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The Effect of Nitrogen Level and Form on the Growth and Development of Vetiver Grass (*Chrysopogon zizanioides*)

Suneetha Mondyagu[1], David E. Kopsell[1*], Richard W. Steffen[1], Dean A. Kopsell[2], and Robert L. Rhykerd[1]
[1] Department of Agriculture, Illinois State University, Normal, IL
[2] Plant Sciences Department, The University of Tennessee, Knoxville, TN
[*]Corresponding author email: dkopsell@ilstu.edu

ABSTRACT

Vetiver grass (*Chrysopogon zizanioides*) is a warm season perennial grass grown as a phytoremediation tool and recently proposed as a plant material source for biofuel production. However, limited information exists on Vetiver grass fertility management practices in cropping systems. Therefore, the effects of nitrogen (N) level and N-form on Vetiver growth and development were investigated. Plant slips of *C. zizanioides* Sunshine were greenhouse-grown using nutrient solution culture. In the first experiment, N-level treatments were 26.3, 52.5, 105, 210 and 410 mg $N·L^{-1}$ at a ratio of 3:1 nitrate-N: ammonium-N. In a second experiment, the ratio of NH_4:NO_3 was changed from 0:100, 25:75, 50:50, 75:25, and 100:0 while keeping a total N level of 210 mg $N·L^{-1}$. At 12 weeks after transplanting (WAT), plant height, tiller number, accumulated shoot, root, and total fresh weights and dry weights, total leaf number, and chlorophyll content were recorded. Leaf number decreased linearly ($P \leq 0.10$) from 11.2 per plant at 26.3 mg $N·L^{-1}$ to 9.7 per plant at 210 mg $N·L^{-1}$ while leaf chlorophyll content increased, then decreased quadratically ($P \leq 0.10$) over the same N level treatment range. Shoot dry weight and total plant dry weight increased, then decreased ($P \leq 0.10$, respectively) as N level was increased in nutrient solution. In the second experiment, plant height decreased linearly ($P \leq 0.05$) from a mean of 100.9 cm per plant at 1:100 NH_4:NO_3 ratio to 90.5 cm per plant at 100:1 NH_4:NO_3 ratio treatment. Linear increases were observed for tiller number ($P \leq 0.10$) and leaf number ($P \leq 0.001$) per plant as the ratio of NH_4:NO_3 was changed from 1:100 to 100:0. Limited effects of form were identified for plant biomass parameters. Results demonstrate that Vetiver grass may successfully be cultured under lower N fertility and has a tolerance to ammonium-N in managed cropping systems.

Key words: chlorophyll, hydroponics, macronutrient, micronutrient, SPAD

INTRODUCTION

Vetiver grass (*Chrysopogon zizanioides* L. Roberty) is a perennial aromatic grass native to southern India. Vetiver cultivation is possible in all regions with a warm and moist climate, and it is intensively cultivated in many semitropical areas including China, Indonesia, Angola, Somalia, Congo, Brazil, Guatemala, Haiti, and the southern U.S. (Peyron, 1989). Vetiver grass can tolerate extreme climatic variations such as prolonged drought, flooding and temperatures between -14°C to 55°C. In China, it has survived at -22°C and in the U.S. state of Georgia it was able to tolerate temperatures to -10°C (Troung, 1996). Vetiver is highly tolerant to saline conditions, having a critical salt toxicity level of ECse = 8 $dS \cdot m^{-1}$ (Troung, 1996). Two genotypes of Vetiver have been identified; namely a seeded north Indian type and a sterile, or very low fertility south Indian type. Sunshine Vetiver is a domesticated South India type which is non-fertile, has received a low risk score for the potential to become invasive by the USDA-Natural Resources Conservation Service, and requires asexual propagation (Joy, 2009). Vetiver grass is currently grown in 100 different countries as a result of World Bank extension efforts (Slinger, 1997).

Vetiver grass has a wide range of ecological and economic applications (Wilde et al., 2005). Applications of Vetiver include use in agriculture land and slope stabilization, soil erosion control, and incorporation in ornamental landscape plantings (Maffei, 2002). It has be inter-cropped as a contour fence with sugar cane (*Saccahrum officinalis*), maize (*Zea mays*), and tea (*Camellia sinennsis*) to conserve soil moisture and restrict soil runoff (Daffron, 1993). Recently Vetiver grass has been proposed as a plant material source for biofuel production (Boucard, 2005). Vetiver grass holds annual biomass production potential of 100 to 120 $tonnes \cdot ha^{-1}$ (Troung and Smeal, 2003) and has an energy value of 16.3 $MJ \cdot kg^{-1}$ compared to petroleum (41.9 $MJ \cdot kg^{-1}$), coal (27.9-30.2 $MJ \cdot kg^{-1}$), dry wood (19.8 $MJ \cdot kg^{-1}$), and sugarcane bagasse (9.3 $MJ \cdot kg^{-1}$; Grimshaw, 2008). It also has potential as a carbon sequester and Grimshaw (2008) reported that Vetiver grass can sequester 4.5 times more carbon per year than a fast growing poplar tree (*Populus* sp.) per unit area.

The role of using green plants is a promising biological technique for phytoremediation (Schroder et al., 2002). Vetiver has unique morphological and physiological characteristics that make it an excellent phytoremediation tool (Troung, 2000). In fact, Vetiver has proved to be a more successful phytoremediation plant species than Bermudagrass (*Cynodon dactylon*), Bahiagrass (*Paspalum notatum*), Rhodes grass (*Chloris guyana*), tall wheatgrass (*Thynopyron elongatum*), marine couch (*Sporobolus virginicus*) and samphire (*Sarcocrina* spp.; Sinha et al., 2007).

Nitrogen (N) is an essential element and has a major influence on a number of plant responses including pigmentation, shoot and root growth, cold and drought tolerance, wear tolerance, thatch accumulation and recovery potential (Carrow et al., 1987). An awareness of differences in response to N between economically important grass species underlies much of the present day grassland management and experiments suggest that differences between species in response to N may be considerable (Lovvorn, 1945). Trenholm et al. (1998) reported that N also affects the development of tillers in grasses. Over fertilization of N can cause grasses to become susceptible to environmental and biological stresses like summer drought, winter desiccation, and heat and cold damage (Dunn et al., 1995). Although most managed crops have recommended fertilizer rates for targeted optimal yield and performance, information on N rate application for Vetiver grass is limited (Joy, 2009). Therefore, this current study was undertaken to investigate the optimal N fertilization rate and form for improved growth of Vetiver grass.

MATERIALS AND METHODS

Experimental Design

Two experiments were carried out in a controlled greenhouse environment (22°C day/14°C night set points) under natural lighting conditions at Illinois State University, Normal, IL (Lat. 40° 30'N) between February and May, 2009. The first study investigated the effect of nitrogen (N) fertilization level and the second study investigated the effect of N fertilization form on Vetiver grass growth and quality. The experimental design was a randomized complete block design with four replications for each experiment. Generally, Vetiver grass propagules are available in bundles of rooted slips bearing 3-5 tillers each. For the two experiments, approximately 200 mature plant slips of Sunshine Vetiver grass bearing 3-5 tillers each were purchased from Florida Vetiver Systems LLC (Maitland, FL). Mature plant slip shoots were cut back to 30 cm and roots were trimmed to 20 cm to make them uniform in size at the time of planting. Plastic 38 L containers (Sterilite® Lapis Blue; Townsend, MA) with lids having five 4 cm diameter holes equally spaced 16 cm apart were used for growing plant slips. Each container was filled with 34 L of a modified half strength nutrient solution (Hoagland and Arnon, 1950).

In the N fertilization level study, plants were grown at increasing N treatment concentrations of 26.3, 52.5, 105, 210, 420 mg N·L^{-1}. The two dominant N forms were balanced in all of the N treatments to achieve a ratio of 25% NH_4^+:75% NO_3^-. In the N fertilization form study, the total N level was held constant at 210 mg N·L^{-1} and plants were grown under increasing NH_4^+ at 0, 25, 50, 75 and 100% of total N. Elemental concentrations of nutrient solutions were (mg·L^{-1}): phosphorus (P) (89.9); potassium (K) (132); calcium (Ca) (80); magnesium (Mg) (24); iron (Fe) (0.5); boron (B) (0.125); molybdenum (Mo) (0.0025); manganese (Mn) (0.125); and zinc (Zn) (0.0125). Solutions were aerated with an air blower (Model VB-007S, Sweetwater, Ft. Collins, CO) connected to two air stones in each container. Nutrient solutions were replaced every two weeks throughout the experiment to refresh the solution to the initial nutrient concentrations.

Data Collection & Statistical Analysis

After twelve weeks of hydroponic culture, plant height, number of tillers per plant, total number of leaves per plant, and treatment-wise average chlorophyll content of mature leaves using a Minolta SPAD-502 Chlorophyll Meter (Model 2900; Konica Minolta, Japan) were recorded. Plants were then harvested and shoot and root tissues were washed, towel dried, and separated and fresh weights were recorded. Plant tissues were then dried in a forced-air oven at 60°C. When the tissues achieved a constant mass, dry weights were recorded. All data collected were analyzed by the GLM procedure of SAS 9.2 (Cary, NC) to perform analysis of variance and regression analysis to determine relationships between dependent variables and N treatments.

RESULTS AND DISCUSSION

Experiment 1: Nitrogen Fertilization Level Study

Plant height of Vetiver was within previously reported ranges (Xia and Bing, 2000). Increasing N fertilization level influenced Vetiver plant height (P=0.029). Plant height reached a maximum of 105.0 cm after 12 weeks after transplanting under the 210 mg

$N{\cdot}L^{-1}$ treatment (Table 1). After 14 months of growth, Xia and Bing (2003) reported 'Sunshine' Vetiver plant height ranged from 160 cm to 170 cm. However, in the current study, no significant trends were observed for plant height in response to increasing N treatments from 26.3 to 420 mg $N{\cdot}L^{-1}$ (Table 1). Non-significant plant height over time of Vetiver was also reported by research performed by Muensangk (2000) which investigated ecotype differences. Our results indicate that Vetiver can be cultured under N fertility as low as 26.3 mg $N{\cdot}L^{-1}$ and still attain previously reported average plant height.

The number of tillers per plant was also within previously reported ranges. Increasing N fertilization level influenced the number of tillers per Vetiver plant ($P \leq 0.001$). Twelve weeks after transplanting, the maximum tiller number was found to be 11.2 at 26.3 mg N·L-1 (Table 1). Xia and Bing (2003) reported tiller number per plant ranged from 7 to 59 for twelve different Vetiver ecotypes. Although no significant trends were observed in our current study, the number of tillers decreased from 11.2 at 26.3 mg $N{\cdot}L^{-1}$ to 9.7 at 420 mg $N{\cdot}L^{-1}$. Abbaszadeh et al. (2009) reported that N level also influenced tiller number in balm (*Mellissa officinalis*) and that tiller number per plant decreased as the N level increased.

Increasing N fertilization level influenced Vetiver leaf number per plant ($P=0.10$) and chlorophyll leaf content ($P=0.10$). Average leaf number per plant decreased linearly ($P \leq 0.10$) from 56.0 under 26.3 mg $N{\cdot}L^{-1}$ to 46.9 under 420 mg $N{\cdot}L^{-1}$ (Table 1). Chlorophyll content displayed a quadratic trend ($P \leq 0.10$), first increasing then decreasing with increasing N rate concentrations and showed a maximum of 52.7 $g{\cdot}m^{-2}$ at 105 mg $N{\cdot}L^{-1}$ (Table 1). Buah and Mwinkaara (2009) reported a linear increase in chlorophyll SPAD value content at increasing N levels from 0, 40, 80 and 120 kg N· ha-1 in sorghum (*Sorghum* sp.). Differences in regression trends between Buah and Mwinkaara (2009) and our current study may be attributed to differences in plant genus and level of N fertilization.

Increasing N fertilization level influenced shoot fresh weight (SFWT; $P=0.099$), shoot dry weight (SDWT; $P=0.077$), root dry weight (RDWT; $P=0.084$), and total plant dry weight (TDWT; $P=0.096$), but not root fresh weight (RFWT; $P=0.205$) and total plant fresh weight (TFWT; $P=0.228$). Shoot fresh weight and SDWT were maximum at 52.5 mg $N{\cdot}L^{-1}$ (Table 2). Bradshaw et al. (1964) showed that among seven grasses, yields were significantly varied as the N level concentration increased and matgrass (*Nardus stricta*) produced maximum yield at only 27 ppm ($mg{\cdot}L^{-1}$) N. Vetiver appears to be another grass species which produces maximum yield under lower total N fertilization. However, Vetiver root dry weight and TDWT were maximum at 210 mg $N{\cdot}L^{-1}$. Shoot dry weight increased, then decreased quadratically ($P \leq 0.10$) in response to increasing N levels (Table 2). Total plant dry weight also increased, then decreased quadratically ($P \leq 0.10$) in response to increasing N levels (Table 2). Muir et al. (2001) reported that total plant biomass of switchgrass (*Panicum virgatum*) increased, then decreased quadratically as total N fertilization was increased from 0 kg $N{\cdot}ha^{-1}$ to 224 kg $N{\cdot}ha^{-1}$.

Experiment 2: Nitrogen Fertilization Form Study

Plant height of Vetiver in this experiment was also within previously reported ranges (Xia and Bing, 2000) and similar to experiment 1 (Table 1). Changing the ratio of $NH_4^+{:}NO_3^-$ influenced Vetiver plant height ($P=0.001$). Plant height reached a maximum of 103.4 cm 12 weeks after transplanting under the 50:50 $NH_4^+{:}NO_3^-$ (Table 3). Plant height decreased linearly ($P \leq 0.05$) as the ratio of NH_4^+:NO_3^- was changed from 0:100 to 100:0 (Table 3). Increasing ammonium-N fertilization has been reported to decrease plant height in many different plant species (Brown et al., 2010; Muniz et al., 2009; Kim et al., 2006). This is because carbon used for plant growth is reallocated and utilized in the detoxification of absorbed ammonium (Mills and Jones, Jr., 1996).

The number of tillers per plant was also within previously reported ranges (Xia and Bing, 2000) and similar to experiment 1 (Table 1). Changing the ratio of NH_4^+:NO_3^- influenced Vetiver tiller number ($P=0.001$) and tiller number increased linearly ($P \leq 0.10$) from 8.2 tiller per plant at 0% NH_4^+ to 10.4 tillers per plant at 100% NH_4^+ 12 WAT (Table 3). Nitrogen form has been demonstrated to affect tillering in grasses (Assuero and Tognetti, 2010), however maximum tillering has been reported in grasses fertilized with a 50:50 NH_4^+:NO_3^-. Leaf number per plant also increased linearly ($P \leq 0.01$) in response to increasing NH_4^+ in solution (Table 3). A similar trend was reported by McInenly et al. (2010) who showed that aboveground biomass of rough fescue (*Festuca campestris*) was increased when foliar N was supplied as 100% ammonium as opposed to 100% nitrate. Cholorphyll leaf content, as measured by SPAD, did not significantly change in response to changing the ratio of NH_4^+:NO_3^- in solution culture (Table 3).

Changing the ratio of NH_4^+:NO_3^- influenced RFWT ($P=0.10$) but did not influenced SFWT ($P=0.537$), TFWT ($P=0.359$), SDWT ($P=0.566$), RDWT ($P=0.184$), or TDWT ($P=0.636$). Twelve weeks after transplanting, RFWT decreased linearly ($P \leq 0.10$) in response to increasing NH_4^+ in solution (Table 4). When N was supplied as NH_4^+, biomass allocation to roots decreased in thickspike wheatgrass (*Agropyron dasystachyum*; Li and Redmann, 1992). Similar results were also reported in creeping bentgrass (*Agrostis palustris*) where root weight was found to be highest at a 1:3 ratio of NH_4^+:NO_3^- (Glinski et al., 1990). Total fresh shoot and root yields of napiergrass (*Pennisetum purpureum*), a tropical grassland species, did not differ when N fertilization treatment were changed from solely nitrate to solely ammonium (Rahman et al., 2010). Bowler and Press (1996) reported that total dry weight of colonial bentgrass (*Agrostis capillaris*) was not affected by N form. The response of Vetiver grass growth to the ratio of NH_4^+:NO_3^- from 0:100 to 100:0 follows reported trends in other grass species.

Vetiver grass growth and biomass parameters showed varied responses and were mostly non-significant in both the N fertilization level and N fertilization form experiments. This lack of significance may indicate that Sunshine Vetiver has a higher amount of genetic variability than its cultivar classification. According to Grimshaw (2009), Sunshine is a Vetiver cultivar but is also referred to as an upland ecotype. Igbokwe et al. (1991) reported the extent of the fertilizer response among four Vetiver accessions depended on factors such as seasonal variation and genetic variability and Muensangk (2000) reported non-significant trends over time for several growth factors among Vetiver ecotypes. A similar situation has been identified in switchgrass, where two main ecotypes differ in ploidy, morphology, growth pattern, and climatic adaptation (Zalapa et al., 2011). Adams and Daffron (1997) reported that Monto Vetiver from Australia and Sunshine Vetiver from North America have no morphological and physiological differences and Joy (2009) lists Sunshine as genetically similar to the Vetiver cultivars Boucard, Fort Polk, Haiti, Huffman, Monto, and Vallonia. An ecotypic Sunshine Vetiver population may not respond uniformly to N fertility treatments due to genetic variations. Therefore, further refinement of the current Sunshine population and its related cultivars may be needed prior to large scale production consideration. The same recommendations are being proposed for switchgrass in order to produce significant heterotic increases in biomass yield in managed cropping systems (Zalapa et al., 2011).

CONCLUSION

Due to its unique physical and morphological characteristics, its tolerance to environmental extremes and low risk of becoming an invasive plant species, Vetiver grass may have production potential in the U.S. as a plant material source for biofuel production or carbon sequestration. Plant height and total plant fresh weight were not significantly different between 26.3 and 420 $mg{\cdot}L^{-1}$ N treatments. Therefore, our results indicate that Vetiver grass may successfully be cultured under lower N fertility. Vetiver also showed tolerance to NH_4^+-N. Although plant height decreased as NH_4^+-N increased, results were still within reported ranges for Vetiver cultivation. No significant differences between 0% and 100% NH_4^+-N, at a luxuriant total N level, for total plant fresh weight were observed. These results can be used to better predict the optimal N fertilization rates for investigating Vetiver grass culture under managed field conditions for large scale production. However, despite the wide range of environmental adaptation and non-fertile reproductive characteristics of Sunshine Vetiver, caution should be exercised when introducing a non-native plant species over a wide geographic area. Additional research is also needed to confirm if Sunshine Vetiver grass is actually an ecotype which warrants further genetic refinement through breeding to produce the uniformity of a true cultivar.

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LITERATURE CITED

Abbaszadeh, B., H.A. Farahani., S.A. Valadabadi and H.H. Darvishi. 2009. Nitrogenous fertilizer influence on quantity and quality values of balm (*Mellissa officinalis* L.). Journal of Agricultural Extension and Rural Development 1(1):31-33.

Adams, R.P. and M.R. Daffron. 1997. DNA fingerprints (RAPDs) of the pan-tropical grass, *Vetiveria zizanioides* L. The Vetiver Newsletter 18:28-33.

Assuero, S.G. and J.A. Tognetti. 2010. Tillering regulation by endogenous and environmental factors and its agricultural management. The Americas Journal of Plant Science and Biotechnology 4(1):35-48.

Boucard, G. 2005. Vetiver Network Discussion Board. (http://www.vetiver.org/cgi-bin/discus/discus.cgi).

Bowler, J.M. and M.C. Press. 1996. Effects of elevated CO_2, nitrogen form and concentration on growth and photosynthesis of a fast- and slow-growing grass. New Phytologist 132(3):391-401.

Bradshaw, A.D., M.J. Chadwick, D. Jowett and R.W. Snaydon. 1964. Experimental investigations in to the mineral nutrition of several grass species. JSTOR: Journal of Ecology 52(3):665-676.

Brown, H.E., M.S. Watt, P.W. Clinton, and E.G. Mason. 2010. Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of *Pinus radiata* seedlings. Trees 24(6):1097-1107.

Buah, S.S.J. and S. Mwinkaara. 2009. Response of sorghum to nitrogen fertilizer and plant density in the guinea savanna zone. Journal of Agronomy 8(4):124-130.

Carrow, R.N., B.J. Johnson and R.E. Burns. 1987. Thatch and quality of Tifway bermudagrass in relation to fertility and cultivation. Agronomy Journal 79:524-530.

Daffron, M. 1993. Question and Answers, p. 59-70. In: Vetiver grass: A thin green line against erosion. National Academy Press, Washington, D.C.

Dunn, J.H., D.D. Minner, B.F. Fresenburg, S.S. Bughrara, and C.H. Hohnstrater. 1995. Influence of core aerification, topdressing, and nitrogen on mat, roots, and quality of Meyer zoysiagrass. Agronomy Journal 87(5):891-894.

Glinski, D.S., H.A. Mills, K.J. Karnok and R.N. Carrow. 1990. Nitrogen form influences root growth of sodded creeping bentgrass. HortScience 25(8):932-933.

Grimshaw, R. 2008. First National Indian Vetiver Workshop. The Vetiver Network International. (www.vetiver.org).

Grimshaw, R. 2009. Personal communication. (http://www.vetiver.org/cgi-bin/discus/discus.cgi).

Hoagland, D.R. and D.J. Arnon. 1950. The water culture method for growing plants without soil. California Agricultural Experimental Station Circular 347. Berkeley, CA.

Igbokwe. P.E., S.C. Tiwari, J.L. Burton and R.E. Waters, Jr. 1991. Influence of accession variability and fertilization on the establishment and growth of Vetiver grass in a field nursery. The Vetiver Network Newsletter 7:113-125.

Joy, R.J. 2009. 'Sunshine' vetivergrass *Chrysopogon zizanioides* (L.) Roberty. USDA-NRCS Pacific Island Area Plant Materials Program, Hoolehuam HI.

Kim, S.J., C. Kawahaarada, and G. Ishii. 2006. Effect of ammonion:nitrate nutrient ratio on nitrate and glucosinolate contents of hydroponically-grown rocket salad (*Eruca sativ*a Mill.). Soils Science and Plant Nutrition 52(3):387-393.

Li, Y.S. and R.E. Redmann. 1992. Growth-responses to ammonion-N, nitrate-N and defoliation in *Agropyron dasystachyum* from the Canadian mixed grassland. American Midland Naturalist 127(2):300-308.

Lovvorn, R.L. 1945. The effect of defoliation, soil fertility, temperature and length of day on growth of some perennial grasses. Journal of American Society Agronomy 37:570-582.

Maffei, M. 2002. Introduction to genus Vetiveria, p. 1-18. In: M. Maffei (ed.). Vetiveria: The genus Vetiveria. Taylor & Francis, London, UK.

McInenly, L.E., E.H. Merrill, J.F. Cahill, and N.G. Juma. 2010. *Festuca campestris* alters root morphology and growth in response to simulated grazing and nitrogen form. Functional Ecology 24(2):283-292.

Mills, H.A. and J.B. Jones, Jr. 1996. Essential plant nutrients, p. 6-62. In: Plant analysis handbook II. MicroMacro Publishing Inc., Athens, Georgia, USA.

Muensangk, S. 2000. The use of Vetiver grass in the reclamation and improvement of acid sulphate soils in Thailand: A pre-experiment study. In: Proceedings of the second international Vetiver conference, Thailand. Part 4:279-282.

Muir, P.J., M.A. Sanderson, W.R. Ocumpaugh, R.M. Jones and R.L. Reed. 2001. Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus and row spacing. Agronomy Journal 93:896-90.

Muniz, M.A., J.G. Barbosa, J.A.S. Grossi, M.Y. Orbes, and P.G. Sa. 2009. Production and quality of pot chrysanthemum fertigated with different nitrate/ammonium relations. Bioscience Journal 25(1):75-82.

Peyron, L. 1989. Vetiver in perfumery. Quintessenza 13:4-14.

Rahman, M.M., Y. Ishii, M. Niimi, and O. Kawamura. 2010. Effect of application form of nitrogen on oxalate accumulation and mineral uptake by napiergrass (*Pennisetum purpureum*). Grassland Science 56(3):141-144.

Schroder, P., P.J. Harvey and J.P. Schwitzguebel. 2002. Prospects for the phytoremediation of organic pollutants in Europe. Environmental Science Pollution R 9(1):1-3.

Sinha, R.K., H. Sunil, and P.K. Tandon. 2007. Phytoremediation: Role of plants in contaminated site management, p. 315-330. In: S.N. Singh and R.D. Tripathi (eds.). Environmental bioremediation technologies. Springer-Verlag, New York, NY.

Slinger, V. 1997. Introduction to genus Vetiveria, p. 1-18. In: M. Maffei (ed.). Vetiveria: The genus Vetiveria. Taylor & Francis, London, UK.

Trenholm, L.E., A.E. Dudeck, J.B. Sartian, and J.L. Cisar. 1998. Bermudagrass growth, total nonstructural carbohydrate concentration, and quality as influenced by nitrogen and potassium. Crop Science 38:168-173.

Troung, P.N. 1996. An overview of research, development and application of the VGS overseas and in Queensland, p.6-7. In: Proc. R, D and A of VGS in Queensland, Australia.

Troung, P.N. 2000. The global impact of Vetiver grass technology on the environment, p. 46-47. In: Proceedings of the second international Vetiver conference, Thailand.

Troung, P.N. and C. Smeal. 2003. Pacific Rim Vetiver Network Tech. Bull. No. 2003/3.

Wilde, E.W., R.L. Brigmon, D.L. Dunn, M.A. Heitkamp, and D.C. Dagnan. 2005. Phytoextraction of lead from firing range soil by Vetiver grass. Chemosphere 61:1451–1457.

Xia, H.P. and Y. B. Bing. 2003. Study of screening better ecotypes of Vetiver grass, p. 517-523. In: Proceedings of third international conference on Vetiver and exhibition. China Agriculture Press, Guangzhou, China.

Zalapa, J.E., D.L. Price, S.M. Kaeppleer, C.M. Tobias, M. Okada, and M.D. Casler. 2011. Hierarchical classification of switchgrass genotypes using SSR and chloroplast sequences: Ecoytpes, ploides, gene pools, and cultivars. Theoretical and Applied Genetics 122:805-817.

Table 1. Mean values[z] of Vetiver grass plant height, tiller number, leaf number, and chlorophyll content 12 weeks after transplanting (WAT) into a modified Hoagland's nutrient solution with nitrogen levels (N LEVEL) varying from 26.3 $mg \cdot L^{-1}$ to 420 $mg \cdot L^{-1}$ with ratio of 1:3 NH_4:NO_3.

N LEVEL ($mg \cdot L^{-1}$)	Plant height[y] (cm)	Tiller Number	Leaf Number	Chlorophyll[x] ($mg \cdot m^{-2}$)
26.3	100.1 ± 17.6	11.2 ± 4.8	56.00 ± 6.13	48.42 ± 3.42
52.5	100.4 ± 13.4	10.5 ± 4.9	49.25 ± 2.93	48.62 ± 1.37
105	102.4 ± 13.0	10.7 ± 5.7	53.05 ± 11.68	52.72 ± 1.69
210	105.0 ± 8.7	10.4 ± 3.3	47.25 ± 5.20	48.52 ± 2.89
420	99.8 ± 12.2	9.7 ± 3.9	46.95 ± 10.35	48.37 ± 2.85
Contrast[w]				
Linear	NS	NS	*	NS
Quadratic	NS	NS	NS	*

[z] Mean of 5 plants per treatment replication ± standard deviation.

[y] Plant height was measured from base of the crown to leaf tip in centimeters (cm).

[x] Mean chlorophyll content of mature leaves measured in $mg \cdot m^{-2}$ using a hand-held SPAD meter.

[w] Significance for linear and quadratic orthogonal contrasts for N LEVELS.

[NS,*,**,***] Non-significant or significance at $P \leq 0.10$, $P \leq 0.05$, $P \leq 0.01$, respectively.

Table 2. Mean values[z] of Vetiver grass plant biomass 12 weeks after transplanting into a modified Hoagland's nutrient solution with nitrogen levels (N LEVEL) varying from 26.3 $mg \cdot L^{-1}$ to 420 $mg \cdot L^{-1}$ with a ratio of 1:3 NH_4:NO_3.

N LEVEL	Plant biomass[y] (g)					
($mg \cdot L^{-1}$)	SFWT	RFWT	TFWT	SDWT	RDWT	TDWT
26.3	387.5 ± 75.0	233.9 ± 50.1	621.4 ± 119.9	77.2 ± 14.0	35.3 ± 5.5	112.5 ± 18.5
52.5	406.4 ± 62.0	215.8 ± 14.2	622.1 ± 101.2	85.2 ± 14.9	34.7 ± 2.3	119.9 ± 16.6
105	391.3 ± 73.5	264.1 ± 69.6	655.5 ± 141.5	82.1 ± 12.9	37.2 ± 6.3	119.3 ± 18.9
210	388.7 ± 21.4	263.8 ± 24.7	652.5 ± 19.6	82.9 ± 5.3	37.7 ± 1.9	120.6 ± 5.4
420	345.7 ± 12.4	246.9 ± 27.7	592.7 ± 33.9	71.5 ± 2.8	34.1 ± 2.0	105.6 ± 3.8
Contrast[x]						
Linear	NS	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	*	NS	*

[z] Mean of 5 plants per treatment replication ± standard deviation. Abbreviations: SFWT=shoot fresh weight, RFWT=root fresh weight, TFWT=total plant fresh weight, SDWT=shoot dry weight, RDWT=root dry weight, and TDWT=total plant dry weight.

[y] Mean plant biomass measured in grams (g).

[x] Significance for linear and quadratic orthogonal contrasts for N LEVELS.

[NS,*,**,***] Non-significant or significance at $P \leq 0.10$, $P \leq 0.05$, $P \leq 0.01$, respectively.

Table 3. Mean values^z of Vetiver grass plant height, tiller number, leaf number, and chlorophyll content 12 weeks after transplanting (WAT) into a modified Hoagland's nutrient solution with varying NH_4:NO_3 ratios of 0:100, 25:75, 50:50, 75:25, 100:0 while keeping total N level constant at 210 mg $N \cdot L^{-1}$.

RATIO of NH_4:NO_3	Plant height^y (cm)	Tiller number	Leaf Number	Chlorophyll^x $(mg \cdot m_{-2})$
0:100	100.9 ± 14.1	8.2 ± 3.2	36.2 ± 2.6	45.4 ± 3.6
25:75	102.5 ± 11.3	9.2 ± 4.2	40.6 ± 5.1	46.0 ± 2.9
50:50	103.4 ± 9.8	9.1 ± 3.8	42.1 ± 8.2	48.9 ± 4.2
75:25	100.6 ± 9.3	9.5 ± 3.7	42.3 ± 3.1	48.8 ± 3.3
100:0	90.5 ± 14.5	10.4 ± 4.2	44.9 ± 3.3	47.1 ± 4.3
Contrast^w				
Linear	**	*	***	NS
Quadratic	***	NS	**	NS

[z] Mean of 5 plants per treatment replication ± standard deviation.

[y] Plant height was measured from base of the crown to leaf tip in centimeters (cm).

[x] Mean chlorophyll content of mature leaves measured in $mg \cdot m^{-2}$ using a hand-held SPAD meter.

[w] Significance for linear and quadratic orthogonal contrasts for N RATIOS.

$^{NS, *, **, ***}$ Non-significant or significance at P≤0.10, P≤0.05, P≤0.01, respectively.

Table 4. Mean values^z of Vetiver grass plant biomass 12 weeks after transplanting into a modified Hoagland's nutrient solution with varying NH_4:NO_3 ratios of 0:100, 25:75, 50:50, 75:25, 100:0 while keeping total N rate constant at 210 mg $N \cdot L^{-1}$.

RATIO of	Plant biomass^y (g)					
NH_4:NO_3	SFWT	RFWT	TFWT	SDWT	RDWT	TDWT
0:100	357.3 ± 45.2	208.5 ± 25.5	565.7 ± 69.3	75.9 ± 6.7	32.5 ± 0.9	108.3 ± 7.6
25:75	383.9 ± 53.6	236.2 ± 66.8	620.2 ± 109.8	84.9 ± 13.5	34.2 ± 5.9	119.1 ± 17.6
50:50	418.7 ± 68.6	204.1 ± 47.8	622.8 ± 112.2	88.3 ± 11.9	31.6 ± 6.1	119.9 ± 17.1
75:25	351.5 ± 35.1	202.2 ± 73.8	553.7 ± 108.7	75.9 ± 5.9	31.1 ± 6.7	107.1 ± 10.0
100:0	349.8 ± 43.4	161.3 ± 9.2	511.1 ± 45.6	82.6 ± 13.4	29.2 ± 1.0	111.8 ± 12.9
Contrast^x						
Linear	NS	*	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS

[z] Mean of 5 plants per treatment replication ± standard deviation. Abbreviations: SFWT=shoot fresh weight, RFWT=root fresh weight, TFWT=total plant fresh weight, SDWT=shoot dry weight, RDWT=root dry weight, and TDWT=total plant dry weight.

[y] Mean plant biomass measured in grams (g).

[x] Significance for linear and quadratic orthogonal contrasts for N RATIOS.

$^{NS, *, **, ***}$ Non-significant or significance at P≤0.10, P≤0.05, P≤0.01, respectively.

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Flora of Twin Shelters and Twin Mounds Hill Prairies, Pere Marquette State Park, Jersey County, Illinois, Changes Since 1963

William E. McClain[1], John E. Ebinger[2]
[1]Illinois State Museum, Springfield, Illinois 62706; [2]Department of Biological Sciences, Eastern Illinois University, Charleston, Illinois 61920

ABSTRACT

The vascular flora of Twin Mounds and Twin Shelters hill prairies within Pere Marquette State Park, Jersey County, Illinois was studied during the 2009 and 2010 growing seasons. These two prairies are located on southwest-facing slopes approximately 10 km from Grafton, Illinois. Community composition was analyzed using m^2 quadrats placed at one-meter intervals along two randomly located transect lines within each prairie. Frequency, mean cover, and importance value (I. V. total = 200) were determined from the data collected. A total of 59 vascular plant taxa was collected in the prairies with 32 encountered in plots. *Schizachyrium scoparium* (Michx.) Nash (little bluestem) had the highest importance value followed by *Aster oolentangiensis* (sky blue aster), *Sorghastrum nutans* (L.) Nash (Indian grass), and *Solidago nemoralis* Ait. (gray goldenrod). Changes in the vegetation within the last 47 years include the extirpation of two vascular plant species, *Lespedeza violacea* (L.) Pers. (violet lespedeza) and *Asclepias hirtella* (Pennell) Woodson (tall green milkweed) and the moss *Wiessia controversa* Hedwig. The lichen *Dermatocarpon hepaticum* (Ach.) Th. Fr. and the vascular plant *Senecio plattensis* Nutt. (prairie ragwort) experienced significant population declines while the combined biomass of *Andropogon gerardii* Vitman (big bluestem) and *Sorghastrum nutans* (L.) Nash (Indian grass) increased.

INTRODUCTION

Pere Marquette State Park lies within the Driftless Section of the Middle Mississippi Border Natural Division (T6N, R13W, Sec. 9). This division is characterized by steep topography and numerous outcrops of limestone, dolomite, and shale, especially within Pere Marquette State Park (Schwegman 1973). The most widespread geologic feature is Pleistocene loess which forms a thick mantle over the underlying bedrock (Rubey 1952). This loess is the parent material for Hamburg silt, the grass-covered, calcareous soil characteristic of hill prairie communities in the western portion of Illinois. Though most of Pere Marquette State Park is forested, hill prairie communities are conspicuous features of south- to southwest-facing bluffs bordering the Illinois River floodplain (McFall & Karnes 1995). These hill prairies have been the focus of several studies, including Kilburn and Ford (1963) who studied the flora of Twin Mounds hill prairie, McClain (1983) who documented the loss of hill prairie at five locations in Pere Marquette State Park, and McClain and Anderson (1990) who studied woody invasion on Twin Mounds hill prairie. In addition, Evers (1955) examined numerous hill prairies in the Mississippi and Illinois river systems. More than 50 hill prairies throughout Illinois were examined in his extensive study and dominant plant species, disturbances, and general characteristics were recorded.

These previous studies, like most hill prairie surveys, were not designed to detect long term trends in the vegetation. The purpose of the present study was to document the flora of Twin Mounds and Twin Shelters hill prairies, and to determine changes in the vegetation of Twin Mounds hill prairie since the study of Kilburn and Ford (1963).

DESCRIPTION OF THE STUDY SITE

Land purchases for the establishment of Pere Marquette State Park began in 1932. Building and trail construction, including the shelter at the crest of Twin Shelters Hill Prairie, were completed by the Civilian Conservation Corps (CCC) under the direction of the National Park Service during the period April 1, 1933 to June 30, 1939. The work of the CCC also included 96 days for fighting “weed fires”, the construction of four miles of firebreaks, 11 miles of fire hazard reduction, and 286 days of fire suppression training (McClain and Anderson 1990).

Fire suppression was practiced at Pere Marquette State Park and none of the hill prairies are known to have burned during the forty year period from 1932 until 1972. Woody vegetation made considerable advances onto the hill prairies during this time. Twin Mounds and Twin Shelters hill prairies are currently about 100 m apart, but were part of one large prairie in the1930s based on 1936 aerial photographs (McClain 1983). Woody invasion reduced the size of this large prairie from 8.7 ha in 1936 to 1.5 ha in 1983 (McClain and Anderson 1990). A “thin thread” of prairie still connected these two sites in 1979, but this narrow remnant has since been obscured by woody vegetation (McClain, personal observations). Recent studies indicate a reduction of 50% to 65% in the size of many hill prairies during the 50 year period from 1936 to 1986 (McClain and Anderson 1990, Robertson et. al. 1996, Schwartz et. al. 1997).

Prescribed fire was introduced to Pere Marquette in 1973 when a burn was conducted on Twin Mounds hill prairie by the Illinois Department of Conservation (now the Department of Natural Resources). Subsequent prescribed burns were conducted on the Twin Mounds hill prairie in 1975, 1977, 1982, 1985, 1987, 1996, 1999, 2002, 2007, 2009, and 2011. Prescribed burns were conducted on Twin Shelters hill prairie in 1974, 1987, 1996, 2007, and 2009.

METHODS

Twin Mounds and Twin Shelters hill prairies were visited during the growing seasons of 2009 and 2010 to collect vascular plant specimens and study the composition and structure of the prairie vegetation. Voucher specimens were deposited in the Stover-Ebinger Herbarium of Eastern Illinois University in Charleston (EIU). Exotic species were identified using Gleason and Cronquist (1991) and Mohlenbrock (2002) while nomenclature follows Mohlenbrock (2002).

The ground layer vegetation was surveyed along two 25 m long transects (n = 50 at each site) located on the mid-slope of Twin Mounds and Twin Shelters hill prairies. The transect location at Twin Mounds hill prairie is within the area studied by Kilburn and Ford (1963). One m^2 quadrats were located along each transect at 1 m intervals (n = 25). Odd-numbered quadrats were placed to the right and even-numbered quadrats were placed to the left. A random numbers table was used to determine the number of meters (0-9) a quadrat was placed from transects. Cover was determined using the Daubenmire cover class system (Daubenmire 1959) as modified by Bailey and Poulton (1968). From these data, frequency (%), relative frequency, mean cover (% of total cover), relative cover, and Importance Values (relative frequency + relative cover) were determined for each species found in the plots.

RESULTS

A total of 59 vascular plant species representing 25 families and 49 genera were documented on the prairies, including 11 monocot species representing 3 families and 48 dicot species representing 22 families (Appendix 1). The most common families were the Asteraceae with 13 species, Poaceae (9), and Fabaceae (9). No threatened or endangered species was discovered during the survey, while five exotic species were encountered.

Schizachyrium scoparium (Michx.) Nash. (little bluestem) dominated both prairies, accounting for 38.5 percent of the importance value at Twin Mounds hill prairie and 44.6 percent at Twin Shelters hill prairie (Table 1). *Sorgastrum nutans* (L.) Nash (Indian grass) was third in importance at Twin Mounds hill prairie and second at Twin Shelters hill prairie. Other common native grasses included *Bouteloua curtipendula* (Michx.) Torr. (side-oats grama) and *Andropogon gerardii* Vitman (big bluestem), both species with importance values of 8 or more. *Aster oolantangiensis* Riddell (sky-blue aster) was the most important forb encountered, being second in importance value (36.6) at Twin Mounds hill prairie and third in importance value (28.3) at Twin Shelters hill prairie. *Solidago nemoralis* Ait. (gray goldenrod), *Desmodium sessilifolium* (Torr.) Torr. & Gray (sessile-leaved tick trefoil), and *Dalea purpurea* Vent. (purple prairie clover) were other common forbs. Only 26 species, with a mean total cover of 60.4%, were recorded for the plots on Twin Mounds hill prairie, while 25 species, with a mean total cover of 54.9%, were reported on Twin Shelters hill prairie. Bare ground and litter cover values were nearly identical at both sites, varying from 41% to 43 % (Table 1). Small numbers of an unidentifiable species of the lichen *Dermatocarpon*, possibly *D. hepaticum* (Ach.) Th. Fr., and clumps of the moss *Barbula convuluta* Hedwig were present near the base of Twin Mounds hill prairie. No moss or lichen species were encountered in study plots on either site.

DISCUSSION

The vegetation of the study sites is not diverse. Only 59 vascular plant species were found during this study (9 trips over a two year period) compared to 65 recorded by Kilburn and Ford (1963). Four vascular plant species listed in 1963, *Desmodium panicula-*

tum (Nutt.) DC (panicled tick trefoil), *Spiranthes gracilis* (Bigel) Beck (slender ladies tresses) *Lespedeza violacea* (L.) Pers. (violet bush clover) and *Asclepias hirtella* (Pennell) Woodson (tall green milkweed), could not be located in 2010 and appear to be extirpated. Sessile-leaved tick trefoil, a species present on both hill prairies in 2010, may have been confused with panicled tick trefoil. *Spiranthes magnicamporum* Sheviak (prairie ladies' tresses), a species found in the present study, was not recognized until 1973 (Sheviak 1973). It is a fall blooming species characterized by two to three spiraling rows of flowers compared to just one row for slender ladies' tresses (Mohlenbrock 2002).

The two hill prairies have nearly identical vascular plant species compositions. All but one of the 59 species, including five exotic taxa, recorded for Twin Shelters hill prairie were also present on Twin Mounds hill prairie (Appendix 1). Gray goldenrod, sessile-leaved tick trefoil, and purple prairie clover were more common on Twin Mounds hill prairie while *Aster oblongifolius* Nutt. (aromatic aster), *Hedyotis nigricans,* and big bluestem were more abundant on Twin Shelters hill prairie. Twin Mounds hill prairie occupies the highest, most exposed part of the bluff and has experienced more management (twelve prescribed burns compared to five), factors that could contribute to the higher wildflower densities at this site (Table 1).

Kilburn and Ford (1963) did not report any *Aster* species. However, *Asters* were abundant in 2010 and they were reported from the early 1950s (Evers 1955). Four *Aster* species were collected in 2010, including three in study plots. *Aster oolantangiensis* Riddell (sky blue aster) was most common, having importance values of 36.6% at Twin Mounds hill prairie and 28.3% at Twin Shelters hill prairie (Table 1). The reasons for their absence in the 1963 study are not known, but *Aster* species may have been overlooked if sampling was conducted when plants were not flowering.

The increase in biomass of the tall prairie grasses, big bluestem and Indian grass, since 1963 may be affecting the abundance of small-statured plants (rosettes of vascular plants, mosses and lichens) that inhabit open, sunny spaces between clumps of prairie grasses. Big bluestem and Indian grass had combined cover values on Twin Mounds hill prairie of only 3.4% in 1963 compared to 14.3 % in 2010 (Table 1). Prairie ragwort, listed as *S. pauperacalus* Michx. (balsam ragwort) by Kilburn and Ford (1963), was common in quadrats on Twin Mounds hill prairie in 1963 with a frequency of 87 %. Its frequency dropped to 27 % by 1990 (McClain & Anderson 1990), and no plants were present in study plots on either prairie in 2010 (Table 1). Only nine plants were found growing in small patches of open soil near the base of Twin Mounds hill prairie

Nonvascular plants have also been affected by changes in the prairie community. The moss *Weisia controversa* Hedwig and the lichen *Dermatocarpon hepaticum* were prominent in 1963, having frequencies of 87.8 % and 68.9 %. However, neither species was present in plots in 2010 (Kilburn & Ford 1963, Table 1). *Weisia controversa* Hedwig appears to be extirpated, but a small population of *Dermatocarpon,* possibly *D. hepaticum*, was found at the base of Twin Shelters hill prairie in the same eroded area as prairie ragwort.

Prescribed fire programs have been implemented on hill prairies to mitigate the loss of wildland fire. However, most prescribed burns are conducted during the dormant season, a practice that favors grasses such as big bluestem and Indian grass over wildlflowers. The hill prairies at Pere Marquette State Park were among the first sites burned when the Illinois Department of Conservation (Now the Department of Natural Resources) began its prescribed burning program in 1973. The cumulative effects of dormant season burning over a period of nearly forty years may be contributing to the prominent increase in biomass of tall prairie grasses (Anderson 2006).

The abundance of whitetail deer may also be affecting herbaceous wildflower density in these two hill prairies. These animals have become abundant in Illinois in the last 50 years and evidence of whitetail deer was observed in both hill prairies during this study. Deer selectively graze wildflowers such as *Amorpha canescens* Pursh. (lead plant), but rarely graze grasses or other graminoids. Deer browsing, which has the potential to shift herbaceous prairie plant populations in favor of grasses, may be contributing to the prominence of tall prairie grasses (Anderson et. al. 2001).

The hill prairies of Illinois are sites of rapid change. Woody invasion, fragmentation, size reduction, and the complete disappearance of these grasslands have taken place in the last 60 years (Robertson et. al. 1996, Schwartz et. al. 1997, McClain et. al. 2009). This study of hill prairies at Pere Marquette State Park suggests that other vegetation changes are occurring along with fragmentation and woody invasion, including changes in the density of grasses and wildflowers and species loss (McClain et. al. 2004).

LITERATURE CITED

Anderson, R. C., E. A. Corbett, M. R. Anderson, G.A. Corbett, and T. M. Kelley. 2001. High white-tailed deer density has negative impact on prairie forbs. Journal of the Torrey Botanical Club 128(4): 381-392.

Anderson, R. C. 2006. Evolution and origin of the grassland of Central North America: climate, fire, and mammalian grazers. Journal of the Torrey Botanical Society 133(4): 626-647.

Bailey, A. W. and C. E. Poulton. 1968. Plant communities and environmental relationships in a portion of Tillamook burn, northwestern Oregon. Ecology 49: 1-13.

Daubenmire, R. 1959. A canopy coverage method of vegetation analysis. Northwest Science 33: 43-64.

Evers, R. A. 1955. Hill Prairies of Illinois. Illinois Natural History Survey Bulletin 26: 367-446.

Gleason, H. A. and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. Second Edition, The New York Botanical Garden, Bronx, New York.

Kilburn, P. D. and C. D. Ford, Jr. 1963. Frequency distribution of hill prairie plants. Transactions of the Illinois Academy of Science 56:94-97.

McClain, W. E. 1983. Photodocumentation of the loss of hill prairie within Pere Marquette State Park, Jersey County, Illinois. Transactions of the Illinois State Academy of Science 76:343-346.

McClain, W. E. and E. A. Anderson. 1990. Loss of hill prairie through woody plant invasion at Pere Marquette State Park, Jersey County, Illinois. Natural Areas Journal 10:69-75.

McClain, W. E., Moorehouse, A., Ebinger, J. E. 2009. Loss of Hulsebus Hill Prairie, Henderson County, Illinois, to woody plant invasion. Transactions of the Illinois State Academy of Science 102 (3& 4): 141- 147.

McClain, W. E., Phillippe, L. R., Ebinger, J. E. 2004. Vascular Flora of Manito Prairie Nature Preserve, Tazewell County, Illinois. Transactions of the Illinois State Academy of Science 97(2): 83-94.

McFall, D. and J. Karnes. 1995. (Editors). A directory of Illinois Nature Preserves. Volume 2. Northwestern, Central and Southern Illinois. Illinois Department of Natural Resources, Division of Natural Heritage, Springfield, Illinois.

Mohlenbrock, R. H. 2002. Vascular flora of Illinois. Southern Illinois University Press, Carbondale, Illinois.

Robertson, K. R., M. W. Schwartz, J. W. Olson, B. K. Dunphy, and H. D. Clarke. 1996. Fifty years of change in Illinois Hill Prairies. Erigenia 14:41-52.

Rubey, W. W. 1952. Geology and mineral resources of the Hardin and Brussels Quadrangles. U. S. Government Printing Office. Washington, D. C.

Schwartz, M. W., K. R. Robertson, B. K. Dunphy, J. W. Olson, and A. M. Trame. 1997. The biogeography of and habitat loss on hill prairies. Pages 267-283. in M. M. Schwartz, ed., Conservation of Highly Fragmented Landscapes. Chapman & Hall. New York.

Schwegman, J. E. 1973. Comprehensive plan for the Illinois Nature Preserves System, Part 2. The Natural Divisions of Illinois. Illinois Nature Preserves Commission, Rockford. 32 pp + map.

Sheviak, C.J. 1973. A new *Spiranthes* from the grasslands of Central America. Botanical Museum Leaflet 23: 285-297.

Table 1. Frequency (%), mean cover (% of total area), and importance value (IV) for the species encountered at Twin Mounds and at Twin Shelters Loess Hill Prairies, Pere Marquette State Park, Jersey County, Illinois (*exotic species)

	Twin Mounds Prairie			Twin Shelters Prairie		
Species	Freq. %	Mean Cover	I. V.	Freq %	Mean Cover	I.V.
Schizachyrium scoparium	100	16.11	38.5	94	17.43	44.6
Aster oolentangiensis	100	15.00	36.6	84	9.24	28.3
Sorghastrum nutans	92	11.83	30.5	84	10.91	31.4
Solidago nemoralis	80	6.43	20.2	40	2.78	10.6
Desmodium sessilifolium	74	3.11	13.8	42	0.71	7.0
Dalea purpurea	92	1.64	13.6	44	0.62	7.1
Bouteloua curtipendula	96	1.03	13.1	78	0.74	11.9
Andropogon gerardii	34	2.46	8.1	52	5.40	16.9
Aster ericoides	42	1.53	7.5	12	0.06	1.7
Brickellia eupatorioides	24	0.22	3.2	24	0.27	3.8
Desmanthus illinoensis	16	0.18	2.2	12	0.11	1.8
Ruellia humilis	16	0.13	2.1	14	0.17	2.2
Hedyotis nigricans	14	0.07	1.8	52	2.07	10.9
Desmodium ciliare	12	0.16	1.7	8	0.09	1.3
Agalinus aspera	10	0.05	1.3	4	0.02	0.5
Asclepias viridiflora	6	0.08	0.8	--	--	--
Dalea candida	6	0.08	0.8	2	0.01	0.3
Dichanthelium oligosanthes	6	0.03	0.8	--	--	--
Spiranthes magnicamporum	6	0.03	0.8	--	--	--
Asclepias verticillata	4	0.07	0.6	4	0.02	0.5
Linum sulcatum	4	0.02	0.5	4	0.02	0.5
Lithospermum incisum	4	0.02	0.5	--	--	--
Cornus drummondii	2	0.06	0.3	6	1.11	2.8
Eupatorium altissimum	2	0.06	0.3	--	--	--
Amorpha canescens	2	0.01	0.2	--	--	--
Antennaria plantaginifolia	2	0.01	0.2	--	--	--
Aster oblongifolius	--	--	--	34	2.02	8.3
Lespedeza virginica	--	--	--	10	0.49	2.3
Rhus aromatic	--	--	--	8	0.43	1.9
Carex pensylvanica	--	--	--	10	0.10	1.6
Pycnanthemum pilosum	--	--	--	8	0.09	1.3
Lithospermum canescens	--	--	--	4	0.02	0.5
Totals		60.42	200.0		54.93	200.0
Bare ground and litter		41.24			43.11	

APPENDIX I

Vascular plant species encountered on two loess hill prairies at the Pere Marquette State Park, Jersey County, Illinois are listed alphabetically by family under the major plant groups. Collecting numbers are preceded by the initial of the collector (E = John E. Ebinger; M = William E. McClain). Specimens are deposited in the Stover/Ebinger Herbarium (EIU), Eastern Illinois University, Charleston, Illinois. (*exotic species)

DICOTS
Acanthaceae
Ruellia humilis Nutt.: E32349; E32356
Anacardiaceae
Rhus aromatica Ait. E32348; E32359
Asclepiadaceae
Asclepias verticillata L.: E32329; E32372
Asclepias viridiflora Raf. E32333; E32368
Asteraceae
Antennaria plantaginifolia (L.) Hook. E32679
Aster ericoides L.: E32675
Aster oblongifolius L.: E32684
Aster oolentangiensis Riddell: E32683
Aster pilosus Willd.: E32676
Brickellia eupatorioides (L.) Shinners: E32345; E32355
Erigeron strigosus Muhl.: E32228; E32222
Eupatorium altissimum L.: E32680
Eupatorium serotinum Michx.: E32353
Helianthus divaricatus L.:E32331
Senecio plattensis Nutt. M2829
Solidago nemoralis Ait.: E32330; E32367
Vernonia missourica Raf.: E32350
Boraginaceae
Lithospermum canescens (Michx.) Lehm.: E32685
Lithospermum incisum Lehm.: E32678
Caeslpiniaceae
Chamaecrista nictitans (L.) Moench.:E32335
Campanulaceae
Lobelia spicata Lam.: E32334: E32376; M2763
Cornaceae
Cornus drummondii C.A. Mey.: E32342 ; E32375
Euphorbiaceae
Chamaesyce nutans (Lag.) Small: E32363
Croton monanthogynus Michx.: E32361
Fabaceae
Amorpha canescens Pursh: E32336; E32371
Dalea candida (Michx.) Willd.: E32346
Dalea purpurea Vent.: E32338; E32358
Desmodium ciliare (Muhl.) DC.: E32340; E32362
Desmodium sessilifolium (Torr.) Torr. & Gray: E32339; E32360
Lespedeza capitata Michx.: E32677
Lespedeza virginica (L.) Britt.: E32682
**Melilotus albus* Medic.: E32343; E32370
Psoralidium tenuiflorum (Pursh) Rydb.: E32229; E32223
Hypericaceae
**Hypericum perforatum* L.: M2762
Lamiaceae
Monarda fistulosa L.: E32351
Pycnanthemum pilosum Nutt.: E32337
Linaceae
Linum sulcatum Riddell.: E32347: E32373
Mimosaceae
Desmanthus illinoensis (Michx.) MacM.: E32369
Onagraceae
Oenothera biennis L.: E32366
Oxalidaceae
Oxalis stricta L.: E32224
Ranunculaceae
Anemone virginiana L.: E32230
Rubiaceae
Hedyotis nigricans (Lam.) Fosb.: E32354; E32357
Scrophulariaceae
Agalinus aspera (Doug.) Britt.: E32687
Penstemon pallidus Small: E32231; E32225
Simaroubaceae
**Ailanthus altissima* (Mill.) Swingle: E32344
Solanaceae
Physalis heterophylla Dunal.: E32341; E32364
Physalis virginiana Mill.: E32232
Verbenaceae
Verbena stricta Vent.: M2761

MONOCOTS
Cyperaceae
Carex pensylvanica Lam.: E32686
Orchidaceae
Spiranthes magnicamporum (L.) Rich.: E32688
Poaceae
Andropogon gerardii Vitman: E32712
Bouteloua curtipendula (Michx.) Torr.: E32332; E32374
Dicanthelium oligosanthes (Schult.) Gould: E32226; E32219
Elmus canadensis L.: E32365
Elymus virginicus L.: E32352
**Poa pratensis* L.: E32227; E32220
Schizachyrium scoparium (Michx.) Nash: E32711
**Setaria faberi* F.Herrm.: E32377
Sorghastrum nutans (L.) Nash: E32710

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Influence of Culture Conditions on Hydrogen Peroxide Production by *Lactobacillus jensenii*

Adonica S. Cosgrove Pohren and Scott M. Holt*
Western Illinois University, Department of Biological Sciences
1 University Circle, Macomb, IL 61455, USA
*Corresponding Author

ABSTRACT

The purpose of this study was to determine how changes in certain culture conditions influence hydrogen peroxide production rate by the vaginal isolate *Lactobacillus jensenii*. Hydrogen peroxide production was detected for all variables, but the highest rates occurred at 13.8 mM glucose, pH 7, 40°C, and during early logarithmic growth stage when cell density was taken into account. Hydrogen peroxide was produced from *L. jensenii* regardless of the experimental variable; however, significant differences in production rate were detected within each variable. Since vaginal lactobacilli may play a crucial role in prevention of disease, it is important to know how *in vitro* environmental factors, which may emulate the vaginal environment or aid in the development of potential vaginal probiotic strains, influence production of hydrogen peroxide.

INTRODUCTION

Lactobacillus spp., which inhabit mucus membranes of humans and animals, are known to be dominant microorganisms in the reproductive tract of healthy women (Brown, 1978; Hill et al. 1984) and may provide protection from certain vaginal infections (Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Spurbeck and Arvidson, 2008). Disturbance or alteration of the *Lactobacillus*-dominated vaginal microbiota has been linked to an increased susceptibility to certain infections (Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Wilks et al. 2004). *Lactobacillus* spp. are thought to maintain or regulate the mucus-membrane microbial ecosystem by production of antimicrobial substances such as bacteriocins (Jack et al. 1995), lactic acid, (Boskey et al. 1999; Ogawa et al. 2001) and hydrogen peroxide (Eschenbach et al. 1989, Wilks et al. 2004). Hydrogen peroxide-producing lactobacilli seem to play a crucial role in regulating a healthy vaginal microbiota (Cherpes et al. 2008; Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999 Wilks et al. 2004). The absence of hydrogen peroxide-producing vaginal *Lactobacillus* spp. has been associated with an increased risk of bacterial vaginosis (Cherpes et al. 2008; Hawes et al. 1996), certain urinary tract infections (Gupta et al. 1998), and acquisition of human immunodeficiency virus (Martin et al. 1999). Wilks et al. (2004) and Cherpes et al. (2008) found that the presence of hydrogen peroxide-producing *Lactobacillus* spp. in the vagina of pregnant women was linked to a lower risk of bacterial vaginosis and uterine infection. Among the numerous *Lactobacillus* species isolated from the human vagina (*L. casei*, *L. gasseri*, *L. iners*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L.delbrueckii*, *L. vaginalis*, *L. salivarius*), *L. jensenii* is among the most abundant species (Antonio et al. 1999; Martin et al. 2008; Martin and Suarez, 2010; McGroarty et al. 1992; Vallor et al. 2001; Vásquez et al. 2002). In addition to abundance, nearly all of the vaginal *L. jensenii* strains reported in the literature make hydrogen peroxide (Antonio et al. 1999; Eschenbach et al. 1989; Martin et al; 2008; Martin and Suarez, 2010; McGroarty et al. 1992; Wilks et al. 2004) and at higher levels than most other vaginal *Lactobacillus* species (Martin and Suarez, 2010; Wilks et al. 2004). The level of hydrogen peroxide synthesis is an important characteristic of vaginal lactobacilli since there appears to be a relationship between its production and a reduced risk of genitourinary infections (Cherpes et al. 2008; Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Wilks et al. 2004). Therefore, hydrogen peroxide production is an important physiological characteristic for endogenous vaginal lactobacilli and for assessing the potential of a vaginal probiotic strain (Bolton et al. 2008; McLean and Rosenstein, 2000). Since vaginal lactobacilli may play a crucial role in prevention in disease, it is important to know which *in vitro* environmental factors will influence the rate of hydrogen peroxide production and to what extent. Very little information has been published about the influence of environmental conditions or growth phase on hydrogen peroxide production rate from important vaginal *Lactobacillus* species such as *L. jensenii*. A few other studies have also found that certain environmental or culture conditions such as aeration (Martin and Suarez, 2010; Otero and Nader-Macias, 2006; Tomas et al. 2003), incubation temperature, and culture pH (Tomas et al. 2003) can impact hydrogen peroxide production from vaginal *Lactobacillu*s isolates. Recently, Martin and Suarez (2010) observed that vaginal *L. jensenii* cultures grown in media containing Fe^{3+} did not make hydrogen peroxide. The Fe^{3+} in the growth medium activated a peroxidase made by *L. jensenii* which degraded the hydrogen peroxide (Martin and Suarez, 2010). It has been previously reported that lactobacilli possess an atypical manganese-containing catalase/peroxidase system (Barynin et al. 2001; Yoder et al. 2000). Considering that lactobacilli possess a catalase/peroxidase and that complex growth media may contain Fe^{3+}, it is important to remove spent medium components from lactobacilli cells prior to measuring hydrogen peroxide synthesis. Most of the published literature dealing with vaginal lactobacilli, however, measure hydrogen peroxide production directly from spent growth media without using a wash step. In addition to a wash step, it is also important to measure the rate of hydrogen peroxide synthesis by lactobacilli rather than by end-point determination. Barnard and Stinson (1999) reported that rates of hydrogen peroxide were a more accurate assessment of the potential *in vivo* antagonistic ability of lactic acid bacteria rather than a simple end-point determination. As hydrogen peroxide diffuses away from the producing bacterial strain *in vivo*, the concentration is reduced and loses its killing effectiveness (Barnard and Stinson,1999). Therefore, a vaginal lactobacillus isolate that can produce hydrogen peroxide at a high rate may be most effective for antagonism. Most of the published literature dealing with vaginal lactobacilli, however, measure hydrogen peroxide by using an end-point determination which is not a true kinetic assessment. *L. jensenii* was used for this study because it has been frequently isolated from the vagina of healthy women and most strains are strong producers of hydrogen peroxide (Antonio et al. 1999; Martin et al. 2008; Martin and Suarez, 2010; McGroarty et al. 1991; Wilks et al. 2004; Vallor et al. 2001; Vasquez et al. 2002). *L. jensenii* is not yet considered a probiotic strain and needs to be further evaluated based on the other characteristics mentioned above. The purpose of this study was to determine how changes in glucose concentration, pH, temperature, and stages of growth influence hydrogen peroxide production rates by the important vaginal isolate and potential probiotic species *L. jensenii*.

MATERIALS AND METHODS

Hydrogen peroxide assay

The assay for hydrogen peroxide was adapted from Barnard and Stinson (1999). All assay reagents were prepared in sodium phosphate buffer (0.1 M, pH 7.8). The assay consisted of 20 µl horseradish peroxidase (2 U, HRP, Sigma, St. Louis), 900 µl of 2,2'-azino-bis-(3-ethylbenzthiazoline)-6-sulfonic acid (90 µg, ABTS, Sigma), and 50 µl of cell supernatant. The assay reagents were immediately mixed by inversion in a one ml cuvette and the absorbance (A_{414nm}) was measured using a spectrophotometer (Genesys 20, Thermospectronic, Madison, WI). The spectrophotometer was adjusted to zero absorbance with a blank which consisted of 920 µl buffer and 50 µl supernatant. Two controls were used for each reading to adjust for background from the enzyme or substrate. The enzyme control consisted of all components of the assay with the exception of ABTS. The substrate control consisted of all components of the assay with the exception of HRP. An additional control (reagent control) was tested with all of the components of the assay except the cell supernatant to insure that the assay was only detecting hydrogen peroxide production, and not background from the buffer. Cells from each trial were frozen and later counted with a Petroff-Hausser Chamber (data not shown). A standard curve of A vs hydrogen peroxide concentration was prepared using Microsoft Excel. The equation of the regression line for the standard curve was $y\ (A_{414nm}) = 0.094x\ (H_2O_2\ mM) - 0.0959\ (r^2 = 0.998)$ and was used to calculate hydrogen peroxide concentrations from each sample for all experiments. Initial rates of hydrogen peroxide production were used in this study because it is a more accurate assessment of the ability of certain lactic acid bacteria to control competing bacteria (Barnard and Stinson, 1999).

Data analysis

Four main experiments were performed in this study and included determination of the influence of glucose concentration, pH, temperature, and growth stage on hydrogen peroxide formation in *L. jensenii*. For each experimental variable, a plot of hydrogen peroxide concentration (mM) vs time (min) was prepared and a linear regression line was determined using Microsoft Excel. The slope of this linear regression line was used to determine units of activity for each experimental variable. One unit (U) of activity was defined as one mM of H_2O_2 produced per min under the assay conditions. Each variable was performed in triplicate and activity was reported as the mean U ±standard deviation (SD). ANOVA and Tukey multiple comparison tests were used to determine if differences existed among activity means within each experiment and were reported as *P* values. All statistical analyses were performed with Systat 8.0 software (SPSS Inc., Chicago, IL).

Bacterial strain, growth conditions, and cell preparation

Lactobacillus jensenii NRRL B-4550 was obtained from the United States Department of Agriculture, Agriculture Research Service Culture Collection in Peoria, IL. The *L. jensenii* strain used in this study (B-4550) was originally isolated from human vaginal dis-charge as reported by Glasser et al. (1970) and is the *L. jensenii* type strain (Skerman et al. 1980). The strain has been used extensively in research related to the topic of vaginal lactobacilli and hydrogen peroxide (Antonio et al. 1999; Rabe and Hillier, 2003; Song et al. 1999; Spurbeck and Arvison, 2008; Zhong et al. 1998;). For preparation of stock cultures, isolated colonies were inoculated into Difco^{TM} MRS Broth (10 ml) (Becton, Dickinson, & Co., Sparks, MD) and incubated overnight at 37°C. Cells from the overnight cultures were collected by centrifugation, resuspended in 1 ml fresh MRS Broth containing 20% (v/v) glycerol, and were stored frozen at -80°C until needed. For each experiment, a single vial of frozen stock cells was thawed, gently mixed, and 50-ul was inoculated into the 10 ml MRS broth. Each spent frozen stock culture was not re-frozen but instead destroyed. A new vial of frozen stock culture was used for each experiment. This approach provided for very consistent inocula, much more consistent than performing multiple cultivations (two or three cultivations or transfers) prior to the assay. The inoculum provided consistent results for lag, log, and stationary phases and for enumerating cell numbers by microscopic cell count using Petroff-Hausser Chamber before each hydrogen peroxide assay was performed. The growth curve showed the expected lag, log, and stationary phases (Fig 1). The inoculated test tube culture (10 ml MRS broth) was subsequently incubated overnight at 37°C. Growth of the culture was measured by A_{600nm} using a spectrophotometer (Spectronic 21, Bausch and Lomb). Cells were collected by centrifugation to a pellet (Sorvall angle centrifuge, Sorvall L.L.C., New Castle, DE) and washed three times with sodium phosphate buffer (0.1 M, pH 7.8, Breznak and Costilow, 1994) to remove medium components and glucose. The cells were resuspended in fresh phosphate buffer and adjusted to a A_{600} value of 1.0. This prepared culture was then used to determine the influence of environmental factors (glucose, pH, and temperature) on hydrogen peroxide production.

Influence of glucose on hydrogen peroxide production by *L. jensenii*

Samples (200 µl) from a prepared culture were distributed into each of five pre-warmed (37°C water bath) microcentrifuge tubes containing 200 µl of 0.1 M sodium phosphate buffer (pH 7.8) and varying concentrations of glucose (mM). The lids were closed and the tubes were incubated for up to 40 min at 37°C. One tube was removed from the water bath at each time interval (0, 10, 20, 30, 40 min). At each time interval, the tube was centrifuged for three min at 14,000× g (Eppendorf 5415C, Westbury, NY) to prepare a clarified supernatant, which was assayed for hydrogen peroxide.

Influence of pH on hydrogen peroxide production by *L. jensenii*

Cell preparation and the assay conditions were the same as previously described except that glucose concentration was maintained at 13.8 mM and the pH of the test buffers varied. The buffers used to test the effects of pH included citrate-phosphate buffer (pH 7 and pH 6) and acetate buffer (pH 5 and pH 4).

Influence of temperature on hydrogen peroxide production by *L. jensenii*

Cell preparation and assay conditions were the same as previously described except that glucose concentration was maintained at 13.8 mM, pH was maintained at 7.0, and the incubation temperatures were 30°C, 35°C, 40°C, and 45°C.

Influence of *L. jensenii* growth stage on hydrogen peroxide production.
A growth curve was prepared (data not shown) by inoculation of a *L. jensenii* thawed stock culture (65 µl) into a 16-mm screw-cap test tube containing 13 ml of MRS broth which was incubated at 37°C in a water bath. Growth (A_{600nm}) was measured using a spectrophotometer (Spectronic 21) at three-h intervals for 15 h, and again at 20 h. Hydrogen peroxide formation was immediately detected from each sample collected during the growth assessment as described previously. The cells were washed and resuspended in fresh phosphate buffer using optimum conditions (13.8 mM, glucose, pH 7). Samples were assayed during early logarithmic (3 h), late logarithmic (6 h), early stationary (9 h), and late stationary phase (15 h) of growth.

RESULTS

Hydrogen peroxide was produced regardless of the glucose concentrations tested (Figure 2). The highest rate of hydrogen peroxide production occurred at 13.8 mM glucose (0.27 U ± 0.08) and the lowest rate was at 55.5 mM glucose with an activity of 0.06 U ± 0.01 (Figure 2). Hydrogen peroxide production at 55.5 mM glucose and when no glucose was added to the assay was significantly different than at 13.8 mM glucose ($P = 0.031$, $P = 0.003$, respectively) (Figure 2). In addition, hydrogen peroxide production at 55.5 mM glucose was significantly different from 27.7 mM glucose and 41.6 mM glucose ($P = 0.028$, $P = 0.025$) (Figure 2).

Hydrogen peroxide was produced regardless of pH conditions tested (Figure 3). The highest rate of hydrogen peroxide production was detected at pH 7, with an activity of 0.31U ± 0.09 and the lowest rate of production was at pH 5 (0.07 U ± 0.00) (Figure 3). There was a significant difference in hydrogen peroxide production between pH 7 and pH 5 (P = 0.004), pH 7 and pH 4 ($P = 0.021$), and pH 5 and pH 6 ($P = 0.020$) (Figure 3).

Hydrogen peroxide was produced regardless of the assay temperatures tested (Figure 4). The highest rate of hydrogen peroxide production occurred at 40°C (0.19 U ± 0.01) and the lowest rate of production occurred at 30°C (0.05 U ± 0.01) (Figure 4). A significantly lower rate of hydrogen peroxide production occurred at 30°C when compared to the other temperatures tested (35°C, $P = 0.003$; 40°C, $P = 0.000$, and 45°C, $P = 0.000$) (Figure 4). A significant difference in hydrogen peroxide production also occurred between 40°C and 35°C ($P = 0.003$) (Figure 4).

The relationship between growth stage and hydrogen peroxide production by *L. jensenii* is shown in figures 5 and 6. Hydrogen peroxide production was calculated as mM L^{-1} min^{-1} (U) in figure 5 and as mM L^{-1} min^{-1} / A in figure 6. Hydrogen peroxide assays calculated as mM L^{-1} min^{-1} / A value were determined to account for differences in hydrogen peroxide production due to cell density (Figure 6). Hydrogen peroxide was produced regardless of the growth stages tested (three h, mid log; six h, late log; nine h, early stationary; and 15 h, late stationary, Figures 5 and 6). When calculated as mM L^{-1} min^{-1}, the highest rate of hydrogen peroxide production occurred after six h growth (late log phase, 0.24 U ± 0.05) and the lowest rate occurred after three h growth (mid log phase, 0.07 U ± 0.02) (Figure 5). A significant difference in hydrogen peroxide production occurred between three h and six h growth ($P = 0.0002$), six h and nine h growth ($P = 0.024$), and three h and 15 h growth ($P = 0.007$) (Figure 5). When cell density was taken into account as mM L^{-1} min^{-1} / A, the highest hydrogen peroxide production occurred at three h growth (mid log phase, 1.11 ± 0.35) and the lowest activity occurred at nine h growth (early stationary phase, 0.10 ± 0.01) (Figure 6). A significant difference in hydrogen peroxide production occurred between three h and six h growth ($P = 0.006$), three h and nine h growth ($P = 0.002$), and three h and 15 h growth ($P = 0.002$) (Figure 6).

DISCUSSION

The purpose of this study was to determine how certain *in vitro* culture conditions influence the rate of hydrogen peroxide production by *L. jensenii*. It was apparent from this study that many factors can influence *in vitro* hydrogen peroxide production by *L. jensenii*. For example, hydrogen peroxide production by *L. jensenii* was lowest at the highest glucose concentration (55.5 mM) and at the lowest glucose concentration (0 mM) tested. These data were similar to findings by Barnard and Stinson (1999) who studied hydrogen peroxide synthesis in *Streptococcus gordonii*. *S. gordonii* is a lactic acid bacterial species associated with oral health. Barnard and Stinson (1999) showed that there was essentially a bell shaped curve associated with hydrogen peroxide production as it related to glucose concentration. In their study, hydrogen peroxide synthesis was lowest at the lowest (0.01 mM) and highest (1000 mM) glucose concentrations tested and was higher at the intermediate glucose concentrations (0.1mM, 1.0 mM, and 10 mM). The fact that both *L. jensenii* (in this study) and *S. gordonii* (Barnard and Stinson (1999) displayed lower hydrogen peroxide synthesis at the highest glucose concentrations tested in each study may reflect a common mechanism used by lactic acid bacteria to efficiently regulate bacterial competition in a vaginal or oral environment. Competition for nutrients among various microbial groups in the oral or vaginal environment would be less important when carbohydrate concentration is abundant. Consequently, a high rate of hydrogen peroxide synthesis by lactic acid bacteria is not needed to suppress competing bacteria and the cells can devote resources to more important metabolic processes. *L. jensenii* and *S. gordonii* (Barnard and Stinson 1999) both produced hydrogen peroxide when glucose was not added to the assay conditions. The energy needed for hydrogen peroxide synthesis by lactic acid bacteria in the absence of extraneous glucose may have come from metabolism of intracellular polysaccharide stores (Barnard and Stinson, 1999; Minah and Loesche, 1977). Minah and Loesche (1977) showed that up to 14% of the carbohydrate consumed by hydrogen peroxide-producing oral streptococci is converted into intracellular carbohydrate. The ability of *L. jensenii* to produce an intracellular polysaccharide reserve, however, was not determined in this study. Although not statistically significant, *L. jensenii* made twice as much hydrogen peroxide with no exogenous glucose (0 mM) than with the highest glucose concentration tested (55.5 mM). The ability to make hydrogen peroxide without exogenous carbohydrate present may reflect the need for certain lactic acid bacteria (*L. jensenii* or *S. gordonii*, Barnard and Stinson, 1999) to inhibit competing bacteria in their particular environment (vaginal or oral) under low carbohydrate conditions when competition for nutrients would be more intense (Barnard and Stinson, 1999). Vaginal glucose in the form of glycogen (Gregoire et al. 1971; Paavonen, 1983) is used by lactic acid bacteria as an energy source for hydrogen peroxide synthesis (Barnard and Stinson, 1999; Minah and Loesche, 1977) and is also metabolized to form lactic acid (Boskey et al. 2001). Glycogen content will vary based on the cycle stage and vaginal location but has been determined to be 1122 µg to 1667 µg of glycogen per 100 mg of tissue wet weight epithelial tissue (Gregoire et al. 1971).

Vaginal glycogen levels are regulated by estrogen (Gregoire et al. 1971; Paavonen, 1983). Consequently, fluctuations in estrogen such as menarche, menopause, oral contraception, and hormone replacement therapy could conceivably alter the vaginal biota including hydrogen peroxide-producing lactobacillus species (Cauci et al. 2002). Increased estrogen production can cause higher amounts of glycogen to be deposited into the vagina (Boskey et al. 2001; Gregoire et al. 1971; Paavonen, 1983). It is hypothesized that decreased vaginal glycogen levels may increase the antimicrobial competitive activities (lactic acid and hydrogen peroxide production) of vaginal lactobacilli.

Production of hydrogen peroxide by *L. jensenii* in this study was also influenced by pH. The higher production rates of hydrogen peroxide detected in this study at pH 6 and pH 7 than at pH 4 and 5 may be a response by *L. jensenii* to inhibit microbial competition in the vaginal environment as the pH increases. The healthy vagina should have a pH range of 4.0-4.7 (Gardner and Dukes 1955) and an abundance of the lactobacilli morphotype (large gram-positive rod) (Nugent et al. 1991; Spiegel, 1991). An increase in vaginal pH above 4.5, a decrease in lactobacilli, and the appearance of non lactobacilli bacterial morphotypes (gram-negative rods, gram-variable rods and curved rods, gram positive cocci) are among the clinical signs associated with bacterial vaginosis (Amsel et al. 1983; Nugent et al. 1991; Spiegel, 1991). The lowest rates of hydrogen peroxide synthesis obtained in this study were detected at pH 4 and pH 5. This response to pH may be a mechanism for *L. jensenii* to conserve energy during times of lower pH because the normal acidic vaginal environment (pH 4.0-4.7) itself would help inhibit the growth of pathogenic organisms (Ogawa et al. 2001; Stamey and Timothy, 1975).

Temperature was also an important factor in the production of hydrogen peroxide by *L. jensenii*. The peak rates of production (0.19 mM L^{-1} min^{-1} and 0.16 mM L^{-1} min^{-1}) occurred at the two highest temperatures tested (40°C and 45°C), indicating that increased temperature enhances the production of hydrogen peroxide in *L. jensenii in vitro*. Although the difference in rates between the two highest temperatures was not significant, cells assayed for hydrogen peroxide production at 45°C may have been slightly temperature stressed resulting in the lower rate. In general, higher hydrogen peroxide synthesis rate coordinated with optimum growth temperatures (37°C-44°C) associated with *Lactobacillus* species isolated from the vagina (McLean and Rosenstein, 2000; Tomás et al. 2003). The coordination of high hydrogen peroxide synthesis with optimum growth temperatures that mimic the human vagina (37°C) is an important physiological characteristic for endogenous vaginal lactobacilli and for assessing the potential of a vaginal probiotic strain (McLean and Rosenstein, 2000).

In addition to the other environmental factors mentioned in this study, hydrogen peroxide production by *L. jensenii* coordinated with growth stage (Fig 6) rather than by cell numbers alone (Fig 5). That is, actively growing cells of *L. jensenii* and not necessarily high cell numbers produced the highest rates of hydrogen peroxide. For example, when measured as mM L^{-1} min^{-1}, hydrogen peroxide synthesis was assessed by cell numbers during the growth cycle (Fig 5). For this cell number assessment, hydrogen peroxide synthesis was lowest during mid-log (0.07 mM L^{-1} min^{-1}) but peaked during late-log growth phase

(0.24 mM L^{-1} min^{-1}). When calculated as mM L^{-1} min^{-1} / A value, hydrogen peroxide synthesis peaked during mid-log growth (1.11 mM L^{-1} min^{-1} / A) followed by a sharp reduction in activity as the growth cycle proceeded into late log (0.29 mM L^{-1} min^{-1} / A) and stationary phase (0.10 mM $L^{-1}$$min^{-1}$ / A; 0.13 mM L^{-1} min^{-1} / A) (Fig 6). This activity assessment (mM L^{-1} min^{-1} / A value) is an indication of hydrogen peroxide synthesis efficiency by *L. jensenii*. This value may be a more accurate assessment of the hydrogen peroxide capability of a probiotic culture since lactobacilli cell numbers in the healthy vagina (Boskey et al. 1999) would not reach the concentration or density as is typically obtained *in vitro* (Tomás et al. 2003). In addition, these data may also indicate that hydrogen peroxide production in *L. jensenii* coordinates with the synthesis of cell enzymes needed for primary metabolism such as NADH oxidases (Marty-Tysset et al. 2000; Talwalkar and Kailasapathy, 2003).

This *in vitro* study provides the first evidence that hydrogen peroxide synthesis rate by the vaginal isolate *L. jensenii* is not static but is highly influenced by environmental factors. *L. jensenii* can adjust hydrogen peroxide synthesis rate in response to changes in glucose concentration, pH, temperature, and growth phase. Parameters such as glucose concentration and pH are important environmental conditions that can influence composition of the vaginal microbiota. It is not ideal to correlate results of an *in vitro* study to an *in vivo* environment such as the vagina. The response in hydrogen peroxide synthesis by *L. jensenii* to glucose and pH fluctuations, however, may reflect a common mechanism used by lactic acid bacteria to efficiently regulate bacterial competition in the vaginal environment. This study may help to establish a scientific basis for the selection of vaginal probiotic bacterial strains and also may be used as a guideline for studies focused on differential gene expression in response to changes in environmental conditions.

LITERATURE CITED

Amsel, R. Totton, P.A., Spiegel, C.A., Chen, K.C.S., Eschenbach, D.A., and Holmes, K.K. (1983) Nonspecific vaginitis: diagnostic criteria and microbial and epidemiological associations. Am J Med 74:14-22.

Antonio, M.A.D., Hawes, S.E., and Hillier, S.L. (1999) The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by the species. J Infect Dis 180:1950-1956.

Barnard, J.P. and Stinson, M.W. (1999) Influence of environmental conditions on hydrogen peroxide formation by *Streptococcus gordonii*. Infect Immun 67:6558-6564.

Barynin, V.V., Whittaker, M.M., Antonyuk, S.V., Lamzin, V.S., Harrison, P.M., Artymiuk, P.J. and Whittaker, J.W. (2001) Crystal structure of manganese catalase from *Lactobacillus plantarum*. Structure 9:725-738.

Bolton M., Van Der Straten, A. and Cohen, C.R. (2008) Probiotics: potential to prevent HIV and sexually transmitted infections in women. Sex Trans Dis 35:214-225.

Boskey, E.R., Telsch, K.M., Whaley, K.J., Moench, T.R. and Cone, R.A. (1999) Acid production by vaginal flora *in vitro* is consistent with the rate of extent of vaginal acidification. Infect Immunology, 67:5170-5175

Boskey, E.R., Cone, R. A., Whaley, K.J. and Moench, T.R. (2001) Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Hum Reprod 16:1809-1813.

Breznak, J.A. and Cosilow, R.N. (1994) Physicochemical factors in growth. In: Gerhardt, P., Murray, R.G.E., Wood, W.A. and Krieg N.R. (ed), Methods for General and Molecular Bacteriology, Washington, D.C.: American Society for Microbiology, pp.137-154.

Brown, W.J. (1978) Microbial ecology of the normal vagina. In Hafez, E.S.E. and Evans, T.N. (ed), The Human Vagina, North-Holland Publishing Co., Amsterdam. pp. 407-422.

Cauci, S., Driussi, S., De Santo, D., Penchioni, P., Iannicelli, T., Lanzafame, P., De Seta, F., Quadrifoglio, F., deAloysio, D and Guaschino, S. (2002) Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. J Clin Microbiol 40:2147-2152.

Cherpes, T.L., Hillier, S.L., Meyn, L.A., Busch, J.L. and Krohn, M.A. (2008) A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. Sex Transm Dis 35:78-83.

Eschenbach, D.A., Davick, P.R., Williams, B.L., Klebanoff, S.J., Young-Smith, K., Critchlow, C.M., and Holmes, K.K. (1989) Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. J Clin Microbiol 27:251-256.

Gardner, H.L and Dukes, C.D. (1955) *Heamophilus vaginalis* vaginitis. A newly defined specific infection previously classified “nonspecific” vaginitis. Am J Obstet Gynecol 69:962-976.

Gasser F, Mandel, M, and Rogosa, M. (1970) *Lactobacillus jensenii* sp. nov., a new representative of the subgenus *Thermobacterium*. J Gen Microbiol 62:219-222.

Gregoire, A.T., Kandil, O. and Ledger, W.J. (1971) The glycogen content of human vaginal epithelial tissue. Fertil Steril 22:64-68.

Gupta, K., Stapleton, A.E., Hooton, T.M., Roberts, P.L., Fennell, C.L. and Stamm, W.E. (1998) Inverse association of H_2O_2-producing lactobacilli and vaginal *Escherichia coli* colonization in women with recurrent urinary tract infections. J Infect Dis 178:446-450.

Hawes,S.E., Hillier, S.L., Benedetti, J., Stevens, C.E., Koutsky, L.A., Wolner-Hanssen, P. and Holmes, K.K. (1996) Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. J Infect Dis 174:1058-1063.

Hill, G.B., Eschenbach, D.R. and Holmes, K.K. (1984) Bacteriology of the vagina. Scand J Urol Nephrol Suppl 86:23-39.

Jack, R.W., Tagg, J.R. and Ray, B. (1995) Bacteriocins of gram-positive bacteria. Microbiol Rev 59:171-200.

Martin, H.L., Richardson, B.A., Nyange, P.M., Lavreys, L., Hillier, S.L., Chohan, B., Mandaliya, K., Ndinya-Achola, J.O., Bwayo, J. and Kreiss, J. (1999) Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infec Dis 180:1863-1868.

Martin, R., Soberon, N., Vaneechoutte, M., Florez, A.B., Vazquez, F. and Suarez, J.E. (2008) Characterization of indigenous vaginal lactobacilli from healthy women as probiotic candidates. Int Microbiol 11:261- 266.

Martin, R., and Suarez, J.E. (2010) Biosynthesis and degradation of H_2O_2 by vaginal lactobacilli. Appl Environ Microbiol 76:400-405.

Marty-Teysset, C., de la Torre, F. and Garel, J.R. (2000) Increased production of hydrogen peroxide by *Lactobacillus delbrueckii* subsp. *bulgaricus* upon aeration: involvement of an NADH oxidase in oxidative stress. Appl Environ Microbiol 66:262-267.

McGroarty, J.A., Tomeczek, L., Pond, D.G., Reid, G. and Bruce, A.W. (1992) Hydrogen peroxide production by *Lactobacillus* species: correlation with susceptibility to the spermicidal compound nonoxynol-9. J Infect Dis 165:1142-1144.

McLean, N.W. and Rosenstein, I.J. (2000) Characterization and selection of a *Lactobacillus* species to re-colonize the vagina of women with recurrent bacterial vaginosis. J Med Microbiol 49:543-552.

Minah, G.E. and Loesche, W.J. (1977) Sucrose metabolism by prominent members of the flora isolated from cariogenic and non-cariogenic dental plaques. Infect Immun 17:55-61.

Nugent, R.P., Krohn, M.A. and Hillier, S.L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 29:297-301.

Ogawa, M., Shimizu, K., Nomoto, K., Tanaka, R., Hamabata, T., Yamasaki, S., Takeda, T. and Takedam, Y. (2001) Inhibition of *in vitro* growth of Shiga toxin-producing *Escherichia coli* O157:H7 by probiotic *Lactobacillus* strains due to production of lactic acid. Int J Food Microbiol 68:135-140.

Otero, M. C. and Nader-Macias, M.E. (2006) Inhibition of *Staphylococcus aureus* by H_2O_2-producing *Lactobacillus gasseri* isolated from the vaginal tract of cattle. Anim Reprod Sci 96:35-46.

Paavonen, J. (1983) Physiology and ecology of the vagina. Scand J Infect Dis Suppl 40:31-35.

Rabe, L.K. and Hillier, S.L. (2003) Optimization of media for detection of hydrogen peroxide production by *Lactobacillus* species. J Clin Microbiol 41:3260-3264.

Skerman, V.B.D., McGowan, V., and Sneath, P.H.A. (1980) Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30:225-420.

Song, Y.L, Kato, N., Matsumiya, Y., Liu, C.X., Kato, H. and Watanabe, K. (1999) Identification of and hydrogen peroxide production by fecal and vaginal lactobacilli isolated from Japanese women and newborn infants. (1999) J Clin Microbiol 37:3062-3064.

Spiegel, C.A. (1991) Bacterial vaginosis. Clin Microbiol Rev 4:485-502.

Spurbeck, R.R. and Arvidson, C.G. (2008) Inhibition of *Neisseria gonorrhoeae* epithelial cell interactions by vaginal *Lactobacillus* species. Infect Immun 76:3124-3130.

Stamey, T.A. and Timothy, M.M. (1975) Studies of introital colonization in women with recurrent urinary infections. I. The role of vaginal pH. J Urol 114:262-263.

Talwalkar, A. and Kailasapathy, K. (2003) Metabolic and biochemical responses of probiotic bacteria to oxygen. J Dairy Sci 86:2537-2546.

Tomas, M.S., Bru, E. and Nader-Macias, M.E. (2003) Comparison of the growth and hydrogen peroxide production by vaginal probiotic lactobacilli under different culture conditions. Am J Obstet Gynecol 188:35-44.

Vallor, A.C., Antonio, M.A.D., Hawes, S.E. and Hillier, S.L. (2001) Factors associated with acquisition of, or persistent colonization by vaginal Lactobacilli: role of hydrogen peroxide production. J Infect Dis 184:1431-1436.

Vásquez A., Jakobsson, T., Ahrné, S., Forsum, U. and Molin, G. (2002) Vaginal *Lactobacillus* flora of healthy Swedish women. J Clin Microbiol 40:2746-2749.

Wilks, M., Wiggins, R., Whiley, A., Hennessy, E., Warwick, S., Porter, H., Corfield, A. and Millar, M. (2004) Identification and H_2O_2 production of vaginal *Lactobacilli* from pregnant women at high risk of preterm birth and relation with outcome. J Clin Microbiol 42:713-717.

Yoder, D.W., Hwang, J. and Penner-Hahn, J.E. (2000) Manganese catalases. Met Ions Biol Syst 37:527-557.

Zhong, W., Millsap, K., Bialkowska-Hobrzanska, H., and Reid, G. (1998) Differentiation of *Lactobacillus* species by molecular typing. Appl Environ Microbiol 64:2418-2423.

Figure 1. Growth curve of *L. jensenii* after inoculation into a 10 ml MRS broth culture. The 10-ml MRS culture was started from a frozen stock culture (50 ul) of *L. jensenii*. Each time point represents data from three growth trials. Absorbance values were exactly the same for each time point.

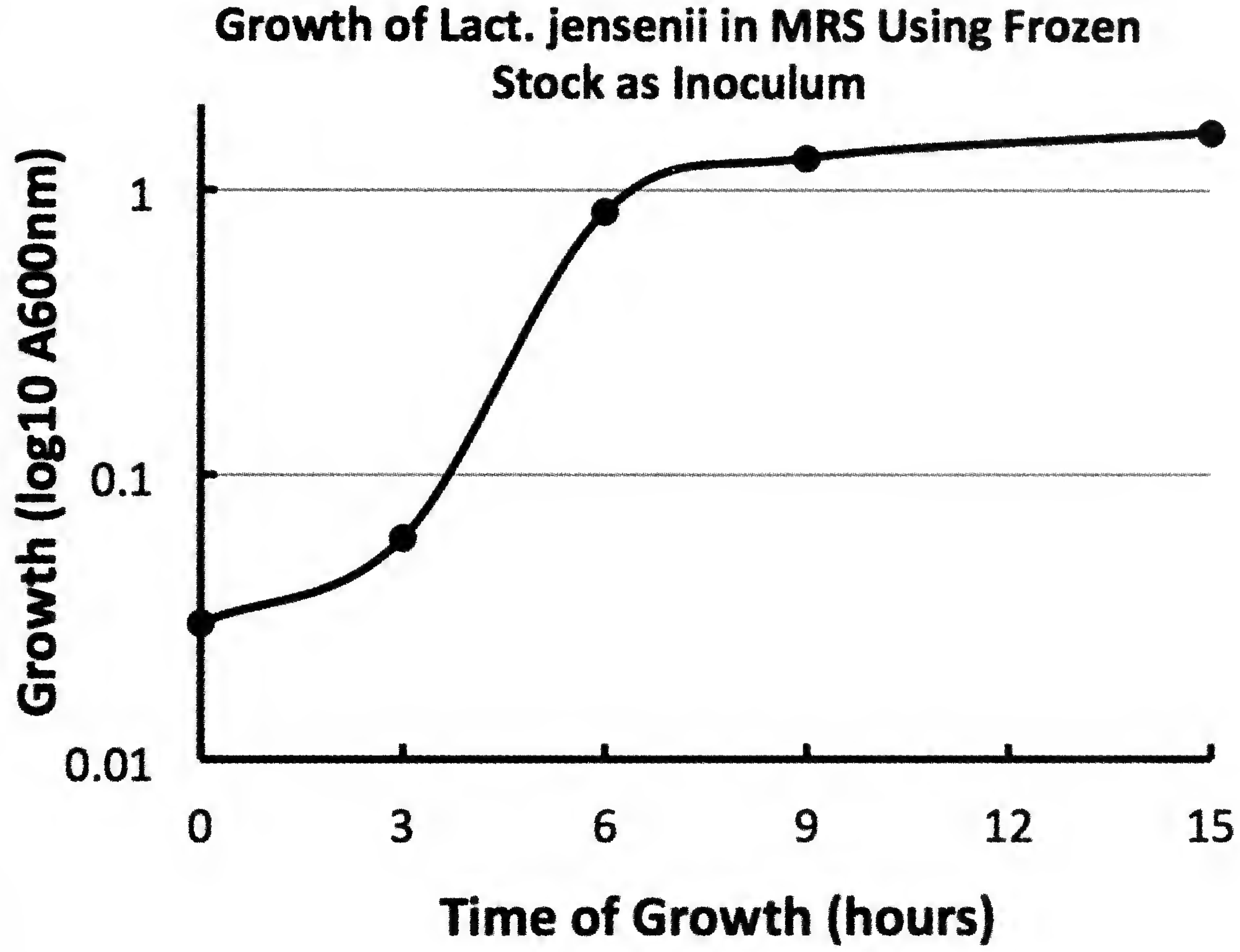


Figure 2. Influence of glucose concentration on hydrogen peroxide production by *L. jensenii*. Hydrogen peroxide production rate (mM L^{-1} min^{-1}) was determined from three trials for each glucose concentration and reported as mean ± SD.

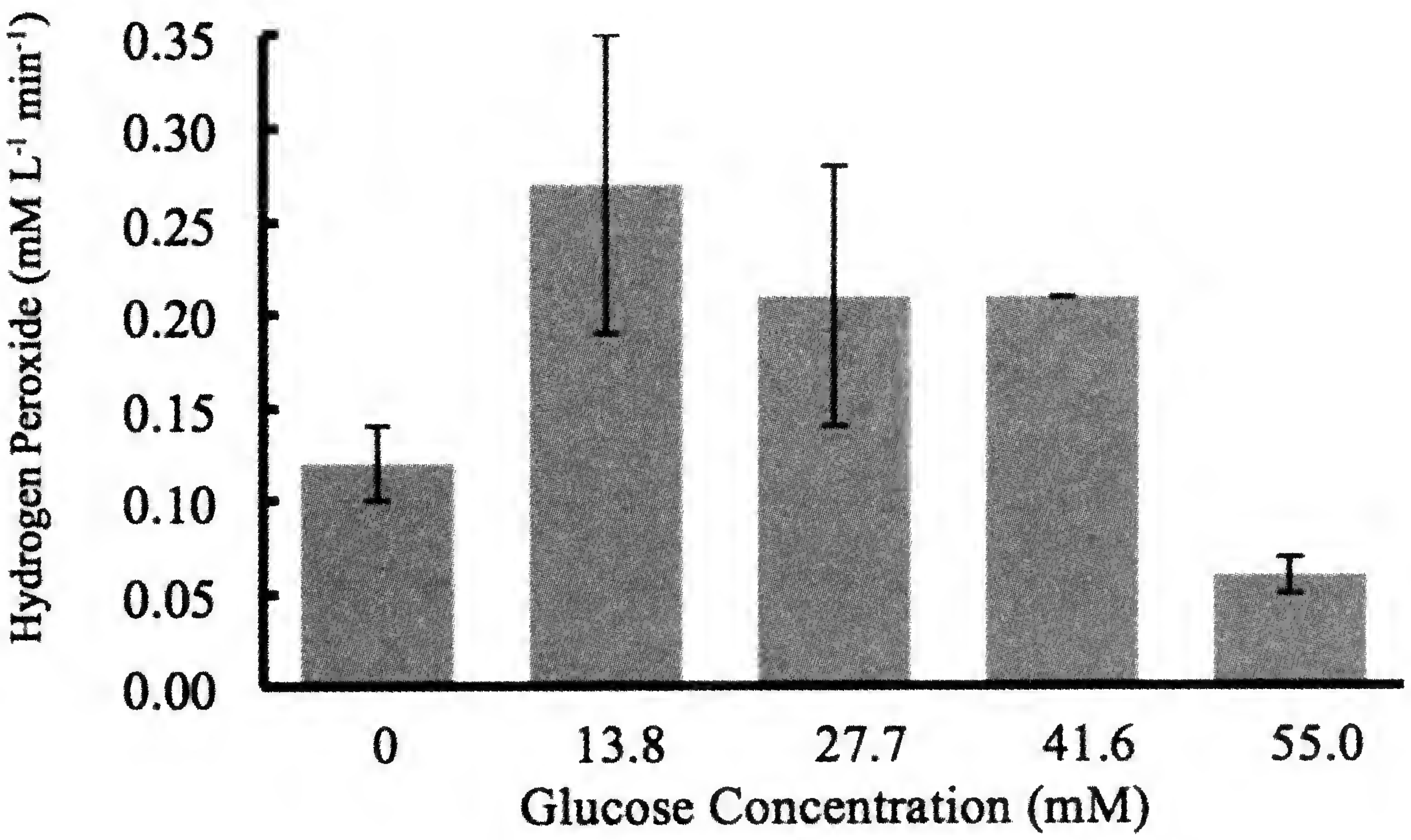


Figure 3. Influence of pH on hydrogen peroxide production by *L. jensenii*. Hydrogen peroxide production rate (mM L^{-1} min^{-1}) was determined from three trials for each pH and reported as mean ± SD.

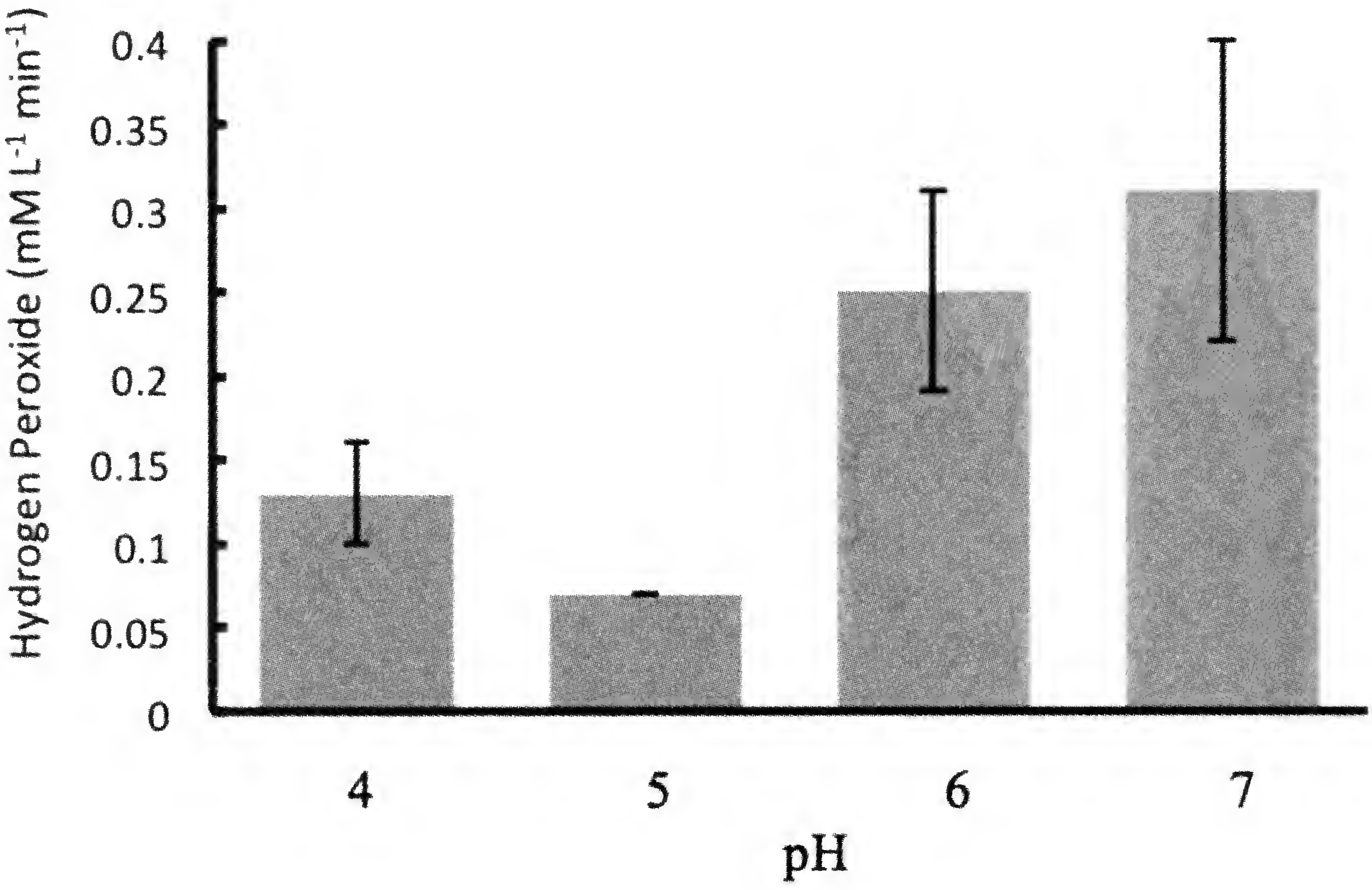


Figure 4. Influence of temperature on hydrogen peroxide production by *L. jensenii*. Hydrogen peroxide production rate (mM L^{-1} min^{-1}) was determined from three trials for each temperature and reported as mean ± SD.

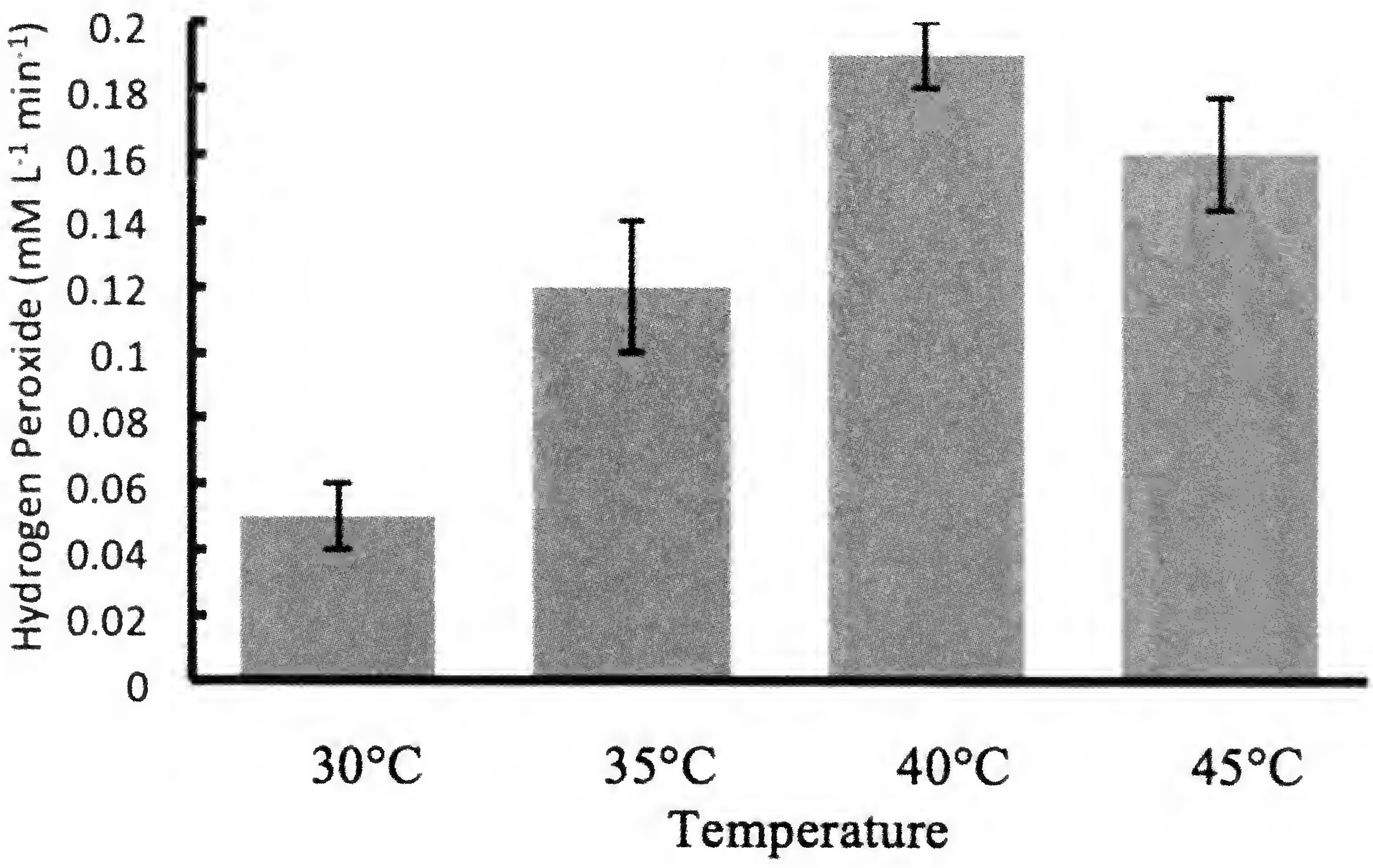


Figure 5. Influence of *L. jensenii* growth stage on hydrogen peroxide production measured as mM L^{-1} min^{-1}. Hydrogen peroxide production rate (mM L^{-1} min^{-1}) was determined in triplicate for each growth stage and reported as mean ± SD.

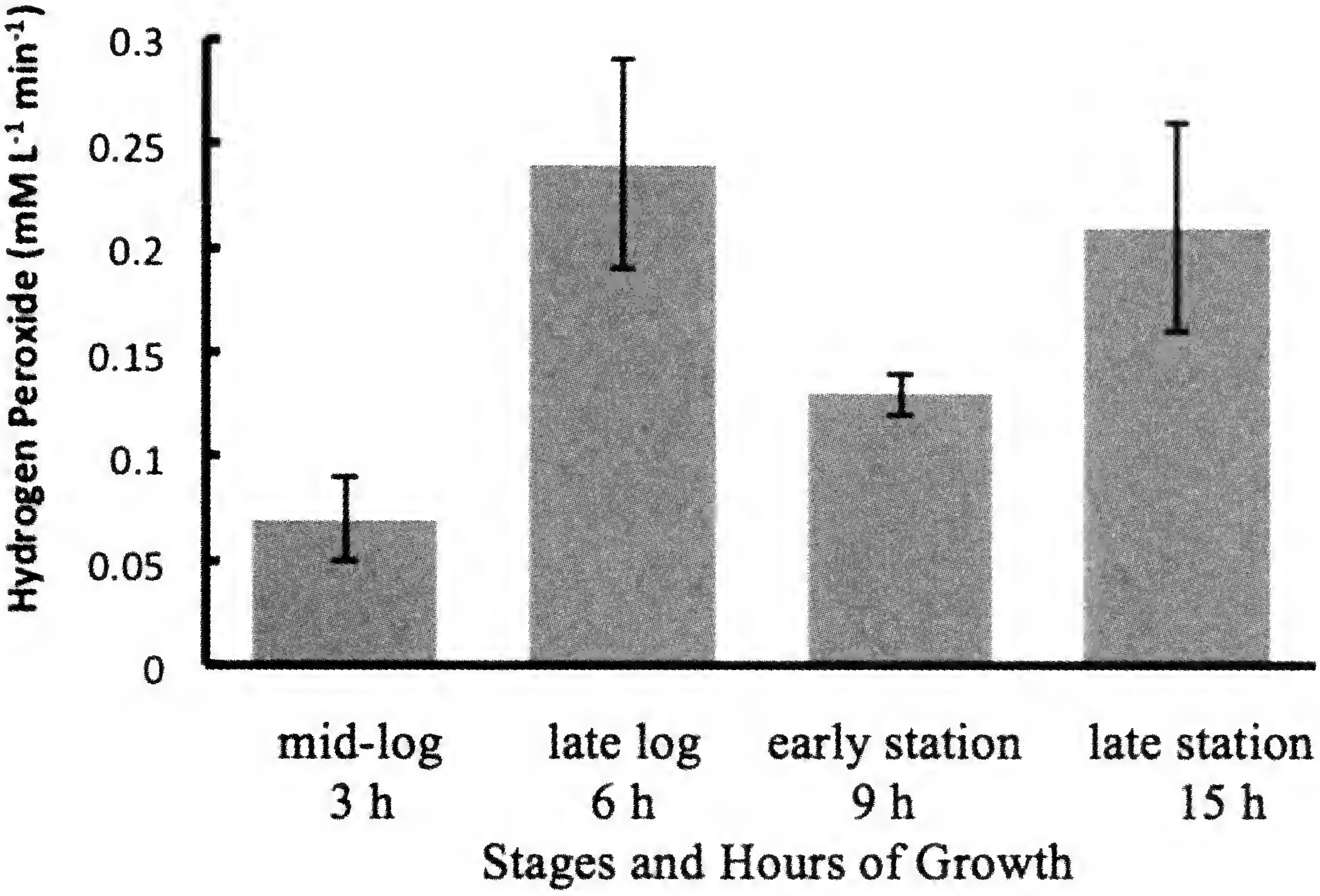
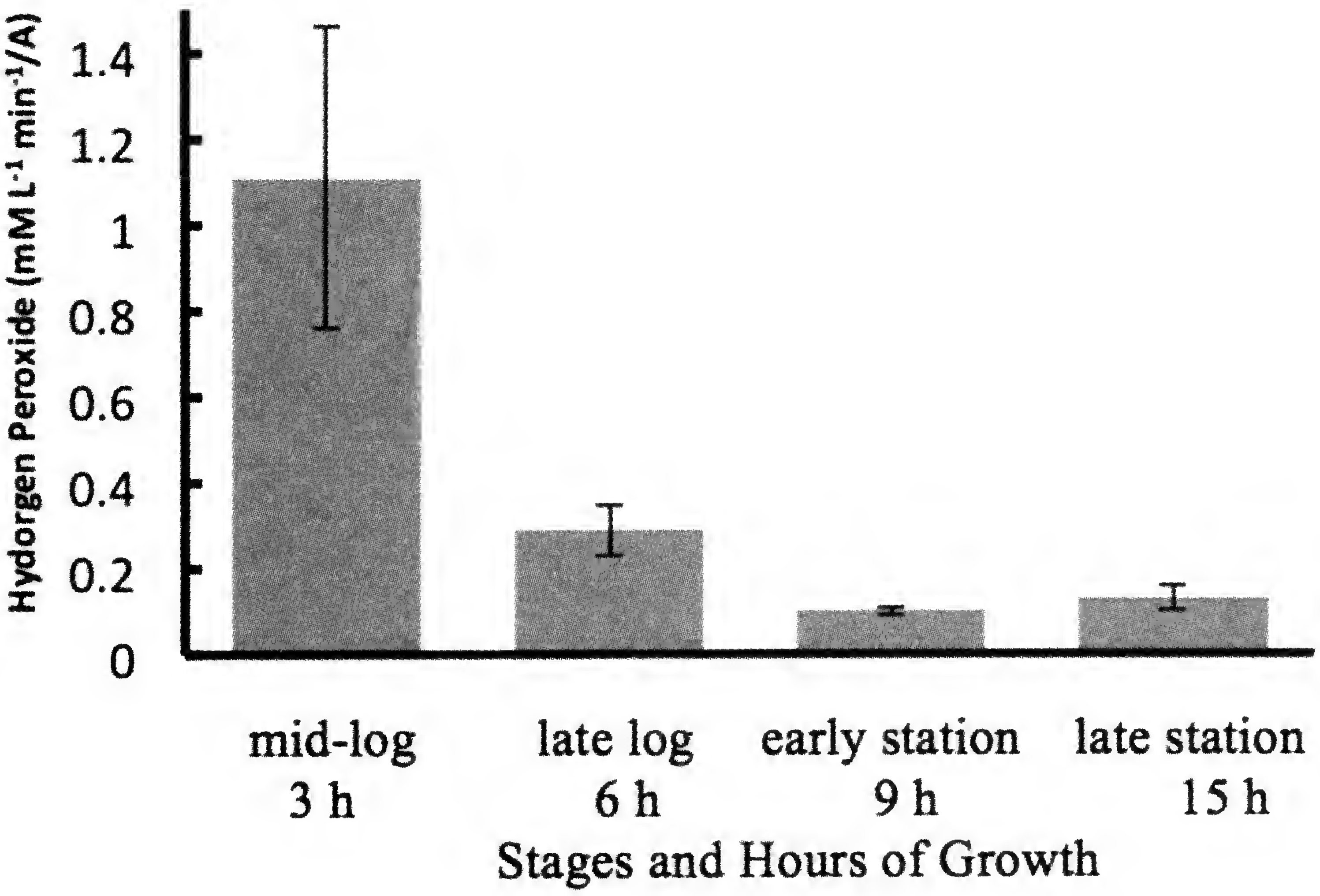


Figure 6. Influence of *L. jensenii* growth stage on hydrogen peroxide production measured as (mM L^{-1} min^{-1} / A). Hydrogen peroxide production rate (mM L^{-1} min^{-1} / A) was determined from three trials for each growth stage and reported as mean ± SD.



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Prevalence of *Baylisascaris procyonis* and Implications for Reintroduced Woodrat Populations in Southern Illinois

Simon R. Bade, F. Agustín Jiménez, Aaron K. Poole, and George A. Feldhamer
Department of Zoology, Southern Illinois University, Carbondale, IL 62901-6501

ABSTRACT

The roundworm *Baylisascaris procyonis* (Nematoda: Ascaridae) is carried and spread by raccoons (*Procyon lotor*). In intermediate hosts the parasite can cause brain damage and death. The prevalence of *B. procyonis* in raccoon populations is a concern in areas where the state-endangered eastern woodrat (*Neotoma floridana*) occurs or has been reintroduced because the foraging and hoarding strategies of woodrats ("packrats") make them particularly susceptible to infection. To determine the prevalence of this parasite and the possible implications for reintroduced eastern woodrats, we established 10 transects from Garden of the Gods (Saline County) east to Rim Rock/Pounds Hollow (Gallatin County) where woodrats have been reintroduced in Illinois. Raccoon latrine sites were visually located along these transects and scat piles sampled and analyzed using Fecalyzer flotation kits. Of 79 total fecal samples, only 1 tested positive for *B. procyonis*. This low prevalence (1.3%) for *B. procyonis* suggests that it likely poses little risk to the reintroduced woodrat populations within the study area.

Key words: *Baylisascaris procyonis*, eastern woodrats, raccoons, reintroductions

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INTRODUCTION

Raccoons (*Procyon lotor*) have a wide geographic distribution in North America and population densities are often quite high (Gehrt 2003). They thrive in proximity to humans and low pelt prices and reduced trapping effort have increased their abundance. Their large population sizes and ubiquitous distribution make raccoons an important vector for parasites and diseases including distemper, rabies, and the roundworm *Baylisascaris procyonis* (Mitchell et al. 1999).

Raccoons develop communal latrine sites used by various individuals (Gehrt 2003, Page et al. 2009a, b). Rodents often forage on scats at latrine sites for undigested seeds (LoGiudice 2001). This behavior presents a strong potential method of transmission of *B. procyonis* (Page et al. 1998). The parasite has little to no effect on the raccoon host and is rarely fatal except in cases of severe infection (Kazacos 2001, Gehrt 2003). In an accidental host infected with the parasite, the larvae migrate to ocular and brain tissue, encyst there and can cause listlessness, emaciation, loss of motor skills, coma, and ultimately death (Kazacos et al. 1984).

The prevalence of raccoons and their latrine sites may have a direct impact on other wildlife species, including woodrat populations. Whereas eastern woodrats (*Neotoma floridana*) once ranged throughout much of southern Illinois, today only four remnant populations remain. These show evidence of inbreeding (Monty et al. 2003) and the species is listed as endangered in the state. Reintroduction efforts in the Shawnee Hills region began in 2002 (Feldhamer and Poole 2008), with woodrats from Arkansas and Missouri released in formerly occupied sites at Garden of the Gods, Buzzard's Point, High Knob, and Pounds Hollow/Rim Rock. Reasons for the decline of woodrats in Illinois are unknown, but may have included habitat loss, degradation, and fragmentation; predation; competition; and mortality due to parasitism from *B. procyonis* (Birch et al. 1994; Feldhamer and Poole 2008). A high incidence of *B. procyonis* could have a negative impact on population viability of reintroduced woodrats in Illinois because the foraging strategies of eastern woodrats make them particularly susceptible to infection. Woodrats routinely collect and store items from their habitat, including raccoon scat. Consumption of fresh *B. procyonis* eggs is not harmful because they are not infective until they embryonate in 2 to 4 weeks. Woodrats, however, store scats and may not eat the seeds, giving eggs time to become infective (Page et al. 1999). Furthermore, the eggs remain viable for several years, creating the possibility for increased risk of infection as the bank of eggs increases.

The primary objective of this study was to determine the prevalence of *B. procyonis* in the local raccoon population inhabiting areas of woodrat reintroductions. The secondary objective was to determine significant microhabitat variables associated with location of raccoon latrines.

MATERIALS AND METHODS

Study Area

Between 2002 and 2009, 422 woodrats were reintroduced into 4 sites within the Shawnee National Forest, from Garden of the Gods (Saline County) east to Buzzard's Point, High Knob, and Rim Rock (Gallatin County) (Figure 1). These sites were selected for reintroduction based on documented past presence of the species and their bluff escarpments that provide rocky habitat (see Novosak 2004). Dominant overstory is oak (*Quercus* spp.)-hickory (*Carya* spp.) forest, although Rim Rock is primarily beech (*Fagus* spp.)-maple (*Acer* spp.) forest. The forest above the bluffs is xeric, while below the bluffs the habitat is more mesic. Self-sustaining populations of woodrats have been established at these sites (Feldhamer and Poole 2008, Ing 2008).

Latrine/Scat Analysis

We established 10 1-km transects between Garden of the Gods and Pounds Hollow to sample raccoon latrine sites. Five transects were on the upland slopes of the bluff line, where reintroduced woodrats may have dispersed, and 5 were below the bluff line in areas known to harbor reintroduced woodrats. Transects were unbounded and all latrines that could be located visually were sampled. Likely sites for raccoon latrines (downed logs and prominent rocks) were examined along the route, flagged, and a GPS location determined. Each identified site constituted an individual sampling unit. Size of latrines was classified as small (1 scat), medium (2 scats), or large (≥3 scats) based on amount of scat. Number of samples taken was dependent upon the size of each scat pile—small piles were sampled once, medium piles twice, and large piles three times. Sampling began March 2008, and a circuit of all 10 transects was made monthly for 20 months except August 2008. Different transects were visited weekly, with even effort among all transects. During June and July 2008, transects were walked twice each month. Checking continued through October 2009 for a total of 210 km monitored.

A sample of about 3 g was collected from individual scats in each latrine. Samples were stored at -70° C. Presence/absence of *B. procyonis* ova was determined by flotation using sodium nitrate and the Fecalyzer system (Evsco Pharmaceuticals, Bueno, NJ) following manufacturers recommendations. Eggs were identified using guidelines for morphology and size from Page et al. (2005). Eggs of *B. procyonis* are oval-shaped, generally between 65 and 80 μm in length, and have thick, granulated shells. They differ from eggs of *Toxocara* spp. in shape, membrane thickness and appearance (Kazacos and Boyce 1989).

Habitat Measurements

Microhabitat variables were measured at each latrine site and included: number of downed trees (coarse woody debris) in a 5-m radius surrounding the latrine, substrate (bare stone, log, or soil), and distance to nearest tree > 30 cm diameter at breast height (DBH). These habitat variables were also collected at random sites by choosing a cardinal direction at each latrine site and pacing 50 steps in that direction.

Statistical Analyses

We used a 1-tailed, paired t-test to determine differences in the amount of coarse woody debris and distance to the nearest tree at latrine sites vs. random sites. Chi-square analyses were used to test for differences in location of latrine sites above or below bluff line, and on substrate types. Level of significance was $\alpha = 0.05$.

RESULTS

We collected 79 fecal samples from 54 latrine sites. Most of the scat found was in single piles. However, raccoons used several latrine sites repeatedly during the course of the sampling period. Thus, there were only 40 unique latrine sites, or 1 for every 5.25 km monitored. Of the 79 samples, only 1 (1.3%)—from Garden of the Gods—contained eggs of *B. procyonis*.

Four substrate types contained latrines: rock (n = 18), downed log (n = 13), leaf litter (n = 8), and live tree (n = 1). There was a significant difference among substrate types for occurrence of latrines ($\chi^2 = 15.8$, df = 3, $P < 0.05$). Essentially the same substrate types occurred on the 40 randomly selected sites as the identified latrine sites, with the exception of one random site with grass substrate. The mean amount of coarse woody debris at latrine sites (28.12%) was greater ($t_{39} = 2.46$, $P < 0.009$) than at random sites (16.62%). The mean distance to a tree > 30 cm DBH was 5.08 m at the latrine sites and 5.05 m at the random sites; these were not significantly different ($t_{39} = 0.72$, $P = 0.473$).

Considering location of the 40 latrines with respect to the bluff line, there were 20 above and 20 below. However, when each woodrat release site was considered independently, there were significant differences. At Garden of the Gods, there were 13 latrine sites above the bluff line and only 3 below ($\chi^2 = 6.5$, df = 1, $P < 0.05$). At Rim Rock, there were 3 latrine sites above the bluff line and 10 below ($\chi^2 = 3.76$, df = 1, $P < 0.05$). Sample sizes at High Knob (3 above and 4 below) and Buzzard's Point (2 above and 2 below) were too small to test statistically.

Seasonal variation in scat presence was apparent. Scat was most frequently detected in autumn [Sep.-Nov.] ($\bar{x} = 4.2$ scats/month), winter [Dec.-Feb.] ($\bar{x} = 6.3$ scats/month), and spring [Mar.-May] ($\bar{x} = 5.3$ scats/month), while an average of only 0.6 scats/month was found during June, July, and August.

DISCUSSION

Occurrence of *Baylisascaris procyonis*

Previous studies have determined the prevalence of infection of *B. procyonis* among raccoon populations in southern Illinois. Birch et al. (1994) found only 5.0% prevalence of *B. procyonis* among a sample of 60 raccoons in southern Illinois. This was much lower than the 64% prevalence found by Barnstable and Dyer (1974) in a sample of 36 raccoons. A more recent study by Nielsen et al. (2007) at Union County Conservation Area (UCCA)—with a very high density of raccoons—found *B. procyonis* at 16% of latrine sites. Prevalence of *B. procyonis* is positively correlated with the density of raccoons (Gompper and Wright 2005) and increased raccoon contact rates lead to increased rates of internal parasitism (Wright and Gompper 2005). Anthropogenic impacts that increase resources also can increase parasite prevalence, as can age structures of raccoon populations (Prange et al. 2003, Page et al. 2009b).

Given the well-documented ubiquitous nature of raccoons (Gerht 2003, Nielsen et al. 2007), we expected that scat would be abundant and easy to find on our study area. The number of scats we found was much lower than expected, however, and may indicate a low density of raccoons. The study area is removed from most development and the sites generally have little human use. Garden of the Gods is the only site that permits camping; Rim Rock has extensive hiking and picnic use, whereas High Knob and Buzzard's Point are more remote and less accessible to people with only hiking and equestrian use permitted. Locations of scats relative to the bluff line paralleled human activity. At Garden of the Gods most use is above the bluff line, whereas at Rim Rock picnic tables are only below the bluff line. High Knob has mostly equestrian use below the bluff line, whereas few people visit Buzzard's Point.

Raccoons are well known for their tendency to gravitate toward urban and suburban areas for easier access to food, with densities often higher than in rural areas (Prange et al. 2003). The study site at Garden of the Gods had the highest number of latrines and also the most human use. Likewise, UCCA experiences higher human use for camping, boating, fishing, and hiking, and high densities of raccoons; Nielsen et al. (2007) reported 1.67 raccoons/ha there. The apparent seasonal variation in scat prevalence we found, with fewer scats during summer, also was unexpected. Whether this was because of seasonal variation in raccoon habitat, increased decomposition rate, increased scat foraging by rodents, or some other factor is unknown.

Site Selection of Latrines

Raccoons choose specific latrine sites, often rocky, open areas (Page et al. 1998). Our results found 18 of the 40 unique latrines (45%) were on rocky locales. However, we did not determine relative availability of substrates or selection by raccoons. Latrines on our study area may have been deposited in proportion to available substrate without regard to selection—particularly because proportion of substrates at random sites was similar. The amount of coarse woody debris was significantly related to the occurrence of latrines, but unexpectedly, the distance to a large tree had no significant bearing on selection of sites. This is in contrast to Nielsen et al. (2007), who found that 61% of latrine sites were deposited at the base of large trees. Also contrary to most accounts in the literature, there was little proclivity for raccoons on our study area to form large communal latrine sites. We found only one large communal latrine at Garden of the Gods, located near a campsite, which had the only sample to test positive for *B. procyonis*.

Management Implications

Periodic monitoring of eastern woodrat and raccoon populations in southern Illinois should continue. Control of raccoon populations that are near reintroduction sites may be necessary should their numbers significantly increase. Raccoon populations state-wide have remained stable for the past 15 years (Bluett 2010), but as noted, they attain their highest densities in proximity to humans and/or agricultural land, where food is easy to obtain. It is in the best interest of the reintroduced woodrat populations if human impact remains at current low levels. Increased camping and recreational use may lead to increases in litter and garbage, resulting in increased raccoon abundance. The rarity of scat (and tracks) on our study area suggests a low density of raccoons and corresponding low occurrence of *B. procyonis*. The reintroduced woodrat populations in this area appear to be expanding, with documented reproduction and dispersal (A. K. Poole, SIUC, unpublished data), which is further indicative of low levels of *B. procyonis*. As such, we conclude that *B. procyonis* is not now a major threat to the population of woodrats in the study area.

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LITERATURE CITED

Barnstable, R. W. and W. G. Dyer. 1974. Gastrointestinal helminths of the raccoon, *Procyon lotor*, in southern Illinois. Transactions of the Illinois State Academy of Science 67:451-460.

Birch, G. L., G. A. Feldhamer, and W. G. Dyer. 1994. Helminths of the gastrointestinal tract of raccoons with management implications of *Baylisascaris procyonis* occurrence. Transactions of the Illinois State Academy of Science 87:165-170.

Bluett, R. 2010. 2010 spotlight survey: wildlife diversity program note #10-2. Illinois Department of Natural Resources, Office of Resource Conservation, Springfield, IL.

Feldhamer, G. A. and A. K. Poole. 2008. Status and conservation of other at-risk species of North American woodrats. Pages 193-206 *in* J. Wright and J. D. Peles, editors. The Allegheny woodrat: ecology, conservation, and management of a declining species. Springer Publishing, New York, NY.

Gehrt, S. D. 2003. Raccoon (*Procyon lotor* and Allies). Pages 611-634 *in* G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America: biology, management, and conservation. Second edition. Johns Hopkins University Press, Baltimore, MD.

Gompper, M. E. and A. N. Wright. 2005. Altered prevalence of raccoon roundworm (*Baylisascaris procyonis*) owing to manipulated contact rates of hosts. Journal of Zoology, London 266:215-219.

Ing, D. M. 2008. The eastern woodrat (*Neotoma floridana*) in southern Illinois: assessment of pre-existing and reintroduced populations. M. S. Thesis, Southern Illinois University, Carbondale.

Kazacos, K. R. 2001. *Baylisascaris procyonis* and related species. Pages 301-341 *in* W. M. Samuel, M. J. Pybus, and A. A. Kocan, editors. Parasitic diseases of wild mammals. Iowa State University Press, Ames.

Kazacos, K. R., W. A. Vestre, and E. A. Kazacos. 1984. Raccoon ascarid larvae (*Baylisascaris procyonis*) as a cause of ocular larval migrans. Investigative Ophthalmology and Visual Science 25:1177-1183.

Kazacos, K. R. and W. M. Boyce. 1989. *Baylisascaris* larva migrans. Journal of the American Veterinary Medical Association 195:894-903.

LoGiudice, K. 2001. Latrine foraging strategies of two small mammals: implications for the transmission of *Baylisascaris procyonis*. American Midland Naturalist 146:369-378.

Mitchell, M. A., L. L. Hungerford, C. Nixon, T. Esker, J. Sullivan, R. Koerkenmeier, and J. P. Dubey. 1999. Serologic survey for selected infectious disease agents in raccoons from Illinois. Journal of Wildlife Diseases 35:347-355.

Monty, A-M., E. J. Heist, E. R. Wagle, R. E. Emerson, E. H. Nicholson, and G. A. Feldhamer. 2003. Genetic variation and population assessment of eastern woodrats in southern Illinois. Southeastern Naturalist 2:243-260.

Nielsen, C. K., A. Defore, and E. Bade. 2007. Survey of *Baylisascaris procyonis* and canine distemper virus in southern Illinois raccoons. Transactions of the Illinois State Academy of Science 100: 169-176.

Novosak, B. A. 2004. Reintroduction of the eastern woodrat (*Neotoma floridana*) in southern Illinois: habitat assessment and release. M. S. Thesis, Southern Illinois University, Carbondale.

Page, L. K., R. K. Swihart, and K. R. Kazacos. 1998. Raccoon latrine structure and its potential role in transmission of *Baylisascaris procyonis* to vertebrates. American Midland Naturalist 140:180-185.

Page, L. K., R. K. Swihart, and K. R. Kazacos. 1999. Implications of raccoon latrines in the epizootiology of *Baylisascaris*. Journal of Wildlife Diseases 35:474-480.

Page, L. K., S. D. Gehrt, K. K. Titcombe, and N. P. Robinson. 2005. Measuring prevalence of raccoon roundworm (*Baylisascaris procyonis*): a comparison of common techniques. Wildlife Society Bulletin 33:1406-1412.

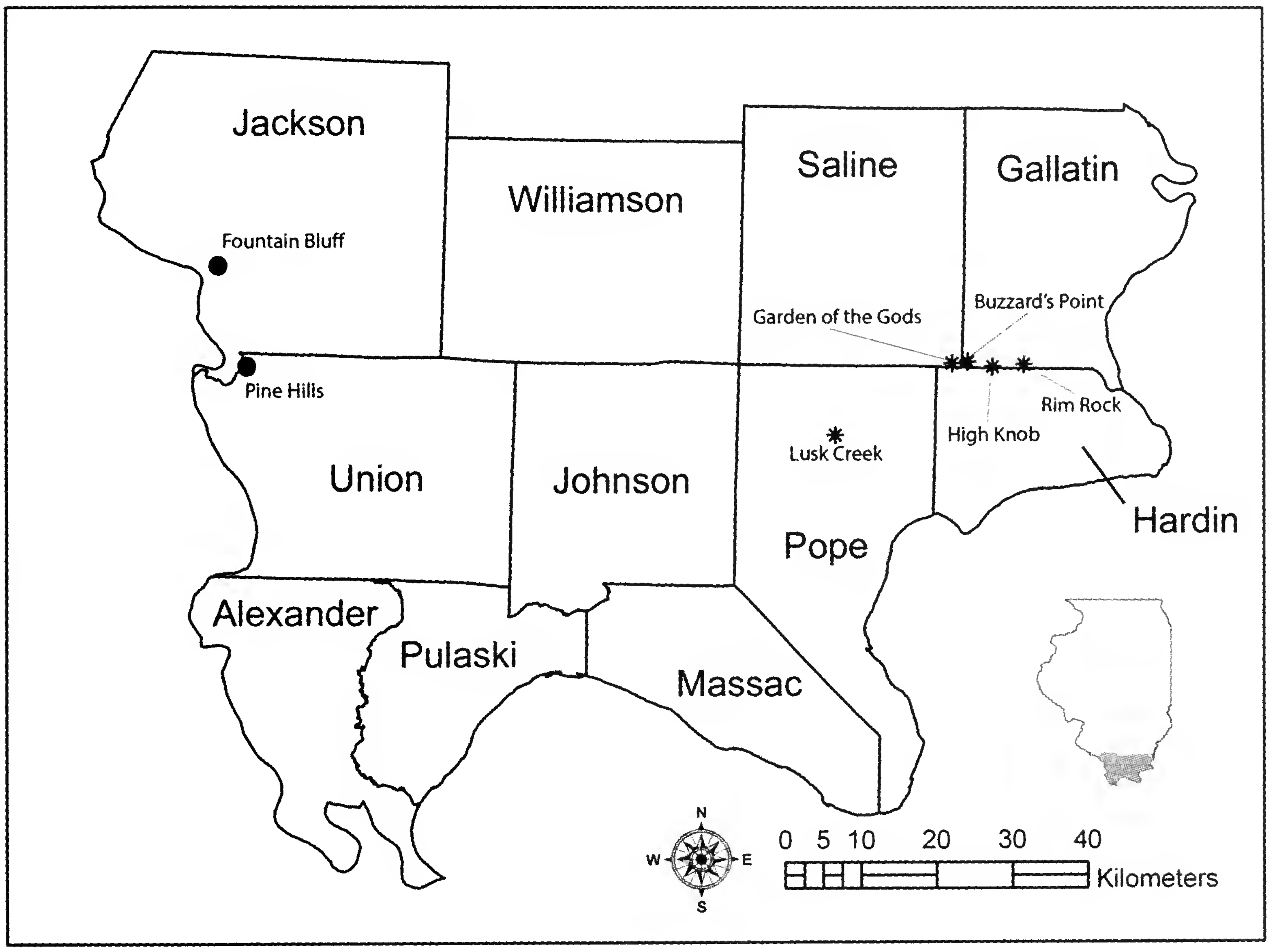
Page, L. K., C. Anchor, E. Luy, S. Kron, G. Larson, L. Madsen, K. Kellner, and T. J. Smyser. 2009a. Backyard raccoon latrines and risk for *Baylisascaris procyonis* transmission to humans. Emerging Infectious Diseases 15:1530-1531.

Page, L. K., S. D. Gehrt, A. Cascione, and K. F. Kellner. 2009b. The relationship between *Baylisascaris procyonis* prevalence and raccoon population structure. Journal of Parasitology 95:1314-1320.

Prange, S. D., S. D. Gehrt, and E. P. Wiggers. 2003. Demographic factors contributing to high raccoon densities in urban landscapes. Journal of Wildlife Management 67:324-333.

Wright, A. N. and M. E. Gompper. 2005. Altered parasite assemblages in raccoons in response to manipulated resource availability. Oecologia 144:148-156.

Figure 1. Location of eastern woodrat (*Neotoma floridana*) reintroduction sites (*) in southern Illinois. The 4 sites studied for *Bayliascaris procyonis* prevalence were Garden of the Gods, Buzzard's Point, High Knob, and Rim Rock. Woodrats were also introduced to Lusk Creek but that area was not included in this study. Previous extant woodrat populations (●) are at Pine Hills and Fountain Bluff.



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Efficacy of *Hoodia gordonii* Extract as a Weight Loss Supplement: A Comparative Study Between an Invertebrate, *Tenebrio molitor* (Coleoptera: Tenebrionidae), and a Vertebrate, *Rattus norvegicus* (Rodentia: Muridae)

[1]Justin Brohard, [1]Marianne Robertson, and [2]Anne Rammelsberg
Millikin University, [1]Department of Biology & [2]Department of Biochemistry
1184 West Main St., Decatur, IL 62522

ABSTRACT

Despite legal constraints, *Hoodia gordonii* is marketed in many over-the-counter forms claiming to promote weight loss by reducing appetite. The belief that this plant is an efficacious appetite suppressant is based in traditional knowledge from the San tribes of the Namibian desert. We tested the efficacy of one commercial form of this plant extract to reduce feeding behavior, and therefore promote weight loss, in two different organisms. We used Sprague-Dawley rats, *Rattus norvegicus*, as a human analog to predict the usefulness of this plant as a dietary supplement for humans. We also used the adult mealworm beetle, *Tenebrio molitor*, to compare an invertebrate species with the vertebrate results. *T. molitor* is a close relative of many beetle species native to the same region in which *H. gordonii* naturally grows. The control group contained organisms not exposed to *H. gordonii*. The experimental group received a body mass equivalent dosage of *H. gordonii* in solution with distilled water for a month. We monitored food consumption and body weight for one month to compare the control group results with those of the organisms that ingested *H. gordonii* solution. This study does not provide support that the commercial plant product used is efficacious as a hunger suppressing dietary supplement in either species.

INTRODUCTION

Hoodia gordonii is a desert grown plant native to South Africa and the Namib Desert that has been utilized for generations by the San people of South Africa for its supposed ability to suppress hunger. This plant has recently been exploited and processed into a highly popular diet strategy marketed and sold in a variety of forms through multiple providers (Consumer Reports on Health, 2006). In 2003, an economical and legally binding arrangement was reached between the San tribes and the Council for Scientific and Industrial Research (CSIR) of South Africa, which allows these parties to monitor and control the production and sale of any products containing *H. gordonii* in an effort to prevent over-exploitation of this resource (WHO, 2006). This treaty also gave the San people rights to a portion of the profit and royalties associated with the production and sale of *H. gordonii*-containing products (Vermeylen, 2007). With an emphasis placed on the maintenance of sufficient population levels, strict regulations have been placed on the harvesting and trade of this plant for commercial use. This limitation has often led suppliers to dilute the concentration of extract within the marketed forms (Vermaak et al., 2010).

Despite the popularity of this plant extract, little scientific data have been collected with regards to the actual effects of this plant compound. Larvae of the cabbage looper moth, *Trichoplusia ni*, have been used to determine the carry-over of larval *H. gordonii*-containing diet to the adult stage after pupation (Chow et al., 2005; Shikano and Isman 2009). Not only was this species studied to determine the effects of ingested *H. gordonii*, but also to determine the effects of topical application of the plant extract to the larval, pupal, and adult stages of *T. ni* (Akhtar et al., 2009). Ingestion of *H. gordonii* caused a significant change in oviposition site preference; topical application of *H. gordonii* had no effect on this behavior. Rader et al. (2007) determined the chemical composition of the plant extract, mainly as a method of determining the purity of marketed forms, and identified P57, an oxypregnane steroidal glycoside found in many weight loss supplements, as one of the primary components in *H. gordonii*.

To study the efficacy of *H. gordonii* on a close relative of the native South African beetle species, we chose to study the adult mealworm beetle, *Tenebrio molitor*. Mealworm beetles, family Tenebrionidae, are commonly referred to as darkling beetles. By testing the effects of *H. gordonii* extract on the feeding behavior of a close relative to the native species of tenebrionid beetle, we hope to develop results that can be compared to the species of beetle that would potentially feed on this plant in the wild (Hamilton et al., 2003). If *H. gordonii* does act as a hunger suppressant, and the tenebrionid beetles naturally exposed to this plant ingest its material, the overall food intake over the lifetime of an individual beetle may be significantly reduced. This reduction in caloric intake, and thereby reduction in energy gain, may result in decreased reproductive success of those that consume the plant. The small size of this beetle makes it an ideal organism to study because it is cost and space efficient. This efficiency allows us to increase the sample size of our test groups, allowing the study to more accurately represent the variability present in natural settings.

We also studied the efficacy of *H. gordonii* on Sprague-Dawley rats, *Rattus norvegicus*, because of the common usage of this organism as a human analog species for pre-clinical research (Boozer and Herron, 2006). It is important to test the efficacy of pharmaceutical candidates on a human analog prior to performing clinical research, and the Sprague-Dawley rat is historically a popular choice for this type of research (Fallon et al., 2008). *R. norvegicus* is a relatively accessible and affordable species making it a popular choice as a research organism. Not only does research on rats allow us to predict the efficacy of *H. gordonii* as a weight loss supplement for humans, but it also allows us to conduct a comparative study on two organisms that are not closely related. This comparative analysis should provide information about any possible differences in the efficacy of *H. gordonii* as a hunger suppressant in invertebrates versus vertebrates. Although the size of these rats limits the sample size, it also increases the accuracy of our techniques for administration of *H. gordonii* solution.

Our hypothesis, based on the popularity of commercialization and traditional use by the San tribes of South Africa, is that ingestion of *H. gordonii* will suppress the appetite of both *T. molitor* and *R. norvegicus*. This suppressing effect should be represented by a decrease in average daily food consumption over the trial period and subsequent weight loss in the organisms whose diet has been supplemented with *H. gordonii* solution at the end of the trial period.

MATERIALS AND METHODS

We conducted this research in the Leighty-Tabor Science Center at Millikin University in Decatur, Illinois. The control group received no plant extract solution and the experimental group received a body mass equivalent dosage of *H. gordonii* extract in solution with distilled water. Although the concentrations of the solution used for each trial differed based on the average starting weight of the animals, we prepared these solutions using the same *H. gordonii* extract, in capsule form, purchased from Mari-Mann Herb Farm in Decatur, Illinois.

We separated the beetles and rats into control and experimental groups based on relative body size by alternately placing the lightest individual of each species into a control group and the next lightest into an experimental group. This separation method ensured that the weight distribution between the two groups was almost equivalent, thereby eliminating body size as a variable on feeding rates. Although the measurement technique used for each organism differed because of the size of each organism used, we collected the same data for each trial. In order to test the efficacy of *H. gordonii* as an appetite suppressant, we measured the mass of food consumed. We then calculated the mean of these measurements to represent the average daily food consumption for each organism. We also recorded the body weight of each animal for the duration of each trial. At the end of the trials, we calculated the average final mass of each group of organisms and used this measurement to determine if the *H. gordonii* solution had caused a difference in weight. Since the average initial weights of each group were nearly equivalent, we were able to use only the final body mass to determine the efficacy of *H. gordonii* as a weight loss dietary supplement.

Due to the inconsistency present in the dietary supplement market, future research could address a chemical analysis of the *H. gordonii* extract used in this experiment. Since P57 has been identified as the active ingredient in *H. gordonii*, it would be useful determine the amount of compound, if any, present in the commercial extract used. High-performance thin layer chromatography (HPTLC) can be used to determine the presence of P57 (Vermaak et al., 2010). We used this method of biochemical analysis to determine that P57, the suspected active ingredient of *H. gordonii*, is present within the extract used in this research (Fig. 1). High-performance liquid chromatography (HPLC) can also be used to form a chemical fingerprint of the plant extract for comparison to the known chemical composition of pure *H. gordonii* (Avula et al., 2007). We are currently pursuing further methods of sample authentication.

T. molitor protocol

We separated 100 male and female adult *T. molitor* beetles into two groups, control (n = 50) and experimental (n = 50). We supplied control beetles with distilled water and experimental beetles with a constant dose of *H. gordonii* plant extract in solution with distilled water. We prepared water dishes by taping the bottoms of two 60.0 mm polystyrene petri dishes together to form a sealed container, which we filled with two cotton balls prior to sealing. We then drilled a hole in the side of this container just big enough for the beetles to fit their heads inside to access the water supply. These containers were constructed for the purpose of controlling levels of evaporation; preliminary test results confirmed that this container design limited evaporation to an insignificant level.

We used the average weight of an adult human, 70 kg, to determine the dosage of *H. gordonii* per body weight ratio. We used this ratio to determine the daily dosage of *H. gordonii* extract to administer to the beetles based on the average mass of the beetles. We determined the total dosage for the entire group of beetles for the length of the trials and created a 1.0 L stock solution containing 0.024 g of powdered *H. gordonii* extract. Once we created this stock, we used a glass pipette to fill each water container in the experimental group with 1.0 mL of this solution. We filled each container in the control group with 1.0 mL of distilled water. We prepared the food for the beetles by mixing equal weights of wheat flour and whole oatmeal.

We individually housed each beetle in a 475 mL plastic container with approximately 100 mL of the prepared food mixture and covered the container with a ventilated plastic lid. We maintained each beetle in the same container for the duration of the experiment to ensure that any changes in the weights of the food were attributed to the appropriate beetle. To weigh the beetles, we removed them from their housing containers, one at a time, made sure they were free of any food particles or other debris, then placed them on their dorsal side in a plastic petri dish on the electronic balance. While the water containers were removed, and the lid was off, we weighed the entire food container. Prior to the trials, we tested the excrement production rates of the beetles by housing an individual beetle in an empty container for 24 hours. We found that, due to evaporation of waste, this production rate was insignificant when compared to the mass of the container. Therefore, we were able to attribute any changes in the mass of the container to consumption by the beetle being housed in that container. We tracked the weight of each container throughout the trial and calculated any change in the mass of the container that had occurred at each measurement period. We repeated this measurement process three times a week throughout the trial period of one month, recording each of the values to the nearest 0.001 g. We dispatched all remaining beetles by feeding them to a pet bearded dragon.

R. norvegicus protocol

We separated 22 juvenile male *R. norvegicus* into two separate groups, control (n = 11) and experimental (n = 11). We gavaged the control rats three times a week with distilled water and the experimental rats three times a week with *H. gordonii* solution (see description below and in Talpur et al., 2001). We housed rats in individual plastic containers with base dimensions of 30 x 50 x 30 cm. These containers had grated stainless steel lids, equipped with food and water dispensing mechanisms, which allowed appropriate air flow and resource accessibility for the rats. We supplied the rats with *ad libitum* rat chow pellets. We labeled each housing container using a number between 1 and 22 and either an 'A' or a 'B'. The rats in the group labeled with an 'A' were in the control group and those with a 'B' were in the experimental group. In order to measure the weights of the rats throughout the trial, we placed a foam bucket onto a balance to restrain the rats, eliminating the need for anesthesia, without affecting the accuracy of the measurements. The same balance was used to measure food consumption by measuring the mass of the remaining food at each measurement period and calculating the difference from the previous measurement. We weighed rats and food three times a week and recorded data to the nearest 0.01 g. At the conclusion of the trial, we dispatched all rats using an approved method of carbon dioxide euthanasia and post-euthanasia freezing.

The gavaging process is a modification of the protocol used by Talpur et al. (2001) who tested the efficacy of a mixture of Chinese herbal supplements as a weight loss supplement. We used the average weight of an adult human, 70 kg, to determine the *H. gordonii* dosage per body weight ratio. We used this ratio to determine the proper dosage to be administered to the rats in the experimental group based on their average initial body mass. We determined that the appropriate daily dosage, compared to 400 mg in humans, should be 12 mg per day. This dosage calculation allowed us to prepare a stock *H. gordonii* solution that contained 30 mg of *H. gordonii* plant extract per 1 mL of distilled water. This mixture, administered via a 5.08 cm curved stainless feeding needle three times a week, ensured an average dosage of 12 mg of *H. gordonii* per day per rat for the one month trial period.

Statistical Analysis

We calculated the mean weights for each of the groups, control and experimental, after each day of measurement, and then compared the means between the two groups using standard deviation calculations. The standard deviation of a data set is the root mean of the variance within the data set. Standard deviation utilizes the square root of the average of the deviation of each measurement from the mean; a small standard deviation indicates a data set in which most values fall close to the mean, while a large standard deviation indicates data that are spread out across the range. The standard deviation is used to create error bars when plotting the mean values between the two groups. If the error bars for each mean overlap one another, then there is no significant difference between the control and experimental groups. Additionally, we used a two-tailed t-test to determine the probability that any overlap was caused by random chance alone. This test determines a *P*-value, which is used to determine significance of any differences that may be present within the data while assuming unequal variance between the populations. A *P*-value greater that 0.05 indicates no significant difference in the data.

RESULTS

We omitted data collected for beetles that died before the end of the trials, leaving data for a total of 7 trial sets for 73 of the initial 100 beetles, which were able to be analyzed and compared between the two groups. The number of beetles remained close between the two groups throughout the experiment, with a total of 13 control beetles and 14 experimental beetles dying. There were no deaths in either rat groups. Both groups of *T. molitor* started with a mean body mass of 0.11 g. Both groups of *R. norvegicus* started with an initial mean body mass of approximately 210 g. This similarity in initial body mass between the control and experimental groups allowed us to use the mean final mass to compare any change in body mass that might be due to ingestion of *H. gordonii* extract. There were no significant differences in either average final body mass or mean daily food consumption for either species. However, the average final body mass (Fig. 2) and average daily food consumption (Fig. 3) were slightly lower in the experimental group for the beetles. Similarly, the average final body mass (Fig. 4) and average daily food consumption (Fig. 5) were slightly lower in the experimental group for the rats.

DISCUSSION

Our hypothesis, that ingestion of *H. gordonii* plant extract would decrease food consumption resulting in a decrease in total body weight, was not supported. Although there was a trend toward decreased average daily food consumption in the experimental groups, as well as a trend toward decreased final body weight in these groups, these trends do not represent significant differences in the data. Therefore *H. gordonii* extract, when administered in a body mass equivalent dosage, does not seem to cause any significant change in feeding behavior or body weight in adult mealworm beetles or Sprague-Dawley rats.

Although *H. gordonii* extract did not produce significant differences in the feeding rates of *T. molitor* or *R. norvegicus*, it is still important to compare the results between the invertebrate and vertebrate subjects. In *T. molitor*, the difference between average daily food consumption by the experimental and control groups is larger than the difference in average final body mass of the two groups. In *R. norvegicus*, the difference between average daily food consumption by the experimental and control groups is smaller than the difference in average final body mass of the two groups. Although this variation is not represented by statistically significant differences, it does shed light on a potential difference in the type of effect, if any, which may be caused by ingestion of *H. gordonii*. This variation indicates that the invertebrate species might be exhibiting a behavioral response to the plant extract while the vertebrate species might be exhibiting a physiological response. The variation could also be attributed to differences in the metabolism of the two species. Although *H. gordonii* ingestion did not cause significant decrease in the feeding behavior of mealworm beetles, it did affect the oviposition preference of the cabbage looper moth, *Trichoplusia ni* (Chow et al., 2005; Shikano and Isman, 2009). Ingestion of *H. gordonii* by larval moths increased ingestion of *H. gordonii* in the adult moths. This indicates that *H. gordonii* is capable of producing an effect in an invertebrate species. Repeating our protocol on *T. ni* should allow us to further determine the efficacy of *H. gordonii* as an appetite suppressant in invertebrates.

We have anecdotal evidence that some of the rats, in both the control and experimental groups, exhibited behavioral changes throughout the duration of the trial. Some of the rats showed increased avoidance behavior and others showed increased aggression. Additionally, following gavaging, several rats in both groups moved quickly and randomly about the cage. Since we only have anecdotal evidence of the occurrence of these behavioral changes we cannot be sure of their cause. We can only speculate that they may be a result of associative learning.

We recognized two potential sources of error in the beetle protocol. It is possible that the large ventilation holes in the lid of the beetles' housing containers could have affected the overall mass of the containers by allowing moisture to move freely in and out of the container. Additionally, although the electronic balance used is designed for extreme precision, other individuals were also using the same balance which could have resulted in calibration errors.

Although this research does not provide information about possible long-term effects of *H. gordonii* extract, it does provide some insight to the short-term effects of this plant on *T. molitor* and *R. norvegicus*. Based on our results, members of family Tenebrionidae that are naturally exposed to *H. gordonii* are not likely to be affected by ingestion of this plant material. Additionally, based on the lack of efficacy of this plant extract as a weight loss supplement in rats, it is likely that *H. gordonii* would not have a hunger suppressing effect on humans. It would be interesting to test this plant extract on humans to determine whether the San people were accurate in their predictions, but for the adult mealworm beetle and Sprague-Dawley rats, *H. gordonii* does not cause a significant decrease in body weight or feeding behavior.

LITERATURE CITED

Akhtar, Y., I. Shikano, and M.B. Isman. 2009. Topical application of a plant extract to different life stages of *Trichoplusia ni* fails to influence feeding or oviposition behavior. Entomologia Experimentalis et Applicata. 132: 275-282.

Anonymous. 2006. Hoodia: Worth trying for weight loss? Consumer Reports on Health. February: 10.

Anonymous. 2006. Protecting traditional knowledge: the San and Hoodia. Bulletin of the World Health Organization. 84:345.

Avula, B., Y.H. Wang, R.S. Pawar, Y.J. Shukla, I.A. Khan. 2007. Chemical Fingerprinting of *Hoodia* Species and Related Genera: Chemical Analysis of Oxypregnane Glycosides Using High-Performance Liquid Chromatography with UV Detection in *Hoodia gordonii*. Journal of AOAC International. 90:1526-1532.

Boozer, C.N., and A.J. Herron. 2006. Simmondsin for weight loss in rats. International Journal of Obesity. 30:1143-1148.

Chow, J.K., Y. Akhtar, and M.B. Isman. 2005. The effects of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: *Trichoplusia ni* (Lepidoptera: Noctuidae). Chemoecology. 15:129-133.

Fallon, E., L. Zhong, J.K. Furne, M.D. Levitt. 2008. A mixture of extracts of black and green teas and mulberry leaf did not reduce weight gain in rats fed a high-fat diet. Alternative Medicine Review. 13:43-49.

Hamilton, W.J., J.R. Henschel, and M.K. Seely. 2003. Fog collection by Namib Desert beetles. South African Journal of Science. 99:181.

Rader, J.I., P. Delmonte, and M.W. Trucksess. 2007. Recent studies on selected botanical dietary supplement ingredients. Analytical and Bioanalytical Chemistry. 389:27-35.

Shikano, I., and M.B. Isman. 2009. A sensitive period for larval gustatory learning influences subsequent oviposition choice by the cabbage looper moth. Animal Behaviour. 77:247-251.

Talpur, N.A., B.W. Echard, V. Manohar, H.G. Preuss. 2001. Influence of a combination of herbs on appetite suppression and weight loss in rats. Diabetes, Obesity, and Metabolism. 3:181-185.

Vermaak, I., J.H. Hamman, and A.M. Viljoen. 2010. High performance thin layer chromatography as a method to authenticate *Hoodia gordonii* raw material and products. South African Journal of Botany. 76:119-124

Vermeylen, S. 2007. Contextualizing 'fair' and 'equitable': The San's reflections on the *Hoodia* benefit-sharing agreement. Local Environment. 12:423-436.

Figure 1. Comparison of HPLC profiles of P57 standard solution (A), *H. gordonii* extracted with acetonitrile (b), and *H. gordonii* extracted with acetonitrile and water (50:50), at a detection wavelength of 220 nm.

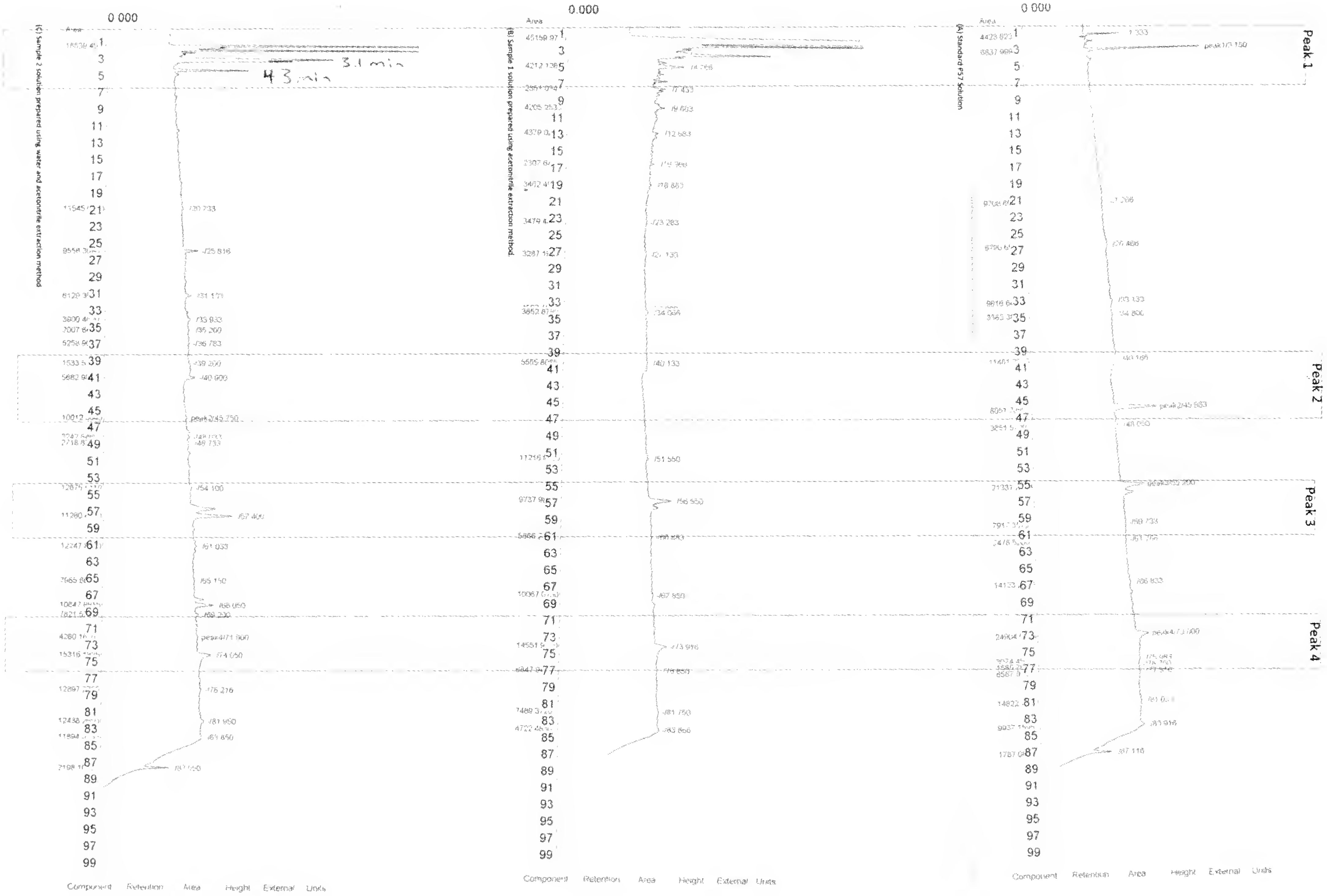


Figure 2. Comparison of average final body mass (± SD) of adult *Tenebrio molitor* beetles in the control group receiving distilled water versus an experimental group receiving 0.024g of *Hoodia gordonii*/L of distilled water (*P* = 0.141).

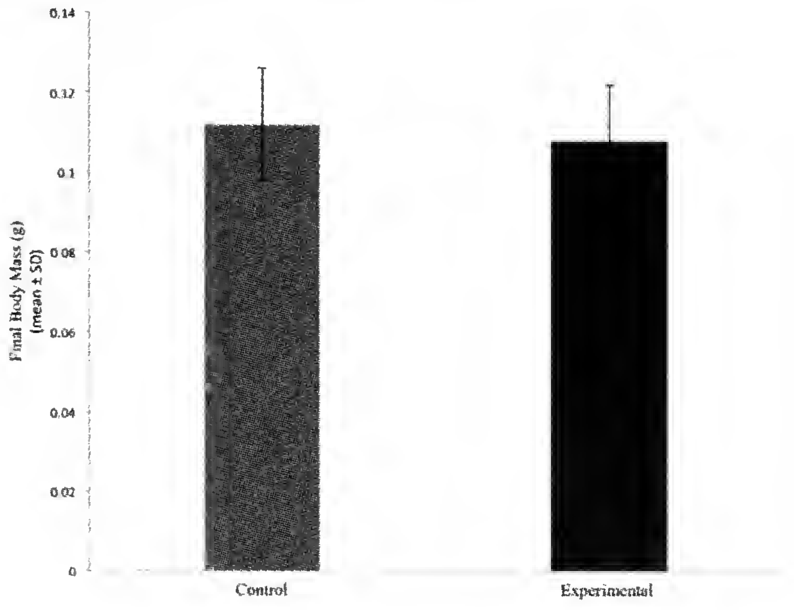


Figure 3. Comparison of mean daily food consumption by control adult *Tenebrio molitor* receiving distilled water versus an experimental group receiving 0.024g of *Hoodia gordonii*/L of distilled water (*P* = 0.405).

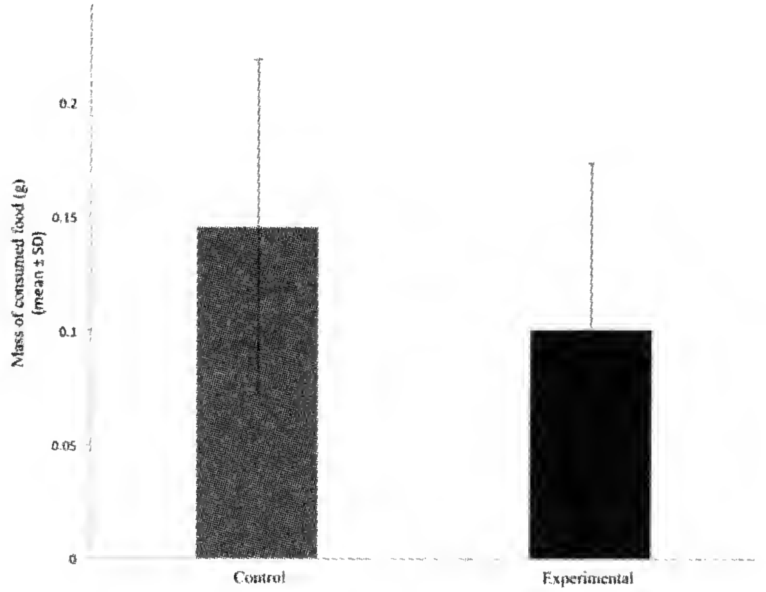


Figure 4. Comparison of average final body mass (± SD) of control *Rattus norvegicus* gavaged with distilled water versus an experimental group gavaged with 30mg of *Hoodia gordonii*/mL of distilled water (*P* = 0.198).

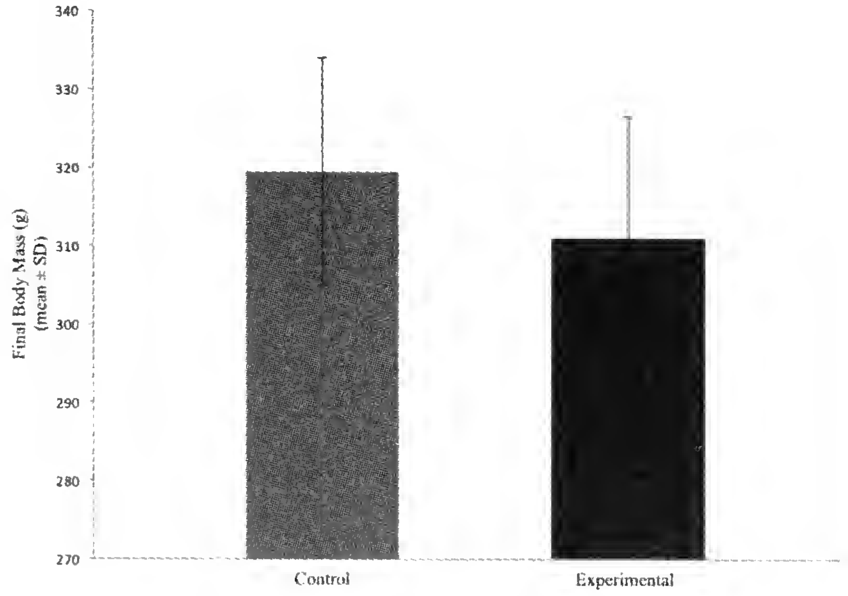
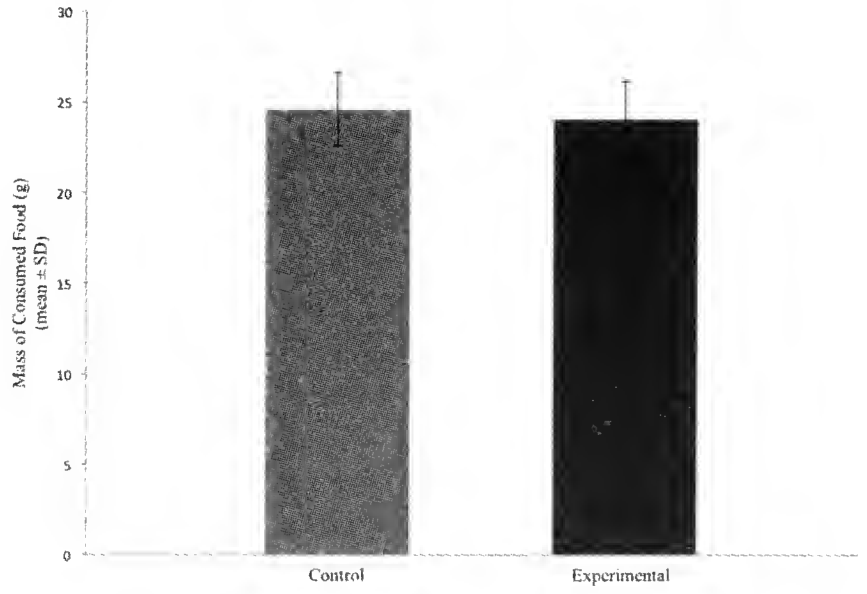


Figure 5. Comparison of average final body mass (± SD) of mean daily food consumption by control *Rattus norvegicus* gavaged with distilled water versus an experimental group gavaged with 30mg of *Hoodia gordonii*/mL of distilled water (*P* = 0.364).



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Mass Death of Wintering American Robins (*Turdus migratorius*) in Decatur, Illinois

Max S. Huschen and David Joseph Horn
Department of Biology, Millikin University
1184 West Main Street, Decatur, Illinois 62522

ABSTRACT

A flock of American Robins (*Turdus migratorius*) congregated around a fruiting tree and nearby building on the campus of Millikin University in Decatur, Illinois in early 2011. Thirty-four robins were found dead around campus buildings during 8-17 February, after a blizzard passed through central Illinois. We investigated potential causes for the high number of deaths including examinations of gizzard contents and feces for both food eaten and internal parasites, and observed robin behavior. The robins apparently congregated around one of the few food resources available in the area during this blizzard. They were subject to starvation intensified by poor nutritional quality, parasitic infection, and aggressive defense by conspecifics, and were more susceptible to other forms of mortality including bird-window collisions.

INTRODUCTION

Mass deaths of birds are not uncommon, but largely go unnoticed. Mass deaths can be regional or local in scope and are a result of both human and natural causes including collisions with human structures (Klem 1990), poisoning (Dolbeer et al. 1995), and disease (LaDeau et al. 2007). Poor weather is also a factor causing mass deaths (Jehl 1998). For example, while rare, starvation deaths during the winter can occur during extreme winter storms (Bednekoff and Houston 1994). We investigated the mass death of American Robins (*Turdus migratorius*) that had congregated around a single fruiting crabapple tree (*Malus* spp.) at a building on the Millikin University campus in Decatur, Illinois. We hypothesized that starvation was the primary cause of robin mortality, and secondary factors including nutritional quality, parasitism, and aggressive defense by conspecifics intensified starvation.

METHODS

We observed American Robins at Millikin University in Decatur, Illinois starting on 8 February 2011 when a blizzard occurred in central Illinois with temperatures reaching a low of -12°C and snow accumulation of 30.5 cm. We began receiving notifications of dead robins along the south side of Staley Library, as well as surrounding buildings, immediately preceding the blizzard and the days after. We collected dead robins and observed the flock of several dozen robins that continued to congregate around the crabapple tree next to the library until the snow receded on 17 February.

Dead robins were weighed, gizzard and intestine dissected, and feces analyzed. Identification of internal parasites was performed following McQuistion and Wilson (1989). The nutritional content of the crabapples was analyzed by Eurofins Nutrition Analysis Center (Des Moines, IA, USA; % carbohydrates determined via CFR 21-calculation, % lipids calculated via acid hydrolysis – AOAC 954.02, % protein calculated via combustion – AOAC 990.03, and calories determined via CFR Atwater calculation). Ten-min observations were made of the behavior of the robins twice each day. Feeding behavior, resting behavior, and social interactions including aggressive defense of the food resource among robins were noted. We defined any instance where one robin would chase another robin from the food resource as aggressive defense (Pietz and Pietz 1987).

RESULTS

Thirty-four dead robins were found over the 10-day period. Twenty-six robins were weighed, gizzard and intestine contents analyzed, and feces examined for internal parasites. The other eight robins had been scavenged or decomposed prior to collection. The mean weight of 26 robins was 50 g (range from 37 to 67 g). Eighteen of 26 robins (69%) had empty gizzards. Four of eight robins with full gizzards had whole undigested crabapples within the gizzard. The other four robins had unidentifiable food in the gizzard. The crabapples contained 30.9% carbohydrates, 1.2% lipids, 2.5% protein, and an energy content of 1.45 kcal/g. Thirteen of 26 robins (50%) had internal parasites. Six robins had parasites of the phylum Acanthocephala. We found parasites of the genus *Capillaria* in five robins and in one robin we found *Hymenolepis* (spp.). Three of 26 robins were in a position directly underneath windows where window collisions have been observed in the past (DJH, unpubl. data). We noted at least three instances of aggressive defense of the food resource during our behavioral observations.

DISCUSSION

On average, American Robins weighed 77 g throughout the year in Pennsylvania and 86 and 84 g for males and females, respectively, during winter in Ithaca, New York (Sallabanks and James 1999). The weight of every dead robin we found was at least 10% under the average weight of 77 g recorded for robins in Pennsylvania. Many species, particularly smaller birds, maintain fat reserves below their physiological capacity in winter and have only enough reserves to survive the coldest night at a certain location at a certain time (Lima 1986, Krams et al. 2010). Thus, American Robins at our location may not have had the fat reserves necessary to survive multiple days in below-zero temperatures with limited access to food. This may be a result of a trade-off between the benefits of fat reserves to prevent starvation and the risk of predation (Lima 1986). Birds with higher fat reserves may be more likely to survive cold winter nights, but they are also more vulnerable to predation due to increased body weight and decreased take-off speed (Lima 1986). We conclude the mass kill was primarily a result of starvation. Other factors possibly contributed including the nutritional quality of the food source, parasitism, aggressive defense, and collisions with windows.

Nutritional Quality

The robins' main food source was apparently limited to crabapples because of the blizzard, and particularly the ice and snow on the ground. These fruits may not have met their nutritional requirements. Studies have found robins will consume more fruits with higher lipid concentrations during the autumn (Lepczyk et al. 2000). Lipid-rich fruits may be important for fattening, as thrushes achieve the highest energy assimilation rates when choosing fruits high in lipid content (Witmer and Van Soest 1998, Lepczyk et al. 2000). At a time when energetic demands were high in the robins we studied, the amount of lipid-rich fruits in their diet and presumably energy assimilation rates was low.

A 55-g robin requires 30.7 kcal/day (Hazelton et al. 1984). Thus, a similar-sized robin in our study would have needed to eat 21 g, or 74 crabapples, per day to meet nutritional requirements. Crabapple seeds also contain cyanide (Seigler 1976), and this poison can cause death when consumed in large amounts by Cedar Waxwings (*Bombycilla cedrorum*, Woldemeskel and Styer 2010). We found evidence in the gizzards of partially digested crabapple seeds and it is possible these robins may have been exposed to cyanide.

Parasitism

American Robins are known to be infected by a large number of parasites including coccidia, trematodes, nematodes, cestodes, and acanthocephalans (Welte and Kirkpatrick 1986). Collectively, over 40 helminth parasites have been identified in robins (Cooper and Crites 1976). In our study, parasites were found in 50% of the dead robins, and three different types of parasites were discovered. Robins with parasitic infections may have reduced metabolism, inactivity, and weight loss at the time where increased metabolism and activity were most needed. Acanthocephalans are known to reduce metabolic rate in European Starlings (*Sturnus vulgaris*, Atkinson et al. 2008). *Capillaria* spp. are digestive tract parasites that have been found to cause inactivity, anorexia, weight loss, vomiting, and death in guinea fowl (De Rosa and Shivaprasad 1997). Rates of parasitic infection in other studies were similar to ours and ranged from 33-77% (Welte and Kirkpatrick 1986, McQuistion and Holmes 1988).

Aggressive Defense

We observed multiple instances of defense of the food source. Typically, one robin would chase other robins to gain access to fruits on the ground. In robins, defense of fruits is rare except when temperatures are extremely low in which case it will last for a few days (Sallabanks 1993). Robins with winter territories have feeding bouts that are five times longer, ingest twice as much food per bout, but forage half as fast as robins that intrude onto a territory (Sallabanks 1993). Given the large food requirements and defense displayed, some robins may not have had access to the food source and subsequently did not meet their nutritional requirements.

Window Collisions

The number of bird-window collisions is approximately 7-8 birds per building per year on the Millikin campus, and the library is one of the buildings with the most collisions (DJH, unpubl. data). The proximity of the crabapple tree to the building may have influenced the number of window collisions. Birds can become intoxicated from eating large amounts of overripe fruit (Woldemeskel and Styer 2010), which may in turn make them more vulnerable to collisions.

There were several factors that we were unable to control in this study. Given the cold weather, we were not able to capture living birds in the area and weigh them to compare the weights of living birds with the dead ones we found. For the same reason, we could not compare parasitism rates between surviving birds with those that died. Finally, future studies should compare the number of birds that died to the number of living birds in the area, and perform additional behavioral observations.

Singularly, factors such as starvation, low nutritional quality, cyanide poisoning, parasitism, resource defense, and window collisions can result in bird death. Collectively, we suggest that these factors, and particularly starvation, resulted in a kill of 34 American Robins under harsh and stressful weather conditions at Millikin University in February 2011. While the death of 34 robins is small relative to the overall population of robins in central Illinois, this study can provide insight into the causes of future localized mass deaths.

ACKNOWLEDGMENTS

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LITERATURE CITED

Atkinson, C. T., N. J. Thomas, and D. B. Hunter. 2008. Parasitic diseases of wild birds. Wiley-Blackwell, Ames, Iowa, USA.

Bednekoff, P. A. and A. I. Houston. 1994. Optimizing fat reserves over the entire winter: a dynamic model. Oikos 71:408-415.

Cooper, C. L. and J. L. Crites. 1976. A check list of the helminth parasites of the robin, *Turdus migratorius* Ridgway. American Midland Naturalist 95:194-198.

De Rosa, M. and H. L. Shivaprasad. 1997. Capillariasis in Vulture Guinea Fowl. American Association of Avian Pathologists 43:131-135.

Dolbeer, R. A., D. F. Mott, and J. L. Belant. 1995. Blackbirds and starlings killed at winter roosts from PA-14 applications, 1974-1992: implications for regional population management. Proceedings of the Eastern Wildlife Damage Management Conference 7:77-86.

Hazelton, P. K., R. J. Robel, and A. J. Dayton. 1984. Preferences and influence of paired food items on energy intake of American Robins and Gray Catbirds. Journal of Wildlife Management 48:198-202.

Jehl, J. 1998. Conspecific collisions can precipitate mortality in migrating Eared Grebes. Wilson Bulletin 110:409-411.

Klem Jr., D. 1990. Collisions between birds and windows: mortality and prevention. Journal of Field Ornithology 61:120-128.

Krams, I., D. Cirule, V. Suraka, T. Krama, M. J. Rantala, and G. Ramey. 2010. Fattening strategies of wintering Great Tits support the optimal body mass hypothesis under conditions of extremely low ambient temperature. Functional Ecology 24:172-177.

LaDeau, S. L., A. M. Kilpatrick, and P. P. Marra. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 447:710-714.

Lepczyk, C. L., K. G. Murray, K. Winnet-Murray, P. Bartell, E. Geyer, and T. Work. 2000. Seasonal fruit preferences for lipids and sugars by American Robins. Auk 117:709-717.

Lima, S. L. 1986. Predation risk and unpredictable feeding conditions: determinants of body mass in birds. Ecology 67:377-385.

McQuistion, T. E. and B. B. Holmes. 1988. *Isospora robini* sp. n., a new coccidian parasite (Apicomplexa: Eimeriidae) from the American Robin (*Turdus migratorius*). Proceedings of the Helminthological Society of Washington 55:324-325.

McQuistion, T. E. and M. Wilson. 1989. *Isospora geospizae*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the Small Ground Finch (*Geospiza fuliginosa*) and the Medium Ground Finch (*Geospiza fortis*) from the Galapagos Islands. Systematic Parasitology 14:141-144.

Pietz, M. A. J. and P. J. Pietz. 1987. American Robin defends fruit resource against Cedar Waxwings. Journal of Field Ornithology 58:442-444.

Sallabanks, R. 1993. Fruit defenders vs. fruit thieves: winter foraging behavior in American Robins. Journal of Field Ornithology 64:42-48.

Sallabanks, R. and F. C. James. 1999. American Robin (*Turdus migratorius*). The Birds of North America. Number 462.

Seigler, D. S. 1976. Plants of the northeastern United States that produce cyanogenic compounds. Economic Botany 30:395-407.

Welte, S. C. and C. E. Kirkpatrick. 1986. Syngamiasis in juvenile American Robins (*Turdus migratorius*), with a note on the prevalence of other fecal parasites. Avian Diseases 30:736-739.

Witmer, M. C. and P. J. Van Soest. 1998. Contrasting digestive strategies of fruit eating birds. Functional Ecology 12:728-741.

Woldemeskel, M. and E. L. Styer. 2010. Feeding behavior-related toxicity due to *Nandina domestica* in Cedar Waxwings *(Bombycilla cedrorum)*. Veterinary Medicine International 2010:2042-2048.

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Conservation Review of the Longnose Dace *Rhinichthys cataractae* (Valenciennes) in Northwestern Illinois

Jeremy S. Tiemann[1*], Christopher A. Taylor[1], and Jason Knouft[2]
[1]Illinois Natural History Survey, Prairie Research Institute, University of Illinois
1816 South Oak Street, Champaign, IL 61820
[2]St. Louis University, Department of Biology
3507 Laclede Avenue, St. Louis, MO 63103
[*]Correspondence: jtiemann@illinois.edu

ABSTRACT

The longnose dace *Rhinichthys cataractae* (Valenciennes) is a small, elongated, slightly dorsoventrally compressed minnow that possesses the widest distribution of any North American cyprinid. In Illinois, it is considered rare and currently is known from streams in the Wisconsin Driftless Area and the shoreline of Lake Michigan and some of its tributaries. We examined the distribution, population status, and habitat requirements of *R. cataractae* in the Wisconsin Driftless Area in Illinois. The dace was collected at 12 of 33 sites sampled, and catch-per-unit-effort (number of individuals per hour collecting) varied from 0.8-52 per site. It was found most often in gravel/cobble riffles in small- to medium-sized streams. Although its range is limited in Illinois, the dace is locally abundant in several basins and we feel it does not warrant listing at this time.

Key Words:
Longnose dace, *Rhinichthys cataractae*, Wisconsin Driftless area, endangered species

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INTRODUCTION

The longnose dace *Rhinichthys cataractae* (Valenciennes) is a slightly dorsoventrally compressed minnow (Cyprinidae) with a long fleshy snout. The species can reach 15 cm in length (Smith, 1979; Becker, 1983) and possesses the widest distribution of any North American minnow, generally occurring in areas above 40°N (Page and Burr, 1991). *Rhinichthys cataractae* is distributed from north of the Arctic Circle south to the Appalachian Mountains and west to the Rocky Mountains. Individuals occupy gravel/cobble riffles in small- to medium-sized cool-water streams and in wave swept shallows of the Great Lakes. In Illinois, the species is considered rare (Smith, 1979) and has been found only in a few of the streams of the Wisconsin Driftless Area (e.g., Menominee, Little Menominee, Sinsinawa, and Plum river basins) in Jo Daviess and Carroll counties, along the shores of Lake Michigan and in a few streams that feed into the Lake in Cook and Lake counties. Records also exist from Union County but the dace is considered extirpated from southern Illinois (Smith, 1979).

While *R. cataractae* has been a known component of the Illinois fish fauna since 1884 (Forbes, 1884), little is known about its range and habitat preferences in the Wisconsin Driftless Area of the state. The species was reported to be "very rare" in inland waters by both Forbes and Richardson (1920) and Smith (1979). To address these uncertainties, we conducted the first targeted status survey of *R. cataractae* in northwestern Illinois. The objectives of our study were to 1) gather distribution and abundance data for the Illinois Endangered Species Protection Board to assist in listing decisions under the Illinois Endangered Species Act; 2) provide valuable natural history data (e.g., habitat requirements) on one of Illinois' rarest fishes; and 3) use geographic information systems to determine if geologic, hydrologic, or landscape variables can predict the distribution of *R. cataractae*. Such data can assist natural resource agencies in determining geographic regions that may hold the highest potential for long-term protection of the longnose dace.

METHODOLOGY

Fish sampling – Thirty-three sites were sampled in streams within the Wisconsin Driftless Area (Table 1; Fig. 1) from 18 June 2008 – 18 June 2009. Sites were selected based on either historical records for *R. cataractae* or habitat characteristics (e.g., gravel/cobble riffles in small- to medium-sized cool-water streams) that looked suitable for the dace. Historical records were defined as collection locations from unpublished literature (e.g., internal reports from Illinois Department of Natural Resources, Western Illinois University, or U.S. Fish and Wildlife Service) or locations for which vouchered specimens exist at a museum. Of the 12 fish collections contacted, only three (Field Museum of Natural History [FMNH], Chicago; Illinois Natural History Survey [INHS] Fish Collection, Champaign; Milwaukee Public Museum [MPM], Milwaukee) had records from our study area. At most sites, fishes were collected for 45 minutes using a barge electro-shocker set at 200 volts; however, a few sites were too small to accommodate the barge and therefore were sampled using a 3.05 m minnow seine for 45 minutes. Fishes were identified, counted, and released upon completion of sampling at a site, and at least one dace was vouchered from each site and deposited in the INHS Fish Collection. Four historical sites (Table 2) were not visited because access could not be gained or *R. cataractae* had been collected at the site within the past five years.

Geographic Information Systems – The potential distribution of *R. cataractae* was predicted using georeferenced locality data, GIS environmental layers, and the Maxent species distribution algorithm (Phillips et al., 2006). Maxent is a general-purpose machine learning approach to modeling of species distributions using presence-only data (Phillips et al., 2006). Maxent predicts potential distribution of a species by estimating probability distribution of maximum entropy across a specified region, subject to a set of constraints that represent incomplete information about the target distribution (Phillips et al., 2006). Locality data across the range of *R. cataractae* in Illinois was integrated with landcover and physical GIS data to predict potential areas of occurrence in the state. GIS data included measures of elevation, slope, flow accumulation, drift thickness, and landcover characteristics (e.g., riparian forest density). The predicted distribution of *R. cataractae* was then compared to sites not containing the species to further explore the importance of abiotic and biotic factors regulating the distribution and presence of *R. cataractae*.

RESULTS

Distribution and population status – *Rhinichthys cataractae* was collected at 12 of the 33 sites sampled, and catch-per-unit-effort (standardized by number of individuals collected per hour of collecting effort) varied from 0.8-52 per site (Table 1). The species was collected throughout the Menominee, Little Menominee, Sinsinawa, and Plum river basins and in two sites in the Galena River basin (Fig. 1). The species was most often collected in areas with swift flows over gravel/cobble riffles with depths ranging from 0.1-0.5 m. Even though there are unconfirmed records available (e.g., internal reports from Illinois Department of Natural Resources, Western Illinois University, or U.S. Fish and Wildlife Service), we failed to collect *R. cataractae* in the Apple or Rock river basins and voucher specimens do not exist for this species in these basins. We believe the dace does not occur in the Apple or Rock river basins and all records have been based on misidentified blacknose dace *Rhinichthys atratulus*. However, we cannot rule out that *R. cataractae* might have possibly become extirpated in these basins.

Geographic Information Systems – Fish localities were evenly divided for development and testing during Maxent species distribution model generation. The Maxent model produced a significant prediction of the distribution of *R. cataractae* across the study area ($AUC = 0.742$, $P = 0.041$). Landcover classification (66.3%) and flow accumulation (33.6%) contributed 99.9% of the explanatory power of the model prediction, suggesting these two variables are of primary importance in predicting presence of the species. Finally, the distribution model generated for *R. cataractae* was tested using sites where no dace were collected. In this case, the Maxent model was not able to predict areas without *R. cataractae* ($AUC = 0.635$, $P = 0.092$). This result suggests sites where the fish was absent in our collections do not contain suitable habitat for *R. cataractae* based on the GIS data sets used to generate the models.

DISCUSSION

Rhinichthys cataractae appears stable in Illinois. Our field survey found the species at a total of 12 sites (Table 1). Of those sites, four were ones at which the species had been previously collected and eight were new. Of the eight new sites, two were in the Galena River drainage. These two records represent the first vouchered records for the species in this drainage in Illinois. While population sizes in the Galena River drainage sites were small (<1.5 individuals per hour), habitat at those sites was identical to that found at other locations with larger populations. Further work will be needed in the Galena River drainage to determine if significant population changes are occurring.

Habitat at sites containing *R. cataractae* was relatively uniform. The species was always found in areas with moderate to strong flow and substrates of clean, cobble sized (0.1-0.3 m diameter) rocks. There was variation in stream width (8-20 m) and water depth (0.1-0.5 m) among sites.

The results of the Maxent species distribution models suggest presence of *R. cataractae* can be predicted by landscape-level variables, particularly flow accumulation (essentially a measure of how far downstream the species occurs) and riparian landcover type. The dace primarily was found at sites with upland forest (41.7% of sites) and rural grassland

(25.0% of sites) riparian landcover classifications and in more upstream locations within drainage basins. These habitat characteristics are consistent with those reported for the species from across its range (Smith, 1979; Becker, 1983; Aadland, 1993; Goldstein, 2009).

With the exception of the two Galena River drainage populations discussed above and one Carroll Creek site located in Mt. Carroll, longnose dace were relatively common in suitable habitat at most sampling sites (Table 1). Number of individuals collected per hour of sampling effort ranged from 0.8-52.0. During our survey, we not only documented the species in a new basin, but also showed the dace continues to occur at multiple sites in the Menominee, Little Menominee, Sinsinawa, and Plum river drainages. Also, *R. cataractae* occurs at several sites in the Wisconsin side of these basins (data from MPM). When combining these factors, we feel the longnose dace does not warrant listing at the state level as threatened or endangered at this time. We did not assess the status of Lake Michigan populations in Illinois. However, the fish has been recently (post-1980) collected throughout the area including the Lake and some of its tributaries in Cook and Lake counties, Illinois, Lake County, Indiana, and Kenosha County, Wisconsin (Retzer and Batten, 2005; data from FMNH, INHS, and MPM).

The longnose dace is considered a cool-water, sensitive species (Lyons et al., 2010). The fish's preference for clean cobble substrates in northwestern Illinois streams highlights one potential threat for the species. Lyons et al. (2010) predicted climate warming could affect the distribution of *R. cataractae*. Also, non-point source pollution by siltation can overlay cobble and prevent fish from taking shelter and/or feeding in interstitial spaces. While agricultural and livestock activities have been prevalent in northwestern Illinois for 100-150 years and some degradation of streams in that region has occurred, continued efforts must be made to limit suspended solid input into streams to protect populations of longnose dace and other aquatic taxa.

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LITERATURE CITED

Aadland, L.P. 1993. Stream habitat types: their fish assemblages and relationship to flow. North American Journal of Fisheries Management 13:790-806.

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison. 1052 p.

Forbes, S. A. 1884. A catalogue of the native fishes of Illinois. Report of the Illinois State Fish Commissioner for 1884:60-89.

Forbes, S. A. and R. E. Richardson. 1920. The fishes of Illinois. 2nd Edition. Illinois Natural History Survey. 357 p.

Goldstein, R.M. 2009. Interpreting stream physical characteristics Index of Biotic Integrity classifications: general similarities and specific differences. North American Journal of Fisheries Management 29:151-162.

Lyons, J., J.S. Stewart, and M. Mitro. 2010. Predicted effects of climate warming on the distribution of 50 stream fishes in Wisconsin, U.S.A., Journal of Fish Biology 77: 1867-1898.

Phillips, S.J., R.P. Anderson, and R.E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231-259.

Retzer, M.E. and B. Batten. 2005. Fishes of the Chicago region: A review of the Dennison and Illinois Natural History Survey Collections. Transactions of the Illinois State Academy of Science 98:63-73.

Smith, P.W. 1979. The Fishes of Illinois. University of Illinois Press, Urbana. 314 p.

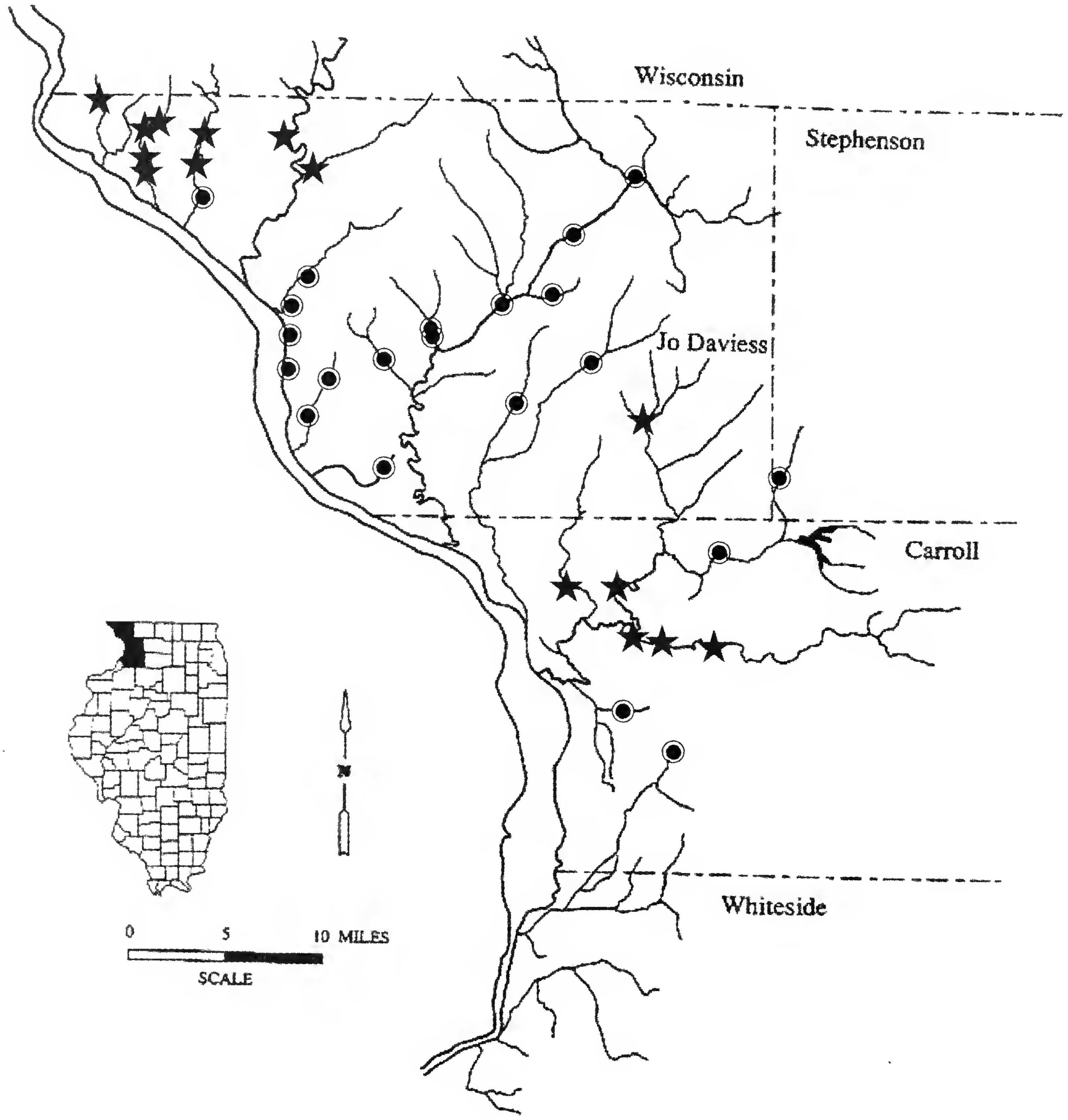
Table 1. Sites sampled during the 2008-2009 *Rhinichthys* survey. Dace column indicates which *Rhinichthys* species were present at a given location and catch-per-unit-effort is standardized by number of *R. cataractae* per hour collecting. Asterisk (*) indicates that two individuals were vouchered but CPUE was not determined.

Stream	State: County	Common location	Lat/Long	Dace	CPUE
Menominee River	IL: Jo Daviess	3.7 mi E of Dubuque, IA	N42.50456, W90.59150	R. cataractae	4.0
Little Menominee River	IL: Jo Daviess	6.8 mi E of Dubuque, IA	N42.48716, W90.53232	*R. cataractae*	8.0
Little Menominee River	IL: Jo Daviess	6.8 mi WNW of Galena, IL	N42.46167, W90.54689	*R. cataractae*	14.7
Sinsinawa River	IL: Jo Daviess	5.2 mi NNW of Galena, IL	N42.47952, W90.48580	*R. cataractae*	*
Sinsinawa River	IL: Jo Daviess	4.3 mi NW of Galena, IL	N42.45670, W90.49397	*R. cataractae*	4.0
Sinsinawa River	IL: Jo Daviess	3.2 mi WNW of Galena, IL	N42.43277, W90.48820		
Galena River	IL: Jo Daviess	4.3 mi NNE of Galena, IL	N42.47666, W90.40684	*R. cataractae*	0.8
East Fork Galena River	IL: Jo Daviess	3.6 mi NE of Galena, IL	N42.45323, W90.37774	*R. cataractae*	1.3
Smallpox Creek	IL: Jo Daviess	3.8 mi SE of Galena, IL	N42.37408, W90.38259	*R. atratulus*	
Trib Smallpox Creek	IL: Jo Daviess	4.7 mi SSE of Galena, IL	N42.35261, W90.39854		
Trib Mississippi River	IL: Jo Daviess	7.7 mi S of Galena, IL	N42.30674, W90.40259		
Trib Mississippi River	IL: Jo Daviess	6.1 mi SSE of Galena, IL	N42.33134, W90.39968		
Beaty Hollow Creek	IL: Jo Daviess	5.1 mi NW of Hanover, IL	N42.29883, W90.36081	*R. atratulus*	
Beaty Hollow Creek	IL: Jo Daviess	5.4 mi WNW of Hanover, IL	N42.27174, W90.38270	*R. atratulus*	
Trib Mississippi River	IL: Jo Daviess	2.1 mi SW of Hanover, IL	N42.23384, W90.30610		
Apple River	IL: Jo Daviess	7.3 mi NNW of Stockton, IL	N42.44879, W90.05540		
Apple River	IL: Jo Daviess	8.1 mi NE of Elizabeth, IL	N42.40484, W90.11684		
Apple River	IL: Jo Daviess	3.0 mi NNE of Elizabeth, IL	N42.35366, W90.18855		
Irish Hollow Creek	IL: Jo Daviess	4.2 mi NNW of Hanover, IL	N42.31338, W90.30627	*R. atratulus*	
Welsh Hallow	IL: Jo Daviess	4.9 mi NE of Elizabeth, IL	N42.36036, W90.14499	*R. atratulus*	
Furnace Creek	IL: Jo Daviess	2.3 mi NW of Elizabeth, IL	N42.33646, W90.25866	*R. atratulus*	
Furnace Creek	IL: Jo Daviess	2.0 mi WNW of Elizabeth, IL	N42.32993, W90.25776		
Rush Creek	IL: Jo Daviess	6.3 mi SE of Elizabeth, IL	N42.31229, W90.09727		
Little Rush Creek	IL: Jo Daviess	3.5 mi SE of Elizabeth, IL	N42.28083, W90.17325		
Plum River	IL: Jo Daviess	7.0 mi SSW of Stockton, IL	N42.25284, W90.03830	*R. cataractae*	6.6
Plum River	IL: Carroll	5.3 mi N of Mount Carroll, IL	N42.17094, W89.97069		
Crane Grove Creek	IL: Stephenson	3.75 mi SSE Freeport	N42.23691, W89.60449	*R. atratulus*	
Carroll Creek	IL: Carroll	1.6 mi E of Mount Carroll, IL	N42.09490, W89.94827	*R. atratulus R. cataractae*	40.0
Carroll Creek	IL: Carroll	Mt. Carroll, IL city park	N42.10077, W89.97618	*R. cataractae*	2.0
Carroll Creek	IL: Carroll	4.1 mi WNW Mount Carroll, IL	N42.10870, W90.05673	*R. cataractae*	52.0
Camp Creek	IL: Carroll	3.9 mi NNE of Savanna, IL	N42.14525, W90.12274	*R. cataractae*	20.3
Deer Creek	IL: Carroll	5.3 mi WSW of Mount Carroll, IL	N42.05363, W90.06570	*R. atratulus*	
Johnson Creek	IL: Carroll	5.2 mi SSW of Mount Carroll, IL	N42.02494, W90.01644	*R. atratulus*	

Table 2. Historical sites where *Rhinichthyes cataractae* has been collected but were not sampled because sites were either inaccessible (I) or a recent (R) record exists for the fish since 2005. Specimens housed at the Illinois Natural History Survey Fish Collection, Champaign.

Stream	State: County	Common location	Lat/Long	Last observed	Reason
Little Menominee River	IL: Jo Daviess	6.1 mi E of Dubuque, IA	N42.48306, W90.54625	22 June 2005	R
Little Menominee River	IL: Jo Daviess	6.3 mi WNW of Galena, IL	N42.45096, W90.54439	28 April 1992	I
Plum River	IL: Carroll	6.0 mi NW of Mount Carroll, IL	N42.14581, W90.07369	17 July 1963	I
Carroll Creek	IL: Carroll	2.7 mi WNW of Mount Carroll, IL	N42.10474, W90.02817	23 June 1995	I

Figure 1. Map of the study area. Stars indicate sites where *R. cataractae* has been vouchered (data taken from the Illinois Natural History Survey Fish Collection, Champaign) and circles designate those sites where we failed to collect the dace during our survey.



BOOK REVIEW 2012 – #1

Colorado's Spanish Peaks Region: An Exploration Guide to History, Natural History, Trails and Drives by Dr. Richard Keating. 350 pp. The Missouri Botanical Garden Press, St. Louis, MO. USD $24.99 ISBN 9781930723856.

If a person were to require one book about this area of southeastern Colorado as an introduction to its physical and historic marvels, that book should be the newly-released Colorado's Spanish Peaks Region: An Exploration Guide to History, Natural History, Trails, and Drives by Richard Keating.

Perhaps it is our "orphan" status that keeps this area from so-called complete guides to geology, trails, history, etc., of Colorado, even those claiming to cover the Front Range or the Sangre de Cristos. If covered at all, Huerfano County usually merits a paragraph or two, possibly a page at most, and it is not always accurate information. In one fairly recent publication, for instance, the author could not tell the Devil's Stairsteps from Profile Rock, with a picture of one and the name of the other beneath it.

Keating's book, all 350 pages of it, is nearly all about Huerfano County, though it includes a bit of information about the region from the Great Sand Dunes National Park clear east to the dinosaur tracks of the Purgatoire Valley. Perhaps five percent of the text covers Las Animas County, which is just the way Huerfanos like it!

The author explains that his purpose for writing the book was the paucity of information about the Spanish Peaks area and his goal to fill that vacuum. He and his late wife have been regular visitors for some years, and so he has, alas, fallen in with the local disregard for correct names. Thus, the Cucharas river, pass and valley have become the Cuchara, and Mt. Mestas evolves into Mt. Maestas. These localisms in no way detract from the text.

Colorado's Spanish Peaks Region covers historic, geologic, and ecologic topics, giving a synopsis of inhabitants and travelers from prehistoric to frontier times, answering the common question of how the stone dykes were formed and other geologic mysteries (complete with a glossary of terms), offering guidelines on how to avoid predators, "real and rumored." (No, he says, there are no wolves or grizzlies.) A very simple and straightforward explanation of why higher altitudes have higher radiation levels and thus different vegetation is offered. An understatement, at least this week, tells us "wind...is rather constant in this region," giving fair warning to lady tourists worried about their coiffures and "local small pet advisories."

Keating describes 12 scenic drives, from the all-paved Highway of Legends to the four-wheel-drive Medano Pass. Again, warnings are included to the uninitiated to the weather and its effect on dirt roads, noting road "conditions vary year by year" (a nice thing to say when often, they vary hour by hour). He further explains that much of the territory these roads cross is private property and goes so far as to include the state regulations on trespassing.

His thoughtful coverage of these tours includes a mile by mile itinerary of scenic, geologic, recreation, historic and biologic highlights, explaining how the monumentless Monument Lake got its name and even why not to go on a side road from Mosca Pass (because it only leads to a microwave radio tower).

A respectful explanation for our roadside memorials, called descansos in Spanish, is given and is an education in itself.

There are 20 hiking trails described here with their conditions and more warnings about weather – which cannot be overstressed in the mountains – trespassing, and rules of etiquette such as keeping pets on leashes and packing out trash. He cautions readers about the occasionally difficult task of locating trailheads, and sometimes, even trails. His list of 10 basic necessities to take on a hike include a map and or compass to tackle some of these more elusive paths. One of these trails is the Baker Creek, which he says, “gives a close-up view of nature. Few area residents know it exists.” He also tells us there is construction trash dumped there. Keating includes the North Fork trail as a leisurely three-and-half hour walk with spectacular views and a less rigorous grade than some of the others, like the old road to the Bullseye mine on the West Spanish Peak.

He thoughtfully adds a section of topographic maps to show the roads and trails covered in the text.

Keating dedicates more than 100 pages to the descriptions and locations of flora and fauna, from prairie to peaks. Interesting trivia is included, such as the use by native tribes of a certain type of pine for lodgepoles, and thus the meaning of the tree’s name. At the end of the book, a separate index is provided for plant and animal names.

The author provides an extensive list of books, maps, pamphlets and other resources where more information about the Spanish Peaks area may be found. This is subtitled “The wood pulp section. Read so that trees won’t have died in vain.” This might give the impression that the section is extraneous, but it isn’t. Current web site addresses are included.

Keating’s text is accompanied by sharp photos, recent photos [!], of scenes and living things, to illustrate his points of why so-and-so is scenic, or a hard climb, or a unique plant. He shares his knowledge of photography so the reader can take equally clear pictures.

All in all, this is a fine book. The author deserves all congratulations for fulfilling his self-avowed aim of writing a comprehensive guide book to the seldom mentioned Spanish Peaks area. Bravo!

Review by Nancy Christofferson. Reprinted with permission from Huerfano World Journal 127(15): 7. Editor: Gretchen Lorr.

2012 ISAS STUDENT GRANT AWARD RECIPIENTS

Kelly Commons – Millikin University
How Does Wild Bird Feeding Affect the Wild Bird Community?

Madeline Knott – Millikin University
Comparison of IL-6 Levels and *Staphylococcus aureus* BKA in Central Illinois Songbirds

Grant Woods – Eastern Illinois University
Geospatial Investigation of the Occurrence of Confluent Meander Beds in East Central Illinois

Amanda Wildenberg – Eastern Illinois University
Fish, Macroinvertebrate, and Mussel Community Responses to Sanitary Effluent in an Illinois River

Kayla Little – Southern Illinois University – Edwardsville
Avian Hematozoa of Illinois and Their Impacts on Health

Kate Koepp – Southern Illinois University – Edwardsville
Habitat Preferences of *Fundulus notatus* and *F. olivaceus* in an Illinois Stream Contact Zone

2012 BOTANY TRAVEL GRANT RECIPIENTS

Jennifer O'Brien – Eastern Illinois University

Roxane Krutsinger – Southern Illinois University – Edwardsville

2012 ANNUAL MEETING STUDENT PRESENTATION AWARDS

BOTANY DIVISION

Oral Presentations

1st Place: Eun Sun Kim – University of Illinois – Chicago
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Presence and Intensity of Pre-Dispersal Seed Predation in Response to Habitat Quality

2nd Place: Shanna David – Illinois College
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Poster Presentations

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Determining the DNA Content of a Unique Population of *Schoenoplectus hallii* in Howell County, MO

2nd Place: Roxane Krutsinger – Southern Illinois University – Edwardsville
Rising Productivity Through Time in a Tallgrass Prairie Restoration

Jonathan Clark – Southern Illinois University – Edwardsville
Germination of Field-Collected Japanese Hops (*Humulus japonicus,* Cannabinaceae): Lab Techniques and Between Population Variation

CELL, MOLECULAR, & DEVELOPMENTAL BIOLOGY DIVISION

Oral Presentations – Undergraduate

1st Place: Bhargay Patel – Southern Illinois University – Edwardsville
Preservation of Beta-Cell Function by Peroxynitrite Scavenger Under Chronic Nutrient Overload

Oral Presentations – Graduate

1st Place: Stephanie B. Vernier – Southern Illinois University – Edwardsville
Role of Lipids and Peroxynitrite in Beta Cell Defects Under Chronic Nutrient Overload

John M. Anderson – Eastern Illinois University
Protective Mechanisms Against Water Stress Evaluated in Insect Cells

CELL, MOLECULAR, & DEVELOPMENTAL BIOLOGY DIVISION – CONT'D

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Poster Presentations – Graduate

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CHEMISTRY DIVISION

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Transactions Information

Transactions Editor
Teresa (Tere) North
Western Illinois University
teren1956@gmail.com

Executive Secretary
Robyn Meyers
Illinois State Museum
rmyers@museum.state.il.us

DIVISION CHAIRS

Agriculture
Vacant

Anthropology & Archaeology
Bonnie W. Styles
Illinois State Museum
styles@museum.state.il.us

Botany
Barbara Carlsward
Eastern Illinois University
bscarlsward@eiu.edu

Cell, Molecular, & Developmental Biology
Tom Fowler
Southern Illinois University Edwardsville
tfowler@siue.edu

Chemistry
Dean Campbell
Bradley University
campbell@bumail.bradley.edu

Computer Science
Jim McQuillan
Western Illinois University
jm-mcquillan@wiu.edu

Earth Science
Jim Riley
Eastern Illinois University
jdriley@eiu.edu

Engineering & Technology
Vacant

Environmental Science
Elizabeth Walton
Southern Illinois University Edwardsville
elwalto@siue.edu

Health Sciences & Microbiology
Vance McCracken
Southern Illinois University Edwardsville
vmccrac@siue.edu

Physics, Mathematics, & Astronomy
Casey R. Watson
Millikin University
crwatson@millikin.edu

Science, Mathematics, & Technology Education
Kelly Barry
Southern Illinois University Edwardsville
kbarry@siue.edu

Zoology
David Duvernell
Southern Illinois University Edwardsville
dduvern@siue.edu
Paul Brunkow
Southern Illinois University Edwardsville
pbrunko@siu.edu

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Transactions Information

***Transactions* Editor**
Teresa (Tere) North
Western Illinois University
teren1956@gmail.com

Executive Secretary
Robyn Meyers
Illinois State Museum
rmyers@museum.state.il.us

DIVISION CHAIRS

Agriculture
Vacant

Anthropology & Archaeology
Bonnie W. Styles
Illinois State Museum
styles@museum.state.il.us

Botany
Barbara Carlsward
Eastern Illinois University
bscarlsward@eiu.edu

Cell, Molecular, & Developmental Biology
Tom Fowler
Southern Illinois University Edwardsville
tfowler@siue.edu

Chemistry
Dean Campbell
Bradley University
campbell@bumail.bradley.edu

Computer Science
Jim McQuillan
Western Illinois University
jm-mcquillan@wiu.edu

Earth Science
Jim Riley
Eastern Illinois University
jdriley@eiu.edu

Engineering & Technology
Vacant

Environmental Science
Elizabeth Walton
Southern Illinois University Edwardsville
elwalto@siue.edu

Health Sciences
Vance McCracken
Southern Illinois University Edwardsville
vmccrac@siue.edu

Microbiology
Vance McCracken
Southern Illinois University Edwardsville
vmccrac@siue.edu

Physics, Mathematics, & Astronomy
Casey R. Watson
Millikin University
crwatson@millikin.edu

Science, Mathematics, & Technology Education
Kelly Barry
Southern Illinois University Edwardsville
kbarry@siue.edu

Zoology
David Duvernell
Southern Illinois University Edwardsville
dduvern@siue.edu

Paul Brunkow
Southern Illinois University Edwardsville
pbrunko@siu.edu

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The Varying Phenology and Growth Patterns of *Baptisia bracteata* (Fabaceae) in Reconstructed Prairie

Chris E. Petersen, Sharon M. Bauzys, Felicia A. Speranske, and Barbara A. Petersen
College of DuPage, Glen Ellyn, IL 60137

ABSTRACT

First flowering time and flowering duration of *Baptisia bracteata* Muhl. ex. Ell (cream wild indigo), were examined in the Russell R. Kirt Tallgrass Prairie, Glen Ellyn, IL, during 2008, 2009, and 2011. While some *B. bracteata* delay flowering, most bloom during May, just prior to the blooming period of the taller and more prolific congener, *B. alba*. Both congeners are host to the pre-dispersal seed predator, *Apion rostrum* Say (Apionidae), although the reproductive cycle of the weevil is synchronized to *B. alba*. Factors relating to seed yield and the growth of the rhizomatous *B. bracheata* were also investigated. *B. bracteata* with more shoots, flowers and racemes tended to flower earlier and longer than those with fewer counts, evidence of the importance of plant size to flowering phenology. Still, as indicated by flower count/plant, a larger size was only positively correlated to seeds matured/plant during 2011. Counts of *A. rostrum* showed positive relationships to pods and seeds matured/plant during 2008 and 2011. Mean shoot count/plant was not significantly different between 2008 and 2011 among 39 tracked individuals. Growth was largely limited to smaller individuals while larger individuals sometimes declined in size. Selective pressures favoring larger size may include competition for resources. However, rewards of seeds matured/plant based on a larger inflorescence size, may not be realized every year. Larger plants may be more prone to resource limitations and weevil attacks. Variances in plant sizes can be just explained by age. Alternatively, size plasticity may occur due to changing environmental conditions and reproductive effort from year to year. It remains unknown if low output of mature pods was due to a lack of pollinators. By subsequently having lower seed output, the species may lower its' attractiveness to *A. rostrum*.

INTRODUCTION

One basic principle of life history theory explains that there is a cost to reproduction which is minimized by trade-offs among components of reproductive yield (Sánchez-Humanes, et al. 2011; Williams, 1966). Flowering phenology may be under the influences of trade-offs within a species, e.g., when the timing of first flowering or flowering duration show asynchrony as a response to changing environmental constraints (Elzinga et al., 2007). Larger plants are often found to bloom earlier and longer than smaller plants, presumably to attract pollinators and because they have greater nutrient reserves available for flowering (Bolmgren and Cowan, 2008; Bustamante and Burquez, 2008; Pettersson, 1994). By flowering off-peak, smaller plants may be able to avoid consumers such as seed predators, but perhaps at the cost of less favorable weather (Albrectsen, 2000; Evans et al., 1989; Mahoro, 2002; McIntosh, 2002; Mduma et al., 2007).

Baptisia bracteata Muhl. ex. Ell (cream wild indigo; = *B. leucophaea*) is a native tall-grass perennial of the Midwest which shows variance in flowering phenology. At our study site, the reconstructed Russell R. Kirt Prairie, IL, the species blooms in May when a new season of emergent prairie growth has just begun to show (Petersen et al., 1998). Most plants tend to flower at the same time while a few delay. Using data collected during 2008, 2009, and 2011, we investigated factors that could explain the asynchrony. We also examined factors relating to seed yield and the change in size of 39 plants over a 4-year span.

Each *B. bracteata* consists of subterranean rhizomes from which multiple aerial shoots develop to form a concentric cluster measuring up to a half meter in diameter. Racemes along shoots radiate outward from the cluster, exposing conspicuous displays of yellow flowers. Aging flowers along the indeterminate racemes shift from being staminate to carpellate. This shift allows for a degree of self pollination (Haddock and Chaplin, 1982). *Bombus* are the major pollinators. Pods inflate with pollination and can bear over 14 seeds. Unlike the congener, *B. alba* L. (Vent)(white wild indigo) which also grows in the prairie, inflated pods of *B. bracteata* remain attached regardless of seed number, and the ones that fail to mature seeds appear to ripen at the same rate as those that retain seeds. *B. alba* often aborts many to all of its inflated pods in apparent response to environmental extremes and low counts of seeds in pods (Petersen et al., 2011).

At our study site, *B. bracteata* is infested by the pre-dispersal seed predator, *Apion rostrum* Say (Apionidae). The weevil also infests and is more synchronized to pod development of the later blooming *B. alba* (Petersen and Wang, 2006). Overwintering adult weevils oviposit into inflating pods of both congeners. Resulting larvae consume seeds as their only source of nutrition. Maturation is reached by August, and the weevils disperse when pods dehisce or fragment.

METHODS

Reconstruction of the 7.1 ha Russell R. Kirt Tallgrass Prairie began in 1984. Today, the dominant tall grasses are *Andropogon gerardii* Vitman (big bluestem), *Sporobolus heterolepsis* Gray (prairie dropseed), and *Sorghastrum nutans* (L.) Nash (Indian grass). Over 100 species of forbs contribute to the flora. The prairie was burned annually until 2006. It has not been burned since.

Sampling methods in 2008, 2009, and 2011 followed the same protocol. A concentric cluster of *B. bracteata*, with shoots being within 10 cm of one another, was assumed to be one individual based on soil excavation of individuals not otherwise included in sampling. Fifty to 51 plants were randomly selected for sampling each year. First and last flowering dates of each *B. bracteata* were recorded along with counts of shoots, racemes, and flowers. When pods matured, they were counted along with their contents of seeds and weevils. If pods had holes from which weevils could escape, *A. rostrum* count/plant was prorated using mean counts from intact pods. Shoot and flower counts per plant provided alternative measures of plant size. Seeds matured/plant and counts of *A. rostrum*/plant provided measures of reproductive output and weevil infestation, respectively.

All statistical analyses were done using Statistica 6 (Statsoft, 2001). Due to failure in meeting assumptions of parametric testing, relationships between flowering phenology, other reproductive allocations to include seeds matured, and weevil infestation were explored using Spearman Rank Correlation (Zar, 1984). Rhizomatous growth was estimated by comparing the ratio of 2011:2008 shoot counts to 2008 shoot counts using Spearman Rank Correlation.

RESULTS

The first *B. bracteata* flowered on May 11th during 2008 and 2011, and on May 26th during 2009. Plant measurements according to year are presented in Table 1. For individuals, an earlier flowering start, a longer flowering duration, and a higher flowering count/plant were positively correlated for all years, except in 2011 between flowering initiation and duration (Table 2). However, as indicated by flower count/plant, a larger size was only positively correlated to seeds matured/plant during 2011. Plants producing more flowers also tended to show higher shoot counts; but more shoots did not correlate consistently with an earlier time and duration of flowering. It also did not translate to a higher yield of seeds matured. Finally, mean counts of *A. rostrum*/plant were positively correlated to pods and seeds matured per plant in 2008 and 2011, but not in 2009.

Table 1. Summary (mean ± standard error; n) of variables associated with reproductive yield of *Baptisia bracteata* and infestation by *Apion rostrum* according to year. Flowering time is in reference to the mean date plants flowered after the first *B. bracteata* bloomed.

Variable	Year		
	2008	2009	2011
Time of first flowering	9.6 ± 0.8 (51)	9.2 ± 0.8 (50)	14.0 ± 1.0 (42)
Flowering duration (days)	16.8 ± 0.6 (51)	17.2 ± 0.5 (50)	16.5 ± 1.1 (42)
Shoots/plant	8.0 ± 1.0 (51)	9.6 ± 0.9 (50)	10.9 ± 1.3 (42)
Flowers/plant	114.4 ± 16.2 (51)	95.1 ± 11.9 (50)	122.9 ± 23.8 (42)
Pods matured/plant	9.8 ± 3.1 (51)	32.3 ± 6.4 (50)	19.2 ± 5.9 (42)
Seeds matured/plant	28.1 ± 16.3 (51)	62.5 ± 20.6 (50)	8.5 ± 6.5 (42)
Apion rostrum/plant	10.8 ± 4.4 (20)	13.6 ± 4.3 (32)	26.7 ± 8.3 (24)
% of *Baptisia bracteata* bearing mature pods	37.3% (51)	80.0% (50)	59.5% (42)

While shoot count among tracked *B. bracteata* did not change significantly from 2008 to 2011 (t = 1.876; df = 38; $P < 0.068$), plants with fewer shoots accounted for most increases (Figure 1). The ratio in shoot count between 2011 and 2008 was negatively correlated with shoot count in 2008 ($r_s = -0.54$; $P < 0.05$). From 2008 to 2011, 22 plants showed an increase in shoot count, 4 showed no change, while 13 decreased in shoot count.

Table 2. Spearman rank correlation comparing variables of flowering phenology, reproductive yield, and Apion rostrum infestations presented sequentially for years 2008, 2009, and 2011. AC = Counts of Apion rostrum/plant. The AC sample sizes (n's) for 2008, 2009, and 2011, were 20, 32, and 24, respectively. Otherwise, n = 51 for 2008, 50 for 2009, and 42 for 2011. Bold type denotes significance (P ≤ 0.05).

	FD	SC	FC	PM	SM	AC
Time of first flowering (FF)	**-0.85**	-0.21	**-0.36**	<-0.00	0.23	-0.33
	-0.55	**-0.35**	**-0.55**	-0.28	0.02	-0.17
	-0.23	-0.07	**-0.32**	<0.00	0.17	-0.08
Flowering duration (FD)		0.13	**-0.85**	-0.06	-0.20	0.30
		0.36	**0.43**	**0.52**	0.20	-0.12
		0.45	**0.54**	**0.39**	**0.43**	0.14
Shoot count/plant (SC)			**0.65**	0.15	-0.06	0.21
			0.41	**0.43**	0.10	0.12
			0.42	**0.44**	0.27	**0.51**
Flower count/plant (FC)			0.21	<0.00	0.43	
			0.59	0.20	0.31	
			0.48	**0.42**	**0.42**	
Pods matured/plant (PM)				**0.56**	**0.52**	
				0.52	0.34	
				0.72	**0.78**	
Seeds matured/plant (SM)					**0.56**	
					0.25	
					0.43	

DISCUSSION

Flower count per *B. bracteata* provided a better correlating factor to flowering phenology than shoot count/plant. The discrepancy may reflect the additional function of shoots in photosynthesis. Shoot number may vary due to disproportionate allocations of energy to non-reproductive and reproductive structures as a reaction to changing environmental conditions. Regardless, results are consistent with larger *B. bracteata* blooming earlier and longer than those smaller.

Plant size can indicate age. Alternatively, plant size may be influenced by the local environment and changes in reproductive effort from year to another. With larger *B. bracteata* being more likely to become smaller, and smaller individuals larger between 2008 and 2011, such changes in size can function to promote the fitness of a perennial over the course of its lifetime (Salguero-Gómex and Casper, 2010). Larger size may offer competitive benefits to acquisition of resources not investigated in this study. Nevertheless, greater rewards of seeds matured/plant, where plant size is based on flower count, may not be realized every year. Larger plants may also be more prone to resource limitations and attack by consumers like *A. rostrum* (Hainsworth et al., 1984; Ohasi and Yahara, 2000). A longer term study should offer insights in explaining size differences in *B. bracteata*.

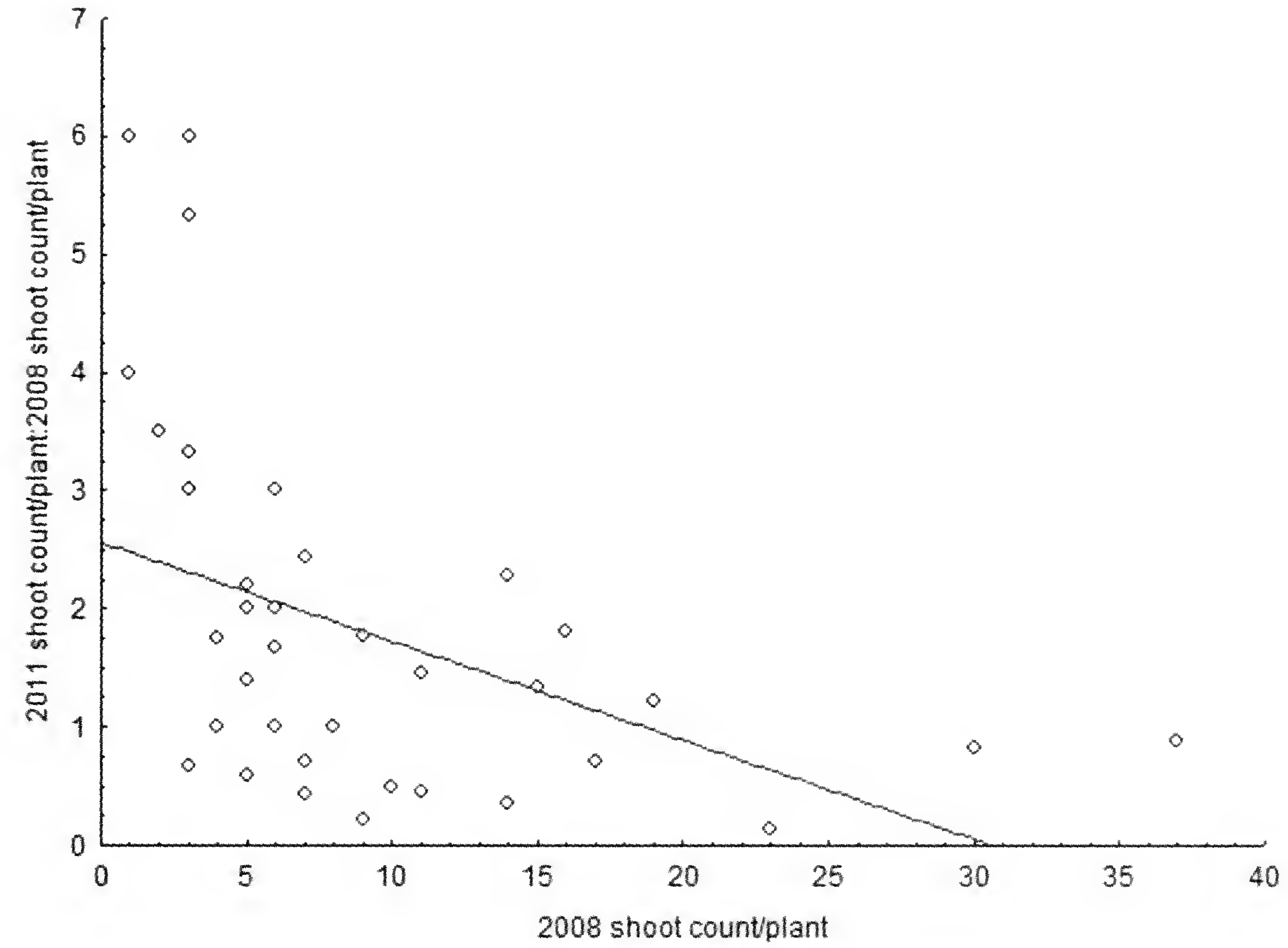

Figure 1. Shoot count ratio of *B. bracteata* comparing years 2011 to 2008, plotted against the 2008 shoot count. Best-fit linear regression line is provided to help visualize trend.

Despite the seemingly prolific flowering by *B. bracteata*, few pods were matured/plant. It remains unknown if pollen limitations are due to a lack of pollinators at the reconstructed site. Mature pods bear seeds. By having a seed output much lower than *B. alba* (Petersen et al., 2007), the species may be less attractive to *A. rostrum* explaining the synchronization of the weevil's reproductive cycle around the taller and more prolific congener.

LITERATURE CITED

Albrectsen, B. R. 2000. Flowering phenology and seed predation by a tephritid fly: Escape of seeds in time and space. Ecoscience 7: 433-438.

Bolmgren, K. and P. D. Cowan. 2008. Time-size tradeoffs: a phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperate flora. Oikos 117:424-429.

Bustamante, E. and A. Burquez. 2008. Effects of plant size and water on the flowering phenology of the Organ Pipe Cactus (*Stenocereus thurberi*). Ann. Botany 102:1019-1030.

Elzinga, J. A., A. Atlan, A. Biere, Luc Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. Trends Ecol. Evol. 22:432-439.

Evans, E., C. C. Smith, and R. P. Gendron. 1989. Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. Oecologia 78:220-230.

Haddock, R. C., and S.J. Chaplin. 1982. Pollination and seed production in two phenologically divergent prairie legumes (*Baptisia leucophaea* and *B. leucantha*). Am. Midl. Nat. 108: 175-186.

Hainsworth, F. R., L.L. Wolf, and T. Mercier. 1984. Pollination and pre-dispersal seed predation: net effects on reproduction and inflorescence characteristics in *Ipomopsis aggregate*. Oecologia 63:405-409.

Mduma, S. A., A. R. E. Sinclair, and R. Turkington. 2007. The role of rainfall and predators in determining synchrony in reproduction of savanna trees in Serengeti National Park, Tanzania. J. Ecol. 95:184-196.

Mahoro, S. 2002. Individual flowering schedule, fruit set, and flower and seed predation in *Vaccinium hirtum* Thunb. (Ericaceae). Can. J. Bot. 80:92-92.

McIntosh, M. E. 2002. Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). Plant Ecol. 159:1-13.

Ohashi, K. and T. Yahara. 2000. Effects of flower production and predispersal seed predation on reproduction in *Cirsium purpuratum*. Can. J. Bot. 78:230-236.

Petersen, C.E., S. C. Lindsey, D. M. Dudgeon, and R. A. Pertell. 1998. The effect of seed predation on pod abortion by the prairie legume, *Baptisia leucophaea*. The Trans. Ill. State Acad. Sci. 91:47-52.

Petersen, C. E. and W. Wang. 2007. Congener host selection by the pre-dispersal seed predator, *Apion rostrum* (Coleoptera: Apionidae). Gt. Lakes Entomol. 39:68-74.

Petersen, C. E., A. A. Mohus, B. A Petersen, and B. A. McQuaid. 2010. Multiyear study of factors related to flowering phenology and reproductive yield of *Baptisia alba* in Northeastern Illinois. Trans. Ill. State Acad. Sci. 103:109-117.

Pettersson, M. W. 1994. Large plant size counteracts early seed predation during the extended flowering season of a *Silene uniflora* (Caryophyllaceae) population. Ecography 17:264-271.

Salguero-Gómex R. and B. B. Casper. 2010. Keeping plant shrinkage in the demographic loop. J. Ecol. 98: 312-323.

Sánchez-Humanes, B. V. L. Sork, J. M. Espelta. 2011. Trade-offs between vegetative growth and acorn production in *Quercus lobata* during a mast year: the relevance of crop size and hierarchical level within the canopy. Oecologia 166:101-110.

StatSoft. 2001. Statistica AX 6.0. 2001. StatSoft, Tulsa, OK.

Williams, G. C. 1966. Natural selection costs of reproduction and a refinement of Lack's Principle. Am. Nat. 100:687-690.

Zar, J.H. 1984. Biostatistical Analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ, USA.

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Vascular Flora of Capel Glacial Drift Hill Prairie Natural Area, Shelby County, Illinois

William E. McClain[1], John E. Ebinger[2]*, Roger Jansen[3], and Gordon C. Tucker[2]
[1]Illinois State Museum, Springfield, IL 62706
[2]Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920
[3]Illinois Department of Natural Resources
1660 West Polk Avenue, Charleston, IL 61920
*corresponding author (jeebinger@eiu.edu)

ABSTRACT

The vascular flora of Capel Glacial Drift Hill Prairie Natural Area, Shelby County, Illinois was studied during the 2009 and 2010 growing seasons. The 1.10 ha glacial drift hill prairie is located on a southwest-facing slope associated with Lake Shelbyville, Wolf Creek State Park 4 km east of Findley, Illinois. Plant community structure was determined using m^2 square quadrats located at one-meter intervals along two randomly located transect lines. Frequency, mean cover, relative frequency, relative cover, and importance value (I. V. total = 200) were determined from the data collected. A total of 106 vascular plant taxa were observed on the site, with 39 encountered in the plots. *Andropogon gerardii* (big bluestem) had the highest importance value followed by *Schizachyrium scoparium* (little bluestem), *Echinacea pallida* (pale coneflower), and *Dalea purpurea* (purple prairie clover). Exotic species were represented by six taxa.

Key Words: *Andropogon gerardii,* glacial hill prairie formation, soil slumping.

INTRODUCTION

Small prairie openings in the forested landscapes of east-central Illinois were first described and named “hill prairies” by Vestal (1918). These small prairies (mostly < 1 ha), now refer to as glacial drift hill prairies, developed near the crest of slopes or spurs on mostly Wisconsin age glacial till. In east-central Illinois these prairie openings have been studied in Coles County (Ebinger 1981, Reeves et al. 1978, Owens and Cole 2003, Behnke and Ebinger 1989), Vermilion County (Owens and Ebinger 2008) and on Illinoian glacial till in Jasper County (Edgin et al. 2010). Glacial drift hill prairies have also been studied in central Illinois in Moultrie County (Owens et al. 2006), Macoupin County (McClain et al. 2002), and are not uncommon in the Illinois River valley from Peoria and Tazewell counties north through Marshall, Woodford, and Putnam counties (McFall and Karnes 1995).

Glacial drift hill prairies have many edaphic, physical, and floristic similarities. They occur on steep south- to southwest-facing slopes with well-drained soils that are low in organic content and nutrients (Ebinger 1981, McClain et al. 2002, Owens et al. 2006, Owens and Ebinger 2008). The predominant vegetation is native warm season clump grasses complemented by prairie forbs with a sparse vegetation ground cover. Drying winds, unstable soil, fire, cutting, and grazing were thought to play significant roles in the development and maintenance of these sites (Vestal 1918, Reeves et al. 1978). The present study was undertaken to describe the composition and structure of the vegetation of Capel Glacial Drift Hill Prairie Natural Area. This prairie was added to the Illinois Natural Areas Inventory in 1999 and is considered to be of high natural quality.

DESCRIPTION OF THE STUDY SITE

Capel Glacial Drift Hill Prairie Natural Area is located on a southwest-facing slope overlooking Lake Shelbyville in Wolf Creek State Park, 4 km east of Findley, Shelby County, Illinois (SW1/4, NE1/4, Section 7, T12N, R5E). This 1.10 ha prairie is located at an elevation of about 190 m on the Shelbyville terminal moraine of Wisconsin glaciation in the Grand Prairie Section of the Grand Prairie Natural Division (Schwegman 1973). Tallgrass "black soil" prairie dominated the extensive flat to gently rolling uplands of this natural division, the forests being mostly restricted to floodplains and dissected topography of river valleys and other hilly areas (Anderson 1991, Ebinger and McClain 1991). Lake Shelbyville was formed when the Kaskaskia River was dammed just east of the town of Shelbyville in 1970 by the Army Corps of Engineers. Small prairie opening are occasionally encountered in the rough topography surrounding the lake where edaphic and microclimatic conditions combined to produce excessively droughty sites (Reeves et al. 1978, Edgin et al. 2010). The forest communities found at Wolf Creek State Park were discussed by Newell et al. (1991).

The soil of the glacial drift hill prairie is Miami loam, a highly eroded soil with little of the original A horizon present, the original loess deposits having eroded away (Gotsch 1996). This soil, which is on 18 to 30 % slopes, is well drained, low in organic content, slightly acid, and commonly low in available phosphorus and potassium. Many gravel size pebbles are imbedded in the soil. In this part of Illinois annual precipitation averages 97.5 cm, with April having the highest rainfall (9.4 cm). Mean annual temperature is 11.8 °C with July being the hottest month (average of 24.6 °C), the coldest being January (-3 °C). The average number of frost-free days is 171 (Midwestern Regional Climate Center 2011).

First examined in 1998 by personnel from the Illinois Department of Natural resources (Smith1998), Capel Glacial Drift Hill Prairie was first burned in the spring of 1999. Subsequent management efforts have focused on control of *Melilotus albus* (white sweet clover) and *Securigera varia* (crown vetch). Prescribed burning was conducted in April of 2005 and 2010, and a wild fire burned the site in the spring of 2011.

MATERIALS AND METHODS

Collections of the vascular flora of the prairie and adjacent open woodland margin were made periodically during the 2009 and 2010 growing seasons. Voucher specimens were collected for most taxa and deposited in the Stover-Ebinger Herbarium of Eastern Illinois University, Charleston (EIU). Nomenclature follows Mohlenbrock (2002), and the assignment of exotic species status was determined using Taft et al. (1997) and Mohlenbrock (2002).

Quantitative sampling was conducted in late summer of 2010 using m^2 quadrats located at one-meter intervals along two randomly placed 25 m transects oriented perpendicular to the slope (n = 25 per transect). Even-number quadrats were placed to the right, odd-numbered to the left of each transect. Percent cover of each species was determined using Duabenmire cover class system (Daubenmire 1959) as modified by Bailey and Poulton (1968): class 1 = 0-1%; class 2 = 1-5%; class 3 = 5-25%; class 4 = 25-50%; class 5 = 50-75%; class 6 = 75-95%; class 7 = 95-100%. From these data, frequency (%), mean cover (%), relative frequency, relative cover, and Importance Value [relative frequency + relative cover) were determined.

The Floristic Quality Index (FQI) was determined using the coefficient of conservatism (CC) assigned to each species by Taft et al. (1997). As used here, the FQI is a weighted index of species richness (N) and is the arithmetic product of the mean coefficient of conservatism (C-value), multiplied by the square root of species richness (√N) of the site [FQI = C-value (√N)].

RESULTS

Floristic Composition

At total of 106 vascular plant species were collected or observed in and at the edge of the prairie (Appendix I). Of the species present 26 were monocots in five families while 80 were dicots in 31 families. Seven non-native (exotic) taxa were found with only *Melilotus albus* occurring in the plots. Predominant plant families were Asteraceae (24 species), Poaceae (19), and Fabaceae (10). No state endangered or threatened species were observed (Illinois Endangered Species Protection Board 2005). The C-value and FQI for all species were 4.54 and 46.7 respectively with 21 species having a CC of seven or more.

Ground Layer

Of the 106 species encountered on the prairie 39 were found in the plots (Table 1). Prairie grasses were the most important species. *Andropogon gerardii* (big bluestem) dominated with the highest importance value (I.V. of 38.5) and mean cover (24.57%), followed by *Schizachyrium scoparium* (little bluestem) (I.V. of 25.3 and a mean cover of 13.99%). Other species with importance values ±10.0 included *Echinacea pallida* (pale coneflower), *Dalea purpurea* (purple prairie clover), *Aster oolentangensis* (sky-blue aster), and *Asclepias viridiflora* (green milkweed). Five woody species were recorded in the plots, *Rosa carolina* (Carolina rose) with an I.V. of 6.1, *Cornus drummondii* (rough-leaved dogwood) with an I.V. of 1.3, and seedlings of three tree species (*Carya ovata*, *Quercus stellata*, and *Sassafras albidum*).

Table 1. Frequency (%), mean cover (% of total area), relative frequency, relative cover, and importance value (I.V.) for the ground layer species at Capel Glacial Drift Hill Prairie Natural Area, Wolf Creek State Park, Shelby County, Illinois. (*exotics)

Species	Freq. %	Mean Cover	Rel. Freq.	Rel. Cover	I. V.
Andropogon gerardii	90	24.57	8.6	29.9	38.5
Schizachyrium scoparium	88	13.99	8.4	16.9	25.3
Echinacea pallida	98	11.92	9.3	14.4	23.7
Dalea purpurea	82	4.91	7.8	5.9	13.7
Aster oolentangensis	70	5.35	6.7	6.5	13.2
Asclepias viridiflora	84	2.78	8.0	3.4	11.4
Euphorbia corollata	68	1.81	6.5	2.2	8.7
Brickellia eupatorioides	56	1.56	5.3	1.9	7.2
Aster patens	38	2.76	3.6	3.3	6.9
Rosa carolina	34	2.36	3.2	2.9	6.1
Lithospermum canescens	48	0.59	4.6	0.7	5.3
Lespedeza virginica	36	1.40	3.4	1.7	5.1
Coreopsis palmata	30	1.22	2.9	1.5	4.4
**Melilotus albus*	34	0.56	3.2	0.7	3.9
Hypericum sphaerocarpum	34	0.47	3.2	0.6	3.8
Aureolaria grandiflora	14	1.33	1.3	1.6	2.9
Sporobolus cryptandrus	26	0.28	2.5	0.3	2.8
Solidago nemoralis	16	1.00	1.5	1.2	2.7
Silphium integrifolium	10	1.02	1.0	1.2	2.2
Carex pensylvanica	12	0.69	1.1	0.8	1.9
Cornus drummondii	6	0.61	0.6	0.7	1.3
Ruellia humilis	12	0.06	1.1	0.1	1.2
Comandra umbellata	10	0.10	1.0	0.1	1.1
Ipomoea pandurata	6	0.42	0.6	0.5	1.1
Physostegia virginiana	8	0.14	0.8	0.2	1.0
Pycnanthemum pilosum	6	0.37	0.6	0.4	1.0
Tree seedlings (3 species)	8	0.09	0.8	0.1	0.9
Elymus virginicus	4	0.02	0.4	--	0.4
Taenidia integerrima	4	0.02	0.4	--	0.4
Eupatorium serotinum	2	0.06	0.2	0.1	0.3
Helianthus divaricatus	2	0.06	0.2	0.1	0.3
Helianthus mollis	2	0.06	0.2	0.1	0.3
Antennaria plantaginifolia	2	0.01	0.2	--	0.2
Aster turbinellus	2	0.01	0.2	--	0.2
Galium circaezans	2	0.01	0.2	--	0.2
Polygala senega	2	0.01	0.2	--	0.2
Scutellaria leonardii	2	0.01	0.2	--	0.2
Totals		82.63	100.0	100.0	200.0
Bare ground and litter		26.24			

DISCUSSION

The glacial drift hill prairies of east-central Illinois are characterized by similar soils (type, structure, stability, slope, exposure), flora (common prairie grasses and forbs as well as few conservative prairie species), and bare ground (25 to 50% of the total cover), but commonly differ in size, species richness, and distribution and abundance of the dominant grass species. These differences are likely related to edaphic characteristics of the sites. Some of the glacial drift hill prairies, particularly those in Macoupin County (McClain et al 2002) and Jasper County (Edgin et al. 2010) are on more shallow slopes, are larger, and have higher species richness. In these hill prairies *Andropogon gerardii* commonly dominates and the soils are stable, soil slumping being uncommon. The glacial drift hill prairies of the Middle Fork of the Vermilion and the upper Embarras Rivers (Coles and Vermilion Counties), in contrast, are usually on steeper slopes, tend to be somewhat smaller, and have lower species richness. Also, *Schizachyrium scoparium* and *Sorghastrum nutans* are the most abundant grasses while *Andropogon gerardii* is apparently a minor component (Edgin et al. 2010). In these hill prairies soil slumping is common.

Slumping, the down-hill movement of large masses of soil, is a characteristic of many glacial drift hill prairies in central Illinois. The sparse prairie vegetation can not hold the tremendous weight of the saturated clay soil on the steep slopes. In wet years, soil breaks away and slide down hill, taking prairie vegetation, shrubs, and sometimes large trees with it. On Capel Hill Prairie excessive soil slumping, due to shoreline erosion of Lake Shelbyville, has limited the size of the prairie. The slumping has helped maintain the hill prairie by eliminating surrounding trees and by creating bare ground which rapidly succeeds to a community containing mostly prairie grasses and forbs (Owens et al. 2006). Slumping facilitates an early succession stage that seems to be favorable to *Schizachyrium scoparium* (Vestal 1918). This sun-loving, early to mid-successional species is usually associated with drier sites with poorer soils and tends to decrease with succession.

Capel Hill Prairie is located 11 km southwest of Coneflower Glacial Drift Hill Prairie in Moultrie County (Owens et al. 2006). This prairie, which is located on the Cerro Gordo recessional moraine of Wisconsin glaciation, occurs on a moderate slope with eroded, well-drained Miami silt loam soil that is slightly acidic and has low organic content. On both Coneflower and Capel hill prairies *Andropogon gerardii* was the dominant grass while *Schizachyrium scoparium* ranked second among the grasses. Though similar, Capel Hill Prairie has low species diversity (106 taxa) and has excessive soil slumping due to its position on the Lake Shelbyville shoreline, Coneflower Hill Prairie, in contrast, has high species diversity (164 taxa), and minimal soil slumping. Excessive disturbances are probably responsible for some of this lack of diversity. It is very possible that previous to the formation of Lake Shelbyville, Capel Hill Prairie had minimal slumping. The undercutting of the shoreline by wave action is undoubtedly responsive for the present excess slumping, and may be responsible for the low species diversity. Five species originally reported as occurring on this hill prairie (Smith 1998) (*Calystegia spithamaea*, *Helenium autumnale*, *Liatris aspera*, *Polygala verticillata, Pycnanthemum tenuifolium*) were not observed during the two years of our study.

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LITERATTURE CITED

Anderson, R. C. 1991. Presettlement forests of Illinois. Pp. 9-19. in: G. V. Burger, J. E. Ebinger, and G. S. Wilhelm (editors.). Proceedings of the oak woods management workshop. Eastern Illinois University, Charleston, Illinois.

Bailey, A. W. and C. E. Poulton. 1968. Plant communities and environmental relationships in a portion of Tillamook burn, northwestern Oregon. Ecology 49: 1-13.

Behnke, G. and J. E. Ebinger. 1989. Woody invasion of glacial drift hill prairies in east-central Illinois. Transactions of the Illinois State Academy of Science 82: 1-4.

Daubenmire, R. 1959. A canopy coverage method of vegetation analysis. Northwest Science 33: 43-64.

Ebinger, J. E. 1981. Vegetation of glacial drift hill prairies in east-central Illinois. Castanea 46: 115-121.

Ebinger, J. E. and W. E. McClain. 1991. Forest succession in the prairie peninsula of Illinois. Illinois Natural History Survey Bulletin 34:375-381.

Edgin, B., S. J. Adams, E. J. Stork, and J. E. Ebinger. 2010. Flora and species dominance of Green Prairie, Jasper County, Illinois – A glacial drift hill prairie on Illinoian till. Erigenia 23:24-33.

Illinois Endangered Species Protection Board. 2005. Checklist of Endangered and Threatened Animals and Plants of Illinois. Endangered Species protection Board, Springfield, Illinois.

Gotsch, K. A. 1996. Soil Survey of Shelby County. U.S.D.A. Natural Resources Conservation Service and Illinois Agricultural Experiment Station. U.S. Government Printing Office, Washington DC.

McClain, W. E., M. A. Phipps, H. H. Eilers, and J. E. Ebinger. 2002. Vascular plants of glacial drift prairies in Macoupin County, Illinois. Castanea 67: 54-60.

McFall, D. and J. Karnes. 1995. (editors). A directory of Illinois Nature Preserves. Volume 2. Northwestern, Central and Southern Illinois. Illinois Department of Natural Resources, Springfield, Illinois.

Midwestern Regional Climate Center. 2011. http://mcc.sws.uiuc.edu

Mohlenbrock, R. H. 2002. Vascular flora of Illinois. Southern Illinois University Press, Carbondale, Illinois.

Newell, S. A., L. L. Horton, and J. E. Ebinger. 1991. Vegetation of Wolf Creek State Park, Shelby County, Illinois. Erigenia 11:22-26. 1991.

Owens, N. L. and G. N. Cole. 2003. 25 years of vegetational changes in a glacial drift hill prairie community in east-central Illinois. Transactions of the Illinois State Academy of Science 96: 265-269.

Owens, N. L. and J. E. Ebinger. 2008. Windfall Glacial Drift Hill Prairie, Vermilion County, Illinois: Present vegetation and changes since 1977. Transactions of the Illinois State Academy of Science 101: 157-165.

Owens, N. L., G. C. Tucker, and J. E. Ebinger. 2006. Flora and vegetation of Coneflower Glacial Drift Hill Prairie Natural Area, Moultrie County, Illinois. Rhodora 108:370-386.

Reeves, J. T., U. D. Zimmerman, and J. E. Ebinger. 1978. Microclimatic and soil differences between hill prairies and adjacent forests in east-central Illinois. Transactions of the Illinois State Academy of Science 71: 156-164.

Schwegman, J. E. 1973. Comprehensive plan for the Illinois Nature Preserves Commission: part 2. The Natural Divisions of Illinois. Illinois Nature Preserves Commission, Rockford, Illinois.

Smith, E. L. 1998. Nomination Form, Wolf Creek State Park - Capel Hill Prairie.

Taft, J. B., G. S. Wilhelm, D. M. Ladd, and L. A. Masters. 1997. Floristic quality assessment for vegetation in Illinois, a method for assessing vegetation integrity. Erigenia 15: 1-95.

Vestal, A. G. 1918. Local inclusions of prairie within forest. Transactions of the Illinois State Academy of Science 11: 12-126.

Appendix I. Vascular plant species encountered Capel Glacial Drift Hill Prairie Natural Area, Wolf Creek State Park, Shelby County, Illinois are listed alphabetical by family under the major plant groups. Collecting numbers (JEE) are listed after each species. Specimens are deposited in the Ebinger/Stover Herbarium (EIU), Eastern Illinois University, Charleston. Species followed by INAI (Illinois Natural Areas Inventory) were observed on the site during the original survey by the INAI. During the present study most of these species were observed. (*exotics)

DICOTS
Acanthaceae
Ruellia humilis Nutt.:33032

Apiaceae
Taenidia integerrima (L.) Drude:33074

Asclepiadaceae
Asclepias purpurascens L.:32912
Asclepias tuberosa L.:32407
Asclepias viridiflora Raf.:32911

Asteraceae
Achillea millefolium L.:32913
Ambrosia artemisiifolia L.:32689
Antennaria plantaginifolia (L.) Hook.:32760
Arnoglossum atriplicifolium (L.) H. Robins.:32409
Aster oolentangiensis Riddell:32690
Aster patens Ait.:32384
Aster turbinellus Lindl.:32385
Brickellia eupatorioides (L.) Shinners:32410
Coreopsis palmata Nutt.:32411
Echinacea pallida (Nutt.) Nutt.:32412
Erigeron strigosus Muhl.:32914
Eupatorium altissimum L.:32691
Eupatorium serotinum Michx.:32692
Helenium autumnale L.:INAI
Helianthus divaricatus L.:32413
Helianthus mollis Lam.:INAI (observed)
Liatris aspera Michx.:INAI
Parthenium integrifolium L.:32414
Ratibida pinnata (Vent.) Barnh.:33075
Silphium integrifolium Michx.:32415
Solidago nemoralis Ait.:32386
Solidago speciosa Nutt.:32693
Solidago ulmifolia Muhl.:32437
Verbesina helianthoides Michx.:32416
Boraginaceae
Lithospermum canescens (Michx.) Lehm.:32756

Caprifoliaceae
Viburnum prunifolium L.:32694
Viburnum rafinesquianum Schultes:33031

Cistaceae
Lechea tenuifolia Michx.:32417

Convolvulaceae
Calystegia spithamaea (L.) Pursh:INAI
Ipomoea pandurata (L.) G.F.W. Mey.:32418

Cornaceae
Cornus drummondii C.A. Mey.:32439
Cornus florida L.:32766

Ebenaceae
Diospyros virginiana L.:32440

Elaeagnaceae
**Elaeagnus umbellata* Thunb.:32696

Euphorbiaceae
Euphorbia corollata L.:32419

Fabaceae
Dalea candida (Michx.) Willd.:32441
Dalea purpurea Vent.:32420
Desmodium sessilifolium (Torr.) Torr. & Gray.:32442
Lespedeza capitata Michx.:32698
**Lespedeza cuneata* (Dum.-Cours.) G. Don:32699
Lespedeza violacea (L.) Pers.:32700
Lespedeza virginica (L.) Britt.:32421
**Melilotus albus* Medic.:32422
**Melilotus officinalis* (L.) Pallas:32915
**Securigera varia* (L.) Lassen:32697

Fagaceae
Quercus muhlenbergii Engelm.:32701
Quercus stellata Wangh.:32916

Gentianaceae
Frasera caroliniense Walt.:32755

Hypericaceae
Hypericum sphaerocarpum Nutt.:32423

Juglandaceae
Carya tomentosa (Poir.) Nutt.:33076

Lamiaceae
Hedeoma pulgioides (L.) Pers.:32702
Monarda bradburiana Beck.:32703
Physostegia virginiana (L.) Benth.:32704
Pycnanthemum pilosum Nutt.:32424
Pycnanthemum tenuifolium Schrad.:INAI
Scutellaria leonardii Epling:32443

Lauraceae
Sassafras albidum (Nutt.) Nees:INAI (observed)

Oxalidaceae
Oxalis violacea L.:32759

Polyalaceae
Polygala senega L.:32444
Polygala verticillata L.:INAI

Portulacaceae
Claytonia virginica L.:32761

Primulaceae
Lysimachia lanceolata Walt.:32917

Ranunculaceae
Ranunculus fascicularis Bigel:32758

Rhamnaceae
Ceanothus americanus L.:32425

Rosaceae
Amelanchier arborea (Michx. F.) Fern.:32763
Rosa carolina L.:32445

Rubiaceae
Galium circaezans Michx.:32426
Houstonia lanceolata (Poir.) Britt.:32446

Santalaceae
Comandra umbellata (L.) Nutt.:INAI (observed)

Scrophulariaceae
Agalinus tenuifolia (Vahl.) Raf.:32388
Aureolaria grandiflora (Benth.) Pennell:32427
Penstemon pallidus Small:32428

Violaceae
Viola pedata L.:32764
Viola pratincola Greene:32757

Vitaceae
Parthenocissus quinquefolia (L.) Planch.:INAI (observed)

MONOCOTS
Cyperaceae
Carex brachyglossa Mack.:33030
Carex hirsutella Mack.:32905a
Carex muhlenbergii Schk.:32429
Carex pensylvanica Lam.:32762

Iridaceae
Sisyrinchium albidum Raf.:32765

Liliaceae
Allium canadense L.:32905

Orchidaceae
Spiranthes magnicamporum Sheviak:32389

Poaceae
Agrostis gigantea Roth:32380
Agrostis hyemalis (Walt.) BSP.:32906
Bromus pubescens Muhl.:32907
Andropogon gerardii Vitman:32430
Danthonia spicata (L.) Roem. & Schultes:32705
Dichanthelium acuminatum (Sw.) Gould & Clark:32706
Dichanthelium depauperatum Muhl.:32908
Elymus x ebingeri G.C. Tucker:32382
Elymus canadensis L.:32432
Elymus histrix L.:32707
Elymus virginicus L.:32381
Festuca subverticillata (Pers.) E.B. Alexeev:32909
**Poa compressa* L.:32433
**Poa pratensis* L.:INAI (observed)
Schizachyrium scoparium (Michx.) Nash:32434
Sphenopholis obtusata (Michx.) Scribn.:33910
Sporobolus cryptandrus (Torr.) Gray:32448
Sporobolus heterolepis (Gray) Gray:32708
Tridens flavus (L.) Hitchc.:32709

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The Effects of Beaver-created Wetlands on Surface Water Quality of Lotic Habitats in Northern Illinois

Eric K. Bollinger and Brian Conklin
Department of Biological Science, Eastern Illinois University, Charleston, Illinois 61920

ABSTRACT

We examined 41 chemical parameters in water samples taken upstream and downstream from 7 beaver-created wetlands in DuPage County, IL. Overall, a significant majority of parameters (18 of 26) present in detectable amounts had lower concentrations in downstream samples, suggesting that these wetlands do serve to improve water quality by filtering out some pollutants. However, only 5 of these parameters had statistically significant differences between upstream and downstream samples (manganese, arsenic, sulfate, total solids, and calcium) and 2 of these actually had higher concentrations downstream (manganese and arsenic). This suggests that materials used by beavers in dam construction (e.g., cornstalks, treated lumber) may also serve to reduce certain aspects of water quality.

INTRODUCTION

Wetlands may act as natural filters, removing dissolved and solid pollutants from aquatic ecosystems (e..g., Whigham et al. 1988, Agovina 1990, Verhoeven et al. 2006). For example, artificial wetlands (especially those associated with wastewater treatment) remove significant quantities of various compounds including nitrogen, phosphorus, total suspended solids, and sulfates (Gersberg et al. 1984, 1986, Bowmer 1987, Breen 1990, Shutes 2001, Jiang et al. 2007, Pankratz et al. 2007). Data from natural wetlands also indicate positive effects on downstream water quality, although these effects are often complex (Richardson 1985, Whigham et al. 1988, Montreuil and Merot 2006, Verhoeven et al. 2006).

Beaver-created wetlands are one type of natural wetland that could also improve water quality in downstream areas. For example, Parker et al. (1985) found reduced concentrations of nitrogen, suspended solids, and phosphorus downstream from beaver wetlands. Other studies have also indicated that beaver ponds may reduce downstream transport of sediments, nutrients, and pollutants (e.g., Naiman and Melillo 1984, Francis et al. 1985, Maret et al. 1987, Cirmo and Driscoll 1993).

In this study, 41 chemical parameters were examined from water taken above and below beaver-created wetlands in DuPage County, Illinois. This study was designed to examine the effects that these urban populations of beavers had on water quality of the associated streams and rivers.

MATERIALS AND METHODS

Study Area

This study was conducted on lands owned by the Forest Preserve District of DuPage County in northeastern Illinois. This preserve system encompasses more than 10,100 hectares in 60 preserves. Numerous marshes, rivers, and streams within this preserve system support beaver populations.

Site Selection

Beaver wetlands used in this study were selected based on the following criteria. The site had to (1) have an active beaver colony that was maintaining a dam, (2) be located on land owned or managed by the Forest Preserve District of DuPage County, (3) be associated with a lotic ecosystem with clearly delineated single points of inflow and outflow, and (4) have no close association with other wetlands. Seven beaver-created wetlands were found that met these criteria.

Water Sample Collection

We collected surface water samples in 500 ml high-density polyethylene, wide-mouthed bottles that had been washed with 1 N HCL to remove possible contamination. One sample was taken upstream and one downstream from each beaver wetland. "Upstream" samples were collected approximately 25 m upstream from the impact area of the wetland where significant surface water movement was still occurring. "Downstream" samples were collected at least 5 m but no more than 20 m downstream from the beaver dam. We took all samples from the middle-third of the stream or river.

Analysis of Chemical Parameters (Techniques used for the first two sampling dates, 27 June and 10 July 1991)

The concentration of dissolved calcium (Ca^{++}) was determined using the EPA-approved EDTA titrimetric method with Eriochrome Black T as the indicator (APHA 1985). Concentrations of dissolved chloride (Cl^-) were determined using reagents obtained from Hach Company (Lovelend, CO) utilizing the EPA-approved silver nitrate buret titration method using 0.0141 N $AgNO_3$ (Hach Company 1989). Dissolved nitrate (NO_3^-) concentrations were determined using a hybrid method that utilized Hach's NitraVer 5 nitrate reagent powder pillow. This reagent contains the necessary chemicals to apply the cadmium reduction method for photometric determination of nitrate. The NitraVer 5 was added to 25 ml of the water sample in test tubes that had a path length of 2.5 cm after which samples were analyzed spectrophotometrically (Spec 21) to determine optical density at a wavelength of 400 nm. Samples of known nitrate concentration were used to construct a concentration vs. transmittance curve. Applying this curve, the concentrations of the samples could be determined. Dissolved sulfate (SO_4^{-2}) concentrations were determined with a hybrid method using Hach's SulfaVer 4 powder pillow and test tubes with a 2.5cm path length. Optical density at 450 nm was determined using a spectrophotometer (Spec 21) and a standard curve was constructed utilizing a stock sulfate solution (APHA 1985).

Methods used for the Entire Study. Total phosphorus (tP) concentrations were determined using Standard Methods' Ascorbic Acid Method and persulfate digestion. The pH was determined using a dO_2 meter and a Yellow Springs Instrument Company probe. Total solids (TS) were determined by using a 50 ml porcelain drying dish into which 40 ml of the water sample were added. We dried the sample in an oven overnight at 98°C, then for an additional 15 min at 110°C and then cooled the sample in a desiccator for one hr. The samples were then weighed to the nearest 0.0001 g. Total non-volatile solids were determined by ashing the TS sample in a 510°C muffle furnace for 15 min and then weighing it after it had cooled. Total volatile solids (TVS) were determined by taking the mathematical difference between TS and TNVS.

Methods used for the last 6 sampling dates. Concentrations of dissolved arsenic (As) and dissolved cadmium (Cd) were determined using inductively coupled plasma/mass spectrometry (ICP/MS; method 6010-EPA-5w846). Other dissolved metal concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; method 6020-EPA-5w846). Beginning with the sample collected 16 July 1991 (the third sample), dissolved sulfate, dissolved chloride, dissolved nitrate, dissolved fluoride, and dissolved bromide concentrations were determined using ion chromatography (IC; method 300-Mcaww-EPA).

The EPA-approved ICP/MS, ICP-OES, and IC methods were performed by Dave Roberts of the Enesco Rocky Mountains Analytical Laboratory in Arvada, CO. Samples were sent by overnight express mail in a 0°C cooler and preserved with nitric acid upon receipt by the lab. Table 1 summarized the parameters we examined, the method utilized for analysis, and the detection limits for each.

Table 1. Water quality parameters examined from water samples collected at beaver-created wetlands in DuPage County, IL. Detection limits in ppm except pH.

Parameter (symbol)	Detection limit	Detection Method
Aluminum (Al)	0.009	ICP-OES
Silver (Ag)	0.002	ICP-OES
Arsenic (As)	0.001	ICP/MS
Barium (Ba)	0.003	ICP-OES
Beryllium (Be)	0.002	ICP-OES
Boron (B)	0.005	ICP-OES
Bromide (Br-)	0.2	IC
Cadmium (Cd)	0.001	ICP/MS
Calcium (Ca)	0.1/0.01	Titrimetric/ICP-OES
Chloride (Cl-)	5/3	$AgNO_3$ Titrimetric/OC
Chromium (Cr)	0.006	ICP-OES
Cobalt (Co)	0.004	ICP-OES
Copper (Cu)	0.004	ICP-OES
Iron (Fe)	0.007	ICP-OES
Fluoride (F-)	0.1	IC
Lead (Pb)	0.01	ICP-OES
Lithium (Li)	0.002	ICP-OES
Magnesium (Mg)	0.008	ICP-OES
Manganese (Mn)	0.003	ICP-OES
Molybdenum (Mo)	0.005	ICP-OES

Parameter (symbol)	Detection limit	Detection Method
Nickel (Ni)	0.004	ICP-OES
Nitrate (NO_3^-)	0.1	NitraVer 5/IC
Dissolved Phosphorus (dP)	0.09	ICP-OES
Total Phosphorum (tP)	0.1	Ascorbic Acid
Potassium (K)	0.2	ICP-OES
Antimony (Sb)	0.02	ICP-OES
Selenium (Se)	0.05	ICP-OES
Silica Oxide (SiO_2)	0.2	ICP-OES
Strontium (Sr)	0.02	ICP-OES
Tin (Sn)	0.03	ICP-OES
Sodium (Na)	0.1	ICP-OES
Sulfate (SO_4^{2-})	5	IC
Thallium (Tl)	0.5	ICP-OES
Titanium (Ti)	0.003	ICP-OES
Vanadium (V)	0.004	ICP-OES
Zinc (Zn)	0.002	ICP-OES
Total Solids (TS)	25	Standard Methods
Total Volatile Solids (TVS)	25	Standard Methods
Total Non-volatile Solids (TNVS)	25	TS-TVS
pH	0.1 units	Field pH meter
Dissolved Oxygen (dO_2)	0.1	YSI meter and probe

Data Analysis

We sampled all 7 wetlands for the first two sampling dates (27 June and 10 July 1991), after which the dam associated with one wetland was destroyed. Water levels became low enough to preclude sampling in 3 other wetlands after the second sampling date as a result of a drought. Because of this situation and the fact that certain methods varied between the first two and last 6 sampling dates, we analyzed our data as 2 separate balanced data sets. The first data set ("data set 1") consisted of 7 wetlands sampled over two dates. The second data set ("data set 2") consisted of 3 wetlands sampled over the last 6 sampling dates (for most parameters) and all 8 sampling dates for calcium, chloride, nitrate, sulfate, pH, dissolved oxygen, and total solids. We used repeated-measures ANOVA to analyze both data sets, with sampling date as the repeated measure. Chemical parameters were analyzed separately. However, the following chemical parameters were typically below detection limits and so were excluded from the statistical analyses: silver (Ag), beryllium (Be), chromium (Cr), cobalt (Co), lithium (Li), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), thallium (Tl), titanium (Ti), and vanadium (V).

RESULTS

Data set 1 (7 wetlands, 2 dates)

We failed to find any significant differences between upstream and downstream concentrations for this data set (ANOVA, all $F < 3.75$, $P > 0.05$), although sulfate concentrations were marginally lower downstream ($F = 3.74$, $P = 0.10$).

Data set 2 (3 wetlands, 6 dates)

Five parameters showed significant differences between upstream and downstream concentrations (ANOVA, $P < 0.05$, Table 2). Three parameters (SO_4^{-2}, TS, and Ca) had lower average concentrations downstream and two (Mn and As) had higher average concentrations downstream. Overall, 18 parameters had lower average concentrations downstream, 8 had higher concentrations downstream and one (pH) was the same (chi-square = 3.85, df = 1, $P < 0.05$).

Table 2. Comparison of upstream and downstream concentrations of water quality parameters for 3 beaver-created wetlands over 6 sampling dates. All concentrations are in ppm (except pH); see Table 1 for key to parameter abbreviations.

Parameter	Mean Upstream Concentration	Mean Downstream Concentration	Percent Difference	Sig. of F-Statistic
Mn	0.0205	0.3281	+1500*	0.02**
As	0.022	0.050	+127	0.02**
SO_4^{2-}	114.7	85.5	-25	0.04**
TS	1590	1254	-21	0.05**
Ca	114.4	98.6	-14	0.09**
Fe	0.0292	0.0920	+215	0.16
Al	0.0267	0.0519	+94	0.22
Cl-	258	317	+23	0.024
Na	259.8	197.4	-24	0.27
Mg	55.08	47.88	-13	0.32
Mo	0.0125	0.0105	-16	0.40
Zn	0.0121	0.0092	-24	0.40
tP	0.5194	0.6694	+29	0.41
F-	1.3429	1.1095	-17	0.41
Sr	0.5205	0.4248	-18	0.53
B	0.3240	0.2902	-10	0.58
Ba	0.1920	0.1629	-16	0.59
NO_3^-	1.0343	1.2014	+16	0.65
K	6.7571	6.2095	-8	0.67
Cu	0.009	0.008	-11	0.67
Br-	0.2571	0.2381	-7	0.73
Ni	0.0041	0.0039	-5	0.73
dO_2	5.2	5.1	-2	0.73
dP	0.6743	0.6114	-2	0.76
pH	7.4	7.4	0	0.83
Cd	0.018	0.016	-11	0.84
SiO_2	4.9429	4.9571	+0	0.99

*'+' denotes higher concentration downstream; '-' denotes lower concentration downstream

**P < 0.10, ANOVA

DISCUSSION

Wetlands are thought to act as biological filters, removing dissolved and solid pollutants and improving water quality (Feeney and Morrell 1985, Hair 1986, Agovina 1990, Verhoeven et al. 2006). Thus, our prediction was that beaver wetlands would improve water quality by lowering concentrations of pollutants in our downstream samples. However, most parameters did not differ significantly upstream and downstream from beaver wetlands. Of those parameters that did differ 2 of the 5 (Mn and As) actually had higher concentrations downstream. Arsenic used to be a common preservative added to treated lumber, so if beavers imported treated lumber in the construction of their dams, leaching from this material may explain our results. Given the proximity of our beaver wetlands to human development, this is certainly possible. However, we did not notice any type of finished lumber in the beaver dams in this study, and neither chromium nor copper were similarly elevated downstream (other elements used in treated lumber; Lebow et al. 1999), so the explanation of this result remains problematic. However, arsenic was used in pesticides prior to 1993, and can be taken up by corn in measurable concentrations (Parsons et al. 2008). In addition, manganese is found in corn, and beavers are known to commonly eat corn in the Midwest. Furthermore, we have seen cornstalks used in beaver dam construction.

Although our small sample of wetlands probably contributed to our lack of significant results, we did find that a significant majority of parameters had lower concentrations downstream. This suggests that our beaver-created wetlands do serve to filter out some pollutants. However, items brought into the aquatic system by beavers (e.g., corn, treated lumber?) may also serve to reduce water quality downstream from these wetlands.

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LITERATURE CITED

Agovina, A. V. 1990. Wetland identification: a means to prevent potential public health problems. Journal of Environmental Health 52:280-281.

APHA AWWA WPCF. 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed., Washington, D.C.

Bowmer, K. H. 1987. Nutrient removal from effluents by an artificial wetland: influence of rhizosphere aeration and preferential flow studied using bromide and dye tracers. Water Research 21:591-599.

Breen, P. F. 1990. A mass balance method for assessing the potential of artificial wetlands for wastewater treatment. Water Research 24:689-697.

Cirmo, C. P. and C. T. Driscoll. 1993. Beaver pond biogeochemistry: acid neutralizing capacity generation in a headwater wetland. Wetlands 13:277-292.

Feeney, P. and B. Morrell. 1985. Wetlands disposal is sewage option. American City & County March 1985:91-92.

Francis, M. M., R. J. Naiman, and J. M. Melillo. 1985. Nitrogen fixation in beaver (*Castor canadensis*) influenced subarctic streams. Hydrobiologia 121:193-202.

Gersberg, R. M., B. V. Elkins, and C. R. Goldman. 1984. Use of artificial wetlands to remove nitrogen from wastewater. Journal of Water Pollution Control Federation 56:152-156.

Gersberg, R. M., B. V. Elkins, S. R. Lyon, and C. R. Goldman. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Research 20:363-368.

Hach Company. 1989. Water analysis handbook. Hach Company, Loveland, CO.

Hair, J. D. 1986. Who cares about wetlands? EPA Journal 12:14-15.

Jiang, C., X. Fan, G. Cui, and Y. Zhang. 2007. Removal of agricultural non-point source pollutants by ditch wetlands: implications for lake eutrophication control. Hydrobiologia 581:319-327.

Lebow, S. T., D. O. Foster, and P. K. Lebow. 1999. Release of copper, chromium, and arsenic from treated southern pine exposed in seawater and freshwater. Forest Produces Journal 49:80-89.

Maret, T. J., M. Parker, and T. E. Fannin. 1987. The effect of beaver ponds on the nonpoint source water quality of a stream in southwestern Wyoming. Water Research 21:263-268.

Montreuil, O. and P. Merot. 2006. Nitrogen removal in valley bottom wetlands: assessment in headwater catchments distributed throughout a large basin. Journal of Environmental Quality 35:2113-2122.

Naiman, R. J. and J. M. Melillo. 1984. Nitrogen budget of subarctic stream altered by beaver (*Castor canadensis*). Oecologia 62:150-155.

Pankratz, S., T. Young, H. Cuevas-Arellano, R. Kumar, R. F. Ambrose, and I. H. Suffet. 2007. The ecological value of constructed wetlands for treating urban runoff. Water Science and Technology 55:63-69.

Parker, M., F. J. Wood, Jr., B. H. Smith, and R. G. Elder. 1985. Erosional downcutting in lower order riparian ecosystems: have historical changes been caused by the removal of beaver? *In* Riparian Ecosystems and their Management: Reconciling Conflicting Uses. (Edited by R. R. Johnson, C. D. Ziebell, D. R. Patton, P. F. Ffolliott, and R. H. Hamre), pp. 35-38. First North American Riparian Conference; April 16-18; Tucson, Ariz. Gen. Tech. Rep. RM-120. USDA, Forest Serv., Rocky Mt. Forest and Range Expt. Station. Ft. Collins, CO.

Parsons, J. G., A. Martinez-Martinez, J. R. Peralta-Videa, and J. L. Gardea-Torresday. 2008. Speciation and uptake of arsenic accumulated by corn seedlings using XAS and DRC-ICP-MS. Chemosphere 70:2076-2083.

Richardson, C. J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. Science 226:1424-1427.

Shutes, R. B. E. 2001. Artificial wetlands and water quality improvement. Environment International 26:441-447.

Verhoeven, J. T. A., B. Arheimer, C. Yin, and M. H. Hefting. 2006. Regional and global concerns over wetlands and water quality. Trends in Ecology and Evolution 21:96-103.

Whigham, D. F., C. Chitterling, and B. Palmer. 1988. Impacts of freshwater wetlands on water quality: a landscape perspective. Environmental Management 12:663-671.

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Discovery of New Localities for the Threatened Kirtland's Snake (*Clonophis kirtlandii*) in Central Illinois

Angelo P. Capparella[1*], Todd Springer[2], and Lauren E. Brown[1]
[1]School of Biological Sciences, Illinois State University
Campus Box 4120, Normal, IL 61790-4120
[2]1835 E. Lafayette St., Bloomington, IL 61701
[*]Correspondence: apcappar@ilstu.edu

ABSTRACT

Three localities (two new, one reconfirmed) in central Illinois with habitat and behavioral observations are described for Kirtland's snake which is quite rare throughout its range in the U.S.A.

INTRODUCTION

The Illinois State Threatened Kirtland's snake *Clonophis kirtlandii* (Kennicott, 1856) is one of the rarest snakes in the Midwest because of the nearly complete destruction of native prairie, its primary former habitat. This small snake is semifossorial and tends to occupy crayfish burrows (Anton et al., 2003) in damp, open areas. The species can be easily identified by its red or orange belly bordered by a single row of round black spots on each side (Phillips et al., 1999). The purpose of the red belly is unknown. It is questionable if aposematic coloration is involved as the snake is not known to be poisonous and is reluctant to bite. When first captured, the snake flattens its body like a thin ribbon. Known natural food includes earthworms, slugs, water striders, leeches, and crayfish (Conant, 1943; Minton, 1944; Bavetz, 1993; Thurow, 1993).

The range of *C. kirtlandii* was probably best shown in Conant and Collins (1998, map p. 305), although new records have extended its range southward into northwestern Tennessee (Frymire and Scott, 2012). The species has been found from eastern Missouri to western Pennyslvania, and from northeastern Illinois and southern Michigan to northwestern Tennessee. In Illinois its range encompassed much of the central and northeastern portions of the State. Most of the distribution in Illinois and elsewhere occurred within the historic Prairie Peninsula (Transeau, 1935). By the 1890s the species had become rare in the northern half of Illinois (Garman, 1892). Brown et al. (1975) suggested that the populations in Illinois may be relictual and that the species might be becoming extinct in the western portion of its range. Since 1975, a few additional relictual localities have been reported in Illinois (e.g., Brown, 1987), always based on only one or a few specimens. The purpose of this paper is to report two new records and the confirmation of a nearly half-century old record in central Illinois with habitat notes and behavioral observations.

NEW AND RECONFIRMED LOCALITY RECORDS

Living specimens were captured, photographed, and released unharmed at the following three localities in southern McLean County.

1. NW ¼, NE ¼, Sec. 26, T22N, R2E, Randolph Township, 4 km (2.5 mi) NE Heyworth. One individual was captured and photographed by T. Springer 16 October 2010 in a small grassy area adjacent to a small creek fed by a spring. The creek is a tributary of Kickapoo Creek. The snake was in leaf litter and nearby there were crayfish burrow chimneys. Old field-savannah is north of the site and a nearly pristine mature woodland occurs on a hillside south of the creek. When an attempt was made to pick up the snake, it flattened its body. Another sighting was made in the summer of ca. 2001 in mowed grass about 20 m (65.6 ft) north of the creek.

2. SE ¼, NE ¼, Sec. 19, T22N, R1E, Funks Grove Township, 2.4 km (1.5 mi) SW Funks Grove. One individual was caught and photographed 6 June 2004 by E. Smith and A. Funk in an old field (former pasture) adjacent to the parking lot of the Sugar Grove Nature Center. A small intermittent creek and outhouse are located near the capture site. Subsequent inspection of this creek revealed crayfish burrow chimneys. Another *C. kirtlandii* was seen by R. Carriger on 12 September 2011 along a small intermittent creek that receives flow from the aforementioned creek. Another sighting by S. Marshall occurred on 20 September 2011, not far from the September 12 sighting along this second intermittent creek. This snake was photographed by J. Tobias and measured at 38 cm (14.9 in) in total length. It was moving in a shaded area near a group of tree stumps and logs approximately 9 m (29.5 ft) from the creek and not far from a playground. The last documented sighting was 20 May 2012. It was found in a mulch pile by a young girl near the Marshall site and photographed by M. Litwiller. Habitats in the nearby area include restored prairie, old field in various successional stages, streams, former pasture or tilled areas, and open woodland.

3. SE ¼, SW ¼, Sec. 35, T23N, R4E, Dawson Township, 7 km (4.4 mi) NE LeRoy. K. Harness caught one individual and photographed it on 9 April 2011. Another capture was photographed on 14 August 2011. Both were found under rocks a considerable distance from each other (ca. 222 m [728 ft]) at the dam for Dawson Lake in Moraine View State Recreation Area. Both were estimated to be 20.3-25.4 cm (8-10 in) in total length. The specific habitat at the two sightings is under riprap for erosion control at the dam. A road runs across the dam with dense forest and cleared areas nearby. Numerous other potential habitats (marshes, streams, pond, old fields, former pasture, other prairie-like habitats) occur in the area.

DISCUSSION

Numerous species of snakes in eastern North America flatten their body when alarmed (Conant and Collins, 1998). This behavior is particularly well developed in *C. kirtlandii* in both frequency and thinness of body. Often this is interpreted as a defensive reaction to make the body appear larger in dorsal view, and hence more threatening to a potential predator. The body flattening has also been noted by Tucker (1994) to enhance the ability of *C. kirtlandii* to utilize small subterranean crevices. At the Randolph Township site, T.S. attempted to pick up the newly encountered *C. kirtlandii* which was exhibiting flattening behavior. However, this proved to be rather difficult because T.S. was wearing gloves at the time. Thus, another potential function of flattening behavior may be to inhibit a potential predator from picking up and devouring the *C. kirtlandii*.

There are two other historical localities known for McLean County: (1) Normal: Garman (1892), no voucher specimen; UIMNH 4962, no other information; BMNH 93.1.2.1-2 (Conant 1943); and (2) Lake Bloomington: Holman (1966), no voucher specimen. In addition, there is an older record for Dawson Lake in Moraine View State Recreation Area, ISUC 703, 5 May 1966, J.A. Holman collector. It is likely that the Normal population is extinct because of intense urbanization. However, the Lake Bloomington region encompasses a relatively large area that has numerous habitats which might be suitable for and inhabited by *C. kirtlandii*. We do not know the exact location of Holman's record from Dawson Lake.

Bavetz (1993, 1994) indicated there was a total of 70 known locality records for *C. kirtlandii* in Illinois. He surveyed 19 of these sites, and 14 other sites near known localities that appeared to have appropriate habitat to support the species. Living *C. kirtlandii* were seen at only two of the old sites and only one new locality was found. Bavetz (1994) suggested that only eight localities "support reproducing populations" based on voucher specimens or photographic slides. In light of Bavetz's findings, our report of living *C. kirtlandii* sighted multiple times at two new localities and one older locality in McLean County take on considerable significance because of the Threatened status of the species in Illinois.

In the species description, Kennicott (1856: 96) commented briefly on early habitat of *C. kirtlandii* in northern Illinois: "The few specimens obtained have been found in the woods, generally under logs." Garman (1892: 275) commented further on early habitat in Illinois: "A handsome snake, which ten years ago was not uncommon along prairie brooks, in the central part of the State. Tiling, ditching, and cultivation of the soil have destroyed its haunts and nearly exterminated it...I have never seen it elsewhere than on the open prairie."

Mid-20th century habitat for *C. kirtlandii* was described in Conant's (1943: 328) monograph as: "essentially an inhabitant of open country...does occur in woods, but it is far more abundant in prairie-like situations." Later, Conant's (1958) field guide indicated that the species usually occurs in wet meadows.

We suggest that many present habitats of *C. kirtlandii* may be primarily successional and degraded derivatives (including extremely disturbed urbanized areas) of historical wet prairies. In non-urban regions they may be most frequently found in small, open grassy areas that are wet or adjacent to small watercourses or ponds. Some might also be found in wooded areas (possibly old fields). Presence of crayfish burrows or previously constructed burrows of other animals are also of importance (Tucker, 1994).

The primary cause of the decline of *C. kirtlandii* is probably the pervasive destructive effects of agriculture on the snake's habitat, with urbanization being a secondary cause. However, there are many possible factors that have had a role in the global decline of reptiles (Gibbons et al., 2000). Two or more of these may have acted together in a detrimental manner causing the decline of *C. kirtlandii.*

Clonophis kirtlandii is classified as a State Threatened Species in Illinois and Ohio, and State Endangered in Indiana, Kentucky, Michigan and Pennsylvania (Ernst and Ernst, 2003; Gibson and Kingsbury, 2004). The U.S. Fish and Wildlife Service (Dodd et al., 1985) placed *C. kirtlandii* in its Category 2 (possibly appropriate for federal listing as Endangered or Threatened but more information may be needed). Furthermore, there is apparently no conservation program specifically targeting *C. kirtlandii* anywhere throughout its range (Gibson and Kingsbury, 2004).

McLean County might be an appropriate county to initiate a conservation program, as four of the records (our three plus Lake Bloomington area) occur in areas with suitable, or potentially suitable habitat (nature preserves, undeveloped land owned by the City of Bloomington, sympathetic land owner). Furthermore, the occurrence of two or more sightings at each of the three localities we report suggests the existence of extant populations. Another locality occurs in Woodford County near the McLean County line in a pasture but otherwise relatively undisturbed “wet meadow” (Brown, 1987). Other counties that have a number of locality records (Champaign, Cook) may also have several areas suitable for conservation programs of *C. kirtlandii.* If conservation programs are not soon activated by governmental agencies or the public, this small innocuous snake may become extinct.

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Specimens examined.—ISUC 703, Dawson Lake, McLean Co., IL, 5 May 1966, J. A. Holman; UIMNH 4962 Normal, McLean Co., IL [no date or collector given]. Color photos of the snakes (showing key characters) from the Dawson Lake, Funks Grove and Heyworth localities were deposited with the Illinois Natural History Survey and Illinois State University Collection. Museum abbreviations: BMNH British Museum (Natural History); ISUC Illinois State University Collections; UIMNH University of Illinois Natural History Museum.

LITERATURE CITED

Anton, T.G., D. Mauger, C.A. Phillips, M.J. Drezlik, J.E. Petzing, A.R. Kuhns, and J.M. Mui. 2003. *Clonophis kirtlandii* (Kirtland’s snake). Aggregating behavior and site fidelity. Herpetol. Rev. 34:248-249.

Bavetz, M. 1993. Geographic variation, distribution, and status of Kirtland’s snake, *Clonophis kirtlandii* (Kennicott) in Illinois. M.S. Thesis, Southern Ill. Univ. Carbondale.

Bavetz, M. 1994. Geographic variation, status, and distribution of Kirtland’s snake (*Clonophis kirtlandii* Kennicott) [*sic*] in Illinois. Trans. Ill. St. Acad. Sci. 87:151-163.

Brown, L.E. 1987. A newly discovered population of Kirtland's snake with comments on habitat and rarity in central Illinois. Bull. Chicago Herpetol. Soc. 22:32-33.

Brown, L.E., R.S. Funk, D. Moll, and J.K. Tucker. 1975. Distributional notes on reptiles in Illinois. Herpetol. Rev. 6:78-79.

Conant, R. 1943. Studies on North American water snakes–1 *Natrix kirtlandii* (Kennicott). Am. Midl. Nat. 29:313-341.

Conant, R. 1958. A Field Guide to Reptiles and Amphibians of the United States and Canada East of the 100th Meridian. Houghton Mifflin Co., Boston.

Conant, R. and J.T. Collins. 1998. A Field Guide to Reptiles & Amphibians Eastern and Central North America. 3rd ed. Houghton Mifflin Co., Boston and New York.

Dodd, C.K., Jr., G.E. Drewry, R.M. Nowak, J.M. Sheppard, and J.D. Williams. 1985. Endangered and threatened wildlife and plants; review of vertebrate wildlife; notice of review. Part III. U.S. Dept. Interior, Fish and Wildlife Serv., Federal Register 50:37958-37967.

Ernst, C.H. and E.M. Ernst. 2003. Snakes of the United States and Canada. Smithsonian Books, Washington, D.C. and London.

Frymire, D. and A.F. Scott. 2012. *Clonophis kirtlandii* (Kirtland's Snake). Herpetol. Rev. 43:447.

Garman, H. 1892. A synopsis of the reptiles and amphibians of Illinois. Bull. Ill. St. Lab. Nat. Hist. Vol. III, Art. XIII, pp. 215-389.

Gibbons, J.W., D.E. Scott, T.J. Ryan, K.A. Buhlmann, T.D. Tuberville, B.S. Metts, J.L. Greene, T. Mills, Y. Leiden, S. Poppy, and C.T. Winnie. 2000. The global decline of reptiles, déjà vu amphibians. BioScience 50:653-666.

Gibson, J. and B. Kingsbury. 2004. Conservation assessment for Kirtland's snake (*Clonophis kirtlandii*). U.S.D.A. Forest Service, Eastern Region, Milwaukee, 29 pp.

Holman, J.A. 1966. Herpetological records from northcentral Illinois. Trans. Ill. St. Acad. Sci. 59:298-300.

Kennicott, R. 1856. Description of a new snake from Illinois. Proc. Acad. Nat. Sci. Philadelphia 8:95-96.

Minton, S., Jr. 1944. Introduction to the study of the reptiles of Indiana. Am. Midl. Nat. 32:438-477.

Phillips, C.A., R.A. Brandon, and E.O. Moll. 1999. Field Guide to Amphibians and Reptiles of Illinois. Manual 8. Ill. Nat. Hist. Surv., Champaign.

Thurow, G.R. 1993. *Clonophis kirtlandii* (Kirtland's snake). Diet. Herpetol. Rev. 24:34-35.

Transeau, E.N. 1935. The prairie peninsula. Ecology 16:423-437.

Tucker, J.K. 1994. A laboratory investigation of fossorial behavior in Kirtland's snake, *Clonophis kirtlandii* (Kennicott) (Serpentes: Colubridae), with some comments on management of the species. Bull. Chicago Herpetol. Soc. 29:93-94.

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The Role of Oil Content and Size in Seed Selection by Wild Birds

Kathleen A. Collins and David Joseph Horn[1]
[1]Department of Biology, Millikin University
1184 W. Main St., Decatur, IL 62522, USA

ABSTRACT

We determined whether birds actively select specific varieties of a single seed species, whether seed selection is based on oil content, how birds can distinguish seeds based on oil content, and if seed preferences are equivalent among species. Three trials were conducted from winter 2007-summer 2007 at Rock Springs Environmental Center in Decatur, IL. During each trial, seed consumption and bird abundance of each species at each seed variety of black-oil sunflower (*Helianthus annuus*) were recorded. In all trials, seed consumption of the highest oil content seed was 31-49% greater than any other seed variety. House Finch (*Carpodacus mexicanus*), American Goldfinch (*Spinus tristis*), and House Sparrow (*Passer domesticus*) preferred seeds with a higher oil content when seed size was equivalent, but selected shorter, deeper seeds with a lower oil content at equivalent levels to larger seeds with a higher oil content when both varieties were present. Knowing the factors influencing seed choice allows for the development of seed varieties that will be more attractive to songbirds.

INTRODUCTION

Previous studies have examined seed species preferences of wild birds that use feeders (Geis 1980, Horn et al. 2002). Few studies have investigated whether birds choose among multiple varieties of the same seed species, and why birds prefer the seeds of the variety they select. Factors influencing seed choice may include nutritional content of the food (Schaefer et al. 2003), seed size (Willson 1972), bill size (Hespendeide 1966), and behavior of the bird (Foster 2008). In a study of seed preferences of Henslow's Sparrows (*Ammodramus henslowii*), two of the preferred seed species had higher energy content, while two of the avoided seed species had the lowest content, indicating that nutrition could play a role in seed selection (DiMiceli et al. 2007). Birds have also selected seed species based on the handling time of a particular species, which in turn, was dependent on the size and shape of the bill (Hrabar and Perrin 2002). When selecting among seeds of a single species, Black-capped Chickadees (*Poecile atricapillus*) and Red-breasted Nuthatches (*Sitta canadensis*) selected sunflower seeds based on weight (Heinrich et al. 1997). Weighing sunflower seeds could be beneficial to birds in order to minimize the energy costs of foraging while maximizing the reward (Lima 1985).

We studied four questions associated with why wild birds select specific seeds: (1) Do birds actively select varieties of the same seed species? (2) Do birds select seeds based on nutritional content? (3) Is nutritional content related to seed size? (4) Are there differences among species in regards to seed preference? We hypothesized that birds will distinguish among seed varieties of the same species, bird abundance would be greater at seed varieties with greater oil content, and that seed morphology is related to oil content.

In 2006, over 55 million Americans over the age 16 spent more than $4.1 billion on bird food, feeders, and other products (U.S. Fish and Wildlife Service 2007, 2008). Conducting studies on whether birds prefer certain seed varieties over others may lead to a better bird feeding experience if preferred varieties are introduced to bird seed blends. In turn, attracting a greater diversity of birds to one's yard may elevate awareness for the natural world, increase participation in outdoor recreation, and motivate individuals to become involved in conservation issues at a time when participation in some nature-based activities is declining (Pergams and Zaradic 2008, U.S. Fish and Wildlife Service 2009).

METHODS

Procedures

Our study consisted of three trials conducted from winter–summer 2007 at Rock Springs Environmental Center in Decatur, Illinois. Tubular bird feeders were utilized (Droll Yankees B-7R), and there were three or four feeders used, each filled with a different variety of the same species of black-oil sunflower seed, depending on the trial. Black-oil sunflower was selected as it is one of the most preferred seed types among species that commonly use feeders (Geis 1980, Horn et al. 2002). Feeders were hung from a cable approximately 3 m off the ground, and were placed approximately 1 m apart from each other in a straight line. Feeders were rotated in a systematic manner on a weekly basis to ensure that birds were selecting the seed variety not feeder location (Horn et al. 2003), and the initial location of each seed variety within a feeder was determined randomly. Feeder observations took place while seated on a bench looking through a large viewing window. The feeders were approximately 3 m from the window.

To determine whether birds select seeds based on nutritional content and whether there are differences among species in regards to seed preferences, three trials were conducted. Trial 1 examined how seed consumption and relative abundance of birds differed among bird feeders filled with three varieties of small black-oil sunflower seeds (where small seeds are defined as seeds that fit through a 0.5 cm hole [seeds were provided by and sorted at D&D Commodities Ltd., Greeley, CO, USA]). Three oil contents were used: 40.2, 43.6, and 51.4% (oil contents were determined at Eurofins Scientific Inc., Des Moines, IA, USA). Trial 1 ran from 26 Feb.–6 Apr. 2007.

Trial 2 examined seed consumption and bird abundance at feeders filled with large black-oil sunflower seeds of varying oil contents (where large seeds are defined as seeds that cannot fit through a 0.5 cm hole). Three oil contents were used: 37.8, 41.1, and 48.1%. The trial ran from 6 Apr.–18 May 2007.

Trial 3 examined bird use of sunflower seeds of varying size and oil content. Four seed types were used: small 43.6, large 41.1, small 51.4, and large 48.1%. The trial ran from 18 Jun.–9 Aug. 2007.

Consumption of each seed variety was recorded as the difference in total mass of the seed at the beginning and end of each trial. For each trial, bird abundance was determined through monitoring sessions. Specifically, the number of each bird species present at each feeder was recorded every 5-min during 60-min monitoring sessions, and the means for each session were calculated. Approximately 3 monitoring sessions took place each week with 18, 15, and 24 monitoring sessions being conducted for Trials 1, 2, and 3, respectively. Observations took place from 08:00–17:00. Because relationships between bird abundance and preference, and seed consumption and preference, are similar (Horn 1995), measures of both bird abundance and seed consumption were used as indicators of preference for each trial.

To determine if nutritional content is related to seed size, we took multiple measures of each seed variety. We measured the depth, length, width, and weight of 75 seeds of each seed variety using digimatic calipers (Mitutoyo CD-6" CSX) and an analytical balance (Mettler AJ100).

To examine whether birds actively select varieties of the same seed species, we conducted another study concurrent with Trial 3. The study involved observing seed choice of individual birds immediately after feeders were rotated (Fridays) compared to seed choice after birds were adjusted to the feeders' new positions (Mondays and Thursdays), allowing us to determine if birds change the feeder they visit when the preferred seed variety is moved to a different feeder location. Recordings of seed choice by individual birds were made every 30 sec in 45-min sessions performed every Friday, Monday, and Thursday. To determine which bird to observe, six species were placed into a priority list in random order, and the species of the highest priority present in the feeder area was selected to be observed during the 30-sec interval. Upon selecting a bird to monitor, the first seed choice of the individual was recorded.

For our study, we did not put color bands on birds, and it is possible that some birds were double counted. The number of birds frequenting a feeding station, however, can be very large. Geis and Pomeroy (1993) captured birds in a single yard using mist nets and funnel traps within a lightly developed area in Clarksville, Maryland. In the summer of 1989, they established population estimates ranging from 14 for Tufted Titmouse (*Baeolophus bicolor*) to 5,798 for House Finch (*Carpodacus mexicanus*) with an estimated population size of all feeder birds combined of 6,937 individuals. In our study, the mean number of bird visits per monitoring session in Trial 3 (conducted during summer) was 151. Thus, if Geis and Pomeroy's estimate of 6,937 individuals was representative of our study site, a minimum of 46 monitoring sessions would need to be conducted each trial to record each individual within the population.

Statistical analysis

Only species with > 30 observations per trial were used in analyses. To determine whether seed choice was equivalent among the four seed types before and after feeders were rotated, chi-square tests were performed to compare the frequency each seed variety was selected by individual birds immediately after feeders were rotated (Fridays) compared to the period when birds had time to adjust to the new positions (Mondays and Thursdays combined). Chi-square tests were used because our response variable was the number of times individual birds selected a particular seed variety first as opposed to a response variable such as the mean number of bird visits to a seed variety immediately after rotation compared to 3-5 days after rotation. *P*-values < 0.05 were considered significantly different. To determine whether bird abundance of each species, total number of individuals of all species combined, and species richness per 60-min monitoring session differed among seed types, 95% confidence intervals (CIs) were calculated (Di Stefano 2004), as CIs provide both measures of uncertainty and effect size among treatments (where the seed varieties are the treatments, Johnson 1999). CIs were also calculated to identify differences in seed shape and weight among seed varieties. Treatments with non-overlapping CIs were considered significantly different.

RESULTS

Do birds actively select varieties of the same seed species?

During our examination of whether birds actively select seed varieties (performed concurrently with Trial 3), six species had > 30 observations. All six species, Black-capped Chickadee ($N = 83$, $X^2 = 166.4$, df = 3, $P < 0.001$), Tufted Titmouse (*Baeolophus bicolor*, $N = 38$, $X^2 = 45.7$, $P < 0.001$), White-breasted Nuthatch (*Sitta carolinensis*, $N = 48$, $X^2 = 11.6$, $P = 0.009$), House Finch ($N = 106$, $X^2 = 19.4$, $P < 0.001$), American Goldfinch ($N = 96$, $X^2 = 35.8$, $P < 0.001$), and House Sparrow ($N = 49$, $X^2 = 62.1$, $P < 0.001$), had differences in the frequency of visits to a seed variety after adjusting to the seed variety being in a new position (Table 1, see Fig. 1 as a representative example).

Table 1. For six bird species, frequency of seed choice among four varieties of black-oil sunflower (Small - 43.6, Large - 41.1, Small - 51.4, and Large - 48.1) differed between when feeders were first rotated (Friday) compared to after rotation (Monday and Thursday).

	Small - 43.6		Large - 41.1		Small - 51.4		Large - 48.1	
Species	F[A]	M/T	F	M/T	F	M/T	F	M/T
AMGO[B]	0.19	0.25	0.22	0.06	0.52	0.62	0.07	0.07
BCCH	0.39	0.07	0.26	0.22	0.13	0.22	0.22	0.50
HOFI	0.19	0.28	0.27	0.13	0.35	0.42	0.19	0.17
HOSP	0.14	0.34	0.14	0.23	0.43	0.34	0.29	0.09
TUTI	0.44	0.21	0.00	0.10	0.11	0.28	0.44	0.41
WBNU	0.07	0.11	0.21	0.11	0.21	0.26	0.52	0.53

[A] F = Friday, M/T = Monday/Thursday.

[B] AMGO = American Goldfinch, BCCH = Black-capped Chickadee, HOFI = House Finch, HOSP = House Sparrow, TUTI = Tufted Titmouse, and WBNU = White-breasted Nuthatch.

Do birds select seeds based on nutritional content?

Seed consumption was used to determine whether birds select seeds based on oil content. During Trial 1, the greatest consumption of small seed occurred at the highest oil content seed (51.4%) with consumption of the 51.4% oil content seed at 21 kg, 40.2 at 16 kg, and 43.6 at 13 kg. During Trial 2, the greatest consumption also occurred at the seed with the highest oil content (48.1%) with 20 kg consumed followed by 41.1 at 14 kg, and 37.8 at 11 kg.

During Trial 1, no significant differences in bird abundance were observed among the three small-seed varieties with five species having > 30 observations: Downy Woodpecker (*Picoides pubescens*, N = 106), Black-capped Chickadee (N = 63), House Finch (N = 81), American Goldfinch (N = 691), and House Sparrow (N = 45).

No significant differences in bird abundance were observed during Trial 2 among the three large-seed varieties with three species being observed >30 times: Rose-breasted Grosbeak (*Pheucticus ludovicianus*, N = 61), House Finch (N = 48), and American Goldfinch (N = 197).

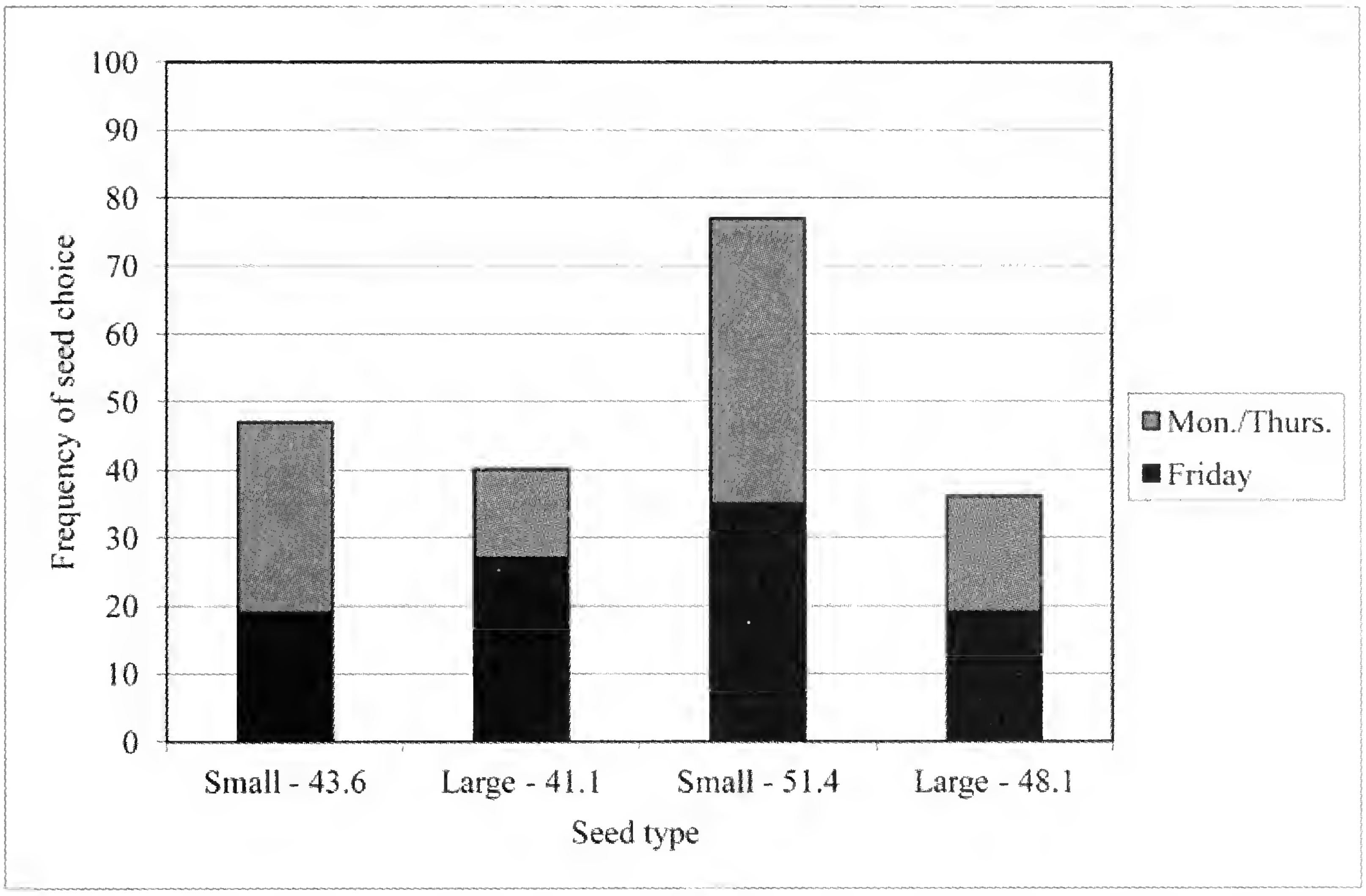


Figure 1. House Finch seed choice differed among four varieties of black-oil sunflower that varied in size and % oil content when feeders were first rotated (Friday) compared to after rotation (Monday and Thursday, N = 106, X^2 = 19.4, df = 3, $P < 0.001$). Changes in the frequency of seed choice (proportion of total bird visits made to each of the four seed varieties) indicate that birds actively choose specific varieties of seed based on size and oil content.

Is nutritional content related to seed size?

Birds may be able to assess the oil content of a seed based on the seed's depth, length, and weight, all of which differed in a predictable manner among seed varieties. Collectively, deep, short, heavy seeds may be cues that birds use to identify high oil content. For example, the depth of small seeds was greatest at the highest oil content seed (Fig. 2a). The length of large seeds, as well as small seeds, decreased with oil content (Fig. 2b). The weight of small, and large seeds, increased with oil content (Fig. 2c), with large seeds being heavier than small seeds regardless of oil content (Fig 2d).

Figure 2. 95% confidence intervals of the depth, length, and weight of black-oil sunflower seed varieties that vary in size and % oil content.

Figure 2A. The depth of small seeds was greatest for the highest oil content variety.

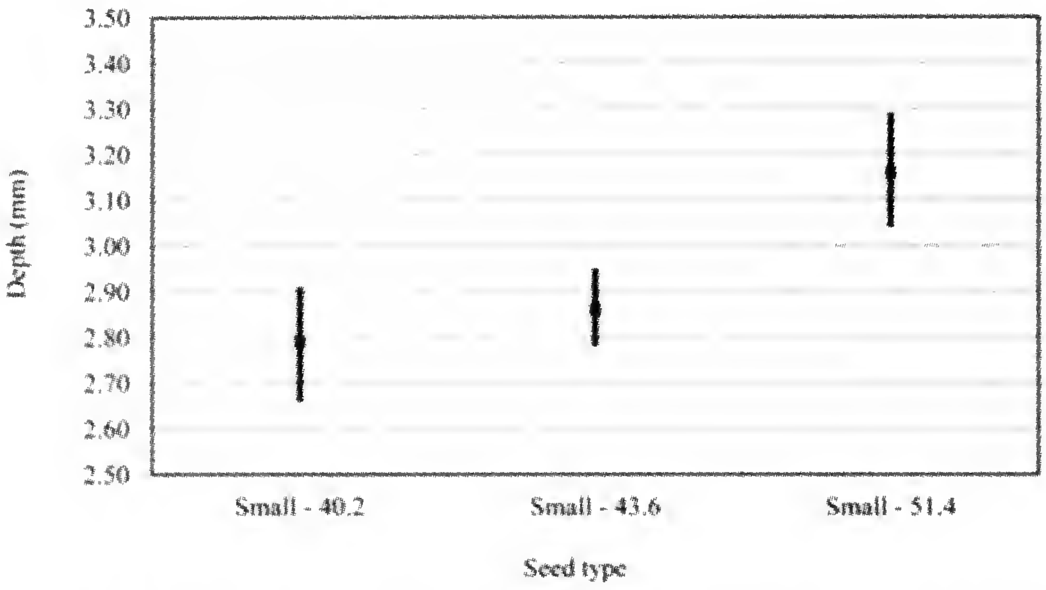


Figure 2B. The length of large seeds, as well as small seeds, decreased as oil content increased.

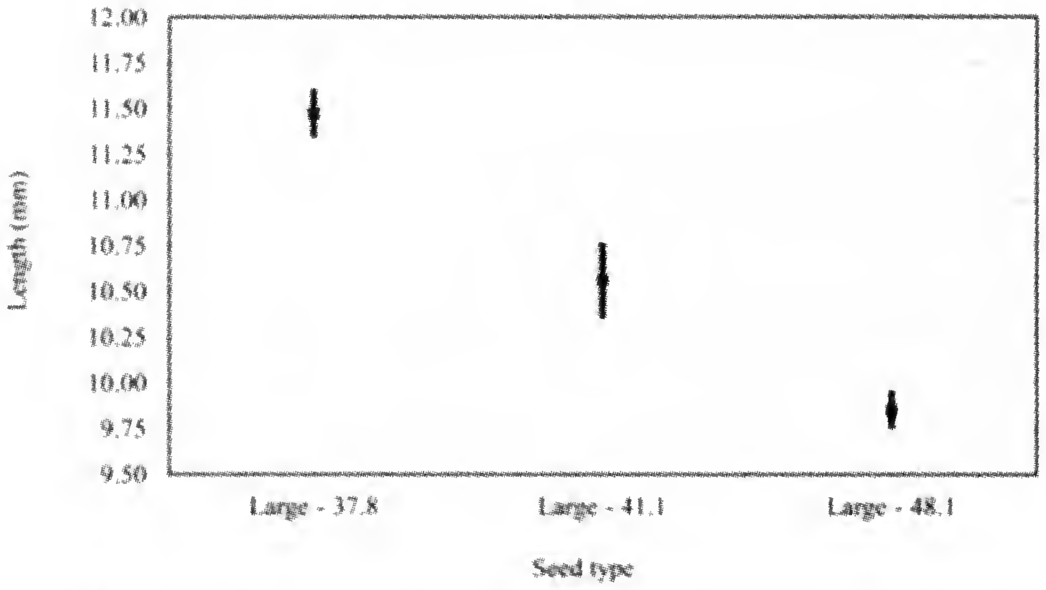


Figure 2C. The weight of small seeds, as well as large seeds, was greatest for the highest oil content variety.

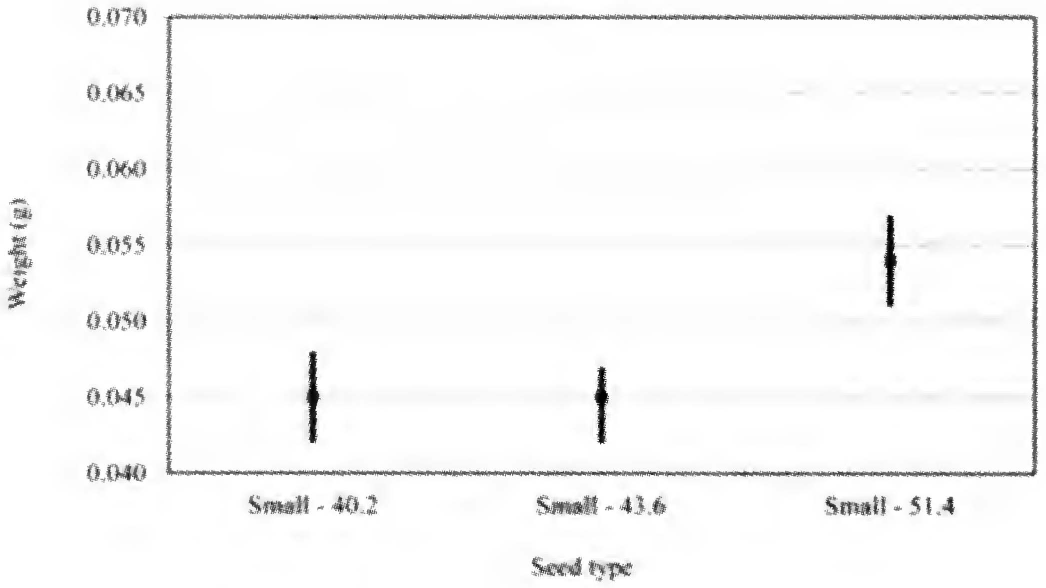
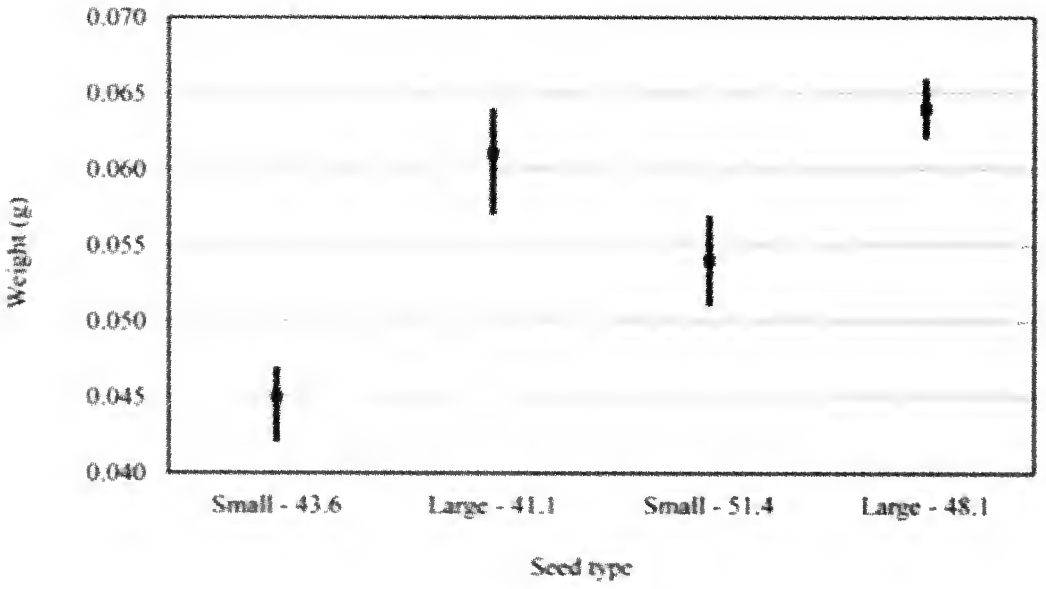


Figure 2D. Large seeds were heavier than small seeds, regardless of oil content.



Are there differences among species in regards to seed preference?

Trial 3 examined seed consumption and bird abundance in relation to both seed size and oil content. The greatest consumption occurred at the highest oil content seed (small 51.4%) at 52 kg. The small seed with the lower oil content (43.6%) was the second most consumed seed at 35 kg followed by large 48.1% at 29 kg and large 41.1 at 26 kg. Thus, while previous trials found that the higher the oil content, the greater the seed consumption; in some cases smaller seeds with lower oil content are preferred over larger seeds with higher oil content.

Small seeds were preferred over larger seeds as 43.6% and 51.4 small seeds had a greater abundance of birds of all species combined than 41.1% and 48.1 large seeds. At Rock Springs, 95% CIs of the mean number of birds per 60-min observation session ranged from 1.43-1.96 at small 43.6% seeds, 0.80-1.35 at large 41.1 seeds, 1.95-2.78 at small 51.4 seeds, and 0.77-1.20 at large 48.1 seeds.

The greater number of individuals of all species combined at small seed varieties is likely a result of differences among species in regards to seed preference. We found that species that sit and eat at a feeder would select smaller seeds with a lower oil content at an equivalent level to larger seeds with a higher oil content. House Finch (*N* = 1031), American Goldfinch (*N* = 536), and House Sparrow (*N* = 155) had their greatest abundance at small seeds, and had significantly greater abundances at the small 51.4% seed compared to the larger seeds (Fig. 3). 95% CIs of the mean number of House Finch per 60-min observation session ranged from 0.69-1.29 at small 43.6% seeds, 0.35-0.80 at large 41.1 seeds, 0.85-1.63 at small 51.4 seeds, and 0.31-0.69 at large 48.1 seeds. 95% CIs of the mean number of American Goldfinch per 60-min observation session ranged from 0.30-0.52 at small 43.6% seeds, 0.22-0.40 at large 41.1 seeds, 0.64-0.90 at small 51.4 seeds, and 0.14-0.32 at large 48.1 seeds. 95% CIs of the mean number of House Sparrow per 60-min observation session ranged from 0.07-0.23 at small 43.6% seeds, 0.03-0.11 at large 41.1 seeds, 0.12-0.31 at small 51.4 seeds, and 0.02-0.11 at large 48.1 seeds. Downy Woodpecker (*N* = 75) abundance was equivalent at each seed variety.

DISCUSSION

We found that wild birds actively choose specific varieties of a single seed, and make choices based on oil content and seed size. In addition, we found that seed characteristics can be used as an indicator of the nutritional content of the seed. Several studies have addressed seed preferences among bird species. A majority of the studies have focused on seed size and shape of multiple seed species (Hespenheide 1966, Willson 1971, Willson 1972, Pulliam 1985). These studies found that birds develop preferences toward certain seed species based on feeding efficiency, or handling time, and the relationship between seed size and bill size. In general, larger-billed birds have the ability to eat larger seeds more effectively than small-billed birds (Hespenheide 1966, Diaz 1990). Studies of a bird's ability to assess the quality of different varieties of the same seed species based on its physical characteristics are fewer, but have been demonstrated in Black-capped Chickadees (Templeton 2011). Specifically, chickadees were able to select seed heads of spotted knapweed (*Centaurea maculosa*) that had greater amounts of gall fly (genus *Urophora*) larvae using seed head size as one indicator of gall fly abundance.

We found that wild birds showed a preference for black-oil sunflower varieties with the highest oil content. Previous studies found that wild birds display preferences for specific micronutrients, and have the ability to distinguish between varying concentrations of sugars and lipids (McWillams et al. 2002, Schaefer et al. 2003, Pierce et al. 2004, Brown et al. 2010). In a study of four tanager species, Schaefer et al. (2003) concluded that birds were able to distinguish 1% differences in sugar concentrations and 2% differences in lipid concentrations, and typically selected foods with the highest concentrations of each. We found that seed-eating birds were able to distinguish differences in oil content of at least 10%.

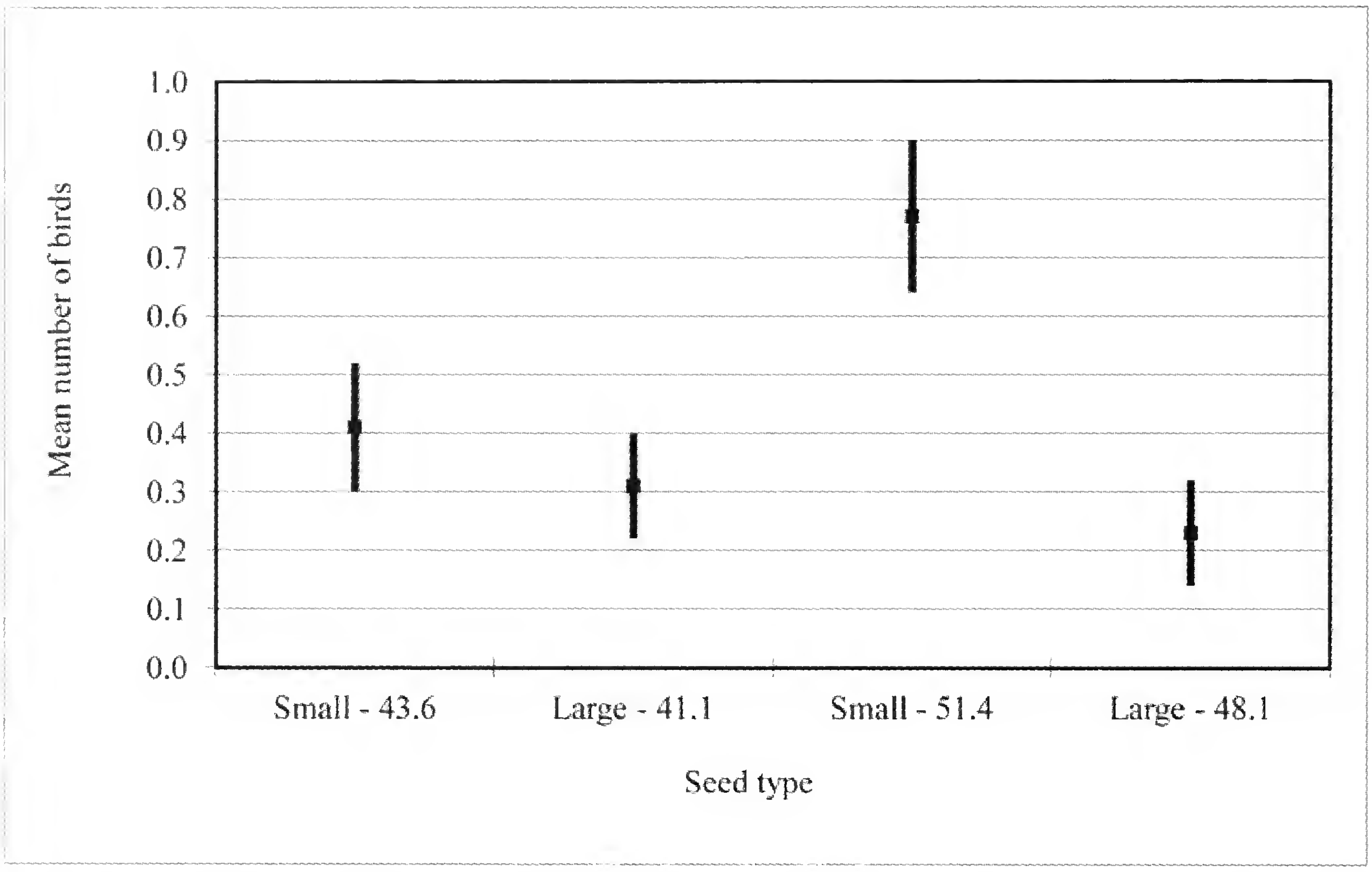


Figure 3. American Goldfinch preferred the small black-oil sunflower seeds with 51.4% oil over the three other varieties of black-oil sunflower that varied in size and/or % oil content. An equivalent number of goldfinches were found at small 43.6% seeds compared to large 48.1% seeds indicating that oil content is not the only factor influencing seed choice.

Seed size serves as both a cue for the oil content of a seed and a factor influencing seed choice. We found oil content of sunflower seeds increased with decreasing length, and increasing depth and weight. Birds "weigh" seeds before making a selection (Heinrich et al. 1997). By weighing seeds, birds may not only be maximizing energy costs of foraging (e.g., Lima 1985), but are also able to assess the seed's nutritional content. We also found that birds used seed size as a factor in seed selection, as some species consumed smaller seeds with less oil content over larger seeds with greater oil content when given a choice. In previous studies, Java Sparrow (*Padda oryzivora*) selected safflower seeds that were deeper (Van der Meij and Bout 2000), and Northern Cardinal selected sunflower seeds that were shorter, but thinner (Willson 1972).

We found differences among species in the seed varieties they choose. Species that sit at a feeder and eat, such as House Finch, American Goldfinch, and House Sparrow selected smaller seeds at equivalent rates to larger seeds, even if larger seeds had higher oil contents. Thus, for birds that eat food where it is found, ease in handling seeds may be a more significant factor (Willson 1972) than for species that take a seed and consume it elsewhere, where maximizing energy gained from each seed may be a more important factor in seed choice (Lima 1985). This may explain why Black-capped Chickadee, Tufted Titmouse, and White-breasted Nuthatch had the greatest frequency of visits at large, high oil content seeds after adjusting to feeder rotation during the study conducted in conjunction with Trial 3.

Knowledge of seed preferences of birds, and the factors that influence preferences, may ultimately result in improved seed varieties and an enhanced experience for people who feed birds. Specifically, packagers of bird seed could select sunflower varieties of certain size and oil content to create specialized blends based on the birds individuals want to attract. Blends used to attract birds that sit and eat at a feeder might include smaller sunflower seeds, while blends for attracting birds that grab a seed and eat elsewhere might be comprised of larger, higher oil content seeds.

Farmers growing black-oil sunflower face a dilemma. While the seeds are developing in the plant in the field, seed varieties that are bird resistant, particularly to Red-winged Blackbirds (*Agelaius phoenicus*) should be planted (Gross and Hanzel 1991, Bullard 1988, Linz et al. 2011). However, the seed traits selected in bird-resistant varieties during the 1980's, including fibrous hulls with high levels of anthocyanins, also created seeds with low oil content and yield (Linz et al. 2011). While these varieties were less attractive to pest birds (Bullard et al. 1989), they may also be unattractive to birds at feeders that prefer high oil content seed. Future research should examine whether lower oil content black-oil sunflower with fibrous hulls would be selected over other seed species found in bird food blends. In addition, studies should examine if birds have different preferences for certain seed varieties during different seasons. For example, do birds consume even more of the higher oil content seeds in the fall and winter in order to meet the demands of their changing metabolic rates and increase in lipid and protein storage (Karasov and Pinshow 1998, Linkes et al. 2002, McWilliams et al. 2002)?

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LITERATURE CITED

Brown, M., C. T. Downs, and S. D. Johnson. 2010. Sugar preferences of a generalist nonpasserine flower visitor, the African Speckled Mousebird (*Colius striatus*). Auk 127:781-786.

Bullard, R. W. 1988. Characteristics of bird-resistance in agricultural crops. Proceedings of the Vertebrate Pest Conference 13:305-309.

Bullard, R. W., P. P. Woronecki, R. A. Dolbeer, and J. R. Mason. 1989. Biochemical and morphological characteristics in maturing achenes from purple-hulled and oilseed sunflower cultivars. Journal of Agricultural and Food Chemistry 37:886-890.

Diaz, M. 1990. Interspecific patterns of seed selection among granivorous passerines: effects of seed size, seed nutritive value and bird morphology. Ibis 132:467-476.

DiMiceli, J. K., P. C. Stouffer, E. I. Johnson, C. Leonardi, and E. B. Moser. 2007. Seed preferences of wintering Henslow's Sparrows. Condor 109:595-604.

Di Stefano, J. 2004. A confidence interval approach to data analysis. Forest Ecology and Management 187:173-183.

Foster, M. S. 2008. Freeze-frame fruit selection by birds. Wilson Journal of Ornithology 120:901-905.

Geis, A. D. 1980. Relative attractiveness of different foods at wild bird feeders. U.S. Department of Interior, Fish and Wildlife Service Special Scientific Report 233. Washington, D.C.

Gross, J. F. and J. J. Hanzel. 1991. Stability of morphological traits conferring bird resistance to sunflower across different environments. Crop Science 31:997-1000.

Heinrich, B., C. C. Joerg, S. S. Madden, and E. W. Sanders Jr. 1997. Black-capped Chickadees and Red-breasted Nuthatches "weigh" sunflower seeds. Auk 114:298-299.

Hespenheide, H. A. 1966. The selection of seed size by finches. Wilson Bulletin 78:191-197.

Horn, D. J. 1995. Perching orientation affects number of feeding attempts and seed consumption by the American Goldfinch (*Carduelis tristis*). Ohio Journal of Science 95:292-293.

Horn, D. J., S. E. Fairbairn, and R. J. Hollis. 2002. Factors influencing the occurrence of birds that use feeders in Iowa. Journal of the Iowa Academy of Science 109:8-18.

Horn, D. J., M. Abdallah, M. K. Bastian, J. R. DeMartini, and R. M. Wilhemi. 2003. Bird abundance at feeders increases with decreasing distance to cover. Transactions of the Illinois State Academy of Science 96:247-254.

Hrabar, H. D. N. K. and M. Perrin. 2002. The effect of bill structure on seed selection by granivorous birds. African Zoology 37:67-80.

Johnson, D. H. 1999. The insignificance of statistical significance testing. Journal of Wildlife Management 63:763-772.

Karasov, W. H. and B. Pinshow. 1998. Changes in lean mass and in organs of nutrient assimilation in a long-distance migrant at a springtime stopover site. Physiological Zoology 71:435-448.

Lima, S. L. 1985. Maximizing feeding efficiency and minimizing time exposed to predators: a trade-off in the Black-capped Chickadee. Oecologia 66:60-67.

Linkes, E. T., S. M. Scott, and D. L. Swanson. 2002. Seasonal acclimatization in the American Goldfinch revisited: to what extent do metabolic rates vary seasonally? Condor 104:548-557.

Linz, G. M., J. H. Homan, S. J. Werner, H. M. Hagy, and W. J. Bleier. 2011. Assessment of bird-management strategies to protect sunflowers. Bioscience 61:960-970.

McWilliams, S. R., S. B. Kearney, and W. H. Karasov. 2002. Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. Journal of Avian Biology 33:167-174.

Pergams, O. R. W. and P. A. Zaradic. 2008. Evidence for a fundamental and pervasive shift away from nature-based recreation. Proceedings of the National Academy of Sciences 105:2295-2300.

Pierce, B. J., S. R. McWilliams, A. R. Place, and M. A. Huguenin. 2004. Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory Red-eyed Vireos (*Vireo olivaceous*). Comparative Biochemistry and Physiology, Part A 138:503-514.

Pulliam, H. R. 1985. Foraging efficiency, resource partitioning, and coexistence of sparrow species. Ecology 66:1829-1836.

Schaefer, H. M., V. Schmidt, and F. Bairlein. 2003. Discrimination abilities for nutrients: which difference matters for choosy birds and why? Animal Behaviour 65:531-541.

Templeton, C. N. 2011. Black-capped Chickadees select spotted knapweed seedheads with high densities of gall fly larvae. Condor 113:395-399.

U.S. Fish and Wildlife Service. 2007. 2006 national survey of fishing, hunting, and wildlife-associated recreation. National overview.

U.S. Fish and Wildlife Service. 2008. Wildlife watching in the U.S.: The economic impacts on national and state economies in 2006. Addendum to the 2006 national survey of fishing, hunting, and wildlife-associated recreation.

U.S. Fish and Wildlife Service. 2009. Wildlife watching trends: 1991-2006 a reference report. Addendum to the 2006 national survey of fishing, hunting, and wildlife-associated recreation.

Van der Meij, M. A. A. and R. G. Bout. 2000. Seed selection in the Java Sparrow (*Padda oryzivora*): preference and mechanical constraint. Canadian Journal of Zoology 78:1668-1673.

Willson, M. F. 1971. Seed selection in some North American finches. Condor 73:415-429.

Willson, M. F. 1972. Seed size preference in finches. Wilson Bulletin 84:449-455.

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Comparison of Methods to Detect Mesocarnivores in Southern Illinois

Clayton K. Nielsen[1,2] and Susan E. Cooper[1]
[1]Cooperative Wildlife Research Laboratory and [2]Department of Forestry
Southern Illinois University, Carbondale, IL 62901-6504

ABSTRACT

We used trapping, track plates, and remote cameras to survey the distribution of gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), and bobcats (*Lynx rufus*) in southern Illinois during 2005-07. Gray fox detection rates were low for all survey techniques when compared to more abundant mesocarnivore species. We captured 9 gray foxes and 21 bobcats in 7,729 trap-nights and 6 coyotes in 1,416 trap-nights. Track plates (n = 883 survey-nights) resulted in the detection of 6 gray foxes, 1 bobcat, and 1 coyote. Cameras (n = 953 survey-nights) resulted in the detection of 4 gray foxes, 5 bobcats, and 4 coyotes. Although the relative effectiveness of detection methods varied by species, our data are generally in agreement with other survey methods that indicate the relative scarcity of gray foxes compared to bobcats and coyotes in southern Illinois.

Key words: bobcat, *Canis latrans*, coyote, gray fox, *Lynx rufus*, remote camera, survey, track plate, southern Illinois, trapping, *Urocyon cinereoargenteus*.

INTRODUCTION

Mesocarnivores often use large home ranges and engage in secretive behavior; these attributes complicate assessments of their abundance and distribution. Three sympatric mesocarnivore species associated with forest cover in southern Illinois are the gray fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), and bobcat (*Lynx rufus*), and wildlife biologists have expended many resources monitoring their populations during the past 20 years (e.g., Nielsen and Woolf 2002*a*,*b*). The most reliable estimate of mesocarnivore population trends in Illinois is the Archery Deer Hunter Survey (ADHS), which each year asks hunters to document harvest effort and wildlife sightings. Since its inception in 1991, the ADHS has quantified a 75% decrease in gray fox sightings (Bluett 2007) versus a 15% increase in coyotes and an almost 600% increase in bobcats (Figure 1). Wildlife biologists are unsure why gray fox populations are declining in Illinois, but important limiting factors may include intraguild predation, competition, and mortality from disease (Cypher 2003).

We used traps, track plates (Drennan and Dodd 1998, Olson et al. 2003) and remote cameras (Silveira et al. 2003, York et al. 2003) to survey for gray foxes, coyotes, and bobcats in southern Illinois, and compared detection rates among survey methods and species. In addition to our assessment of survey methods, we also wished to determine whether field survey methods generally supported mesocarnivore trends observed in the ADHS.

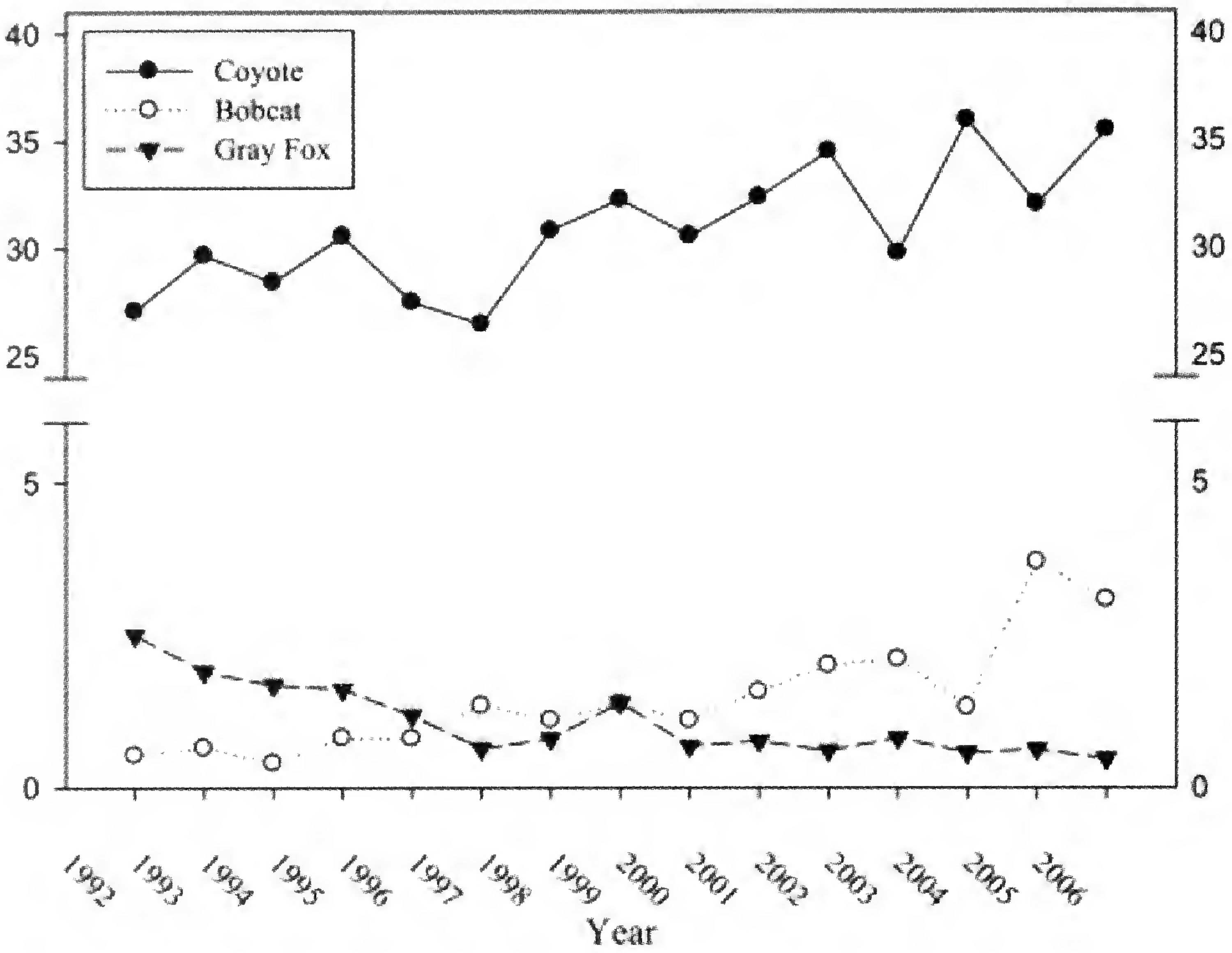

Figure 1. Trends in sighting indices for gray foxes, bobcats, and coyotes based on the Illinois Department of Natural Resource's Archery Deer Hunter Survey, 1996-2006, Illinois, USA.

STUDY AREA

We conducted field research in 5 southern Illinois counties (Jackson, Johnson, Pope, Union, and Williamson) in the Shawnee National Forest, Crab Orchard National Wildlife Refuge, Giant City State Park, Ferne Clyffe State Park, Southern Illinois University Carbondale, City of Carbondale, and on land in private ownership. Elevation in southern Illinois ranges from 91 to 325 m (Netstate 2006) and comprises cropland (39%); upland and bottomland forests (25%) dominated by maples (*Acer* spp.), oaks (*Quercus* spp.), and hickory (*Carya* spp.); and rural grasslands (24%; Luman et al. 1996). Climate in southern Illinois is characterized by 4 distinct seasons with an average annual temperature of 14° C and average annual precipitation of 120 cm (Illinois State Water Survey 2003). Road density (1.4 km/km^2) and human population density (21.5 persons/km^2) are moderately high in this area (Nielsen and Woolf 2002*a,b*).

METHODS

Trapping

We trapped for gray foxes, coyotes, and bobcats during 3 field seasons: 30 November 2005–18 March 2006, 5 June–4 August 2006, and 9 October 2006–28 February 2007. We used Victor #1.5 padded foothold traps and wire-cage box traps (30 x 30 x 72.5 cm), and also used Woodstream #3 padded foothold traps during winter 2005-06. During the winter seasons, we selected trapping areas based on reported animal sightings, evaluation of suitable habitat, historic occurrence, and incidental captures in other studies (Follman 1973, Cypher 1991; C. Nielsen, unpublished data). We used standard dirt-hole and scent-post sets with the foothold traps, and placed box traps under shrubs and concealed them with vegetation. We used a variety of baits including game meat, fatty acid scent discs, commercial scent lures, peanut butter, jelly, and carnivore urine. We checked all traps every morning and immediately released all non-target animals. We immobilized trapped individuals using Telazol® with a dosage rate of 13 mg/kg, or used physical restraint and a blindfold. All animals were marked with individual ear tags. Capture and handling procedures followed Southern Illinois University Carbondale Animal Care and Use Protocol #05-028.

We estimated capture rates for gray foxes, bobcats, and coyotes. Capture rates for gray foxes and bobcats were the total number of individuals captured/100 trap-nights; however, capture rates for coyotes was based solely on the number of individuals captured in #3.0 footholds/100 trap-nights, as box traps and #1.5 footholds were less likely to capture coyotes.

Track Plates and Remote Cameras

During 5 June–5 September 2006, we surveyed southern Illinois for gray foxes, bobcats, and coyotes using track plates and remote cameras. We divided each county into sections (2.6 km^2) and considered sections for surveying if they contained >50% forest and adequate road access. We used forest cover as a criterion for selecting a section based on knowledge of habitat use by gray foxes, bobcats, and coyotes (Anderson and Lovallo 2003, Bekoff and Gese 2003, Cypher 2003). We included only sections with road access across the section to maximize survey efficiency; 117 sections in the 5-county study area met these criteria. From these, we randomly selected approximately 50% of the suitable sections in each county: 19 from Jackson County, 6 from Johnson County, 18 from Pope County, 11 from Union County, and 4 from Williamson County.

We placed 5 stations along the main road of each section (Figure 2). The first station was placed on the road in the middle of the section with 2 additional stations placed in each other direction. Each station was separated by 325 m (Conner et al. 1983), and we placed 1 remotely triggered camera and 1 track plate at each station. We randomly selected the first detection device and placed it 50 m from the road, and then placed the second detection device 25-50 m from the first. Because it is not uncommon to see gray foxes, bobcats, and coyotes on roads or to find their carcasses near roads (Kolowski and Nielsen 2008), we thought selection of sites near roads would not bias the probability of detecting carnivores. We opportunistically placed detection devices near game trails, creeks, and other probable animal travel ways. We conducted surveys for 4 consecutive nights, weather permitting (Roughton and Sweeny 1982, Nottingham et al. 1989, Engeman and Allen 2000). We recorded the location of all detection devices using a global positioning system unit.

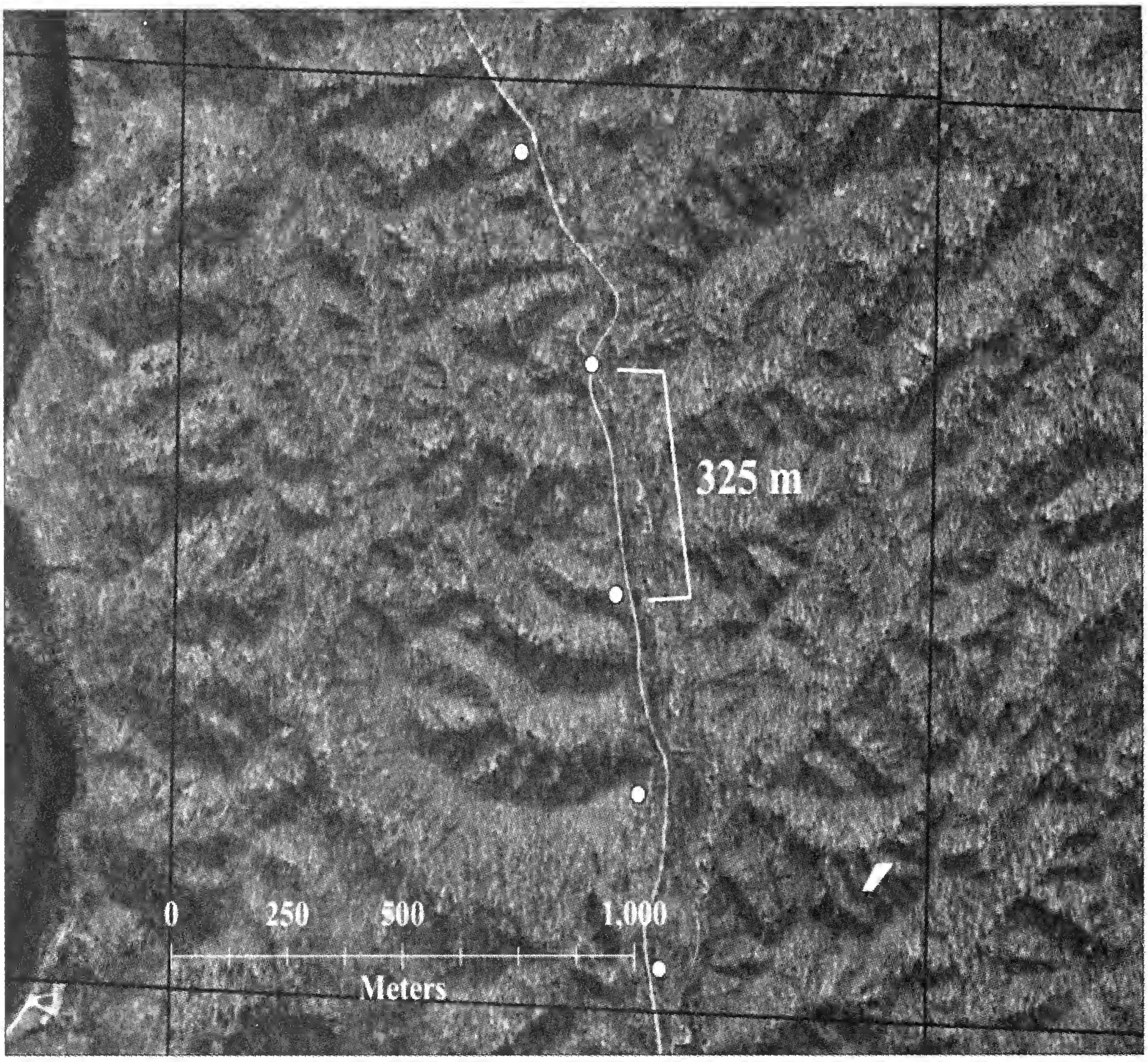

Figure 2. Set up of sections (2.6 km^2) for track plate and camera detection surveys ran during June–September 2006, southern Illinois, USA. One camera and 1 track plate were placed at each station marked by the white dot. Stations were separated by 325 m.

At each detection device, we randomly selected a lure that should appeal to all mesocarnivore species potentially present. We used a commercial food-based lure (Lenon's Fox #1 Super All Call), a mixture of fish oil and shellfish oil, or a fatty-acid-scent disc. We replaced lures if they had been obviously removed by animals visiting the site. As an attractant, we also sprayed the area around each detection device with carnivore urine. We used Moultrie® 100v2 Gamespy (2.1 megapixel) and Moultrie® 200 Gamespy (3.1 megapixel) cameras. We set cameras to take 2 pictures/min when triggered and attached the camera approximately 0.5 m above the ground on a tree. We then dug a dirt hole approximately 2 m from the camera in which the lure was placed. We downloaded all images from the cameras and identified species.

Track plates were 0.64-m^2 treated plywood boards covered with aluminum flashing and sprayed with a 1:4 mixture of dissolved carpenter's chalk and denatured ethyl alcohol (Drennan and Dodd 1998, Olson et al. 2003). We placed a lure in the center of the track plate and identified carnivore tracks to species. We calculated detection rates using cameras and track plates for gray foxes, bobcats, and coyotes based on the number of individuals detected/100 survey-nights.

RESULTS

Trapping

We captured 9 gray foxes and 21 bobcats in 7,729 trap-nights and 6 coyotes in 1,416 trap-nights. Capture rates (individuals/100 trap-nights) were 0.12, 0.27, and 0.42, for gray foxes, bobcats, and coyotes, respectively (Table 1). Other animals captured included Virginia opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), domestic dogs (*C. familiaris*), striped skunks (*Mephitis mephitis*), eastern cottontails (*Sylvilagus floridanus*), squirrels (*Sciurus* spp.), domestic cats (*Felis catus*), an eastern box turtle (*Terrapene carolina*), and a woodchuck *(Marmota monax*; Table 2).

Table 1. Detection rates (individuals/100 survey-nights) of sympatric carnivores based on trapping, track plate, and camera surveys conducted during December 2005–February 2007, southern Illinois, USA.

Device (# survey-nights)	Gray fox	Bobcat	Coyote
Box trap (2,163)	0.05	0.37	N/A
Foothold (5,566)	0.14	0.23	0.42[a]
Track plate (883)	0.68	0.11	0.11
Camera (953)	0.42	0.53	0.42
Totals (9,565)	0.20	0.28	0.34

[a] Capture rate for coyotes was based on total trap nights using #3.0 foothold traps (*n* = 1,416).

Table 2. Capture totals based on 7,756 trap-nights during December 2005–March 2007, southern Illinois, USA.

Species	Footholds	Box trap	Totals
Virginia opossum	104	108	212
Raccoon	89	65	154
Striped skunk	9	14	23
Bobcat	13	8	21
Domestic dog	13	0	13
Eastern cottontail	9	0	9
Gray fox	8	1	9
Coyote	6[a]	0	6
Squirrel	3	0	3
Domestic cat	2	1	3
Woodchuck	1	0	1
Turtle	1	0	1
Totals	258	197	455

[a] Capture total for coyotes was based on total trap nights using #3.0 foothold traps (*n* = 1,416).

Track Plates and Remote Cameras

Track plates (*n* = 883 survey-nights) resulted in the detection of 6 gray foxes, 1 bobcat, and 1 coyote (Table 1). Cameras (*n* = 953 survey-nights) resulted in the detection of 4 gray foxes, 5 bobcats, and 4 coyotes. Other species detected using these methods included raccoons, opossums, domestic dogs, squirrels, eastern cottontails, striped skunks, turkey vultures (*Cathartes aura*), black vultures (*Coragyps atratus*), a woodchuck, a domestic cat, a wild turkey (*Meleagris gallopavo*), and a nine-banded armadillo (*Dasypus novemcinctus*).

Overall, remote cameras (1.36 detections/100 survey-nights) were more effective for detecting these 3 focal carnivore species than track plates (0.91 detections/100 survey-nights). Both bobcats and coyotes were more effectively detected by cameras than the other devices, but gray foxes appeared to be best detected by track plates (Table 1).

DISCUSSION

Our trapping data are in agreement with the ADHS, which indicates the relative scarcity of gray foxes in Illinois compared to bobcats and coyotes (Bluett 2007). We captured twice as many bobcats as gray foxes and coyotes, but the capture rate for coyotes (0.42) was higher than for both gray foxes (0.11) and bobcats (0.27). We based capture rates for coyotes solely on the #3.0 foothold traps because #1.5 foothold traps and the box traps were too small to effectively catch or hold most coyotes. This bias should not exist for gray foxes and bobcats (Zezulak 1980, Fuller et al. 1995, Gabriel 2006). Using these capture rates as coarse indices of relative abundance, gray foxes are scarcer than bobcats or coyotes. However, the species differ in likelihood of capture. For example, coyotes are wary of novel objects, especially within their core home ranges, making them difficult to trap (Sauvajot et al. 2000, Sequin et al. 2003, Mettler and Shivik 2007), whereas gray foxes and bobcats may not possess this trap shyness (Fritzell and Haroldson 1982). Therefore, capture rates from this study actually may underestimate relative abundance of coyotes in southern Illinois.

The relative effectiveness of detection methods used during this study varied by species. Although remote cameras detected the most bobcats and coyotes, cameras did not appear to detect gray foxes as well as did track plates. Again, differential detectability of gray foxes and coyotes may explain some variation in the number of detections obtained by devices such as track plates or cameras. Coyotes are wary of cameras (Sauvajot et al. 2000, Sequin et al. 2003), and it is likely they would have similar reactions to track plates, as concluded by Heske et al. (2011). Track plate surveys could be improved (e.g., by creating a more natural-looking tracking surface such as sand or soil) to make them more attractive to wary species (Heske et al. 2011).

There are many ways to conduct carnivore surveys (Roughton and Sweeny 1982, Gese 2001, Sargeant et al. 2003, Barea-Azcon et al. 2007). The most efficient surveys would allow researchers to run the fewest number of devices in the smallest area for the fewest nights without risking inefficient sampling (Field et al. 2005, Joseph et al. 2006). Complicating matters is that at different times of the year, animals are more or less likely to visit devices (Gompper et al. 2006). For example, during the breeding and juvenile dispersal seasons, home ranges expand (Follman 1973, Andelt and Gipson 1979, Sawyer and Fendley 1990), whereas home ranges contract during the whelping season (Follman 1973, Sawyer and Fendley 1990). There also may be an increased chance of detection during seasons when food is less plentiful, due to animals roaming more widely in search of food.

Altering survey methods in several ways may have produced higher detection rates of mesocarnivores in southern Illinois. Because coyotes may be more reluctant to visit non-natural devices (Sauvajot et al. 2000, Sequin et al. 2003, Mettler and Shivik 2007), dirt-circle scent stations may seem less out of place than track plates. Remote cameras also may appear suspicious to coyotes (Sauvajot et al. 2000, Sequin et al. 2003). Also, we conducted surveys in the summer, at which time the maximum number of gray foxes should be present due to the birth pulse. Juvenile animals may be less wary of survey devices (Windberg and Knowlton 1990). It is also difficult to determine the number of nights to survey for multiple species that have differently-sized home ranges. Because gray foxes inhabit relatively small home ranges when compared to bobcats and coyotes, they should have been more likely to encounter a detection device within the 2.6-km^2 study sections, whereas a coyote or bobcat may never enter that section in the 4 nights we ran surveys.

Our initial assessment of mesocarnivore distribution in Illinois has refined objectives for continuing research on mesocarnivores in Illinois. Specifically, we are using remote cameras (and not the other techniques discussed in this paper) to conduct occupancy surveys (MacKenzie et al. 2006) of gray foxes and sympatric mesocarnivores at 1,118 sites in 357 2.6-km^2 sections in the 16 southernmost counties of Illinois (C. Nielsen, unpublished data). These surveys will provide a more rigorous assessment of factors affecting occupancy and distribution of gray foxes, coyotes, and bobcats, focusing primarily on species interactions and habitat characteristics.

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LITERATURE CITED

Andelt, W. F., and P. S. Gipson. 1979. Home range, activity, and daily movements of coyotes. Journal of Wildlife Management 43:944-951.

Anderson, E. M., and M. J. Lovallo. 2003. Bobcat and lynx (*Lynx rufus* and *Lynx canadensis*). Pages 758-786 *in* G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America: Biology, management, and conservation. John Hopkins University Press, Baltimore, Maryland, USA.

Barea-Azcon, J. M., E. Virgos, E. Ballesteros-Duperon, M. Moleon, and M. Chirosa. 2007. Surveying carnivores at large spatial scales: a comparison of four broad-applied methods. Biodiversity Conservation 16:1213-1230.

Bekoff, M., and E. M. Gese. 2003. Coyote (*Canis latrans*). Pages 467-481 *in* G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America: Biology, management, and conservation. John Hopkins University Press, Baltimore, Maryland, USA.

Bluett, B. 2007. 2006 Archery deer hunter survey. Wildlife Diversity Program Note 06-4. Illinois Department of Natural Resources, Springfield, Illinois, USA.

Conner, M. C., R. F. Labisky, and D. R. Progulske. 1983. Scent-station indices as measures of population abundance for bobcats, raccoons, gray foxes, and opossums. Wildlife Society Bulletin 11:146-152.

Cypher, B. L. 1991. Coyote foraging dynamics, space use, and activity relative to resource variation at Crab Orchard National Wildlife Refuge, Illinois. Dissertation, Southern Illinois University, Carbondale, Illinois, USA.

Cypher, B. L. 2003. Foxes. Pages 511-546 *in* G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America: Biology, management, and conservation. John Hopkins University Press, Baltimore, Maryland, USA.

Drennan, P. B., and N. L. Dodd. 1998. Use of track stations to index abundance of sciurids. Journal of Mammalogy 79:352-359.

Engeman, R. M., and L. Allen. 2000. Overview of a passive tracking index for monitoring wild canids and associated species. Integrated Pest Management Reviews 5:197-203.

Field, S. A., A. J. Tyre, and H. P. Possingham. 2005. Optimizing allocation of monitoring effort under economic and observational constraints. Journal of Wildlife Management 69:473-482.

Follman, E. H. 1973. Comparative ecology and behavior of red and gray foxes. Dissertation, Southern Illinois University, Carbondale, Illinois, USA.

Fritzell, E. K., and K. J. Haroldson. 1982. *Urocyon cinereoargenteus*. Mammalian Species 189:1-8.

Fuller, T. K., S. L. Berendzen, T. A. Decker, and J. E. Cardoza. 1995. Survival and cause-specific mortality rates of adult bobcats (*Lynx rufus*). American Midland Naturalist 134:404-408.

Gabriel, M. W. 2006. Exposure to *Anaplasma phagocytophilum* and ticks in gray foxes (*Urocyon cinereoargenteus*) in northern Humboldt County, California. Thesis, Humboldt State University, Humboldt, California, USA.

Gese, E. M. 2001. Monitoring of terrestrial carnivore populations. Pages 372-396 *in* J. L. Gittleman, S. M. Funk, D. W. MacDonald, and R. K. Wayne, editors. Carnivore Conservation. Cambridge University Press and The Zoological Society of London, Cambridge, Great Britain.

Gompper, M. E., R. W. Kays, J. C. Ray, S. D. Lapoint, D. A. Bogan, and J. R. Cryan. 2006. A comparison of noninvasive techniques to survey carnivore communities in northeastern North America. Wildlife Society Bulletin 34:1142-1151.

Heske, E. J., T. W. Rodges, and T. R. Van Deelen. 2011. Detection bias in noninvasive track surveys of mammalian predators in Illinois. Transactions of the Illinois Academy of Science 104: 137-143

Illinois State Water Survey, State Climatologist Office. 2003. Climate of Illinois. <http://www.sws.uiuc.edu/atmos/statecli/General/Illinois-climate-narrative.pdf#search=%22annual%20average%20temperatures%20southern%20illinois%22>. Accessed 27 Aug 2006.

Joseph, L. N., S. A. Field, C. Wilcox, and H. P. Possingham. 2006. Presence-absence versus abundance data for monitoring threatened species. Conservation Biology 20:1679-1687.

Kolowski, J. M., and C. K. Nielsen. 2008. Using Penrose distance to identify potential risk of wildlife-vehicle collisions. Biological Conservation 141:1119-1128.

Luman, D., M. Joselyn, and L. Suloway. 1996. Critical trends assessment project: landcover database of Illinois, 1995. Illinois Natural History Survey, Springfield, Illinois, USA.

MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, New York, New York, USA.

Mettler, A. E., and J. A. Shivik. 2007. Dominance and neophobia in coyote (*Canis latrans*) breeding pairs. Animal Behaviour Science 102:85-94.

Netstate. 2006. The geography of Illinois. <http://www.netstate.com/states/geography/il_geography.htm>. Accessed 27 Aug 2006.

Nielsen, C. K., and A. Woolf. 2002*a*. Habitat-relative abundance relationship for bobcats in southern Illinois. Wildlife Society Bulletin 30:222-230.

Nielsen, C. K., and A. Woolf. 2002*b*. Survival of unexploited bobcats in southern Illinois. Journal of Wildlife Management 66:833-838.

Nottingham, B. G., Jr., K. G. Johnson, and M. R. Pelton. 1989. Evaluation of scent-station surveys to monitor raccoon density. Wildlife Society Bulletin 17:29-35.

Olson, T. L., J. S. Dieni, F. G. Lindzey, and S. H. Anderson. 2003. Swift fox detection probability using tracking plate transects in southeast Wyoming. Pages 93-98 *in* M. A. Sovada and L. Carbyn, editors. The swift fox: ecology and conservation of swift foxes in a changing world. Canadian Plains Research Center, Regina, Saskatchewan, Canada.

Roughton, R. D., and M. W. Sweeny. 1982. Refinements in scent station methodology for assessing trends in carnivore populations. Journal of Wildlife Management 46:217-229.

Sauvajot, R. M., E. C. York, T. K. Fuller, H. S. Kim, D. A. Kamradt, and R. K. Wayne. 2000. Distribution and status of carnivores in the Santa Monica Mountains, California: preliminary results from radio telemetry and remote camera surveys. Pages 113-123 *in* J. E. Keeley, M. Baer-Keeley, and C. J. Fotheringham, editors. Second interface between ecology and land development in California. U.S. Geological Survey Open-File Report 00-62, Sacramento, California, USA.

Sawyer, D. T., and T. T. Fendley. 1990. Seasonal home range size and movements behavior of the gray fox on the Savannah River site. 1990 Proceedings annual conference of Southeastern Association of Fish and Wildlife Agencies 44:379-389.

Sequin, E. S., M. M. Jaeger, P. F. Brussard, and R. H. Barrett. 2003. Wariness of coyotes to camera traps relative to social status and territory boundaries. Canadian Journal of Zoology 81:2015-2025.

Silveira, L., A. T. A. Jacomo, J. Alexandre, and F. Diniz-Filho. 2003. Camera trap, line transect census and track surveys: a comparative evaluation. Biological Conservation 114:351-355.

Windberg, L. A., and F. F. Knowlton. 1990. Relative vulnerability of coyotes to some capture procedures. Wildlife Society Bulletin 18:282-290.

York, E. C., T. L. Moruzzi, T. K. Fuller, J. F. Organ, R. M. Sauvajot, and R. M. DeGraaf. 2003. Description and evaluation of a remote camera and triggering system to monitor carnivores. Wildlife Society Bulletin 29:1228-1237.

Zezulak, D. S. 1980. Northeastern California bobcat study. California Department of Fish and Game, Nongame Wildlife Investigations, Project E_W_2, Job IV_1.6, Sacramento, California, USA.

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Selection of Parturition Sites by Migrating and Dispersing Female White-tailed Deer in Illinois

Charles M. Nixon,[1,9] Philip C. Mankin,[2,10] Dwayne R. Etter,[3] Lonnie P. Hansen,[4]
Paul A. Brewer,[5] James E. Chelsvig,[6] Terry L. Esker,[7] and Joseph B. Sullivan[8]
[1]Illinois Natural History Survey, Champaign, IL 61820
[2]Department of Natural Resources and Environmental Sciences, University of Illinois
1102 South Goodwin, Urbana, IL 61820
[3]Michigan Department of Natural Resources, 8562 East Stoll Road, Lansing, MI 48823
[4]Missouri Department of Conservation, 1110 South College Ave., Columbia, MO 65201
[5]Illinois Department of Natural Resources, One Natural Resources Way
Springfield, IL 62702
[6]Forest Preserve District of Cook County, 536 North Harlem Ave.,
River Forest, IL 60305
[7]Illinois Department of Natural Resources, 4295 North 1000th St., Newton, IL 62448
[8]RR #3, Box 201A, Mt. Sterling, IL 62353
[9]Corresponding author's present address: 19 Westwood, Monticello, IL 61856
e-mail: cenixon@mchsi.com
[10]Present address: 5 Steele A Way, Brooksville, ME 04617

ABSTRACT

Emigration behavior by female white-tailed deer (*Odocoileus virginianus*) is an important attribute of population dynamics in Illinois. The factors influencing the process of selecting a new home range are largely unknown, yet may affect hunting success, other interactions with human activities, and deer social behaviors. Twenty-five radio-marked dispersing (N = 14) and migrating (N = 11) female white-tailed deer (4 adults, 7 yearlings, 14 fawns when marked) were followed from 3 sites (2 in central and 1 in northern Illinois) to the home ranges selected for parturition and fawn rearing. Dispersing deer (*i.e.*, those making one-way movements to a new home range) moved an average of 44.9 ± SE 6.4 km whereas migrators (*i.e.*, those moving to a new range) moved 14.0 ± SE 7.2 km from their natal ranges. Females moved rapidly in nearly a straight line to their new home range readily crossing roads, rivers, and under high tension power lines on their journey. Females marked in east-central and northern Illinois where forest cover totaled <5% of the landscape, selected sites with greater forest cover, grasslands, forest patch size and density, and total patch richness than were available in random locations. Habitats selected by females in west-central Illinois where more forest cover was available did not differ from random sites. Parturition ranges (used May 15–July 15) at all 3 sites contained fewer houses and roads and more forest patches compared with the natal ranges and the larger new range (used May 15–October 1). Migrating females survived longer than dispersing females, but female life span and fawn survival were not significantly affected by the habitat composition of the new home ranges.

INTRODUCTION

Emigration behavior is an important attribute of white-tailed deer of both sexes within the fragmented landscapes that characterize much of the Great Plains and the glaciated portions of Illinois (VerCauteren and Hygnstrom, 1994; Brinkman *et al.*, 2005; Nixon *et al.*, 2007). Emigration movements usually occur when deer are 10–13 mo old and may involve travel up to 200 km or more from their natal ranges (Sparrowe and Springer, 1970; Gladfelter, 1978; Dusek *et al.*, 1989; Nixon *et al.*, 1991). Emigration by female deer in Illinois has been triggered by mother and sibling movements, orphaning, social position, birth sequence with later born fawns in a social group more likely to emigrate, and, with many fawns breeding, a search for a suitable parturition site (Etter *et al.*, 1995; Nixon *et al.*, 2007; Nixon *et al.*, 2008; Nixon *et al.*, 2010).

Less attention has been devoted to examining the characteristics of home ranges selected by female immigrants or why they stop an emigration and settle on a new range. Possible internal incentives to stop include hunger, fatigue from travel, and changes in reproductive hormone levels associated with impending parturition (Plotka *et al.*, 1977; Ozoga *et al.*, 1982; Stamps, 2001; Wiems, 2001; Andreassen *et al.*, 2002). External factors may also affect final selection of a new home range including an immigrant's attraction to a parturition site if >1 yr old, an attraction to or avoidance of conspecifics, a search for a landscape similar to that of the natal range, and avoidance of large rivers, interstate highways, and urban centers (Nelson and Mech, 1992; Danchin *et al.*, 2001; Stamps, 2001; Klaver *et al.*, 2008; Long *et al.*, 2010). In Illinois, it is likely the sounds, scents, and sights of human activity in and around various sites that are otherwise adequate in terms of nutrition and cover, may also influence the decision on where to stop an emigration movement.

We hypothesized that selection of a stopping point for an emigration movement was a random event, based on the effect of various external and internal stimuli. Selection of a home range is a second order scale of habitat selection, when emigrating females select a landscape mosaic of forest, grasslands, and crop fields supportive of their immediate needs (Orians and Wittenberger, 1991; Morrison *et al.*, 2006; Godvik *et al.*, 2009; Grovenburg *et al.*, 2010). Such a selection affects future survival, reproductive success, and overall fitness. We examine some of the factors that may influence immigrating Illinois breeding females in their selection of a new home range in late spring just before the critical period of parturition and fawn rearing.

METHODS

We used 3 study areas (1 in northern Illinois and 2 in central Illinois) to investigate the demographic characteristics, seasonal movements, social behavior, habitat selection, and the importance of refugia for deer in landscapes shaped by human activities (Nixon *et al.*, 1991). Each area included a wooded public park surrounded by privately owned agricultural fields. The 2,953-ha east-central (EC) study area lies within Piatt County. In 1985, this region was only 2.4% forested, with forests restricted to areas too rough or wet to farm (Iverson *et al.*, 1989). The Sangamon River bisected the study area from NE to SW and provided a forested emigration route for deer. The 5,942-ha west-central area (WC), located in Brown and Adams counties, was more rugged with forests covering about 22%

of the landscape (Iverson *et al.*, 1989) and where farms generally included a livestock component. The 1,648-ha northern (NO) area in DeKalb County lies within a region also intensively row cropped with forests occupying about 1.3% of the landscape. Urban sprawl was evident in this region which includes Chicago and Rockford, the largest cities in Illinois. There was no major river corridor associated with either the WC or NO study area.

The 25 female white-tailed deer reported in this paper were part of a larger comprehensive study that included 286 deer captured during 1980–85 (EC) and 352 captured during 1990–1993 (WC and NO). These deer were captured using rocket-propelled or drop nets (Hawkins *et al.*, 1968) (Table 1). Deer were manually restrained and marked with ear tags and radio transmitters (Wildlife Materials, Carbondale, IL; Telonics, Inc., Mesa, AZ; or Advanced Telemetry Systems, Inc., Isanti, MN). Our capture and marking methods were approved by the University of Illinois Institutional Animal Care and Use Committee.

Table 1. Life history of emigrating female white-tailed deer radio-tracked to parturition sites away from the natal range in Illinois during 1980–1985 and 1990–1993. Contact months represents the period from capture to last contact or death (NO = northern, EC = east-central, WC = west-central study areas).

Tag	Year marked	Age when marked	Type of movement	Radio fixes (N)	Distance moved (km)	Contact (mo)	Life span (mo)	Cause of death
NO Area								
155	1991	Fawn	Dispersal	28	58.5	33		Alive[1]
159	1991	Fawn	Migration	60	22.0	36	67	Auto
212	1992	Fawn	Dispersal	13	51.4	8	28	Auto
178	1992	2 yr	Migration	75	6.4	26		Alive[1]
196	1992	3 yr	Migration	69	8.5	25	77	Train
EC Area								
262	1980	Fawn	Migration	167	6.9	45	53	Bow cripple
276	1980	Fawn	Dispersal	11	57.3	9	17	Gun
218	1983	Fawn	Dispersal	30	25.5	8	17	Gun
507	1983	Fawn	Dispersal	48	65.0	25	29	Gun
577	1984	Fawn	Dispersal	23	36.8	8	17	Gun cripple
636	1985	Fawn	Dispersal	34	13.7	8	17	Gun
286	1980	Yearling	Dispersal	286	20.2	9	30	Gun
325	1981	Yearling	Dispersal	67	52.9	36	54	Auto
338	1981	Yearling	Dispersal	271	12.6	10	30	Gun
542	1984	Yearling	Dispersal	38	31.3	18	41	Unknown
619	1985	Yearling	Dispersal	19	22.8	8	28	Bow
266	1980	Yearling	Migration	157	7.3	44	65	Gun
558	1984	2 yr	Migration	111	3.2	33	65	Gun
238	1980	3 yr	Migration	113	20.8	10	54	Gun cripple
WC Area								
55	1991	Fawn	Dispersal	9	128.0	13		Alive[1]
117	1991	Fawn	Dispersal	7	60.3	10	17	Gun
138	1991	Fawn	Migration	36	5.0	8	17	Gun cripple
109	1991	Fawn	Migration	102	26.7	35	42	Gun
194	1992	Fawn	Migration	28	17.6	21	66	Gun
115	1991	Yearling	Migration	102	26.7	37	65	Gun

[1] Alive at end of study. Cause of death unknown.

Deer were aged using tooth replacement and wear from incisors and premolars observed on live deer as either fawn (<12 mo), yearling (12–23 mo), 2 yr old (24–35 mo), or adult (≥36 mo) (Severinghaus, 1949). Pregnancy was determined for fawn captures using blood progesterone levels (Wood *et al.*, 1986) or from direct observation of all 25 females on the new range. We captured deer in the order they were lured to bait sites with no random capture selection attempted. Radios were allocated according to their availability each year with a priority given to radio-marking family groups *(e.g.*, females with associated fawns and/or yearlings).

Females were radio tracked using 2 truck-mounted, 8-element yagi antennas aligned in a null configuration. Radio locations were validated using an unpublished program for the Apple II computer (L. P. Hansen, Illinois Natural History Survey) for the EC females and the software program LOCATE II (Nams, 1990) for the WC and NO females. Home range boundaries for each female was calculated for each natal range and for the new range selected by each immigrant using all radio fixes and observations (Table 1) and then superimposed on topographic maps (Lesage *et al.*, 2002).

We defined dispersal as the one-way emigration to a new home range at least 12 km from the natal range. Migration was defined as a movement by females at least 3 km away from the natal range to a new home range where parturition took place. These females then returned to their natal range each winter. Migrating females ≥2 yr old when first captured were considered to have already located their summer ranges as fawns or yearlings. We base this assumption on 2 known characteristics of white-tailed deer in Illinois: first, known female emigrations from our study areas have always begun following their ejection from their mother's parturition site in late spring at 10–13 mo old or, for various reasons (such as reproductive state, social status, loss of kin) delayed until 21–24 mo old (Nixon *et al.*, 1991); second, most mother's strong attraction each year to a site of parturition once a successful birth has occurred (Ozoga *et al.*, 1982; Tierson *et al.*, 1985; Dusek *et al.*, 1989; Nixon *et al.*, 1992; Etter *et al.*, 1995) and verified by the behavior of our marked females followed for longer than a single season (14 of 25 deer).

The new home range settled by female deer was occupied from late February until October 1, the date when fawns were considered finally weaned (Clutton-Brock *et al.*, 1982). The part of the new home range that was occupied May 15–July 15 was considered the parturition range, and the habitats within this range were analyzed separately from the total new home range. Generally, only migrating females moved as early as late February, whereas dispersing deer typically started moving in May. Each delineated home range was cover mapped as upland or bottomland forest <40 or ≥40 yr old, permanent grasslands (CRP lands, pastures active or abandoned, roadsides, cultivated hay fields, mowed grasses of cemeteries, lawns, and proposed housing developments), row crops of corn (*Zea maize*) or soybeans (*Glycine max*), and permanent water bodies. Forest overstories at all sites were similar to the natal sites when forest age and flooding frequency was also similar. Upland forest ≥40 yr old were dominated by various oaks (*Quercus sp.*) and hickories (*Carya sp.*). Those upland forests <40 yr old were mixtures of oaks, hickories, elms (*Ulmus sp.*), black walnut (*Juglands niger*), honeylocust (*Gleditsia triacanthos*), osage orange (*Machura pomifera*), and shingle oak (*Quercus imbricaria*). Bottomland forest <40 or ≥40 yr old that was frequently inundated was nearly a monotype of silver maple (*Acer saccharinum*), whereas dryer sites were mixtures of silver maple, hackberry

(*Celtis occidentalis*), sycamore (*Platanus occidentalis*), black walnut, cottonwood (*Populus deltoides*), ashes (*Fraxinus sp.*), and bur oak (*Quercus macrocarpa*).

Data Analysis

Because we used both migrating (N = 11) and dispersing deer (N = 14) for this analysis, we first compared the landscape characteristics of the new home ranges selected by the 2 types of movement behaviors using the t-test. The landscape variables used to analyze landscape use (the natal range, travel corridor used by each deer, parturition range, and new home range) are listed in Table 2. We separated the parturition ranges from the new home ranges because of the importance of cover to early fawn survival (Rohn *et al.*, 2007).

Table 2. Landscape attributes and human presence within the natal ranges, the new home ranges (May 15–October 1), and the parturition ranges (May 15–July 15) selected by 25 emigrating female white-tailed deer in Illinois during 1980–1985 and 1990–1993.

Landscape Variables

Travel Corridors
- Roads — all state and federal highways
- Rivers — all river crossings
- Forest patches — all distinct forest patches encountered
- Power lines — all high tension lines carrying at least 34,500 volts

Home Ranges
- Vegetation stage — dormant or actively growing when emigration began
- Female present — females actually seen on the new home ranges
- Percent forest — percent of home range forested
- Percent cropland — percent of home range in active crops
- Percent grasses — percent of home range in permanent grass
- Richness — sum of all distinct habitat patches
- Nearest neighbor — distance between forest patches (km)
- Forest patch density — number of forest patches per 10 ha
- Average size — average size of forest patches (ha)
- Houses — all occupied dwellings in the home range
- Forest age — <40 or ≥40 years
- Forest type — upland or bottomland forest

Several of these landscape variables were significantly correlated as determined by the nonparametric Spearman's Rho test (Zar, 1999), a result expected because the landscape is finite and higher amounts of 1 habitat mean lower amounts of another. For example, negative correlations included percent cropland with percent forest (Rho = –0.82, $P < 0.0001$), percent grassland (Rho = –0.61, P = 0.001), and forest patch size (Rho = –0.69, P = 0.0002), while positive correlations included forest patch size with percent forest (Rho = 0.70, $P < 0.0001$) and percent grassland (Rho = 0.40, P = 0.047) as well as number of forest patches with distance between forest patches (Rho = 0.63, P = 0.0007). We used stepwise regression (backward selection) to reduce the number of variables, allowing the analysis to sort for the appropriate (less correlated) landscape variables. Backward selection regression was chosen because it is better able to identify significant independent variables than is forward selection when there is multi-collinearity among variables (Zar, 1999).

Travel Characteristics

The deviation in travel direction from a straight line was examined by comparing radio locations along each travel route to a straight line drawn from the center of the natal range to the center of the new home range. We also tested for a deviation in the angle of dispersal for 14 deer from the EC area compared to the general direction azimuth of the river corridor. We used the Wilcoxon 2-sample test to examine for differences in the 2 measurements (Zar, 1999).

The initial direction (degrees azimuth) a female moved away from the natal range was measured and placed on USGS topographic maps (published 1971–1984 for EC, 1971–1980 for NO, 1981 for WC). We then compared this chosen direction with 6 alternative directions that were 30°, 60°, and 90° on either side of this route (and the same length as the actual route) using one-way ANOVA and recorded the number of roads (Illinois Secretary of State highway maps), rivers, forest patches, and high voltage power transmission lines (Illinois Commerce Commission 1971, 1997) encountered along these azimuths. We felt limiting this effort to 90° on either side of the actual route best provided an insight into the possible travel choices available to the marked females as they were leaving their natal range. Forest patches were located along each azimuth using topographic maps and rechecked using LANDSAT satellite imagery through the internet program Google Earth.

Recently there has developed interest in the biological effects of the electromagnetic forces associated with high voltage power lines. We were interested in the biological effects, if any, of electromagnetic forces on deer travel routes and on the selection of a new range. Both roe deer (*Capreolus capreolus*) and domestic cattle (*Bos taurus*) tend to align their resting body axis in the north-south geometric direction, and high voltage lines have been shown to disrupt this body alignment suggesting that these animals can detect and respond to such an electromagnetic force (Burda *et al.*, 2009).

Using topographic maps, we also recorded the amount of forest and cropland each deer encountered (based on radio locations) during an emigration. We then compared the percent forest and cropland present on the natal range with the same variables encountered along the travel corridor and with those within the newly settled home range using one-way ANOVA. Whether distance traveled between ranges affected final landscape selection was examined using distance traveled as a dependent variable in a stepwise regression analysis and using landscape characteristics found on the new range as independent variables (Table 2).

Home Range Characteristics

In an effort to determine the characteristics of the landscape in the 10 counties where our 25 females ultimately settled, we randomly selected 75 sections from these 10 counties using a ratio of 3 times the number of females followed from each study area (EC = 14, NO = 5, WC = 6) (Anderson *et al.*, 2005). The mean area of the new home range for our 25 females was 177.9 ± SE 41.9 ha (Table 3), and we used a circle of this size centered within each selected section on topographic maps to measure the amount of forests, croplands, and grasslands present at the time of emigration. We then compared the percentage of forest, grasslands, and croplands found in these sections with the actual habitats selected by our marked females using one-way ANOVA.

Table 3. Mean values of landscape characteristics measured within the natal ranges, new home ranges, parturition ranges, and random sites of 25 female white-tailed deer in Illinois during 1980–1985 and 1990–1993. The natal and random sites were measured as the same new home range size.

						Percent of total			Forest patches only		
Location	N	Home Range (ha)	Roads (km)	Houses (N)	Richness (all types)	Forest	Grassland	Crop	Size (ha)	Density (per 10 ha)	Nearest Neighbor (km)
Natal											
	25	179.5	1.87	2.9	4.9	39.2	26.9	31.3	52.5[a]	0.12	0.08
New home (May 15–October 1)											
	25	179.5	1.47	4.36	7.2[a]	35.7	19.7	43.4	20.7	0.29	0.13
Parturition (May 15–July 15)											
	20	28.9	0.30[a]	0.72[a]	3.1	42.2	21.3	36.3	10.4	0.65[a]	0.09
Random sites											
	75	179.5	1.22	10.6	1.1	9.4	9.8	75.5	— No Data —		

[a]Value significantly different from other ranges/sites (see text)

We also used ANOVA tests to compare forest characteristics (Table 2) present in the natal ranges with the parturition and new home ranges selected by our marked females. Using one-way ANOVA, we compared similar characteristics of ranges selected by females (N = 15) that appeared to be stopped in their travels by an obstacle such as open agricultural land (*e.g.*, a lack of forest), the presence of a large urban area, or a large river valley dominated by cultivated lands with those females (N = 10) where no obvious obstacle to further travel existed.

Costs of Emigration

Stepwise regression analysis using life span as the dependent variable was compared with 13 independent variables describing the human activities, general landscape characteristics found in the selected ranges, and possible social encounters (females present or not) (Table 2).

The possible effects of landscape selection on subsequent fawn survival was examined using one-way ANOVA to compare the landscape characteristics (total patch richness, forest patch nearest neighbor, forest patch density and average size) chosen by females that successfully raised 1 or more fawns with those that lost 1 or more fawns before fawn independence at 1 yr old.

RESULTS

Twenty-five radio marked females (4 adults, 7 yearlings, 14 fawns when marked) were tracked for an average of 20.9 mo. The usable radio fixes averaged 76.2 (range 7–286) per female (Table 1). The 14 dispersing females moved an average of 44.9 ± SE 6.4 km from their natal range, whereas the 11 migrators moved an average of 14.0 ± SE 7.2 km.

One female moved in February, 5 females moved in March, 5 in April, and 7 each in May and June. The February–April movements were made by females ≥20 mo old. The fawns (10–12 mo old) moved in May or June.

Nineteen of the 25 females were marked on the edge of the study areas where subordinate groups tended to reside (Nixon *et al.*, 2010), and 18 of these 19 females were members of social groups considered subordinate. Only 5 females were offspring of a dominant matriarch or 1 of her daughters (social status was unknown for 2 females). Sixteen females were pregnant when they emigrated (6 pregnant for the first time), 5 were barren, and the reproductive status was unknown for the remaining 4 females. Twelve females left the natal range without kin support, whereas 13 traveled with a relative (2 with mother, 4 with a sibling, 7 with offspring of the previous year).

For the new home ranges selected by dispersing and migrating deer, there were no significant differences in the composition of home ranges selected by migrating (N = 11) versus dispersing (N = 14) females in the total patch richness, nearest neighbor distance, average forest patch density or size, forest type (upland vs bottomland), the percentage of landscape in forest, grassland, or cropland, or in the roads and houses present (t = 0.04–1.65, P = 0.11–0.98). Thus, we treated the 2 groups as a single sample.

Travel Characteristics

A comparison between a straight line drawn from the center of the natal range to the center of the new range indicated that travel deviated only 8.2% from a straight line. However, the matched pair test indicated the actual path deviated by 2.33 ± SE 0.53 km and was significantly different from zero (P = 0.0003). For deer emigrating from the EC study area, with the general river corridor at 45°, the mean direction for the 14 females was 59° or eastward, a significant deviation from the direction of the river (P = 0.002).

Comparing the actual route with the simulated alternate routes (30°, 60°, or 90°), EC females crossed rivers significantly more often (F = 6.9, P = 0.009) and encountered significantly more forest patches (6.6 vs. 1.8, F = 26.8, P < 0.0001), but did not differ in the number of roads (F = 0.74–1.52, P = 0.23–0.39) or power lines crossed (F = 0.04–1.26, P = 0.39–0.85). Routes selected by females from the WC and NO areas, with no river corridor, did not differ from the simulated routes in the number of forests, rivers, roads, and high voltage power lines crossed during their travels.

A comparison between forest and cropland portions of the natal ranges, travel corridors, and new home ranges found that the travel corridor had more cropland (63% in travel corridor, 43% in new home range, 30% in natal range; F = 8.4, df = 2, 72, P = 0.0005), but all 3 locations were similar in the amount of forest cover encountered (35–42%), and all differed from random sites (P < 0.001) (Table 3).

The stepwise regression for distance traveled using 13 variables resulted in 3 significant variables (vegetative stage, movement type, and number of houses encountered) accounting for nearly all the variance (R^2 = 0.83). However, these results were biased by the longer distances moved by dispersing females and the disparity in initiation of movement between fawns (May–June) and older deer (February–April). The farther deer dispersed, the more houses were present in the selected new home range, perhaps an indication that these females were becoming less discriminating as parturition approached.

Home Range Characteristics

Females selected a new home range with significantly more forest (35.7% vs. 9.0%), more permanent grassland (19.7% vs. 2.2%), and less cropland (43.5% vs. 75.5%) than was indicated as available in our random sections from 10 counties where the 25 females selected a new home range ($F = 4.7–34.2$, $P = 0.03–< 0.001$) (Table 3). Females in EC Illinois settled on areas with more total patch richness, forest patch density, and forest patch size compared to random sites ($P = 0.007$). Northern Illinois females also selected sites featuring more forest patches of larger size ($P = 0.01$) compared to random sites. Home range size was positively associated with the percent of cropland present in new home range ($F = 10.6$, $P = 0.003$) and negatively associated with the percent of forest cover present ($F = 7.3$, $P = 0.01$). This result was biased by the large ranges occupied by the 3 females that settled on landscapes entirely devoid of forest cover. However, final home range size was not influenced by patch richness, forest patch number and size, forest type and age, or the distance between forest patches ($P > 0.05$) (Table 3).

On new home ranges, the presence or absence of conspecific females (17 present, 8 absent) did not differ as to patch richness, nearest neighbor distance, size or density of forest patches, or with random sites (Table 3). In a comparison of natal, new home, and parturition ranges, patch richness was significantly higher in the new home range ($F = 7.6$, $df = 2, 65$, $P = 0.001$) (Table 3). There were more forest patches present in the parturition ranges than in the other 2 ranges ($F = 15.7$, $df = 2, 64$, $P < 0.0001$), but the natal ranges contained larger patches ($F = 15.2$, $df = 2, 62$, $P < 0.001$). Parturition sites featured fewer houses ($P = 0.04$) and roads ($P < 0.001$) than the natal or new home ranges.

Five females stopped emigrating because their way was blocked by an obvious obstacle, 3 by an urban area, 1 by the expansive Interstate-72 highway which she never crossed, and 1 female by the 1.6 km wide cultivated Illinois River valley (Table 3). An additional 7 females traveled within a watershed that progressively lost forest cover. These females stopped at the site of the last reasonably abundant forest cover after first probing ahead and then returning along the more open watershed. Six additional females stopped in the first woodlot encountered after leaving the natal range. There was no obvious reason for stopping for 7 females (5 pregnant, 1 barren, 1 reproductive status unknown) other than, in some cases, impending parturition for 5 of 7 females. We compared the landscape composition of home ranges of females stopped by an obvious obstacle ($N = 5$) with those that were not stopped ($N = 20$) to see if females that stopped had home range characteristics different from those with a more open choice. We found no significant difference in the amount of forest, grasslands, and croplands present between the 2 groups ($P > 0.05$).

Costs of Emigration

We used life span and fawn survival to assess the costs of locating away from the natal range and the loss of kin support. Stepwise regression using life span as the dependent variable and 13 landscape and human intrusion variables occurring in each new home range, found only the type of movement (disperse or migrate) significant ($R^2 = 0.50$). We did not find any significant effect of composition in the new home range on whether or not our females lost 1 or more fawns before weaning (P > 0.20). Of 6 pregnancies (by 5 females) where fawns were lost, 4 occurred by the end of August.

DISCUSSION

Emigrating females in WC Illinois did not select habitats significantly different in composition from random sites. Apparently when forest cover is abundant (in this case about 20% of the landscape), dispersing female's selection of a parturition site depends on factors other than habitat composition such as obstacles encountered, impending birth, or avoiding human intrusions. However, the more forested landscape in WC Illinois did not reduce the distance females traveled before settling on a new home range (Table 1).

In areas of Illinois where forest covers <5% of the landscape, emigrating females must search for forest cover sufficient to protect fawns from disturbance and predation. Marked females from EC and NO Illinois selected a landscape with more forest and permanent grassland present than was available in the general landscape. Three NO females (#159, #155, #196) each selected a range with no permanent forest cover except fence lines and narrow hedgerows. Two of these were from subordinate social groups, and both of these deer were pregnant. The remaining female dispersed from a dominant social group, her immediate reproductive status unknown. Selections of forest-free areas indicate some females in Illinois appear to use agricultural lands in a similar fashion as roe deer do in Europe, including settling in areas entirely devoid of forests (Harris and Woollard, 1990).

Home range habitat characteristics selected by migrating and dispersing females differed only in the distance between forest patches, with migrating females selecting the more scattered forest cover. These migrating females did not have to winter on these ranges and could accommodate a summer-fall range with less forest cover. Migratory females live longer than dispersing females, a result of their vacating hunted summer ranges for the more protected natal range (Nixon *et al.*, 2008). We do not know how or when deer determine the suitability of a site for year-round occupancy, but selection of a cover-deficient site necessitates movement away in late fall. We suspect deer do not initially determine this suitability but depend on circumstances to dictate a movement response as the seasons change. The return to the natal range is the easier choice because females are familiar with the route back to the natal range.

Both types of movement behavior used nearly a straight line during emigration, readily crossing rivers, roads, and high voltage power line rights-of-way. Travel corridors traversed significantly more cropland than was present in the selected new home range, an expected result given Illinois' agriculturally dominated landscape. Travel distance was influenced by plant phenology (farther distance after leaf out), but age was the primary cause, with fawns moving later and farther than older deer.

There is almost no literature on how emigrating deer orient themselves during a dispersal movement (Andreassen *et al.*, 2002). Long *et al.* (2010) found that male immigrants were influenced in their selection of a travel route by the presence of mountain ridges on 1 study area in Pennsylvania but not from a second area. These Pennsylvania deer also tended to avoid 4-lane highways, a feature of emigration detected for only 1 of our females (#262). The presence of roads can alter behaviors by affecting home range size, escape responses, and physiology for deer exposed to them (Trombulak and Frissell, 2000), but Illinois deer are exposed to many road crossings almost from birth. The natal ranges for our study females were surrounded by roads with at least moderate traffic.

A forested travel corridor existed within the EC study area but not on the WC and NO study areas, and 10 of 14 EC females used the river corridor to exit their natal range. Four of these females then left the river corridor and traversed agricultural fields to the site of new home range selection. Three of these females selected ranges without other females present, perhaps because of resident female harassment during travel or that 3 of these females were considered subordinate in the social order on their natal range. One female (#507) was born into a dominant group, but also selected a female-free location for a parturition site.

Most of our study females moved rapidly and in nearly a straight line between the natal and final home range, considered the simplest and most efficient way to complete an emigration movement (Zollner and Lima, 1999; Wiems, 2001). Long *et al.* (2010) also observed that emigrating male white-tails moved quickly without much wandering and, because of this behavior, felt that males were not searching for specific habitats. Kilgo *et al.* (1996) found that dispersing deer also traveled rapidly in nearly a straight line while moving through unfamiliar landscapes in Florida. There appeared to be no avoidance of rivers, major roads, or high voltage power lines during female travels between ranges in Illinois. In fact, 10 of 25 females settled on ranges with a high voltage power line present and were seen bedded and feeding within the power line rights-of-way. Except during flooding episodes, rivers encountered were generally <50 m wide and presented no obstacle to continued movement.

Parturition ranges for all 25 females contained fewer houses and roads and more forest patches than either the natal ranges or the larger new home ranges. Storm *et al.* (2007) reported that breeding females avoided occupied houses during the parturition season in southern Illinois. More forest cover has been associated with increased fawn survival in Illinois (Rohn *et al.*, 2007) and a somewhat higher ratio of resident females to migrating females in the northern plains (Grovenburg *et al.*, 2011). Coyotes are less efficient hunters in forest cover, so more forest cover should improve fawn survival (Richer *et al.*, 2002; Rohn *et al.*, 2007). Rohn *et al.* (2007) found that more edge and larger forest patches improved fawn survival by reducing predator risk in southern Illinois. They also noted that smaller forest patches surrounding the larger forest blocks reduced home range size and provided more feeding opportunities (Rohn *et al.*, 2007). Our marked females also selected ranges for increased patch density and fewer human activities in the parturition range. Fawn survival appeared to be independent of landscape composition, similar to observations in Pennsylvania where patch diversity, amount of forest edge, and road density had no significant affect on fawn survival (Vreeland *et al.*, 2004).

Life span of females was affected by the amount of forest cover, with larger forests more likely to offer increased hunting opportunities and a lower life expectancy. This is the reverse situation found for deer in South Dakota where more forest cover improved life span (Klaver *et al.*, 2008). In Illinois, even the larger forest patches were generally <100 ha in size (Iverson *et al.*, 1989). Harden *et al.* (2005) found that the larger blocks of forest cover attracted more deer hunters in southern Illinois.

The new home range selected by our marked females tended to be elongate in shape, considered an optimum shape for exploiting a patchy environment (Ford, 1983). Home range size was affected by the amount of cropland encountered with females encompassing a much larger home range as forest cover declined, similar to that observed in SW Minnesota (Walter *et al.*, 2009). Home range size for the 3 females with no forest cover present ranged up to 1,000 ha with daily movements often 2–3 km, similar to home ranges and movements described for deer in South Dakota (Sparrowe and Springer, 1970; Grovenburg *et al.*, 2009). Two of the 3 females were migrants, returning to their natal range each winter after corn harvest. The third female sheltered in unpicked corn during winter.

A first pregnancy for 6 females that delayed emigration until late May–early June meant that these females did not have a lot of time before parturition to find a new range. As noted by Orians and Wittenberger (1991), the later emigration is delayed before some important event (parturition, winter weather, etc.), the poorer the options available. Estrogen levels continue to increase from 6 wk prepartum to parturition, and the estrogen/progesterone ratio rises steeply during the final weeks of pregnancy (Plotka *et al.*, 1977) which may stimulate pregnant females to disperse quickly close to parturition. Five of these females were dispersers, and 1 was a migrant. Even though forest cover was quite limited for 3 of these females, all 6 reared fawns to weaning age, and 2 of these females survived >2 yr on their new range.

Some of the marked fawns and yearlings departed the natal range without kin support (10 moved alone, 2 with unknown deer), relying on instinct and perhaps the knowledge gained by familiarity with the characteristics of the natal range (forest age and composition) to guide their selection process. Forests surrounding all sites were very similar to the natal ranges for these females in age and flooding frequencies (Nixon *et al.*, 1991). Deer obviously use olfactory cues, sounds emanating from the environment, and their vision to settle on or avoid areas as they travel (Andreassen *et al.*, 2002). Six females had favorable winds blowing from the site of final settlement during at least 2 days while emigrating.

Six of our 25 females were known to have stopped for 1–3 days along the journey, perhaps accessing the qualities of the site for permanent occupancy (Opdam, 1990; Stamps, 2001). Emigration then continued perhaps because of resident deer aggression (Ozoga *et al.*, 1982), insufficient cover before crops are high enough for concealment before a June movement (Nixon *et al.*, 2007), insufficient forage (unlikely in an agriculture-dominated landscape), excessive human disturbance (Etter, 2001), or, for 5 of the 6 females, impending parturition.

Dispersal theory states that dispersing individuals should disperse to the first vacant territory and stop because longer movements incur more risks (Murray, 1967). However, in areas where forests cover is <5% of the landscape and human disturbances are frequent, dispersing females are forced to travel long distances before settling (Sparrowe and Springer, 1970; Zagata and Haugen, 1973; Kernohan *et al.*, 1994; Nixon *et al.*, 2007).

Migrating females move shorter distances and can afford to be less selective as to cover because they return to their natal range in winter. Unlike males, females, because of fawn rearing requirements, must be more selective in order to protect fawns when selecting a new range. Life threatening events such as hunting are not apparent when females select new ranges in late spring-early summer, but even so, life history attributes like dispersal or migratory behaviors that evolved as protection from predation, variable climates, or habitat alterations from the Pleistocene to the present day are still effective options in today's environment in Illinois (Danchin *et al.*, 2001; Stamps, 2001).

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LITERATURE CITED

Anderson, D. P., M. G. Turner, J. D. Forester, J. Zhu, M. S. Boyce, H. Beyer and L. Stowell. 2005. Scale-dependent summer resource selection by reintroduced elk in Wisconsin, USA. *J. Wildl. Manage.,* 69:296–310.

Andreassen, H. P., N. C. Stenseth and R. A. Ims. 2002. Dispersal behavior and population dynamics of vertebrates. p. 237–256. *In:* J. M. Bullock, R. E. Kenwood and R. S. Hails (eds.). Dispersal Ecology. Blackwell Publ., Malden, Massachusetts.

Brinkman, T. J., C. S. Deperno, J. A. Jenks, B. S. Haroldson and R. G. Osborn. 2005. Movement of female white-tailed deer: effects of climate and intensive row-crop agriculture. *J. Wildl. Manage.*, 69:1099–1111.

Burda, H., S. Begall, J. Cerveny, J. Neef and P. Nemec. 2009. Extremely low-frequency electromagnetic fields disrupt magnetic alignment of ruminants. *PNAS,* 106:5708–5713.

Clutton-Brock, T. H., F. E. Guinness and S. D. Albon. 1982. Red deer: behavior and ecology of two sexes. University of Chicago Press, Chicago, Illinois.

Danchin, E. D., D. Heg and B. Doligez. 2001. Public information and breeding habitat selection. p. 243–258. *In:* J. Clobert, E. Danchin, A. Dhondt and J. Nichols. (eds.) Dispersal. Oxford University Press, New York.

Dusek, G. L., R. J. Mackie, J. D. Herriges, Jr. and B. B. Compton. 1989. Population ecology of white-tailed deer along the lower Yellowstone River. *Wildl. Monog.*, 104. The Wildlife Society, Washington, D.C.

Etter, D. R. 2001. Ecology and management of overabundant white-tailed deer from suburban Chicago, Illinois. Ph.D. Dissertation, University of Illinois, Urbana, Illinois.

Etter, D. R., C. M. Nixon, J. B. Sullivan and J. A. Thomas. 1995. Emigration and survival of orphaned female deer in Illinois. *Can. J. Zool.*, 73:440–445.

Ford, R. G. 1983. Home range in a patchy environment: optimal foraging predictions *Amer. Zool.*, 23:315–326.

Gladfelter, H. L. 1978. Movement and home range of deer as determined by radio telemetry. *Iowa Wildl. Res. Bull.*, 23.

Godvik, I. M. R., L. E. Loe, J. O. Vik, V. Veiberg, R. Langvator and A. Mysterud. 2009. Temporal scales, trade-offs, and functional responses in red deer habitat selection. *Ecology,* 90:699–710.

Grovenburg, T. W., J. A. Jenks, R. W. Klaver, C. C. Swanson, C. N. Jacgues and D. Todey. 2009. Seasonal movements and home ranges of white-tailed deer in north-central South Dakota. *Can. J. Zool.*, 87:876–886.

Grovenburg, T. W., J. A. Jenks, R. W. Klaver, C. S. DePerno, T. J. Brinkman, C. C. Swanson and J. A. Jenks. 2011. Influence of landscape characteristics on migration strategies of white-tailed deer. *J. Mammal.*, 92:534–543.

Grovenburg, T. W., C. N. Jacques, C. C. Swanson, R. W. Klaver and J. A. Jenks. 2010. Use of late season standing corn by female white-tailed deer in the northern great plains during a mild winter. *Prairie Nat.*, 42:8–18.

Harden, C. D., A. Woolf and J. Roseberry. 2005. Influence of exurban development on hunting opportunity, hunter distribution, and harvest efficiency of white-tailed deer. *Wildl. Soc. Bull.*, 33:233–242.

Harris, S. and T. Woollard. 1990. The dispersal of mammals in agricultural habitats in Britain. p. 159–188. *In*: R. Bunce and D. Howard (eds). Species dispersal in agricultural habitats. Belhaven Press, New York.

Hawkins, R. E., L. D. Martoglio and G. G. Montgomery. 1968. Cannon netting deer. *J. Wildl. Manage.*, 32:191–195.

Illinois Commerce Commission. 1971. Electric utilities in Illinois, 34,500 or higher voltage. Ill. Commerce Comm., Springfield.

Illinois Commerce Commission. 1997. Electric utilities in Illinois, 34,500 or higher voltage. Ill. Commerce Comm., Springfield.

Iverson, L. R., R. L. Oliver, D. P. Tucker, P. G. Risser, C. D. Burnett and R. G. Rayburn. 1989. The forest resources of Illinois: an atlas and analysis of spatial and temporal trends. *Ill. Nat. Hist. Surv. Spec. Publ.*, 11:181.

Kilgo, J. C., R. F. Labisky and D. E. Fritzen. 1996. Directional long-distance movements by white-tailed deer (*Odocoileus virginianus*) in Florida. *Wildlife Biology*, 3:289–292.

Kernohan, B. J., J. A. Jenks and D. E. Naugle. 1994. Movement patterns of white-tailed deer at Sand Lake National Wildlife Refuge, South Dakota. *Prairie Nat*, 26:293–300.

Klaver, R. W., J. A. Jenks, C. S. Deperno and S.L. Griffin. 2008. Associating seasonal range characteristics with survival of female deer. *J. Wildl. Manage.*, 72:343–353.

Lesage, L., M. Crete, J. Huot and J. P. Ouellet. 2002. Use of forest maps versus field surveys to measure summer habitat selection and sexual segregation in northern white-tailed deer. *Can. J. Zool.*, 80:717–726.

Long, E. S., D. R. Diefenbach, B. D. Wallingford and C. S . Roseberry. 2010. Influence of roads, rivers, and mountains on natal dispersal of white-tailed deer. *J. Wildl. Manage.*, 74:1242–1249.

Morrison, M. L., B. G. Plarcot and R. W. Mannan. 2006. Wildlife-habitat relationships: concepts and applications. 3rd edition. Island Press, Washington, D.C.

Murray, B. G., Jr. 1967. Dispersal in vertebrates. *Ecology*, 48:975–978.

Nams, V. O. 1990. Locate II user's guide. Pacer, Truro, Nova Scotia, Canada.

Nelson, M. E. and L. D. Mech. 1992. Dispersal in female white-tailed deer. *J. Mammal.,* 73:891–894.

Nixon, C. M., L. P. Hansen, P. A. Brewer and J. E. Chelsvig. 1991. Ecology of white-tailed deer in an intensively farmed region of Illinois. *Wildl. Monog.* 118. The Wildlife Society, Washington, D. C.

Nixon, C. M., L. P. Hansen, P. A. Brewer and J. E. Chelsvig. 1992. Stability of white-tailed doe parturition ranges on a refuge in east-central Illinois. *Can. J. Zool.,* 70:968–973.

Nixon, C. M., P. C. Mankin, D. R. Etter, L. P. Hansen, P. A. Brewer, J. E. Chelsvig, T. L. Esker and J. B. Sullivan. 2007. White-tailed deer dispersal behavior in an agricultural environment. *Amer. Midl. Nat.*, 157:212–220.

Nixon, C. M., P. C. Mankin, D. R. Etter, L. P. Hansen, P. A. Brewer, J. E. Chelsvig, T. L. Esker and J. B. Sullivan.. 2008. Migration behavior among female white-tailed deer in central and northern Illinois. *Amer. Midl. Nat.*, 160:178–190.

Nixon, C. M., P. C. Mankin, D. R. Etter, L. P. Hansen, P. A. Brewer, J. E. Chelsvig, T. L. Esker and J. B. Sullivan.. 2010. Characteristics of dominant and subordinate led social groups of white-tailed deer in Illinois. *Amer. Midl. Nat.,* 163:388–399.

Opdam, P. 1990. Dispersal in fragmented populations: the key to survival. p. 3–17. *In*: R. G. H. Bunce and D. C. Howard. (eds.) Species dispersal in agricultural habitats. Belhaven Press, New York.

Orians, G. H. and J. F. Wittenberger. 1991. Spatial and temporal scales in habitat selection. *Amer. Nat.,* 137:529–549.

Ozoga, J. J., L. J. Verme and C. S. Bienz. 1982. Parturition behavior and territoriality in white-tailed deer: impact on neonatal mortality. *J. Wildl. Manage.*, 46:1–11.

Plotka, E. D., U. S. Seal, L. J. Verme and J. J. Ozoga. 1977. Reproductive steroids in the white-tailed deer (*Odocoileus virginianus borealis*). II. Progesterone and estrogen levels in peripheral plasma during pregnancy. *Biol. Reprod.,* 17:78–83.

Richer, M. C., M. Crete, L. P. Rivest and J. Huot. 2002. The low performance of forest versus rural coyotes in northern North America: inequality between presence and availability of prey. *Ecoscience,* 9:44–54.

Rohn, J. H., C. K. Neilsen and A. Woolf. 2007. Survival of white-tailed deer fawns in southern Illinois. *J. Wildl. Manage.*, 71:851–860.

Severinghaus, C. W. 1949. Tooth development and wear as criteria of age in white-tailed deer. *J. Wildl. Manage.*, 13:195–216.

Sparrowe, R. D. and P. F. Springer. 1970. Seasonal activity patterns of white-tailed deer in eastern South Dakota. *J. Wildl. Manage.*, 34:420–431.

Stamps, J. A. 2001. Habitat selection by dispersers: integrating proximate and ultimate approaches. p. 230–242. *In*: J. Clobert, E. Dauchin, A. A. Dhondt and J.D. Nichols (eds.). Dispersal. Oxford University Press, New York.

Storm, D. J., C. K. Nielsen, E. M. Schulier and A. Woolf. 2007. Space use and survival of white-tailed deer in an exurban landscape. *J. Wildl. Manage.*, 71:1170–1176.

Tierson, W. C., G. F. Mattfeld, R. W. Sage, Jr. and D. F. Behrend. 1985. Seasonal movements and home ranges of white-tailed deer in the Adirondacks. *J. Wildl. Manage.*, 49:760–769.

Trombulak, S. C. and C. A. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. *Conserv. Biol.*, 14:18–30.

VerCauteren, K. C. and S. E. Hygnstrom. 1994. A review of white-tailed deer movements in the Great Plains relative to environmental conditions. *Great Plains Research,* 4:117–132.

Vreeland, J. K., D. R. Diefenbach and B. D. Wallingford. 2004. Survival rates, mortality causes, and habitats of Pennsylvania white-tailed deer fawns. *Wildl. Soc. Bull.*, 32:542–553.

Walter, W. D., K. C. VerCauteren, J. M. Gilsdorf and S. E. Hygnstrom. 2009. Crop, vegetation and biofuels: response of white-tailed deer to changing management priorities. *J. Wildl. Manage.*, 73:339–344.

Wiems, J. A. 2001. The landscape context of dispersal. p. 96–109. *In*: J. Clobert, E. Danchin, A. A. Dhondt and J. Nichols (eds.). Oxford University Press, New York.

Wood, A. K., R. E. Short, A. Darling, G. L. Dusek, R. G. Sasser and C. A. Ruder. 1986. Serum assays for detecting pregnancy in mule and white-tailed deer. *J. Wildl. Manage.*, 50:684–687.

Zagata, M. D. and A. O. Haugen. 1973. Winter movement and home range of white-tailed deer at Pilot Knob State Park, Iowa. *Proc. Iowa Acad. Sci.*, 79:74–78.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, New Jersey.

Zollner, P. A. and S. L. lima. 1999. Search strategies for landscape level interpatch movements. *Ecology,* 80:1019–1030.

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Illinois Status Survey of the Redside Dace *Clinostomus elongatus*: The Newest Addition to the State's Native Fish Fauna

Jeremy S. Tiemann* and Mark H. Sabaj Pérez[1]
Illinois Natural History Survey, Prairie Research Institute
University of Illinois, 1816 South Oak Street, Champaign, IL 61820
[1]Present Address: The Academy of Natural Sciences of Drexel University
1900 Benjamin Franklin Parkway, Philadelphia, PA 19103-1195
*Correspondence: jtiemann@illinois.edu

ABSTRACT

The redside dace *Clinostomus elongatus* is a small, laterally compressed cyprinid commonly found in small streams with moderate to high gradients, clear and cool water, and substrates of clean gravel, sand, or bedrock. Fish surveys in Winnebago County, Illinois, and Rock County, Wisconsin, conducted from 1997-2000 and 2010-2011 discovered the dace at a single site in Illinois in East Fork Raccoon Creek (Pecatonica River – Rock River Drainage). This is the first documented record of this species in Illinois and raises the known total of state native fishes to 192. Based on these surveys, *C. elongatus* is expected to periodically occur in the Illinois portion of the Raccoon Creek basin, and therefore should be considered a peripheral species in Illinois.

INTRODUCTION

The redside dace *Clinostomus elongatus* is a small, laterally compressed minnow (Cyprinidae) with a large oblique mouth and long pointed snout. It commonly inhabits small streams with moderate to high gradients, clear and cool water, and substrates of clean gravel, sand, or bedrock (Trautman, 1981; Becker, 1983). The dace is drawn to particular habitat features, and generally orients toward stream positions with slower current velocities, greater depths, and closer to woody debris (Novinger and Coon, 2000; Zimmerman, 2009). *Clinostomus elongatus* is distributed across once glaciated regions of the northeastern United States and southern Ontario in watersheds draining into the Mississippi River, Ohio River, Great Lakes, and Atlantic Ocean (COSEWIC, 2007). The western-most populations occur in a few isolated, disjunct populations throughout the upper Mississippi River basin, including the Raccoon Creek basin (Pecatonica River – Rock River Drainage) in Rock County, Wisconsin (Gilbert, 1980; Becker, 1983). However, the species had never been documented in Illinois. We performed fish surveys in the Raccoon Creek basin to determine the presence of *C. elongatus* in Illinois.

METHODS

Two assessment surveys were preformed in the Raccoon Creek basin (Pecatonica River - Rock River Drainage). The first assessment occurred from November 1997 to April 2000 and was conducted by MHSP (Sabaj, 2000), whereas the second was conducted by JST and occurred from June 2010 to October 2011. During the first assessment, fishes were collected at 10 sites in the basin with a 2.43 m minnow seine and a Smith-Root backpack electrofisher (model 12-B) powered by a 24 volt, 12Ah battery for up to 4 hours per site; each site was visited 1-5 times during the Spring-Summer and/or Fall-Winter (Sabaj, 2000). During the second assessment, fishes were collected at 20 sites (Table 1) for up to 2 hours per site using a barge electro-shocker set at 200 volts, a Smith-Root Model 12 DC backpack electrofisher, or a 3.05 m minnow seine; each site was visited four times. During each site-visit, all fishes collected were identified to species in the field. A subsample of fishes representing the total diversity collected at each site was vouchered and deposited in the Illinois Natural History Survey Fish Collection, Champaign (INHS). Fishes collected but not vouchered were returned without harm to their native habitat. Nomenclature follows Page and Burr (2011). In addition, the Wisconsin Department of Natural Resources' (WDNR) Fish Mapper was accessed (http://infotrek.er.usgs.gov /wdnrfish/map/index), and fishes housed at the following museums were searched for voucher specimens of *C. elongatus* collected from Illinois and Wisconsin: Academy of Natural Sciences, Philadelphia (ANSP), Field Museum of Natural History, Chicago (FMNH), INHS, Milwaukee Public Museum (MPM), University of Kansas Museum of Natural History, Lawrence (KU), Southern Illinois University Fish Collection, Carbondale (SIUC), University of Michigan Museum of Zoology, Ann Arbor (UMMZ), and United States National Museum - Smithsonian Institution, Washington D.C. (USNM). Museum names and codes follow Sabaj Pérez (2012).

RESULTS

The two assessments of the Raccoon Creek basin yielded 55 native species of fishes, including *C. elongatus* (Table 1). However, only once was *C. elongatus* collected in Illinois. Eight juveniles were taken on 31 May 1998 in East Fork Raccoon Creek, 4.3 km NW Rockton, Winnebago County, 42.48508°N, 89.13744°W (Figure 1 – Site 17). Seven specimens were vouchered (INHS 46430) and one was returned live to the stream at the place of capture. All specimens were collected in a relatively deep (ca. 3-3.5 ft.) and partially shaded pool (side pocket of the main channel) with a thick silt and mud substrate. In the previous year, a beaver dam stretched across the channel just below the mouth of the pocket, inundating a short stretch of the creek. The beaver dam had been removed by the landowner prior to the 1998 visit; however, the pool retained water and provided refuge for the redside dace and many other juvenile minnows including bigmouth shiners *Notropis dorsalis*, southern redbelly dace *Chrosomus erythrogaster*, fathead minnows *Pimephales promelas*, creek chubs *Semotilus atromaculatus* and brassy minnows *Hybognathus hankinsoni*. The same pool was sampled on eight additional occasions, once before collecting the redside dace and seven times after, but only the one visit yielded any dace.

Table 1. Sampling sites for the *Clinostomus elongatus* survey. Streams include Raccoon Creek (RC), unnamed tributaries to Raccoon Creek (Trib RC), East Fork Raccoon Creek (EFRC), and unnamed tributary to East Fork Raccoon Creek (Trib EFRC). Latitude and longitude are in decimal degrees. Reference Point is approximate location of site on gazetteer. Point on map (PoM) refers to site number on Figure 1. Asterisks (*) are the sites sampled by Sabaj (2000). Last observed (Last obs.) is when *Clinostomus elongatus* was last observed at the site and includes the 1997-2000 and 2010-2011 surveys as well as those preformed by Fago (1982).

Stream	State: County	Latitude	Longitude	Reference Point	PoM	Last obs.
RC	WI: Rock	42.5751	-89.2079	S Luther Valley Rd	1	Fago (1982)
RC	WI: Rock	42.5416	-89.2072	W Beloit Newark Rd	2	2011
RC	WI: Rock	42.5265	-89.1955	WI Rte 81	3	
RC	WI: Rock	42.5092	-89.1696	Mill Pond Rd	4	Fago (1982)
RC	WI: Rock	42.5038	-89.1609	W St Lawrence Rd	5	
trib RC	WI: Rock	42.5234	-89.1781	WI Rte 81	6	
trib RC	WI: Rock	42.5278	-89.1692	Co Hwy H	7	
EFRC	WI: Rock	42.5628	-89.1446	W Gravedale Rd	8	Fago (1982)
EFRC	WI: Rock	42.5408	-89.1326	W Beloit Newark Rd	9	2011
EFRC	WI: Rock	42.5261	-89.1256	W Spring Creek Rd	10*	2011
EFRC	WI: Rock	42.5041	-89.1173	W St Lawrence Rd	11	2011
EFRC	WI: Rock	42.4974	-89.1202	State line	12*	2011
trib EFRC	WI: Rock	42.5255	-89.0949	W Spring Creek Rd	13*	
RC	IL: Winn.	42.4916	-89.1482	Power line crossing	14*	
RC	IL: Winn.	42.4760	-89.1377	Yale Bridge Rd	15*	
RC	IL: Winn.	42.4557	-89.1270	W Rockton Rd	16*	
EFRC	IL: Winn.	42.4850	-89.1374	Confluence with RC	17*	1998
Trib RC	IL: Winn.	42.4939	-89.1708	Pomeroy Rd	18*	
Trib RC	IL: Winn.	42.4768	-89.1266	Yale Bridge Rd	19*	
Trib RC	IL: Winn.	42.4676	-89.1381	Clover Rd	20*	

The dace was collected throughout the Wisconsin side of the Raccoon Creek basin including <0.5 km from the state line during the second survey (Table 1). Three adults were collected on 1 August 2011 in East Fork Raccoon Creek, 7.3 km W Beloit, Rock County, 42.49969°N, 89.11987°W (Figure 1 – Site 12). One specimen was vouchered (INHS 104899) and two were returned live to the stream at the place of capture. These three individuals were collected in a clear pool containing gravel/cobble substrates. This site was ~1 km upstream of where the Illinois specimens were collected in 1998. Of the museums examined, only INHS and MPM had redside dace specimens from the Raccoon Creek basin. On 8 June 1998, 24 adult specimens were collected in the East Fork Raccoon Creek, 8.0 km W Beloit, in Rock County, Wisconsin, 42.52617°N, 89.12562°W (INHS 46977). MPM had specimens collected by Fago (1982), who reported the dace from another locality in the upper East Fork and 3 localities in upper Raccoon Creek, Rock County, Wisconsin (Table 1; Figure 1). According to the WDNR's Fish Mapper, the only stream flowing into Illinois that contained *C. elongatus* was Raccoon Creek.

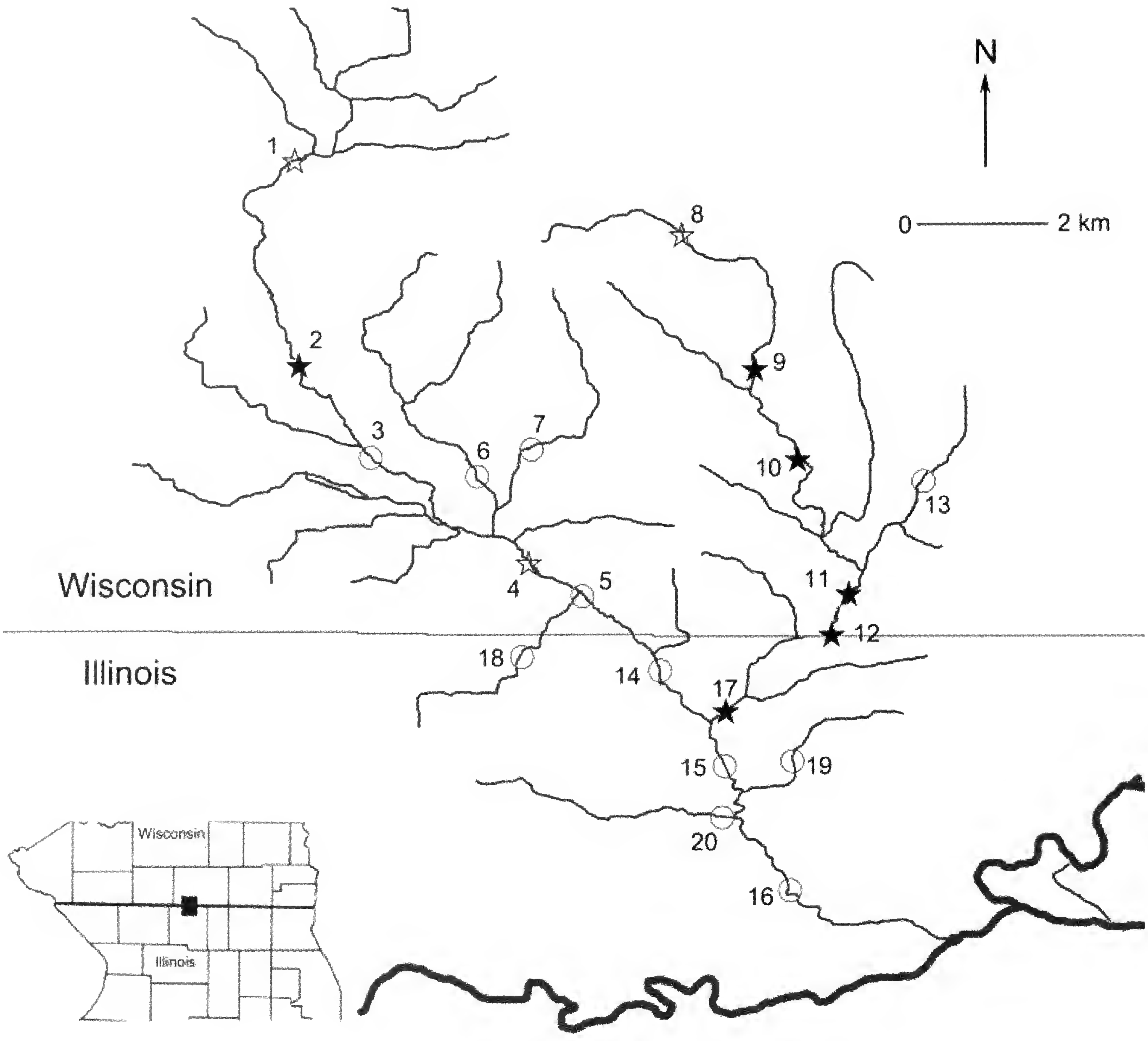

Figure 1. Map of the study area. Solid stars indicate sites where *Clinostomus elongatus* was collected during the 1997-2000 and 2010-2011 surveys, open stars show where Fago (1982) reported the fish, and open circles designate those sites where we failed to collect the dace during our surveys.

DISCUSSION

This study is the first to document *C. elongatus* in Illinois, and increases the total number of fish species reported as native to Illinois waters to 192 (Burr and Page, 2009). Resident populations of *C. elongatus* occur in the Wisconsin headwaters of East Fork Raccoon Creek and Raccoon Creek; however, the dace is evidently a peripheral species in Illinois. The substrate changed from predominantly gravel and cobble over mud and sand on the Wisconsin side of the basin to largely sand with scattered patches of gravel and small cobble in Illinois. Although its occurrence in the state has been confirmed, it remains undetermined whether this species reproduces in Illinois and maintains a resident population or is just an occasional transient that is washed downstream.

As reported by Koster (1939), *C. elongatus* was commonly collected with blacknose dace *Rhinichthys atratulus*, creek chubs, white suckers *Catostomus commersoni*, and Johnny darters *Etheostoma nigrum*. Redside dace typically reproduce over the pebble-nests of other minnows in small headwater streams dominated by gravel substrates (Koster, 1939). Two pebble nest-building species (hornyhead chub *Nocomis biguttatus* and creek chub) were found throughout the Raccoon River basin in Illinois. However, habitat suitable for pebble-nests (e.g., clean gravel substrates) was rather uncommon in the Illinois portion of this system, which is dominated by sand. Two small tributaries to Raccoon Creek (see sites 18 and 20, Figure 1) offered the best habitats for pebble-nests and are thereby the best candidates for supporting viable Illinois populations of *C. elongatus*. However, these streams were often dry and collections in these tributaries yielded no specimens.

Several other state native fishes have been discovered since Smith's (1979) comprehensive summary of the state's ichthyofauna. The bleeding shiner *Luxilus zonatus*, taillight shiner *Notropis maculatus,* and fringed darter *Etheostoma crossopterum* were not reported in Smith (1979) but have been collected in Illinois in the last 30 years (Burr et al., 1988; Poly and Wilson, 1998; Hiland and Poly, 2000). The cypress minnow *Hybognathus hayi*, bigeye chub *Hybopsis amblops*, and crystal darter *Cystallaria asprella* were considered extirpated by Smith (1979), but since have been sporadically collected in Illinois (Warren and Burr, 1989; Burr et al., 1996; Tiemann et al., 2004; Stewart et al., 2005; Steuck et al., 2010). Although the northern studfish *Fundulus catenatus* is listed as part of the state's ichthyofauna, Smith (1979) stated that there was no evidence of a population in Illinois and the species was a "straggler from a Missouri tributary." A single specimen was collected in July 2007 in the West Fork Richland Creek (Kaskaskia River Drainage), St. Clair County (Randy Sauer, Illinois Department of Natural Resources, personal communication). These recent additions to the biodiversity of Illinois provide a refreshing, albeit fleeting departure from more common statewide trends such as species extirpations (e.g., Burr and Warren, 1986) and invasions by non-indigenous fishes (e.g., Laird and Page, 1996; Chick et al., 2003; Irons et al., 2006).

The Pecatonica River drainage, which includes the Raccoon Creek basin, has seen an increase in native fish species richness during the last 100 years (Retzer, 2005). In an evaluation of Illinois streams based on aquatic biodiversity, Page et al. (1992) listed Raccoon Creek as a Biologically Significant Stream so it is no surprise that the basin supports a diverse fish fauna, including the Illinois state-threatened starhead topminnow *Fundulus dispar* and Iowa darter *Etheostoma exile*. Though not formally sampled, we also encountered a diverse mussel fauna with 10 live species, including the Illinois state-threatened slippershell *Alasmidonta viridis*. However, the Pecatonica River basin, including Raccoon Creek, has been threatened by siltation and agricultural pollution (Smith, 1971; Page et al., 1992). Additionally, Raccoon Creek is altered by a headwater impoundment (Mill Pond) near our Site 4. In addition to altering habitat and blocking fish dispersal, dam effects include stocking of sportfish (Taylor et al., 2001; Tiemann et al., 2007). We collected several top predators near Site 4, including largemouth bass *Micropterus salmoides* and northern pike *Esox lucius*, that were not collected elsewhere in the basin.

Multiple authors have noted a decrease in the overall range and abundance of the redside dace, primarily as a result of activities that increase turbidity, silt deposition, and mean water temperature in small streams (either as a result of dams, climate change, or removal of riparian areas), or introduction of top predators (Harlan and Speaker, 1956; Trautman, 1981; Lyons et al., 2000; COSEWIC, 2007). Because of these threats and its limited distribution, the American Fisheries Society listed *C. elongatus* as vulnerable, the status applied to a taxon that is in imminent danger of becoming threatened throughout all or a significant portion of its range (Jelks et al. 2008). Within the upper Midwest, the dace is extirpated from Iowa, listed as endangered in Canada, Michigan, and Indiana, and listed as a species of special concern in Wisconsin (Harlan and Speaker, 1956; Lyons et al., 2000; COSEWIC, 2007). Several populations of *C. elongatus* have disappeared from the upper Rock River basin in Dane County, Wisconsin (Lyons et al., 2000).

The continued occurrence of the redside dace in Illinois is largely dependent upon the integrity of the headwater habitats in Wisconsin that support reproductively viable populations. If the Wisconsin populations remain intact, one might expect the redside dace to periodically appear in Illinois waters. While it is possible that the specimens collected in East Fork Raccoon Creek (all juveniles) had been washed downstream from source populations in Wisconsin headwaters, it is also likely that *C. elongatus* occurs in extremely patchy, isolated schools that are easily missed even during extensive sampling.

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LITERATURE CITED

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison. 1052 pp.

Burr, B.M., K.M. Cook, D.J. Eisenhour, K.R. Piller, W.J. Poly, R.W. Sauer, C.A. Taylor, E.R. Atwood, and G.L. Seegert. 1996. Selected Illinois fishes in jeopardy: new records and status evaluations. Transactions of the Illinois State Academy of Science 89:169-186.

Burr, B.M. and L.M. Page. 2009. Illinois fish communities: more than a century of change. Pp 147-162 *in* C.A. Taylor, J.B. Taft, and C.E. Warwick, editors. Canaries in the catbird seat: the past, present, and future of biological resources in a changing environment. Illinois Natural History Survey Special Publication 30, Champaign. 306 pp.

Burr, B.M. and M.L. Warren, Jr. 1986. Status of the bluehead shiner (*Notropis hubbsi*) in Illinois. Transactions of the Illinois Academy of Science 79:129-136.

Burr, B.M., M.L. Warren, Jr., and K.S. Cummings. 1988. New distributional records of Illinois fishes with additions to the known fauna. Transactions of the Illinois State Academy Science 81:163-170.

Chick J.H., R.J. Maher, B.M. Burr, and M.R. Thomas. 2003. First black carp captured in US. Science 300:1876-1877.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2007. COSEWIC assessment and update status report on the redside dace *Clinostomus elongatus* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa. vii + 59 pp.

Fago, D. 1982. Distribution and relative abundance of fishes in Wisconsin, I. Greater Rock River Basin. Wisconsin Department Natural Resource Technical Bulletin 136:1-120.

Gilbert, C.R. 1980. *Clinostomus elongatus* (Kirtland), redside dace. Pp 148 *in* D.S. Lee, C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr., editors. Atlas of North American freshwater fishes. North Carolina State Museum Natural History, Raleigh. 854 pp.

Harlan, J.R. and E.B. Speaker. 1956. Iowa fish and fishing. 3rd edition. Iowa State Conservation Commission, Des Moines. 377 pp.

Hiland, T.M. and W.J. Poly. 2000. The bleeding shiner, *Luxilus zonatus*, in Illinois (Cyprinidae). Transactions of the Illinois State Academy of Science 93:145-147.

Irons, K.S., M.A. McClelland, and M.A. Pegg. 2006. Expansion of round goby in the Illinois waterway. American Midland Naturalist 156:198-200.

Jelks, H.L., S.J. Walsh, N.M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D.A. Hendrickson, J. Lyons, N.E. Mandrak, F. McCormick, J.S. Nelson, S.P. Platania, B.A. Porter, C.B. Renaud, J.J. Schmitter-Soto, E.B. Taylor and M.L. Warren Jr. 2008. Conservation Status of Imperiled North American Freshwater and Diadromous Fishes. Fisheries 33:372-407.

Koster, W.J. 1939. Some phases of the life history and relationships of the cyprinid, *Clinostomus elongatus*. Copeia 1939:201-208.

Laird, C.A. and L.M. Page. 1996. Non-native fishes inhabiting the streams and lakes of Illinois. Illinois Natural History Survey Bulletin 35:1-51.

Lyons, J., P.A. Cochran, and D. Fago. 2000. Wisconsin fishes 2000: status and distribution. University of Wisconsin Sea Grant Institute, Madison, Wisconsin. 87 pp.

Novinger, D.C. & T.G. Coon. 2000. Behavior and physiology of the redside dace, *Clinostomus elongatus*, a threatened species in Michigan, Environmental Biology of Fishes 57:315- 326.

Page, L.M. and B.M. Burr. 2011. A field guide to freshwater fishes of North America north of Mexico. 2nd Edition. The Peterson Field Guide Series, Houghton Mifflin Company, Boston, Massachusetts. 663 pp.

Page, L.M., K.S Cummings, C.A. Mayer, S.L. Post, and M.E. Retzer. 1992. Biologically significant Illinois streams. An evaluation of the streams of Illinois based on aquatic biodiversity. Illinois Natural History Survey, Center for Biodiversity Technical Report 1992(1). Champaign. 485 pp.

Poly, W.J. and A.K. Wilson. 1998. The fringed darter, *Etheostoma crossopterum*, in the Cache River Basin of Southern Illinois (Percidae: Subgenus *Catonotus*). Ohio Journal of Science 98:6-9.

Retzer, M.E. 2005. Changes in the diversity of native fishes in seven basins in Illinois, USA. American Midland Naturalist 153:128-141.

Sabaj, M.H. 2000. Illinois status survey of the redside dace, *Clinostomus elongatus*, the newest addition to the state's native fauna. Technical Report submitted to the Illinois Department of Natural Resources, Division of Natural Heritage. 12 pp + appendix.

Sabaj Pérez, M.H. (editor). 2012. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Verson 3.0 (23 February 2012). Electronically accessible at http://www.asih.org/, American Society of Ichthyologists and Herpetologists, Washington, DC.

Smith, P.W. 1971. Illinois streams: a classification based on their fishes and an analysis of factors responsible for disappearance of native species. Illinois Natural History Survey Biological Notes 76:1-14.

Smith, P. W. 1979. The fishes of Illinois. University of Illinois Press, Urbana. 314 pp.

Steuck, M.J., S. Yess, J. Pitlo, A. Van Vooren, and J. Rasmussen. 2010. Distribution and relative abundance of upper Mississippi River fishes. Upper Mississippi River Conservation Committee, Onalaska, Wisconsin. 21 pp.

Stewart, J.G., V.B. Barko, D.B. Henry, D.P. Herzog, J.W. Ridings, A.F. Kelley, and J.E. Wallace. 2005. New records of the crystal darter (*Crystallaria asprella*) in the middle Mississippi River. American Midland Naturalist 154:471-473.

Taylor, C.A. J.H. Knouft, and T.M. Hiland. 2001. Consequences of stream impoundment on fish communities in a small North American Stream. Regulated Rivers: Research and Management 17:687-698.

Tiemann, J.S., H.R. Dodd, N. Owens, and D.H. Wahl. 2007. Effects of lowhead dams on unionids in the Fox River, Illinois. Northeastern Naturalist 14:125-138.

Tiemann, J.S., M.E. Retzer, and B.L. Tiemann. 2004. Range expansion of the state endangered bigeye chub *Hybopsis amblops* (Rafinesque) in Illinois. Transactions of the Illinois State Academy of Science 97:255-257.

Trautman, M.B. 1981. The fishes of Ohio with illustrated keys, rev. ed. The Ohio State University Press, Columbus. 782 pp.

Warren, M.L., Jr. and B.M. Burr. 1989. Distribution, abundance, and status of the cypress minnow, *Hybognathus hayi*, an endangered Illinois species. Natural Areas Journal 9:163-168.

Zimmerman, B.J. 2009. Microhabitat use by the redside dace (*Clinostomus elongatus*) in Ohio. Master's thesis. Bowling Green State University, Bowling Green, Ohio. 28 pp.

BOOK REVIEW 2012 – #2

Side Channels. A Collection of Nature Writing and Memoir by Thomas V. Lerczak (2011). 186 pp. Mill City Press, Minneapolis, MN. $15.95.

This book is an unusual hybrid: part birder's field guide, part general natural history, and part memoir. As a study in contradictions we learn that the author grew up in urban Chicago and how he came to live in the open country near the Illinois River. We learn how, from an early age, he was drawn to nature, and especially to observation of birds. Following his ecological graduate studies Lerczak became a state biologist. In his duties as a staff member of the Nature Preserves Commission, he has come into close contact with many habitat types. Through his work and through his writing for Illinois Audubon Magazine, he has emerged as one of Illinois' premier naturalists.

The volume is a collection of 30 chapters, fourteen of which originally appeared in Illinois Audubon magazine. Others are personal observations and reflections seen here for the first time. In the memoir-based chapters he notes that he is especially drawn to rivers. His descriptions of them, and their wildlife, are especially evocative.

Longtime Illinois residents will doubtless find much they did not already know. Lerczak always digs into the back story as he describes topics such as the habits of Great Blue Herons, eagles in winter refuges, the life of the six species of swallows along the Illinois River, the reasons why we have bur oaks in Mason County, or the territories of Red-headed Woodpeckers. We learn why hawks don't glide over large lakes, and of the habitat needs of vultures vs. eagles. We learn why suppression of fires has the unintended consequence of changing the composition of oak forests.

Many chapters are essays on birds of this state. In part II, he travels more widely offering interesting observations of Colorado, Wisconsin, the North Cascades, the Great Lakes including Isle Royale, the Porcupine Mountains wilderness, and New York at Niagara Falls. He feels the contentment that comes from a lifetime of kinship with the earth's community of life.

The sense of peace that one can feel in nature has led many of us to proceed down the road of environmental activism. Lerczak has stood at the entrance of this road but, in the end, rejected the politics and egos of agenda driven groups. This may puzzle those who are more politically engaged with environmental protection. But his reluctance overshadows the fact that, professionally, he has contributed a great deal to the actual preservation of thousands of acres of natural habitat within his home state. This is a major and enduring legacy that few are in a position to claim.

I note, in full disclosure, that Tom Lerczak was once a student in my Colorado field course. His sense of humor and earnest thoughtfulness were evident then as was the fact that he never went anywhere without his binoculars. It was clear in those days that his future career would bear watching.

Lerczak's book displays an understated, companionable style as he introduces habits and habitats in a way that will appeal to novices as well as those more expert. Each chapter is a good stand-alone read. This book should be read by any natural history buff, and by anyone looking for ideas of places to explore, both near and far.

Review by Richard C. Keating, Research Associate at the Missouri Botanical Garden and Professor Emeritus at Southern Illinois University – Edwardsville.

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Kendall Annetti – University of Illinois – Urbana-Champaign
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Oral Presentations

1st Place: Gerry Mestek – Aurora University
A Study on the Arboreal Flora and the Environmental Conditions
in a Bottomland Forest Site, Duck Creek Nature Trail,
Silver Springs State Park, Kendall County, Illinois

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