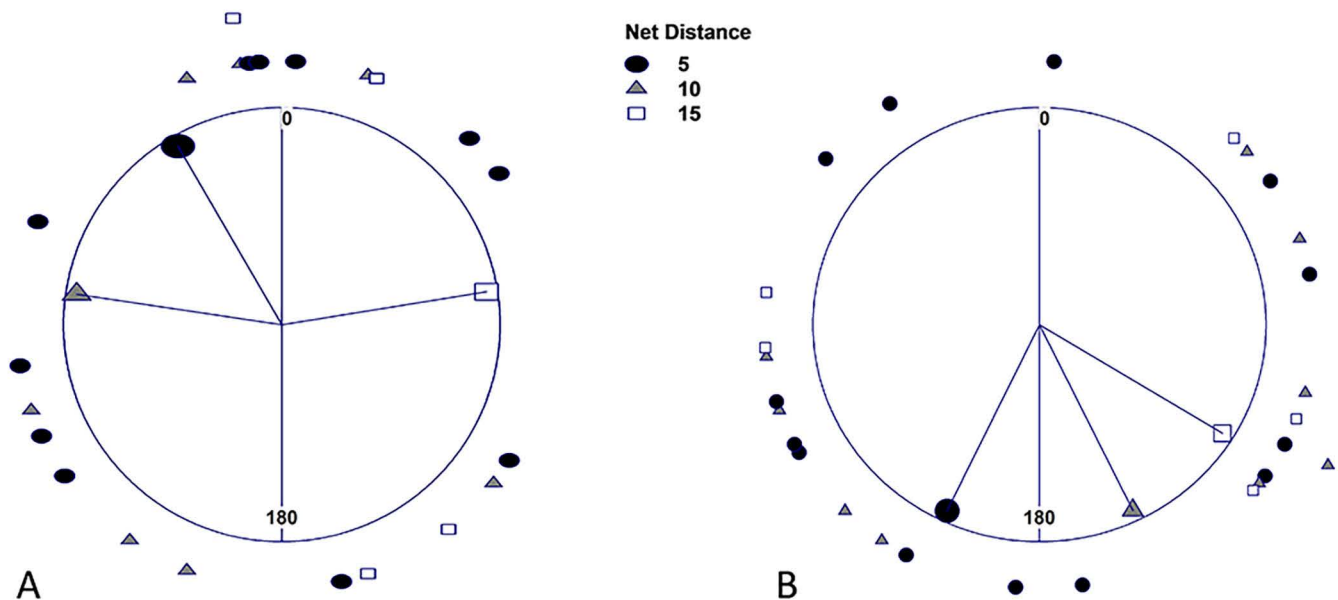


Figure 3. Location of mice after traveling 5, 10, or 15 m from release point. Original capture location is located at the top of each circle. Release locations were in the center of each circle, and the vectors represent the average angle for each group. (A) Positions of mice released away from roads (control releases). (B) Positions of mice released near roads (road releases).



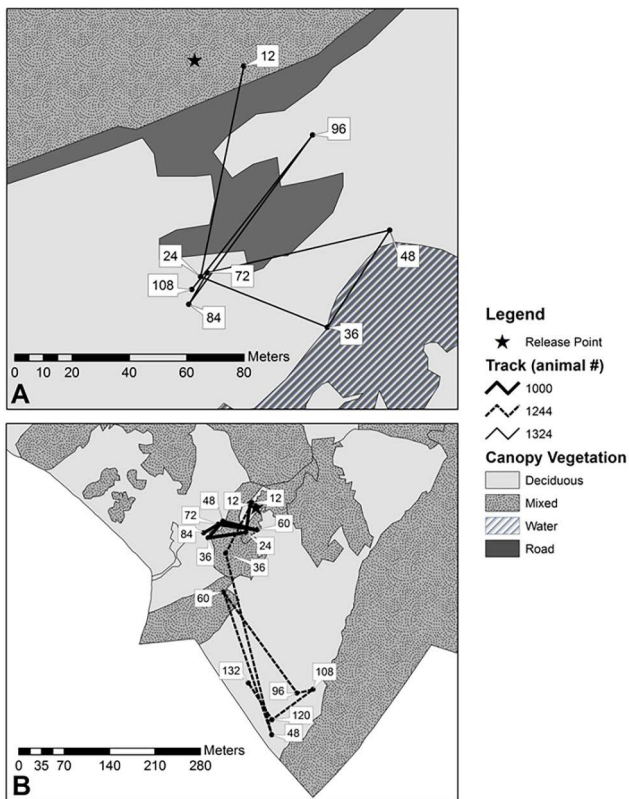


Figure 4. Example data from radio-tracking study. (A) A representative track of an animal released outside the park (road release). (B) Representative tracks from animals released inside the park (control release). Each line style represents a different individual. The numbers along the tracks correspond to the hours since release.

Interestingly, none of the released mice returned to the original trapping grid and the radio-collared mice released from the control sites established home ranges further from the trapping grid indicating a net movement away from home (e.g., Figure 4).

Mice in both types of release may have simply been exploring their new area in a more-or-less random walk manner. However, given the initial tendency of mice released near the road to move away from the road, combined with the radio-tracking data demonstrating road crossing within 12-24 hours, it is clear that white-footed deermice will eventually approach and cross roads, although the behavioral drive for them to cross is still unknown.

Fahrig and coworkers (Ford and Fahrig 2008, McGregor et al. 2008) have previously studied the impacts of roads on small mammal behavior. McGregor et al. (2008) found that translocated rodents will cross roads, but the distance of the translocation and the number of intervening roads impacts the likelihood of return. Interestingly, they found no correlation between traffic volume and the frequency of these behaviors. Similarly, Ford and Fahrig (2008) showed

that eastern chipmunks avoid roads and road verges upon translocation, again independent of traffic volume. Taken together, these studies strongly suggest that it is the road surface and the verge, rather than traffic, that some small mammals avoid. Similarly, Rondinini and Doncaster (2002) argued that hedgehogs appear to avoid the synthetic surface of the road by altering their posture when crossing roads. Our data support the hypothesis that it is the road surface itself that is avoided rather than traffic. Our releases were at night, when few vehicles (<2/hr, pers. obs.) travel the roads, and the roads in our study also lacked any recognizable verge (the forest habitat extended to the paved surface with trees being as close as 0.4 m from the pavement). We found that white-footed deermice behave differently when released near roads as compared to when released far from roads. Specifically, the mice tend to move away from the road (and thus away from home) when near a road, but tend to move towards home when not released near a road. This behavior does not, however, prevent the mice from crossing the road as indicated by our radio-tracking data.

Many studies have concluded that roads, even those with low traffic volumes, will act as barriers to the movement of white-footed deermice (Adams and Geis 1983, Ford and Fahrig 2008, Gerlach and Musolf 2000, McGregor et al. 2008, Merriam et al. 1989, Oxley et al. 1974, Spellerberg 1998, Swihart and Slade 1984). However, all five radio-tracked mice released on the outside of the main park in 2004 returned to the inside of the park to establish new home ranges, crossing a road in the process. Gerlach and Musolf (2000) demonstrated that only larger roads (i.e., multi-lane freeways) actually reduced the genetic connectedness between populations, presumably by reducing dispersal across roads. Similarly, McGregor et al. (2008) showed that small mammals, including white-footed deermice, will readily cross roads. These studies, in conjunction with our data, strongly suggest that narrow roads do not act as a significant barrier to the movement of white-footed deermice. Instead, it is more likely that roads appear to act as barriers because they serve as convenient home range boundaries for many individuals within a population of white-footed deermice (e.g., Merriam et al. 1989).

This study confirms previous work that roads do not form an impervious barrier to the dispersal of white-footed deermice. As stated above, several studies have demonstrated that roads prevent certain individuals from dispersing and, therefore, roads do alter the behavior of mice. However, we argue that roads act as a semi-permeable filter to dispersing mice, although the particular characteristics of individuals and roads that permit dispersal or even simply movement across roads are still poorly understood. Factors such as these need to be further studied to better predict the influence of roads and other infrastructure on small mammal populations.

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## AEROBIC ORAL AND NASAL BACTERIA IN NEW JERSEY BLACK BEARS (*URSUS AMERICANUS*) WITH A REPORT ON ANTIBIOTIC SUSCEPTIBILITY OF *ESCHERICHIA COLI*<sup>1</sup>

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### ABSTRACT

The microbiology of animal bite wound infections is often polymicrobial. Black bear attacks have been a rare occurrence in the past and with few published studies on their oral flora, the bacteria present in black bear bite wounds is largely unknown. This study examines the oral and nasal aerobic bacteria from research trapped bears in northwestern New Jersey during June 2014. Oral swabs from the buccal and lingual supragingival tooth surfaces and nasal swabs from 22 black bears were plated onto Tryptic Soy Agar (TSA), Eosin Methylene Blue agar (EMB), and Columbia Nalidixic Acid agar (CNA) for bacterial isolation. Twelve aerobic bacterial species were identified from the oral and nasal samples. The most frequently isolated bacteria were *Bacillus* sp., *Klebsiella oxytoca*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The diversity in the aerobic oral and nasal flora of black bears in New Jersey suggests the importance of including these organisms in basic health risk assessment protocols and suggests a potential tool for assessment of bear/habitat interactions. To evaluate the role of black bears in the spread of antibiotic resistant *Escherichia coli*, oral, nasal and anal samples were collected from eight black bears (two sows and six cubs). Antibiotic resistance was measured for tetracycline and streptomycin. *E.coli* resistance for tetracycline and streptomycin was 7%. [ J PA Acad Sci 88(2): 95-100, 2014 ]

### INTRODUCTION

New Jersey black bears (*Ursus americanus*) are found primarily in the northern portion of the state but the population has been steadily moving south. Black bears have been sighted in all 21 New Jersey counties (NJDEP, 2014). They den in rock cavities, brush piles, felled trees, and open nests. The oral flora of bears generally reflects their diet,

which is high in plant matter, insects and occasional meat or carrion (Huffman et al., 2010; Thomas and Brook, 2011).

Wound infections due to bites tend to reflect the microorganisms present in the saliva and oral cavity of the human or animal that created the bite wound. The mammalian mouth has been reported to support the growth of over 200 species of facultative organisms and obligate anaerobes, with as many as 100 million organisms per mL of saliva. The oral flora of the animal saliva, rather than the skin flora of the victim, is the source of most bacteria isolated from bite wound cultures. Infections are usually polymicrobial and contain mixed aerobic-anaerobic isolates (Rodriguez, [http://www2.massgeneral.org/id/hms/handouts20032004/martin4\\_04.pdf](http://www2.massgeneral.org/id/hms/handouts20032004/martin4_04.pdf)).

Data for the oral bacteriology of bears as well as bear bite wounds in humans are limited to a few studies and case reports (Rose, 1982; Parry et al. 1983; Herrero and Fleck, 1990; Floyd et al. 1990; Floyd, 1999; Kunimoto et al. 2004; Lehtinen, et al., 2005; Abrahamian and Goldstein, 2011). Bacteria isolated from bear bite wounds in humans include *Aeromonas hydrophila*, *Bacillus cereus*, *Citrobacter diversus*, *Hafnia* sp., *Acinetobacter* sp., *Enterococcus durans*, *Enterobacter* sp., *Escherichia coli*, *Mycobacterium fortuitum*, *Neisseria sicca*, *Proteus vulgaris*, *Serratia fonticola*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus sanguis* (Rose, 1982; Parry et al. 1983; Kunimoto et al. 2004 Lehtinen et al. 2005).

Goatcher et al. (1987) obtained swab specimens from nasal, rectal, and preputial or vaginal areas of 37 grizzly and 17 black bears, to determine the types and frequency of predominant aerobic microflora. Bacterial genera most frequently isolated were *Escherichia*, *Citrobacter*, *Hafnia*, *Proteus*, *Staphylococcus*, and *Streptococcus*, comprising about 65% of the isolates. *Erwinia*, *Xanthomonas*, *Agrobacterium*, *Rhizobium*, and *Gluconobacter/Acetobacter* were also isolated but at lower frequencies (less than 5%). The majority of microorganisms isolated from the plant samples in the study area were also found in bear samples. This observation and the presence of certain water and soil bacteria in samples from bears suggest that the predominant microflora of both grizzly and black bears were transient and probably influenced by their foraging habits and surrounding environments.

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Clarke et al. (2012) examined the anaerobic oral flora of hunter killed black bears (*Ursus americanus*) in eastern North Carolina. Sixteen anaerobic bacterial species, representing nine genera were identified, and a number of organisms grown that could not be identified. The most frequently identified anaerobes were *Peptostreptococcus prevotii*, *Streptococcus constellatus*, and *Porphyromonas gingivalis*. The diversity in the anaerobic oral flora of black bears in eastern North Carolina suggests the importance of including these organisms in basic health risk assessment protocols.

The emergence and dissemination of antimicrobial resistance is a growing concern to public and animal health. The contribution attributable to wildlife remains unclear.

Antibiotic use in clinical, veterinary and agricultural practices has been the major selective force for emergence and spreading of resistant strains and resistance genes, since the 1950s (Bonomo and Rossolini, 2008). Increased overlap between humans and wildlife populations has increased the risk for resistance to antibiotics in wildlife.

The use of antibiotics in agricultural and livestock is believed to increase the selection of resistant bacterial strains. Wildlife species such as black bears are considered as a source for resistant bacteria and significantly contribute to the spread of resistant bacteria from farms and local areas to the environment (Sayah et al., 2005; Cole et al., 2005).

Black bears are driven by their need to eat, so that anything that is accessible and can be eaten is a potential bear attractant. Garbage is a bear attractant in New Jersey. It is not the only food source; bird feeders, pet food and compost often attract bears and bring them in close proximity to humans.

The gram negative bacterium *E. coli* is the most frequently used indicator for addressing antibiotic resistance dissemination in different environments and host species. It is a frequent carrier of different antibiotic resistance genes. It is also a prominent constituent of human, bird and most mammalian gastrointestinal tracts (Martinez, 2009).

There is little information on the bacteria found in the oral and nasal cavities of bears, and no studies have been conducted in New Jersey black bears. The objectives of this study was to determine: (1) the oral and nasal aerobic bacteria from New Jersey black bears during research trapping in June 2014, and (2) the antimicrobial resistance of *Escherichia coli* isolated from oral, nasal and anal samples to tetracycline and streptomycin in denning sows and their cubs.

## MATERIALS AND METHODS

Samples were collected during the den and summer research trapping session from black bears in northwestern New Jersey in March and June 2014. Age (adult, yearling, or cub) and sex were recorded for each bear sampled. Bears were trapped using Aldrich foot snares by New Jersey

Division of Fish and Wildlife personnel. A Dan-inject dart rifle (Wildlife Pharmaceuticals Inc. Fort Collins, CO) was used for injecting the anesthetic. The drug was a mixture of 200 mg/kg ketamine hydrochloride (Ketaset®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) and 450 mg/kg xylazine hydrochloride (Rompun® Mobay Corporation Animal Health Division, Shawnee, Kansas). During den work sows were anaesthetized in the den using the protocol described above.

Oral swabs (BBL™ CultureSwab™ Collection and Transport System ) were taken from the buccal and lingual supragingival tooth surfaces and nasal swabs from 22 black bears, 4 adult and 10 yearling males, and 2 adult and 6 yearling females. Swabs were plated within seven hours. Each swab was plated onto a Tryptic Soy Agar (TSA) plate, an Eosin Methylene Blue (EMB) agar plate, and Columbia Nalidixic Acid agar. All plates were incubated at 36° C for 24 to 36 hours. Colony morphology was recorded and isolated colonies were gram stained. Gram positive cocci were plated onto Mannitol Salts Agar (MSA) and species identified biochemically. Gram positive bacilli were stained for endospores. Gram negative bacteria were identified using the biochemical tests for the Enterobacteriaceae and the Pseudomonadaceae.

The oral, nasal and anal areas were swabbed (Fisher Scientific, Waltham, Maine) from two sows and six black bear cubs (five females and one male). Samples were collected and plated within seven hours during den research work in March 2014.

For isolation of *E. coli* each swab was inoculated onto EMB agar plates and incubated at 36 °C for 18-24 hrs. *E. coli* colonies, indicated by the metallic green color, were isolated for biochemical testing and for antibiotic sensitivity testing. Antibiotic sensitivity testing (Kirby Bauer Method), of the *E. coli* isolates were tested by use of antibiotic impregnated discs, tetracycline (30µg), and streptomycin (10µg) (Becton Dickinson, Cockeysville, MD) on a seeded bacterial lawn. Resistance, intermediate susceptibility and susceptibility was determined by measuring the zone of inhibition (Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement, 2007).

## RESULTS

Gram negative bacteria were isolated from the oral and nasal samples from all 22 black bears during research trapping. The bacterial species and percent positive for each species are found in Table 1. The isolates were *Bacillus mycoides*, *B. subtilis*, *Bacillus* sp., *Micrococcus luteus*, *Staphylococcus aureus*, *S.epidermidis*, *Streptococcus* sp., *Enterobacter* sp., *E. coli*, *Klebsiella oxytoca*, *Proteus* sp., and *Pseudomonas aeruginosa*. Tables 2 and 3 depict the oral and nasal flora that were isolated by sex and age.

A total of 14 *E. coli* isolates were recovered from the oral,

Table 1. Bacterial species and percent positive isolated from the mouth and nasal passages of 22 black bears from northwestern, New Jersey.

Bacterial Species	Oral Samples	Nasal Samples
	Number of bears (percent positive)	Number of bears (percent positive)
<i>Bacillus mycoides</i>	4 (18.2)	4 (18.2)
<i>Bacillus subtilis</i>	0	4 (18.2)
<i>Bacillus</i> sp.	16 (72.7)	14 (63.6)
<i>Micrococcus luteus</i>	9 (40.1)	7 (31.8)
<i>Staphylococcus aureus</i>	2 (9.1)	3 (13.6)
<i>Staphylococcus epidermidis</i>	4 (18.2)	7 (31.8)
<i>Streptococcus</i> sp.	1 (4.5)	1 (4.5)
<i>Enterobacter</i> sp.	2 (9.1)	0
<i>Proteus</i> sp.	0	1 (4.5)
<i>Escherichia coli</i>	1 (4.5)	0
<i>Klebsiella oxytoca</i>	10 (45.5)	4 (18.2)
<i>Pseudomonas aeruginosa</i>	8 (36.4)	6 (27.3)

Table 2. Number of black bears that tested positive for oral bacterial flora by sex and age.

Bacterial Species	Yearling Males (10)	Adult Males (4)	Yearling Females (6)	Adult Females (2)
<i>Bacillus mycoides</i>	1	0	3	0
<i>Bacillus</i> sp.	7	4	3	2
<i>Bacillus subtilis</i>	0	0	0	0
<i>Micrococcus luteus</i>	3	1	3	2
<i>Staphylococcus aureus</i>	1	1	0	0
<i>Staphylococcus epidermidis</i>	2	1	0	1
<i>Streptococcus</i> sp.	0	1	0	0
<i>Enterobacter</i> sp.	0	1	0	1
<i>Escherichia coli</i>	0	0	1	1
<i>Klebsiella oxytoca</i>	3	3	2	2
<i>Proteus</i> sp.	0	0	0	0
<i>Pseudomonas aeruginosa</i>	5	1	2	0

nasal, and anal cavities of denning black bears. Tetracycline resistance (TetR) for *E. coli* was 7%, intermediate resistance was 14% and 79% of the isolated colonies were susceptible to the antibiotic (Table 4). Streptomycin resistance (StrR) for *E. coli* was 7% and 93% were sensitive. No intermediate resistance was detected (Table 4).

## DISCUSSION

Microbial flora can provide insight into the assessment of bear/habitat interactions in that they may reflect what they eat, drink and breathe. In addition the types of microbial flora may aid in understanding of health risks associated with animal bites.

Floyd et al. (1990) reported on the aerobic oral flora of 12 black bears (*Ursus americanus*) with periodontal disease from the northern lower peninsula of Michigan. Bacteria isolated included *Micrococcus* sp., alpha and beta-hemolytic *Streptococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter* sp., nonhemolytic *Streptococcus*,



Table 3. Number of black bears that tested positive for nasal bacterial flora by sex and age.

Bacterial Species	Yearling Males (10)	Adult Males (4)	Yearling Females (6)	Adult Females (2)
<i>Bacillus mycoides</i>	1	0	3	0
<i>Bacillus</i> spp.	7	4	3	2
<i>Bacillus subtilis</i>	0	0	0	0
<i>Micrococcus luteus</i>	3	1	3	2
<i>Staphylococcus aureus</i>	1	1	0	0
<i>Staphylococcus epidermidis</i>	2	1	0	1
<i>Streptococcus</i> sp.	0	1	0	0
<i>Enterobacter</i> sp.	0	1	0	1
<i>Escherichia coli</i>	0	0	1	0
<i>Klebsiella oxytoca</i>	3	3	2	2
<i>Proteus</i> sp.	0	0	0	0
<i>Pseudomonas aeruginosa</i>	5	1	2	0

*Bacillus* sp., *Enterobacter* sp., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus* sp., and *Actinomyces* sp. The results of our study were similar to that of Floyd et al. (1990). The most common isolates of bacteria found in the New Jersey black bears' oral and nasal cavities were *Bacillus* sp., *Klebsiella oxytoca*, *Micrococcus luteus*, and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus* sp. All of these bacteria have the potential to be pathogenic in deep, crushing bite wounds that can be inflicted by a black bear.

The nasal bacteria isolated were similar to the oral samples, *Bacillus* sp. were the most common bacteria found. The most notable difference between the oral and nasal samples is that there were more *Staphylococcus aureus* and *Staphylococcus epidermidis* found in the nasal samples. When comparing the oral and nasal samples it was found that 13 of the 22 samples (59%), had the same bacteria present on both the oral and nasal swabs.

Freer (2004), reported that any bacterium, virus, or fungus can become a contaminant in bite wounds. Crushing bite wounds of black bears cause a particular concern for infection because these wounds tend to be deep and allow bacteria to enter far into the wound. All of the bacteria found in the 22 bears from New Jersey are capable of becoming opportunistic pathogens depending on the immune system of the person attacked and on the severity of the wound (Kunimoto et al., 2004). Freer (2004) recommended that prophylactic antibiotics should be given as soon as possible for patients with crushed or deep bite wounds. Kunimoto et al. (2004) reviewed bear attack case reports and noted that patients that were effectively treated were "treated with a broad spectrum agent with activity against *S. aureus* and aerobic gram-negative rods." A broad spectrum agent, such as streptomycin and amoxicillin, would cover most of the microorganisms found in the 22 New Jersey black bears, as

many were gram negative rods and *S. aureus* was present in both the oral and nasal samples. This study did not attempt to identify anaerobic oral or nasal bacteria, but an early treatment with metronidazole is believed to effectively eliminate anaerobic bacteria from wounds caused by bears (Kunimoto et al., 2004).

Previous studies (Bonnedahl et al. 2009; Hernandez et al. 2010; Simoes et al. 2010) showed that wild birds frequently carry resistant *E. coli* often with human-associated genotypes. Wheeler et al. (2012) evaluated antibiotic resistance as a molecular marker for the intensity of human-wildlife microbial connectivity in the Galápagos Islands. Many of the reptiles harbored *E. coli* bacteria that were resistant to ampicillin, doxycycline, tetracycline, and trimethoprim/sulfamethoxazole. Their findings suggest that reptiles living in closer proximity to humans potentially have higher exposure to bacteria of human origin; however, it is not clear to what extent this potential exposure translates to ongoing exchange of bacterial strains or genetic traits.

The black bear population in New Jersey has greatly increased. Generally a forest species, these opportunistic animals have learned to benefit from human presence. Our study did not find a high percentage of resistant strains of *E. coli* in the denning black bears. This lower percentage of resistance may reflect the small sample size.

The opportunity exists to use wildlife, particularly black bears, because of the close proximity to humans, as sentinels to monitor the spread of resistant bacteria.

Table 4. Antimicrobial resistance for tetracycline (30µg) and streptomycin (10µg). R = resistant, I = intermediate and S = Susceptible, NA = no *E. coli* isolated.

Sample	Tetracycline (30µg)	Streptomycin (10µg)
Sow 1		
Oral	R	R
Nasal	S	S
Anus	I	S
Cub 1 female		
Oral	S	S
Nasal	NA	NA
Anus	S	S
Cub 2 female		
Oral	S	S
Nasal	S	S
Anus	I	S
Sow 2		
Oral	NA	NA
Nasal	S	S
Anus	S	S
Cub 1A female		
Oral	S	S
Nasal	NA	NA
Anus	S	S
Cub 2A female		
Oral	NA	NA
Nasal	NA	NA
Anus	NA	NA
Cub 3A female		
Oral	NA	NA
Nasal	NA	NA
Anus	NA	NA
Cub 4A male		
Oral	NA	NA
Nasal	S	S
Anus	S	S

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## CASE REPORT: FATAL DISSEMINATED TOXOPLASMOSIS IN A BLACK BEAR CUB<sup>1</sup>

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### ABSTRACT

**A black bear (*Ursus americanus*) cub with signs of neurological disease was captured in West Milford, NJ. The animal died in captivity and was examined because of suspected rabies. At necropsy, the lungs were reddened and noncollapsed and had multiple pale round foci. Foci of necrosis were associated with *Toxoplasma gondii* cysts and tachyzoites in several organs. Rabies antigen was not detected. [ J PA Acad Sci 88(2): 101-106, 2014 ]**

### INTRODUCTION

Infection with the coccidian parasite *Toxoplasma gondii* has a worldwide distribution. This obligate intracellular parasite can infect humans as well as virtually all warm-blooded animals, including mammals and birds. Since its first description in the gundi, a rodent from North Africa, by Nicolle and Manceaux in 1908, the parasite was progressively recognized as the agent of a widespread zoonosis. Cats and other feline species are the only recognized definitive hosts of *T. gondii* (Hutchison et al. 1969; Frenkel et al. 1970; Dubey and Frenkel 1972). *Toxoplasma* antibodies are frequently found both in wild and captive felines.

Toxoplasmosis is widespread in wild foxes, raccoons, and skunks according to serologic data, but clinical toxoplasmosis is rarely seen in these species. Black bears have one of the highest *T. gondii* seroprevalences among all known intermediate hosts. Seroprevalence study by Kinyon (2004) in NJ reported a 32% positivity in adult black bears, 42% in yearlings and 20% in cubs. Table 1 lists the seroprevalence of *T. gondii* found in grizzly, black bear and polar bears from various regions.

Disseminated toxoplasmosis (DT) has been defined as

microbiological or histological evidence of disease affecting more than one organ. DT was reported in a red fox (*Vulpes vulpes*) with signs of neurological disease captured in Fairmount Park, Philadelphia, PA. The animal died in captivity. The liver had pale foci up to 4 mm in diameter. Foci of necrosis were associated with *T. gondii* tachyzoites in several organs including liver, lungs and adrenal glands (Dubey et al. 1990). Disseminated toxoplasmosis has also been reported from a captive ring tailed lemur (*Lemur catta*) (Spencer et al. 2004), it was diagnosed in seven captive slender-tailed meerkats (*Suricata suricatta*) according to clinicopathologic findings and immunohistochemistry by Juan-Salles et al. (1997), and in a sand fox (*Vulpes rueppelli*) reported by Pas and Dubey (2008). Lloyd and Stidworthy (2007) reported the first case of disseminated toxoplasmosis in a juvenile cheetah (*Acinonyx jubatus*) which died with rapidly progressive pyrexia, tachypnea, abdominal effusion, and hepatomegaly. Postmortem examination revealed lesions consistent with acute disseminated infection with *Toxoplasma gondii*. The presence of this organism was confirmed in multiple organs by immunohistochemistry and polymerase chain reaction.

Fatal DT has been reported from marine mammals. Migaki et al. (1990) reported fatal DT in a spinner dolphin (*Stenella longirostris*) and Resendes et al. (2002) reported DT in a Mediterranean pregnant Risso's dolphin (*Grampus griseus*) with transplacental fetal infection. The first cases of DT in sirenians was reported by Bossart et al. (2012) in Antillean manatees (*Trichechus manatus manatus*) from Puerto Rico.

Fatal acute to subacute DT was diagnosed as the main cause of death in 16 of 20 4-to 7-month-old Kodiak bears (*Ursus arctos middendorffi*) at Rostock Zoo, Germany (Kiupel et al. 1987). The Kodiak bears were all closely related, which raised the possibility that a genetically-based immunodeficiency was responsible for the high susceptibility. DT was documented in a 6-day-old polar bear cub (*Thalarctos maritimus*) (Roken 1993). We report herein a case of DT in a black bear cub without concurrent infection.

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Table 1. Serological prevalence of *Toxoplasma gondii* antibodies in brown, black and polar bears from different geographical locations. Methods of testing included the Latex Agglutination Test (LAT); Modified Agglutination Test (MAT); Direct Agglutination Test (DAT) and the Indirect Hemagglutination test (IHA).

Ursidae	Number Tested	Prevalence (%)	Assay Used	Locality	Reference
<i>Ursus arctos</i>	480	18.0	LAT	Alaska	Chomel et al. 1995
<i>U. arctos</i>	892	25.0	MAT	Alaska	Zarnke et al. 1997
	149	27.0	LAT	California	Rupanner et al. 1982
	665	80.0	LAT	Pennsylvania	Briscoe et al. 1993
	40	15.0	LAT	Alaska	Chomel et al. 1995
	143	43.4	MAT	Alaska	Zarnke et al. 2000
<i>U. americanus</i>	303	8.0	IHA	Idaho	Binninger et al. 1980
	143	84.0	MAT	North Carolina	Nutter et al. 1998
	328	40.2	LAT	New Jersey	Kinyon 2004
	29	44.8	MAT	Florida	Chambers et al. 2012
	66	25.7	MAT	Maryland	Dubey et al. 2013
	419	20.1	MAT	Norway	Oksanen et al. 2009
	108	11.1	MAT	Greenland	Oksanen et al. 2009
<i>U. maritimus</i>	500	6.0	LAT	US/Russia	Rah et al. 2005
	228	45.6	DAT	Norway	Jensen et al. 2010
	136	13.2	LAT	Alaska	Kirk et al. 2010

## REPORT

In August of 2009, a female black bear cub was found walking aimlessly around the back yard of a residence in West Milford, NJ, where it was captured by animal control personnel. The 8 month old cub died the next day and was submitted to the NJ Division of Fish and Wildlife for necropsy.

The cub weighed 7.03 kg, with a body length of 58.4 cm (Figure 1). Diarrhea was evidenced by wet feces soaking the perianal hairs. No skeletal muscle hematomas, suggestive of trauma, were observed. The stomach was empty except for mucous and a large ascarid (Figure 2). The small intestine contained 3 smaller ascarids.

Blood pooled about the cerebellum and posterior dorsal lobes of the cerebrum (Figure 3). Blood also circumscribed the spinal cord as it passed through the foramen magnum (Figure 4). The enlarged right axillary lymph node was congested with blood. The auricle of the heart was distended with current jelly clots (Figure 5). Blood, in the left lung lobes, contrasted with the many cream foci approximately 1-2 mm diameter scattered throughout the lung (Figure 6). Because the animal had signs of neurologic disease, brain



Figure 1. The cub weighed 7.03 kg, with a body length of 58.4 cm.

tissue was submitted to the NJ Department of Health for fluorescent antibody testing (FAT) for the detection of rabies antigen. No rabies viral antigen was detected.

Tissue from the heart, liver, lungs, kidneys, spleen, brain,





Figure 2. Stomach from the black bear cub was empty with the exception of one ascarid.



Figure 5. Heart auricle distended with blood.

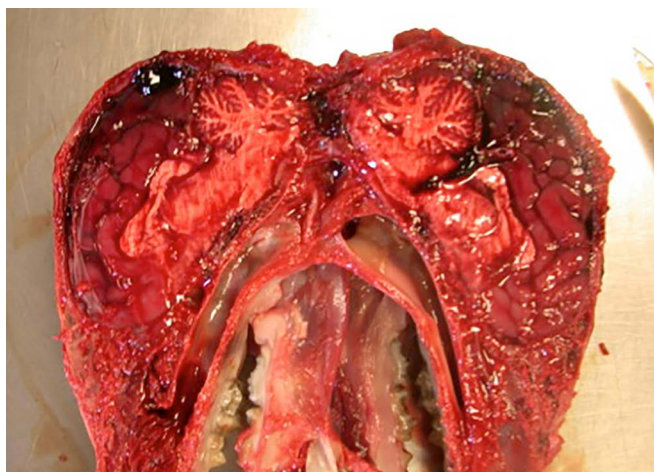


Figure 3. Cerebral and cerebellar hemorrhage.



Figure 6. Multiple pale round cream foci are scattered throughout the blood congested lungs.

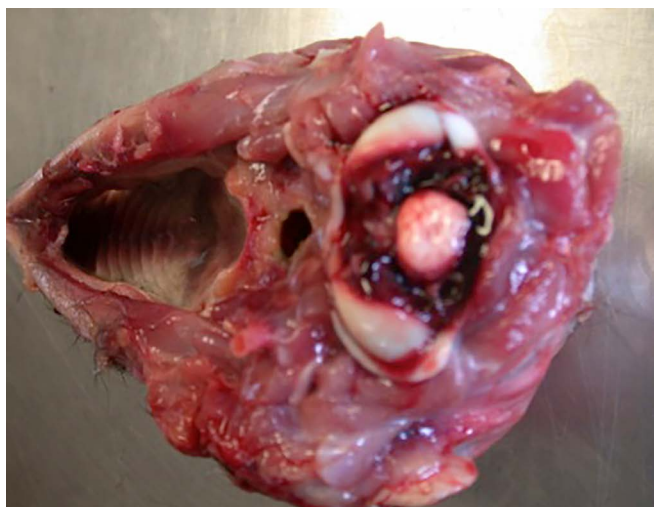


Figure 4. Hemorrhage circumscribing spinal cord passing through the foramen magnum.

stomach, intestines, lymph nodes, were fixed in 10% neutral buffered formalin. Paraffin sections were cut at 6  $\mu$ m and stained with hematoxylin and eosin for examination by light microscopy.

The main features of pneumonia in this bear consisted of nodules, characterized by a fibrotic capsule with central core in the necrotic lung containing free and encysted *Toxoplasma* cysts and tachyzoites with leucocytic infiltration (primarily macrophages and eosinophils) (Figure 7). *Toxoplasma* organisms in the lung were stained positively with anti-*T. gondii* serum.

Cerebral and cerebellar multifocal necrosis was associated with perivascular cuffing of leucocytes (Figure 8), *T. gondii* cysts (Figure 9) and eosinophil infiltration associated with tachyzoites (Figure 10).



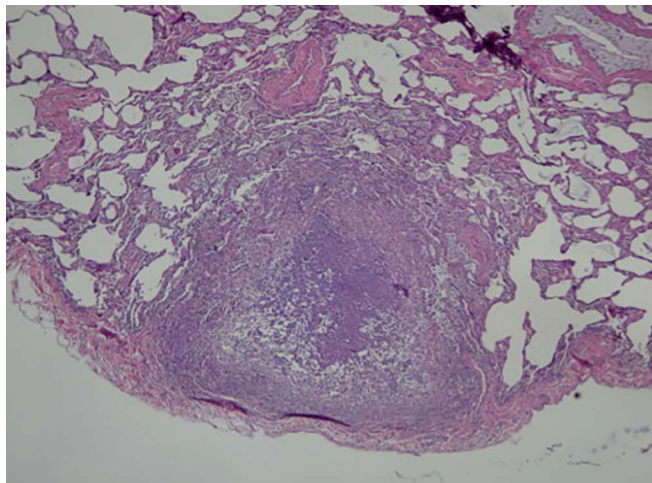


Figure 7. Lung nodules comprised of a fibrotic capsule with central core of leucocytes and cysts containing *Toxoplasma* bradyzoites (Hematoxylin & Eosin stain).

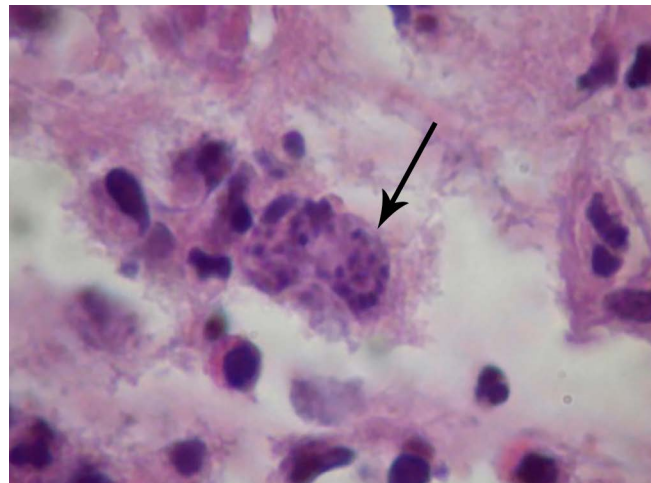


Figure 9. Cerebrum with *Toxoplasma gondii* cyst containing bradyzoites (arrow) (Hematoxylin & Eosin stain).

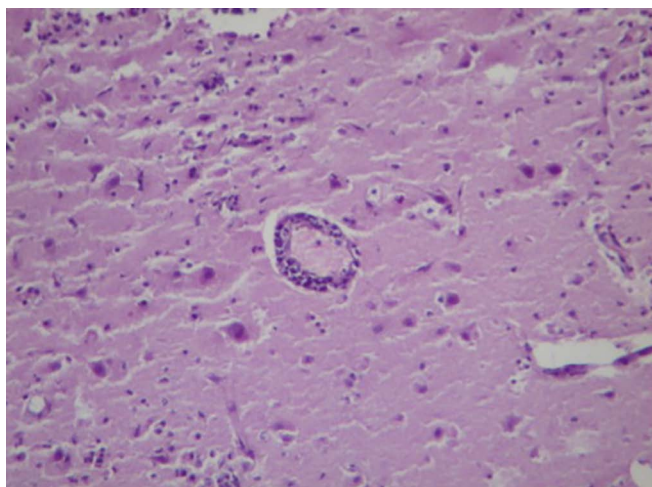


Figure 8. Perivascular cuffing of leucocytes in the brain (Hematoxylin & Eosin stain).

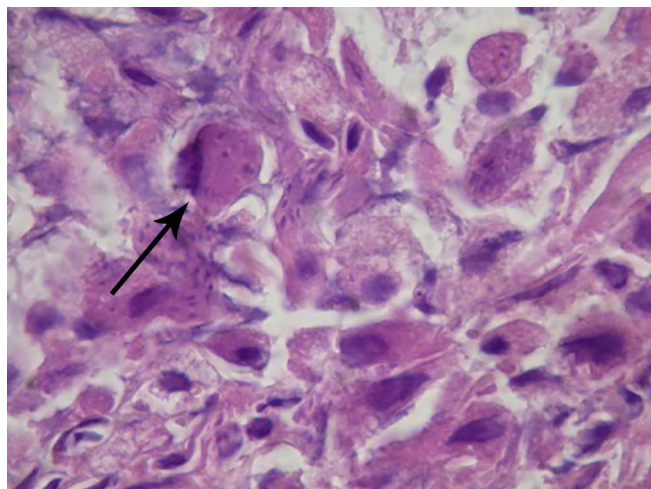


Figure 10. Cerebellum with eosinophilic infiltration (arrow) associated with *Toxoplasma gondii* bradyzoites (Hematoxylin & Eosin stain).

The average weight for an 8-month old black bear cub in NJ is 28.1 kg with a total length of 114 cm (personal communication Michael Madonia, NJ Division of Fish and Wildlife). The cub in this report weighed only 7.03 kg with a total length of 58.4cm.

The key to *T. gondii*'s pathogenesis is its ability to differentiate from a rapidly replicating tachyzoite stage during acute infection to a relatively non-immunogenic, dormant bradyzoite stage contained in tissue cysts. Cardiac dilatation as seen in the black bear cub has been described from animals dying at the Philadelphia Zoo from acute toxoplasmosis. Bhagavati and Choi (2009) reported that frequent hemorrhagic lesions occurred in cerebral toxoplasmosis in AIDS patients. The lesions in the brain and

spinal cord of the cub contributed to the behavioral signs that were exhibited.

Here we report the first case of disseminated toxoplasmosis in a wild black bear cub. Evidence to support disseminated toxoplasmosis as the source of infection include the detection of numerous *T. gondii* cysts in various tissues on histopathology. If there was any underlying, predisposing factor for DT in the black bear cub, it could not be determined in this case study.

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