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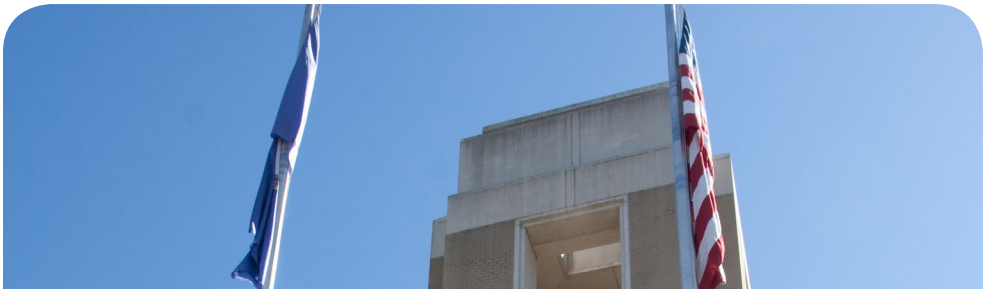

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Vol. 66 **No. 4** **Winter, 2015**

TABLE OF CONTENTS

ARTICLES **PAGE**

Virginia Journal of Science
Volume 66, Issue 4
Winter 2015

Life-history Aspects of *Moxostoma cervinum* (Blacktip Jumprock) in the Roanoke River, Virginia

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ABSTRACT

Life-history aspects of *Moxostoma cervinum* (Blacktip Jumprock) were identified using specimens from recent collections and the Roanoke College Ichthyological Collection. The largest specimen examined was a female 161.27 mm SL and 66 months of age. Spawning appears to occur in May, with a mean of 2477.6 oocytes (SD = 2825.3) up to 1.54 mm diameter in gravid females. Sexual maturity appears to occur by 1-2 years of age in males and 2-3 years of age in females. Male to female ratio was not significantly different from 1:1. Chironomidae composed the bulk of the diet; while detritus, Trichoptera, Ephemeroptera, and Acari were important food items in multiple months. Weight of gut contents and proportion of Chironomidae as food items increased with size of specimens examined.

INTRODUCTION

Moxostoma cervinum (Cope) (Blacktip Jumprock) inhabits upland streams in the James, New, Roanoke, Tar, and Neuse river systems of Virginia and North Carolina (Jenkins and Burkhead 1994). Jenkins (1970), Buth (1978), and Smith (1992) all placed the species in the genus *Scartomyzon* with other small suckers inhabiting faster, shallower waters. However, most recent analyses embed the species within the genus *Moxostoma* (Harris et al. 2002, Doosey et al. 2010, Chen and Mayden 2012) with larger suckers often found in very different habitats. This phylogenetic placement means that understanding the biology and life-history of *M. cervinum* is important in identifying derived and ancestral character states, thus helping to interpret the substantial variation in the biology and life-history of the *Moxostoma*. Despite this importance, our understanding of this species' life history is restricted to three paragraphs in the species account in *Freshwater Fishes of Virginia,* which gives limited details on aspects of diet, size and age at maturity, and timing of spawning (Jenkins and Burkhead 1994). The objective of this study is to document more detailed life-history aspects of *M. cervinum* from specimens collected throughout the year employing methods utilized in similar studies.

MATERIALS AND METHODS

Moxostoma cervinum were collected from the Roanoke River near Salem, VA (Roanoke County) between September 2010 and August 2011 by sampling daylight

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hours near the end of each month using a Smith-Root LR-24 electrofisher and a 3.3-m x 1.3-m seine with 9.5-mm mesh. We supplemented our collections with specimens from the Roanoke College Ichthyological Collection (RC) for months when we collected few specimens ($n < 15$). Specimens were collected following Nickum et al. (2004) protocols, fixed in 10% formalin, rinsed with water and then stored in 45% isopropyl alcohol. A total of 154 specimens were examined in this study. Details on specimens examined (collection sites, collection dates, numbers of specimens taken, collector field numbers) are available from the authors upon request. Standard length (SL) of preserved specimens was measured to the nearest 0.01 mm using digital calipers. Total weight (TW) and eviscerated weight (EW) were measured by blotting the specimens dry and weighing to the nearest 0.001g on a digital analytical scale. Regressions by least sum of squares were performed for EW and SL to examine the relationship between length and weight. A two sample t-test was used to test for difference between male and female standard length. A chi-square test was used to detect a sex ratio different from 1:1. All statistical analyses were performed using Minitab 17 Statistical Software (Minitab, Inc., State College, PA) with alpha equal to 0.05.

Specimens were aged using two methods. For all specimens, three scales were removed from the right dorsolateral portion of the body, mounted on a slide and examined under 40x magnification for annuli (see Bond 1996). If the three scales removed did not have the same number of annuli (e.g. regenerated scales that lack a focus), more scales were removed until a clear consensus number of annuli was identified. For specimens with a standard length >119 mm, a single opercle was removed, prepared and analyzed following Beckman and Hutson (2012). Each opercle was removed from the left portion of the body, set in boiling water for 10 minutes, then set in bleach for another 10 minutes to facilitate the removal of excess tissue and then allowed to air dry until annuli were clearly visible. Annuli were read by locating the presence of an opaque region near the edge of the opercle, and counting each opaque region as a single annulus; the number of annuli present was determined by two observers. This method, in comparison to scale annuli data, revealed that the number of annuli on the scales underestimated the age of specimens three years of age or greater and agreed with the scale data for specimens less than three years of age. Therefore, the number of annuli on the opercle was solely used to estimate the age of specimens three years of age or greater.

Specimens less than 12 months of age were counted as 0+, specimens 12-23 months were counted as age 1+, specimens 24-35 months were counted as age 2+, specimens 36-47 months were counted as age 3+, specimens 48-59 months were counted as age 4+, and specimens greater than 60 months were counted as age 5+. The proportion of all specimens examined represented by each age class was calculated to approximate the age-class distribution of the population. A t-test of age in months was used to test for differences in lifespan among sexes.

Gonads were examined to determine sex, removed from each specimen, and weighed to the nearest 0.001 g. Gonadosomatic Index (GSI) was calculated for all specimens by dividing gonad weight by EW. One-way analysis of variance was performed to test for differences in GSI among specimens of the same sex collected from different months. In gravid females, fully yolked, mature oocytes were counted, and five representative oocytes were measured to provide an approximation of ova size

and number (Heins and Baker 1988). Regression of SL as a predictor of number of mature oocytes was performed to test the influence of specimen size on fecundity. Due to GSI values peaking in May, declining in June, and reaching a minimum level in July, we used May as the month of spawning for estimating age of specimens.

The anterior third of the gastrointestinal tract was opened and its contents were removed and weighed using a digital analytical balance and recorded to the nearest 0.001 g. Weight of gut contents for specimens with empty guts was recorded as 0. Food items were counted and identified to the lowest taxonomic category possible following Thorp and Covich (1991) and Merritt and Cummins (1996). Detritus was noted as being present or absent in an individual specimen. The number of identifiably different food items in each specimen was recorded as variety of food items. One-way analysis of variance was performed on weight of gut contents, variety of food items, and percent Chironomidae to test for differences in feeding throughout the year. Regressions by least sum of squares were performed for SL and weight of gut contents, SL and variety of gut contents, and SL and percent Chironomidae to test the influence of size on feeding.

RESULTS

Eviscerated weight increased with standard length (r^2 = 88.78%, $P < 0.0001$) and is described by the model EW = (SL) x 0.4952 – 29.05. Females were larger than males ($P < 0.0001$) with the mean size of females 105.57 mm SL (SD = 33.26) and males 85.02 mm SL (SD = 25.46). The smallest specimen examined (37.94 mm SL) was collected in January, had zero annuli and appeared to be eight months of age. The largest specimen examined (161.27 mm SL) was a female collected in November, had five annuli, and appeared to be one of the oldest specimens examined at 66 months of age (Figure 1). All specimens examined for annuli had zero to five which corresponded to annuli forming near the end of winter or early spring in specimens up to 66 months of age. Mean lifespan was greater ($P = 0.003$) for females (24.76 months, SD = 16.11) than males (18.08 months, SD = 11.31). There was also a slightly skewed sex ratio of 1:1.69 in favor of females; however, the difference in the number of males and females was not significant ($P = 0.938$). Standard length increased with age in months (r^2 = 83.99, $P < 0.0001$) and is described by the model LOGSL = (LOG age in months) x 0.4906 + 1.3465. Of the 154 collected specimens, 25.97% were age 0+, 35.06% were age 1+, 23.38% were age 2+, 6.49% were age 3+, 7.41% were age 4+, and 1.95% were age 5+ (Figure 2).

Monthly GSI was not uniform for females (females $P = 0.005$), but did not differ significantly for males ($P = 0.116$). Individual GSI was highest in May for females (0.135) (Figure 3) and males (0.052) (Figure 4). Maximum GSI values declined in June to 0.002 for males, and for females reaching a minimum value of 0.00453 in July. Mean GSI values were lowest during June and July for both females (June = 0.01, SD = 0.013; July = 0.008, SD = 0.004) and males (June = 0.003, SD = 0.0005; July = 0.004, SD = 0.004). Mean GSI values were highest for both sexes during November (females = 0.05, SD = 0.04; males = 0.03, SD = 0.008). Elevated GSI values generally persisted from fall months through spring for both sexes (Figures 3 and 4). Mature oocytes were 0.6-1.54 (mean = 0.96, SD = 0.19) mm in diameter and numbered from 560 to 15,441 (mean = 2477.6, SD = 2825.3). The smallest female with mature oocytes was 24 months of age and had a SL of 93.3 mm. All females collected during spring

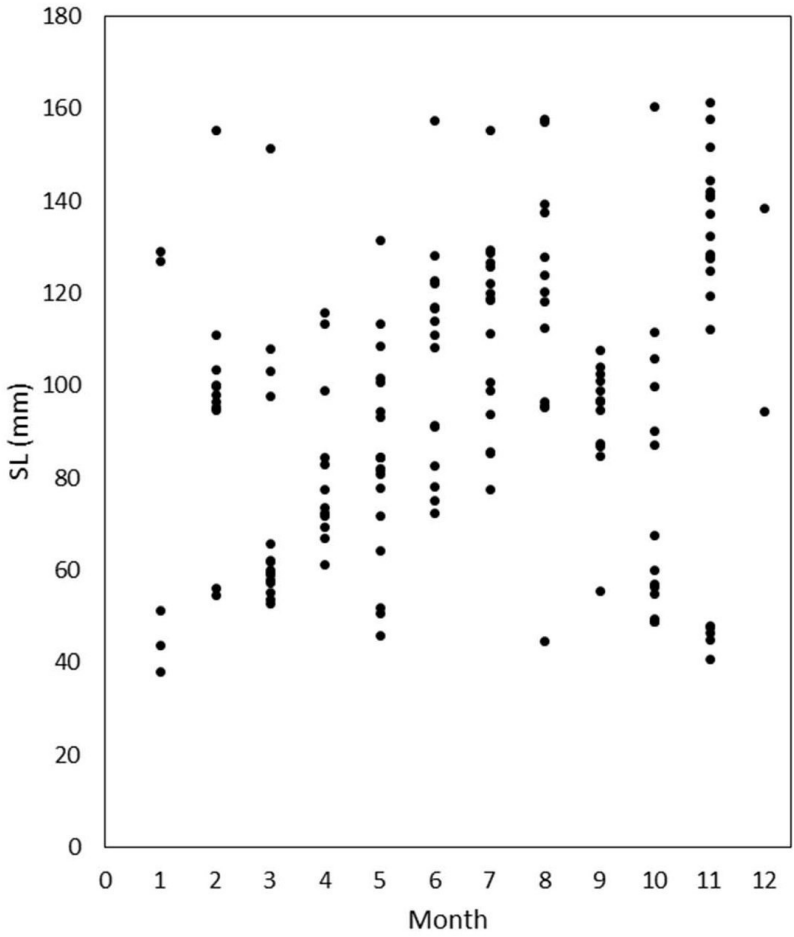

FIGURE 1. Standard length in mm by month of collection for *Moxostoma cervinum*. (1=January, 2 = February, etc.). N = 154

months approximately three years of age had mature oocytes, while 57% of females 24+ months of age had mature oocytes. Number of mature oocytes and SL were significantly correlated ($P = 0.002$) with a modest $r^2$of 29.76%. The smallest sexually mature male (GSI = 0.028) was 65.93 mm SL and 10 months of age. All males approximately two years of age or greater were sexually mature, and 7% of males approximately one year of age were sexually mature.

Due to mastication by pharyngeal teeth, most food items were not identifiable below the order or family level (Table 1). Weight of gut contents was not uniform across all months ($P >.0001$). The highest mean weight of gut contents (0.077 g) was

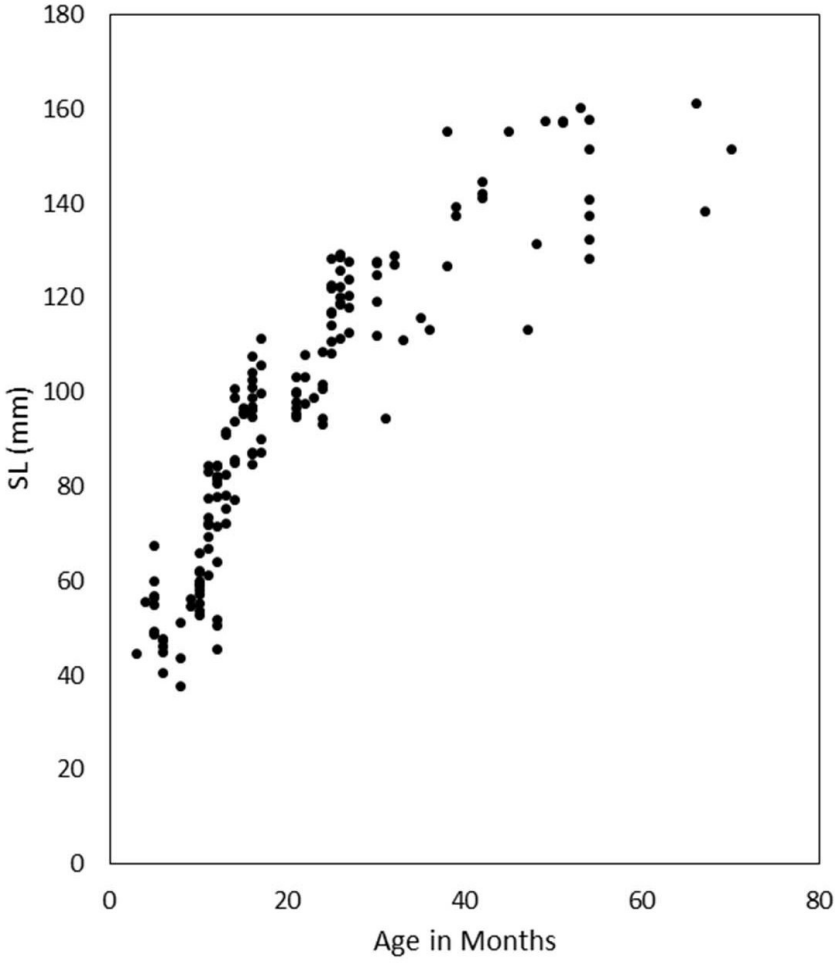

FIGURE 2. Standard length in mm versus hypothesized age in months for *Moxostoma cervinum.* N = 154

in August and was lowest in January (0.000 g). The relationship between SL and weight of gut contents was significant ($P < 0.0001$) and had a modest r^2 value of 24.1%. Chironomidae and detritus were found in 70% and 82% of specimens examined, respectively, and 14% contained no gut contents. Variety of gut contents was not uniform across all months ($P < 0.0001$) and peaked in August (n = 13). The relationship between SL and variety of gut contents was significant ($P < 0.0001$) and had a modest r^2 value of 17.8%. The proportion of gut contents that were Chironomidae

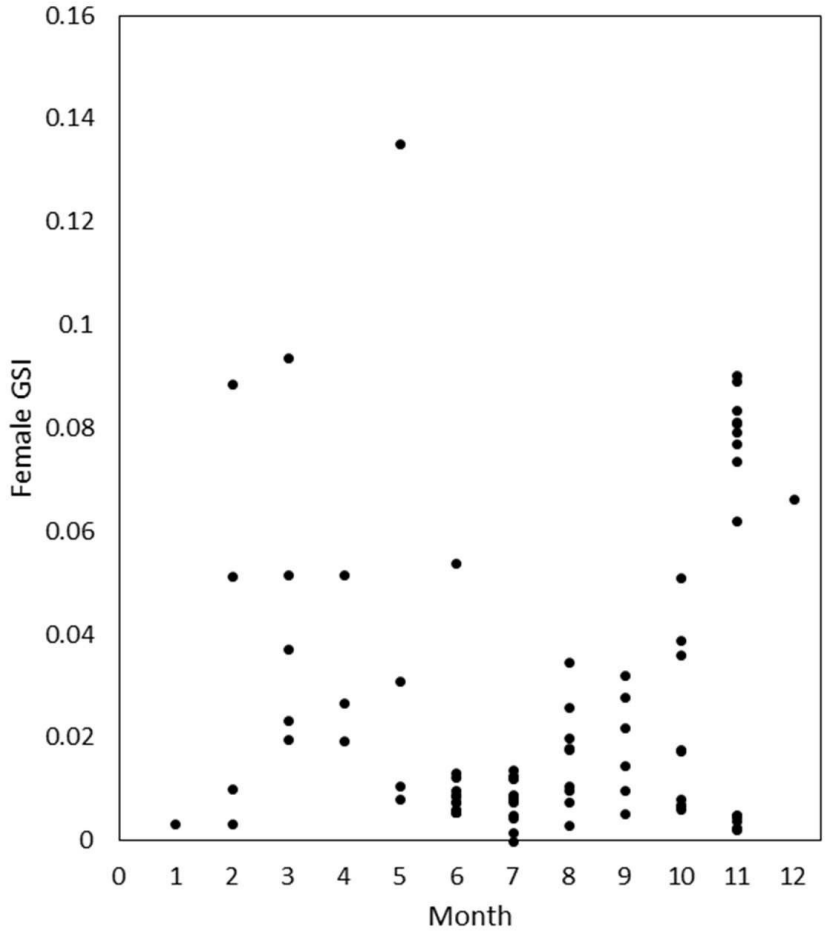

FIGURE 3. Gonadosomatic index by month of collection for female *Moxostoma cervinum*. (1=January, 2 = February, etc.). N = 76

was not uniform across all months ($P < 0.0001$) and was greatest during April and lowest during July. A significant positive relationship ($P = 0.013$) between SL and proportion of gut contents as Chironomidae had a low r^2 value of 3.99%.

DISCUSSION

Moxostoma cervinum appear to grow up to approximately 84 mm SL in their first year, up to 129 mm SL by the end of their second year, up to 156 mm SL by the end of their third year, up to 158 by the end of their fourth year, and up to 161 by the end of their fifth year. The largest specimen examined (161.27 mm SL) for this study is similar in size to the maximum size reported by Jenkins and Burkhead (1994) of 164

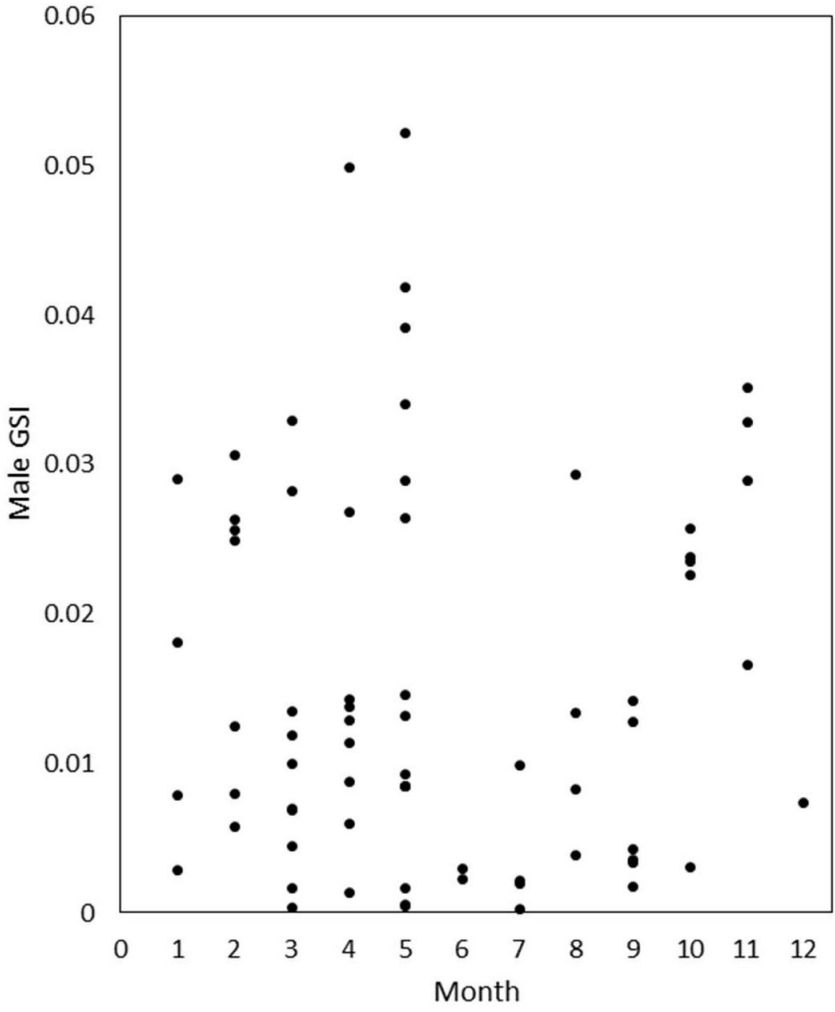

FIGURE 4. Gonadosomatic index by month of collection for male *Moxostoma cervinum*. (1=January, 2 = February, etc.). N = 63

mm SL suggesting that maximum age for the species is five years. The relatively low proportion of specimens less than one year old is likely explained in part by the difficulty capturing small specimens in a seine with 9.5 mm mesh as many are likely to pass through without being captured. The difference in habitat between juvenile and adult specimens noted by Jenkins and Burkhead (1994) may also partly explain the low number of juveniles, as most collections for this study were conducted in flowing habitats over larger substrate preferred by adults. The relatively high proportion (39%)

TABLE 1. Number of individuals of each taxon identified from gut contents of *Moxostoma cervinum* collected in the Roanoke River from corresponding months and total number. Total number of food items from each month is the second row from the bottom, and number of taxa represented each month is the bottom row.

Taxon	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Acari	0	0	0	0	18	3	1	11	3	0	0	0	36
Brachiopoda	0	0	0	0	0	0	0	3	0	0	0	0	3
Chironomidae	0	108	26	350	723	515	99	500	196	28	604	0	3149
Coleoptera larvae	0	0	0	0	0	0	0	2	0	0	0	0	2
Coleoptera adults	0	0	0	0	0	0	0	2	0	0	0	0	2
Diptera adults	0	0	0	9	0	0	0	3	0	0	8	0	20
Ephemeroptera	0	0	0	8	5	0	4	7	4	0	0	0	28
Oligochaeta	0	0	5	0	0	0	0	0	0	0	0	0	5
Prosobranchia	0	0	1	0	0	0	0	0	0	0	0	0	1
Simulidae	0	0	0	0	6	0	0	0	2	0	0	0	8
Trichoptera	0	1	0	1	1	29	16	6	4	54	7	0	119
Unidentified eggs	0	0	0	0	0	0	294	0	0	0	0	0	294
Total	0	109	32	368	753	547	414	534	209	82	619	0	3667
Taxa each month	0	2	3	4	5	3	5	8	5	2	0	0	

of specimens two years of age or greater suggests a high survivorship of adult specimens as would be expected due to few large piscivorous predators in the relatively shallow, flowing habitat preferred by *M. cervinum.* The difference in mean size among sexes is likely due to the difference in lifespan as females appear to outlive males.

A relatively small proportion of males appear to be sexually mature at approximately one year of age, and all appear to be mature by two years of age. Females appear to take longer to mature than males as a majority are mature by two years of age and all are mature by three years of age. Spawning appears to begin in April and continue through May as indicated by GSI values and condition of oocytes (Figures 3 and 4). Jenkins and Burkhead (1994) reported water temperatures during likely spring spawning of *M. cervinum* as 14-23° C, and water temperature during our collections from April and May for this study were within that range. The low GSI values for both sexes in summer months, followed by an increase in fall indicate an early physiological preparation for spring spawning. While this appears to be unusual, other recent studies have documented similar increases in GSI during fall for small Catostomidae species (O'Kelley and Powers 2007, Tarasidis and Powers 2014).

Feeding appears least intense during colder months of winter as indicated by lower values for weight of gut contents and variety of food items from December to March (Table 1). The abundance of Chironomidae and detritus in the gut of specimens indicates a similar diet to that of other small catostomids (Timmons et al. 1983, O'Kelley and Powers 2007, Tarasidis and Powers 2014). The high number of Chironomidae may indicate selective feeding by *M. cervinum*, or may be the result of high densities of chironomids (>20,000 individuals/m^2) in the substrate of streams (Benke et al. 1984). While the proportion of the diet made up by Chironomidae is not uniform across all months, the variation does not appear to be strongly seasonal as the two months with the lowest proportion of food items as Chironomidae are adjacent to months with >90% Chironomidae in the diet. The abundance of Trichoptera, Acari, and unidentified eggs from some months, and complete absence from others suggest that *M. cervinum* may simply be opportunistic benthic feeders. While variety of gut contents does appear to increase with SL, the modest relationship between these variables suggests that there is not a strong shift in feeding throughout the life of *M. cervinum*. Larger specimens may simply be able to ingest a greater variety of food items also suggesting that *M. cervinum* are not particularly selective, but rather opportunistic benthic feeders taking advantage of almost any currently abundant food source. While significant, the low r^2 value suggests size of specimens has little influence on the proportion of food items as Chironomidae also suggesting that changes in diet are slight throughout the life of *M. cervinum.*

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Specimen collection was done primarily by J.S. Bentley and S.L. Powers. Data analyses were done by D.A. Thompson and S.L. Powers.

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Amphibian and Small Mammal Assemblages in a Northern Virginia Forest Before and After Defoliation by Gypsy Moths (*Lymantria dispar*)

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ABSTRACT

The introduced European gypsy moth (*Lymantria dispar*) caused substantial defoliation and mortality of oak trees along the North Fork of Quantico Creek in Prince William Forest Park, Prince William County, Virginia, U.S.A., in 1989 and the early 1990s. Results of a drift fence/pitfall study conducted in 1988 were compared to those obtained from the same technique in the same areas in 1993 to elucidate whether the amphibian and small mammal assemblages had changed over time. Number of *Lithobates sylvaticus* increased significantly in 1993, but the numbers of *Lithobates clamitans* and *Plethodon cinereus* were significantly higher in 1988. Total numbers of amphibians caught in both years was similar. Two species of salamanders caught in 1988 were not caught in 1993, and one salamander and one frog caught in 1993 were absent in 1988. Total numbers of small mammals caught in 1993 were significantly greater than in 1988. The increase was due to greater numbers of *Blarina brevicauda* and *Sorex longirostris*. The hypothesis that no significant differences in amphibian and small mammal species richness and relative abundance before and after gypsy moth defoliation hypothesis was not supported by the results of this study.

Key Words: community ecology, forest ecology, amphibians, small mammals, gypsy moth, *Lymantria dispar*

INTRODUCTION

Defoliation of millions of hectares of hardwood trees in northeastern North America by the introduced European gypsy moth (*Lymantria dispar*) has resulted in substantial ecological and economic damage (Houston 1981a, 1981b; White and Schneeberger 1981). This forest pest was inadvertently introduced in 1869 into Massachusetts and has migrated westward and southward, entering Virginia in or about 1982 (McManus and McIntyre 1981; Gansner et al. 1993; Ravlin and Weidhaas 1991). It has since spread throughout much of the Commonwealth, causing defoliation and tree (primarily oak [*Quercus* spp.]) mortality in northern counties and along the Blue Ridge and Allegheny mountains (Ravlin and Weidhaas 1991; Gansner et al. 1993).

At the request of Prince William Forest Park in Prince William County, Virginia, I conducted a study of the effects of gypsy moth defoliation on an assemblage of

terrestrial vertebrates in 1993. I had previously evaluated the amphibian and small mammal assemblages at this site in 1988, a year before initial defoliation in 1989 (Mitchell and Pague 2016). In this paper, I elucidate the changes in the forest floor vertebrate community that may have occurred in response to the defoliation and mortality of forest trees between 1988 and 1993. Specifically, I evaluated the null hypothesis that there were no significant differences in amphibian and small mammal species richness and relative abundance before and after gypsy moth defoliation.

MATERIALS AND METHODS

Study site

The study site was located in a 14 ha portion of Prince William Forest Park (PWFP), Prince William County, Virginia, U.S.A., at an elevation of 73.2 m. The study area was approximately 125 m south of the confluence of an unnamed tributary and the North Fork of Quantico Creek, about 6 km northwest of the town of Triangle.

The forest canopy at the study site was dominated by American beech (*Fagus grandifolia*), white oak (*Quercus alba*), and mockernut hickory (*Carya tomentosa*). Flowering dogwood (*Cornus florida*) was the primary understory tree. White oak and tulip poplar (*Liriodendron tulipifera*) were the most common trees in the forest surrounding the site. The understory consisted of wild azalea (*Rhododendron nudiflorum*), American holly (*Ilex opaca*), and flowering dogwood. Herbaceous plants were not abundant, but included aster (*Aster* spp.), hayscented fern (*Dennstaedtia punctilobula*), bindweed (*Convolvulus* spp.), and sedge (Cyperaceae). The forest floor was generally open with a moderate leaf cover and variable amounts of coarse woody debris ranging from small limbs to dead trees and scattered patches of grasses.

Vegetation changes 1988–1993

The primary change in the forest community was the loss of all oak trees on the site and in the surrounding area from mortality caused, at least in part, from defoliation by the gypsy moth. This was accompanied by a substantial (unmeasured) increase in the amount of coarse woody debris. Numerous limbs and several of the fallen dead trees near the 11–12 ha study sites littered the area in 1993. Coarse woody debris was the only category of forest floor cover that appeared to have increased between 1988 and 1993.

The amount of canopy closure was determined by electronically comparing forest canopy density in aerial infrared photographs taken by the National Park Service (NPS) in June 1983 (5 yr before the initial study), 1989, and 1991. Photographs were scanned at 200 dots per inch with a hand-held 256 gray-scale scanner. The digitized data were then entered into the Geographic Resource Analysis Support System 4.0 (GRASS) at the Center for Urban Ecology, Washington, D.C. The resulting geographic reference points were registered to the existing PWFP forest cover database to ensure accurate placement of plots on the aerial photographs. Two areas were selected for comparison: 12.2 ha centered over the study site (aerial photographs available for 1989 and 1991), and 11.4 ha adjacent to the study site (photographs available for 1983, 1989, and 1991). A densiometer was used to obtain an estimate (average of 5 readings) of canopy cover over the study site in August 1993.

Canopy cover data were unavailable for 1983 in the study site, but assumed to be similar to that in the adjacent site; the same assumption was made for 1993 cover in the adjacent site. Canopy cover before gypsy moth defoliation (1983) on the adjacent plot

was 81.1% compared to 54.6% in 1989 (the first year of defoliation) and 82.8% in 1991. The amount of canopy cover over the study site was 67.7% in 1989, 83.8% in 1991, and 90.9% in 1993. Recovery of canopy cover in 1991 may have been due to generation of new foliage by oaks, most of which died after 1991, however. The densiometer readings in 1993 were taken under beech trees that were apparently unaffected by gypsy moths. Thus, forest canopy cover decreased on the study and adjacent sites in 1989, but appeared to have recovered by 1993, despite loss of oak trees. The NPS conducted aerial pesticide treatments of the microbial pesticide *Bacillus thuringiensis* (Bt) and the species-specific virus Gypchek® from 1989 to 1995 to kill the moths and reduce the infestation.

Weather patterns were similar in 1988 and 1993 (Figure 1). Average monthly minimum and maximum temperatures did not differ significantly in all pairwise comparisons ($t = -1.11–1.60$, $P = 0.115–0.915$). Monthly precipitation totals were higher in 1993 for March, April, August, and September, whereas totals were higher in May, June, and July in 1988.

Field methods

Data on species richness and relative abundance of amphibians and small mammals were obtained in 1988 and 1993 with the use of a drift fences and pitfalls technique (Campbell and Christman 1982; Mitchell et al. 1997). Four lengths of aluminum flashing (0.61 x 7.5 m) were installed upright in a cross configuration, each arm separated from the center point by about 7.5 m, leaving an open center. We sunk a 3.8 l (#10) tin can in the ground on each side at each end of each arm and a 19 l (5 gallon) plastic bucket in the middle of the array. Thus, each drift fence arm consisted of four 3.8 l cans and one 19 l bucket; 20 pitfalls total for the array. A total of 3920 trap days were recorded in 1988 during the period of 22 March - 3 October, and 3680 trap days in 1993 during the period of 1 April - 1 October.

Analysis

Descriptive statistics and comparisons among sample means for parametric data were obtained using Statistix programs (version 4.0, Analytical Software, St. Paul, MN). Nonparametric comparisons were made with chi-square tests following Zar (2009). Because the chi-square statistic is calculated using actual frequencies or numbers observed rather than percentages (Zar 2009), I adjusted the 1993 capture numbers to account for the fewer number of trap days that year. Significance was accepted at alpha = 0.05. Estimates of community diversity, e.g., Shannon diversity index (H') and evenness (J), followed procedures in Brower et al. (1989). Herpetofaunal names follow Crother (2012) and small mammal names follow Bradley et al. (2014).

RESULTS

In addition to amphibians and small mammals, five species of reptiles (*Plestiodon fasciatus, Plestiodon laticeps, Carphophis amoenus, Diadophis punctatus, Thamnophis sirtalis)* were captured in both years combined (15 individuals in 1988, 19 in 1993). The small sample sizes precluded any statistical analysis.

Twelve species of amphibians occurred on the site in 1988 and 1993 combined (Table 1). Total numbers of individuals was similar in the two samples (228 vs 206).

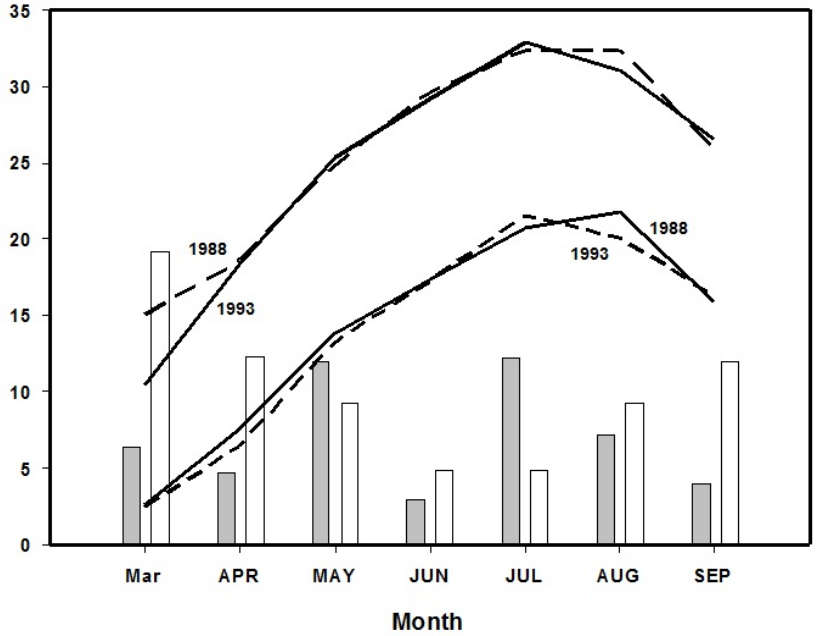

FIGURE 1. Monthly precipitation totals (bars, cm) for March-September 1988 and 1993 and monthly minimum and maximum temperatures (lines, ºC) from the U.S. Marine Corps Airfield at Quantico, Virginia, located approximately 13.5 km southeast of the study site. Open bars represents 1993 and solid bars 1988. The scale on the Y-axis is the same for precipitation and temperature.

The number of anurans was significantly greater in 1993 than in 1988. Numbers of *Lithobates clamitans* and *L. sylvaticus* were significantly greater in 1993 than in 1988 (Table 1). Salamander abundance was significantly higher in 1988 than in 1993, but this difference was due to the large numbers of one species (*Plethodon cinereus*). Two species (*Acris crepitans*, *Ambystoma opacum*) caught in 1988 were not encountered in 1993, and two species (*Eurycea bislineata*, *E. guttolineata*) caught in 1993 were not found in 1988. Amphibian community diversity was similar between years (Table 1). The relative numbers of individuals among species were more evenly distributed in 1993 than in 1988.

A total of six species of small mammals (3 insectivores, 3 rodents) occurred in the combined samples (Table 2). The total number of individuals was significantly higher in 1993 than in 1988. The total number of insectivores was significantly higher in 1993 than in 1988, whereas the difference was not significant for rodents (Table 2). The difference in shrew numbers was due to the significantly higher numbers of *Blarina brevicauda* and the number of *Sorex longirostris* caught in 1993. *Sorex longirostris* was not caught in 1988 and *Zapus hudsonius* was not caught in 1993. The distribution of

TABLE 1. Species richness and relative abundance of amphibians in a northern Virginia hardwood forest before and after defoliation by the gypsy moth. Upper number is the number of adults and lower number is number of juveniles. #/per trap day is number per trap day x 100. NT = not tested due to small sample size. Total numbers of adults and juveniles were used for the statistics.

Species	1988	#/trap day	1993	#/trap day	χ^2	P
Anurans						
Acris crepitans	1/0	0.026	0		NT	
Bufo americanus	8/21	0.740	4/13	0.462	3.13	0.077
Lithobates catesbeianus	0/1	0.026	0/4	0.109	NT	
Lithobates clamitans	0/41	1.046	2/14	0.415	10.97	0.0009
Lithobates palustris	1/35	0.198	1/30	0.842	0.37	0.541
Lithobates sylvaticus	4/7	0.281	5/86	2.473	62.75	<0.001
Number of individuals	119		159		5.76	0.0164
Salamanders						
Ambystoma opacum	1/0	0.026	0			
Eurycea bislineata	0		1/0	0.027	NT	
Eurycea guttolineata	0		3/0	0.082	NT	
Notophthalmus viridescens	0/4	0.102	0/1	0.027	NT	
Plethodon cinereus	72/31	2.628	36/4	1.087	27.755	<0.001
Plethodon cylindraceus	1/0	0.026	1/0	0.027	NT	
Number of individuals	109		46		26.61	<0.001
Species richness	10		10			
Total number of individuals	228	5.816	206	2.598	1.12	0.2910
Anurans/trap day x 100		3.036		4.321		
Salamanders/trap day x 100		2.781		1.250		

individuals among species in both years provided the similar estimates of species diversity (H') and evenness (J) for the small mammal assemblages (Table 2).

DISCUSSION

Differences in species richness and relative abundances in amphibians and small mammals in the samples from 1988 and 1993 indicated a mixed response to the temporary reduction in canopy cover and loss of oak trees at this site. There were significant differences in the composition of these vertebrate assemblages before and after gypsy moth defoliation because some species were more abundant in 1988 and others were more abundant in 1993. Three alternative hypotheses may account for the differences observed.

(1) The changes in species on the study sites and differences in numbers may have been due to different weather patterns in 1988 and 1993. Average monthly minimum and maximum temperatures did not differ significantly in all pairwise comparisons (t = -1.11–1.60, P = 0.115–0.915). Monthly precipitation totals were higher in 1993 for

TABLE 2. Species richness and relative abundance of small mammals in a northern Virginia hardwood forest before and after defoliation by the gypsy moth. The raw number refers to all adults. #/per trap day is number per trap day x 100. NT = not tested due to small sample size. Total numbers of adults and juveniles were used for the statistics.

Species	1988	#/trap day	1993	#/trap day	χ^2	P
Insectivores						
Blarina brevicauda	5	0.128	16	0.435	5.76	0.016
Sorex hoyi	9	0.230	6	0.163	0.60	0.439
Sorex longirostris	0		7	0.190	NT	
Number of inividuals	14		29		5.23	0.022
Rodents						
Microtus pennsylvanicus	2	0.051	2	0.054	NT	
Peromyscus leucopus	2	0.051	3	0.082	NT	
Zapus hudsonius	2	0.051	0		NT	
Number of individuals	6		5		0.09	0.763
Species richness	5		5			
Total number of individuals	20	0.510	34	0.924	3.63	0.057
Shrews/trap day x 100	14	0.357	29	0.788		
Rodents/trap day x 100	6	0.153	5	0.136		
H'	0.607		0.594			
J	0.868		0.849			

March, April, August, and September, whereas totals were higher in May, June, and July in 1988. Variation in monthly precipitation between years and among months and years suggests that the different patterns of summer rainfall had little effect on assemblage structure. Thus, variation in weather patterns between study years cannot account for the differences in the terrestrial vertebrate assemblages observed in 1988 and 1993.

(2) Modification of the habitat due to changes caused by gypsy moth defoliation could have influenced changes in assemblage composition and numbers of individuals caught in 1993. The principle differences between 1988 and 1993 in the amphibian and small mammal assemblages were in numbers *Plethodon cinereus* and *Blarina brevicauda*, respectively. Loss of canopy cover in 1989 could have increased the amount of solar radiation reaching the forest floor causing drying and a reduction in the number of invertebrate prey, and consequently the reproductive potential of the salamanders. Alternatively, additional sunlight may have stimulated herbaceous plant growth and an increase in invertebrate abundance. However, the *P. cinereus* population most likely inhabited the area shaded by the American beech on the study site and may not have been as affected by changes in the forest floor as those in areas dominated by oaks. Recovery of the forest canopy from 68% in 1989 to 91% in 1993 should have also allowed the salamander's microhabitat to recover. In addition, the increased

amount of coarse woody debris provided additional surface retreats for the territorial *P. cinereus*.

Small mammals, specifically two species of shrews, showed the clearest increase in numbers from 1988 to 1993. Shrews, especially *Blarina brevicauda*, are frequent inhabitants of forest habitats, unlike some rodents (with the exception of *P. leucopus*) which prefer old fields with grass cover (Pagels et al. 1992; Bellows and Mitchell 1999; Bellows et al. 2001). The increase in shrew numbers suggests that habitat changes (e.g., increased openness, oak mortality, downed woody debris) that occurred in 1989 and in the years immediately following were more optimal than these habitats were in 1988. Tomblin and Cranford (1993) showed that habitat quality increased for small mammals in chestnut oak communities following high mortality in response to gypsy moth infestations.

The microbial insecticide *Bacillus thuringiensis* (Bt) is used widely to control gypsy moth damage. The protein crystals specific to lepidopteran guts are not known to directly harm vertebrates (Holmes 1998). Because lepidopteran larvae occurred in low frequencies in terrestrial salamander diets in West Virginia, Raimondo et al. (2003) inferred that salamander populations were not affected directly or indirectly. Although the numbers of *Blarina brevicauda* increased significantly by 1993 in this study, it is not possible to clearly attribute the change to Bt because of the small sample sizes.

(3) The inventory technique used in this study, drift fences with pitfall traps, randomly samples terrestrial vertebrates moving across the forest floor (Bennett et al. 1980). Many of the animals encountering the drift fence were likely transients moving through the study area and many of these were juveniles. Frogs in particular are well known for dispersing widely from breeding sites and can move up to a kilometer or more from their natal site (e.g., Willis et al. 1956; Berven and Grudzein 1990). One anuran (*Anayxrus americanus*) was found about 20 cm underground when digging the pit for one of the buckets, suggesting that it was at least a temporary resident. Salamanders in the genus *Plethodon* (woodland salamanders) remain within small home ranges in hardwood forests and rarely disperse more than a few meters (e.g., Madison 1969; Wells and Wells 1996). The low-density small mammal assemblages that characterized the PWFP study site in 1989 and 1993 may have been comprised of transient animals, as was shown for assemblages in eastern Virginia (Rose and Stankavich 2008).

The null hypotheses that there were no significant differences in amphibian and small mammal species richness and relative abundance before and after gypsy moth defoliation or its control were not supported by the results of this study. Alternative hypotheses that may account for the differences in species composition and species numbers were the changes in habitat caused by oak mortality from gypsy moth defoliation, indirect effects of Bt treatment, and the transient behavior of the frogs and small mammals. The variation in numbers of species and individuals between years was likely due to a combination of all of these factors.

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A Comparison of Survey Methods for Documenting Presence of *Myotis leibii* (Eastern Small-Footed Bats) at Roosting Areas in Western Virginia

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ABSTRACT

Many aspects of foraging and roosting habitat of *Myotis leibii* (Eastern Small-Footed Bat), an emergent rock roosting-obligate, are poorly described. Previous comparisons of effectiveness of acoustic sampling and mist-net captures have not included Eastern Small-Footed Bat. Habitat requirements of this species differ from congeners in the region, and it is unclear whether survey protocols developed for other species are applicable. Using data from three overlapping studies at two sampling sites in western Virginia's central Appalachian Mountains, detection probabilities were examined for three survey methods (acoustic surveys with automated identification of calls, visual searches of rock crevices, and mist-netting) for use in the development of "best practices" for future surveys and monitoring. Observer effects were investigated using an expanded version of visual search data. Results suggested that acoustic surveys with automated call identification are not effective for documenting presence of Eastern Small-Footed Bats on talus slopes (basal detection rate of 0%) even when the species is known to be present. The broadband, high frequency echolocation calls emitted by Eastern Small-Footed Bat may be prone to attenuation by virtue of their high frequencies, and these factors, along with signal reflection, lower echolocation rates or possible misidentification to other bat species over talus slopes may all have contributed to poor acoustic survey success. Visual searches and mist-netting of emergent rock had basal detection probabilities of 91% and 75%, respectively. Success of visual searches varied among observers, but

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detection probability improved with practice. Additionally, visual searches were considerably more economical than mist-netting.

INTRODUCTION

There has been an estimated mortality of more than 6 million bats in the genus *Myotis* in White-Nose Syndrome (WNS) affected areas (Blehert et al. 2009; Ford et al. 2011; Francl et al. 2011; Minnis and Lindner 2013; Puechmaille et al. 2011). This disease has continued to spread across the Northeast into the Appalachians, Midwest and mid-South (Francl et al. 2012), and now is present throughout much of the eastern United States and Canada (U.S. Fish & Wildlife Service 2016a). Undoubtedly, this increased geographic footprint has led to higher overall mortality than original estimates.

Biologists have long relied on capture methods such as mist-netting near roosts or water sources and along flyways to document presence of bats (Kunz et al. 2009). Declines in bat populations due to WNS have made previous standard capture methods largely ineffective for some bat species of conservation concern in WNS-impacted areas (Coleman et al. 2014; Ford et al. 2011). As early as 1994, long before the WNS emergence, the U.S. Geological Survey (USGS) acknowledged a need to resolve questions about bat population status, recognizing that data available from state and federal agencies were insufficient to provide population estimates and assess trends, thereby recommending new sampling strategies (Loeb et al. 2015). Threats of additional population declines and regional extirpation of some bat species from WNS have heightened the need to effectively monitor long-term trends in population status, distribution, and structure of species assemblages within both WNS and presumed future WNS-impacted areas.

The distribution, use of hibernacula, and foraging and roosting habits during the maternity season by *Myotis leibii* (Eastern Small-Footed Bat) were poorly documented prior to WNS, compared to its congeners (Krutzsch 1966; Best and Jennings 1997; Chapman 2007; Johnson et al. 2011). In Virginia, lack of targeted survey efforts and research has led to considerable variability in conclusions about the species' conservation status; including designations as locally abundant in western Virginia (Dalton 1987), uncommon in Virginia (Webster et al. 2003), and greatest conservation need, Tier I Virginia Wildlife Action Plan (Virginia Department of Game and Inland Fisheries 2016). Moreover, reports of declines in population sizes associated with WNS vary among bat species (Hayes 2012). It has been difficult to precisely document declines for Eastern Small-Footed Bats because they often hibernate alone, in small groups, and often in obscure locations opposed to aggregative hibernators such as *Myotis lucifugus* (Little Brown Bats) and *Myotis sodalis* (Indiana Bats; Veilleux 2007:Turner et al. 2011; Francl et al. 2012).

In 2013, the U.S. Fish & Wildlife Service (USFWS) was petitioned to consider listing Eastern Small-Footed Bat as threatened or endangered under the Endangered Species Act (U.S. Fish & Wildlife Service 2014). After reviewing the available scientific information, USFWS (U.S. Fish & Wildlife Service 2013) determined that listing the Eastern Small-Footed Bat was not warranted; however, numerous data gaps were noted that need to be addressed to better understand Eastern Small-Footed Bat ecology and true conservation status.

For most *Myotis* in WNS-impacted areas, acoustic monitoring has emerged as an increasingly-used method to detect presence. Acoustic monitoring requires less effort and mitigates the higher costs, low detection probabilities, and potential false negatives from surveying with mist-nets (Coleman et al. 2014). Accordingly, USFWS now allows acoustic surveys to document presence or presumed absence of the endangered Indiana Bat (Niver et al. 2014) and is currently developing similar guidelines for the threatened *Myotis septentrionalis* (Northern Long-Eared Bat; Mike Armstrong, U.S Fish & Wildlife Service, personal communication). Although mist-netting allows gathering of information on sex ratios, body condition, and reproductive condition (Kunz et al. 2009), acoustic detectors are an attractive alternative sampling tool because they are relatively simple to operate and can collect large amounts of data for extended periods (Morris et al. 2011). Acoustic detectors also are capable of sampling a much larger area than nets (O'Farrell and Gannon 1999), and detection should be less sensitive to abundance, adding to the technique's utility. Even prior to WNS, a combination of sampling methods had been proposed as the most effective monitoring strategy, as this maximized information collected and leveraged the strengths of each method (O'Farrell and Gannon 1999; Patriquin et al. 2003; Flaquer et al. 2007; Robbins et al. 2008). Although acoustic monitoring is effective for many species, a post-WNS study on bat detection probabilities in northwestern New York using opportunistic capture and acoustic methods found that Eastern Small-Footed Bats had substantially lower detection probabilities than other species in that area (Coleman et al. 2014). Because Coleman et al. (2014) focused on Indiana and Little Brown Bats' foraging habitats, the efficacy of acoustic surveys in habitats more likely to be used by Eastern Small-Footed Bats (i.e., emergent rock formations and nearby 1st and 2nd order streams) largely is unknown.

To address the lack of comparisons of detection methods within Eastern Small-Footed Bat roosting areas in the central Appalachians and to aide in the development of "best practices" for future surveys and monitoring, a *post-hoc* comparison of detection probabilities of three survey methods was performed: acoustic surveys with automated identification of calls, visual searching for roosts on emergent rock formations, and mist-netting at sites where Eastern Small-Footed Bats were known to occur. Secondary benefits of each survey method also were considered.

MATERIALS AND METHODS

This *post-hoc* study used Eastern Small-Footed Bat detection data collected during three separate studies from sites in Virginia where Eastern Small-Footed Bats were known to occur. To maximize comparability, the original datasets were reduced to two local sites utilized by all three studies and where Eastern Small-Footed Bats previously had been detected (Moosman et al. 2015). The study sites were post-Pleistocene colluvial fields (talus slopes) in western Virginia. Sites differed in their specific geology and physical setting. Site one, Devil's Marbleyard (hereafter DMY), is a 3.0 ha field of large Antietam quartzite boulders located in the George Washington and Jefferson National Forest in Rockbridge County (37.581332°N, 79.471420°W, datum WGS 84). The DMY is surrounded by a mixed deciduous forest predominated by *Quercus prinus* L. (Chestnut Oak), *Quercus rubra* L. (Northern Red Oak), *Quercus coccinea* (Scarlet oak), *Pinus virginiana* (Virginia Pine), and *Acer rubrum* L. (Red Maple) (Mengak and Castleberry, 2008). Site two is a 3.34 ha talus slope of smaller

scree composed of quartzite with some larger boulders located within the Sherando Lake's Recreation Area (hereafter Sherando) of the George Washington and Jefferson National Forest in Augusta County (37.929370°N, -79.004356°W, datum WGS 84). Sherando is surrounded by a mixed deciduous forest similar to that surrounding DMY.

As a capture baseline, mist-net data were collected during June 2009 and July 2014 (Moosman et al. 2015), and visual search and acoustic data were collected between June and August 2014. Mist-nets were deployed with 38-mm mesh in two manners. Two 12-m-long x 3-m-high nets end to end directly on the talus slope were deployed at DMY because the location lacked corridors conventionally considered suitable for surveys with mist-nets. Mist-nets were placed perpendicular to the forest edge extending toward the center of the boulder field. Mist-nets were deployed 15 min before sunset for a duration of 1.5 hours. Mist-nets at Sherando followed conventional placements. Two stacked 6-9-m-long nets were placed more than 30 m apart adjacent to the talus slope where Eastern Small-Footed Bats had access to the stream corridor. Mist-nets were deployed 15 min before sunset for a duration of 4.25 hours. Captured Eastern Small-Footed Bats were individually weighed to the nearest 0.1 g using a spring scale (Pesola AG, Baar, Switzerland[1]). Sex, age, and reproductive state were recorded for each Eastern Small-Footed Bat and a numbered aluminum band (Porzana Limited, East Sussex, UK) was placed on the forearm of each Eastern Small-Footed Bat and then subsequently released.

Occurrence data were gathered using visual surveys. The survey team visually searched for Eastern Small-Footed Bats in crevices using penlights (Energizer Holdings, Inc., St. Louis, Missouri) over the length and width of the survey area by means of belt transects. Belt transects followed a defined azimuth between two points, yet were adapted to allow transects to be bent in response to impassable areas (e.g. large gaps, rock faces, dangerous footing). The survey team performed simultaneous visual searches on different transects separated by 3 m and walked laterally across slopes from tree edge to adjacent edge. Once the adjacent edge was reached, the survey team started a new transect 3 m above or below the outmost completed transect. This was repeated until the entire rock slope was surveyed. Eastern Small-Footed Bats were not handled during visual surveys.

Passive acoustic surveys were conducted using Song Meter SM2BAT+ detectors set on zero-crossing/frequency division recording (Wildlife Acoustics Inc., Maynard, Massachusetts). Recordings were started an hour before dusk, and ended an hour after dawn. Talus slopes at DMY and Sherando were acoustically sampled and independence was maintained among detectors. Two detectors were placed on DMY. Both detectors were placed on the forest edge each fastened to a tree at a height of 2 m using bungee cords (The Original Bungee Cord Company, Anaheim, California). One detector was placed on the southeast forested edge of DMY with the microphone facing northwest towards the talus slope. A second DMY detector was placed on the western forested edge facing northeast towards the talus slope. Five detectors were placed at Sherando. Three detectors were placed adjacent to the colluvial field on the forest edge each fastened to a tree at a height of 2 m using bungee cords. One Sherando detector was

[1] Use of trade, product, or firm names does not imply endorsement by the US government.

placed on the northeastern edge of the talus slope facing southwest towards the talus slope. A second detector was placed on the southernmost edge of the talus slope facing northwest towards the talus slope. The third detector was placed on the southwestern edge with the microphone facing east towards the talus slope. Two additional Sherando detectors were placed on their sides secured with bungee cords to boulders directly on the talus slope. One was placed within the northern one-third of northeastern talus slope roughly 50 m from either forest edge facing east. The other was placed within the middle of the northeastern talus slope 20 m from either edge facing south.

Bat calls were analyzed using Kaleidoscope Pro 2.2.2 software (Wildlife Acoustics Inc., Maynard, Massachusetts) using the U.S. Fish & Wildlife (Ford 2014) standards with sensitivity set at negative 1, signal parameters at 5-120 kHz and 2-500 ms. Minimum pulses were 3 with species classifier pool set to include Eastern Small-Footed Bat, Northern Long-Eared Bat, Little Brown Bat, Indiana Bat, *Eptesicus fuscus* (Big Brown Bat), *Lasiurus borealis* (Eastern Red Bat), *Lasiurus cinereus* (Hoary Bat), and *Perimyotis subflavus* (Tri-colored Bat).

Detection methods were compared by calculating detection probability for each data type using a single-season, single-species occupancy model; detection probability models were fit using program PRESENCE version 8.0 (Hines and McKenzie 2002; MacKenzie et al. 2002). Considering Eastern Small-Footed Bats were known to occur at the two study areas, occupancy (Φ) was fixed to one. An exploratory analysis of an expanded version of the visual detection dataset was performed to examine interpersonal variance in detection rates, also using a single-season, single-species occupancy model.

RESULTS

Visual surveys found 62 Eastern Small-Footed Bats, 10 at Sherando and 52 at DMY during the summer of 2014. No other bat species were found by visual searches in rock crevices at Sherando and DMY. The three-person survey team visually searched 13.5 hours at Sherando and 37.8 hours at DMY for a total of 51.33 hours. Mist-netting efforts captured a total of 39 Eastern Small Footed-Bats between the two sites between the summers of June of 2009 and July of 2014. At Sherando, mist-netting efforts captured 6 Eastern Small-Footed Bats, 10 Northern Long-Eared Bats, 2 Big Brown Bats, 4 Eastern Red Bats, 1 Hoary Bat, 1 Tri-Colored Bat and 2 *Lasionycteris noctivagans* (Silver-haired Bats). At DMY, 33 Eastern Small-Footed Bats were captured. No other bat species were captured at DMY. The time spent mist-netting was 43.22 hours at Sherando and 12.24 hours at DMY for a total of 55.46 hours mist-netting with two people netting (Moosman et al. 2015). Lastly, analysis of the calls recorded by the 5 detectors at Sherando and the 2 detectors at DMY did not yield definitive detection of Eastern Small-Footed Bats in 392 total detector-hours over 7 nights per accepted USFWS acoustic monitoring guidance (U.S. Fish & Wildlife Service 2016b). A total of 4446 echolocation passes at 7 survey points between DMY and Sherando were recorded including 15 Big Brown Bat passes, 183 Eastern Red Bat passes, 21 Hoary Bat passes, 9 Little Brown Bat passes, 927 Northern Long-Eared Bat passes, 24 Tri-Colored Bat passes, and 3267 passes not identified because of poor call quality or insufficient call duration.

Detection probabilities varied among sampling methods. Basal detection probabilities of 91% for visual searches, 75% for mist-netting, and 0% for acoustic

surveys were found (Figure 1). Visual detection probability varied among surveyors, but all improved with each subsequent site visit.

DISCUSSION

Visual surveys produced the highest detection probability of any of the sample methods used. It should be noted that mist-netting on the rocks was conducted for 1.5 hours at Sherando and 4.25 hours at DMY rather than sitting with nets open for multiple hours as is typical protocol at both sites when mist-netting corridors.

Prior to this study, research by Coleman et al. (2014) suggested that passive acoustic sampling was more efficacious than active acoustic sampling or mist-netting when surveying for Indiana Bats or Little Brown Bats. Similarly, Murray et al. (1999) noted that passive detection using bat detectors to determine site-level species richness values was typically more effective than mist-netting and generally documented more extant species at a location. Although accurate for the species detected, the general recommendation of passive acoustics by Coleman et al. (2014) and Murray et al. (1999) clearly is not supported for Eastern Small-Footed Bats, at least in or near emergent rock habitats. Eastern Small-footed Bats are challenging to detect acoustically as supported by the lack of acoustic detection at known occupied roosts and the lack of detection by Coleman et al. (2014).

Misidentification by Kaleidoscope Pro 2.2.2 software also could have occurred. There was a large number of Northern Long-eared bat calls identified by Kaleidoscope. Northern Long-eared bats have similar echolocation call characteristics to Eastern Small- footed Bat calls, and it is possible that some of these calls were Eastern Small-Footed Bat calls that were misidentified as Northern Long-eared bats. However, Ford (2014) showed that overall correct classification rates of Eastern Small-Footed Bats generally exceed 90% with low mis-classification overlap for Northern Long-eared Bats – the species we would presume from our findings to have been the plausible source for errors of omission.

A suite of reasons is likely to have contributed to the lack of acoustic detection including variability among detector sites (e.g. vegetative clutter, wind), atmospheric attenuation, frequency and amplitude of the bat, and the directionality of the bat call itself (Griffin 1971; Lawrence and Simmons 1982; Fricke 1984; Fenton et al. 1998; Larson and Hayes 2000; Murray et al. 2001; Scott et al. 2010; Adams et al. 2012). The high frequency echolocation calls of this species (Mukhida et al. 2004) increase the difficulty of its detection, as high frequency echolocation calls attenuate more than lower frequency calls (Griffin 1971; Lawrence and Simmons 1982; Fricke 1984), and emergent rock habitats with complex and angular shapes probably promoted signal reflection that degraded call quality (Winkler and Murphy 1995; Agranat 2014). Approximately 42% of echolocation passes recorded were unable to be assigned to bat species which is strongly indicative poor call quality. Moreover, it also is unknown if Eastern Small-Footed Bats engage in search-phase echolocation over rocks when they emerge. There is ample evidence that bats employ visual cues when navigating (Ellins and Masterson 1974; Horowitz et al. 2004), so it may be that Eastern Small-Footed Bats navigate primarily by sight or memory when exiting roosts and commuting over the large open expanses of talus/colluvial fields to forest edges to commence foraging activity. Because talus slopes are relatively more reflective and less shaded at night as compared to the surrounding forest edge, there may be less need to

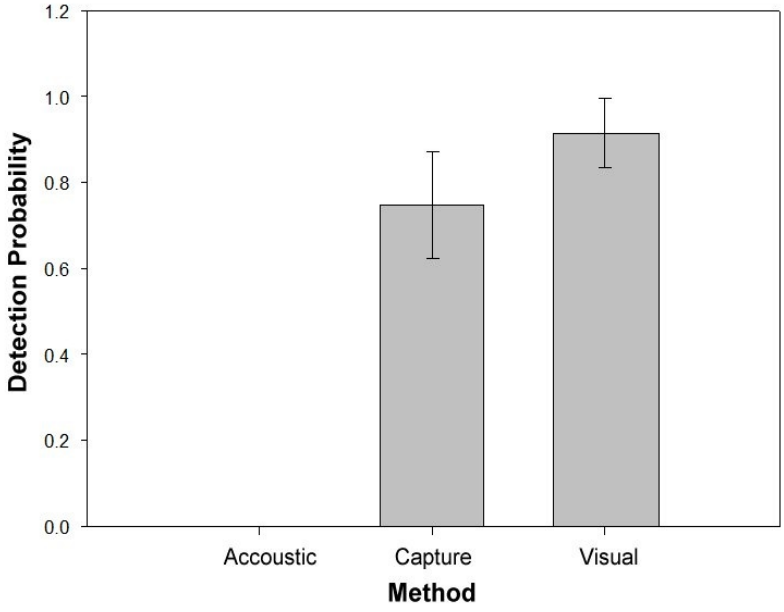

FIGURE 1. Detection probability estimates by method from single-season, single species models for Eastern Small-Footed Bat (*Myotis leibii*) at 2 sites in western Virginia. 'Acoustic' refers to passive sampling using acoustic detection meters. 'Capture' refers to mist-netting conducted June 2009 and July 2014. Visual searches and acoustic recordings were conducted June-August 2014. Overlapping error bars between 'Capture' and 'Visual' depict no significant difference between these two methods. Occupancy (Ψ) was fixed to 1 because Eastern Small-Footed Bat were known to exist at the 2 study areas.

echolocate to navigate around the large rock obstacles although this merits additional work to fully demonstrate.

Detection of rare bat species often requires considerable efforts and incurs substantial monetary costs (Weller 2008). Detection of changes in population status of bats also is difficult due to the limited recapture rates (Schorr et al. 2014). Although acoustic monitoring is a more efficient and cost effective tool for estimating occupancy and detection probability than traditional netting, these results strongly suggest that acoustic monitoring Eastern Small-Footed Bat and automated call identification software such as Kaleidoscope may not be the most accurate technique for determining Eastern Small-Footed Bat presence in these habitat types.

Mist-netting is an adaptable bat survey technique, but it is necessary to consider roosting habits, movement and bat ecology to choose the correct deployment strategy that maximizes the chance of capture (Carroll et al. 2002; Brack Jr et al. 2004; Kunz et al. 2009). In the eastern United States, bat assemblages often have been documented

using Indiana Bat survey protocols (Winhold and Kurta 2008) leading to possible netting bias. Using Indiana Bat survey protocols reduces the chances of collecting other bat species with disparate foraging habits or habitat associations (Larsen et al. 2007). Currently no such standardized protocol exists for documenting Eastern Small-Footed Bat occurrence.

Visual surveys in this study had the highest detection probability, and had an added utility in that it is relatively non-invasive to examine crevices to determine whether Eastern Small-Footed Bats are present. Visual surveys likely reduce the stress to individual bats because they are not handled. Likewise, visually confirming Eastern Small-Footed Bat presence at roosting sites provides an opportunity to accrue additional data about Eastern Small-Footed Bat day-roost ecology and habitat that otherwise would be impossible to obtain without radio-tracking subsequent to mist-net capture. In addition, visual searches provide the potential for development and deployment of population size estimation and mark-recapture efforts (Moosman and Warner 2014). Success during visual searches varied among observers, but detection probability during visual searches improved with additional site visits. As is supported by cognitive theory, visual searchers become more proficient and efficient with practice (Lawson and Shen 1998). Techniques used in this study are similar to avian nest-searching methods described widely in the literature (Nichols et al. 1986). For example, ornithologists became more efficient at finding nests over time (Powell et al. 2005; Gervasi et al. 2014). Furthermore, increasing the skill of visual searchers would improve cost effectiveness of the technique through reduction in-person hours necessary to denote occurrence at a given location.

CONCLUSION

The results suggest that visual searches are an efficient way to detect and monitor Eastern Small-Footed Bats. The utility of visual searches depends on specific monitoring needs, with visual searches potentially offering a more efficient method, particularly if the objective is to document occurrence and habitat associations of this species. However, detection probabilities for this species probably will vary with the size, configuration and accessibility of the talus slope. Because many aspects of the roosting behavior of Eastern Small-Footed Bats have not been extensively studied, numerous questions remain. Visual searches were effective for the talus slopes we surveyed, but many emergent rock formations in the Appalachians are not conducive to this survey method. For instance, using visual searches of cliffline habitats in the central Appalachians, e.g., New River Gorge in West Virginia, will not be possible without specialized rock climbing equipment, and thus a change in method and additional personnel training. These results highlight the need to continue to refine Eastern Small-Footed Bat survey protocols. Since the use of acoustic monitoring has gained acceptance, and Eastern Small-Footed Bats were listed as a species of greatest conservation need Tier I rank in the Virginia Wildlife Action Plan (Virginia Department of Game and Inland Fisheries 2016), this is particularly relevant for managers relying on acoustics to understand potential biases resulting from false negatives in their surveys.

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Statement of Responsibility: John K. Huth was the primary investigator and involved in all aspects of the project. Alexander Silvis was involved in data collection and running statistical analyses. Paul R. Moosman, Jr. provided mist-net data for comparison with the other methods and editing. W. Mark Ford provided advice about study design, statistical analysis and editing. Sara Sweeten provided guidance and analysis with program PRESENCE.

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Pesticide Analysis in Vegetables Using QuEChERS Extraction and Colorimetric Detection

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ABSTRACT

A novel combination of extraction and detection methods is demonstrated for pesticide residue analysis in vegetable samples. Acetylcholinesterase (AChE) inhibition was used as a simple colorimetric test for organophosphates/carbamates (OP/C), and was tested with extracts from the widely-used QuEChERS extraction method. In the absence of pesticide, diluted (50% with water) acetonitrile did not inhibit enzyme activity, demonstrating the compatibility of this extraction solvent with the AChE inhibition test. QuEChERS extraction of chlorpyrifos-spiked tomato, spinach and lettuce samples indicated a high sensitivity to OP/C, with AChE inhibition occurring in the ppb range. The applicability of this method combination was tested by screening tomatoes from 18 different sources, including private gardens, farmer's market venders, and local supermarkets. Tomatoes from one private garden, three "certified naturally grown" farmer's market venders and two "organic" supermarket source had AChE inhibition significantly above nominally pesticide-free controls, suggesting the presence of OP/C residue. These residues were likely below levels of health concern, as indicated by lack of complete AChE inhibition, and the absence of inhibition upon sample dilution. This study demonstrates that the combination of QuEChERS extraction and AChE-inhibition detection provides a relatively simple and inexpensive alternative for detection of OP/C in vegetable samples.

Keywords: QuEChERS, vegetable, pesticides, acetylcholinesterase

INTRODUCTION

Although organic food production has become more prevalent, the production of vegetables is still largely dependent on the use of pesticides such as organophosphates (e.g., Jaipieam et al. 2009), which can clearly present a health risk to consumers (Kamanyire and Karalliedde 2004). In an attempt to keep consumer exposure to these pesticides below levels of health concern, monitoring programs have been established in many countries. While routine, residue monitoring does remain a relatively expensive and complex process, typically relying on chromatographic techniques for

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detection. This limits the ability to effectively monitor pesticides in situations with inadequate resources, such as in developing countries. Unfortunately, these are locales where risks from pesticide exposure are of greatest concern due to increased use (Nweke and Sanders 2009), weak regulation, poor education about safe application practices (Williamson et al. 2008), and/or increased reliance on crop foods (rather than meat) as a critical dietary component. Studies of pesticide residues in developing countries illustrate situations where pesticide residues are routinely found on market vegetables (e.g., Amoah et al. 2009; Srivastava et al. 2011; Hossain et al. 2015). Thus, pesticide monitoring is most difficult in countries where that monitoring is arguably most needed. As has been noted by other authors, an effective, simple, and inexpensive method is needed to enable environmental analysis in situations of limited resources (Hennion and Barcelo 1998; Mallat et al. 2001; Qian et al. 2009; Xu et al. 2012).

Assessment of pesticide levels is a two-step process: extraction & detection. Extraction is now relatively simple and cheap due to the recent development of the QuEChERS extraction method ("Quick, Easy, Cheap, Effective, Rugged, Safe") (Anastassidoes et al. 2003). As a consequence of its advantages over conventional extraction techniques, QuEChERS has now become the extraction method of choice, and numerous studies exist demonstrating its utility for the extraction of a wide array of chemical compounds from many foodstuffs (e.g., Lehotay 2007; Lesueur et al. 2008; Nguyen et al. 2008; Koesukwiwat et al. 2010). While QuEChERS has simplified the extraction process, the pesticide detection step remains a relatively expensive and complex process. Two detection methods are typically used: gas (GC) or liquid chromatography (LC) coupled with mass spectrometry (MS) (e.g., Lesueur et al. 2008; Nguyen et al. 2008). While routine, specific and precise, these detection methods are less feasible in situations of limited resources or where a faster screening process is desired.

Enzyme-based detection methods, such as ELISA or acetylcholinesterase (AChE) inhibition tests, present an alternative method for monitoring pesticides, and have been used for monitoring pesticides in vegetables (Watanabe et al. 2006; Graber Neufeld et al. 2010), water samples (Mallat et al. 2001), and in human samples (Nweke and Sanders 2009; Worek et al. 2012). In some cases such tests are used as pre-screening tests, reducing the number of samples tested using more complex means (Moris et al. 1995; Hennion and Barcelo 1998). Enzyme-based tests are typically faster and less expensive, and often have high specificity and sensitivity (e.g., Qian et al. 2009; Wang et al. 2011). However, enzyme-based tests with vegetables generally utilize external washes (which do not detect pesticides accumulated inside the plant tissue), or crude extracts which can result in more pronounced matrix effects. Matrix effects vary with both the type of vegetable tested and the specific test kit used, and the resulting dilution of samples to avoid these matrix effects reduces the limit of detection for this assay (Xu et al. 2012). The applicability of enzyme-based tests with unprocessed extracts is thus limited. This limitation could be circumvented by applying a clean-up procedure, such as QuEChERS. However, to date none of the published studies on enzyme-based detection methods for pesticides have utilized these two techniques in concert to simplify the process of pesticide monitoring. The present study verifies the utility of combining the QuEChERS extraction method with the AChE inhibition test, a broad-specificity test for OP/C pesticide, and demonstrates its applicability in the screening of market vegetables.

MATERIALS AND METHODS

Samples

Single samples of supermarket tomato, spinach and lettuce that were certified organic were used as our control samples. These samples were used as nominally pesticide-free samples to assess the effect on enzyme activity of spiking with chlorpyrifos, and to measure enzyme activity from extracts of other organic and nonorganic vegetable samples. Tomato samples for our local survey were purchased from the Harrisonburg (Virginia) farmer's market, from local supermarkets, or collected from local gardens (private residence, and campus garden for Eastern Mennonite University). Samples were frozen (-20ºC) until analysis.

Extraction Method

All samples were extracted using the QuEChERS extraction technique (Anastassiades et al. 2003), which resulted in collection of both surface and internal pesticide residues. In brief, samples were ground in a mortar, and 5g aliquots of the ground sample were then transferred to 50 ml Eppendorf tubes. In rapid succession, 5 ml acetonitrile (HPLC grade), 2 g anhydrous $MgSO_4$, and 0.5 g anhydrous sodium acetate were added to the sample. After capping and shaking the sample vigorously for 1 minute, the sample was spun at 1500 rpm for 2 min. Dispersive solid phase extraction (SPE) was performed by combining 182.5 mg SPE sorbent (Supelco PSA/ENVI-Carb 55233-U) with 1 ml aliquot of the top acetonitrile layer. After vortexing 20 seconds, vials were centrifuged at 3000 rpm for 1 minute. At this point vegetable pigment was no longer visible in the extraction solution (Figure 1). The supernatant was transferred to a clean tube and analyzed immediately with the AChE test (see below).

Detection Method

Pesticide detection was performed using a colorimetric commercial test kit (Organophosphate / Carbamate Screen Kit, PN 550055; Abraxis LLC; Warminster, PA) based on AChE inhibition. The test, a modification of the standard Ellman method (Ellman 1960), produces a yellow color in the presence of AChE activity. Color was quantified with a spectrophotometer; a decrease in absorbance at 405 nm indicated AChE inhibition in the presence of pesticide residue. Two controls (provided with the kit) were run with each sample batch: a negative control (no pesticide), and a positive control (5 ppb diazinon). Percent inhibition was calculated as (absorbance of negative control – absorbance of sample) / (absorbance of negative control – absorbance of positive control). All QuEChERS extractions (in acetronitrile) were diluted to 50% with HPLC-grade water.

RESULTS

Extraction solvent

QuEChERS typically uses acetonitrile as an extraction matrix (Lehotay et al. 2010), whereas the AChE inhibition assay is based the use of a 50% methanol extract. AChE activity was therefore tested in the presence of acetonitrile to establish its compatibility with this typical QuEChERS solvent. Enzyme activity was significantly inhibited ($p<0.05$; paired t-test) by the presence of 100% acetonitrile (Figure 2). When this extract was diluted to 50% with HPLC-grade water, enzyme inhibition was not significantly different from that occurring in the stock solvent (50% methanol). Dilution of QuEChERS extracts to 50% acetonitrile is therefore compatible with using the

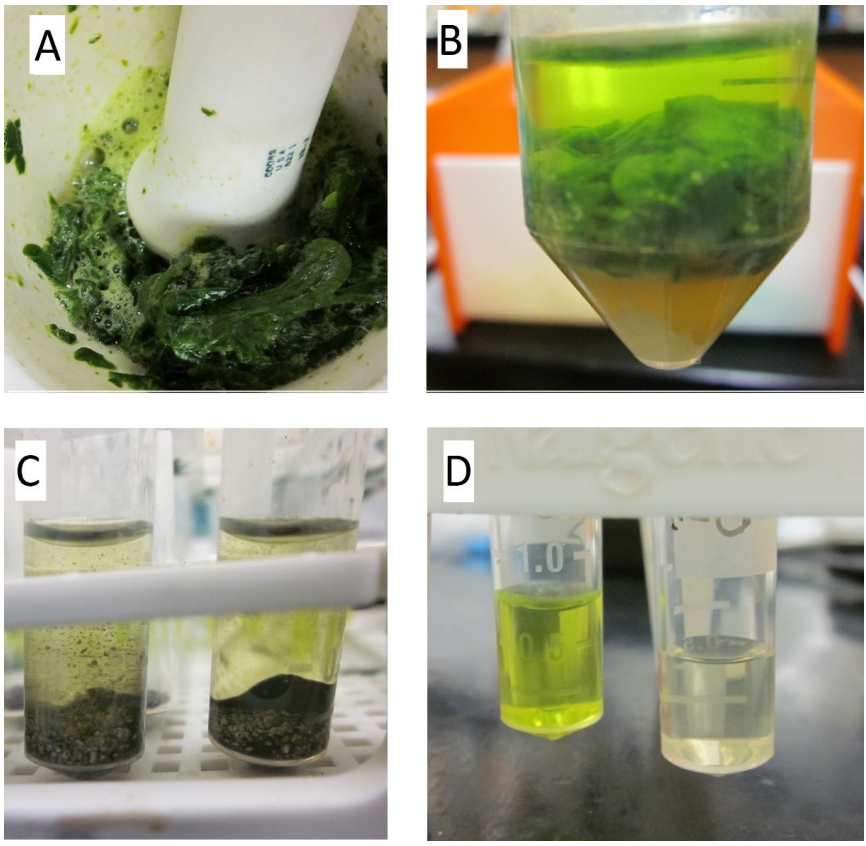

FIGURE 1. Example of QuEChERS procedure as applied to spinach sample. The sample is ground (A), extracted in acetonitrile (B), and placed in SPE sorbent (C). The final extract (D, right tube; cf left tube prior to sorbent exposure) has pigments removed which would otherwise interfere with the colorimetric assay.

AChE inhibition assay, and all subsequent tests (samples, blanks and standards) were performed with 50% acetonitrile.

Assay Sensitivity

Nominally pesticide-free spinach and tomato samples ("certified organic" labeled vegetables from the supermarket) were extracted using QuEChERS and tested with the AChE inhibition assay. A low, but significant ($p<0.05$; paired t-test, extracts compared with negative control), level of enzyme inhibition was observed in these samples (Figure 3; "0 ppb chlorpyrifos"). AChE was inhibited when tomatoes, spinach, or lettuce were spiked (prior to extraction) with chlorpyrifos, a representative OP/C. Significant inhibition ($p<0.05$; Dunnett's test) occurred down to the ppb range (Figure 3).

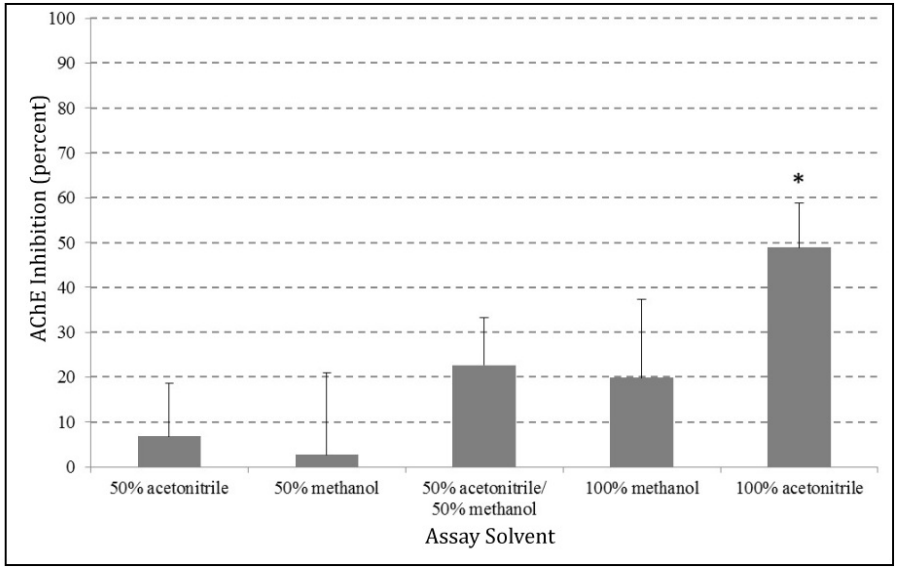

FIGURE 2. Inhibition of acetylcholinesterase activity by extraction solvents, in absence of pesticide residues, demonstrating assay compatibility with typical QuEChERS solvent (acetonitrile) when diluted to 50% with water (Asterisk indicates statistically significant inhibition of enzyme activity in indicated solvent relative to stock negative control, N=3-9 for each category).

Duplicate and triplicate tests of samples suggest that the use of the AChE inhibition assay with QuEChERS samples has a precision comparable to that of QuEChERS used with traditional chromatography techniques, as indicated by calculated relative standard deviation (RSD) and relative percent difference (RPD). The enzyme assay alone had RSDs of 4.75%, 8.57%, and 7.77% when testing 50% methanol negative controls, 50% acetonitrile negative controls, and a 50% methanol positive control, respectively. Market samples of tomatoes measured in duplicate with the enzyme assay had an average RPD of 19.5%. Thus, measured differences in samples (e.g., different tomatoes from a single source) reflect both real differences in those tomatoes, and some difference associated with this estimated level of precision.

Background signal

Nominally pesticide-free samples exhibited inhibition of AChE ("0 ppb" samples; Figure 3). We did not have access to reference standards in which a vegetable sample would be laboratory certified as pesticide free; we therefore could not be assured that pesticides were in fact absent from the nominally pesticide-free samples. However, serial dilutions of tomato samples from three venders that sold certified naturally grown ("organic") tomatoes still had baseline inhibition, even when diluted by 1000x with acetonitrile (Figure 4). Duplicate blank QuEChERS samples (samples processed without the addition of any vegetable) had an inhibition of 19.3%. These results strongly suggest that there is a normal background inhibition of AChE in these samples which is associated with QuEChERS processing (perhaps solvent), and that pesticides

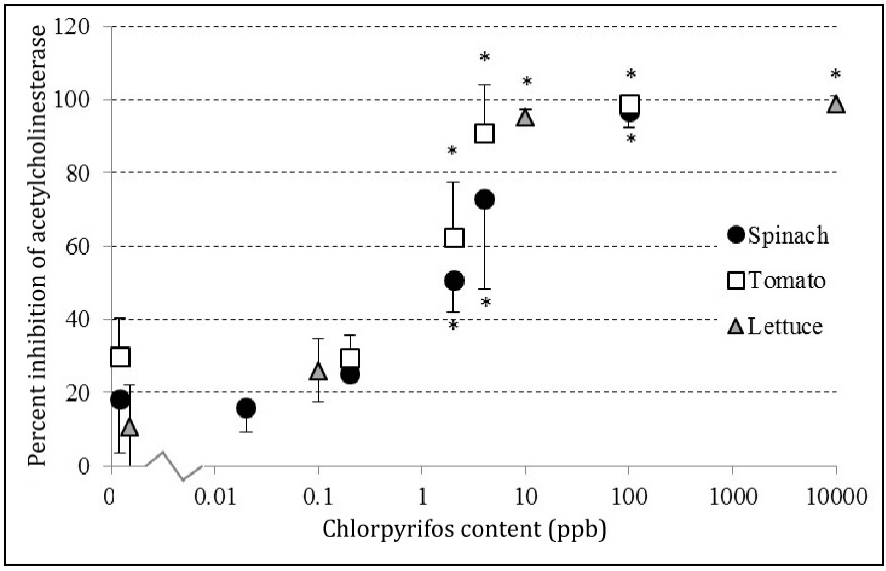

FIGURE 3. Inhibition of AChE by varying concentrations of chlorpyrifos, a representative organophosphate pesticide (Asterisks indicate samples with significantly more enzyme inhibition than unspiked samples; N=3-4 for each category).

were below the detection limit in the nominally pesticide-free samples used for creating a calibration curve (Figure 3). Pesticide presence is therefore indicated by inhibition above a baseline level, rather than simply the presence of any inhibition.

Comparing pesticides in market tomatoes

To demonstrate the applicability of this combination of techniques, tomatoes from several sources in the Harrisonburg, Virginia area were analyzed for pesticide residues. The sampling focused in particular on testing whether pesticide residues would be detected in tomatoes from sources that would be expected to be pesticide free (private gardens where pesticides were not used, from the local farmers market, or organically labeled tomatoes from local grocery stores). Screening of multiple tomatoes from each site indicated that 6 out of the 18 sources had AChE inhibition (Figure 5) significantly above control levels ($p<0.05$, Dunnett's test). Elevated AChE inhibition in 6 "organic" sources suggests the presence of pesticide residues in samples from some growers that do not themselves use pesticides. One sample (campus garden) had a marginally ($p=0.04$) lower signal than control; likely this was due to a single anomalously low sample.

The average relative standard deviation between tomatoes from the same source was 29.8%, suggesting that tomatoes from the same source often have similar levels of pesticides. However, individual tomatoes from a source varied considerably in some cases (e.g., Vender G), suggesting that individual tomatoes can vary in their residue levels even from a single source, and sampling design for screening from vegetable

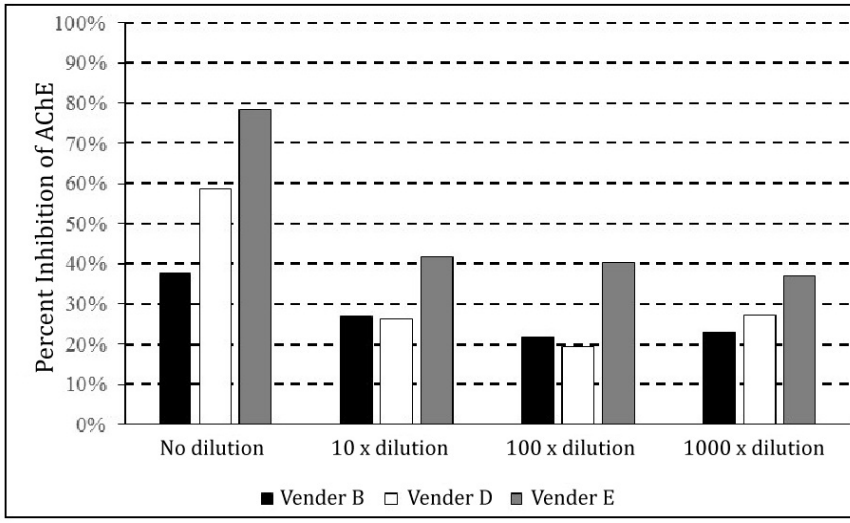

FIGURE 4. Serial dilutions of samples (with acetonitrile) versus percent inhibition, depicting the percent inhibition as samples are diluted. Each data point is the average of duplicate samples.

sources should therefore include multiple samples from each source to account for this variability.

DISCUSSION

This is the first demonstration that the widely-used QuEChERS extraction technique can be utilized in combination with a relatively simple enyzme-based detection assay for a pesticide. The AChE assay was compatible with QuEChERS acetonitrile extracts, and showed a sensitivity and precision comparable to traditional chromatography methods. Clean-up with QuEChERS, followed by dilution to 50% acetonitrile, provided an extract that could be used directly for a relatively quick and inexpensive detection of OP/C using the AChE inhibition test. ELISA assays have been used for a range of specific pesticides (Hennion and Barcelo 1998), and further studies should investigate the similar application of QuEChERS extracts with these tests.

The level of sensitivity is comparable to that of chromatographic methods (Malik et al. 2010; Srivastava et al. 2011), and similar to that found both in pesticide ELISA tests (e.g., Qian et al. 2009) and in other tests of AChE inhibition (Xu et al. 2012). It is also one or two orders of magnitude lower than established maximum residue limits for chlorpyrifos in vegetables (0.05 to 0.5 ppm, European Commission, 2008; 0.01 to 2 ppm, Codex, 2010). Partial inhibition of enzyme occurs over a relatively narrow range of concentrations; total inhibition occurred by the time concentrations reach 10 ppb. Thus, although it is a sensitive technique that easily detects the presence of low concentrations of chlorpyrifos, the lack of a large linear range of response makes it less straightforward for determining exact concentrations. Multiple tests with serial dilution of samples could be used to estimate the concentration in a sample (for example, see

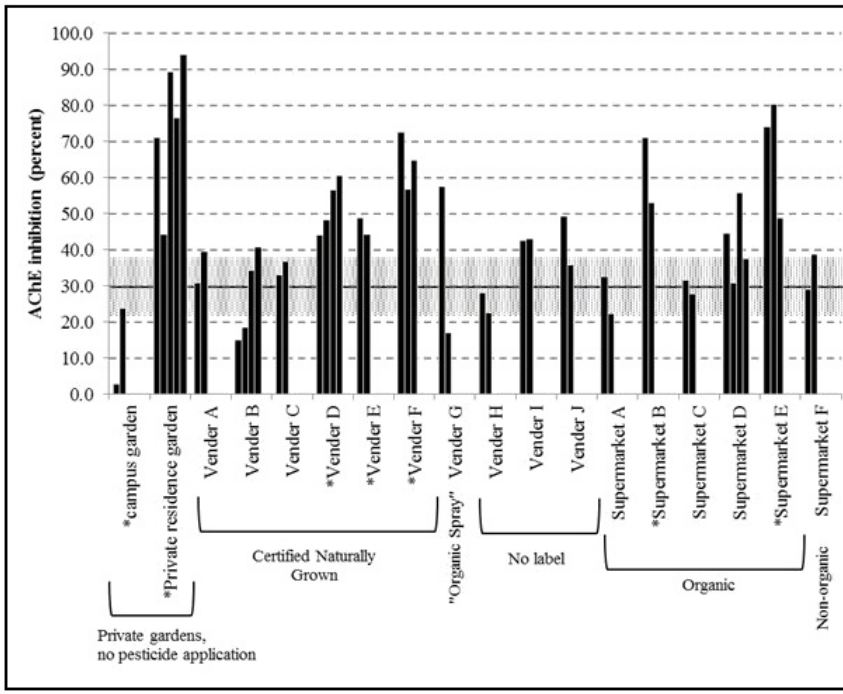

FIGURE 5. AChE inhibition in individual tomatoes from 18 sources in the Harrisonburg area, grouped according to location (private gardens, venders at farmer's market or supermarket), and labelling. The mean and standard deviation for control (nominally no pesticides) is indicated by the horizontal line and shaded box; asterisks indicate sites significantly different from these control samples.

Figure 4), or the technique may be used as a non-quantitative manner either for initial screening (e.g., identifying samples for later more detailed analysis), or for indicating the general presence/absence of pesticide residues. In addition, the precision (as indicated by the RSD) of the current technique is similar to the precision in studies utilizing QuEChERS with GC/MS and HPLC (Lesueur et al. 2008; Lehotay et al. 2010). Taken together, the combined QuEChERS and ELISA method is thus comparable to the QuEChERS used with chromatography methods.

Although residues were indicated from six sources, the presence of residues indicated by this test do not necessarily indicate residue levels are of health concern. In fact, none of the samples showed full inhibition of AChE, suggesting that residue levels were actually quite low, less than what would be equivalent to 10 ppb chlorpyrifos. For two sources that had significant residue levels (Venders D and E), we took a semi-quantitative approach to further estimating the residue levels which further suggested relatively low pesticide levels. Dilution of these samples by 10x reduced AChE inhibition to control levels (Figure 4) and dilution to 100x and 1000x the original concentration did not cause any further decrease in inhibition (consistent with the

observation of a background level of inhibition). The results indicate the utility of serial dilutions in using this assay in a semi-quantitative manner that could match screening results with levels of health concern.

It is somewhat surprising to detect pesticides in these tomato samples, given that all samples where pesticides were detected were labeled as being free of pesticides. The route of pesticide contamination for tomato samples in this study is not known, but there are multiple possible sources, including pesticide drift from neighboring fields, transport-related contamination, and inaccurate product labeling. While there is considerable emphasis on production of vegetables without pesticide use, the presence of residue on certified “pesticide-free” tomatoes suggest that there should be more consideration given to other avenues by which pesticide residues may lodge on vegetables. For instance, the large amount of pesticide that does not reach its target (Pimentel 1995) could represent a significant source of contamination for organic vegetables. Overall, our screening of 18 tomato samples demonstrates the successful application of this combination of rapid and inexpensive extraction and detection methods.

There are several potential limitations to the current methodology. The AChE inhibition test is non-specific for OP/C, and the specific identity of residues is therefore not indicated. The assay responds to all OP/C, but to a greater or lesser amount depending on the specific compound (Xu et al. 2012). Specific concentrations can only be reported if the exact pesticide is known, or if concentrations are reported as (for instance) "chlorpyrifos equivalent". However, the degree of AChE inhibition is arguably the more relevant parameter, as an indicator of actual toxicity regardless of the specific compound. A second limitation is the general reliance of QuEChERS on acetonitrile as an extractant. Acetonitrile may be more difficult to obtain in developing countries, especially given the worldwide fluctuations in acetonitrile availability. Further work should be done on other solvents that have been used with the QuEChERS method, such as ethyl acetate, which is more widely available and less toxic than acetonitrile (Lehotay et al. 2010). Finally, additional work might help to refine the technique for additional precision at these low concentrations.

While chromatographic techniques are recognized as the "gold standard" for pesticide analysis, we suggest that enzyme-based assays (AChE or ELISA) with cleaned-up samples provide distinct advantages under certain circumstances. Under conditions where resources are limited (e.g., developing countries), this method has the potential to be used with a lower investment of resources and training. Even in situations where chromatographic detection is possible, initial screening of samples with the assay test would reduce the time and expense associated with monitoring efforts (Mallat et al. 2001). The use of simple and inexpensive analytical techniques such as demonstrated in this study could thus facilitate monitoring in situations where pesticide residues are of concern.

ACKNOWLEDGEMENTS

Work for this study was performed in the laboratory of Dr. D. S. G. Neufeld, who is the primary author for this article. All authors contributed substantially to data collection and analysis.

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Virginia Academy of Science 2015
Fall Undergraduate Research Meeting

The Fall Undergraduate Research Meeting is a research grant proposal competition which has been held annually since 2001. Undergraduate students, in conjunction with their faculty mentors, develop and submit research grant applications in early October and subsequently present posters outlining their proposed research projects at the Fall Undergraduate Research Meeting in late October. The VAS President-Elect serves as the coordinator for the Fall Undergraduate Research Meeting.

This year 34 Undergraduate Research Grant Applications were submitted by 52 students, in conjunction with their mentors (20 total), from 11 Virginia colleges and universities. Approximately half of the applications were submitted by individual students; the remainder of the applications were submitted by teams of 2 or 3 students.

The Fall Undergraduate Research Meeting was held in the L. Douglas Wilder Building at Virginia State University in Petersburg, VA on Saturday, October 24, 2015. The total attendance at this meeting was 85 (a record for the Fall Meeting). During the morning session, the judges met with the student presenters at each of the 34 posters. The students gave a brief summary of their proposed research project/poster and then responded to questions from the judges.

During the Lunch Break, the judges met to select the recipients of the 2015-2016 Undergraduate Research Grant Awards ($500 each). The final selection of the recipients was based on the judges' evaluations of the previously submitted grant applications and the posters presented at the Fall Meeting, as well as the responses of the students to their questions.

At the beginning of the afternoon session, attendees were welcomed to Virginia State University by David Crosby (Cooperative Extension and VAS Immediate Past President) and Franklin Jackson (Associate Dean, Cooperative Extension). Craig Bayse from the Dept. of Chemistry & Biochemistry at Old Dominion University was the invited speaker. His presentation topic was *Crossing between Art and Science: How Chemistry Can Answer Questions about a 16th Century Painting.*

At the end of the afternoon session, VAS President-Elect and Program Chair for the 2015 Fall Undergraduate Research Meeting Deborah Neely-Fisher (Reynolds Community College) announced the recipients of 2015-2016 Undergraduate Research Grants ($500). The recipients of the five grants were also awarded student membership in the Virginia Academy of Science for 2016 and expected to present the results of their completed research at the 2016 VAS Annual Meeting in May at University of Mary Washington in Fredericksburg.

Participating Institutions

Christopher Newport University
Eastern Mennonite University
George Mason University
James Madison University
Liberty University
Longwood University
Northern Virginia Community College
Virginia Commonwealth University
Virginia Military Institute
Virginia State University
Virginia Polytechnic Institute & State University

Judges

Dr. Birkita Bradford, College of Agriculture, Virginia State University
Dr. David Crosby, Cooperative Extension, Virginia State University
Dr. Pieter deHart, Department of Biology, Virginia Military Institute
Dr. Leonard Githinji, Cooperative Extension, Virginia State University
Dr. Sujan Henkanaththegedara, Department of Biological & Environmental Sciences, Longwood University
Dr. Ngowari Jaja, College of Agriculture, Virginia State University
Debra Jones, College of Agriculture, Virginia State University
Dr. Roman Miller, Biology Department, Eastern Mennonite University
Dr. Deborah Neely-Fisher, School of Science, Mathematics & Engineering J. Sargaent Reynolds Community College
Dr. Deborah O'Dell, Department of Biological Sciences, University of Mary Washington
Dr. Vitalis Temu, College of Agriculture, Virginia State University
Dr. David Torain, Department of Mathematics, Hampton University
Dr. Yixiang Xu, College of Agriculture, Virginia State University

VAS also extends special thanks to the administration, faculty and staff of Virginia State University for hosting the VAS 2015 Fall Undergraduate Research Meeting. Catering for this event was provided by Thompson Hospitality at Virginia State University.

Undergraduate Research Grants Awarded

Meghan S. Delp, Department of Animal & Poultry Sciences, Virginia Polytechnic Institute & State University
Mentor: Mark A. Cline

Project title: **Elucidating the Central Anorexigenic Mechanism of Alpha-melanocyte Stimulating Hormone**. We propose to elucidate the central mechanism of alpha-melanocyte stimulating hormone (α-MSH) using the chick as a model. A c-Fos immunohistochemistry assay, whole hypothalamus mRNA extraction, and individual hypothalamic nuclei mRNA extraction will be conducted. These procedures provide insight into the neuronal circuits regulating the anorexigenic effect of α-MSH.

Justin M. Doran, Department of Biology, Virginia Military Institute
Mentor: Pieter deHart

Project title: **Habitat-mediated Differences in the Isotopic Signatures of Arachnids**. Our project aims to determine the consequences that differing habitats have on arachnids' eating behaviors. In order to accomplish this, we will utilize stable isotope analysis to quantify trophic levels on which each is operating. We will also compare varying species in both habitats.

Angel Jair Gutarra-Leon and Vincent Cordrey, Dept. of Engineering, Northern Virginia Community College
Mentor: Walerian Majewski

Project title: **Experiments with the Electrodynamic Wheel.** The objective of the experiment is to find a conductor that is best suited for levitation by measuring differences in the lift to drag ratio of different conductors as material, design, and shape are varied. We will also be measuring how the different conductors respond to changes in temperatures and how much that affects levitation.

Dominique Richburg and Jorge Tovar, Department of Biology & Chemistry, Liberty University
Mentor: Andrew Fabich

Project title: ***Citrobacter rodentium* Competes with Commensal *E. coli* to Cause Inflammation and Alter the Intestinal Biome**. *Citrobacter rodentium* pathogenesis is commonly used as a model for studying *E. coli* in humans, since it shares 67% of its genes with the pathogenic strains of *E. coli* (EPEC and EHEC). By studying the mechanisms and genes involved in pathogenic adhesion in *C. rodentium*, it will be easier to prevent or find a cure for illness (like Crohn's disease, ulcerative colitis and colonic tumorigenesis) caused by pathogenic *E. coli* strains.

Joshua Sellwood and Nicolas Terreri, Department of Biology & Chemistry, Liberty University
Mentor: Michael Price

Project title: **Identifying Phenotypes in Overexpression of Putative Genes in *Cryptococcus neoformans***. To identify the factors responsible in or of *Cryptococcus neoformans* that allow it to successfully utilize carbon via glycolysis after deletion of pyruvate kinase gene PYK1 to broaden understanding of carbon source utilization in this human pathogen.

Instructions to Authors

All manuscripts and correspondence should be sent to the Editor (wwieland@umw.edu). The Virginia Journal of Science welcomes for consideration original articles and short notes in the various disciplines of engineering and science. Cross-disciplinary papers dealing with advancements in science and technology and the impact of these on man and society are particularly welcome. Submission of an article implies that the article has not been published elsewhere while under consideration by the Journal.

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The title, author's name, affiliation, address and e-mail should be placed on a cover page. An abstract (not to exceed 200 words) summarizing the text, particularly the results and conclusions, is required. The text should follow the general format used by professional journals in the author's discipline. The Virginia Journal of Science has an on-line style manual (www.vacadsci.org). In-text references should follow the name-year format: (McCaffrey and Dueser 1990) or (Williams et al. 1990). In the Literature Cited section at the end of the article, each reference should include the full name of the author(s), year, title of article, title of journal (do not abbreviate), volume number and first and last page of the article. For a books, include author(s), year, title, pages or number of pages, publisher and city of publication. Examples:

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