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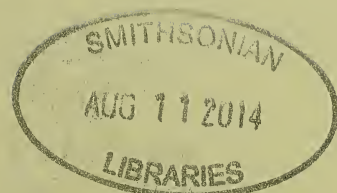
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Luna Moth (*Actias luna*), by Tracy L. Culbertson. See article by Luke E. Dodd and Lynne K. Rieske, page 3, this issue.



# Temporal Variation of Nocturnal Lepidoptera and Other Insects at Robinson Forest, Kentucky

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## ABSTRACT

Lepidoptera are a conspicuous, diverse group of forest insects that are responsive to land management and perturbation. Nocturnal Lepidoptera and other insects were surveyed using blacklight traps during June and July of 1997 at Robinson Forest, a large experimental forest located in the Appalachians of eastern Kentucky. Though the capture of Lepidoptera and other common insect orders varied little between sampling locations, a seasonal trend was detected; fewer Coleoptera, Lepidoptera, and total insects in general were captured in June versus July. Lepidoptera  $\geq 20$  mm in wingspan were further identified and enumerated. A total of 664 Lepidoptera of  $\geq 100$  species and 13 families were tabulated. Species turnover, particularly for the most abundant species, was commonplace between June and July. The food habits of abundant species were varied, suggesting the assemblage of Lepidoptera at Robinson Forest is not only taxonomically rich, but is functionally rich as well.

**KEY WORDS:** Appalachia, diversity, Kentucky, moths, species checklist

## INTRODUCTION

Robinson Forest is an experimental forest managed for research, teaching and extension by the University of Kentucky. It is one of the largest research and educational forests in the eastern United States and is also one of the largest remaining forest stands of any kind on the Cumberland Plateau of Appalachia (Krupa and Haskins 1996). Robinson Forest was originally clearcut between 1908 and 1923, followed by selective cutting of high quality trees in the 1960s (Overstreet 1984). Because of fragmentation, resource extraction, and human land use patterns throughout eastern Kentucky and much of Appalachia (Wickham et al. 2007), Robinson Forest represents a distinct island of second-growth forest with substantial conservation merit (Nieman and Merkin 1995; Krupa and Haskins 1996). To maximize its value with respect to conservation and education, a working knowledge of Robinson Forest's faunal and floral diversity is needed. One conspicuous group of forest fauna that hold promise as indicators of biodiversity and forest

health are the Lepidoptera (Summerville and Crist 2004; Merckx et al. 2012), which are the focus of our study. Our objective was to identify general patterns of nocturnal insect occurrence within a growing season, and provide a species-level diversity assessment for Lepidoptera captured in blacklight traps at Robinson Forest.

## MATERIALS AND METHODS

Robinson Forest is located in Breathitt, Knott, and Perry counties on the Dissected Appalachian Plateau ecoregion of the Cumberland Plateau of the Western Appalachians (Woods et al. 2002). This experimental forest is a composite of seven tracts totaling nearly 6000 ha, with the largest tract over 4000 ha. Our surveys were conducted at three sampling sites ca. 100 m apart within the largest tract of Robinson Forest (37°27'39" N, 83°9'29" W). The terrain consisted of deeply dissected drainages with steep hillsides. Although sites were comparable with respect to woody species composition, stand age, and stocking density (mean basal area = 17.31 m<sup>2</sup> ha<sup>-1</sup>; Overstreet 1984), they varied in slope and aspect and ranged in elevation from 325–475 m (Rieske

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and Buss 2001). The second-growth forest in this area is characterized as mixed mesophytic (Braun 1950). Overstory and mid-story species were predominantly oaks (*Quercus* spp.), hickories (*Carya* spp.), maples (*Acer* spp.), and tulip poplar (*Liriodendron tulipifera* L.). Understory and herbaceous components were dominated by mountain laurel (*Kalmia latifolia* L.) and greenbriar (*Smilax rotundifolia* L.) (Rieske and Buss 2001). Of the three sampling sites considered in this study, two had received treatments to manage defoliating Lepidoptera. A fixed-wing aircraft was used on 7 May 1997 for a single application of diflubenzuron (Dimilin 25W, Uniroyal Chemical Company, Middlebury, CT) at one site applied at the rate of 70 g ai/ha. *Bacillus thuringiensis* variety *kurstaki* (*Btk*, Dipel 4L, Abbott Laboratories, North Chicago, IL) was applied at the other treated site at the label rate of 2.3 L/ha (Rieske and Buss 2002).

We sampled the nocturnal arthropod community at each site over three nights in June–July of 1997 (5 June, 15 July, 31 July,  $n = 8$  trap/nights), using 10 W blacklight traps (Universal Light Trap, Bioquip Products, Gardena, CA). Single blacklight traps were suspended from oak trees ca. 3 m above ground level and were operated from dusk to dawn. Dichlorvos fumigant strips (Roxide International, New Rochelle, NY) were used to subdue trapped insects. Survey nights were fair with minimum night time temperatures of 11.7, 21.1 and 15.6°C, on 5 June, 15 July, and 31 July, respectively. There was no precipitation on any survey night or the day preceding a survey night (UK Ag Weather Center 2012). Following a trap night, specimens were sorted and placed in cold storage (4°C) for identification in the laboratory. Specimens were counted and identified to ordinal level using Triplehorn and Johnson (2005). Lepidoptera were identified to species or genus using Covell (2005), Holland (1903), and reference collections at the University of Kentucky. New voucher specimens were incorporated into an existing reference collection at the University of Kentucky. Reports of food habits were noted for the most common species using Covell (2005), as well as internet-based resources hosted by Iowa State University (Bug Guide 2012).

Classification of noctuoids followed that of LaFontaine and Schmidt (2010). We focused our efforts on macrolepidoptera and those microlepidoptera with wingspans  $\geq 20$  mm (i.e., some Oecophoridae, Yponomeutidae, Tortricidae, Megalopygidae, Limacodidae, and Pyralidae); thus, our study is not an exhaustive assessment. While limited replication prohibited statistical tests for differences in insect occurrence across samples sites (i.e., effect of defoliator suppression), repeated sampling permitted testing for temporal differences in nocturnal insect occurrence. We tested for these differences in the abundance of the most common insect orders, as well as total insects captured per trap using Wilcoxon rank-sum tests in SAS (v. 9.1). We likewise tested for differences between June and July in species richness of Lepidoptera captured per trap, as well as calculated Shannon's diversity and Pielou's evenness indices for comparison between months (Pielou 1975). We used EstimateS (v. 8.2) to calculate ICE (Lee and Chao 1994) and Chao 2 (Chao 1987) richness estimations for our study. Estimations were based on 1000 randomizations (Summerville and Crist 2005).

## RESULTS

A total of 3315 insects were captured. Of these, 1371 were captured at the untreated site, while 707 and 1237 insects were captured at the *Btk*-treated and diflubenzuron-treated sites, respectively. While 8 orders were represented, the vast majority of specimens were Coleoptera (13%), Diptera (29%), and Lepidoptera (50%). Other orders captured were Hemiptera, Hymenoptera, Plecoptera, Psocoptera, and Trichoptera. Seasonal trends for the common orders were evident (Figure 1). Abundance was lower in June than July for Coleoptera, Lepidoptera, and total insects (all  $P \leq 0.05$ ), but there was no difference detected for Diptera ( $P > 0.05$ ).

A total of 1654 Lepidoptera were captured. Of these, 564 were captured at the untreated site, and 478 and 612 were captured at the *Btk*-treated and diflubenzuron-treated sites, respectively. A total of 664 specimens had wingspans  $\geq 20$  mm and were identified to family level. We were able to identify 548 of these specimens further, representing  $\geq 100$  species and 13 families (Table 1). Richness



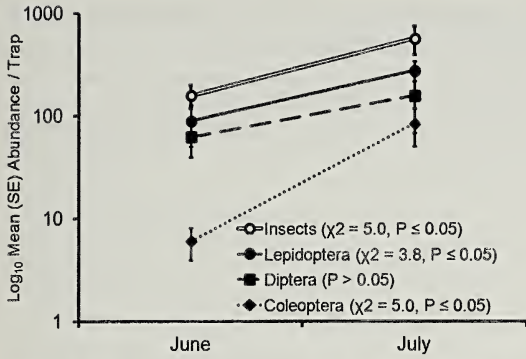


Figure 1. Abundance of nocturnal insects captured in blacklight traps ( $n = 8$  trap-nights) at Robinson Forest, a second-growth forest in southeastern Kentucky. Significance values refer to differences between months.

estimates (Mean  $\pm$  SD) were  $156 \pm 19$  species for Chao 2 and  $183 \pm 21$  species for ICE. The Erebididae and Geometrididae were the most speciose (26 and 25 species, respectively), followed by the Noctuidae, Notodontidae, and Pyralidae (12, 11, and 9 species, respectively). This richness trend across families was consistent for both June and July. A total of  $\geq 69$  species were ubiquitously captured over both months. No difference was detected between months for species diversity, evenness, or richness of Lepidoptera ( $P > 0.05$ ).

Shannon's diversity indices (Mean  $\pm$  SD) were  $2.30 \pm 0.47$  and  $2.58 \pm 0.75$  for June and July, respectively. Pielou's evenness indices (Mean  $\pm$  SD) were  $0.78 \pm 0.06$  and  $0.82 \pm 0.11$  for June and July, respectively. While  $\geq 46$  species were identified in June,  $\geq 75$  species were identified in July. Further, the occurrence of common Lepidoptera shifted between June and July (Table 1). Most species were herbaceous generalists (*Eupithecia* sp., *Virbia* sp.) or generalists of deciduous trees (*Acronicta* sp., *B. ophthalmica*, *H. tessellaris*, and *P. serinaria*). However, an oak-specialist (*H. georgica*) and a smaller variety of detritivores (*A. cuprina* and *Idia* sp.) and lichen-feeding species (*C. albata*) were also common.

## DISCUSSION

This study deepens our understanding of the abundance and diversity patterns of nocturnal insects across Central Appalachia.

We found an increased abundance of Lepidoptera and other forest insects later in the growing season; results consistent with other studies (Butler et al. 2001; Dodd et al. 2012, 2013; but see Burford et al. 1999). Consensus regarding trends in lepidopteran richness and diversity in Appalachia are less clear, however. Dodd et al. (2012) found that diversity increased after May in eastern Kentucky, but did not detect differences later in the growing season. Records from Butler et al. (2001) likewise suggest species richness increased after May in both Virginia and West Virginia. In contrast, Burford et al. (1999) noted a trend towards reduced species richness in eastern Kentucky over their survey period (June to August). While our study suggests a slight increase in lepidopteran diversity from June to July, our data more clearly demonstrate an increase in the overall pool of species recorded later in the growing season; a finding consistent with Dodd et al. (2013). Also similar to the current study, Dodd et al. (2013) estimated overall richness at their study area between  $162 \pm 25$  and  $189 \pm 12$  species (ICE and Chao 2, respectively). This suggests consistency in the pattern of lepidopteran species richness across the Central Appalachians of eastern Kentucky, and further suggests the upland habitats of Robinson Forest possesses a lepidopteran assemblage as rich as those found in portions of the Daniel Boone National Forest (ca. 70 km away; Dodd et al. 2013).

The diversity of food habits we report for Lepidoptera at Robinson Forest suggest this assemblage is not only species rich, but is functionally rich as well. Regardless of the specific species recorded, the abundance of folivores remained relatively constant across June and July. In contrast, strong shifts were seen for Lepidoptera that use other host resources. The lichen-feeder, *C. albata*, was far more abundant in June, whereas detritivores (*A. cuprina* and *Idia* sp.) were far more abundant in July. These data suggest shifts in the dominance of functional groups over the course of a season, presumably with changes in host resources. Broome et al. (2011) note an increase in the dominance of both lichen-feeding and detritivorous Lepidoptera in older coppice-managed woodlots in England; these habitats possessed more closed-in, dense



Table 1. A checklist of Lepidoptera collected in blacklight traps from Robinson Forest, a second-growth forest in southeastern Kentucky. Capture totals are presented across months and survey sites.

| Taxon  | Total captured |      |    |               |           |
|--|----------------|------|----|---------------|-----------|
|  | June           | July | Bk | Diflubenzuron | Untreated |
| <b>ELACHISTIDAE</b>                                |                |      |    |               |           |
| <i>Antaeotricha</i> sp.                            | 2              | 2    | 4  |               |           |
| <b>EREBIDAE</b>                                    |                |      |    |               |           |
| <i>Catocala epione</i> Drury                       |                | 1    |    | 1             |           |
| <i>Catocala ilia</i> Cramer                        |                | 1    |    |               | 1         |
| <i>Catocala</i> sp.                                |                | 2    | 2  |               |           |
| <i>Cisthene</i> sp.                                | 1              |      | 1  |               |           |
| <i>Clemensia albata</i> Packard                    | 73             | 3    | 49 | 9             | 18        |
| <i>Grammia anna</i> Grote                          | 1              |      | 1  |               |           |
| <i>Halysidota tessellaris</i> J.E. Smith           | 2              | 15   | 17 |               |           |
| <i>Haploa clymene</i> Brown                        |                | 1    | 1  |               |           |
| <i>Hypena baltimoralis</i> Guenée                  | 1              |      |    |               | 1         |
| <i>Hypena</i> sp.                                  |                | 1    |    | 1             |           |
| <i>Hyperstrotia pervertens</i> Barnes & McDunnough | 1              | 1    |    | 1             | 1         |
| <i>Hyperstrotia secta</i> Grote                    | 1              | 1    | 1  |               | 1         |
| <i>Idia aemula</i> Hübner                          |                | 5    |    | 5             |           |
| <i>Idia americanalis</i> Guenée                    | 2              | 7    | 4  | 3             | 2         |
| <i>Idia</i> sp.                                    | 1              | 83   |    | 74            | 10        |
| <i>Oruza albocostaliata</i> Packard                |                | 2    |    | 1             | 1         |
| <i>Palthis angulalis</i> Hübner                    |                | 1    | 1  |               |           |
| <i>Pangrapta decoralis</i> Hübner                  | 2              | 4    | 1  | 5             |           |
| <i>Panopoda rufimargo</i> Hübner                   |                | 1    | 1  |               |           |
| <i>Phalaenophana paramusilis</i> Walker            | 1              |      | 1  |               |           |
| <i>Renia</i> sp.                                   | 1              | 2    |    |               | 3         |
| <i>Spilosoma congrua</i> Walker                    | 4              |      | 3  | 1             |           |
| <i>Virbia</i> sp.                                  | 26             |      | 22 |               | 4         |
| <i>Zanclognatha lituralis</i> Hübner               | 4              |      | 1  | 3             |           |
| <i>Zanclognatha ochreipennis</i> Grote             | 1              |      |    |               | 1         |
| <i>Zanclognatha</i> sp.                            | 1              | 1    | 1  | 1             |           |
| <b>GEOMETRIDAE</b>                                 |                |      |    |               |           |
| <i>Besma quercivoraria</i> Guenée                  | 2              |      | 2  |               |           |
| <i>Biston betularia</i> L.                         | 1              |      | 1  |               |           |
| <i>Cabera erythemaria</i> Guenée                   | 1              |      |    |               | 1         |
| <i>Dichordia iridaria</i> Guenée                   | 1              |      | 1  |               |           |
| <i>Euchlaena amoenaria</i> Guenée                  |                | 1    | 1  |               |           |
| <i>Eupithecia</i> sp.                              |                | 36   | 3  | 28            | 5         |
| <i>Glenoides texanaria</i> Hulst                   |                | 5    | 4  |               | 1         |
| <i>Hydrelia inornata</i> Hulst                     | 2              |      |    |               | 2         |
| <i>Iridopsis larvaria</i> Guenée                   |                | 1    | 1  |               |           |
| <i>Lambdina fervidaria</i> Hübner                  | 2              | 4    | 4  | 1             | 1         |
| <i>Lomographa vestaliata</i> Guenée                |                | 3    | 2  |               | 1         |
| <i>Macaria multilineata</i> Grote                  | 2              | 3    | 5  |               |           |
| <i>Metarranthis homuraria</i> Grote & Robinson     | 1              |      |    | 1             |           |
| <i>Orthonama obstipata</i> F.                      | 1              |      |    |               | 1         |
| <i>Pero</i> sp.                                    |                | 1    |    |               | 1         |
| <i>Plagodis alchoolaria</i> Guenée                 |                | 2    |    | 2             |           |
| <i>Plagodis phlogosaria</i> Guenée                 |                | 1    |    | 1             |           |
| <i>Plagodis serinaria</i> Herrich-Schäffer         | 11             |      | 6  |               | 5         |
| <i>Pleuroprucha insularia</i> Guenée               |                | 7    |    | 7             |           |
| <i>Probole amicararia</i> Hèrrich-Schaffer         | 2              | 1    | 2  | 1             |           |
| <i>Scopula limboundata</i> Haworth                 | 1              | 2    | 1  | 1             | 1         |
| <i>Speranza pustularia</i> Guenée                  |                | 1    | 1  |               |           |
| <i>Tetracis cachexiata</i> Guenée                  | 3              |      | 2  |               | 1         |
| <i>Xanthorhoe lacustrata</i> Guenée                |                | 1    |    | 1             |           |
| <b>LIMACODIDAE</b>                                 |                |      |    |               |           |
| <i>Euclea delphinii</i> Boisduval                  |                | 2    |    | 2             |           |

Table 1. Continued.

| Taxon   | Total captured |      |            |               |           |
|---|----------------|------|------------|---------------|-----------|
|   | June           | July | <i>Btk</i> | Diffubenzuron | Untreated |
| <b>LYMANTRIIDAE</b>                           |                |      |            |               |           |
| <i>Dasychira</i> sp.                          | 1              | 4    | 1          | 1             | 3         |
| <b>NOCTUIDAE</b>                              |                |      |            |               |           |
| <i>Acronicta fragilis</i> Guenée              |                | 1    |            | 1             |           |
| <i>Acronicta innotata</i> Guenée              |                | 5    | 3          |               | 2         |
| <i>Acronicta</i> sp.                          | 1              | 19   | 8          | 9             | 3         |
| <i>Athetis tarda</i> Guenée                   | 3              |      |            |               | 3         |
| <i>Callopietria mollissima</i> Guenée         | 1              |      |            |               | 1         |
| <i>Cerma cerintha</i> Treitschke              |                | 3    |            | 2             | 1         |
| <i>Chytonix palliatricula</i> Guenée          |                | 4    |            | 4             |           |
| <i>Colocasia flavicornis</i> J.B. Smith       | 1              | 7    | 6          |               | 2         |
| <i>Feltia</i> sp.                             |                | 1    | 1          |               |           |
| <i>Marimatha nigrofimbria</i> Guenée          |                | 1    |            | 1             |           |
| <i>Pseudeustrotia carneola</i> Guenée         |                | 3    | 1          | 2             |           |
| <i>Polygrammate hebraicum</i> Guenée          |                | 1    | 1          |               |           |
| <b>NOLIDAE</b>                                |                |      |            |               |           |
| <i>Meganola minuscula</i> Zeller              |                | 3    |            | 3             |           |
| <i>Baileya levitans</i> J.B. Smith            |                | 7    |            |               | 7         |
| <i>Baileya ophthalmica</i> Guenée             | 1              | 10   | 7          | 3             | 1         |
| <b>NOTODONTIDAE</b>                           |                |      |            |               |           |
| <i>Dasylophia thyatiroides</i> Walker         |                | 1    | 1          |               |           |
| <i>Datana</i> sp.                             |                | 4    | 4          |               |           |
| <i>Heterocampa biundata</i> Walker            | 3              | 1    | 3          |               | 1         |
| <i>Heterocampa obliqua</i> Packard            |                | 3    | 2          |               | 1         |
| <i>Heterocampa umbrata</i> Walker             |                | 3    | 2          | 1             |           |
| <i>Hyperaeschra georgica</i> Hèrrich-Schaffer | 10             |      | 7          |               | 3         |
| <i>Macrurucampa marthesia</i> Cramer          |                | 1    |            |               | 1         |
| <i>Nadata gibbosa</i> J.E. Smith              | 2              | 4    | 5          | 1             |           |
| <i>Peridea angulosa</i> J.E. Smith            |                | 1    |            | 1             |           |
| <i>Peridea basitriens</i> Walker              |                | 7    | 6          |               | 1         |
| <b>OECOPHORIDAE</b>                           |                |      |            |               |           |
| <i>Machima tentoriferella</i> Clemens         |                | 1    |            | 1             |           |
| <i>Psilocorsis</i> sp.                        | 1              |      | 1          |               |           |
| <b>PYRALIDAE</b>                              |                |      |            |               |           |
| <i>Aglossa cuprina</i> Zeller                 |                | 17   | 4          | 9             | 4         |
| <i>Blepharomastix ranalis</i> Guenée          |                | 7    |            | 5             | 2         |
| <i>Crambus</i> sp.                            |                | 8    | 4          |               | 4         |
| <i>Euzophera ostricolorella</i> Hulst         |                | 1    |            | 1             |           |
| <i>Galasa nigrinodis</i> Zeller               |                | 1    | 1          |               |           |
| <i>Palpita magniferalis</i> Walker            | 2              |      |            |               | 2         |
| <i>Pantographa limata</i> Grote & Robinson    |                | 4    |            | 4             |           |
| <i>Pococera asperatella</i> Clemens           | 2              | 1    |            | 1             | 2         |
| <i>Udea rubigalis</i> Guenée                  |                | 1    |            | 1             |           |
| <b>SATURNIIDAE</b>                            |                |      |            |               |           |
| <i>Actias luna</i> L.                         |                | 2    |            | 2             |           |
| <i>Callosomia promethean</i> Drury            |                | 1    |            | 1             |           |
| <i>Citheronia regalis</i> F.                  |                | 1    |            | 1             |           |
| <i>Eacles imperialis</i> Drury                |                | 2    |            | 2             |           |
| <b>SESIIDAE</b>                               |                |      |            |               |           |
| <i>Synanthedon acerni</i> Clemens             | 1              |      |            |               | 1         |
| <b>SPHINGIDAE</b>                             |                |      |            |               |           |
| <i>Ceratonia undulosa</i> Walker              |                | 1    |            | 1             |           |
| <i>Paonias myops</i> J.E. Smith               |                | 1    |            | 1             |           |
| <b>TORTRICIDAE</b>                            |                |      |            |               |           |
| <i>Clepsis melaleucana</i> Walker             | 2              |      | 2          |               |           |



deciduous canopies. Our observations demonstrate these two dietary groups vary in their dominance within a growing season in North America. We suggest the increased capture of detritivores in July likely relates to increasing vegetative biomass later in the growing season.

While species estimations indicate our sampling effort was far from comprehensive, we documented a wide diversity of Lepidoptera at Robinson Forest. Such species inventories from forested parcels of land that lay within a fragmented landscape are important because they provide records that may: 1) differ from those historically collected in more contiguous forest habitats and 2) establish a baseline for subsequent inventories (Summer-ville et al. 1999). This study adds 78 new records for Robinson Forest on the state-wide lepidopteran database (Covell et al. 2012). Our sampling was restricted to upland hardwood habitats (Rieske and Buss 2002), but species estimations suggest that additional sampling spanning an array of habitats would reveal even more species. Numerous low-order streams with associated riparian and cove habitats are prevalent throughout Robinson Forest; such habitats are known to harbor significant lepidopteran diversity (Dodd et al. 2011, 2013). This inventory is important in not only establishing a benchmark for subsequent insect inventories at Robinson Forest, but also in detailing prey available to the federally-threatened Rafinesque's big-eared bat (*Corynorhinus rafinesquii* Lesson). An isolated population of this moth specialist is found at Robinson Forest (Hurst and Lacki 1997, 1999) and, as such, this study likewise provides a foundation for understanding the prey available to this species.

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# Use of Genetic Markers to Verify the Distribution of Northern Leopard Frogs (*Lithobates pipiens*) and Southern Leopard Frogs (*Lithobates sphenoccephalus*) in Kentucky

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## ABSTRACT

Understanding species distributions is important for determining whether conservation efforts are necessary. In central Kentucky, an area of potential range overlap occurs between northern leopard frogs (*Lithobates pipiens*), which is a species of special concern, and southern leopard frogs (*L. sphenoccephalus*). Their distributions are not completely delineated because of their low abundance in central Kentucky and similarity in morphology. Thus, use of genetic markers is necessary for identification. We surveyed for new, and sampled known, populations with a focus on the area of overlap in central Kentucky. We sequenced a portion of the mitochondrial 16S rRNA gene for 55 individuals from 16 populations. Maximum parsimony analysis separated individuals into two completely unresolved lineages representing the two species. Species were distributed as predicted, and no areas of fine-scale overlap were detected, although we did find a population of southern leopard frogs 10 km north-northwest of a northern leopard frog population. It was difficult to find *L. pipiens* at historically recorded populations, but we documented them in two counties where identification was previously unknown because morphology was intermediate between the species. Although genetic data reliably distinguished the species, they were inconclusive about whether hybridization occurs; future work is necessary to address this question.

KEY WORDS: *Lithobates pipiens*, *Lithobates sphenoccephalus*, genetics, distribution, Kentucky

## INTRODUCTION

Accurately determining a species distribution and understanding the genetic relationships with surrounding heterospecifics can help clarify past evolutionary history, current status, and, if necessary, the need for future conservation efforts (Avice 2004). Several factors affect species distributions, including physical geographic barriers, environmental changes, interspecies competition, and hybridization (Bridle and Vines 2006). In Kentucky, two closely related frog species, northern leopard frogs (*Lithobates pipiens*) and southern leopard frogs (*L. sphenoccephalus*), have distributions that are generally adjacent across their margins (Figure 1), but previous research has not determined distribution along these range margins in Kentucky, or whether these two species hybridize.

The *Lithobates pipiens* species complex consists of more than 25 species of leopard frogs (Hillis 1988). Many species in this complex have overlapping distributions, similar morphology, and similar habitat and breeding requirements, which may lead to

misidentification, inaccurate determination of species distributions, and possible hybridization of the species (Hillis et al. 1983; Hillis 1988). For example, *L. pipiens* and *L. blairi* (plains leopard frogs) hybridize in sympatric areas (Hillis 1988), and *L. sphenoccephalus* and *L. blairi* are documented to hybridize in the laboratory (Parris 2000, 2001). However, no studies have examined whether *L. pipiens* and *L. sphenoccephalus* hybridize.

*Lithobates pipiens* is distributed across Canada and the northern United States and range as far south as northern Kentucky in the eastern portion of their distribution (Rorabaugh 2000). *Lithobates sphenoccephalus* is found in the southern United States, ranging as far north as central Illinois and as far west as Texas (Butterfield et al. 2005). The ranges of *L. pipiens* and *L. sphenoccephalus* typically do not overlap, but in Kentucky, the two distributions meet and potentially overlap along a narrow zone located primarily along the edge of the Outer Bluegrass Ecoregion (Woods et al. 2002). Prior to our study, *Lithobates pipiens* was documented in 23 counties in northern Kentucky (KDFWR 2005; Figure 1), whereas *L. sphenoccephalus* had been found in 68 counties, primarily in

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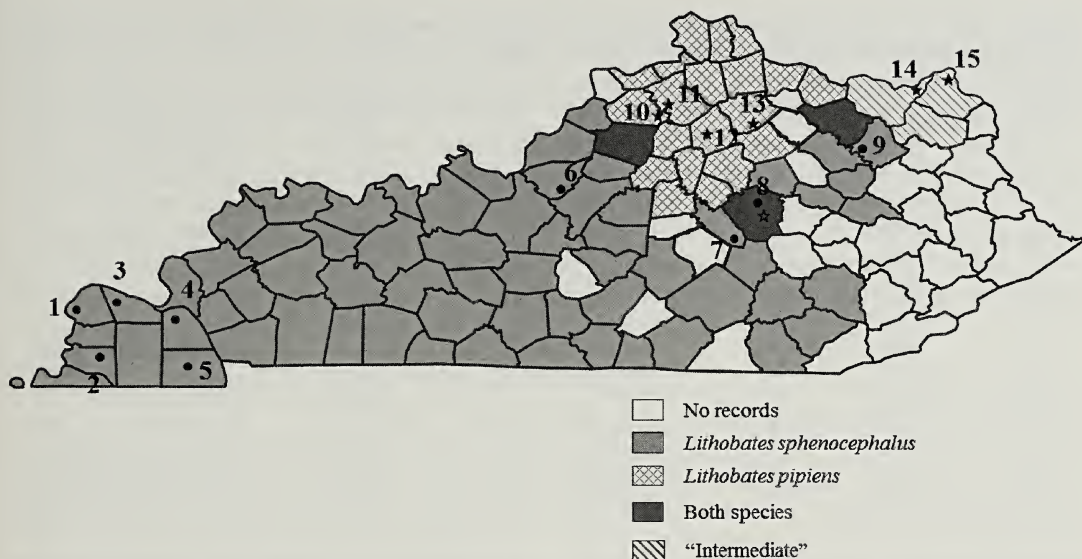


Figure 1. Distribution map of *Lithobates pipiens* and *L. sphenoccephalus* in Kentucky based on all county occurrences, including three counties where frogs were considered to have intermediate morphology (KDFWR 2005). Kentucky specimen locations are numbered corresponding to Table 1. Star = *L. pipiens*; dot = *L. sphenoccephalus*. Unnumbered star in Madison County represents historic population of *L. pipiens*.

southwestern Kentucky (KDFWR 2005; Figure 1). Three of these counties have been documented to contain both species. Twenty-nine counties have not been documented to contain either species; however, most boundaries of their distributions are unknown. Additionally, populations in northeast Kentucky (e.g., Carter, Greenup, and Lewis counties) were considered as intermediate between southern and northern leopard frogs based on morphological assessment (KDFWR 2005; Figure 1). In spring 2006, populations in Greenup and Lewis counties were tentatively designated as northern leopard frogs based on vocalizations (J. MacGregor, Kentucky Department of Fish and Wildlife Resources, pers. comm., 1 March 2007).

Northern and southern leopard frogs are similar morphologically, and identification based upon appearance is difficult. There are two morphological characters that have been used to distinguish the two species from each other, but misidentification is possible, especially in areas where ranges of the species overlap. *Lithobates sphenoccephalus* is commonly identified by the presence of a small white dot on the tympanum, which is absent in *L. pipiens*. Also, dorsal spots are typically not present on the snout of *L. sphenoccephalus*,

while spots are present on the snout of *L. pipiens* (Dodd 2013).

*Lithobates pipiens* and *L. sphenoccephalus* are not federally recognized as endangered or threatened. However, *L. pipiens* populations have experienced declines in the western portion of its range (USFWS 2009). Because of its limited distribution in Kentucky, *L. pipiens* is considered a species of concern by the state and has an S3 rank (vulnerable) (NatureServe 2011). Previous research indicated populations of both *L. pipiens* and *L. sphenoccephalus* are declining in portions of their ranges because of climate change, habitat loss and fragmentation, environmental pollutants, and disease (Hillis 1988; Hoffman et al. 2004; Daszack et al. 2005; USFWS 2009). Understanding the distribution of species is essential for accurate determination of its status and potential need for federal or state listing and for success in conservation efforts.

The primary objective of our study was to resolve the distribution of *L. pipiens* and *L. sphenoccephalus* along adjoining range margins in Kentucky while the secondary objective was to determine if the two species are interbreeding. Determining the genetic relationship between *L. pipiens* and *L. sphenoccephalus* in Kentucky will give us a better understanding



Table 1. Leopard frog specimens collected during the study, their morphological identification, collecting localities. See Figure 1 for distribution of sites, with the exception of Butler County, OH.

| Site No. | ID          | N  | County            | Life stage | Location               |
|----------|-------------|----|-------------------|------------|------------------------|
| 1        | <i>Lsph</i> | 1  | Ballard Co., KY   | Adult      | Swan Lake Road         |
| 2        | <i>Lsph</i> | 5  | Hickman Co., KY   | Larva      | Bugg Road              |
| 3        | <i>Lsph</i> | 1  | McCracken Co., KY | Larva      | Western KY WMA         |
| 4        | <i>Lsph</i> | 1  | Marshall Co., KY  | Adult      | Cypress Creek          |
| 5        | <i>Lsph</i> | 1  | Calloway Co., KY  | Adult      | Hancock BFS            |
| 6        | <i>Lsph</i> | 6  | Bullitt Co., KY   | Adult      | Knobs State Forest     |
| 7        | <i>Lsph</i> | 1  | Garrard Co., KY   | Adult      | Maywoods EEL           |
| 8        | <i>Lsph</i> | 1  | Madison Co., KY   | Juvenile   | Red House Road         |
| 9        | <i>Lsph</i> | 5  | Rowan Co., KY     | Adult      | Minor Clark Hatchery   |
| 10       | <i>Lpip</i> | 9  | Henry Co., KY     | Larva      | Kentucky River WMA     |
| 11       | <i>Lpip</i> | 5  | Owen Co., KY      | Egg        | Kentucky River WMA     |
| 12       | <i>Lpip</i> | 6  | Scott Co., KY     | Egg        | Long Lick Pike         |
| 13       | <i>Lpip</i> | 3  | Harrison Co., KY  | Larva      | Silas Pike             |
| 14       | <i>Lpip</i> | 2  | Lewis Co., KY     | Adult      | Lewis County WMA       |
| 15       | <i>Lpip</i> | 3  | Greenup Co., KY   | Adult      | South Shore WMA        |
| 16       | <i>Lpip</i> | 10 | Butler Co., OH    | Adult      | Rush Run Wildlife Area |

of the distribution and status of leopard frog populations in the state.

## MATERIALS AND METHODS

### Field Sampling

A total of 55 tissue samples were collected from 15 sites across the ranges of *L. pipiens* and *L. sphenoccephalus* in Kentucky and one reference *L. pipiens* site from Ohio (Figure 1). When present, eggs, tadpoles, or adults were collected from each site. Species identification based on morphology was determined for juveniles and adults (Table 1). Surveying and sampling took place from February 2007 to July 2009.

Specimens were collected by hand or with an aquatic dip net. If egg masses were present, five eggs were removed from each mass. If tadpoles or adults were present, a 5-mm section of tail was clipped from each tadpole and one toe from each adult. Sample size ranged from 1 to 10 samples per site depending upon availability of specimens (Table 1). All tissue samples were preserved in 95% ethanol.

### Laboratory Data Collection

DNA was extracted from eggs, tails and toe clippings using DNeasy® Blood and Tissue Kits (Qiagen, Inc., Valencia, California). The mitochondrial 16S rRNA gene region was selected for this study because previous research had found this region to successfully resolve relationships among ranid species including *L. pipiens* and *L. sphenoccephalus* (Hillis and Wilcox 2005). This region was

amplified with polymerase chain reaction (PCR) using primers and conditions of Hillis and Wilcox (2005) along with internal primers developed by our lab (Table 2).

Following verification of successful PCR via gel electrophoresis, PCR products were purified using the Millipore 96-well MultiScreen protocol (Millipore Corp., Billerica, Massachusetts). The purified PCR product was then used in a sequencing reaction using BigDye Version 3 protocol (Life Sciences Corp., Carlsbad, California). Removal of unincorporated dye-terminators was performed using a 95% ethanol and 3M sodium acetate (pH 5.2) mixture in a ratio of 17:1 combined with centrifugation to precipitate each sample. Samples were dried and resuspended in 12 µl of formamide for DNA sequencing with an ABI 310 Genetic Analyzer (Life Sciences Corp., Carlsbad, California). All sequence data were deposited in GenBank with accession numbers KJ573770–KJ573785.

### Phylogenetic Analysis

The nucleotide sequences of a 250-base fragment of the 16S rRNA gene of 55 tissue

Table 2. Internal primers we developed to amplify and sequence a portion of the 16S rRNA gene region in this study (see also Hillis and Wilcox 2005).

| Primer name | Primer sequence (5' → 3') |
|-------------|---------------------------|
| 16Spip-L    | CTAYCGACTTAGAGATAGC       |
| 16Sa*       | ATGTTTTGGTAAACAGGCC       |

\* 16Sa is a reverse primer.

samples were aligned and compared using Sequencher v. 5.0 (Gene Codes Corp., Ann Arbor, Michigan). Our sample sequences were aligned using sequences from Hillis and Wilcox (2005) downloaded from GenBank (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>) and used for reference and outgroup comparison. Consensus sequences were made from individuals that had identical nucleotide sequences.

A maximum parsimony analysis (MP) was performed via a heuristic search with 1000 replications of random stepwise additions using PAUP\* v.4.0b10 (Swofford 2001). Bootstrapping used 1000 replications and heuristic searching with ten random stepwise additions. Based on Hillis and Wilcox (2005), *Lithobates sierramadrensis*, *L. psilonota*, *L. zweifeli*, and *L. tarahumarae* were chosen as outgroup taxa to root trees.

## RESULTS

All adults and juveniles identified to species based on morphology were confirmed by molecular analyses, and no intermediate morphologies were detected (Table 1). The DNA sequences of *L. pipiens* from all locations were identical, and *L. sphenocephalus* from six of nine locations were identical. Other *L. sphenocephalus* samples differed by only one to five bases. Based on the maximum parsimony analysis, all *L. pipiens* and *L. sphenocephalus* individuals were separated into two distinct monophyletic groups with bootstrap values greater than 85. There was no evidence in our DNA data that suggested hybridization between the species (i.e., DNA sequences were clean and did not indicate mixed mitochondrial genomes within individuals). DNA sequences from our study grouped with sequences from Hillis and Wilcox (2005) for both *L. sphenocephalus* and *L. pipiens*. Placements of sequences from outgroup species of Hillis and Wilcox (2005) corroborated the topology of their study.

The majority of the *L. pipiens* sampling localities fell inside the previously known distribution of *L. pipiens* in Kentucky (KDFWR 2005; Figure 1). However, there were two counties (Lewis and Greenup) not represented as having *L. pipiens* by the KDFWR (2005) that we documented to have *L. pipiens*. *Lithobates sphenocephalus* sample locali-

ties matched previously known distributions (KDFWR 2005; Figure 1). Interestingly, one population of *L. sphenocephalus* in Madison County was located approx. 10 km north-northwest of a previously documented *L. pipiens* population, based on vocalizations and morphology, in Madison County (Figure 1). Unfortunately, this *L. pipiens* population went locally extinct prior to our study, so we were unable to genotype those individuals.

## DISCUSSION

We found that the distribution of *L. pipiens* and *L. sphenocephalus* across Kentucky was fairly consistent with previously known state distributions. However, we were able to positively identify *L. pipiens* populations in Lewis and Greenup counties whose taxonomy were previously unknown. We found it relatively difficult to find new populations of leopard frogs in central Kentucky. Many sites historically recorded as having *L. pipiens* were no longer found to have active leopard frog populations. The difficulty in finding leopard frog populations of each species across our focal landscape (i.e., area of potential overlap of the two species) could be due to several reasons, the most likely of which is that populations at the periphery of the species range are typically sporadic in distribution and small in size (Bridle and Vines 2006).

Using morphology as the only species identification method for leopard frog taxa is not always reliable (Hillis et al. 1983). The *L. pipiens* complex is itself a group that has a tortured taxonomic history, with species numbers fluctuating from four distinct species to 25 using a combination of morphology, auditory, biochemical, and reproductive data. In Kentucky, the similar appearance of *L. pipiens* and *L. sphenocephalus*, along with the possibility of hybridization, makes it difficult to accurately identify each species, which in turn makes it difficult to determine an accurate distribution of each species. Over the past decades, inconsistencies have been discovered in using morphological identifiers of each species when compared to vocalizations, and some individuals had intermediate morphologies (J. MacGregor, Kentucky Department of Fish and Wildlife Resources, pers. comm., 1 March 2007), which suggest a genetic interaction might be occurring.



We were able to accurately identify leopard frog species in Kentucky using the 16S rRNA gene region of the mitochondrial genome. Our phylogenetic analysis separated specimens into two monophyletic groups; however, the relationship of the individuals in each group is unresolved. This is because of the selected region of the mtDNA genome that was used for species identification. The 16S rRNA gene of the mtDNA was selected because it has high resolution among leopard frog species (Hillis and Wilcox 2005). Ability to resolve relationships among populations within species was unknown. Based on our study, a more variable region of the mtDNA genome (e.g., the control region) or use of nuclear DNA is needed to clarify relatedness among populations.

*Lithobates pipiens* is listed as a species of concern and S3 in Kentucky; therefore, the fact that we had difficulty finding populations is consistent with its state listing status. In Kentucky, range-wide population monitoring and surveys for *L. pipiens* and targeted population surveys for *L. sphenocephalus* in the central and eastern portions of the state continue to occur (J. MacGregor, Kentucky Department of Fish and Wildlife Resources, pers. comm., 9 May 2013). In addition to broad-scale population surveys, fine-scale studies are needed along their range edges to define the exact distributional limits and potential overlap between the two species. The primary questions remaining to be addressed include determining (1) relationships among populations within each species and how this relates to geography and history and (2) what interactions (if any) occur between individuals of each species, what the outcomes are (i.e., interspecies competition, hybridization, etc.), and how this affects their distributions. This research will help to clarify the evolutionary and ecological relationship between these species, which will inform conservation efforts.

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# Potential of Kentucky Freeway Rights-of-Way to Displace Fossil Fuel Consumption through Production of Switchgrass (*Panicum virgatum* L.)

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## ABSTRACT

The grassland area of freeway rights-of-way in Kentucky was estimated to determine its potential to produce switchgrass (*Panicum virgatum* L.) for bioenergy feedstock. Kentucky's freeways include  $9.2 \times 10^3$  ha of mowed grassland within their rights-of-way. This area could produce approximately  $64 \times 10^3$  t/yr of switchgrass, sufficient to make  $21 \times 10^6$  L of cellulosic ethanol, or generate 64 GWh of electricity annually. Switchgrass grown on freeway rights-of-way could offset about 0.5% of fossil fuel currently used on Kentucky freeways if converted to ethanol, or 0.8% if converted to electricity for vehicle propulsion. Future changes in vehicle efficiency and traffic volume will have much greater impact on the volume of fossil fuel used on Kentucky freeways than conversion of freeway-grown switchgrass to alternative fuel. Kentucky's state government's projection of a 42% increase in traffic volume by 2025 contrasts with this study's projection of 28% decline derived from extrapolation of linear trends observed since 1983. If such a decline is realized in combination with federally mandated vehicle efficiency improvements then freeway-grown switchgrass used for electric vehicle propulsion could offset up to 2% of freeway fossil fuel use in Kentucky by 2025. The contrast between the implications of these estimates reflects considerable uncertainty, but emphasize the importance of conservation in conjunction with alternative fuel production.

KEY WORDS: biofuel, ethanol, switchgrass, highway, right-of-way, conversion

## INTRODUCTION

Interstate freeways account for 4.1% of road distance in the United States, but one-third of vehicle distance traveled (USDOT 2012). While rights-of-way are 95% state-owned and are maintained by state authorities, their construction and maintenance is almost entirely funded by the federal government. Federal guidelines dictate the use of guard rails or roadside recovery areas, free of fixed objects, and a clear median (>11 m in rural areas) between opposing lanes of traffic (USDOT 2007). Medians and roadside recovery areas are normally seeded to grass mixtures and mowed regularly.

Freeway rights-of-way are being examined by federal and state departments of transportation as potential zones for renewable energy production (Poe et al. 2012). The Utah Department of Transportation has assessed the potential for biodiesel feedstock production on its public road rights-of-way (Whitesides and Hanks 2011). Photovoltaic panels have been successfully installed along freeway rights-of-way in the state of Oregon and in several European countries, leading at least 25 states, including Kentucky, to seek information on establishing similar facilities (Ponder et al. 2011). A 1.5 MW wind turbine is being constructed in the Massachusetts Turnpike right-of-way (Poe et al. 2012). Kansas, Nebraska, and Tennessee allow hay harvest and baling in highway rights-of-way (KDOT 2009; NDOR 2013; TDOT 2003). These bales are

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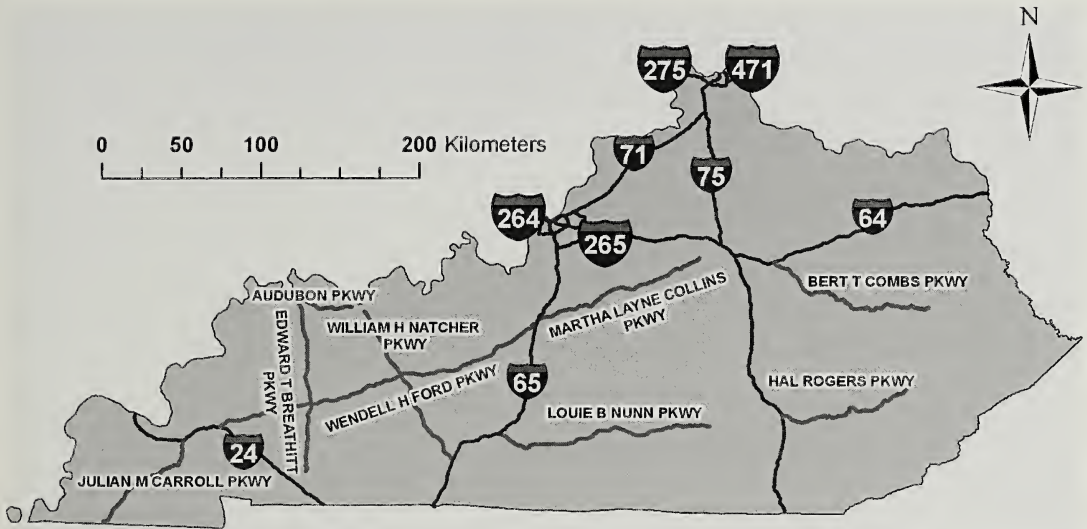


Figure 1. Kentucky's freeway system is composed of federally-funded interstates (numbered) and state-funded parkways (named).

currently used primarily for animal feed and bedding, but could also serve as cellulosic feedstock for combustion, gasification, or fermentation-based energy conversion processes.

Nine interstate freeways pass through Kentucky (Figure 1). Kentucky also has a network of nine state-funded controlled-access freeways, called "parkways," built to similar standards as interstate freeways. Harvesting biomass from freeway rights-of-way might be one way for Kentucky to approach its 2008 commitment to triple renewable energy production and offset 12% of its motor fuel demand with biofuels by 2025 (Beshear 2008; Anderson et al. 2009).

Economic, social and environmental arguments can be made for using freeway rights-of-way for renewable energy production. From an economic perspective, mowing rights-of-way is expensive. Kentucky's roadside vegetation management costs are among the highest in the country, on an area basis (Berger 2005), with annual expenditures of 4.4–5.5 million dollars to mow rights-of-way 4–5 times annually (Open Records request, Kentucky Transportation Cabinet, 2008). Renewable energy production may reduce the need to mow, or provide revenue to offset mowing costs. From a social perspective, renewable energy projects located on rights-of-way result in minimal competition with

crop production, and offer a highly visible reminder of state efforts to reduce fossil fuel use and combat global warming. From an environmental perspective, freeway rights-of-way are disturbed and polluted environments, characterized by abundant light, runoff water, and periodic nutrient flushes from adjacent lands, making them particularly susceptible to colonization by invasive species unless native species are maintained (Coffin 2007). Greenhouse gas (GHG) emissions from freeway travel account for approximately 10% of total US GHG emissions (USEPA 2013), and might be partially offset through renewable fuel production from freeway-grown switchgrass (Campbell et al. 2009; USDOE 2010a).

Switchgrass, *Panicum virgatum* L., has potential as a bioenergy feedstock crop (McLaughlin and Walsh 1998). It is a drought-tolerant C4 warm-season perennial, native to North America, that can be harvested as hay (Weaver 1968). It develops an extensive root system that controls erosion and sequesters carbon belowground (Turhollow 1994). The lowland cultivars 'Alamo' and 'Kanlow' produced an average of 15 t/ha dry biomass under low input conditions in Kentucky between 1999 and 2001 (Fike et al. 2006).

The objectives of this study were to: 1) estimate the area available for switchgrass production on Kentucky's freeway rights-of-way; 2) estimate the potential energy available from



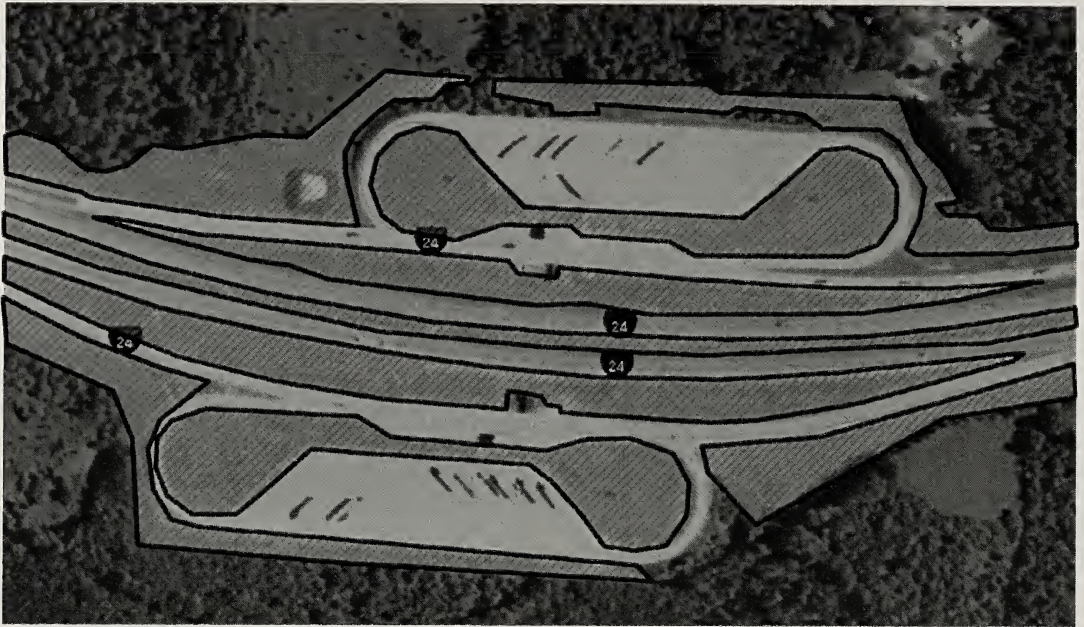


Figure 2. A high resolution aerial image of a Kentucky freeway segment with cross-hatched polygons superimposed over mowed grassland within the right-of-way.

mature freeway-grown switchgrass convertible into ethanol or electricity; 3) estimate the proportion of fuel currently consumed on Kentucky freeways that could be offset by this conversion; and 4) project the future offset potential.

#### METHODS

The total area of mowed grassland in medians and shoulders of interstate and parkway rights-of-way was calculated for each of Kentucky's freeways using Geographic Information System software (ArcGIS v. 9, Esri, Redlands CA) and 1 m resolution ortho imagery, rectified to true ground within  $\pm 6$  m (USDA-FSA 2009) (Figure 2). Fence rows and tree lines separating government from private property were used to indicate right-of-way boundaries. The total mowed area ( $A$ ) and freeway length ( $D$ ) was calculated for each freeway in Kentucky.

Annual traffic volume ( $V$ ) for each freeway was derived from the most recent daily traffic counts conducted by the Kentucky Transportation Cabinet. Counts are conducted at stations located between each freeway interchange. Each station's daily count was multiplied by the distance between adjacent interchanges then divided by the total length of the

freeway to calculate a weighted daily traffic volume for the entire freeway, which was multiplied by 365 days to calculate annual traffic volume ( $V$ ).

Switchgrass was assumed to yield 7.0 t/ha/yr ( $Y$ ). This is approximately half the average annual single-cut dry matter yield observed for established low input plantings of *P. virgatum* var. 'Alamo' in replicated plots at Princeton, KY over two years (Fike et al. 2006) and equal to the average annual yield over 10 years on marginal cropland in North Dakota, South Dakota and Nebraska (Schmer et al. 2010).

Switchgrass was assumed to be converted to ethanol at 376 L/t (USDOE 2010a; Campbell et al. 2009). Energy used for switchgrass production, transportation, and conversion to ethanol was estimated at 0.33, 0.25, and 0.42 GJ/t, respectively (Schmer et al. 2008) excluding energy derived from lignin, which was assumed to provide the heat and electricity used for ethanol refining (Campbell et al. 2009). The 1.0 GJ/t energy input was assumed equivalent to 47 L/t of refined ethanol on an energy content basis. This was subtracted from the gross ethanol conversion rate to give a net ethanol conversion rate of 329 L/t ( $E$ ).



Table 1. Variables used in calculations.

|                | Variable | Unit        | Source     |
|----------------|----------|-------------|------------|
| Freeway length | <i>D</i> | km          | This study |
| Mowed area     | <i>A</i> | ha          | This study |
| Traffic volume | <i>V</i> | vehicles/yr | KTC 2010   |

Annual freeway ethanol consumption was estimated at 0.17 L of ethanol fuel equivalent per km traveled, based on the 2008 national average fuel economy of 0.14 L/km (Peters 2011) and assumptions that ethanol energy density is 34% less than the 2008 fuel mix (USDOT 2011) and freeway fuel economy is 22% better than the national average, as for the current vehicle fleet in the U.S. (USDOE 2010b) ( $0.14/0.66/1.22 = 0.17$  L/km).

Electricity used by electric vehicles was estimated at 0.32 kWh/km, based on the assumption that replacing ethanol fuel consumes 1.9 kWh/L, the average ratio of highway electricity consumption by production model electric vehicles released in the United States and liquid fuel consumption of the nearest internal combustion engine equivalent, stated in ethanol equivalent volume (Campbell et al. 2009).

Freeway-specific variables (Table 1) and energy production and consumption efficiency estimates (Table 2) were used to calculate estimates of potential production and consumption of bioenergy from switchgrass for each freeway in Kentucky (Table 3).

Annual change in total distance traveled by vehicles in Kentucky was calculated for each year between 1983 and 2010. Total vehicle distance traveled (VDT) was divided by state population for each year (USDOC 2002; USDOT 2012; USDOC 2012) to obtain vehicle distance traveled per capita.

Linear regression was used to estimate average annual change in Kentucky VDT between 1983 and 2010. The calculated regression line was extrapolated to 2025 to project future changes. The projection was

compared with the Kentucky Transportation Cabinet's projection of 2.2% annual growth in VDT until 2025, derived from the average annual change in VDT between 1983 and 2009 (Beshear 2008).

## RESULTS

Kentucky's freeway rights-of-way have  $9.2 \times 10^3$  ha of mowed area along 2260 km of roadway (Table 4). Harvesting switchgrass hay from this area would increase the  $1.0 \times 10^6$  ha currently devoted to hay production in Kentucky by less than 1% (USDA 2009). Switchgrass established in all currently mowed areas on freeway rights-of-way would yield about  $64 \times 10^3$  t of harvestable dry biomass annually, once established (Table 4). This could be converted into about 64 GWh of electricity, or  $21 \times 10^6$  L of ethanol (Table 4).

Kentucky's interstate network is only 18% longer than its parkway network, but has 67% more mowed area (Table 4). Parkways and interstates could yield about 23 and 41 t/km/yr, respectively. A typical Kentucky freeway right-of-way could yield about 28 t/km/yr of switchgrass, which could generate 28 MWh/km/yr of electricity, or  $9.3 \times 10^3$  L/km/yr of ethanol fuel.

Liquid transportation fuel accounted for 23% of energy consumed and 43% of energy expenditures in Kentucky in 2009 (Peters 2011). Each year, the equivalent of approximately  $4.0 \times 10^9$  L of ethanol is burned on Kentucky's freeways –  $1.8 \times 10^6$  L per freeway km (Table 5). This represents 17% of the  $23 \times 10^9$  L of ethanol equivalent burned by Kentucky's transportation system annually (Peters 2011).

Assuming a similar mix of vehicle types on each freeway, fuel consumption is directly related to traffic volume, which ranges from 5800 to 91,000 vehicles/day, with a mean of 28,700 (Table 5). Interstate and parkway volumes are 56% above and 66% below this

Table 2. Estimates of bioenergy production and consumption efficiency used in calculations.

|                                 | Constant | Value | Unit    | Source                                   |
|---------------------------------|----------|-------|---------|--|
| Switchgrass yield               | <i>Y</i> | 7.0   | t/ha/yr | Schmer et al. 2008, Patzek 2010          |
| Net ethanol yield               | <i>E</i> | 330   | L/t     | Campbell et al. 2009, USDOE 2010a        |
| Net electricity yield           | <i>L</i> | 1.0   | MWh/t   | Campbell et al. 2009, USDOE 2010a        |
| Highway ethanol consumption     | <i>F</i> | 0.17  | L/km    | USDOE & USEPA 2010, USDOE 2010b          |
| Highway electricity consumption | <i>W</i> | 0.32  | kWh/km  | Schmer et al. 2008, Campbell et al. 2009 |



Table 3. Calculations used to estimate potential production and consumption of switchgrass, ethanol, and electricity from Kentucky freeway rights-of-way.

|                                    | Calculation                                      | Unit   |
|------------------------------------|--|--------|
| Switchgrass production             | $A \times Y$                                     | t/yr   |
| Ethanol production                 | $A \times Y \times E$                            | L/yr   |
| Electricity production             | $A \times Y \times L$                            | MWh/yr |
| Vehicle distance traveled          | $D \times V$                                     | km/yr  |
| Ethanol production/consumption     | $A \times Y \times E / D \times V \times F$      | %      |
| Electricity production/consumption | $A \times Y \times L / 10^6 D \times V \times W$ | %      |

mean, respectively (Table 5). Kentucky’s interstate network carries five times as much traffic as its parkway network. Annual VDT on Kentucky freeways accounts for 32% of the  $76 \times 10^9$  km traveled in the state each year (USDOT 2011).

The  $21 \times 10^6$  L of ethanol that could be produced from switchgrass grown on freeway rights-of-way would offset about 0.5% of the fuel consumed on the Kentucky freeways annually (Table 5), or fuel all passing traffic for less than two days of each year. There is an inverse relationship between a freeway’s traffic volume and potential to offset fuel consumption with switchgrass grown in its right-of-way (Figure 3). Kentucky’s parkways tend to have less traffic than its interstates

(Figure 3), and could produce switchgrass feedstock for enough ethanol to offset about 1.2% of the fuel used to move this traffic (Table 5). The state’s highest and lowest volume freeways, I-264 and the Louie B. Nunn parkway, could offset 0.2% and 3.9% of their fuel use, respectively, if the mowed sections of their rights-of-way were used to grow switchgrass for ethanol feedstock (Table 5, Figure 3). The right-of-way for I-24, the interstate with the lowest traffic volume and the most mowable area per linear distance, could offset 1.1% of the fuel currently used on the road (Tables 4, 5).

Conversion of switchgrass to electricity for electric vehicle propulsion would offset 61% more fuel than conversion to ethanol (Ta-

Table 4. Estimated annual production potential of electricity and ethanol from mature switchgrass following establishment in currently mowed areas of Kentucky freeway rights-of-way.

| Route                | Length (km) | Mowed area (ha) | Switchgrass ( $10^3$ t) | Ethanol ( $10^6$ L) | Electricity (GWh) |
|----------------------|-------------|-----------------|-------------------------|---------------------|-------------------|
| Total freeway        | 2260        | 9151            | 64.1                    | 21.1                | 64.1              |
| Interstates          |             |                 |                         |                     |                   |
| I-24                 | 150         | 1039            | 7.3                     | 2.4                 | 7.3               |
| I-64                 | 296         | 1397            | 9.8                     | 3.2                 | 9.8               |
| I-65                 | 221         | 1077            | 7.5                     | 2.5                 | 7.5               |
| I-71                 | 156         | 575             | 4.0                     | 1.3                 | 4.0               |
| I-75                 | 278         | 1139            | 8.0                     | 2.6                 | 8.0               |
| I-264                | 37          | 153             | 1.1                     | 0.4                 | 1.1               |
| I-265                | 39          | 232             | 1.6                     | 0.5                 | 1.6               |
| I-275                | 39          | 253             | 1.8                     | 0.5                 | 1.8               |
| I-471                | 8           | 31              | 0.2                     | 0.1                 | 0.2               |
| Total interstate     | 1223        | 5898            | 41.3                    | 13.6                | 41.3              |
| Parkways             |             |                 |                         |                     |                   |
| Audubon              | 38          | 179             | 1.3                     | 0.4                 | 1.3               |
| Bert T. Combs        | 121         | 208             | 1.5                     | 0.5                 | 1.5               |
| Edward T. Breathitt  | 112         | 537             | 3.8                     | 1.2                 | 3.8               |
| Hal Rogers           | 97          | 217             | 1.5                     | 0.5                 | 1.5               |
| Louie B. Nunn        | 81          | 485             | 3.4                     | 1.1                 | 3.4               |
| Martha Layne Collins | 143         | 372             | 2.6                     | 0.9                 | 2.6               |
| Julian M. Carroll    | 115         | 277             | 1.9                     | 0.6                 | 1.9               |
| Wendell H. Ford      | 219         | 552             | 3.9                     | 1.3                 | 3.9               |
| William H. Natcher   | 113         | 425             | 3.0                     | 1.0                 | 3.0               |
| Total parkway        | 1037        | 3524            | 22.8                    | 7.5                 | 22.8              |

Table 5. Traffic volume and fuel consumption on Kentucky freeways, with proportion of fuel potentially offset by ethanol or electricity made from switchgrass grown in their rights-of-way.

| Route                | Average daily volume (10 <sup>3</sup> vehicles) | Annual vehicle distance traveled (10 <sup>6</sup> km) | Annual consumption              |                          | Production/consumption |                 |
|----------------------|---|---|---------------------------------|--------------------------|------------------------|-----------------|
|                      |   |   | EtOH equiv. (10 <sup>6</sup> L) | Electricity equiv. (GWh) | EtOH (%)               | Electricity (%) |
| All freeways         | 28.7  | 23.71   | 4031                            | 7588                     | 0.5                    | 0.8             |
| <b>Interstates</b>   |   |   |                                 |                          |                        |                 |
| I-24                 | 24.3  | 1.33  | 227                             | 427                      | 1.1                    | 1.7             |
| I-64                 | 35.0  | 3.78  | 643                             | 1210                     | 0.5                    | 0.8             |
| I-65                 | 51.3  | 4.14  | 704                             | 1326                     | 0.4                    | 0.6             |
| I-71                 | 30.5  | 1.73  | 294                             | 554                      | 0.4                    | 0.7             |
| I-75                 | 55.0  | 5.57  | 947                             | 1783                     | 0.3                    | 0.4             |
| I-264                | 95.2  | 1.28  | 217                             | 409                      | 0.2                    | 0.3             |
| I-265                | 63.6  | 0.90  | 153                             | 289                      | 0.3                    | 0.6             |
| I-275                | 69.4  | 0.99  | 169                             | 318                      | 0.3                    | 0.6             |
| I-471                | 91.0  | 0.26  | 44                              | 84                       | 0.2                    | 0.3             |
| All interstates      | 44.8  | 20.00   | 3400                            | 6400                     | 0.4                    | 0.6             |
| <b>Parkways</b>      |   |   |                                 |                          |                        |                 |
| Audubon              | 8.6   | 0.12  | 20                              | 38                       | 2.1                    | 3.3             |
| Bert T. Combs        | 7.9   | 0.35  | 60                              | 112                      | 0.8                    | 1.3             |
| Edward T. Breathitt  | 13.7  | 0.56  | 95                              | 179                      | 1.3                    | 2.1             |
| Hal Rogers           | 7.4   | 0.26  | 44                              | 84                       | 1.1                    | 1.8             |
| Louie B. Nunn        | 5.8   | 0.17  | 29                              | 54                       | 3.9                    | 6.3             |
| Martha Layne Collins | 13.2  | 0.69  | 117                             | 220                      | 0.7                    | 1.2             |
| Julian M. Carroll    | 9.3   | 0.39  | 66                              | 125                      | 1.0                    | 1.6             |
| Wendell H. Ford      | 9.7   | 0.77  | 132                             | 248                      | 1.0                    | 1.6             |
| William H. Natcher   | 9.8   | 0.40  | 68                              | 129                      | 1.4                    | 2.3             |
| All parkways         | 9.8   | 3.71  | 631                             | 1188                     | 1.2                    | 1.9             |

ble 5). This result is between the 80% advantage to electricity calculated by Campbell et al. (2009), based on mixed city and highway driving of electric vehicles with internal combustion engine (ICE) analogues available by 2003; and the 48% advantage

calculated by Chaves (2009) for an electric Ford Explorer, relative to its ICE counterpart.

Kentucky's annual VDT increased most years between 1983 and 2010, but the annual rate of change, calculated by linear regression, declined by  $0.18 \pm 0.04\%$  per year during this period ( $n = 28, r^2 = 0.46, P < 0.001$ ), from 4.7% in 1984 to  $-0.1\%$  in 2010 (Figure 4). Distance traveled per capita peaked in 2000 and has declined most years since, such that per capita distance was similar in 2010 and 1997 (Figure 4). Kentucky's population growth has equaled or exceeded growth in VDT in eight of the past 10 years.

Extrapolation of this linear trend to 2025 projects that VDT in Kentucky will be similar to that in 1992, or 20% lower than its peak in 2007 (Figure 5). This projection contrasts with the Kentucky Transportation Cabinet's forecast of 42% growth in VDT between 2009 and 2025, based on an assumption of 2.2% annual growth (Strait et al. 2010). A 2.2% annual growth rate in Kentucky's interstate VDT was forecast for the period between 2000 and 2020, based on trends observed between 1980 and 2000 (Barrett et al. 2001).

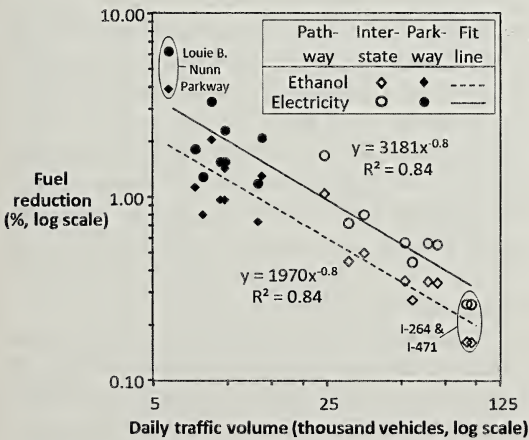


Figure 3. Relationship between daily freeway traffic volume and proportion of fuel consumed on a freeway that could be replaced by ethanol or electricity produced from switchgrass grown in its right-of-way.



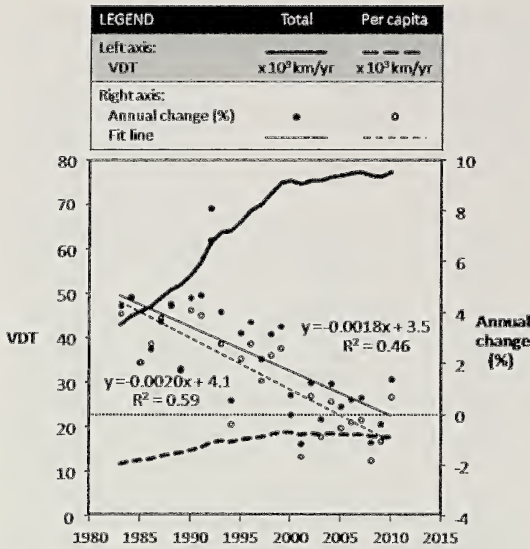


Figure 4. Annual total and per capita vehicle distance traveled (VDT) in Kentucky, 1983–2010 (left axis), with linear regression of annual change (right axis). Points above and below the dotted line show annual increases and decreases in VDT, respectively.

The forecast included assumptions of increasing travel per capita, and a growing population. The assumption of increasing travel per capita has not been realized since 2000 (Figure 4), yet the projected growth rate remains unchanged. Annual federal government projections of growth in VDT, based on state projections such as those developed in Kentucky, have consistently and substantially over-estimated realized VDT since 2006 (Dutzik 2013).

Observed reductions in growth of Kentucky’s annual traffic volume may be driven by demographic changes, rising fuel costs, reduced growth in real income, or cultural change (Polzin 2006; Dutzik 2013). Demographic changes that slow growth in travel include an increasing median age, stabilization in the female proportion of the labor force, and reduced participation in the labor force overall (Polzin 2006; Dutzik 2013). Between 1999 and 2011, gasoline and motor oil expenditures rose from a low of 2.8% of US household expenditures to an unprecedented high of 5.3% of household expenditures (USDOD 2013). The average annual growth in real income of Kentucky residents fell from 2.4% between 1980 and 2000, to just 0.6% between 2000 and 2010 (FRED 2013).

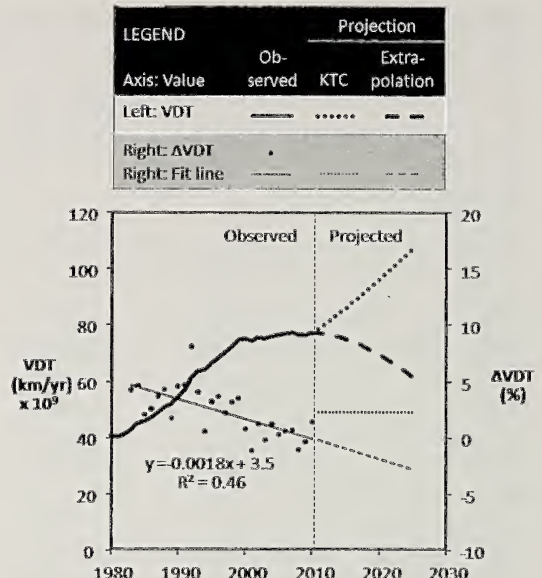


Figure 5. Projections of annual vehicle distance traveled (VDT) in Kentucky to 2025 based on Kentucky Transportation Cabinet (KTC) assumption of 2.2% annual growth (dotted lines), or extrapolation (dashed lines) of linear regression of annual changes observed between 1983 and 2010.

Federal fuel EPA efficiency mandates requiring a 25% increase in the efficiency of light vehicles manufactured between 2011 and 2016 will dampen any effect of increasing traffic volume on fossil fuel consumption on Kentucky freeways, or amplify effects of decreasing traffic volume (USEPA 2010). If the trend in annual traffic volume change established between 1983 and 2010 continues, and vehicles on Kentucky’s freeways are 24% more efficient than today, then Kentucky’s consumption of fossil fuel for freeway transportation in 2025 could be half the present amount. In this scenario, freeway-grown switchgrass could offset ~1% of fossil fuel consumption if converted into ethanol, and almost 2% if converted into electricity to power electric vehicles.

Future changes in traffic volume and vehicle efficiency will have greater impact on fossil fuel consumption than the modest reduction that could be achieved by converting mowed areas of freeway rights-of-way to switchgrass production. The 1.1% reduction in traffic volume observed in the energy price shock year of 2008 likely reduced fossil fuel



consumption on Kentucky freeways more than could be achieved by producing ethanol from switchgrass grown on all currently mowed areas of Kentucky's freeway rights-of-way.

## DISCUSSION

Our estimates of freeway length are within 0.2% of independent estimates (KTC 2010); we are not aware of previous estimates of the mowed area of Kentucky's freeway rights-of-way. Our estimate of mowed area in freeway rights-of-way is roughly twice that of a similar estimate of the amount of land available for biofuel feedstock production in Michigan's highway rights-of-way (Schools *et al.* 2010). Kentucky's interstate network (1223 km) is only half as long as Michigan's (2017 km), suggesting that the method used in Michigan was more conservative than ours.

Estimates of potential switchgrass yield and potential to reduce fuel consumption, presented to provide context for our mowable area estimates, incorporate a large degree of uncertainty. Our assumed switchgrass yield of 7.0 t/ha/yr is equivalent to the average yield reported for ten established switchgrass fields in Eastern Nebraska, South Dakota, and North Dakota (Schmer *et al.* 2010). This estimate is similar to that of Patzek (2010), who used a Monte Carlo simulation to estimate the most likely continuous switchgrass yield, with standard deviation, at  $6.8 \pm 4.5$  t/ha/yr, based on all field data available at the time. Kentucky has a longer growing season and greater rainfall than most Great Plains locations, which could improve yield. Indeed, switchgrass yield observed from small research plots in Kentucky were twice as high as the yield assumed here (Fike *et al.* 2006). Since the mowed areas of freeway rights-of-way tend to be marginal land with severely compacted soil (Whitesides and Hanks 2011), we chose to retain the more conservative yield assumption.

Estimates of the efficiency of conversion of switchgrass to ethanol or electricity are also extrapolated from small-scale trials because this process has not been demonstrated on a large scale. Actual conversion efficiency may be less than half the value assumed here (Patzek 2010). Since each of the yield assumptions is directly related to potential to reduce fossil fuel consumption, over-estimating switchgrass yield

and ethanol conversion efficiency each by a factor of two would over-estimate potential ethanol production by a factor of four.

The amount of fuel presently consumed on Kentucky freeways is likely a poor indicator of future consumption, and different methods of projecting future consumption give very different results. We demonstrate that two different methods of projecting VDT in Kentucky result in a high estimate for 2025 that is twice the low estimate.

Future change in distance traveled is unlikely to be evenly distributed across freeways. Kentucky's most used freeway currently carries 16 times more traffic than its least used freeway. Freeways with lower traffic volume have the greatest potential for switchgrass grown in their rights of way to offset a substantial portion of their fuel use.

This analysis represents an attempt to estimate the volume of transportation fuel that could be offset by switchgrass produced in Kentucky's freeway rights-of-way relative to the fuel volume consumed on those freeways. It does not consider the logistics or economics of such an enterprise, or assume that fuel produced from freeway-grown switchgrass would actually be utilized by vehicles traveling on the same freeway. Elements of Kentucky's freeway network are familiar to most of those who travel through the Commonwealth, offering a broad general awareness of traffic volumes and right-of-way areas. Recognizing that fuel produced from switchgrass grown on all mowed areas of Kentucky's freeway rights-of-way for a year could be consumed by passing traffic in less than two days provides some indication of the productivity and land requirements of switchgrass to transportation fuel pathways, relative to current fuel consumption patterns. Our analysis emphasizes that renewable fuel production from switchgrass grown on right-of-ways in Kentucky freeways cannot substitute for conservation efforts.

## SUMMARY

1. The currently mowed area of Kentucky's freeway rights-of-way is over  $9.2 \times 10^3$  ha. Interstates and parkways account for about two-thirds and one-third of this area, respectively.
2. Switchgrass harvested from this area could produce enough ethanol to replace  $\sim 0.5\%$



- of the fuel currently consumed on Kentucky freeways, or fuel all traffic on the freeway network for less than two days each year.
3. Conversion of switchgrass to electricity to power electric vehicles would reduce fossil fuel consumption on Kentucky freeways by ~60% more than conversion to ethanol. Freeway-grown switchgrass could generate enough electricity to power all freeway traffic for about three days each year, assuming electrification of vehicles.
  4. The annual rate of change in Kentucky's traffic volume declined between 1983 and 2010. Per capita traffic volume has decreased most years since 2000, suggesting that the typical individual in Kentucky drives less each year. Extrapolation of this trend suggests future decline in total traffic volume, in contrast to state government projections of future growth.
  5. Our estimates of the potential of Kentucky freeway rights-of-way to displace fossil fuel consumption through production of switchgrass incorporate considerable uncertainty and may be optimistic. Even so, we find the potential to reduce consumption by this method is trivial compared to the potential for reductions through increased fuel efficiency or lower traffic volume. Conservation and efficiency measures that reduce fuel use increase the potential to displace fossil fuel use through conversion of freeway-grown switchgrass.

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# New County Distribution Records for Butterfly Species in Eastern Kentucky

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## ABSTRACT

Although Kentucky has an active Lepidoptera community, there are still several regions of the state with very sparsely documented butterfly faunas. While conducting surveys and obtaining personal collection materials for Lepidoptera throughout Kentucky, we documented new county distribution records for 16 butterfly species between Bath, Martin, and Rowan Counties in eastern Kentucky. In addition, we documented the second recorded multiple occurrence of *Parrhasius m-album* in Kentucky and a new earliest sighting date for *Anthocharis midea* in Kentucky.

KEY WORDS: butterflies, butterflies in eastern Kentucky, county records, Lepidoptera

## INTRODUCTION

While Kentucky has a diverse butterfly fauna as well as an active Lepidoptera community, there are still several regions of the state with very sparsely documented butterfly faunas. Because of this, there are several counties that lack records for even the most common butterfly species. This is especially true in eastern Kentucky. The majority of recorded species from this region have centered on areas with important or significant natural features such as Carter Caves State Park, Daniel Boone National Forest, and Pine Mountain. While such areas are ecologically important, adding to our knowledge of both the distribution and status of even the most common butterfly species in other areas of eastern Kentucky is essential in order to fully understand and manage our state's diverse butterfly fauna.

While conducting surveys and obtaining personal collection materials for Lepidoptera throughout Kentucky from August 1995 through August 2009, we documented new county distribution records for 16 butterfly species in three counties in eastern Kentucky. We present here an annotated list of the new county distribution records observed, including five

new species records from Bath County, four new species records from Rowan County, and nine new species records from Martin County.

## MATERIALS AND METHODS

Although a number of habitat types were sampled, an effort was made to search areas in each county that were likely to yield large numbers of butterflies including old-field habitats, wooded road corridors (forest service roads, mining/logging roads, etc.) and natural openings. Such areas typically have more solar exposure than forested habitats, leading to larger concentrations of nectar sources for butterflies. All collections were made with the use of a standard dacron chiffon Lepidoptera collecting net (15 inches in diameter), and a standard entomological collecting jar with ethyl acetate added to subdue insects. All specimens collected were pinned and stored as voucher specimens in our personal Lepidoptera collections. Butterflies were considered new county distribution records if they were not listed in Covell (1999) or on the Kentucky Butterfly Net database (Covell et al. 2009).

## SPECIES ACCOUNTS

Bath County

*Sheltowee Trace Trail at Stoney Cove Recreation Area.* 38°06'41.5" N, 83°31'49.7" W.

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Upland mixed mesophytic forest dominated by *Quercus alba* (L.), *Quercus rubra* (L.), and *Acer rubrum* (L.) with scattered *Carya alba* (L.) Nutt., and *Pinus strobus* (L.). Forest understory is dominated by sapling overstory species. Gravel forest service road with several disturbed areas regularly mowed and managed for wildlife.

*Atalopedes campestris* (Boisduval) – One individual was collected on 11 June 2007 while searching for hairstreaks. Specimen was collected puddling on the side of a gravel road along with several individuals of *Erynnis juvenalis* (Fabricius).

*Satyrium calanus* (Hubner) – According to Covell (1999) the subspecies occurring in Kentucky is *falacer* (Godart). One individual was collected on 14 June 2007 nectaring on *Asclepias tuberosa* (L.). In addition, three individuals were observed flying in the same area. Several *Speyeria cybele* (L.) and *Pterourus glaucus* (L.) were observed nectaring with the *Satyrium calanus* specimen.

*Daniel Boone National Forest, off Clear Creek Road, approximately 4.5 kilometers southeast of junction of State Highway 211.* 38°02'22.2" N, 83°35'00.72" W. Wetland complex dominated by *Carex* spp. Area is maintained yearly with mowing by Forest Service to manage for game species. Areas is surrounded by mature forest dominated by *Quercus alba*, *Quercus rubra*, *Quercus prinus* (L.), and *Acer rubrum*. Understory dominated by *Sassafras albidum* (Nutt.) Nees and sapling overstory species.

*Satyrodes appalachia* (R. L. Chermock) – One individual was collected on 6 August 2006 flying near a wetland with multiple *Carex* spp. present.

*Eurema nicippe* (Cram.) – One individual was collected on 9 August 2009 nectaring on a roadside near the edge of a dense hardwood forest.

*Cave Run Lake near Stoney Cove Recreation Area.* 38°06'10.1" N, 83°32'30.05" W. Open old-field areas regularly mowed and managed for game species. Surrounded by upland mixed mesophytic forest dominated by *Quercus alba*, *Quercus rubra*, and *Acer rubrum* with scattered *Carya alba* and larger stands of *Pinus strobus*.

*Phoebis sennae* (L.) – One individual was collected on 9 August 2009 flying through an

old-field habitat near Cave Run Lake. Several other individuals were spotted at this site. Although this collection represents the first record for this county, this species has become increasingly common in the area in recent years.

#### Rowan County

*Martins Branch Trail #119, approximately 2.4 km east of Morehead.* 38°12'15.2" N, 83°24'47.1" W. Upland mixed mesophytic forest dominated by *Quercus alba*, *Quercus rubra*, *Quercus prinus*, and *Carya alba*. Understory dominated by *Amelanchier arborea* (Michx.), *Cornus florida* (L.), and sapling overstory species.

*Parrhasius m-album* (Boisduval and LeConte) – Two males were collected on 24 April 2007 nectaring on *Amelanchier arborea* approximately 2 m from ground level. In addition, five individuals were observed nectaring on the same tree approximately 3.5 m from ground level. This sighting represents only the second recorded multiple occurrence of this species in Kentucky and the only multiple occurrence in eastern Kentucky (Covell 1999).

*Forest Service Road 918A, off Kentucky Route 1274, approximately 5.3 km from junction of Kentucky Route 519.* Mature forest dominated by *Quercus alba*, *Quercus rubra*, *Quercus prinus*, and *Acer rubrum*. Understory dominated by *Sassafras albidum* and sapling overstory species.

*Satyrium edwardsii* (Grote and Robinson) – One individual was collected on 27 May 2005 from a trailhead at the beginning of Forest Service Road 918A. The individual was perched on a dead limb of a *Quercus* snag on the forest edge.

*Daniel Boone National Forest, off Rockfork Road (Kentucky Route 799), approximately 2.2 km from junction of Kentucky Route 377.* 38°17'38.0" N, 82°26'31.1" W. Small stream with scattered open areas surrounded by mature mixed mesophytic forest dominated by *Acer rubrum*, *Quercus alba*, and *Quercus rubra* with *Amelanchier arborea* and *Cornus florida* common understory species.

*Eurema nicippe* – one individual was collected on 1 August 1995 while puddling near a seep from the stream.



*Open disturbed old-field habitat in the city of Morehead, Kentucky.* 38°11'10.4" N, 82°29'04.0" W. Area surrounded by streets and businesses. Disturbed old-field area dominated by forbs and herbaceous vegetation.

*Phoebis sennae* – One individual was collected on 1 August 1995 flying through field habitat.

#### Martin County

*Junction of Evans Hill Road and Kentucky Route 292, approximately 9.6 km southeast of Warfield.* 37°48'07.6" N, 82°22'39.8" W. Old-field habitat next to road leading to reclaimed strip mine. Roadside and old-field habitat dominated by large patches of *Asclepias syriaca* (L.), *Asclepias tuberosa*, and *Apocynum cannabinum* (L.). Surrounding forest is disturbed third growth mixed mesophytic forest dominated by young *Quercus alba*, *Quercus rubra*, and *Acer rubrum*.

*Danaus plexippus* (L.) – One individual was collected on 25 July 2007 while nectaring on *Asclepias syriaca*. Several *Pterourus glaucus* were observed nectaring on the same group of plants.

*Evans Hill Road, reclaimed strip mine approximately 1.7 km from Evans Hill Road and Kentucky Route 292 junction.* 37°47'48.5" N, 82°23'46.2" W. Upland disturbed mixed mesophytic forest dominated by young *Quercus alba*, *Quercus rubra*, and *Acer rubrum*. Several open old-field/reclaimed mine sites with scattered *Juniperus virginiana* (L.) and *Ellaeagnus umbellata* (Thunberg).

*Callophyrus gryneus* (Hubner) – One individual was collected on 7 July 2007 from a *Juniperus virginiana* approximately 2.5 m from ground level while resting on limbs of the tree. Two additional individuals were observed in the same tree. This occurrence represents the first record from the extreme eastern region of the state.

*Mount Sterling Hollow (County Road 1110), Gas Well and Mining Road, 1.4 km from County Road 1110 and Kentucky Route 292 junction, approximately 16.9 km southeast of Warfield.* 37°45'53.2" N, 82°20'33.8" W. Upland mixed mesophytic forest dominated by *Quercus alba*, *Quercus rubra*, *Quercus prinus*, *Acer rubrum*, and *Carya alba*. Understory dominated by *Cornus florida* and sapling overstory trees.

*Anthocharis midea* (Hubner) – One male was collected on 23 March 2007 patrolling the forested edge of a utility line corridor. This occurrence of *Anthocharis midea* represents the earliest date in the season that this species has been observed in Kentucky, as the earliest recorded sightings before this record occurred on 24 March 2000 and 24 March 2007 (Covell 1999; Covell et al. 2009).

*Callophrys henrici* (Grote and Robinson) – Two individuals were collected on 23 March 2007 by shaking the limbs of a *Cercis canadensis* (L.) on the forested edge of a gas well road. The individuals were spotted perched approximately 2.5 m from ground level.

*Calycopis cecrops* (Fabricius) – Five individuals were collected on 7 July 2007 from the forested edge of an old-field nectaring on *Asclepias tuberosa*. At least 10 other individuals were observed along forested edges and near streams where *Asclepias tuberosa* and *Rhus copallinum* Linnaeus were abundant.

*Glaucopsyche lygdamus* (Doubleday) – One male was collected on 23 March 2007 patrolling near the forested edge of a gas well road.

*Pieris virginianensis* Edwards – One individual was collected on 23 March 2007 patrolling inside the forested edge of a gas well road. Two additional individuals were observed near large patches of *Cardamine concatenata* (Michx.).

*Long Branch Hollow, just off unimproved dirt road, approximately 3 km from junction of Long Branch Road and Kentucky Route 292.* 37°46'05.4" N, 82°21'37.9" W. Upland mixed mesophytic forest dominated by *Quercus alba*, *Quercus rubra*, *Carya alba*, and *Acer rubrum* with scattered *Tsuga canadensis* (L.) Carriere near stream. Understory dominated by *Cornus florida* and sapling overstory species.

*Celastrina neglecta* (Edwards) – Seven individuals were collected on 7 July 2007 puddling on a small sandbar on Long Branch Creek. Many other (20+) individuals were observed puddling on the same sandbar and an adjacent one in Long Branch Creek. Other species observed puddling in the same area include *Pterourus glaucus*, *Battus philenor* (L.), *Limnitis arthemis* (Drury), and *Erynnis juvenalis*.

*Poanes zabulon* (Boisduval and LeConte) – One male was collected on 7 July 2007 patrolling the edge of an unimproved dirt

road near a small area of recently logged forest. Several *Erynnis juvenalis* were collected with this specimen, but this was the only *Poanes* collected.

#### DISCUSSION AND CONCLUSIONS

Although most of the species presented here are considered very common within Kentucky, basic distribution and life history information on them are sorely lacking in some areas of the state. For example, both *D. plexippus* and *C. neglecta* are two of the most commonly encountered butterfly species in Kentucky, yet they were both absent from the list of Lepidoptera occurring in Martin County.

Some other species, such as *P. m-album* are typically thought of as uncommon or widespread and rarely seen among Kentucky Lepidopterists (Covell 1999; Loran Gibson pers. comm.). The observations of *P. m-album* given here represent only the second recorded multiple occurrence of this species in Kentucky (all other occurrences are of single individuals at one time). This finding is significant in that it provides evidence that this species may be locally common during peak flight times in certain areas of the state.

This type of information is essential in providing the proper management and con-

servation methods for our diverse wildlife species, including the most common ones. Without information such as that presented in this paper, some of our very common species may be thought of as rare, while populations of the species that are rare may go undiscovered. In order to continue to further the study of Kentucky Lepidoptera, it is imperative that all areas of the state receive attention and that this basic distribution and life history data not be overlooked.

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## Comparison of Schindler-Patalas Traps and Wisconsin Nets for Monitoring Zooplankton in a Large, Shallow Reservoir

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### ABSTRACT

Zooplankton collection methods can differ substantially in the information produced; therefore, determining the best method or methods for a particular ecosystem is essential in understanding limnological processes. We compared results of two sampling methods, Wisconsin net vertical tows and Schindler-Patalas traps, that have been used in a long-term monitoring program in a large, shallow reservoir, Kentucky Lake, USA. Although there were differences in net mesh size and volume of water sampled, statistically similar cladoceran and copepod communities were captured by both methods. Population densities and the number of taxa collected did differ between methods, with many smaller rotifer taxa being found only in the vertical tows, but there were higher densities of larger taxa in the traps. Annual patterns were similar for most larger taxa, except that Wisconsin net tows revealed an autumn density peak for *Bosmina longirostris* not well-detected in the trap samples. Given the biases of each method, the Schindler-Patalas trap appears to be more efficient overall in long-term monitoring studies, particularly in shallow systems where multiple samples are taken frequently at a number of sites.

KEY WORDS: Zooplankton, shallow reservoir, Kentucky Lake, Schindler-Patalas trap, Wisconsin net, method comparisons

### INTRODUCTION

A wide variety of sampling gear is used to assess zooplankton populations (Wetzel and Likens 2000), and comparisons among the methods have been conducted in marine, lake, reservoir, and river ecosystems (e.g., Kankaala 1984; Cook and Hays 2001; Sluss et al. 2011). Typically, zooplankton are captured in traps that sample a known volume of water or in nets designed to be towed over some distance, e.g., from the bottom to the top of the water column. Traps might be preferred when one is interested in data for zooplankton population spatial and temporal distributions, either horizontal or vertical. Nets may be preferred when more qualitative data are desired or when zooplankton populations are sparse requiring larger volumes of water to be filtered (Kankaala 1984). More comprehensive studies may use several methods to capture representative samples of zooplankton communities (Wetzel and Likens 2000). Not surprisingly, different methods often give different results in species composition, densities, and the sizes of organisms captured. Differences may be further magnified by filter mesh sizes. Such differences may be significant in quantitative studies of life histories,

secondary production, food webs, or community structure (Kankaala 1984).

In 1988, a long-term limnological monitoring program began on Kentucky Lake, USA, the terminal and largest reservoir on the Tennessee River system (White et al. 2007). Kentucky Lake averages 6 m deep and is considered mesotrophic. The 16-17 monitoring sites cover approximately 30 km of the lower portion of the lake and are located in the main channel and several shallower embayments (White et al. 2007). Monitoring cruises occur every 16 days in spring through autumn and 32 days in winter. Objectives of the monitoring program have been to follow spatial and temporal patterns over time as they relate to seasonal, longitudinal, lateral and reservoir operational variations. Particular emphasis is placed on understanding processes in sidearm bays of a variety of sizes and watershed uses. For zooplankton the goal has been to understand population density, phenology, and species composition, primarily for the cladocerans and copepods, in relation to other parameters, e.g., temperature, dissolved oxygen, hydrology, invasive species, etc. (Yurista et al. 2001; White et al. 2007). Two types of zooplankton samples have been taken at each site, with slightly different objectives. The primary sampling uses triplicate 15-L

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Schindler-Patalas trap collections to examine aerial and seasonal distributions at a single depth. These samples are supplemented with a single finer meshed Wisconsin net bottom to top of the water column tow to examine size fractions or collect species that might have been missed.

To date, only the Schindler-Patalas samples have been analyzed consistently, while most of the net tows have been archived. Thus, the objectives of this study were 1) to compare overall results between traps and nets, 2) to determine if traps and nets gave consistent and similar results across monitoring sites, and 3) to determine if greater effort should be placed on analyzing net tow samples.

## METHODS

Kentucky Lake monitoring cruises are conducted during the first half of the day, between approximately 0700 and 1300 hr. At each site, three replicate 15-L Schindler-Patalas trap samples (243  $\mu\text{m}$  mesh sieve) are taken at 5 m deep or half the water column depth for shallower sites to approximate the 1% light depth (Yurista *et al.* 2004). One unmeasured Wisconsin net tow (13 cm diameter mouth, 153  $\mu\text{m}$  mesh net) is taken from just off the bottom to the top of the water column. The net is towed at approximately 1 m/sec. All zooplankton samples are rinsed into 70-ml tissue culture flasks (TFCs), kept on ice until returned to the lab, relaxed with tonic water, and preserved with 4% formalin solution. Because the numbers of organisms captured in net tows usually are considerably higher than in the traps, most have not been enumerated while all Schindler-Patalas trap samples have been processed. We analyzed a subset of both sample types that were taken in 2008 from 6 sites, 3 in the main channel and 3 in shallower embayments. This included 22 sampling dates and a total of 108 matched net and trap samples. The year was selected because there were fewer hydrologic anomalies, such as floods or drought conditions that could affect sampling efforts and species composition.

All organisms from both trap and net samples were counted and identified to the lowest convenient taxon by placing TFCs on a gridded plate and using a stereo dissecting microscope. Data were entered into the

Kentucky Lake Monitoring Program database (White *et al.* 2007). First, we made qualitative observations on differences between sampling results that included smaller taxa such as rotifers. To account for the differences in mesh sizes in quantitative comparisons, we limited the analysis to those taxa with larger individuals that would have been caught in the mesh of both traps; *i.e.*, cladocerans, copepods, and some larger rotifers. Densities in trap samples were calculated by compositing data from the three replicate 15-L samples and dividing by 45. Densities per liter in net tows were calculated by multiplying the area of the mouth of the net ( $\pi \times 0.13 \text{ m}^2$ ) times the water depth times 1000. All summary statistics and graphics were generated using the R project (R Core Development Team 2010). Site-by-date pairs for a taxon were excluded from analyses when a taxon was not captured by either method, however when one sample caught a given taxon and the other did not, we assigned a zero to the latter.

## RESULTS

The sum of all the Schindler-Patalas traps at the six sites for the year 2008 captured a total of 23,478 organisms in 4185 L of water, an average of 5.6  $\text{L}^{-1}$ . Net tows captured an estimated 236,604 total organisms (including small rotifers) in 65,302 L, an average of 3.6  $\text{L}^{-1}$ . Excluding the small rotifer taxa, net tows captured an average of 3.0  $\text{L}^{-1}$ . Taxonomic richness differed significantly between sampling methods ( $t_{107} = 17.049$ ,  $p\text{-value} < 0.001$ ) because of the larger variety of small rotifers in the net tows. For the larger taxa alone, the community represented by both methods was similar (Figure 1). Proportionately more *Bosmina longirostris* (Müller) and *Daphnia* spp. (primarily *D. retrocurva* (Forbes)) were collected in the trap samples, while higher numbers of the larger rotifers *Synchaeta*, *Keratella*, and *Asplanchna* were present in the tows. This also was evident for cyclopoid and calanoid copepod nauplii that were proportionately much less abundant in the trap samples.

*Bosmina longirostris* was the most common cladoceran collected by both methods, followed by calanoid and cyclopoid copepods, *D. retrocurva* (Forbes), *Leptodora kindtii* (Focke), *Ceriodaphnia* (two species), and *D.*



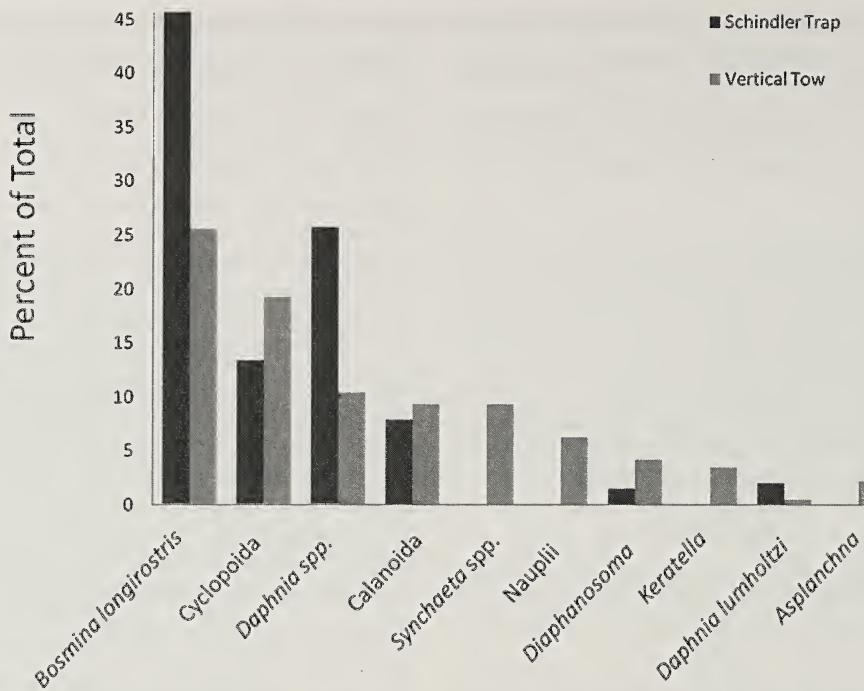


Figure 1. Histograms of dominant larger zooplankton taxa showing the proportions of the total number of larger organisms captured in each sampling method. Data represent >90% the total larger organisms for each method. *Daphnia* spp. is primarily *D. retrocurva*.

*lumholtzi* (Sars) (Figure 1). Other zooplankton taxa occurred in one or both sample types, but were relatively uncommon. These uncommon taxa included *Taphromysis louisianae* (Banner), *Chaoborus punctipennis* (Say), *Holopedium amazonicum* (Stingelin), and species of *Alona*, *Leydigia*, *Chydorus*, harpacticoid copepods, and ostracods. These taxa did not occur in sufficient densities to make statistical comparisons.

Part of our objective was to determine if any differences occurred between nets and traps among specific monitoring sites. Traps captured up to 7 of the larger taxa in a sample, and net tows collected up to 13. Analysis of variance (ANOVA) showed no significant differences in species richness among sites based on trap samples ( $F_5 = 1.185$ ,  $P = 0.32$ ). Net tow data, however, did show significant differences among sites ( $F_5 = 5.13$ ,  $P < 0.001$ ) with the northernmost channel site being more species rich, on average 9.68, than either of the other two channel sites that averaged 7.1 and 7.4 taxa. Aside from slightly greater depth we are unaware of any features

that distinguished this site from others that could cause higher species richness.

Paired *t*-tests revealed significantly different zooplankton density estimates between the sampling methods. *Daphnia* spp. (primarily *D. retrocurva*) occurred in high densities, and estimates were significantly different between methods (Schindler trap mean = 3.02/L; Vertical tow mean = 1.48,  $t_{58} = 4.78$ ,  $P < 0.001$ ), likewise *D. lumholtzi* density estimates were significantly different between the methods ( $t_{37} = 3.6$ ,  $P = 0.001$ ). The most common taxon, *B. longirostris* (Schindler trap mean = 4.59; Vertical tow = 3.17) exhibited higher densities in Schindler traps but differences were not significant between collection methods ( $t_{33} = 0.726$ ,  $P = 0.47$ ). Density estimates for several other taxa were significantly higher in vertical tows, including *Ceriodaphnia* spp. ( $t_{10} = 2.44$ ,  $p$ -value = 0.034), *Diaphanosoma* spp. ( $t_{37} = 4.94$ ,  $P < 0.001$ ), calanoid ( $t_{94} = 5.86$ ,  $P < 0.001$ ) and cyclopoid ( $t_{65} = 9.18$ ,  $P < 0.001$ ) copepods and *L. kindtii* ( $t_{23} = 3.24$ ,  $P = 0.003$ ). Our density estimates for these taxa were generally

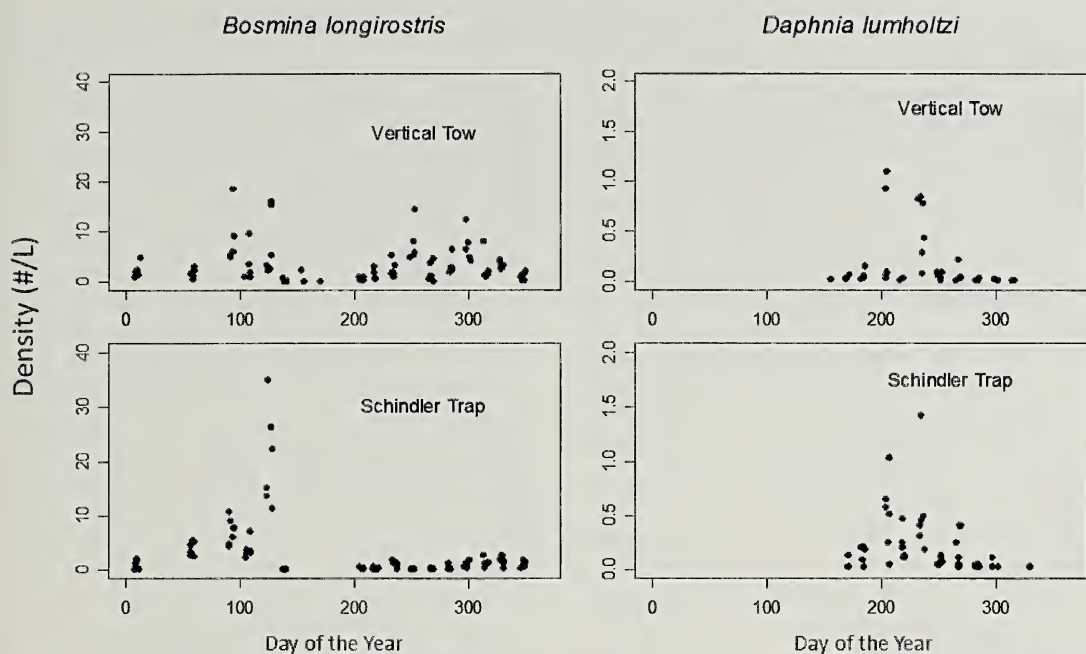


Figure 2. Comparison of *Bosmina longirostris* and *Daphnia lumholtzi* densities in paired vertical tow and trap samples in 2008. Each dot represents the mean number of individuals per sampling site. Dots are “jittered” horizontally to add random variation on the x-axis and to allow overplotted points to show up separately.

lower, ranging from cyclopoid copepods (Schindler trap = 1.03; Vertical tow = 2.19) to the predaceous *L. kindtii* (Schindler trap = 0.03; Vertical tow = 0.07). Other taxa occurred too infrequently to compare statistically.

Timing of spring and summer peak densities was qualitatively similar in both methods for most species (e.g., *D. lumholtzi*, Figure 2). *Bosmina longirostris* had similar intra-annual patterns in the two methods but differed in that there was a broad autumnal density peak from about day 220 to 340 not seen in the traps. Net tows also indicated a much longer spring peak for *B. longirostris* (lasting over 3, 16-day sampling events). Overall similarities suggested that, while absolute densities may be different between the two methods, densities in one sampling method should predict densities in the other method. We tested this using regressions of density estimates from the two sampling methods for each of several dominant species (Figure 3). *Bosmina longirostris* exhibited statistically related densities between the two methods ( $r^2 = 0.89$ ,  $P < 0.001$ ) when the autumn samples were excluded. Similarities between

estimates produced from these methods were observed in other taxa, including calanoid copepods ( $r^2 = 0.44$ ,  $P < 0.001$ ), *D. retrocurva* ( $r^2 = 0.11$ ,  $P = 0.01$ ), *D. lumholtzi* ( $r^2 = 0.30$ ,  $P < 0.001$ ). No similar relationships could be detected in the remaining taxa, most likely because of low densities in both collection methods.

## DISCUSSION

With the exception of *T. louisianae* and the invasive, *D. lumholtzi*, the larger species of cladocerans, copepods, and rotifers of Kentucky Lake are dominated by a small set of easily distinguishable taxa common to the Midwest and Great Lakes regions that have become established in most Midwestern reservoirs (Balcer et al. 1984; Yurista and White 2001; Havel and Shurin 2004). The net tows did not produce any additional or novel taxa for Kentucky Lake that had not been previously identified in the trap samples.

One determinant of the success of nets is the speed at which they are towed and their potential for clogging (Kane and Anderson 2007). However, studies have found that even substantial differences in tow speeds



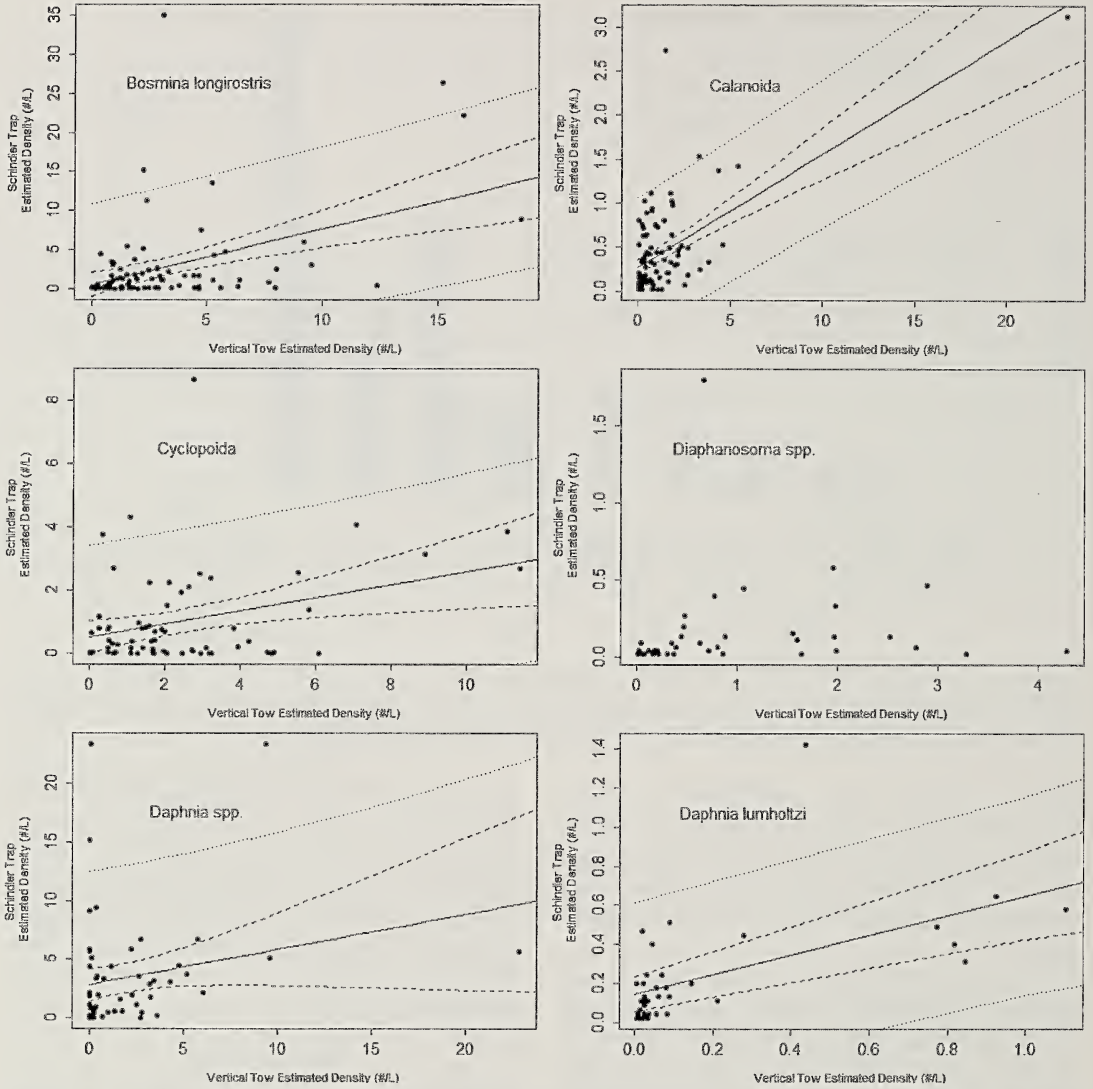


Figure 3. Density comparisons of the six most common taxa of zooplankton captured by the two methods. Regression estimates (solid line), prediction intervals (dashed lines) and confidence intervals (dotted lines). There were no significant similarities for *Diaphanosoma*.

for bongo nets towed horizontally do not yield significant differences in the zooplankton community nor in population data (Kankaala 1984). Likewise, DeVries and Stein (1991) had similar results in a comparison of Schindler-Patalas traps, nets, and tube samplers. The overall lower average densities of organisms per L in the net tows for Kentucky Lake may in part be due to net clogging by blooms of filamentous diatoms, green algae, and blue-green cyanobacteria that are located primarily in the upper 1-2 m of the water

column during spring and summer months; thus the actual volume of water filtered might be much lower than determined by net tow distance alone. Because the mesh size was smaller in the vertical tow nets, we assumed that they would capture many smaller and possibly different organisms than traps. The larger number of rotifers in the net tows most likely reflected the differences in net mesh size and, consistent with previous studies, that the greatest concentrations of small rotifers occur directly on or above the bottom

in Kentucky Lake (Albritton and White 2006).

The most significant difference between the two sampling methods was the discovery of a broad autumnal density peak for *B. longirostris* that had not been observed in the trap collections. *B. longirostris* is the only cladoceran collected year-round from Kentucky Lake (Schram and Marzolf 1993; Yurista et al. 2000). The distinct spring peak usually occurs at approximately the 120<sup>th</sup> day of the year in trap samples (Figure 2, also see Yurista et al. 2000). *Bosmina* exhibits similar autumn peaks in other systems (e.g., Mason and Abdul-Hussein 1991), but an autumn peak had not been documented for Kentucky Lake. Zooplankton may migrate considerably throughout the water column on a daily basis (Kerfoot 1980; DeVries and Stein 1991; Hays 2003), so it is somewhat surprising that so much difference between sampling methods would be observed, unless the *Bosmina* population remains closer to the bottom particularly during colder months while other taxa continue to remain further up in the water column.

Schindler-Patalas trap and Wisconsin net samples produced slightly different views of the Kentucky Lake zooplankton community. Vertical net tows captured more species, particularly those living near the bottom of the water column, and revealed the presence of an additional density peak for *B. longirostris*, but most likely underestimated water column densities because of potential clogging. Schindler-Patalas traps most likely overestimated water column populations because samples were taken at a depth where greater densities were expected. Because traps capture at a discrete mid-water depth, they avoid clogging algae and allowed us to focus on the primary goal of monitoring the cladoceran/copepod community. Further, as the number of organisms captured in the nets was much greater, there was a substantial effort in analyzing these collections, particularly for long-term monitoring programs over large areas such as ours.

Our data suggest that Schindler-Patalas trap samples, despite sampling a relatively smaller portion of the water column, present a reasonably accurate representation of most features of the zooplankton community (Mason and Abdul-Hussein 1991), particularly in Kentucky

Lake. In summary, the advantages of using the Schindler-Patalas trap in a large, shallow mesotrophic reservoir are 1) similar water volumes at similar depths are sampled at each monitoring site independent of the total water depth, 2) the larger mesh size of the filter is effective in capturing cladocerans and other larger taxa, a primary focus of the long-term monitoring program, and 3) samples are fairly easy and quick to process. A disadvantage of traps is that may miss components of the populations that are smaller or that have different depth requirements during the year. Nets do have the advantage of capturing near-bottom taxa. Primary disadvantages of nets are the potential for clogging and that a different volume of water is sampled at each site. Even at a single site, the water depth may differ from one sampling date to the next altering the volume of water filtered. Kentucky Lake is regulated to have a summer pool 2 m deeper than the winter pool. Flood and drought conditions over the past few years have produced a 5 m range in surface elevation.

Collecting Wisconsin net vertical tows at each site does not require much time, but processing them is labor-intensive compared with trap samples. Net samples will continue to be collected and archived but not processed unless there is a specific need. Net samples along with trap samples and the zooplankton database are available to researchers. Further information is available through the Hancock Biological Station website: [www.murraystate.edu/hbs](http://www.murraystate.edu/hbs).

#### ACKNOWLEDGMENTS

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## NOTE

**An Integer Programming Model of Fire Station Allocation in Murray, Kentucky**—A key factor in providing fire protection coverage is the location of the fire stations. The objective of this paper is to address the allocation of fire stations in Murray, Kentucky. We offer an implementation scheme that allows the City of Murray to gradually build additional fire stations in a manner that optimizes coverage of the city.

While the use of geographical factors is the common standard, a more meaningful (and more complex) measure of performance is the response time to an incident. We are concerned particularly with travel time because it is the greatest component of response time to a particular incident. An accurate understanding of the relationship between travel distance and travel time aids in modeling response time. In-depth analysis with respect to fire trucks has been conducted (Kolesar and Blum 1973; Kolesar 1975; Kolesar et al. 1975). Estimates for response time can be calculated from empirical data (Hogg 1968). The entire time distribution of an emergency service system can be found (Chaiken and Larson 1972).

A classical approach is to divide a city into sub-regions and to treat each sub-region as a single point (Toregas et al. 1971). Once the city and its fire stations have been transformed into a discrete set of points, the approach has been to develop a binary integer linear program from which an optimal number of fire stations can be determined.

We view the fire station allocation problem as an issue of *coverage*. We begin by selecting an upper bound for response time  $s$ , and proceed with the objective of determining the location for the minimal number of fire stations that allowed the fire department to respond within time  $s$  (Toregas et al. 1971).

For a given geographical region  $R$ , we divide  $R$  into  $n$  districts. We represent the  $i^{\text{th}}$  district by a GPS coordinate  $d_i$  within the district called the *district center*. We define the set of all district centers as

$$D = \{d_i\} \text{ for } i = 1, \dots, n.$$

The set  $D$  represents the region  $R$  by a finite collection of points within  $R$ . Next we select  $m$  locations within  $R$  as potential fire station sites. The potential fire station sites constitute the set

$$F = \{f_j\} \text{ for } j = 1, \dots, m.$$

Each element of  $F$  corresponds to the GPS coordinate of a location that has been selected as a possible fire station site. We define the decision variables  $x_j$  for  $j = 1, \dots, m$  as

$$x_j = \begin{cases} 1, & \text{if a fire station is to be located at site } f_j \\ 0, & \text{otherwise.} \end{cases}$$

Note that  $x_j$  is a zero-one or *binary integer variable*. We define  $t_{ij}$  as the shortest travel time between district center  $d_i$  and fire station  $f_j$ . Finally, we define the set  $C_i$  as

the set of fire stations  $f_j$  that cover, or are within  $s$  minutes of, district  $d_i$ ,

$$C_i = \{f_j \in F \mid t_{ij} \leq s\} \text{ for } i = 1, \dots, n,$$

where  $n$  is the number of districts in the region  $R$ . Using this decision variable, the coverage requirement of a district  $i$  can be written as

$$\sum_{f_j \in C_i} x_j \geq 1.$$

Notice that if  $C_i = \emptyset$ , then there is no fire station  $f_j$  that is within  $s$  minutes of district  $d_i$  and we say that district  $d_i$  is not covered.

We wish to minimize the number of fire stations in the city subject to the constraint that each district, represented by district center  $d_i$ , is covered by at least one fire station  $f_j$  within the maximum travel time of  $s$  minutes. We can formulate this optimization problem as the binary integer program

$$\begin{aligned} &\text{Minimize } \sum_{f_j \in F} x_j \\ &\text{Subject to : } \begin{cases} \sum_{f_j \in C_i} x_j \geq 1 \text{ for all } i = 1, \dots, m \\ x_j = 0 \text{ or } 1 \text{ for all } j = 1, \dots, m. \end{cases} \end{aligned}$$

The implementation of the program requires that for a given value of  $s$ , the sets  $C_i$  be determined for all  $i = 1, 2, \dots, n$ . This requires that a matrix of the shortest travel times from district center  $d_i$  to any fire department  $f_j$  be computed. The existence of a solution of this binary integer program is guaranteed provided that each of the sets  $C_i$  is non-empty. However, the solution is not necessarily unique.

The National Fire Protection Association ([www.nfpa.org](http://www.nfpa.org)) provides the industry standard for regulations pertaining to fire protection services. Code 5.2.4.1.1 of NFPA 1710, (Safer Grant Act 2010), recommends that the initial responding fire company arrives within a 240-second travel time to at least 90% of the incident calls. This allows one minute for dispatch to sound the alarm and one minute for the firefighters to leave the station, (Nadile 2008). Thus the four minute travel time is to optimize a total response time of six minutes. For the purposes of this study, we select the NFPA standard of a 4 minute travel time as the maximum travel time for the Murray Fire Department.

The City of Murray currently has two fire stations. One of the authors had several meetings with the Murray Fire Marshall (D. Walls, Murray Fire Department, pers. comm., December 2011). The Fire Marshall had already researched six potential sites for new fire stations. An additional site was suggested. Based on these discussions



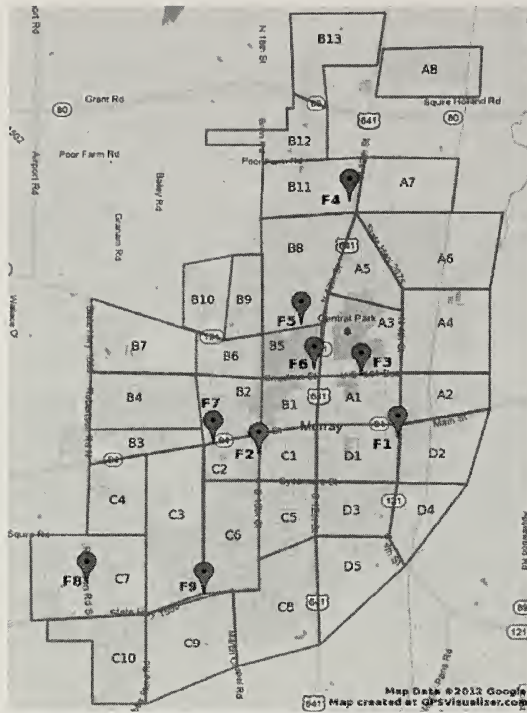


Figure 1. The proposed fire stations and districts.

we consider nine fire station sites, including the two existing fire stations: see Figure 1.

We begin by dividing the city into sub-regions or districts. A natural criterion for creating the districts is the existing road system. We are able to cover all of Murray with 36 districts. We define each district by the set of its vertices. For the districts that follow the road system we can determine the geographic coordinates of its vertices by geocoding the respective intersections. The website GPS Visualizer (Schneider 2003–2012) offers tools for geocoding. Vertices outside the road system must be set manually. Google Maps (Google 2012a) allows us to create latitude-longitude markers. The next objective is to reduce each district to a single latitude-longitude coordinate which we call the district center. We define the *geographic center* as the mean of the latitude-longitude coordinates of the district vertices. We define the *demand center* as the mean of the latitude-longitude coordinates of all the incidents that have occurred within a district in our data set. The data set was provided by the Murray Fire Marshall (D. Walls, Murray Fire Department, pers. comm., December 2011) and contained 2252 incidents that occurred within the City of Murray between 1 January 2004 and 23 August 2011.

We can use the travel time estimate provided by Google Maps (Google 2012a) as the travel time between each fire station to each district center. Google's Distance Service Matrix function allows the user to calculate the travel distance and travel time between multiple origin locations

to multiple destinations while suppressing the driving directions (Google 2012b).

We note that the distance between each origin and destination is measured in miles. The travel time is measured in minutes. The time matrix for each of the center types has 36 rows and 9 columns since we have selected 36 districts and 9 proposed fire stations. Each row of the matrix corresponds to a specific district and each column corresponds to a specific fire station site. The entries in the matrix represent travel time measured in minutes.

We can now establish the specific set-covering model for Murray. We have  $D = \{d_i\}$  for  $i = 1, \dots, 36$  since there are 36 district centers. There are 9 fire station sites so  $F = \{f_j\}$  for  $j = 1, \dots, 9$ . We select the maximum travel time to be 4 minutes. Thus  $s = 4$  and we have that  $C_i = \{f_j \mid t_{ij} \leq 4\}$  for  $i = 1, \dots, 36$ . With these specific conditions we have the following model for Murray.

$$\begin{aligned} & \text{Minimize } \sum_{j=1}^9 x_j \\ & \text{Subject to: } \begin{cases} \sum_{f_j \in C_i} x_j \geq 1 \text{ for all } i=1, \dots, 36 \\ x_j = 0 \text{ or } 1 \text{ for all } j=1, \dots, 9. \end{cases} \end{aligned}$$

Before we can solve this binary integer program, we must ensure that all of the districts are covered by at least one fire station so that all of the sets  $C_i$  are non-empty. This guarantees a feasible solution. For the geographic centers, districts A8, B12, and B13 are not covered. We assume that these districts are covered by station F4 since it is closest to these districts. For the demand centers, districts B10, B12, and B13 are not covered. In this case, we assume that station F5 covered district B10 and that station F4 covers districts B12 and B13 (R. Walls 2012).

Our computations were carried out using the MATLAB's Optimization Toolbox. Scenario 1 seeks to solve the problem by treating all stations as equal. Table 1 contains the solutions for Scenario 1 for both types of district centers. A value of 1 indicates that station is used, while a value of 0 indicates otherwise. The most striking result of Scenario 1 is the exclusion of an existing station, F2, for both types of district centers. This means that station F2 should be closed and four or five additional stations must be constructed to cover all of Murray with respect to geographic and demand centers. In addition, we see that five of the proposed station sites are included in the results of both district center types. We can be confident of the choice of these stations, while station F3 is a weaker choice because it is only in the solution for the geographic centers.

Scenario 2 seeks to determine where new stations should be built with the constraint that the existing stations F1 and F2 will continue to be used. Table 2 contains the results for Scenario 2 for maximum travel time of 4 minutes. The results imply that the City of

Table 1. The MATLAB results of Scenario 1 for  $s = 4$ .

| Stations                | Geographic | Demand   |
|-------------------------|------------|----------|
| F1                      | 1          | 1        |
| F2                      | 0          | 0        |
| F3                      | 1          | 0        |
| F4                      | 1          | 1        |
| F5                      | 1          | 1        |
| F6                      | 0          | 0        |
| F7                      | 1          | 1        |
| F8                      | 0          | 0        |
| F9                      | 1          | 1        |
| <b>Num. of Stations</b> | <b>6</b>   | <b>5</b> |

Murray needs four or five additional stations to cover all of Murray at the four minute maximum travel time. Five of the proposed station sites are included in the results of both district center types. Two of these are the current fire stations. We can be confident of the choice of these stations. Stations F3, F8 and F9 are weaker choices because they are each only in the solution for one of the center types.

Based on the results shown in Table 1 and Table 2, the City of Murray needs at least 5 fire stations to cover all of the districts within four minutes. It is unlikely the City of Murray will build all of these stations at one time. Next, we determine a prioritization of the fire station sites that reduces the maximum travel time required to cover all of the districts after the addition of each new station.

While the set covering model sought to cover all of the districts of Murray, we now seek to ensure that at least 90% of the districts are covered within 4 minutes so that the City is within reach of compliance with the NFPA standard (Safer Grant Act 2010). The prioritization of the fire station sites is determined by the algorithm in Figure 3.

The algorithm shows that starting with the existing fire stations, the number of uniquely covered districts within a maximum time of 4 minutes is determined. The coverage of the existing fire stations together with one of each of the proposed fire stations is determined. The proposed fire station which, when added to the existing fire stations, produces the greatest coverage is selected for construction. The process is repeated with the new set of existing fire stations.

Table 2. The MATLAB results of Scenario 2.

| Stations                | Geographic | Demand   |
|-------------------------|------------|----------|
| F1                      | 1          | 1        |
| F2                      | 1          | 1        |
| F3                      | 1          | 0        |
| F4                      | 1          | 1        |
| F5                      | 1          | 1        |
| F6                      | 0          | 0        |
| F7                      | 1          | 1        |
| F8                      | 1          | 0        |
| F9                      | 0          | 1        |
| <b>Num. of Stations</b> | <b>7</b>   | <b>6</b> |

Table 3. The station implementation scheme.

| Number of stations | Stations within 4 minutes | Number of districts covered | Percentage of districts covered |
|--------------------|---------------------------|-----------------------------|---------------------------------|
| 2                  | F1, F2                    | 17                          | 47.22%                          |
| 3                  | F1, F2, F4                | 26                          | 72.22%                          |
| 4                  | F1, F2, F4, F8            | 31                          | 86.11%                          |
| 5                  | F1, F2, F4, F5, F8        | 34                          | 94.44%                          |

Table 3 gives the results of this implementation. With the addition of three fire stations, we can increase the coverage to 94.44%. The station implementation scheme is approaching the set-covering model solution for Scenario 2. The addition of Stations F3 and F7 increases the coverage to 100%. Figure 2 shows the coverage provided by stations F1 (yellow), F2 (blue), F4 (red), F5 (purple) and F8 (orange). Note that some districts are covered by more than one fire station which leads to combined coloring.

It should be noted that this discrete covering-based approach only produces an approximation of reality. The models do not include the financial requirements of both the construction of a new station nor the labor costs to staff the new facility. The political variables, while difficult to quantify, include such concepts as a city's willingness to build a new station and the city's determination to complete such a project once it has decided to do so.

Analysis of this nature can only hope to be used as a tool for decision makers. While it is an approximation of reality, these models can help to illuminate specific



Figure 2. Fire Coverage of the City of Murray. Four minute maximum travel time. 94.44% coverage.



$n \leftarrow$  number of districts

$S \leftarrow \{f_j \mid f_j \text{ is an existing or pre-chosen station location}\}$

$D(S) \leftarrow \{d_i \mid d_i \text{ is covered by a fire station in } S\}$

**Repeat**

**For**  $d_i \notin D(S)$  and  $f_j \notin S$  **do**

$$L_{ij} \leftarrow \begin{cases} 1, & \text{if } t_{ij} \leq 4 \\ 0, & \text{otherwise} \end{cases}$$

$$h_j \leftarrow \sum_i L_{ij} \quad (\text{number of new districts covered by } f_j)$$

**Endfor**

$$J \leftarrow \{f_i \mid h_i = \max_{f_j \in S} (h_j)\}$$

$$S \leftarrow S \cup J$$

$$D(S) \leftarrow \{d_i \mid d_i \text{ is covered by a fire station in } S\}$$

$$N(S) \leftarrow |D(S)| \quad (\text{number of districts covered by the set } S)$$

**Until**  $N(S) > 0.9n$  (until over 90% of all districts are covered)

Figure 3. Algorithm to prioritize fire station sites.

aspects within the problem of allocating fire stations and assist the decision maker in making more informed public policy.

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## NOTE

**Seasonal Occurrence and Habitat Affiliations of Lampyridae at Mammoth Cave National Park, Kentucky**—Lampyridae are ephemeral forest insects. With few exceptions, temperate species only appear at specific times throughout the year and within the course of the day/night (Faust and Weston 2009). Further, most adults only live ca. 3–4 weeks, and may show a strong affiliation with a specific habitat. Thus, while this bioluminescent assemblage is in general quite conspicuous in eastern North America throughout the growing season, the turnover of particular species is dramatic. Bearing this in mind, we sought to identify the seasonal patterns of lampyrids at Mammoth Cave National Park (MCNP). A series of blacklight surveys were conducted opportunistically across the entirety of MCNP from April–September of 2011. This national park lies in Barren, Hart, and Edmonson counties on the edge of the Crawford-Mammoth Cave Uplands of the Interior Plateau of Kentucky (Woods et al. 2002). MCNP itself is a 23,000 ha parcel; the entirety of our survey efforts took place across an array of upland habitats away from the Green River. Lampyrid specimens were collected across multiple sites in a given night ( $\geq 50$  m apart) using 10 W blacklight traps (Universal Light Trap, Bioquip Products, Gardena, CA). As per recommendations by Yela and Holyoak (1997) for sampling Lepidoptera, survey nights were fair with temperatures  $\geq 16^\circ\text{C}$  at sunset, no precipitation, and low wind. Traps were suspended at 2.5 m and a ca.  $2 \times 6$ -cm dichlorvos-based ‘pest strip’ was placed within traps to subdue specimens. Following a trap night, lampyrid specimens (and associated mimics) were sorted and placed in cold storage ( $4^\circ\text{C}$ ) for identification in the laboratory. Specimens were identified using morphological characteristics and by making comparisons to existing voucher specimens from the region. Identifications were based on accepted aedeagal groups and/or

species complexes level using Brown et al. (2009), Fender (1970), Forrest and Eubanks (1995), Green (1956), Lloyd (1966a, 1966b, 1969), and other relevant literature. We offer the most likely species of these complexes according to previously identified specimens from the Cumberland Plateau region of northern Tennessee and Kentucky. The collection of specimens was deposited with MCNP.

We captured 134 lampyrids on 15 of the 21 nights we surveyed throughout 2011 ( $n = 46$  of 150 trap-nights). A total of 10 species groups were identified using morphological identification (Table 1). We found three species groups to be most common (Figure 1). *Ellychnia corrusca* complex (L.), a relatively large lanternless species, dominated capture in April and May ( $n = 33$ ). This taxon gave way to the genus *Photinus* later in the year. *Photinus punctulatus* Leconte complex [or *P. brimleyi* Green] dominated June and July ( $n = 34$ ) whereas *P. consanguineus* Leconte complex [or *P. macdermotti* Lloyd] dominated August ( $n = 46$ ). Additional species were captured less frequently. *Lucidota atra* (G. Oliver) was captured in August ( $n = 1$ ). *Photinus cookii* Green was captured in July ( $n = 1$ ). *Photinus marginellus* LeConte was captured in August ( $n = 1$ ). *Photinus pyralis* (L.) was captured in June–August ( $n = 5$ ). The genus *Photuris*, whose females are often predatory mimics, were captured at low levels across all months in which we captured other lampyrids. A longer-bodied *Photuris versicolor* complex (F.) was captured in May–August ( $n = 3$  females, 1 male) and a shorter-bodied *P. versicolor* complex (F.) with a dark dorsum and interrupted pronotal vitta was captured in June–July ( $n = 4$  females). *Pyraetomena lucifera* (Melsh.) was captured in June ( $n = 1$ ). Aside from captures, we noted the flight of *Pyraetomena borealis* (Randall) in the forest canopy on May 7, as well as the flight of *Photuris versicolor quadrifulgens* (Barber) in an open field. These species are two of the earliest-flashing

Table 1. A checklist for lampyrids recorded at Mammoth Cave National Park.

| Taxon  | Specimen size range (mm) <sup>1</sup> | Capture period    | Recorded habitat <sup>2</sup> |
|--|---------------------------------------|-------------------|-------------------------------|
| <i>Ellychnia corrusca</i> (L.) complex   | 11–15                                 | 18 April–10 May   | D, M                          |
| <i>Lucidota atra</i> (G. Oliver)   | 11                                    | 20 August         | D                             |
| <i>Photinus consanguineus</i> Leconte complex [or <i>P. macdermotti</i> Lloyd] | 7–10                                  | 24 May–30 August  | D, M                          |
| <i>Photinus cookii</i> Green   | 7                                     | 11 July           | D                             |
| <i>Photinus marginellus</i> Leconte  | 6.5                                   | 20 August         | D                             |
| <i>Photinus punctulatus</i> Leconte complex [or <i>P. brimleyi</i> Green]      | 7.5–11                                | 20 June–20 August | C, D, M                       |
| <i>Photinus pyralis</i> (L.)   | 11–13                                 | 20 June–30 August | C, M                          |
| <i>Photuris versicolor</i> complex (F.); longer-bodied                         | 13–18                                 | 24 May–20 August  | D, M                          |
| <i>Photuris versicolor</i> complex (F.); shorter-bodied                        | 11–12                                 | 29 June–25 July   | C, D                          |
| <i>Photuris versicolor quadrifulgens</i> (Barber)                              |                                       | 7 May             | P                             |
| <i>Pyraetomena borealis</i> (Randall)  |                                       | 7 May             | C                             |
| <i>Pyraetomena lucifera</i> (Melsh.)   | 9                                     | 20 June           | C                             |

<sup>1</sup> Taxa with missing values were not captured in traps but rather identified from flash data.

<sup>2</sup> Nomenclature follows the Kentucky State Nature Preserves Commission system: mixed coniferous-dominant/deciduous forest (C), mixed deciduous-dominant/coniferous forest (D), mesic upland deciduous (M), and prairie/open areas (P).



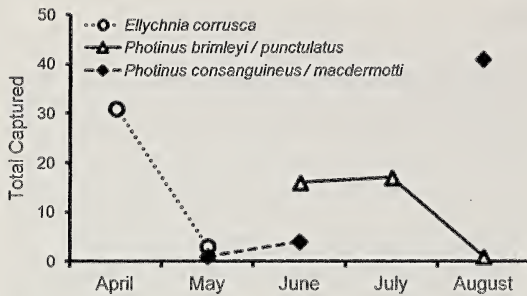


Figure 1. Monthly occurrence of common lampryids at Mammoth Cave National Park.

lampryids of the season in southeastern North America, with *P. borealis* occurring as early as March and *P. quadrimaculatus* by mid-late April (Faust 2012).

Lampryid mimics were also captured during our surveys. A large number of *Atalantycha neglecta* (Fall), a cantharid, were captured from April–May ( $n = 272$ ). Another cantharid, *Atalantycha bilineata* Say was captured on 18 April. The elaterid *Denticollos denticornis* (Kirby) was captured on 10 May. These species were collected in fewer numbers ( $n = 2$  and 1, respectively).

These data provide a benchmark for understanding patterns of emergence and species turnover for the Lampryidae in central Kentucky as well as surrounding states. Blacklight surveys conducted from 1946–2014 by Carl Cook in Center, Kentucky, document ten of the same species as in our study (pers. comm.). Published records (Green 1956, 1957; Lloyd 1966) of Cook's surveys occurring ca. 38 km east of MCNP lack the *Pyrractomena lucifera* and *Pyrractomena borealis* documented in our study. Cook's collections include three species not yet recorded at MCNP, namely *Photinus consimilis* Green 1956, *Photinus sabulosus* Green 1956, and *Pyrractomena angulata* (Say 1825).

Our identification efforts suggest some intergrade among species at MCNP, as not all characters agreed with those in the literature. *Photinus* were particularly problematic; *P. consanguineus* possessed pale coxae and metafemora and the *P. punctulatus* complex lacked the wide, blurred lateral elytral margins typical of *P. brimleyi*. Future studies including DNA-based techniques, as well as flash data and observations of wild flight would likely elucidate species more reliably.

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monies from the Joint Fire Sciences Program. The information reported in this paper (No. 12-09-108) is part of a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

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**Kentucky Academy of Science  
Annual Business Meeting Minutes  
Eastern Kentucky University  
October 20, 2012**

**Outgoing President's Report**

Dawn Anderson called the meeting to order at 6:15 P.M. and welcomed the KAS members present. Dawn stated the KAS Governing Board approved the initiation of a plan to establish a Kentucky Natural History Survey. The Board voted to form an ad hoc committee chaired by Judy Voelker and Ron Jones which will work on a plan and report back to the KAS Governing Board.

Dawn stated Ken Crawford, KAS Treasurer was unable to attend the meeting and to contact Jeanne Harris for questions regarding the Treasurer report.

Dawn asked for reports from KAS Officers and Committees.

**Elections**

Sean Reilley, Chair of the Committee on Elections, reported the very close election results as tabulated using Survey Monkey:

2013 Vice President-David White, Physical Sciences Division Representative-Doug Chatham, Social Sciences Divisional Representative-David Butz.

**Recognition of Board members completing terms in office**

Sean Reilley and Ted Porter were both thanked for their service to the Board by President Anderson and acknowledged with a plaque to commemorate their service. Outgoing President Anderson presented the gavel and the customary donation to the President's fund to Incoming President Cheryl Davis.

**New President's Remarks**

Cheryl Davis thanked the outgoing President and expressed gratitude to Dawn Anderson for her dedication to KAS.

**Meeting Adjourned at 6:30**



**Kentucky Academy of Science  
Annual Business Meeting Minutes  
Morehead State University  
November 9, 2013**

The meeting was called to order by President Cheryl Davis.

### **Approval of Minutes**

Minutes of the KAS Business Meeting from 20 October 2012 were approved.

### **President's Report**

Cheryl Davis welcomed our new Executive Director Amanda Fuller, our new Program Coordinator Melanie Stambaugh, and new journal editor Jerzy Jaromczyk.

### **JKAS Report**

Although Jerzy Jaromczyk was not present, he asked Cheryl to deliver his report. Technical issues are largely fixed, and he is catching up on review of paper submissions.

### **Officers' Reports**

#### **Outgoing President's Report (Cheryl Davis)**

The Next Generation Science Standards (NGSS) have been adopted in Kentucky. Cheryl explained that Nancy Martin had written a statement of endorsement of NGSS, which was adopted by the Board in July, taken to legislature by Blaine Ferrell. Although not accepted by the Legislative Committee, NGSS were approved by Governor Steve Beshear. KAS is sending a thank-you letter to the Governor for his vision on this issue.

#### **Incoming President's Report (K.C. Russell)**

K.C. thanked Cheryl Davis for her capable leadership during the past year. He announced that the Centennial Meeting of KAS will be held 14-16 November a 3-day event, at the Convention Center in Lexington.

#### **Treasurer's Report (Ken Crawford)**

Ken Crawford reported a positive financial balance, and said this annual meeting is on

track to come out ahead. The Athey investments generated an all-time low income, but our Wells Fargo securities are generating enough income to allow 3 percent withdrawal per year, which meets our financial needs. The 2014 annual meeting will cost more than usual, since it will be held at the Hyatt Regency in Lexington. Next year's registration fee will be increased to cover costs. Cheryl then reminded the Board that Ken is retiring from his many years of service as our treasurer. The members conveyed their appreciation.

### **Nominations Committee Report**

Cheryl passed along the information that next year, David White will be our Vice President, and K.C. Russell will be our President.

She also reminded the attending members that a survey monkey election is coming up.

### **Program Coordinator's Report**

Melanie Stambaugh told the membership that we have 479 abstracts submitted for the annual meeting this year, and set a goal of 600 for the Centennial meeting next year. She repeated the call for newsletter articles as well.

### **Executive Director's Report**

Amanda Fuller reported that registration for the meeting was about 825, with 355 banquet tickets sold. She said she hopes for more sponsors next year. Current membership is 2633. Amanda told the members that we have been offered office space for KAS at the Kentucky Science Center in exchange for KAS members serving as an *ad hoc* science advisory council for the museum. She said participants will be solicited in the coming year.

### **Local Arrangements**

Eric Jerde said everything has gone well. He accepted the members' applause, and thanked Assistant Dean Gabriele Sexton for her support of the meeting.

**Natural History Museum**

Ron Jones reported that he and Judy Voelker are assembling an inventory of natural history collections in Kentucky.

**President's Last Action**

Outgoing President Cheryl Davis recognizes outgoing board members Ron Jones (Biological Sciences) and Mary Jansen (Rep-

resentative at large). She then passed the gavel to K.C. Russell, and presented the traditional check to the President's fund. Incoming President K.C. repeated thanks to Cheryl, with congratulations for a successful year for KAS.

Respectfully submitted,  
Robert W. Kingsolver  
KAS Board Secretary



## Abstracts of Some Papers Presented at the 2012 Annual Meeting of the Kentucky Academy of Science

Edited by Robert J. Barney

### AGRICULTURAL SCIENCES

Yield of Baby Corn as Influenced by Individual Plants, Cultivars, Planting Dates, and Harvesting Sequences at Bowling Green, Kentucky in 2012. YAO XUE\*, CHRISTOPHER G. FERGUSON, and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Baby Corn, *Zea mays* L., consists of young ears harvested at silk emergence and prior to fertilization. Baby corn (BC) involved different cultivars, planting dates, and harvesting regimes. Multiple harvests may include subsequent ears from the same plants or come from different plants that are later in maturity. In general, quality of BC decreases with advancing plant maturity and/or season. A preliminary study was conducted to elucidate further the practices of BC production. Treatments included: two planting dates (17<sup>th</sup> May and 4<sup>th</sup> June), four cultivars (sweet corn 'Peaches n, Cream' and 'Incredible'; and field corn 'N68B-3000 GT' and 'N77P-3000 GT'), and five or six harvests on a twice-weekly frequency. Each plant was identified permitting the number of ears per plant to be counted. Average ear weight and length were determined. The present report is limited to ears per plant that ranged from zero to nine with an average of three. The number was higher for the earlier planting date, but not consistently different for successive harvests within dates or for cultivars. The highest number of ears was produced by the sweet corn Incredible in the earlier planting date; the lowest number was produced by the field corn N68B-3000 GT in the later planting date. Weather conditions were adverse for both temperatures and precipitation, thus the study must be repeated.

Evaluation of Cultivars of Sunflower and Selected Environments for Production of Cut Flowers. YAO XUE\*, CHRISTOPHER G. FERGUSON, PAVANI G. VUPPALAPATI, MARTIN J. STONE, and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Sunflower, *Helianthus annuus* L., is native to the United States and was domesticated by American Indians many centuries ago. Sunflowers exhibit wide variation and are grown worldwide for multipurposes. More recently, a cut flower industry has begun to flourish. The ongoing goal of sunflower research at Western Kentucky University is to identify genotypes and environmental conditions that are suitable for cut flower production in South Central Kentucky and that produce floral characteristics sought by area gardeners and florists. The present study involved a total of 23 cultivars and 3 local environments and performance data included in seed company catalogs.

Plant development data included: seedling emergence, days to flowering, height at flowering, number of heads (marketable and non-marketable) per plant, head diameter, vase life, and personal preference. There were evidences of both genetic and environmental variation for the plant characteristics. Flowing heads were rated as pleasing by florists and by laypersons. The number of marketable heads produced by some combinations of cultivars and environments would support profitable production. Cultural practices applicable to commonly grown garden and field crops were suitable for sunflower production. Further refinement is needed in the harvesting schedule and handling to reduce non-marketable heads, to extend vase life, and to permit shipping greater distances. Overall, the results indicated that growing sunflowers for cut flowers is a viable option as niche crop for Central Kentucky.

Cultivar, Mowing Height, and Herbicide Effects on Bermudagrass, *Cynodon dactylon*, Suppression in Tall Fescue, *Schedonorus phoenix*. DAN SANDOR\*, and PAUL WOOSLEY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42104.

Current research at the Western Kentucky University Farm is addressing the effects of mowing height and herbicide treatment in the suppression of bermudagrass, *Cynodon dactylon* [L.] Pers. var. *dactylon* × *C. transvaalensis* Burt-Davy, in plots of tall fescue, *Schedonorus phoenix* (Scop.) Holub. The experimental design was a split plot design. 'KY 31' and 'Bullseye' tall fescue was seeded into an established stand of bermudagrass on 9/08/2011. Mowing Heights were 7.62 cm, 10.16 cm, and 12.70 cm. The size of each plot was 1.22 m × 1.83 m. Treatments consisting of Glyphosate (Roundup) at a rate of 1.169 L/ha were applied one day prior to fescue seeding. On 4/07 and 4/30/2012, herbicide treatments consisting of Mesotrione (Tenacity), Fenoxaprop (Acclaim Extra), and Fluazifop (Fusilade II) were applied at the rates of 0.584 L/ha, 1.46 L/ha, and 0.44 L/ha, respectively, to selected plots. On 7/11/2012, significant differences in turf quality occurred between herbicide treatments, with Fusilade II treatment resulting in lower turf quality. However, Fusilade II resulted in greatest bermudagrass suppression. Fusilade reduced bermudagrass cover by 37% in 'KY 31' and 32% in 'Bullseye'. Treatments receiving Tenacity alone resulted in no bermudagrass suppression. Initially, plots mowed at 12.70 cm resulted in significantly less bermudagrass cover, however, by July there were no significant differences observed in bermudagrass cover among mowing heights within each cultivar. Significant differences in broadleaf weed cover were observed with Tenacity treated plots resulting in greatest broadleaf weed control. 'KY 31' tall fescue exhibited



greatest turf quality at a mowing height of 12.70 cm. In contrast, 'Bullseye' exhibited greatest turf quality at the 7.62 cm mowing height. First year data suggests that mowing height and herbicide treatments affect turf quality and bermudagrass suppression. Further information is expected to be ascertained prior to the conclusion of this research.

Breeding a More Cold Tolerant Ornamental. LOYD BRITT\*, and MARTIN STONE, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

There is a potential market for introduction of cold hardiness traits in tropical plants. *Alocasia macrorrhiza* (L.) G. Don is a large tropical plant native to southeastern Asia. *Arisaema triphyllum* (L.) Schott is native to eastern North America and tolerant of subzero temperatures. Both species are members of the Araceae family, possess the same number of base chromosomes, and taxonomic evidence suggests a high probability of compatibility. Our goal is to hybridize *A. macrorrhiza* with *A. triphyllum* to enhance the cold hardiness of *A. macrorrhiza*. *A. macrorrhiza* is being grown under warm greenhouse conditions. To induce floral production, they were treated with Gibberellic acid (300 mg/L). *A. triphyllum* is being grown under more temperate conditions to slow growth rate. Reciprocal crosses will be made and the progeny tested for cold hardiness.

An Economic Analysis of Grape Production in Kentucky. STEPHEN A. KING\*, TODD WILLIAN, MARTIN J. STONE, and NATHANHOWELL, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The local foods movement and alternatives to traditional agriculture are gaining considerable interest, not only in Kentucky, but throughout the country. Our research provides information that may aid farmers in the decision to invest or not in the alternative agricultural enterprise of viticulture. The primary objective of the research was to determine the expected profitability for a 1-acre table grape vineyard in Kentucky. Data on production relationships, costs, and returns came from the Western Kentucky University demonstration vineyard, published enterprise budgets, and market observations. Annual pro-forma budgets were estimated for a 20-year planning horizon. It was assumed that the vineyard would not yield until the third year and that full production would begin in the fourth year and continues through the 20<sup>th</sup> year. It was further assumed that the farmer would market production via local farmers' markets, school districts, and wholesale markets. Two pre-tax net present values were estimated using a 5% discount rate. The first assumed no compensation to family labor, which indicated a break-even point in the 4<sup>th</sup> year, a benefit-cost ratio of 2.39, and net present value of \$91,059. The second analysis assumed compensation for family labor and indicated a break-even point in the 6<sup>th</sup> year, a benefit-

cost ratio of 1.54, and a net present value of \$54,724. Excluding taxation, the results indicate that table grape production in Kentucky is potentially economically viable.

## BOTANY

Population Trends of the Federally Endangered Running Buffalo Clover (*Trifolium stoloniferum* Muhl. ex. A. Eaton) in the Blue Grass Army Depot from 2003–2012. ALEXI DART-PADOVER\*, DAVID BROWN, and JENNIFER KOSLOW, Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475.

Running buffalo clover (*Trifolium stoloniferum* Muhl. ex. A. Eaton) is an endangered species with the highest density of extant populations at the Blue Grass Army Depot (BGAD) near Richmond, KY. It was discovered at BGAD in 1992 and surveyed every one or two years from 2003 to present. *T. stoloniferum* has occurred in 163 patches since 1992, however only 57 of these patches still persist today. Its peak abundance was recorded in 2006 with 9404 rooted crowns counted in 86 patches. Four years later (2010) the number of rooted crowns had dropped to a quarter of the 2006 number at 2367 in only 52 patches. The latest survey, conducted in summer 2012, has seen a moderate increase to 3751 rooted crowns in 61 patches. Flower production also varied highly among years and was not strongly correlated with trends for rooted crowns. It is unclear what causes the *T. stoloniferum* population to fluctuate from year to year. Previous research suggests that *T. stoloniferum* depends on ground-level habitat disturbance such as grazing, trampling, and flood scouring, and these processes have undoubtedly changed at BGAD over the past two decades. Based on our own observations and other research, we suspect competition from plants such as coralberry (*Symphoricarpos orbiculatus* Moench) and the invasive Japanese stiltgrass (*Microstegium vimineum* A. Camus) threatens recovery. Recently we have initiated controlled landscape scale experiments to investigate potential management strategies, including combinations of grazing, herbicide application and mowing.

## CELLULAR AND MOLECULAR BIOLOGY

Bioaccumulation and Effects of Gold Nanoparticles After Their Prolonged Administration in Mice. PRANAY CHANDRA\*, NILESH SHARMA, SINILAL BHASKARAN, and SHIVENDRA SAHI, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

With the rapid development of engineered nanomaterials, the need for evaluation of their long-term fate in biological systems is being increasingly felt. Particularly, applications of gold nanoparticles in diagnostics, therapeutics and biotechnology products are ever rising, and thus risk potential needs to be assessed. In this investigation, lab mice (C57BL/6) were administered intraperitoneally 40 nm Au-NP at the rate of 333 µg/kg mice over a period of 1–4 weeks. Significant accumula-



tions (2–33.4  $\mu\text{g/g}$  tissue) of Au-NP were found across organs (liver, heart, lung, kidney, spleen and brain). Greater accumulations were noticed in spleen (up to 33  $\mu\text{g/g}$  tissue) and liver (up to 6.7  $\mu\text{g/g}$  tissue). This study also looked at the effect of Au-NP uptake on the tissue levels of essential elements (Cu, Zn and Fe) for competition effects. It was interesting to note that liver levels of Cu and Zn decreased in treatment groups relative to control. General health of mice was monitored by recording their body weight and activity. No significant differences were recorded in the body weight and activity of treatment groups relative to control. Further investigations are continuing to determine the toxicity of gold nanoparticles.

### GEOLOGY

A Geologic Timeline at EKU's New Science Building. WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, Richmond, KY 40475.

Geologic time is so vast that it is beyond true human comprehension, hence the reason for referring to it as "deep time." Geologists have long used analogies in attempts to grasp the breadth and nature of geologic time, and one such analogy is the use of geologic timelines. With the construction of Eastern Kentucky University's New Science Building, the main venue for the 2012 KAS meeting, we developed a timeline along the main walkway paralleling the length of the building. At critical and interesting points in the history of Earth, plaques occur along the walkway at their proportionate position relative to the age of Earth, 4.55 Ga. The plaques give the absolute age of the event, describe it, and explain its significance. A companion document gives added information for each plaque to those who wish to explore the timeline. My presentation will involve a walk along the timeline – please do join us!

### HEALTH SCIENCE

Therapeutic Role of Natural Coconut Oil in Experimental Ulcerative Colitis. PRANAY CHANDRA\*, and NILESH SHARMA, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Ulcerative colitis (UC) is a chronic disease of the colon or large intestine that causes inflammation and ulceration of the inner lining of the colon and rectum. Patients experience a flare of symptoms such as bloody diarrhea, cramping, abdominal pain and loss of body weight. Chronic conditions lead to colon cancer in some patients. Highest incidences are seen in the United States, Canada, the United Kingdom, and Scandinavia. Since the etiology of UC remains unclear, successful treatment strategies targeting large sections of affected population have not been found. Lack of efficacious drugs to treat patients with inflammatory bowel disease underscores the need for the development of a new and effective alternative therapy. Natural coconut (*Cocos nucifera*) oil is a rich source of medium-chain saturated fatty acid (MCFA),

main constituent being lauric acid. In this experiment, mice were chemically induced with chronic ulcerative colitis and treated with coconut oil-containing chow. Treatment with coconut oil produced significant improvement in clinical symptoms and inflammation. It was found to be more effective in a preventive group where mice were fed oil 4-weeks prior to the disease onset. Early exposure to coconut oil improved the severity of disease and significantly reduced systemic inflammatory cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-6) and restored colon size. Inflammatory monocytes (CD14+/CD16+) were significantly reduced in the treatment groups. Limited knowledge of inflammatory conditions coupled with a narrow range of therapeutic options necessitates investigating the role of natural products. Therefore, the present study focuses on the anti-inflammatory role of natural fatty acids derived from *Cocos nucifera* in the murine model of ulcerative colitis.

### PHYSIOLOGY AND BIOCHEMISTRY

Experimental Analysis of the Relationship Between Parasite Burden and Cognitive Abilities in House Mice (*Mus musculus*). CARLI WHITTINGTON\*, CARLYNN REKOSH, CHESIKA CRUMP, and TERRY DERTING, Department of Biological Sciences, Murray State University, Murray, KY 42071.

During early mammalian development, the energetic cost of mounting an immune response may deplete available energy allotments from other biological processes such as brain development. Eppig *et al.* reported that parasitic infections may affect development of an organism's nervous system, resulting in reduced cognitive ability. Our goal was to determine whether parasitic burden is related with cognitive ability. We tested the null hypothesis that parasitism does not affect the cognitive ability of lab mice. Using *M. musculus*, adults were bred and neonates from 10 litters were infected with the gastrointestinal nematode *Heligmosomoides polygyrus*. Control neonates from those same 10 litters were not infected for comparison. At 21 days of age, both groups were subjected to parasite load enumeration through fecal egg counts. At 35 days of age, both groups were subjected to a spatial memory test using a T-maze. After T-maze completion, each mouse was euthanized and the brain removed and mass recorded after drying. There was no significant difference in the dry brain mass of the parasitized and non-parasitized mice. We also failed to see any significant difference between the percent of successful T-maze trials for the parasitized compared with the non-parasitized group. Likewise, within the parasitized mice, parasite egg count and percent success on the T-maze was not correlated significantly. Our work showed that parasitic infection prior to sexual maturation had no effect on the cognitive ability of *M. musculus*. These results did not support a relationship between parasitic burden during post-natal development and cognitive ability.

## PSYCHOLOGY

Racial Identity Development: Examination of the Cross Nigrescence Theory and the Experience of Black Students Attending Predominately White Institutions. QUANTÁ D. TAYLOR, Cultural, Racial and Ethnic Studies & Psychology, Transylvania University, Lexington, KY 40508.

In a study I examined the development of personal and social identity of thirty Black students attending predominately White institutions. A mixed method analysis was used to assess students' current stage of racial identity development. Results indicate that Black students, regardless of class rank, are in constant fluctuation on the categorical development stages as measured by Cross' 2001 Nigrescence Theory. Furthermore, students' interview responses provide examples to reflect these developmental stages as they deal with issues of racism and campus sense of belonging. The results support the idea that Black students on predominantly White campuses face specific stresses associated with being in the minority population. The results further imply the need for campus faculty, staff, administrators, and students to pay greater attention to the experiences of members of minority populations on campus as they adjust and progress through their college experience.

## SCIENCE EDUCATION

Reducing Absenteeism in Community College Biology and Other Courses: a Case for Establishing an Institution-wide Attendance Policy. JOHN G. SHIBER, Division of Biology & Allied Health, KCTCS – Big Sandy District, Prestonsburg, KY 41653.

1689 student attendance and grade records for biology courses under this investigator's instruction (2007–2012) were reviewed and scrutinized in order to build a persuasive argument for establishing a workable, positive, college-wide attendance policy similar to the one employed in these courses. College/university administrators and teachers have hesitated to endorse institution-wide attendance policies, in large part due to a fear of infringing upon student rights and/or sensibilities, but the data presented here give credence to the long-held belief that students who attend classes regularly usually wind up with successful grades, while dispelling the idea that an attendance policy must be punitive to be effective.

How students feel about the issue must be considered in such an initiative, so the investigator enlisted eight colleagues to help poll our Fall-2012 students about their opinions on it. Of 419 responding, 98% agreed that attending classes regularly is important for learning and college success and 75% said that all classes should have an attendance policy. Another 19% thought that most classes should have one, listing only a few they believed didn't need one. Only 6% of students felt that attendance policies are not at all necessary. The results suggest that establishing a uniform, college-wide (if not system-wide), non-punitive attendance policy might be a very positive step toward reducing absenteeism and, thus, increasing student chances for academic success.

## SOCIOLOGY

An Analysis of the Contradictions Between Food Advertising and Healthy Food Guidelines. MICHELLE KROGER, Northern Kentucky University, Highland Heights, KY 41099.

In the last few decades, the American public has begun to experience an increasing public health crisis. Obesity is on the rise, as are other chronic diseases including diabetes and heart disease. The federal government, health experts, employers, insurance companies, the current First Lady, and public service announcements have issued multiple warnings concerning healthy diets and lifestyles. Yet, obesity is becoming pervasive. Why the contradiction between the calls for healthier eating habits and the resistance of the public? What role might food advertisements play? Previous research has focused on the consequences of diets with high levels of salt and sugar and the relationships between various dietary factors and chronic diseases. The present study, using ads from a big box retailer, a national food chain, a regional store, as well as a local grocer, examines the contradictions between advertising and healthy living. The exploratory study analyzes to what extent healthy food choices are being marketed to consumers. The results indicate that processed meats, snack foods, desserts, and candy are advertised more often than fresh fruits and vegetables. The results were the same for coupons. The study concludes that further research is needed to fully analyze the contribution of unhealthy advertisements and coupons in the public health crisis.











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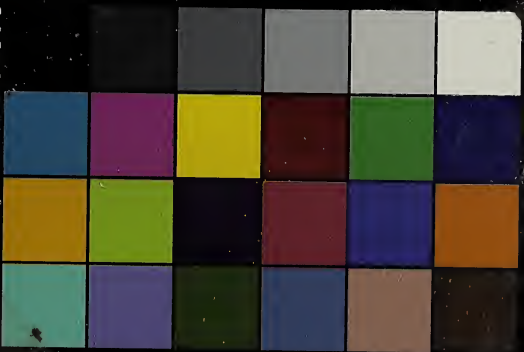
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